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Modélisation de la propagation du virus de l'hépatite E dans la filière porcine et évaluation de stratégies de réduction du risque d'exposition humaine

Morgane Salines

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Par

Morgane SALINES

Modélisation de la propagation du virus de l'hépatite E dans la filière porcine et évaluation de stratégies de réduction du risque d'exposition humaine

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Liste des abréviations utilisées

ACLF	<i>acute-on-chronic liver failure</i>
ADN	
(DNA)	acide désoxyribonucléique (<i>desoxyribonucleic acid</i>)
AIM	<i>Animal Introduction Model</i>
ALT	alanine aminotransférase
ANIA	Association Nationale des Industries Agro-alimentaires
Anses	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
AOM	
(MDA)	anticorps d'origine maternelle (<i>maternally-derived antibodies</i>)
AP	alcaline phosphatase
ARN	
(RNA)	acide ribonucléique (<i>ribonucleic acid</i>)
AST	aspartate aminotransférase
BPH	Bonnes Pratiques d'Hygiène
CAP	
(KAP)	connaissances-attitudes-pratiques (<i>knowledge-attitudes-practices</i>)
CDC	<i>Center for Disease Control and prevention</i>
CIMAP	Comité Interministériel de Modernisation de l'Action Publique
CNR	Centre National de Référence des hépatites à transmission entérique
CSH	cellule souche hématopoïétique
DAOA	denrée alimentaire d'origine animale
DGAI	Direction Générale de l'Alimentation
DILI	<i>drug-induced liver injury</i>
ECDC	<i>European Centre for Disease Control</i>
ECP	effet cytopathique
ECVPH	<i>European College of Veterinary Public Health</i>
EFSA	<i>European Food Safety Authority</i>
ELISA	<i>enzyme-linked immunosorbent assay</i>
EOPS	
(SPF)	Exempts d'Organismes Pathogènes Spécifiques (<i>Specific Pathogen Free</i>)
FCPR	Formation Complémentaire Par la Recherche
FICT	Fédération française des Industriels Charcutiers traiteurs et Transformateurs de viande
GBPH	Guide de Bonnes Pratiques d'Hygiène
HACCP	<i>Hazard Analysis - Critical Control Points</i> (Analyse des dangers - points critiques de contrôle)
HAI	hépatite auto-immune
HAV	virus de l'hépatite A
HBV	virus de l'hépatite B
HEV	
(VHE)	virus de l'hépatite E
HR	hazard ratio
IAA	industrie agro-alimentaire
IBM	modèle individu-centré (<i>individual-based model</i>)

IF	interféron
Ig	immunoglobuline
IMV	
(VIM)	virus immunomodulateur (<i>immunomodulating virus</i>)
INAPOR	
C	Interprofession nationale porcine
ISPV	Inspecteur de Santé Publique Vétérinaire
LAMP	<i>loop-mediated isothermal amplification assay</i> avec immunité maternelle - sensible - exposé - infecté - retiré (<i>maternally immune - susceptible - exposed - infected - retired</i>)
MSEIR	
OIE	Organisation Mondiale de la Santé animale
OMS	
(WHO)	Organisation Mondiale de la Santé (<i>World Health Organisation</i>)
OR	odds ratio
ORF	<i>open-reading frame</i>
PCR	<i>polymerase chain reaction</i>
PCV2	circovirus porcin de type 2
PMS	Plan de Maîtrise Sanitaire
PNH	primate non humain
PS	psychologie sociale
PSPC	plans de surveillance et de contrôle
R0	nombre de reproduction de base (<i>basic reproduction number</i>)
RIVM	<i>Dutch National Institute for Public Health and the Environment</i>
RR	risque relatif
RT-PCR	<i>reverse transcriptase polymerase chain reaction</i>
SDRP	syndrome dysgénésique et respiratoire porcin (<i>porcine reproductive and respiratory syndrom</i>)
(PRRS)	
SEIR	sensible - exposé - infecté - retiré (<i>susceptible - exposed - infected - retired</i>)
SIR	sensible - infecté - retiré (<i>susceptible - infected - retired</i>)
SNA	analyse de réseaux sociaux (<i>Social Network Analysis</i>)
SO	sociologie des organisations
SVA	<i>Swedish National Veterinary Institute</i>
TAR	
(TRA)	théorie de l'action raisonnée (<i>theory of reasoned action</i>)
TCP	
(TPB)	théorie du comportement planifié (<i>theory of planned behaviour</i>)
TIAC	toxi-infection alimentaire collective
TM	<i>Transit Model</i>
TMA	<i>transcription-mediated amplification</i>
VIH	virus de l'immunodéficience humaine
γGT	gamma-glutamyl transférase

Contextualisation professionnelle du projet de recherche

En complément du contexte et des objectifs scientifiques présentés dans le Chapitre I, il apparaît opportun de replacer le présent travail dans sa trajectoire professionnelle, permettant ainsi d'éclairer l'orientation stratégique et le caractère transversal du projet.

La thèse s'est déroulée dans le cadre de la Formation Complémentaire Par la Recherche (FCPR) proposée aux Inspecteurs de Santé Publique Vétérinaire (ISPV). Il s'agit de la possibilité pour des cadres du Ministère en charge de l'Agriculture d'être formés dans et par un institut de recherche dans une discipline ou sur une thématique d'intérêt pour le Ministère. Les objectifs pour le Ministère sont¹ :

- de disposer de cadres scientifiques de haut niveau pour l'administration ou les métiers d'interface comme dans les agences de sécurité ou de régulation ;
- d'alimenter en spécialistes les réseaux techniques ou scientifiques, les agences et organismes actifs en matière de transferts scientifiques et technologiques ;
- de favoriser des filières de recrutement de cadres scientifiques de haut niveau, formés en France, dans les organisations européennes et internationales ;
- d'enrichir et de développer la diversité des approches et des méthodes de travail dans les communautés de travail ;
- de comprendre et de diffuser l'effort de recherche des établissements d'enseignement supérieur au sein desquels servent traditionnellement ces cadres ;
- de former des chercheurs proches de la sphère publique, dont ces établissements ont besoin pour maintenir la diversité des cultures et des approches ;
- d'assurer une reconnaissance internationale grâce au diplôme de doctorat.

C'est dans cette perspective que s'ancre le présent travail de recherche. Outre les attentes précitées du Ministère en charge de l'Agriculture, le choix du sujet de recherche est doublement motivé. D'une part, l'émergence de la problématique dans les pays industrialisés, la gravité de

¹ Ministère de l'Agriculture, de l'Agroalimentaire et de la Forêt, Manuel des procédures à l'usage des candidats à une Formation Complémentaire Par la Recherche, décembre 2015.

la maladie sur tous les continents, et le manque de connaissances sur l'épidémiologie de ce virus dans la filière porcine font de l'hépatite E une thématique passionnante à explorer. Contribuer, même modestement, à la compréhension d'un phénomène biologique encore peu documenté ne peut qu'être épanouissant. D'autre part, ce projet allie des disciplines vastes et variées, notamment l'épidémiologie, la modélisation mathématique, et les sciences humaines et sociales. Si la première est assez naturellement étudiée par les vétérinaires, se plonger dans les suivantes constitue un véritable challenge professionnel et personnel. Ces trois années sont ainsi l'opportunité d'une prise de risque et d'une ouverture stimulantes et enrichissantes. Si la finalité de cette thèse n'est pas de devenir tout à la fois épidémiologiste, modélisateur, et sociologue, il s'agit ici d'utiliser l'épidémiologie, la modélisation et les sciences humaines et sociales pour répondre de la manière la plus exhaustive et pragmatique possible à une problématique multifactorielle de terrain. Ainsi, au-delà de ses objectifs scientifiques, les objectifs professionnels de ce projet de recherche participent sûrement d'une plus grande vivacité intellectuelle, d'une adaptabilité accrue à la diversité et la complexité des thématiques qu'un ISPV peut être amené à aborder, d'une plus forte aptitude à des prises de décision éclairées.

Ce projet de recherche s'inscrit plus largement dans une volonté d'acquérir une vaste culture scientifique dans le domaine de la santé publique vétérinaire. C'est la raison pour laquelle le diplôme de spécialiste du *European College of Veterinary Public Health* (ECVPH, Collège européen de santé publique vétérinaire) est préparé en parallèle de cette thèse. Ce résidanat se déroule sur une durée prévisionnelle de quatre ans, dans la spécialité *Food Science* afin de compléter les compétences méthodologiques acquises en médecine des populations dans le cadre de la présente thèse. La cohérence de mener de front thèse universitaire et résidanat vétérinaire réside dans la complémentarité des deux projets : la thèse, par son côté technique et appliqué, constitue une parfaite porte d'entrée dans le champ très étendu de la santé publique vétérinaire.

Introduction

Le **virus de l'hépatite E (HEV)**, virus à ARN simple brin non enveloppé, est l'agent étiologique de l'hépatite E chez l'homme. Il peut être responsable d'une **hépatite aiguë, fulminante ou chronique** en fonction, entre autres, du statut immunitaire du patient (Emerson et Purcell, 2003; Kamar *et al.*, 2014). Il n'existe à ce jour **aucun traitement de routine** de l'hépatite E et **les possibilités de vaccination sont également limitées**. Le HEV était historiquement reconnu comme étant responsable d'épidémies massives dans les pays en voie de développement avec l'implication des génotypes 1 et 2 et de quelques cas dans les pays industrialisés, majoritairement contractés à l'étranger lors de séjours en zone endémique. Ces dernières années, de plus en plus de **cas sporadiques autochtones** d'hépatite E ont été rapportés dans les pays industrialisés. Ils sont liés aux **génotypes 3 et 4**, partagés par l'homme et différentes espèces animales (Purcell et Emerson, 2008; Dalton et Izopet, 2018). En particulier, le génotype 3 est **fortement prévalent chez les suidés domestiques et sauvages** (Salines *et al.*, 2017a). La **transmission zoonotique** est prouvée et est principalement causée par les **produits à base de foie de porc lorsqu'ils sont consommés crus ou insuffisamment cuits**. Dans les pays industrialisés, l'hépatite E est ainsi une **thématique Une santé (One Health)**, en tant qu'infection zoonotique transmise majoritairement par voie alimentaire (Pavio *et al.*, 2017).

En France, le nombre de cas d'hépatite E diagnostiqués chez l'homme est en **constante augmentation**, notamment en raison de la plus grande sensibilisation des médecins à la maladie et donc de l'accroissement des demandes diagnostiques. Ainsi, **plus de 2 200 cas autochtones** ont été rapportés au Centre National de Référence des hépatites à transmission entérique (CNR) en 2017 contre seulement une dizaine en 2002², ce qui rend la situation préoccupante, notamment en l'absence de traitement et de vaccination efficaces et sûrs à ce jour. La séroprévalence du HEV chez les donneurs de sang peut aller jusqu'à plus de 70 % dans certaines régions de France, suggérant ainsi une forte exposition et un nombre important d'infections asymptomatiques (Mansuy *et al.*, 2016). Le HEV peut ainsi être considéré comme **endémique** en France, voire hyper-endémique dans certaines régions du pays (Mansuy *et al.*, 2016). C'est

² CNR, 2015. "Plan du rapport annuel d'activité. Année d'exercice 2014. Centre national de référence - virus des hépatites à transmission entérique.", disponible à <http://www.cnrvha-vhe.org/>

dans ce contexte que le rapport sur l'évaluation de la politique sanitaire et de l'alimentation, demandé par le comité interministériel de modernisation de l'action publique (CIMAP) en 2014, souligne l'**importance de développer les programmes de recherche relatifs au HEV**.³ En particulier, étant donné la **forte prévalence du HEV dans les élevages porcins français** (avec une circulation virale dans 65 % des élevages et chez plus de 30 % des porcs) et le **risque lié à la consommation de certains produits à base de porc** (avec 4 % des foies de porc dans les abattoirs français contenant du HEV) (Rose *et al.*, 2011), il apparaît essentiel d'**approfondir les connaissances scientifiques sur l'épidémiologie du HEV dans les élevages de porcs** afin de **proposer un plan de maîtrise du virus dans la filière porcine**.

L'élaboration de stratégies de gestion d'un pathogène dans une filière de production animale nécessite une **approche transdisciplinaire** incluant des domaines d'expertise variés : (i) une **expertise épidémiologique** avec une description de la propagation de l'agent pathogène et une compréhension de l'infection grâce à l'identification des facteurs d'introduction, de diffusion et de persistance ; (ii) une **dimension économique** avec une évaluation des coûts liés à la mise en place d'un programme de contrôle ; (iii) une **approche sociale** prenant en compte les opinions et les contraintes des différentes parties prenantes et les conséquences sociétales des mesures mises en place.

C'est dans ce contexte qu'il est apparu nécessaire et pertinent :

- ✓ de **comprendre les modalités de la persistance et la propagation du HEV dans un élevage de porcs naisseur-engraisseur** et d'identifier les facteurs de variation. Cette partie du projet s'est appuyée conjointement sur des essais expérimentaux, des études observationnelles en conditions réelles, et un modèle mathématique multi-pathogènes représentant les interactions entre l'infection par le HEV et des pathogènes impliqués en santé animale ayant une activité immunomodulatrice.
- ✓ de **comprendre la diffusion du HEV au sein de la filière porcine** (tout au long de la pyramide de production sélectionneur - multiplicateur - producteur et jusqu'aux abattoirs), d'identifier les facteurs de persistance du virus au sein de cette filière et de prédire le risque d'entrée de produits contaminés par le HEV dans la chaîne alimentaire. Un modèle de

³ Babusiaux C., Guillou M., 2014. "La politique de sécurité sanitaire des aliments. Diagnostic et propositions à l'attention de Mesdames et Messieurs les ministres de l'Economie et des Finances, de l'Economie sociale et solidaire et de la Consommation, des Affaires Sociales et de la Santé, de l'Agriculture, l'agroalimentaire et de la forêt."

diffusion inter-troupeaux du HEV a ainsi été développé en s'appuyant sur des données rétrospectives de mouvements d'animaux entre élevages et vers l'abattoir.

✓ **d'élaborer un plan de maîtrise efficace et réalisable du HEV dans la filière porcine.**

L'efficacité de stratégies de maîtrise applicables à chaque maillon de la filière a été évaluée grâce aux modèles développés dans les deux premières parties. Conjointement, des enquêtes ont été réalisées auprès des différentes parties prenantes de la filière pour évaluer la faisabilité technique et l'acceptabilité de différents scénarii de maîtrise identifiés.

Dans le premier chapitre du manuscrit, une **synthèse de la bibliographie** apporte, dans un premier temps, des éléments de caractérisation du HEV, de la maladie chez l'homme et des risques de transmission zoonotique, et dans un second temps des données quant à l'épidémiologie descriptive et analytique du HEV dans la filière porcine. Les trois chapitres suivants (chapitres II à IV) s'attachent à rapporter les **études conduites durant la thèse** et correspondent chacun à un volet du projet de recherche. Le dernier chapitre présente une **discussion générale** mettant en perspective les différents volets de la thèse et dégageant des pistes de réflexion générale sur les suites pouvant être données à ces résultats.

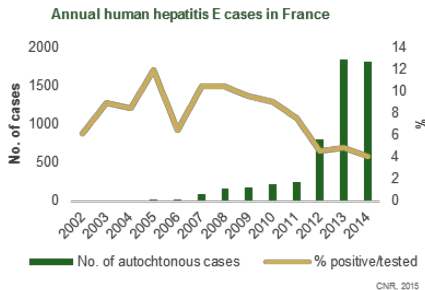
Pour faciliter la lecture et la compréhension de ce manuscrit, des **résumés en français et en anglais** ont été établis à la fin de chaque grande sous-partie au sein des chapitres. Les **publications** dans des revues internationales à comité de lecture ont été insérées dans le corps du manuscrit, tandis que les publications en français et autres communications sont présentées en annexes. La liste de l'ensemble des valorisations associées à la thèse est présentée en **Annexe 1**. Une introduction graphique en anglais est proposée en **Figure 1**.

HEPATITIS E: SAFER FARMS FOR SAFER FOOD

Deciphering the epidemiology of hepatitis E virus within the swine reservoir to support public health risk mitigation strategies



ANSES | French agency for food, environmental & occupational health and safety



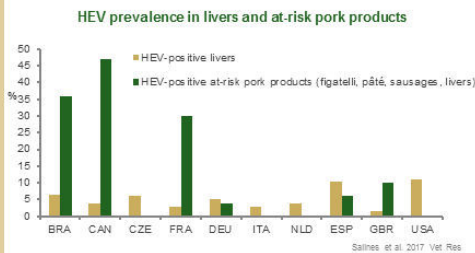
Abstract. Hepatitis E is an **emerging foodborne zoonosis** for which the number of cases constantly increases, with **more than 2,000 human cases reported in France in 2016**. Consumption of raw or undercooked pork products is responsible for the majority of hepatitis E cases in humans, **pigs being therefore considered as the main reservoir**. This research project aims at **managing HEV in pig farms in order to reduce the risk of human HEV exposure** through the consumption of pork products. Combining two epidemiological models and a socio-economical study on the whole pork chain, this **innovative research project** will enable to **gain insight on this still little-known issue** and to **support risk management decisions**.



CONTEXT

Hepatitis E Virus (HEV) is responsible for an **emerging and severe foodborne zoonosis** related to the consumption of at-risk pork products:

- **High HEV prevalence in the swine reservoir**
- **Unexplained variability** of infection dynamics in pigs
- **Worrying prevalence in pork products**



➔ **Critical research needs to understand HEV epidemiology and to develop efficient control measures in the pork production sector**



METHODS

1



Within-herd epidemiological model

Individual-based multi-pathogen model

- ➔ Describe HEV infection dynamics
- ➔ Understand factors influencing its variation
- ➔ Focus on interactions between HEV and immunomodulatory swine diseases (e.g. PRRSV)

2



Between-herd epidemiological model

Social network analysis

- ➔ Understand and forecast HEV spread and persistence along the pig production chain
- ➔ Predict quantitatively the presence of HEV-positive foodstuffs in the food chain

3



Risk mitigation strategies

Modelling & socio-economic approaches

- ➔ Assess the efficacy of several surveillance and control strategies
- ➔ Evaluate the feasibility and the acceptability of these measures
- ➔ Support food policies



EXPECTED IMPACT

- Addressing HEV issue with a **“One health” approach from farm to fork, combining epidemiological modelling and social sciences**, will enable to **efficiently support risk mitigation policies**.

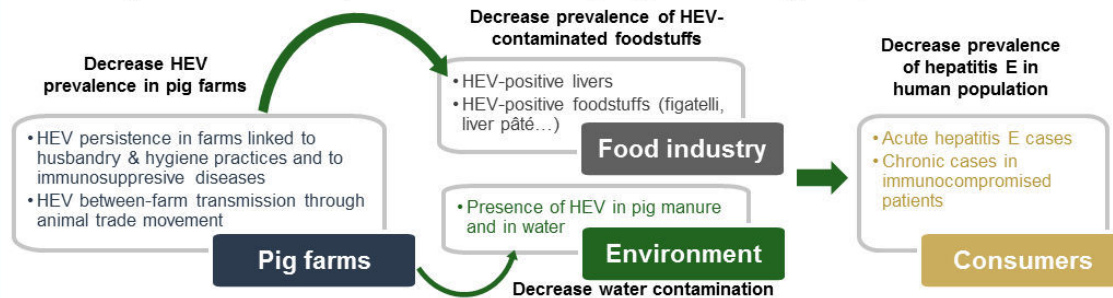


Figure 1 - Présentation du projet de recherche : introduction graphique



Chapitre I

Etat de l'art et stratégie d'approche

PARTIE 1.1. Le virus de l'hépatite E, du porc à l'homme

I. Le virus de l'hépatite E : caractéristiques générales et maladie associée chez l'homme

I.1. Brève approche historique de l'hépatite E

Des premières descriptions de jaunisse 2 000 ans avant notre ère à la caractérisation des hépatites virales au XX^{ème} siècle, l'histoire des hépatites est longue. Dès 1947, McCallum différencie deux grands types d'hépatites : l'une transmissible par voie orale, l'autre par voie parentérale, qu'il nomme respectivement hépatite A et hépatite B. L'identification du virus de l'hépatite B (HBV) par Baruch Blumberg dans les années 1960 et du virus de l'hépatite A (HAV) par Stephen Feinstone dans les années 1970 permet de mettre en évidence l'existence de cas d'hépatites virales causés ni par le HAV ni par le HBV (Feinstone *et al.*, 1975). Ces **hépatites dites « non A - non B »** se présentent elles-mêmes sous deux formes différentes : (i) les hépatites non A - non B à transmission parentérale et à évolution chronique, présentes dans les pays industrialisés et dans les pays en voie de développement, et dont l'agent responsable, le virus de l'hépatite C, est identifié en 1989 ; (ii) les hépatites non A - non B à transmission féco-orale, circulant majoritairement sous forme de larges épidémies dans les pays en voie de développement, et dont l'agent responsable, **le virus de l'hépatite E (HEV), est décrit en 1983 par Mikhail Balayan** (Balayan *et al.*, 1983). Virologue russe, Balayan conduit une expérience consistant en l'ingestion volontaire d'un filtrat de selles provenant d'un patient ayant présenté une hépatite de type épidémique non-A à Tashkent en Asie centrale. Balayan développe alors une hépatite aiguë et observe des particules de 27 à 32 nm de diamètre en microscopie électronique dans ses propres selles. Il classe alors le virus dans la famille des Picornaviridae, en le décrivant comme filtrable et transmissible. Un modèle animal est alors développé, avec transmission de l'agent infectieux chez des primates non humains (PNH) (Abe *et al.*, 1986). En 1989, l'équipe de Daniel Bradley, qui avait développé le modèle animal d'infection du virus non A - non B chez le macaque, publie la découverte de l'agent viral responsable des hépatites

non A - non B de type entérique : le virus de l'hépatite E (Krawczynski et Bradley, 1989; Purcell, 1993). C'est en 1991 que la structure génomique du HEV est caractérisée par des techniques de clonage et de séquençage par Tam *et al.* (1991). En 2002, éclairée par la structure et l'organisation génomique du virus, l'équipe d'Emerson classe le HEV dans la famille des Hepeviridae (Emerson et Purcell, 2004).

Si la caractérisation moléculaire et génomique du HEV a été tardive, le virus était déjà responsable à la fois d'épidémies d'hépatites aiguës mais également de cas sporadiques depuis plusieurs décennies (Kamar *et al.*, 2014). Les **zones dites « endémo-épidémiques »** se caractérisent par un faible niveau d'hygiène et un accès rare à l'eau potable. Par exemple, en octobre 1955, des pluies diluviennes entraînèrent une contamination d'une station de pompage de la banlieue de New Delhi, alimentant un million d'habitants en eau potable. Entre décembre 1955 et janvier 1956, 29 300 habitants de la région développèrent une hépatite aiguë (Viswanathan, 2013). Rétrospectivement, il a été montré qu'il s'agissait d'un virus d'hépatite non A - non B, probablement le HEV (Chuttani *et al.*, 1966; Wong *et al.*, 1980). Le HEV a ensuite été incriminé dans d'autres épidémies, notamment au Cachemire, en Afrique et au Mexique. Plus récemment, en 2004, une épidémie d'hépatite E a eu lieu au Soudan et au Tchad, avec 6 861 cas et 87 décès, et 1 442 cas et 46 décès, respectivement.⁴ Des **cas sporadiques importés** ont également été décrits dans les pays industrialisés, généralement liés aux voyages et à l'immigration depuis les zones endémiques (Kamar *et al.*, 2014).

Des **cas sporadiques autochtones** ont également été rapportés en Europe, au Japon et aux Etats-Unis (Kamar *et al.*, 2014). Des souches génétiquement différentes des souches endémiques ont été isolées et des souches animales ont été caractérisées. En particulier, en 1995, des anticorps anti-HEV et des ARN (acide ribonucléique) du HEV ont été mis en évidence chez des porcs au Népal (Clayson *et al.*, 1995), puis une souche animale du HEV a été isolée et caractérisée chez un porc aux Etats-Unis (Meng *et al.*, 1997). C'est dans ce contexte que l'hypothèse de « maladie exotique » a progressivement été remise en cause, pour laisser place à celle de « **maladie émergente** ». Ainsi, alors que les épidémies d'hépatite E dans les pays en voie de développement sont liées à l'eau souillée, les cas sporadiques semblent s'expliquer par la présence d'une transmission inter-espèces du virus et l'existence d'un ou de plusieurs réservoirs animaux (Pavio *et al.*, 2017).

⁴ World Health Organization, 2004. Hepatitis E in Sudan - update 3. Hepatitis E in Chad - update 4. Disponibles à : http://www.who.int/csr/don/2004_09_28/en/ et http://www.who.int/csr/don/2004_09_27a/en/

I.2. Etiologie de l'hépatite E

I.2.a. Caractéristiques générales du virus de l'hépatite E

- (i) Structure du virus de l'hépatite E et de son génome

Le HEV est un **virus sphérique, non enveloppé, à symétrie icosaédrique, de 32 à 34 nm de diamètre** (Tam *et al.*, 1991). Le génome du HEV est constitué d'un **ARN monocaténaire de polarité positive** d'une longueur d'environ **7,2 kilobases**, terminé aux extrémités par deux séquences non codantes impliquées probablement dans la régulation de la réplication virale (Figure 2). Il comporte **trois Open-Reading Frame (ORF, Cadre Ouvert de Lecture)** (Purcell et Emerson, 2008). L'ORF1 code des protéines non structurales (environ 1 700 acides aminés) impliquées dans la réplication virale, la transcription et la coupure des polyprotéines (Kaur *et al.*, 1992; Koonin *et al.*, 1992; Sehgal *et al.*, 2006; Suppiah *et al.*, 2011). L'ORF2 code la protéine constituant la capsid (660 acides aminés). Elle est impliquée dans l'assemblage du virus, l'encapsidation et la réponse immunitaire de l'hôte au virus (Jameel *et al.*, 1996; Zafrullah *et al.*, 1999; Surjit *et al.*, 2004). L'ORF3 code, lui, pour une petite phosphoprotéine de 113 acides aminés, VP13 (Tang *et al.*, 2011). Le rôle de la protéine VP13 n'est pas encore complètement élucidé mais, en plus d'être associée au cytosquelette, elle **réduirait la réponse inflammatoire de l'hôte** et protégerait les cellules infectées par le virus (Graff *et al.*, 2005; Tang *et al.*, 2011). Elle interagit avec différentes protéines cellulaires et jouerait un rôle dans les voies d'induction de l'interféron et la libération des virions (Takahashi *et al.*, 2008; Dong *et al.*, 2012; Nan *et al.*, 2014; Xu *et al.*, 2014; Osterman *et al.*, 2015; Ding *et al.*, 2017). L'ORF3 pourrait également inhiber la réponse inflammatoire en **réduisant la phagocytose par les macrophages** via la régulation négative de l'expression des protéines CD14 et CD64 (Lei *et al.*, 2019). Récemment, un **nouvel ORF** a été découvert dans le génome des souches de HEV-1 : l'ORF4 code une protéine exprimée en cas de stress du réticulum endoplasmique ; associée à d'autres protéines, elle forme un complexe capable d'augmenter la réplication virale (Nair *et al.*, 2016).

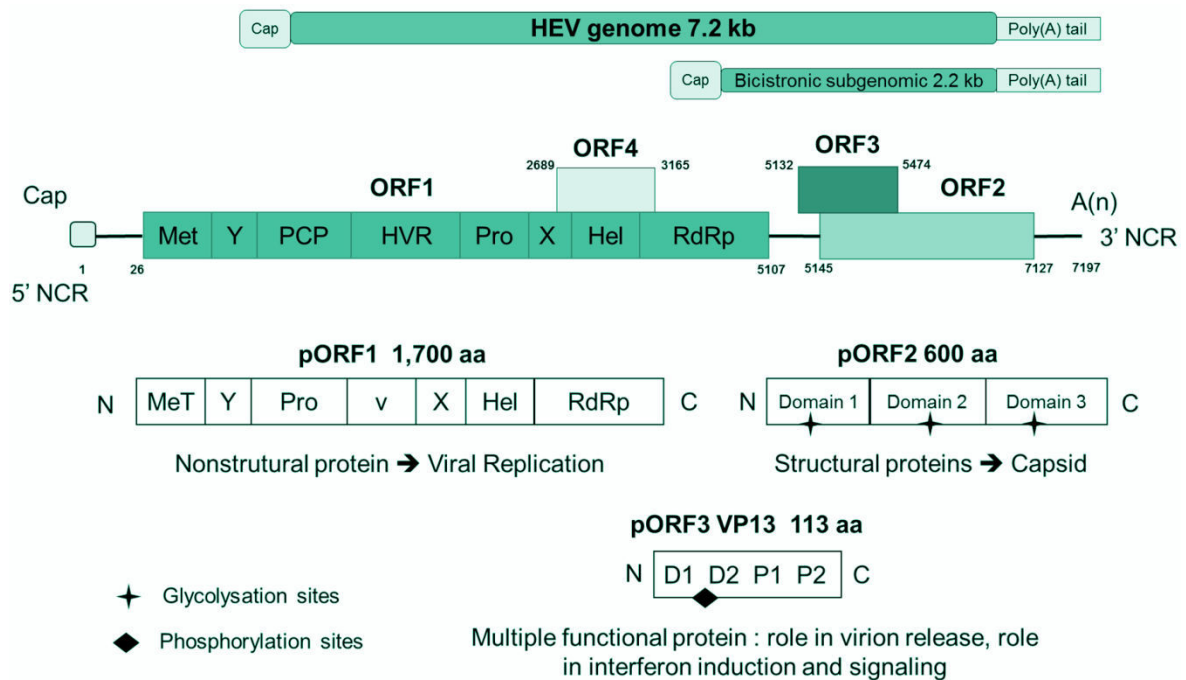


Figure 2 - Organisation du génome du virus de l'hépatite E et structure protéique associée

Source : d'après Capai *et al.* (2018)

(ii) Cycle de réplication virale

Les mécanismes hypothétiques de réplication virale du HEV sont présentés dans la Figure 3. Le virus se multiplie **principalement dans les hépatocytes** mais un intermédiaire de la réplication (ARN de polarité négative) a été détecté dans le **colon, l'intestin et les nœuds lymphatiques de porcs** (Williams *et al.*, 2001) ainsi que dans le **liquide cérébro-spinal et le cœur de patients infectés** (Kamar *et al.*, 2010b). Le récepteur cellulaire du HEV n'est pas encore connu mais la présence de **protéoglycanes à héparane sulfate**, associée à des protéines de stress (*Heat Shock Proteins Hsc 70*), apparaissent nécessaires pour l'attachement du virus aux cellules cibles (*Etapes 2 et 3*) (Zhou et Emerson, 2006; Kalia *et al.*, 2009). Ensuite, le HEV entre dans la cellule par endocytose (*Etape 4*) (Kapur *et al.*, 2012; Holla *et al.*, 2015). L'ARN viral est libéré dans le cytoplasme (*Etape 5*) et est traduit en une protéine non structurale de l'ORF1 (pORF1) (*Etape 6*). L'ARN génomique de polarité positive est ensuite copié en un brin d'ARN de polarité négative (*Etape 7*), qui permet ensuite la synthèse d'ARN génomiques et sous-génomiques (*Etapes 8a et 8b*) (Purdy *et al.*, 1993b). Les ORF2 et ORF3 sont traduits (*Etape 9*) pour produire les protéines structurales qui permettent l'encapsidation des brins d'ARN nouvellement produits (*Etape 10*) (Graff *et al.*, 2006; Yamada *et al.*, 2009). Les virions

quittent alors la cellule par des protéines de l'ORF3 fixées sur les membranes endoplasmiques (*Etape 11a*) ou sur la paroi cellulaire (*Etape 11b*). Les virions sont libérés à l'extérieur de la cellule (*Etape 12*), soit sous **forme quasi-enveloppée** en étant attachés à des protéines de l'ORF3 et des lipides (dans le sang), soit sous forme libre (dans la bile) (Nagashima *et al.*, 2011; Nagashima *et al.*, 2014).

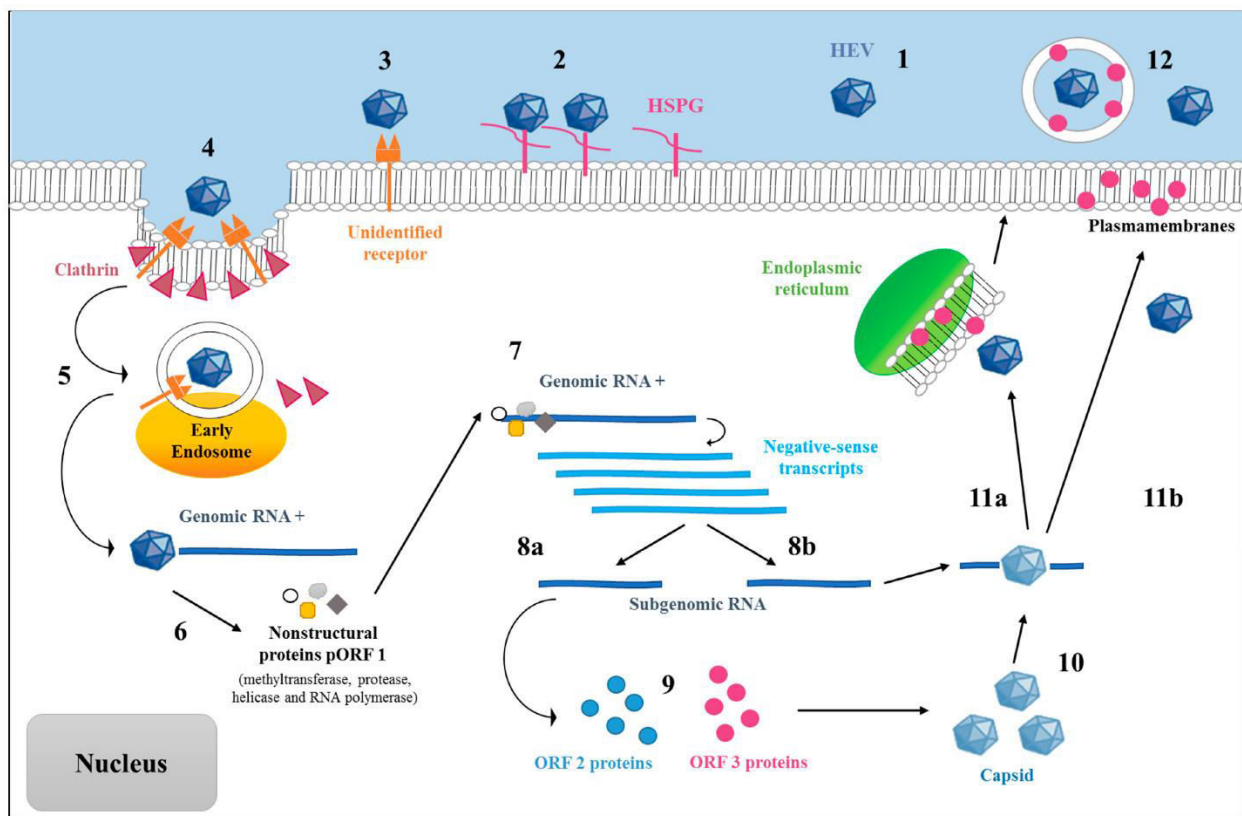


Figure 3 - Cycle de réplication supposé du virus de l'hépatite E

Source : Capai *et al.* (2018)

1.2.b. Diversité du virus de l'hépatite E et taxonomie

Le virus de l'hépatite E appartient à la famille des **Hepeviridae** et au genre des *Orthohepevirus*, qui comprend quatre espèces (A à D). Les souches de HEV humaines et zoonotiques font partie de l'espèce *Orthohepevirus A*, qui comprend huit génotypes (Figure 4) (Smith *et al.*, 2014).

Les génotypes 1 et 2 n'infectent que l'homme, ils sont génétiquement proches (76 % d'homologie). Si le HEV-1 est bien décrit et comprend six sous-types (1a à 1f), le HEV-2 est

moins bien connu et est divisé en deux sous-types (2a et 2b) (Smith *et al.*, 2016). Les souches de génotype 1 circulent principalement en Asie, en Afrique et au Mexique, celles de génotype 2 sont principalement retrouvées au Mexique, au Tchad et au Nigeria (Huang *et al.*, 1992; Buisson *et al.*, 2000; Nicand *et al.*, 2005; Lu *et al.*, 2006; Zehender *et al.*, 2014).

Les génotypes 3 et 4 sont partagés par l'homme et plusieurs espèces animales. Le **génotype 3** est le mieux décrit et le plus documenté dans Genbank. Il comprend dix sous-types (3a à 3j) et deux clades (3abchij et 3efg) (Oliveira-Filho *et al.*, 2013; Ijaz *et al.*, 2014; Smith *et al.*, 2015; Vina-Rodriguez *et al.*, 2015; Smith *et al.*, 2016). D'autres souches de génotype 3 forment un sous-type nommé sous-type 3ra, car elles infectent le lapin (Smith *et al.*, 2016). Ce sous-type inclut aussi une souche isolée à partir d'un cas humain d'hépatite E en France (Izopet *et al.*, 2012). De **nouveaux isolats** sont régulièrement séquencés et **la classification des sous-types du HEV est évolutive**. Ainsi, très récemment, deux nouveaux sous-types ont été proposés. Le premier, dénommé 3l, correspond à des souches de HEV isolées chez des porcs italiens (De Sabato *et al.*, 2018) ; le second, dénommé 3s, correspond à une souche de HEV isolée du foie d'un porc en Suisse (Wist *et al.*, 2018). Au vu des dernières avancées moléculaires et phylogénétiques, le **réseau HEVnet** a d'ailleurs été récemment créé. Il s'agit d'un réseau de laboratoires européens, coordonné par le RIVM (*Dutch National Institute for Public Health and the Environment*), qui a pour objectif le partage des données moléculaires relatives au HEV. Cette collaboration vise notamment à étudier les évolutions et la diffusion du virus, à conduire des études d'attribution de source et d'épidémiologie moléculaire, à mieux comprendre les relations entre le type de souches et les différentes formes cliniques d'hépatite E décrites chez l'homme (Mulder *et al.*, 2019).

Le **génotype 4** est divisé en neuf sous-types (4a à 4i), principalement isolés chez le **sanglier, le porc et l'homme** (Pavio *et al.*, 2017). Le HEV-4 a aussi été détecté chez les ovins, les bovins et les caprins en Chine mais il n'est pas encore déterminé si ces espèces sont des réservoirs du HEV-4 ou des hôtes accidentels (Wu *et al.*, 2015b; Wu *et al.*, 2015a).

Les génotypes 5 et 6 n'ont été détectés que chez les sangliers, aucune infection humaine par ces génotypes n'a été rapportée (Doceul *et al.*, 2016). Néanmoins, Li *et al.* (2019) ont récemment montré qu'il était possible d'infecter expérimentalement des macaques crabiers avec une souche de HEV-5. Le génotype 7 comprend trois séquences, incomplètes. Deux d'entre elles ont été isolées chez des chameaux (Woo *et al.*, 2014), la troisième provient d'un

patient humain transplanté consommant régulièrement de la viande et du lait de chameau (Lee *et al.*, 2016). D'autres souches isolées chez des chameaux en Chine ont été classifiées comme appartenant au génotype 8 (Woo *et al.*, 2016).

Les souches d'*Orthohepevirus B* ont été isolées chez des poulets ; elles sont divisées en quatre génotypes, avec une large répartition géographique et peu de divergence génétique (Huang *et al.*, 2004; Bilic *et al.*, 2009; Smith *et al.*, 2016). Les souches d'*Orthohepevirus C* ont été détectées chez le rat, des pérégrins et la musaraigne des maisons (C1) ainsi que chez des furets et des visons (C2) (Guan *et al.*, 2013; Krog *et al.*, 2013; Li *et al.*, 2013; Smith *et al.*, 2016). L'espèce *Orthohepevirus D* n'inclut que le HEV de la chauve-souris (Drexler *et al.*, 2012). Récemment, une autre souche de HEV a été caractérisée chez des faucons en Europe, et pourrait constituer une nouvelle espèce d'*Orthohepevirus* (Reuter *et al.*, 2016).

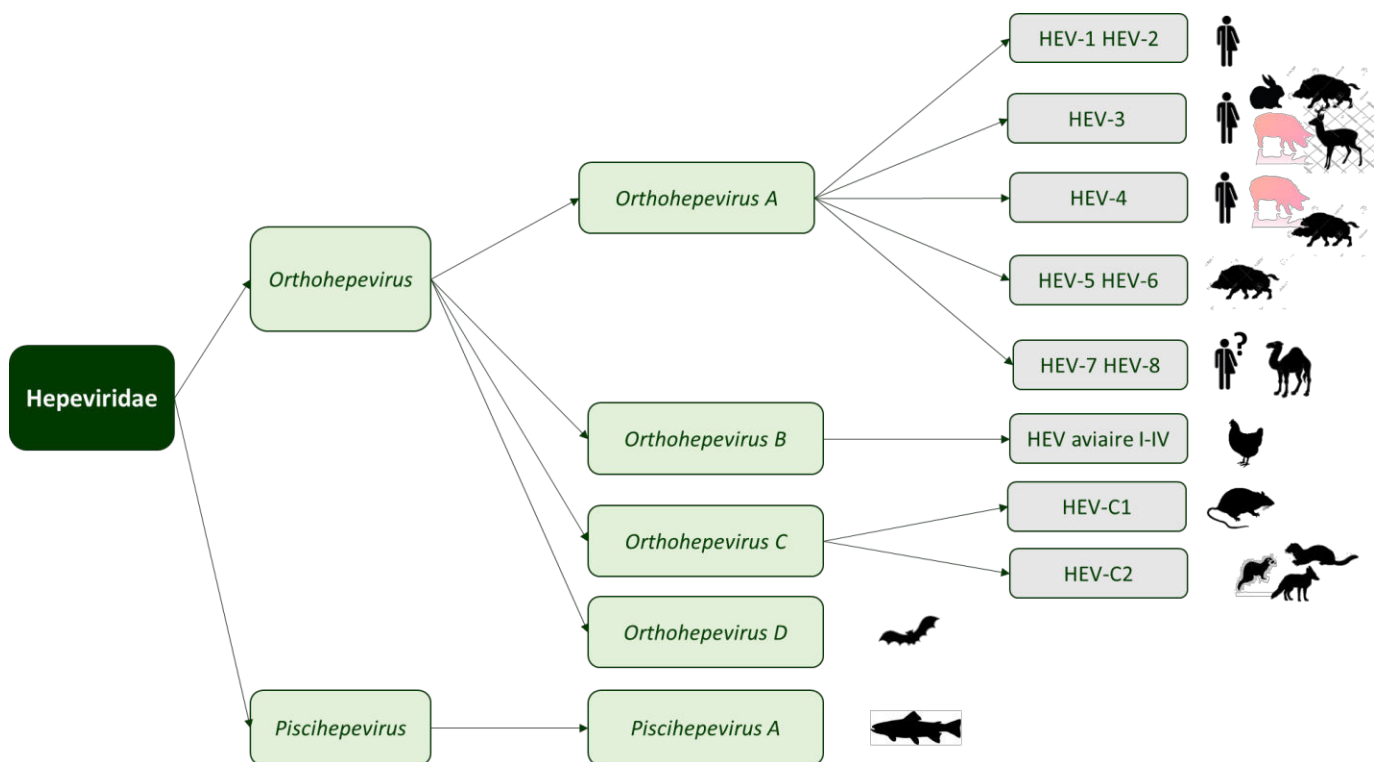


Figure 4 - Classification phylogénétique des génotypes de la famille des Hepeviridae

I.2.c. Méthodes d'étude in vitro et in vivo du virus de l'hépatite E

(i) Culture cellulaire du virus de l'hépatite E

Le HEV est un virus **extrêmement difficile à cultiver *in vitro***, notamment parce qu'il se réplique à des titres très bas. Des essais plus ou moins fructueux de production de HEV *in vitro* ont été réalisés par de nombreuses équipes de recherche, d'abord en utilisant des hépatocytes primaires de primates non humaines (PNH, chimpanzés, macaques crabiers, tamarins) puis à partir de diverses lignées cellulaires humaines, comme des cellules hépatiques embryonnaires (WRL68), des cellules d'hépatomes (PLC/PRF/5, HepG2 et Huh-7), des cellules de carcinomes de colon (Caco-2), des cellules diploïdes embryonnaires pulmonaires (2BS), des cellules de fibroblastes embryonnaires pulmonaires (MRC-5), des cellules cancéreuses pulmonaires (A549). Les deux lignées les plus utilisées sont les **lignées A549 et PLC/PRF/5**.

La réplication du HEV dans les cellules de culture peut être suivie par différentes techniques de détection, notamment l'**observation de l'effet cytopathique (ECP)**, la **détection des ARN viraux ou des protéines virales**. Lorsqu'un ECP peut être observé visuellement, l'essai est relativement facile à mettre en œuvre. Néanmoins, avec certaines lignées cellulaires, il n'y a pas d'ECP, ou la mise en place de l'ECP est longue, ce qui nécessite d'amplifier l'ARN viral par RT-PCR (*Reverse Transcriptase Polymerase Chain Reaction*) ou de détecter des protéines virales par immunofluorescence ; l'essai est alors plus **long et coûteux** à mettre en place. Bien que certaines lignées cellulaires (PLC/PRF/5 et A549) aient été utilisées avec succès dans plusieurs publications indépendantes, **des études de répétabilité et de reproductibilité inter-laboratoires manquent encore actuellement**. De plus, la plupart des systèmes de culture cellulaire du HEV **ne permet pas d'obtenir des concentrations élevées de HEV infectieux** dans le surnageant.

L'une des principales applications de la culture *in vitro* du HEV est **l'évaluation de l'infectiosité des particules virales détectées dans les aliments**. En effet, les systèmes de culture cellulaire pourraient permettre de mesurer de manière non-ambiguë l'infectiosité du HEV ; l'utilisation de réplicats et d'analyses simultanées d'un grand nombre d'échantillons pourrait conduire à un plus grand degré de confiance dans les résultats qu'en utilisant des expérimentations animales. De plus, la culture *in vitro* devrait être plus facile à standardiser que les essais sur animaux, tout en étant moins coûteuse et plus éthique. Néanmoins, les cellules

cultivées peuvent être détériorées par des substances co-extraites des produits à base de porc lors du traitement des échantillons (Cook *et al.*, 2017), et seulement trois études ont réussi à isoler du HEV de produits porcins en utilisant des systèmes de culture cellulaire (Takahashi *et al.*, 2012; Berto *et al.*, 2013a; Berto *et al.*, 2013b). En particulier, Berto *et al.* (2013b) ont construit un système sophistiqué de culture cellulaire en trois dimensions, grâce auquel ils ont pu détecter la réplication virale par RT-PCR ; cette méthode s'est avérée reproductible dans deux laboratoires. A ce jour, les systèmes de culture *in vitro* du HEV sont donc considérés comme une **approche très prometteuse** pour évaluer le potentiel infectieux du HEV détecté dans les aliments. Les efforts de recherche sont actuellement dirigés vers le **développement de systèmes de culture cellulaire validés, répétables et reproductibles** (Cook *et al.*, 2017).

(ii) Modèles animaux de l'infection humaine par le virus de l'hépatite E

Notamment du fait de la difficulté des approches *in vitro*, de nombreux modèles animaux du HEV ont été développés, soit chez des **PNH**, soit chez des **porcs** ou **petits animaux**. Les modèles animaux⁵ présentent de nombreux avantages : ils permettent d'étudier la pathogénicité, de comprendre les mécanismes de réplication virale, de tester des vaccins ou traitements.

Plusieurs espèces de PNH sont sensibles à l'infection par le HEV (chimpanzés, singes rhésus, tamarins, *etc.*) (Vital *et al.*, 1998). L'infection et la transmission naturelles inter-singes du HEV dans un groupe de PNH ont été montrées au Japon (Yamamoto *et al.*, 2012). De plus, des infections expérimentales par des souches HEV-1 à HEV-4 ont été réalisées chez des macaques rhésus et des macaques crabiers et ont servi à développer les **premiers modèles de l'infection par le HEV** et à **caractériser les réponses clinique et immunitaire** à l'infection, qui sont **similaires chez les PNH et l'homme** (Bradley *et al.*, 1987; Longer *et al.*, 1993; Tsarev *et al.*, 1993; Tsarev *et al.*, 1995; Erker *et al.*, 1999; Aggarwal *et al.*, 2001; Li *et al.*, 2006; Ma *et al.*, 2009). Ces modèles ont également été utilisés pour **tester l'efficacité de vaccins** (Purdy *et al.*, 1993a; Tsarev *et al.*, 1994) et **évaluer le potentiel zoonotique de différentes souches virales** (Meng *et al.*, 1998; Arankalle *et al.*, 2006; Liu *et al.*, 2013; Li *et al.*, 2015).

⁵ « En recherche biomédicale, un modèle animal est un modèle permettant l'étude de données de référence sur la biologie ou le comportement, ou chez lequel on peut étudier un processus pathologique spontané ou induit, celui-ci ayant un ou plusieurs aspects communs avec un phénomène équivalent chez l'humain ou d'autres espèces animales. » *American National Research Council Committee on Animal Models for Research and Aging.*

Des souches porcines de HEV ont été identifiées pour la première fois en 1997 (Meng *et al.*, 1997) ; le porc est un **hôte naturel** du HEV-3 et HEV-4. Les **modèles porcins** servent essentiellement à étudier la **transmission inter-espèces** du HEV – expérimentale ou naturelle, par exemple de l’homme au porc (Meng *et al.*, 1998; Halbur *et al.*, 2001; Feagins *et al.*, 2008b), du sanglier au porc (Schlosser *et al.*, 2014; Schlosser *et al.*, 2015), du lapin au porc (Cossaboom *et al.*, 2012). En revanche, l’infection par le HEV chez le porc diffère de celle chez l’homme : par exemple, le porc ne présente aucun signe clinique après une infection par le HEV, ni d’élévation des enzymes hépatiques. **Le modèle porcin est donc peu approprié pour l’étude de la pathogénie du HEV ni pour le développement de solutions thérapeutiques ou prophylactiques.** Le modèle porcin pourrait néanmoins permettre d’étudier les **hépatites E chroniques** chez l’homme, puisqu’il a été montré que, dans certaines circonstances, le porc pouvait développer une infection chronique par le HEV (Salines *et al.*, 2015b). De même, le lapin ne développe pas d’hépatite clinique après une infection par le HEV. Ainsi, de tels modèles sont utilisés principalement pour l’étude de l’infection causée par des souches cunicoles de HEV et pour l’évaluation de vaccins adaptés à ces souches (Cheng *et al.*, 2012). La pertinence des modèles rat et furet est également discutée, notamment car les souches de HEV infectant ces espèces sont éloignées des souches humaines, ce qui limite les perspectives en termes thérapeutiques et prophylactiques (Doceul *et al.*, 2016).

D’autres modèles ont été développés en utilisant des animaux qui ne sont pas des hôtes naturels du HEV. Par exemple, la **gerbille de Mongolie** (*Meriones unguiculatus*), espèce couramment utilisée en expérimentation animale, pourrait constituer un modèle animal intéressant pour l’étude de la réplication du HEV et de sa pathogénie. En effet, plusieurs études ont conduit avec succès des infections expérimentales de gerbilles de Mongolie avec une souche de HEV-4 provenant de foie de porc contaminés (Li *et al.*, 2009b; Yang *et al.*, 2015). Le virus a été détecté dans le sang et les fèces mais aussi dans le foie, les reins, la rate, et le petit intestin. De plus, les modifications histopathologiques du foie des gerbilles infectées se sont révélées similaires à celles décrites chez l’homme ; de même, l’augmentation des enzymes hépatiques s’est avérée cohérente avec les analyses biochimiques humaines. Il a aussi été montré que les souches porcines de HEV-4 pouvaient se répliquer dans le cerveau et la moelle épinière des gerbilles, ce qui pourrait permettre d’utiliser ces animaux pour étudier les manifestations neurologiques de l’infection par le HEV chez l’homme (Shi *et al.*, 2016). Récemment, Hong *et al.* (2015) ont réussi à infecter des gerbilles de Mongolie avec une souche humaine de HEV-1 isolée chez un patient atteint d’hépatite E aiguë ; le tableau clinique et histopathologique

présenté par les gerbilles était alors cohérent avec les descriptions chez l'homme. Ainsi, **la gerbille de Mongolie pourrait être un modèle animal prometteur pour étudier l'infection par le HEV-1 et HEV-4 chez l'homme.**

Les souris sont également souvent utilisées comme modèles d'infections virales. Cependant, comme les porcs, les souris ne présentent pas d'hépatite clinique après inoculation par le HEV (Doceul *et al.*, 2016). Récemment, des **modèles murins ayant un foie humanisé (*human liver chimeric mice*)** ont été développés. Ainsi, Allweiss *et al.* (2016) ont repeuplé le foie de souris avec des hépatocytes humains, et les souris ont ensuite été inoculées avec des souches de HEV-1 ou HEV-3 provenant de patients infectés par le HEV. Les auteurs décrivent une virémie et une excrétion fécale du HEV chez ces souris chimériques, ainsi qu'une transmission virale entre les souris. De plus, il a été montré que des souris humanisées pouvaient développer une hépatite E chronique (van de Garde *et al.*, 2016; Sayed *et al.*, 2017). Enfin, plusieurs études ont montré que le traitement de souris humanisées infectées par le HEV avec de la ribavirine conduisait à une diminution de la charge virale dans le sang, les fèces et le foie (Allweiss *et al.*, 2016; Sayed *et al.*, 2017). Ainsi, **les modèles murins humanisés ouvrent des perspectives prometteuses pour l'étude des hépatites chroniques et l'évaluation de solutions thérapeutiques.**

1.2.d. Résistance du virus de l'hépatite E

Non enveloppé, le HEV est malgré tout relativement **résistant dans le milieu extérieur.**⁶ Il est **sensible aux désinfectants usuels** (hypochlorite de sodium, glutaraldéhyde), bien que la présence de matières organiques diminue de manière significative l'efficacité de ces désinfectants. Il résiste également à l'acidité gastrique et aux sels biliaires de l'homme et des animaux.

La **résistance thermique du HEV** a été évaluée de différentes manières, notamment par des approches *in vivo* du fait de la difficulté à cultiver le virus *in vitro*. En suivant la

⁶ EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Fernandez Escamez PS, Herman L, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Di Bartolo I, Johne R, Pavio N, Rutjes S, van der Poel W, Vasickova P, Hempen M, Messens W, Rizzi V, Latronico F and Girones R, 2017. Scientific Opinion on the public health risks associated with hepatitis E virus (HEV) as a food-borne pathogen. EFSA Journal 2017;15(7):4886, 89 pp. <https://doi.org/10.2903/j.efsa.2017.4886>

séroconversion de porcs inoculés expérimentalement avec une suspension de cubes de foie contenant une souche de HEV-3 et ayant subi différents traitements thermiques, Feagins *et al.* (2008a) ont montré que l'incubation à 56°C pendant une heure n'affectait pas l'infectiosité du HEV, mais que la suspension n'était plus infectieuse après un traitement thermique à 191°C pendant 5 minutes (frit, température interne de 71°C) ou à 100°C (dans l'eau bouillante). En chauffant des suspensions fécales de HEV à des températures comprises entre 45°C et 70°C et après inoculation dans des systèmes de culture cellulaire, Emerson *et al.* (2005) ont obtenu des résultats similaires. Récemment, Imagawa *et al.* (2018) ont prouvé que des souches de HEV présentes dans de la **viande de porc** pouvaient être inactivées par un chauffage à **70°C pendant 5 minutes** et que la cuisson au bain-marie était plus efficace que la cuisson au grill. L'inactivation était plus rapidement obtenue à partir des échantillons de foie de porc que dans la longe de porc, et pour les souches de génotype 3 que les souches de génotype 4. Une autre étude, conduite sur une **matrice plus complexe**, un pâté de foie préparé selon une recette industrielle, montre qu'un **traitement à 71°C pendant 20 minutes** est nécessaire pour obtenir une inactivation complète du HEV (Barnaud *et al.*, 2012). En utilisant des modèles de culture cellulaire, Johne *et al.* (2016) ont montré que des particules virales infectieuses pouvaient résister jusqu'à **28 jours à température ambiante, et jusqu'à 56 jours à 4°C**. De plus, un traitement thermique à **70°C pendant 2 minutes** permet d'éliminer le HEV de ces cultures cellulaires.

A ce jour, **l'efficacité du séchage et de la salaison des produits alimentaires n'a pas été évaluée.**



Ce qu'il faut retenir

Le virus de l'hépatite E est un virus à ARN non enveloppé, qui présente une grande diversité génétique. Parmi les huit génotypes majeurs, les génotypes 3 et 4 sont les principaux génotypes zoonotiques. Les méthodes d'étude de ce virus sont délicates et les modèles *in vivo* et *in vitro* sont en constant développement.

Le virus est relativement résistant dans l'environnement et aux traitements thermiques.



Take home message

Hepatitis E virus is a non-enveloped RNA virus with a high genetic diversity. Of the eight major genotypes, genotypes 3 and 4 are the main zoonotic ones. The methods for studying this virus are tricky and *in vivo* and *in vitro* models are constantly being improved. The virus is relatively resistant in the environment and to heat treatments.

I.3. L'hépatite E chez l'homme

I.3.a. Epidémiologie descriptive de l'hépatite E chez l'homme

(i) Distribution du virus de l'hépatite E dans le monde

La distribution du HEV dans le monde est **hétérogène**, à la fois en termes de génotype circulant, de forme épidémiologique, et de prévalence (Figure 5). Les génotypes 1 et 2 circulent sous **forme endémo-épidémique** dans les pays en voie de développement où la fourniture en eau potable et l'assainissement ne sont pas maîtrisés. Le génotype 1 touche principalement l'Asie, l'Afrique et l'Amérique centrale, tandis que le génotype 2 affecte majoritairement les populations du Mexique, du Nigéria et du Tchad (Emerson et Purcell, 2003). Les génotypes 3 et 4, quant à eux, sont majoritairement responsables de **cas sporadiques autochtones** dans les pays développés (Etats-Unis, Europe, Taïwan, Japon, *etc.*), survenant principalement par consommation de produits contaminés provenant d'animaux réservoirs du HEV (Pavio *et al.*, 2017; Dalton et Izopet, 2018).

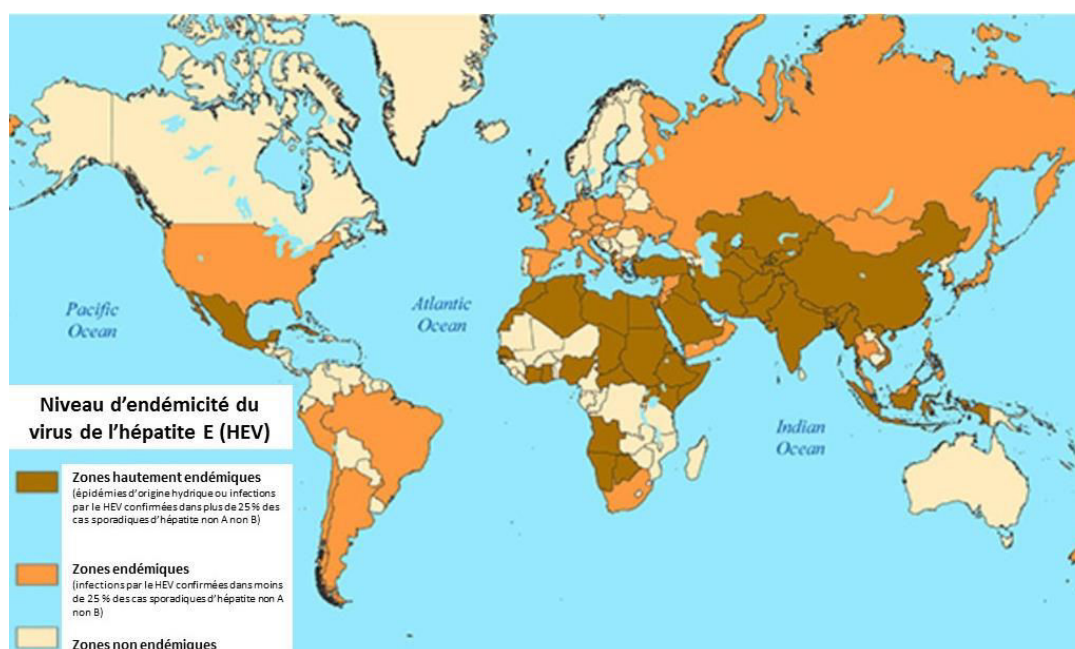


Figure 5 - Niveau d'endémicité du virus de l'hépatite E dans le monde

Source : d'après Center for Disease Control and prevention⁷

⁷ CDC, Center for Disease Control and prevention, disponible à : <https://www.cdc.gov/hepatitis/hev>

(ii) Données de séroprévalence et prévalence dans la population générale dans les pays industrialisés

La séroprévalence du HEV (Immunoglobulines G (IgG) anti-HEV) dans la population générale est classiquement estimée à partir d'échantillons de sera de donneurs de sang. A partir de ces données, Dalton et Izopet (2018) ont proposé de classer les pays industrialisés en **trois catégories selon leur niveau d'endémicité** : élevée (séroprévalence > 20 %), intermédiaire (10-20 %), faible (< 10 %). Une recherche bibliographique sur la période 2010-2018 met en évidence une **grande disparité** de la prévalence des IgG anti-HEV dans la population de donneurs de sang de divers pays industrialisés (Figure 6). Dans certains pays, la circulation du HEV peut ainsi être qualifiée d'**endémo-sporadique**. Néanmoins, ces études ont utilisé des tests sérologiques différents, ainsi que des tailles d'échantillons variables ; leur comparaison doit ainsi se faire avec prudence. Une récente méta-analyse, conduite à partir de 26 études provenant de 15 pays, a permis de calculer une séroprévalence globale de 19 % [14-25] dans les pays industrialisés (Capai *et al.*, 2019). La **prévalence chez les donneurs de sang** a été évaluée dans plusieurs pays, par exemple en Angleterre, où **2,6 pour 10 000** échantillons de sang contenaient de l'ARN du HEV sur la période 2016-2017 (Harvala *et al.*, 2019).

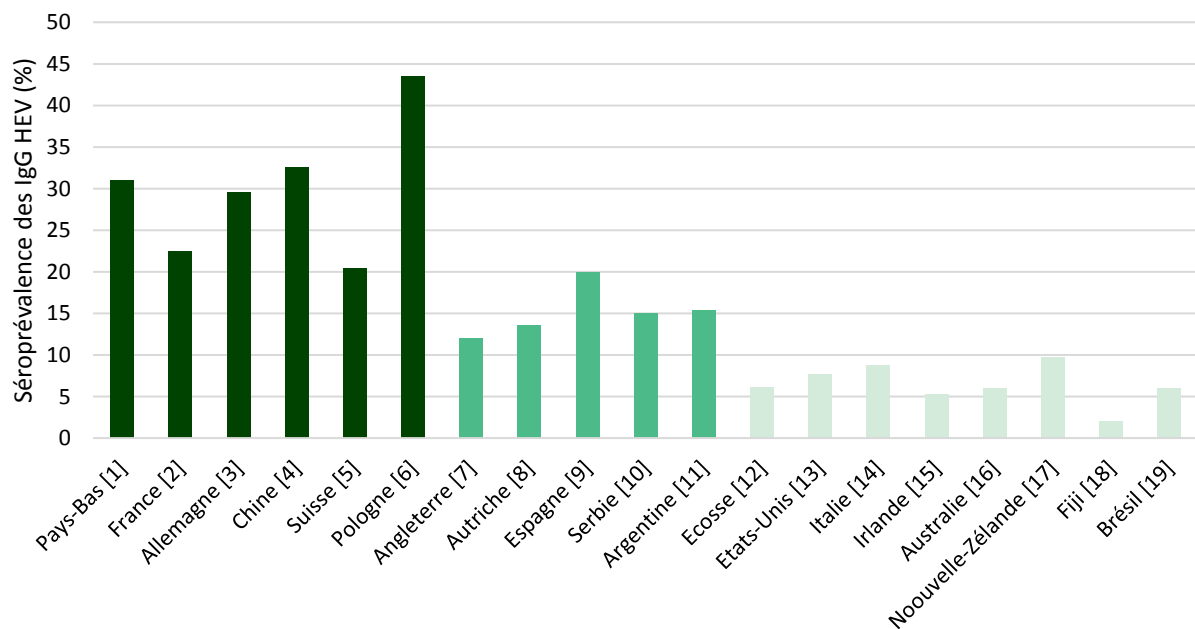


Figure 6 - Séroprévalence du virus de l'hépatite E chez les donneurs de sang dans les pays industrialisés de niveau d'endémicité élevé (séroprévalence > 20 %), intermédiaire (10-20 %) et faible (< 10 %)

Sources : [1] Mooij *et al.* (2018) [2] Mansuy *et al.* (2016) [3] Vollmer *et al.* (2012) [4] Guo *et al.* (2010) [5] Niederhauser *et al.* (2018) [6] Grabarczyk *et al.* (2018) [7] Beale *et al.* (2011) [8] Fischer *et al.* (2015) [9] Sauleda *et al.* (2015) [10] Petrovic *et al.* (2014) [11] Munne *et al.* (2014) [12] Thom *et al.* (2018) [13] Stramer *et al.* (2016) [14] Spada *et al.* (2018) [15] O’Riordan *et al.* (2016) [16] Shrestha *et al.* (2014) [17] Hewitt *et al.* (2018) [18] Halliday *et al.* (2014) [19] Tengan *et al.* (2019)

Par ailleurs, la plupart des études montre que la séroprévalence HEV est plus élevée chez les **hommes de plus de 50 ans** que dans le reste de la population générale (Dalton *et al.*, 2007b; Mansuy *et al.*, 2009a). Aucune explication n’est à ce jour avancée.

L’ECDC (*European Centre for Disease Control*) rapporte une augmentation du nombre de cas en Europe de 514 en 2005 à 5 617 en 2015, avec un total de 21 018 cas déclarés sur l’ensemble de la période. La France, le Royaume-Uni et l’Allemagne concentrent à eux seuls 80 % des cas (Aspinall *et al.*, 2017).

(iii) Quelle situation en France ?

En France, la **séroprévalence du HEV** dans la population générale a récemment été estimée à **22,4 %** [21,6-23,2] à partir de 10 569 échantillons de sérum de donneurs de sang (Mansuy *et al.*, 2016). Plusieurs études montrent l’**existence d’un gradient Nord-Sud**. En effet, la séroprévalence des donneurs de sang est comprise entre 34,0 et 52,5 % dans les régions du Sud-Ouest de la France (Mansuy *et al.*, 2011; Izopet *et al.*, 2015) contre 3,2 % pour les régions Ile-de-France et Pays de la Loire (Boutrouille *et al.*, 2007). Il convient cependant de noter que les tests sérologiques utilisés dans les deux études ne sont pas identiques, ceux de Boutrouille *et al.* (2007) se révélant moins sensibles. Ces données conduisent à qualifier la France de **pays endémique** vis-à-vis du HEV, voire **hyper-endémique dans certaines zones géographiques comme le Sud-Ouest**.

En 2014, une étude réalisée en France à partir de 57 101 échantillons de sérum de donneurs de sang a estimé la **prévalence du HEV à 2,65 pour 10 000** [1,6-3,7] soit 1/3800 dons de sang retrouvés ARN-HEV positifs (Pillonel *et al.*, 2014).

En France, le **nombre de cas rapportés au CNR en 2017** s’élève à 2 245, dont **2 219 cas autochtones** (Figure 7). Depuis 2007, plus de 90 % des souches autochtones étaient de

génotype 3 (Couturier *et al.*, 2018). De 2002 à 2011, le nombre total de cas rapportés au CNR a régulièrement augmenté (13 *versus* 266), avec ensuite une très forte augmentation entre 2012 et 2017. L'augmentation portait essentiellement sur le nombre de cas autochtones (**9 en 2002 *versus* 2 219 en 2017**). Cette incidence croissante du HEV est à mettre en relation avec le nombre de tests diagnostiques réalisés. En effet, en 15 ans de surveillance de l'hépatite E (2002-2016), le nombre de personnes pour lesquelles des échantillons ont été adressés pour un diagnostic d'hépatite E a augmenté de façon exponentielle (209 *versus* 76 000). Ainsi, à partir de 2010, la disponibilité des tests diagnostiques, une meilleure connaissance de l'hépatite E, et la nomination d'un nouveau CNR en 2012 ont entraîné une **augmentation considérable du nombre de personnes testées**, d'où une incidence croissante du nombre de cas autochtones. Au cours de la période 2002-2011, la **proportion de cas** diagnostiqués parmi les personnes testées est restée relativement stable, entre 6 et 10 % puis à partir de 2012, une **tendance à la diminution** a été observée, de 5 à 3 %.

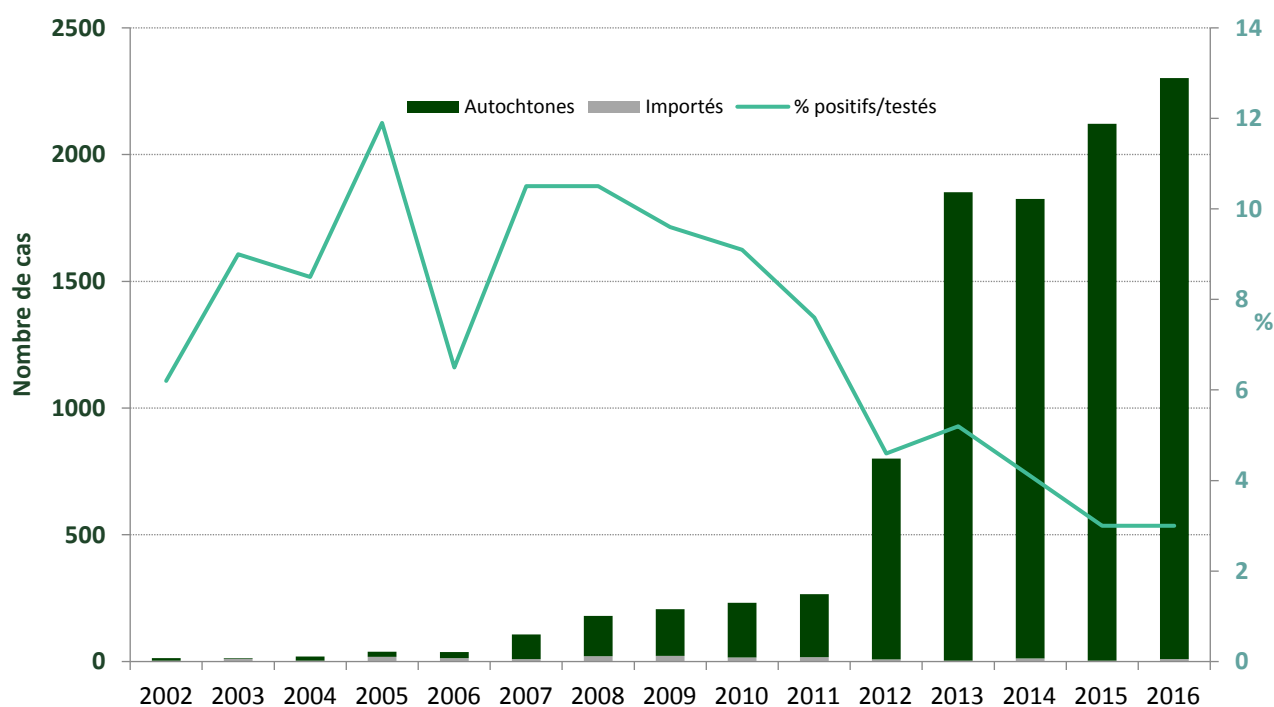


Figure 7 - Nombre de cas d'hépatite E diagnostiqués par an, en France métropolitaine, entre 2012 et 2016

Source : Centre National de Référence des hépatites à transmission entérique⁸

⁸ CNR, Centre National de Référence des hépatites à transmission entérique, disponible à : <http://www.cnrvhvhe.org/>

Le nombre de personnes hospitalisées pour cas d'hépatite E en France a augmenté sur la période considérée (de 57 en 2002 à 653 en 2016) (Couturier *et al.*, 2018). Les caractéristiques des cas autochtones en termes de sexe ou d'âge ont peu évolué depuis 2002. Les cas autochtones étaient plutôt des **hommes, âgés de 50 ans** et plus en moyenne. Tout comme les données de séroprévalence, les cas d'hépatite E n'ont pas une répartition géographique homogène sur le territoire français et suivent un **gradient Nord-Sud (Figure 8)**.

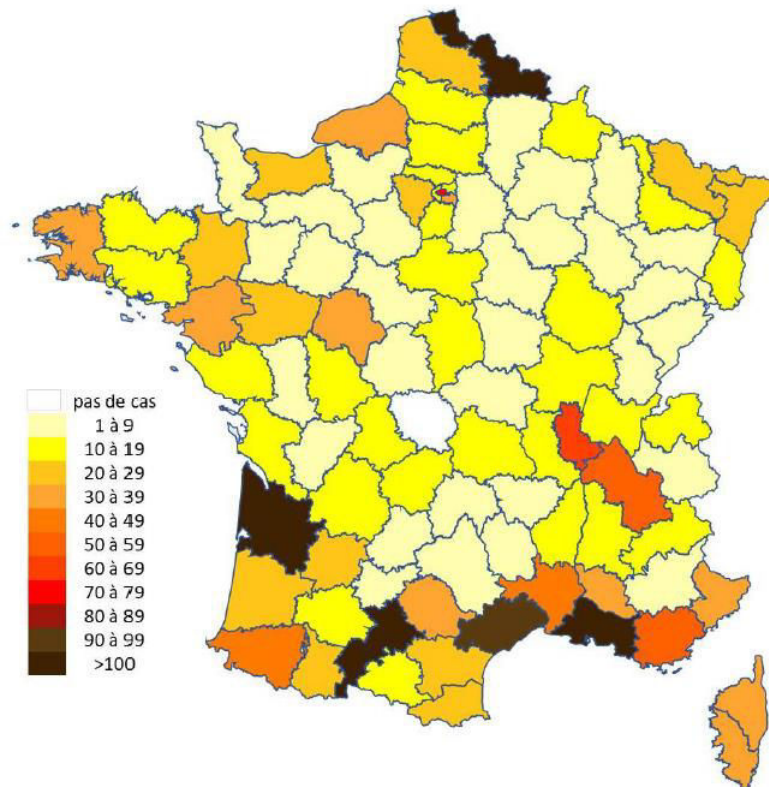


Figure 8 - Répartition géographique des cas d'hépatite E rapportés au CNR en 2017

Source : Rapport annuel d'activité du Centre national de référence des virus des hépatites à transmission entérique, 2018, année d'exercice 2017

Récemment, une équipe de chercheurs a développé un modèle statistique ayant pour objectif d'estimer le nombre de cas, d'hospitalisation et de décès dus, chaque année, à des pathogènes d'origine alimentaire (Van Cauteren *et al.*, 2017). Dans le cas du HEV, ce modèle utilise, entre autres, les données de prévalence et séroprévalence issues des enquêtes nationales précitées ainsi que la proportion d'infections asymptomatiques lors de toxi-infections alimentaires collectives (TIAC). A partir de ce modèle, les auteurs ont estimé le nombre de cas d'hépatite E en France sur la période 2008-2013 à **68 007 infections par an** [46 032-101 279],

dont **546 hospitalisations** [540-553] et **20 décès** [18-22]. Le fossé entre le nombre de cas rapportés au CNR et le nombre d'infections estimé dans cette publication peut notamment s'expliquer par la forte proportion d'infections asymptomatiques et la sous-déclaration des cas (méconnaissance, confusion avec une hépatite médicamenteuse...).

1.3.b. Manifestations cliniques de l'hépatite E

L'infection par le HEV peut conduire à différentes formes d'hépatite E, comme présenté dans la Figure 9.

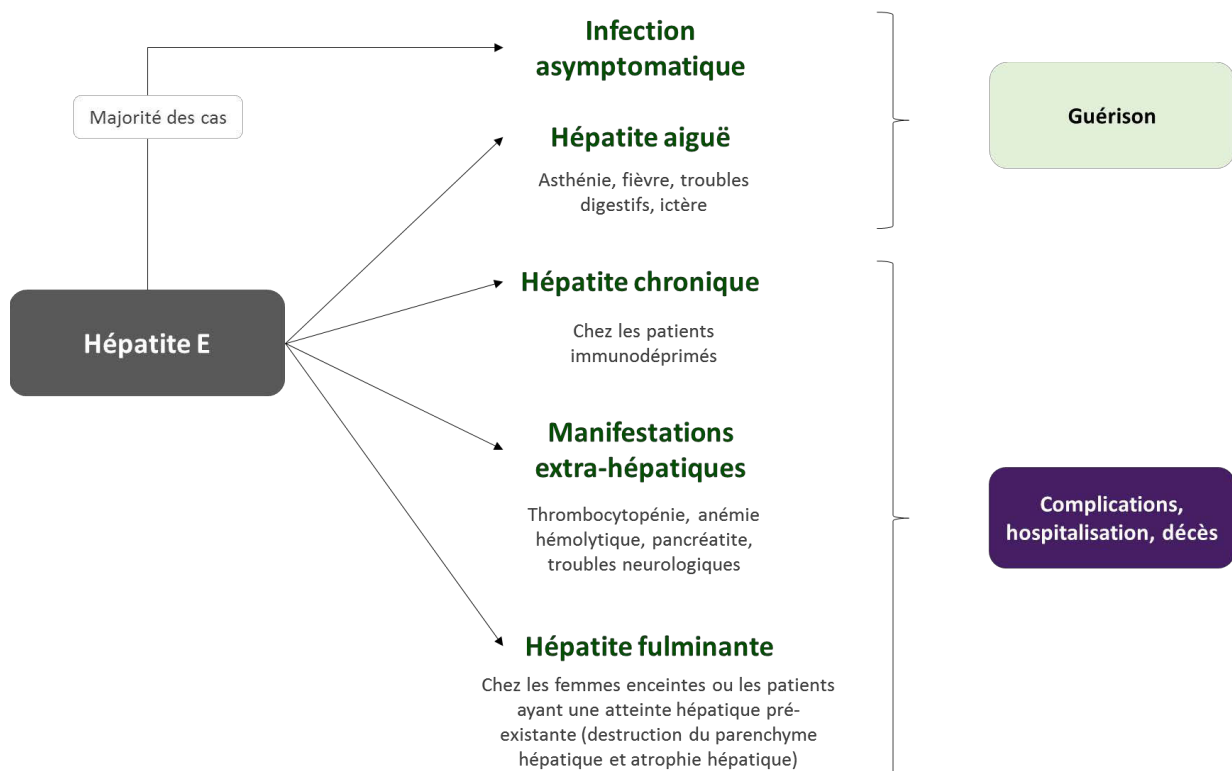


Figure 9 - Formes cliniques possibles d'une infection par le virus de l'hépatite E

(i) Formes asymptomatiques et aiguës

L'infection par le HEV est le **plus fréquemment asymptomatique**. Plusieurs publications ont estimé la proportion d'infections asymptomatiques lors de cas groupés d'infections par le HEV : en Chine, Zhang *et al.* (2016) rapportent 78,6 % d'infections

asymptomatiques (11/14) lors d'un cas groupé dans la province de Shandong ; lors d'un cas groupé sur un bateau de croisière, 66,6 % des personnes infectées (22/33) ne présentaient pas de manifestation clinique d'hépatite (Said *et al.*, 2009) ; en France, des infections groupées lors d'un repas de mariage montrent que 70,6 % des infections (12/17) étaient asymptomatiques (Guillois *et al.*, 2016).

L'infection par le HEV peut également se traduire par une **hépatite aiguë**. La période d'incubation est comprise entre 15 jours et 9 semaines, avec une **incubation moyenne de 40 jours**. Les symptômes suivants sont généralement décrits pendant la phase prodromique : **asthénie, fièvre, arthralgie, troubles digestifs** (diarrhée, vomissements, douleurs abdominales) pendant plusieurs jours. Ensuite, dans 75 % des cas, une **phase ictérique** survient pendant deux semaines. Les tests de laboratoire détectent généralement une augmentation des niveaux d'alanine et aspartate aminotransférase (ALT, AST), ainsi que de l'alcaline phosphatase (AP), des gamma-glutamyl transférases (γ GT) et de la bilirubine. Dans la majorité des cas, la maladie est **spontanément résolutive**, avec une **évolution favorable dans les 4 à 6 semaines** (Wedemeyer *et al.*, 2012).

Par ailleurs, il a été récemment montré que des auto-anticorps pouvaient être produits lors d'hépatite E aiguë (Terziroli Beretta-Piccoli *et al.*, 2018), ce qui peut conduire à envisager de manière erronée un diagnostic d'hépatite auto-immune (HAI). La part des hépatites E dans les HAI est encore inconnue, mais l'hépatite E doit être exclue avant de traiter une HAI avec des traitements immunosuppresseurs, car de tels traitements peuvent conduire à une hépatite E chronique (*cf. infra*).

(ii) Formes fulminantes

Dans 2 à 3 % des cas, les hépatites aiguës peuvent évoluer vers une **forme fulminante** (Kamar *et al.*, 2014). Les premiers cas d'hépatite fulminante ont été rapportés en Italie, en Espagne, en France et au Japon (Suzuki *et al.*, 2002; Sainokami *et al.*, 2004; Mateos Lindemann *et al.*, 2010; Mateos-Lindemann *et al.*, 2013; Doudier *et al.*, 2014; Festa *et al.*, 2014; Doudier *et al.*, 2015). Les hépatites fulminantes surviennent généralement chez des patients présentant une **pathologie hépatique préexistante et chez les femmes enceintes** (Kamar *et al.*, 2014). Ces formes peuvent nécessiter une transplantation hépatique et conduisent parfois au décès. En Inde, l'étude d'une large cohorte de patients souffrant d'une pathologie hépatique chronique a

montré que le pronostic de patients ayant une décompensation hépatique du fait d'une infection par le HEV était significativement moins bon que pour des patients dont la décompensation était due à une autre cause (Kumar Acharya *et al.*, 2007). Une étude française a montré que l'infection d'un patient par le génotype 4 du HEV conduisait à des signes cliniques particulièrement sévères (Jeblaoui *et al.*, 2013), mais des études incluant un plus grand nombre de cas seraient nécessaires pour étudier l'existence éventuelle d'un lien entre le génotype impliqué et la gravité de la maladie.

Les formes aiguës et fulminantes d'hépatite E peuvent être confondues avec une hépatite médicamenteuse, ou *drug-induced liver injury* (DILI), car les manifestations cliniques sont similaires. Néanmoins, le diagnostic d'une DILI repose sur plusieurs critères : une relation temporelle entre le démarrage d'un traitement et le développement d'une hépatite (entre cinq et 30 jours), une relation temporelle entre l'arrêt du traitement et la résolution de l'hépatite, et l'exclusion des autres causes possibles d'hépatite (Kamar *et al.*, 2014). Une étude anglaise et une étude américaine ont montré qu'une DILI avait été diagnostiquée de manière erronée chez 13 % et 3 % respectivement d'un groupe de patients qui étaient en fait atteints d'hépatite E (Dalton *et al.*, 2007a; Davern *et al.*, 2011).

(iii) Formes chroniques

Les hépatites E chroniques se caractérisent par une **virémie persistant** entre 3 et 6 mois après le diagnostic (Kamar *et al.*, 2011b). Les niveaux d'AST et d'ALT sont également moins élevés chez les patients développant une hépatite E chronique que ceux atteints d'une forme aiguë (Murali *et al.*, 2015). Les formes chroniques d'hépatite E sont principalement décrites chez les **patients immunodéprimés en raison d'une greffe d'organe, d'une hémopathie maligne ou d'autres pathologies avec immunodépression** (infection par le virus de l'immunodéficience humaine (VIH), *etc.*) (Peron *et al.*, 2006; Tamura *et al.*, 2007; Kamar *et al.*, 2008; Dalton *et al.*, 2009; Behrendt *et al.*, 2014). La majorité des hépatites E chroniques sont dues au génotype 3 mais récemment, des formes chroniques liées à des souches de génotype 4 ont été rapportées, avec une évolution rapide vers la cirrhose et le rejet de greffe (Geng *et al.*, 2014).

(iv) Manifestations extra-hépatiques

Le HEV peut également être responsable de **symptômes extra-hépatiques**. Des **troubles neurologiques** ont notamment été décrits lors d'infections par le HEV-1 et le HEV-3 : **syndrome de Guillain-Barré, de Parsonage-Turner**, paralysie faciale *a frigore*, méningoencéphalite aiguë, myélite transverse aiguë (Kamar *et al.*, 2014). Belbezier *et al.* (2018) ont récemment conduit une revue systématique de la littérature et rapportent 130 cas de troubles neurologiques décrits entre 2000 et 2017. La majorité des cas ont été rapportés en Europe ou en Asie et étaient secondaires à une infection par une souche de génotype 3. L'âge médian était de 52 ans, il s'agissait principalement d'hommes, non immunodéprimés. Les syndromes de Guillain-Barré étaient les plus fréquemment rapportés (54 cas), suivis par les syndromes de Parsonage-Turner (35 cas), les méningites et/ou méningoencéphalites (9 cas) et diverses mononeuropathies (6 cas). Une analyse rétrospective de 126 patients infectés par le HEV a aussi montré que 7 patients (5,5 %) avaient présenté des symptômes neurologiques, dont 3 patients immunocompétents, 3 ayant reçu une greffe et un patient séropositif VIH (Kamar *et al.*, 2011a). Récemment, une étude rétrospective conduite à partir des cas rapportés au CNR français en 2015 a montré que 16,5 % des patients atteints d'hépatite E (33/200) avaient présenté des symptômes neurologiques, dont 14 avec des douleurs neuropathiques suggérant une neuropathie des petites fibres, 9 avec des troubles sensoriels, 6 présentant le syndrome de Parsonage-Turner, 1 le syndrome de Guillain-Barré, 1 une méningite, 1 une encéphalite et 1 une diplopie. Les manifestations neurologiques étaient plus fréquentes chez les patients immunocompétents (22,6 % *versus* 3,2 %) (Abravanel *et al.*, 2018b). En étudiant les séquences retrouvées dans le fluide cébrospinal et le sérum d'un patient atteint d'hépatite E chronique associée à des troubles neurologiques, une étude a mis en évidence une compartimentation de quasi-espèces (*i.e.* des souches présentant des différences génomiques retrouvées dans le sang et le fluide cébrospinal) ; ceci suggère que les manifestations neurologiques liées à l'infection par le HEV pourraient être liées à l'**émergence de variants neurotropiques** (Kamar *et al.*, 2010b).

Des **troubles rénaux** sont également décrits à la fois lors d'hépatite E aiguë et chronique, avec une atteinte glomérulaire (glomérulonéphrite membrano-proliférative ou membranaire) (Kamar *et al.*, 2005; Kamar *et al.*, 2012). Ces manifestations rénales ont été observées tant chez des patients immunocompétents que chez des patients ayant reçu une transplantation rénale ou hépatique. Des **pancréatites aiguës** ont également été décrites chez des patients infectés par le

HEV-1 (Bhagat *et al.*, 2008; Thapa *et al.*, 2009; Deniel *et al.*, 2011). Des **troubles hématologiques** (thrombocytopénie et anémie aplastique) peuvent aussi survenir (Colson *et al.*, 2008; Fourquet *et al.*, 2010; Shah *et al.*, 2012).

(v) L'infection par le HEV chez les femmes enceintes

Dans les pays en voie de développement, les infections par le HEV-1 et HEV-2 entraînent des formes graves d'hépatite chez les femmes enceintes. Pour exemple, Labrique *et al.* (2012) ont estimé le nombre de décès de femmes enceintes attribuables au HEV au Bangladesh à 1 000 par an. Dans ces zones hautement endémiques, **la mortalité en cas d'infection par le HEV est de 20-25 %** et survient généralement pendant le troisième trimestre (Labrique *et al.*, 2010). Le taux de transmission verticale est également élevé (Khuroo *et al.*, 1995). Dans les pays industrialisés, quelques cas d'hépatite E liés au HEV-3 ont été rapportés chez des femmes enceintes. **Plusieurs cas ont été décrits en France, sans complications** chez les mères ni chez les bébés (Anty *et al.*, 2012; Bouthry *et al.*, 2018). Une étude prospective en France à partir d'une cohorte de 315 femmes enceintes a estimé la prévalence du HEV chez les femmes enceintes à 7,7 % (Renou *et al.*, 2014a). Le HEV-3 et HEV-4 ne semblent cependant pas conduire à des infections fatales ou des hépatites fulminantes chez les femmes enceintes contrairement au génotype 2 (Lachish *et al.*, 2015).

1.3.c. Pathogénie et réponse immunitaire lors d'infection par le virus de l'hépatite E

Lors d'hépatite E aiguë, la virémie persiste généralement moins d'un mois (Figure 10). L'apparition des anticorps anti-HEV et des signes cliniques suit la phase de virémie (Walker, 2018). Plusieurs études ont suggéré que les signes cliniques de l'hépatite E seraient davantage liés à la réponse immunitaire qu'aux dommages cellulaires causés par le virus (Krain *et al.*, 2014). Ceci serait également confirmé par l'augmentation du titre en anticorps et la décroissance de la charge virale lors de l'apparition des symptômes. De plus, il a également été montré que l'activité des **cellules Natural Killer et des lymphocytes T** était réduite chez les patients infectés par le virus de l'hépatite E (Krain *et al.*, 2014). La concentration en **cytokines pro-inflammatoires** est, elle, très élevée chez des patients souffrant d'hépatite E aiguë (Saravanabalaji *et al.*, 2009). Toutes ces modifications immunitaires semblent être responsables des manifestations cliniques de l'hépatite E. Sur le plan histopathologique, la structure lobulaire

du foie est modifiée, on observe une infiltration de lymphocytes et de leucocytes polynucléaires, une prolifération des cellules de Kupffer et une nécrose focale des hépatocytes (Malcolm *et al.*, 2007). Lors d'hépatite E fulminante, une expansion et une prolifération des capillaires biliaires sont en outre rapportées, avec une accumulation de bile et une infiltration interstitielle massive de lymphocytes (Agrawal *et al.*, 2012).

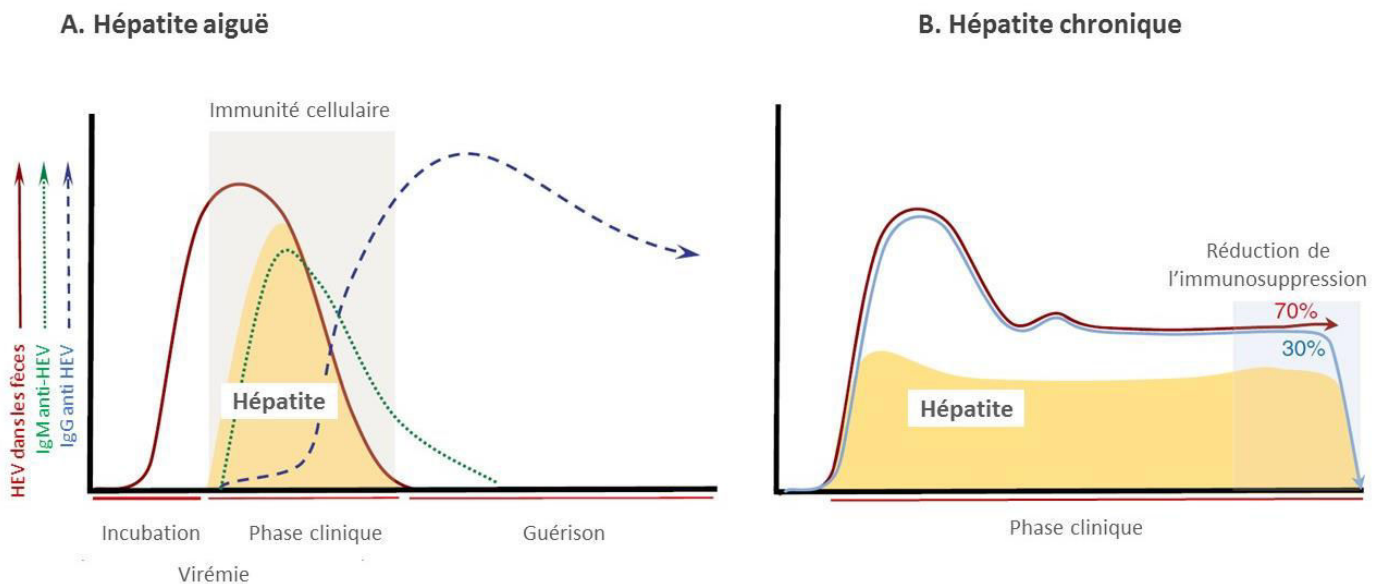


Figure 10 - Représentation schématique d'une hépatite E aiguë (A) ou chronique (B) chez l'homme

Source : d'après Walker (2018)

Les mécanismes immunopathogéniques entrant en jeu lors d'hépatite chronique sont encore mal compris (Figure 10). Le délai et l'amplitude des réponses humorales IgM et IgG chez des patients ayant une hépatite E chronique est très variable et difficilement généralisable, et la réponse IgM n'est donc pas représentée sur la Figure 10. La persistance du HEV en l'absence de séroconversion a été décrite chez certains patients, tandis que d'autres développaient une réponse IgM et IgG détectable (Dalton *et al.*, 2009; Legrand-Abravanel *et al.*, 2010; Pas *et al.*, 2012; Suneetha *et al.*, 2012; Kamar *et al.*, 2013; Moal *et al.*, 2013a). Des études conduites chez des patients ayant reçu une transplantation montrent que la séroconversion IgM peut prendre des mois à se mettre en place, et que les IgM persistent ensuite tout au long de l'infection chronique (Legrand-Abravanel *et al.*, 2010; Choi *et al.*, 2018). Le rôle de l'immunité cellulaire dans le développement de la chronicité a aussi été montré (Kenfak-Foguena *et al.*, 2011; Suneetha *et al.*, 2012; Moal *et al.*, 2013b). Une étude a été conduite chez des patients infectés par le VIH et ayant une hépatite E chronique ; l'un d'entre eux présentait

un faible taux de lymphocytes T CD4+ anti-HEV, une virémie persistante (plus de 24 mois) et une séroconversion anti-HEV retardée (Kenfak-Foguena *et al.*, 2011). Même si les mécanismes immunitaires méritent encore d'être clarifiés, la littérature suggère ainsi qu'**une réponse immunitaire humorale et cellulaire déficiente pourrait conduire à une hépatite E chronique** chez l'homme.

1.3.d. Diagnostic, traitement et prophylaxie des infections par le virus de l'hépatite E

(i) Méthodes et défis diagnostiques

Les méthodes de diagnostic de l'infection par le HEV sont celles communément employées pour détecter les infections virales.

L'infection par le HEV peut être diagnostiquée indirectement par la **recherche des anticorps anti-HEV**, qui reflètent la réponse immunitaire humorale qui suit l'exposition au virus. La détection des **IgM** révèle une infection récente, tandis que celle des **IgG** est le marqueur d'une infection ancienne, les IgG pouvant persister jusqu'à plusieurs années après une infection par le HEV (Al-Sadeq *et al.*, 2018). La recherche des **IgA** peut également appuyer le diagnostic d'une hépatite E aiguë, en complément de la recherche des IgM ou lorsque les tests IgM sont négatifs (Takahashi *et al.*, 2005; Zhang *et al.*, 2009). Notons que si la matrice utilisée est majoritairement le sérum, un **test salivaire** de détection des IgG et des IgM a récemment été développé (Pisanic *et al.*, 2017). Ceci pourrait permettre un diagnostic plus rapide et facile de l'hépatite E, notamment dans les zones dans lesquelles le système de santé est encore peu développé. Les tests sérologiques sont relativement **faciles à réaliser et moins chers** que les tests moléculaires. La réactivité croisée de ces tests entre les différents sous-types de HEV est importante, ce qui conduit à considérer qu'il n'existe qu'**un seul sérotype du HEV** (Engle *et al.*, 2002; Emerson *et al.*, 2006). De nombreux **kits commerciaux** existent, tant pour la détection des IgG et des IgM, les plus fréquemment utilisés étant Wantai et MP diagnostics. Les performances de ces tests sérologiques en termes de **sensibilité et spécificité sont variables**. De nombreuses études ont montré que les tests commerciaux ELISA (*enzyme-linked immunosorbent assay*) disponibles conduisaient à des résultats très différents, même sur des échantillons identiques (Mast *et al.*, 1998; Lin *et al.*, 2000; Bendall *et al.*, 2010; Abravanel *et al.*, 2013; Pas *et al.*, 2013; Avellon *et al.*, 2015; Shrestha *et al.*, 2016). Il faut noter que, chez

des patients immunodéprimés, les tests sérologiques peuvent être faussement négatifs du fait d'une séroconversion retardée (*cf. supra*). C'est très récemment que le premier test sérologique automatisé, VIDAS® ANTI-HEV IgM et IgG, a été validé pour la détection des IgG et IgM anti-HEV, tant sur des patients immunocompétents que des patients immunodéprimés (Abravanel *et al.*, 2019). Notons également que le diagnostic sérologique d'une infection par le HEV peut être délicat car les résultats de ces tests ELISA peuvent être contradictoires ou incertains du fait de la **réactivité croisée avec d'autres virus** (par exemple avec le cytomégalovirus ou le virus d'Epstein-Barr) (Hyams *et al.*, 2014). Pour toutes ces raisons, la confirmation d'une infection par le HEV repose de plus en plus sur des méthodes de détection des antigènes viraux ou du génome viral.

L'infection par le HEV peut être mise en évidence en détectant des **antigènes viraux** en utilisant des tests ELISA sandwich. Cependant le diagnostic par détection du génome viral par RT-PCR est le plus communément mis en œuvre. Après une phase d'extraction, la détection et la quantification des acides nucléiques viraux se base sur une **méthode RT-PCR classique ou en temps réel**. Les matrices sont généralement les selles, le foie et/ou le sang. Chez des patients ayant une hépatite E aiguë, le pic de virémie intervient durant la période d'incubation et le début de la phase symptomatique (Aggarwal *et al.*, 2000). Les ARN du HEV ne sont ainsi plus détectables dans le sang environ trois semaines après le début des symptômes mais peuvent encore être détectés dans les fèces pendant deux semaines supplémentaires. La RT-PCR en temps réel présente l'avantage d'être plus rapide, plus sensible et plus spécifique que la RT-PCR classique ; de plus, elle permet de quantifier la charge virale dès lors qu'une gamme de quantification a été établie. Le risque de contamination croisée est également réduit. Plusieurs **kits commerciaux** de RT-PCR en temps réel existent pour le HEV, les plus fréquemment utilisés étant RealStar HEV RT-PCR 1.0, AmpliCube HEV 2.0 et Ceeram kit, **de sensibilités et spécificités variables** (Al-Sadeq *et al.*, 2018). La recherche des ARN peut s'avérer faussement négative lorsque la virémie du patient est particulièrement faible. Un **test salivaire** de détection des ARN viraux dans la salive a également été récemment étudié, avec des résultats prometteurs (Rivero-Juarez *et al.*, 2018).

D'autres techniques de détection des ARN du HEV sont également employées. Par exemple, la **technique LAMP** (*Loop-Mediated Isothermal Amplification assay*), est une technique similaire à la RT-PCR en temps réel, mais réalisée à température constante (60-65°C) et qui ne nécessite pas de thermocycleur. Une étude a montré que la LAMP pour la détection

des ARN du HEV était plus rapide, plus sensible et plus spécifique qu'une RT-PCR nichée (Lan *et al.*, 2009). Les techniques d'**amplification par transcription** (*Transcription-mediated amplification*, TMA) se révèlent également sensibles, spécifiques et rapides pour la détection et la quantification des ARN du HEV dans plusieurs études (Abravanel *et al.*, 2018a).

Le **typage et la caractérisation moléculaire** des souches de HEV sont généralement effectués par une phase de transcription réverse de l'ARN, suivie d'une PCR nichée et d'un séquençage de Sanger de la cible amplifiée. Le génotypage du HEV est classiquement réalisé en séquençant les produits de PCR de l'ORF2 et de l'ORF1 (Al-Sadeq *et al.*, 2018).

Finalement, tous ces éléments montrent que la détection d'une infection par le HEV peut être délicate. Le **développement de tests standards de détection du HEV** est encore une problématique dans ce domaine, notamment pour permettre le screening des dons du sang au regard du risque HEV (*cf. infra*).

(ii) Possibilités thérapeutiques des infections par le virus de l'hépatite E

Il n'existe à ce jour **aucun traitement de routine** de l'hépatite E. Les hépatites E aiguës régressent généralement spontanément et ne nécessitent pas d'hospitalisation prolongée. Un **traitement symptomatique** est administré et du repos est préconisé. Pour les formes graves et chroniques, des recherches prometteuses sont en cours depuis 2010 pour l'utilisation de la **ribavirine**, un analogue nucléosidique de la guanosine à large spectre antiviral. Cet antiviral est principalement utilisé pour traiter les patients atteints d'hépatite C, mais plusieurs essais ont démontré son efficacité sur des cas d'hépatite E (Kamar *et al.*, 2010c; Mallet *et al.*, 2010; Gerolami *et al.*, 2011; Hajji *et al.*, 2013; Junge *et al.*, 2013; Pischke *et al.*, 2013; Debing et Neyts, 2014; Klein *et al.*, 2015). Néanmoins, les traitements à base de ribavirine présentent plusieurs inconvénients, notamment le fait que cette molécule ne peut pas être utilisée chez la femme enceinte, du fait de ses propriétés tératogènes, ainsi que la survenue d'effets secondaires nombreux (anémie hémolytique, insomnie, dyspnée, irritabilité) (Anang *et al.*, 2018).

Des essais de traitement par l'**interféron alpha** ont également été menés et les résultats sont prometteurs (Alric *et al.*, 2010; Haagsma *et al.*, 2010; Kamar *et al.*, 2010a; Dong *et al.*, 2012). Une **bi-thérapie** associant la ribavirine à l'interféron alpha semble particulièrement efficace (Debing et Neyts, 2014). Là encore, des effets secondaires variés peuvent apparaître

lors d'un traitement par l'interféron alpha (syndrome grippal, troubles neuropsychiatriques, neurologiques, cardiovasculaires, insuffisance rénale, *etc.*) (Raison *et al.*, 2005).

De nouvelles pistes thérapeutiques sont explorées depuis quelques années. En particulier, plusieurs études ont montré l'efficacité du **sofosbuvir** (analogue nucléosidique, inhibiteur d'une polymérase utilisé dans le traitement de l'hépatite C), en association avec la ribavirine, tant *in vitro* que chez des patients infectés par le HEV (Dao Thi *et al.*, 2016; van der Valk *et al.*, 2017; Biliotti *et al.*, 2018; Drinane *et al.*, 2018). Une étude récente a aussi montré qu'*in vitro*, les **sels de zinc** étaient capables d'inhiber la réplication du HEV (Kaushik *et al.*, 2017), ce qui pourrait également ouvrir de nouvelles perspectives thérapeutiques.

(iii) Prophylaxie médico-sanitaire des infections par le virus de l'hépatite E

Des **mesures de prévention non spécifiques** peuvent être mises en place pour lutter contre les infections par le HEV. Dans les pays en voie de développement, le **traitement des eaux usées et l'amélioration de la qualité de l'eau de boisson** sont les points clés pour limiter le risque d'infection. Dans les pays industrialisés, la prévention des cas autochtones d'hépatite E repose sur les **mesures générales d'hygiène individuelle** notamment lors de la préparation des aliments ou de leur consommation : **lavage des mains** à la sortie des toilettes, avant de préparer les repas, après un contact avec des animaux ou les produits d'origine animale ; **nettoyage des ustensiles et surfaces** après la manipulation de produits à base de foie de porc cru, de viande de sanglier, de cerf ; **cuisson à cœur** des aliments destinés à être consommés cuits ; respect des consignes de cuisson et de consommation indiquées sur l'étiquette des produits ; non consommation d'eau non traitée (puits, source, torrent, *etc.*). Il est en particulier recommandé de cuire à cœur les produits les plus à risque à base de foie cru de porc (saucisses de foie fraîches ou sèches, figatelli), les produits à base de sanglier ou de cerf (viande et abats) notamment la fressure (cœur, foie, rate, poumons). La consommation de ces produits même cuits est déconseillée chez les personnes à risque de développer une forme grave d'hépatite E (patients immunodéprimés, patient atteint d'une hépatopathie chronique préexistante et les femmes enceintes).⁹

⁹ Santé Publique France, 2014. Hépatite E : point sur les connaissances, disponible à : <http://invs.santepubliquefrance.fr/Dossiers-thematiques/Maladies-infectieuses/Hepatites-virales/Hepatite-E/Points-sur-les-connaissances>

Plusieurs études ont été conduites dans le but de développer un **vaccin** contre le HEV. Au moins 11 vaccins expérimentaux contre le HEV ont été évalués chez les PNH avec une inoculation d'épreuve (WHO, 2015). A ce jour, seuls deux de ces vaccins ont progressé jusqu'au stade de l'essai clinique chez l'homme. Shrestha *et al.* (2007) ont produit un vaccin recombinant et obtenu une efficacité de 95,5 % avec trois doses. Néanmoins, cette étude n'a pas dépassé le stade II des essais cliniques. Le second vaccin développé est un **vaccin recombinant** basé sur un peptide recombinant de l'ORF2 du HEV, dérivé d'une souche chinoise du HEV-1 (Zhang *et al.*, 2013). L'étude clinique conduite auprès de 50 000 personnes montre une efficacité vaccinale de 100 % après 12 mois et 86,8 % après un suivi de quatre ans et demi. Les personnes ayant reçu trois doses (à zéro, un et six mois) ont maintenu leur niveau d'anticorps anti-HEV pendant au moins quatre ans et demi (Zhang *et al.*, 2015). Il n'existe pas de donnée sur la protection spécifique conférée par ce vaccin contre l'infection par les génotypes 2, 3 ou 4 du HEV mais la protection croisée est probable. Ce vaccin contre le HEV a été **homologué en Chine** pour les personnes de plus de 16 ans (**Hecolin®**). Le fabricant, Xiamen Innovax Biotech Co., Ltd., recommande l'administration de ce vaccin aux sujets présentant un risque élevé d'infection par le HEV, notamment les éleveurs, les manipulateurs de produits alimentaires, les étudiants, les membres des forces armées, les femmes en âge de procréer et les voyageurs se rendant dans des zones à risque. Un programme vaccinal accéléré a également été testé en phase IV sur 126 participants et montre une bonne protection, qui pourrait être utile pour des personnes devant se rendre rapidement dans des zones à risque (Chen *et al.*, 2019). Ce vaccin n'est actuellement **pas commercialisé dans le reste du monde**. A ce jour, en raison du manque d'informations sur l'innocuité, l'immunogénicité et l'efficacité du vaccin dans les sous-groupes suivants de la population, **l'Organisation Mondiale de la Santé (OMS) ne recommande pas son utilisation systématique** chez les enfants de moins de 16 ans, les femmes enceintes, les personnes atteintes d'une affection chronique du foie, les patients en attente d'une transplantation et les voyageurs. En revanche, elle recommande d'envisager son utilisation **dans des cas spécifiques**, par exemple pour **combattre ou prévenir une épidémie** d'hépatite E, ainsi que pour en atténuer les effets chez les personnes à haut risque, telles que les femmes enceintes (WHO, 2015). Une étude récente a également montré que ce vaccin était bien toléré et suffisamment immunogène chez les personnes âgées de plus de 65 ans (Yu *et al.*, 2019).

La production de vaccins vivants atténués repose encore sur des techniques empiriques qui ne permettent pas le bon contrôle ou la stabilisation des phénotypes atténués. Les

déterminants moléculaires de l'atténuation sont souvent liés à un petit nombre de modifications protéiques ; ainsi, il y a un risque de réversion phénotypique ou, à l'inverse, les modifications protéiques peuvent conduire à une pathogénie différente de celle de la souche sauvage (Capai *et al.*, 2018). Enfin, le HEV pouvant circuler dans le sang sous forme quasi-enveloppée et ainsi protégé de la neutralisation par les anticorps (*cf. supra*), l'efficacité vaccinale peut être limitée (Feng *et al.*, 2014; Nagashima *et al.*, 2017). Ces problématiques restent des enjeux centraux dans les recherches actuelles en vaccinologie.

1.3.e. Transmission inter-humaine du virus de l'hépatite E

Lors d'épidémies dans les pays en voie de développement, la transmission inter-humaine est possible par contact de main à main ou par l'intermédiaire du réservoir hydrique (par exemple lorsque deux personnes se lavent les mains dans le même contenant) (Teshale *et al.*, 2010). Des cas de transmission de la mère à l'enfant sont également documentés dans les pays en développement (Andersson *et al.*, 2008; Anty *et al.*, 2012; Tabatabai *et al.*, 2014).

Des cas de transmission inter-humaine par l'intermédiaire de **transfusions de produits sanguins** contaminés ont été décrits, notamment dans les pays industrialisés (Matsubayashi *et al.*, 2004; Boxall *et al.*, 2006; Tamura *et al.*, 2007; Matsubayashi *et al.*, 2008; Haim-Boukobza *et al.*, 2012; Coilly *et al.*, 2013; Hauser *et al.*, 2014; Hewitt *et al.*, 2014; Mallet *et al.*, 2016; Riveiro-Barciela *et al.*, 2017; Satake *et al.*, 2017). Parmi les plus récents, en France, un patient hospitalisé en raison d'un grave accident de ski a reçu une transfusion de plaquettes sanguines qui s'est, *a posteriori*, révélée contaminée par le HEV ; le patient a développé une hépatite qui a nécessité l'utilisation prolongée de la ribavirine pendant trois mois (Loyrion *et al.*, 2017). En Australie, un garçon de six ans a également contracté une hépatite E à la suite d'une transfusion sanguine dont le donneur était virémique HEV (Hoad *et al.*, 2017). Une récente étude rétrospective conduite en Allemagne a montré que des produits sanguins contenant du HEV avaient été transfusés à 14 patients, dont 12 immunodéficients. Un patient immunocompétent a développé une hépatite aiguë spontanément résolutive à évolution favorable. Un patient immunodéficient a développé un syndrome *Acute-on-Chronic Liver Failure* (ACLF) caractérisé par la décompensation aiguë d'une hépatopathie chronique qui a conduit à son décès (Westholter *et al.*, 2018). En Espagne, une étude conduite à partir d'échantillons de sang de 11 313 donneurs de sang a montré que de l'ARN du HEV avait été détecté dans 0,035 % des

dons, et que cinq patients avaient reçu des transfusions provenant de donneurs virémiques (Rivero-Juarez *et al.*, 2019). En 2017, au Royaume-Uni, un cas de transmission du HEV par l'intermédiaire d'une transfusion contaminée a été rapporté (Reynolds *et al.*, 2019). Aux Etats-Unis, une étude rétrospective a permis de montrer que, parmi 2 348 patients transfusés, 40 (1,7 %) présentaient des IgG anti-HEV après la transfusion, et qu'un échantillon de sang transfusé contenait de l'ARN du HEV (Ticehurst *et al.*, 2019). Pour l'instant, seuls **le Royaume-Uni et l'Irlande recommandent et commencent à mettre en place le screening des dons de sang. En France, ce sujet est actuellement en discussion** (Domanovic *et al.* 2017). Une étude récente conduite en Allemagne a simulé l'impact du screening des dons de sang et conclut que tester des pools de produits sanguins par des techniques d'amplification des acides nucléiques du HEV conduirait à une réduction de 80 % de la transmission du HEV (Kamp *et al.*, 2018).

Le HEV peut aussi être transmis lors de **transplantation de foie**. Une étude décrit notamment le cas d'un patient ayant développé une grave cirrhose et étant décédé d'une décompensation hépatique après une transplantation d'un foie contenant du HEV (Schlosser *et al.*, 2012). Des cas d'infection par le HEV ont aussi été rapportés après une **greffe de rein** (Pourbaix *et al.*, 2017). Il a aussi été montré que les cellules souches hématopoïétiques (CSH) pouvaient être infectées par le HEV, mais aucun cas de transmission du HEV par transplantation de CSH n'est à ce jour décrit dans la littérature (Koenecke *et al.*, 2014; Frange *et al.*, 2015; O'Donghaile *et al.*, 2017). Récemment, le HEV a été détecté dans la moelle osseuse de macaques crabiers expérimentalement infectés par le HEV (Bottino *et al.*, 2018). Le risque de transmission du virus lors de greffe de moelle osseuse pourrait ainsi être exploré. Un cas de transmission nosocomiale, non lié à une transfusion ou une transplantation, a également été décrit en France (Mansuy *et al.*, 2009b). Enfin, la transmission par voie sexuelle est controversée. Une transmission parentérale et/ou oro-fécale lors de pratiques sexuelles à risque a été décrite et les relations homosexuelles semblent être un facteur de risque de l'infection par le HEV (Bali *et al.*, 2005; Payne *et al.*, 2013). Néanmoins, une étude récente conduite auprès de patients atteints du VIH n'a pas mis en évidence de transmission du HEV par voie sexuelle (Abravanel *et al.*, 2017b), et Heil *et al.* (2018) ont montré que la séroprévalence du HEV était comparable chez les personnes ayant des pratiques sexuelles à risque et dans la population générale.



Ce qu'il faut retenir

La distribution géographique du virus de l'hépatite E est hétérogène, avec une circulation sous forme endémo-épidémique des génotypes 1 et 2 dans les pays en voie de développement, et sous forme endémo-sporadique des génotypes 3 et 4 dans les pays industrialisés.

En France, la séroprévalence du HEV dans la population générale est de 22,4 %. La répartition géographique suit un gradient Nord-Sud, avec des zones hyper-endémiques dans le Sud de la France. Le nombre de cas autochtones rapportés au Centre National de Référence en 2017 était de 2 219. Un modèle statistique a permis d'estimer le nombre d'infections à 68 000 par an.

L'infection par le HEV est le plus souvent asymptomatique. Les manifestations cliniques sont variables : hépatite aiguë, fulminante (principalement chez les femmes enceintes ou patients atteints d'une pathologie hépatique), chronique (principalement chez les patients immunodéprimés), atteintes extra-hépatiques (notamment troubles neurologiques). Le diagnostic de l'infection peut être délicat lors de faibles charges virales. Les possibilités thérapeutiques et prophylactiques sont encore limitées et font actuellement l'objet d'efforts de recherche.

La transmission inter-humaine peut survenir par transfusion de produits sanguins ou transplantation d'organes contaminés. Le screening des dons de sang est actuellement un sujet en discussion dans de nombreux pays Européens.



Take home message

The geographical distribution of hepatitis E virus is heterogeneous, with endemo-epidemic circulation of genotypes 1 and 2 in developing countries and endemo-sporadic circulation of genotypes 3 and 4 in industrialised countries.

In France, HEV seroprevalence in the general population reaches 22.4%. HEV is distributed according to a North-South gradient, with hyper-endemic areas in Southern France. The number of locally-acquired cases reported to the National Reference Centre in 2017 was 2,219. A statistical model estimated the number of infections to 68,000 per year.

HEV infection in humans is most often asymptomatic. If present, clinical manifestations are variable: acute, fulminant (mainly in pregnant women or patients with another liver disease), chronic hepatitis (mainly in immunocompromised patients) or extra-hepatic disorders (particularly neurological disorders). Diagnosis of infection can be difficult at low viral loads. Therapeutic and prophylactic options are still limited and are currently the subject of research efforts.

Human-to-human transmission can occur through the transfusion of blood products or the transplantation of contaminated organs. Screening of blood donations is currently a topic under discussion in many European countries.

I.4. Transmission zoonotique du virus de l'hépatite E

I.4.a. Arguments moléculaires et expérimentaux d'une transmission inter-espèces

Les différents génotypes du HEV possèdent de 72 à 77 % d'homologie de séquences et les sous-types de 85 à 90 % (Pavio *et al.*, 2008). Des homologies de 99 % (séquençage ORF2) entre les séquences virales d'origine humaine et porcine de souches de génotype 3 collectées sur la même période ont été mises en évidence (Bouquet *et al.*, 2011). Ceci est une indication du possible **franchissement de la barrière inter-espèces** par le HEV, **démontré expérimentalement** à de nombreuses reprises (Figure 11). Par exemple, des porcs sains ont pu être infectés expérimentalement avec une souche humaine du HEV de génotype 3 (Halbur *et al.*, 2001). Des primates ont également pu être infectés par des souches porcines du HEV (Meng *et al.*, 1998). Ainsi plusieurs arguments viennent corroborer l'hypothèse d'une transmission inter-espèces du HEV et en particulier du porc à l'homme.

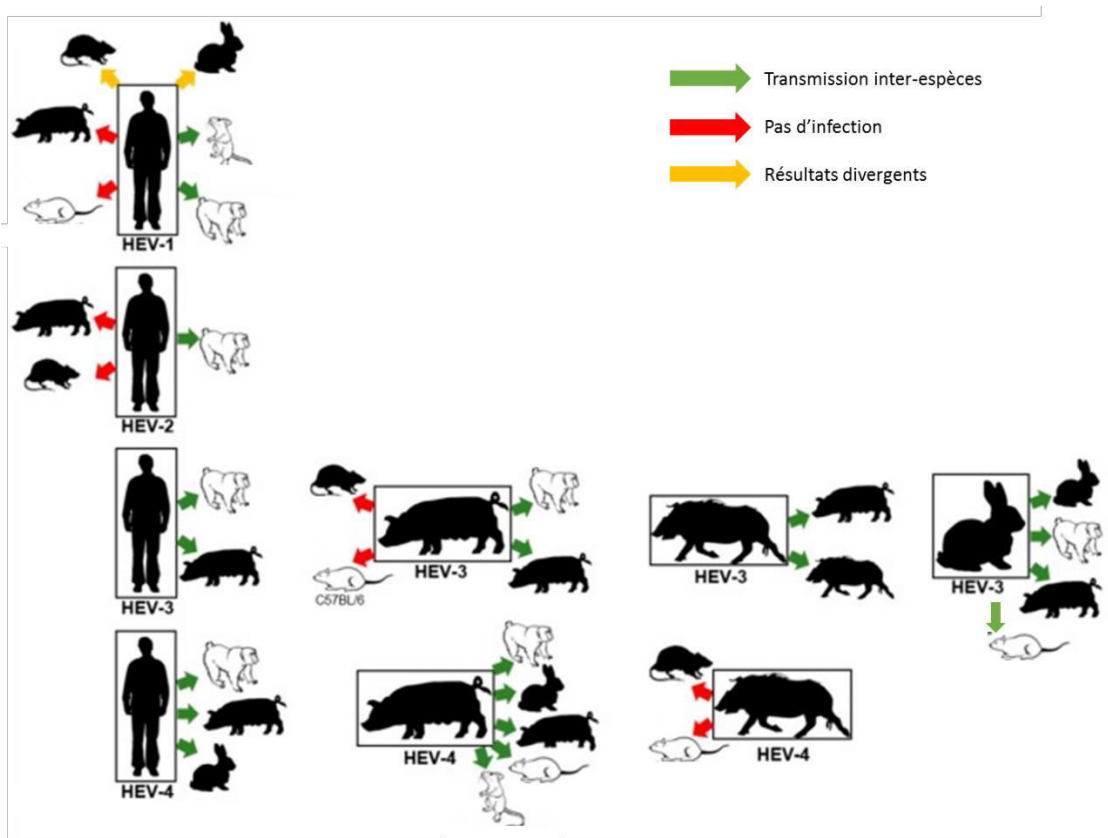


Figure 11 - Preuves expérimentales du passage de la barrière d'espèce par le virus de l'hépatite E

Source : d'après Doceul *et al.* (2016)

I.4.b. Preuves d'une transmission par l'intermédiaire de denrées alimentaires

De nombreux cas d'hépatite E groupés ou sporadiques ont été décrits dans les pays industrialisés (Tableau I). Si la transmission alimentaire est très souvent suspectée, elle est généralement difficile à prouver avec certitude et la source précise de l'infection n'est pas toujours confirmée. Les denrées alimentaires d'origine animale (DAOA) le plus souvent incriminées sont la viande et les produits à base de porc, et la viande et les produits de gibier.

Tableau I - Investigation des cas d'hépatite E groupés ou sporadiques avec une transmission alimentaire suspectée ou avérée¹⁰

Pays	Année	Génotype	Nombre de cas	Source de l'infection	Source / référence
Allemagne	2006		2	Non identifiée	Données ECDC (<i>European Center for Disease Control and Prevention</i>) non publiées
Allemagne	2008		2	Non identifiée	Données ECDC non publiées
Allemagne	2009		2	Non identifiée	Données ECDC non publiées
Allemagne	2011		2 foyers, 4 cas	Non identifiée	Données ECDC non publiées
Allemagne	2012		3 foyers, 6 cas	Non identifiée	Données ECDC non publiées
Allemagne	2013		2 foyers, 4 cas	Non identifiée	Données ECDC non publiées
Allemagne	2014		3 foyers, 8 cas	Non identifiée	Données ECDC non publiées
Allemagne	2015		6 foyers, 14 cas	Non identifiée	Données ECDC non publiées
Autriche	2015		2	Non identifiée	Données ECDC non publiées
Espagne	2014	3	1	Viande de porc *	Riveiro-Barciela <i>et al.</i> (2015)
Espagne	2015	3	7	Viande de sanglier *	Rivero-Juarez <i>et al.</i> (2017)
Espagne	2015	3f	8	Viande de sanglier *	Rivero-Juarez <i>et al.</i> (2017)

¹⁰ EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Fernandez Escamez PS, Herman L, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Di Bartolo I, John R, Pavio N, Rutjes S, van der Poel W, Vasickova P, Hempen M, Messens W, Rizzi V, Latronico F and Girones R, 2017. Scientific Opinion on the public health risks associated with hepatitis E virus (HEV) as a food-borne pathogen. EFSA Journal 2017;15(7):4886, 89 pp. <https://doi.org/10.2903/j.efsa.2017.4886>

France	2007		2	Probablement consommation de viande de porc séchée	Données ECDC non publiées
France	2007		3	Consommation de figatelles crues	Données ECDC non publiées
France	2007-2009	3	7	Consommation de figatelles crues	Colson <i>et al.</i> (2010)
France	2009		1	Consommation de figatelles crues	Données ECDC non publiées
France	2010		2	Consommation de figatelles crues	Renou <i>et al.</i> (2011)
France	2011		7	Non identifiée	Données ECDC non publiées
France	2011		1	Probablement consommation de figatelles crues	Anty <i>et al.</i> (2012)
France	2011	3	1	Consommation de figatelles crues	Moal <i>et al.</i> (2012)
France	2012		4	Non identifiée	Données ECDC non publiées
France	2011-2012	4	4	Consommation de figatelles crues et de viande de porc insuffisamment cuite	Colson <i>et al.</i> (2012), Tesse <i>et al.</i> (2012)
France	2013		2	Non identifiée	Données ECDC non publiées
France	2013	3	17	Consommation de farce à base de foie de porc dans un porcelet rôti insuffisamment cuit	Guillois <i>et al.</i> (2016)
France	2013	3	2	Consommation de figatelles crues *	Renou <i>et al.</i> (2014b)
France	2014	3	1	Probablement consommation de figatelles crues	Doudier <i>et al.</i> (2015)
France	2015		7	Eau de forage privé	Données ECDC non publiées
Hongrie	2004	3	1	Saucisses de porc faites maison	Reuter <i>et al.</i> (2006)
Hongrie	2012		2	Non identifiée	Données ECDC non publiées
Hongrie	2014		2 foyers, 4 cas	Non identifiée	Données ECDC non publiées
Hongrie	2015		2	Non identifiée	Données ECDC non publiées
Italie	2011	3	1	Probablement consommation de figatelles crues	Garbuglia <i>et al.</i> (2015)
Italie	2011	4	5	Non identifiée	Garbuglia <i>et al.</i> (2013)
Japon	2003	3	4	Consommation de sushis de cerf	Tei <i>et al.</i> (2003)

Japon	2003	4	2	Probablement consommation de foie cru de sanglier	Matsuda <i>et al.</i> (2003)
Japon	2003	3, 4	10	Probablement consommation de foie de porc grillé, insuffisamment cuit	Yazaki <i>et al.</i> (2003)
Japon	2004	3	5	Probablement consommation de viande de sanglier cuite au barbecue	Tamada <i>et al.</i> (2004)
Japon	2005	3	1	Consommation de viande de sanglier grillée	Li <i>et al.</i> (2005)
Japon	2005	3	1	Probablement consommation de viande de sanglier cuite au barbecue	Masuda <i>et al.</i> (2005)
République Tchèque	2011		36	Saucisses de tripes faites à la ferme et vendues dans une boucherie	Données ECDC non publiées
République Tchèque	2009		1	Viande de porc insuffisamment cuite	Holub <i>et al.</i> (2009)
République Tchèque	2009-2011		2 foyers, 13 et 8 cas	Probablement consommation de viande de porcs et produits à base de porc lors de fêtes	Trmal <i>et al.</i> (2012)
République Tchèque	2009-2012		27	Probablement consommation de viande de porcs et produits à base de porc	Chalupa <i>et al.</i> (2014)
Royaume-Uni	2008		33	Probablement consommation de mollusques	Said <i>et al.</i> (2009)

* confirmation moléculaire de la source de l'infection

- (i) Viandes et produits à base de porc

Plusieurs cas d'hépatite E ont été attribués avec certitude à la consommation de produits à base de porc contaminés par le HEV (Tableau I). Par exemple, Colson *et al.* (2010) ont décrit des cas groupés d'hépatite E en France chez sept personnes qui avaient consommé des **figatelles** (ou figatelli, saucisses fraîches composées de viande et de foie de porc, spécialités corses). En 2013, des infections groupées par le HEV ont été rapportées à Belle-Île en Mer, parmi lesquelles trois cas cliniques. Les patients avaient consommé un porcelet rôti à la broche farci avec une farce crue contenant le foie du porcelet. Les mêmes souches de HEV que celles impliquées dans les cas humains ont aussi été détectées dans le lisier échantillonné dans l'élevage d'origine du porcelet et dans des eaux usées non traitées provenant

du système d'épuration de la commune (Guillois *et al.*, 2016). Riveiro-Barciela *et al.* (2015) rapportent également le cas d'une infection aiguë par le HEV contractée par voie alimentaire : le HEV a pu être détecté et analysé dans de la viande de porc consommée par le patient, démontrée ainsi avec certitude comme la source de l'infection.

Plusieurs études visant à identifier des **facteurs de risque** expliquant la séropositivité HEV ont été conduites dans différents pays. Par exemple, en France, une récente enquête nationale rapporte les facteurs de risque suivants associés à une sérologie HEV positive : la **consommation de viande de porc ou de gibier** (Risque Relatif (RR) = 1,53), de **saucisses à base de foie de porc** (RR = 1,30), d'**abats** (RR = 1,25) (Mansuy *et al.*, 2016). En Italie, la consommation de saucisses à base de foie de porc est également apparue comme un facteur de risque vis-à-vis de la séropositivité HEV (La Rosa *et al.*, 2011). Une étude américaine montre également que la consommation de viande de porc insuffisamment cuite est un facteur de risque (Odds Ratio (OR) = 12,9) (Cossaboom *et al.*, 2016). Une équipe britannique a aussi montré que, parmi un groupe de 76 donneurs de sang présentant une virémie HEV, la majorité d'entre eux consommait des produits à base de viande de porc provenant d'une même chaîne de supermarchés (Tedder *et al.*, 2016). De manière similaire, une étude allemande conduite auprès de donneurs virémiques rapporte que 89 % d'entre eux consomme régulièrement de la viande de porc, et 67 % d'entre eux ont aussi dit avoir consommé du **tartare de porc** dans les deux mois précédents (Westholter *et al.*, 2018). Récemment, deux études néerlandaises ont également pointé la consommation de viande comme un facteur de risque : Slot *et al.* (2017) ont montré que la séoprévalence HEV chez les donneurs de sang consommant de la viande était significativement plus élevée que chez les végétariens (20,5 % *versus* 12,4 %) ; Mooij *et al.* (2018) ont quant à eux décrit la consommation de **saucisses traditionnelles néerlandaises (de type salami, cervelas, etc.)** comme un facteur de risque (OR = 1,5). Une large étude italienne, menée à partir des données relatives aux cas italiens d'hépatite E sur la période 2012-2016, rapporte aussi la consommation de porc et de saucisses de porc comme facteur de risque (OR = 4,6 et OR = 2,9 respectivement) (Alfonsi *et al.*, 2018).

Quelques **études cas-témoins** liant infection et habitudes de consommation ont également été conduites en Europe. Par exemple, en Allemagne, une étude incluant 45 cas et 135 témoins a mis en évidence que la consommation d'**abats** était associée à l'infection par le HEV (OR = 2,7) (Wichmann *et al.*, 2008). Plus récemment, toujours en Allemagne, Faber *et al.* (2018) ont montré, à partir de 270 cas et 1 159 contrôles, que l'infection par le HEV était

associée, entre autres, à la **consommation de foie de porc insuffisamment cuit** (OR = 5,3), de **viande de porc** (OR = 3), de **saucisses de Francfort** (OR = 1,9) et de **saucisses ou pâté de foie** (OR = 2,1). En Angleterre et en Ecosse, Said *et al.* (2014) ont rapporté, à partir de 25 cas et 75 témoins, que l'infection par le HEV était associée à la consommation de **tourte de porc** (*pork pie*, spécialité britannique, OR = 6,33) et de **saucisses et jambon provenant d'une grande chaîne de supermarchés britannique** (OR = 10,12). Récemment, une étude particulièrement médiatisée des mêmes auteurs a mis en évidence que l'infection par une souche particulière de HEV (phylotype HEV-3-2) était liée à la **consommation de saucisses et de jambons provenant de la chaîne de supermarchés britannique Tesco** (OR = 1,85) (Said *et al.*, 2017). Aux Pays-Bas, une étude cas-témoins impliquant 376 cas d'hépatite aiguë sur la période 2015-2017, montre que la consommation de **saucisses traditionnelles crues à base de viande de porc** est rapportée chez 72 % des patients contre 46 % des témoins, conduisant à un OR de 3 [2,2-4,1] et à un pourcentage de risque attribuable de 47 % (Tulen *et al.*, 2019).

De plus, en France, la comparaison des séquences de HEV présentes chez l'homme (données CNR) et chez le porc (isolement des foies à l'abattoir) sur une même période a permis de montrer une circulation active des mêmes sous-types et dans les mêmes proportions dans ces deux populations. Plusieurs arguments permettent d'écarter une origine locale de l'infection : (i) les séquences identiques entre élevages sont géographiquement proches, (ii) les séquences humaines identiques sont géographiquement très distantes (> 200 km), (iii) les séquences identiques chez l'homme et le porc sont également géographiquement très distantes (> 200 km). Ces résultats suggèrent que **la voie alimentaire serait la voie prédominante de contamination, et non une voie environnementale locale** (Bouquet *et al.*, 2011).

D'autre part, de nombreuses données sont disponibles dans la littérature pour évaluer la contamination des produits à base de porc (Tableau II). Parmi eux, les **foies de porcs** se révèlent être particulièrement à risque, avec une prévalence de foie contaminés à l'abattoir ou en supermarché variant entre 1 et 21 % selon les études et les pays. En France, par exemple, une enquête nationale a montré que 4 % des foies de porc à l'abattoir contenaient de l'ARN viral du HEV (Rose *et al.*, 2011). Par conséquent, les **produits à base de foie de porc** (saucisse de foie, pâté de foie) sont également à risque vis-à-vis du HEV, en particulier s'ils sont consommés crus ou insuffisamment cuits. Par exemple, une étude menée en France à partir de 394 produits achetés en supermarchés a montré que 30 % des figatelles et fitones contenaient de l'ARN viral du HEV, ainsi que 3 % des foies séchés et salés, 25 % des quenelles de foie et 29 % des saucisses

de foie sèches ou fraîches (Pavio *et al.*, 2014). Ces produits constituent des spécialités locales majoritairement consommés dans le Sud de la France, mais des équivalents sont commercialisés dans plusieurs autres pays d'Europe et ont également été testés positifs vis-à-vis du HEV (Tableau II). Il faut noter que les figatelles et préparations apparentées sont constituées de mélanges de plusieurs foies de porc, ce qui augmente considérablement le risque de contamination du produit final, même s'il peut diminuer la charge virale moyenne.¹¹ Le facteur « mélange » joue ainsi un rôle majeur dans le processus de transmission du HEV par voie alimentaire, non compensé par la dilution.¹²

Si le risque représenté par les produits à base de foie de porc est largement admis par la communauté scientifique et les autorités sanitaires, le **risque lié à la consommation de viande de porc ou produits à base de porc ne contenant pas de foie** est plus discuté. La plupart des études conduites récemment n'ont pas détecté d'ARN du HEV dans la viande et produits à base de viande (Tableau II) mais les études d'exposition (*cf. supra*), en mettant en évidence le rôle non seulement des produits contenant du foie mais aussi des produits à base de viande, posent question. Expérimentalement, il a été montré que les muscles des porcs peuvent contenir du HEV (Bouwknegt *et al.*, 2009) et l'enquête de Di Bartolo *et al.* (2012) rapporte 3 % de muscles linguaux de porcs positifs HEV à l'abattoir. La présence du HEV dans les muscles pourrait être liée soit à une répllication du virus dans les muscles, soit à une phase de virémie tardive entraînant la détection du virus dans les muscles à l'abattage. En Allemagne, l'enquête de Szabo *et al.* (2015) a montré que 21,7 % des salamis échantillonnés contenaient du HEV. L'étude de Cossaboom *et al.* (2016) rapporte également que 25 % des « andouillettes » échantillonnées dans un supermarché de Virginie aux Etats-Unis sont positives HEV ; ceci est cohérent avec l'étude de Williams *et al.* (2001) qui montre que le HEV peut se répliquer, entre autres, dans l'intestin du porc. Enfin, une enquête conduite récemment au Pays-Bas décrit une contamination de produits sanguins de porcs, entrant dans la fabrication de produits à base de viande (Boxman *et al.*, 2017). Des données polonaises non publiées suggèrent également un risque lié aux produits de porc contenant du sang de porc en grande proportion, avec 2 % (1/50) d'échantillons de boudin noir positifs HEV (communication personnelle).

¹¹ Par exemple, la fabrication d'un lot d'environ 2 100 figatelles nécessite 75 foies. Sur la base de 4 % de foies contaminés, la probabilité que ce lot soit contaminé (contienne au moins un foie contaminé) est de $1-(0.96)^{75}$, soit 95 %. La charge virale moyenne de ce lot sera 1.8 log plus basse que celle du foie initial.

¹² Avis de l'AFSSA n°2009-SA-0146 du 23 septembre 2009 relatif au virus de l'hépatite E : méthodes de détection, risques pour le consommateur et risque lié à l'environnement.

Tableau II - Données de la littérature relative à la contamination des foies, de la viande et des produits à base de porc

Organe / Produit	Pays	Echantillons positifs (%)	Référence
Foie de porc	Brésil	2/118 (2 %)	Gardinali <i>et al.</i> (2012)
	Burkina Faso	1/157 (1 %)	Traore <i>et al.</i> (2015)
	Cameroun	3/345 (1 %)	de Paula <i>et al.</i> (2013)
	Canada	2/19 (10 %)	Mykytczuk <i>et al.</i> (2017)
	Canada	25/283 (9 %)	Wilhelm <i>et al.</i> (2014)
	Canada	9/43 (21 %)	Leblanc <i>et al.</i> (2007)
	Chine	4/114 (4 %)	Li <i>et al.</i> (2009a)
	Chine	7/114 (6 %)	Geng <i>et al.</i> (2019b)
	République Tchèque	2/40 (5 %)	Di Bartolo <i>et al.</i> (2012)
	France (Corse)	2/24 (8 %)	Jori <i>et al.</i> (2016)
	France	128/3715 (4 %)	Rose <i>et al.</i> (2011)
	Allemagne	8/200 (4 %)	Wenzel <i>et al.</i> (2011)
	Hong Kong	7/479 (2 %)	Chan <i>et al.</i> (2017)
	Inde	2/240 (1 %)	Kulkarni et Arankalle (2008)
	Italie	2/33 (6 %)	Di Bartolo <i>et al.</i> (2012)
	Japon	12/243 (5 %)	Okano <i>et al.</i> (2014)
	Japon	0/110 (0 %)	Sasaki <i>et al.</i> (2013)
	Japon	4/390 (1 %)	Ishida <i>et al.</i> (2012)
	Mexique	26/127 (20 %)	Cantu-Martinez <i>et al.</i> (2013)
	Espagne	1/39 (3 %)	Di Bartolo <i>et al.</i> (2012)
	Thaïlande	3/1090 (1 %)	Intharasongkroh <i>et al.</i> (2017)
	Pays-Bas	4/62 (6 %)	Bouwknegt <i>et al.</i> (2007)
	Pays-Bas	10/79 (13 %)	Boxman <i>et al.</i> (2019)
	Royaume-Uni	1/40 (3 %)	Berto <i>et al.</i> (2012)
	Etats-Unis	14/127 (11 %)	Feagins <i>et al.</i> (2007)
	Viande de porc (muscle)	Canada	0/599 (0 %)
Canada		0/43 (0 %)	Leblanc <i>et al.</i> (2007)
République Tchèque		1/40 (3 %) muscle lingual	Di Bartolo <i>et al.</i> (2012)
Italie		2/33 (6 %) muscle lingual	Di Bartolo <i>et al.</i> (2012)
Espagne		0/39 (0 %)	Di Bartolo <i>et al.</i> (2012)
France		0/1134 (0%)	Feurer <i>et al.</i> (2018)
Thaïlande		2/559 (1 %)	Intharasongkroh <i>et al.</i> (2017)
Royaume-Uni		0/40 (0 %)	Berto <i>et al.</i> (2012)
Chine		0/158 (0 %)	Geng <i>et al.</i> (2019b)
Pays-Bas		0/98 (0 %)	Boxman <i>et al.</i> (2019)
Saucisses ou autres produits contenant du foie de porc		Canada	36/76 (47 %)
	France	68/394 (17 %)	Pavio <i>et al.</i> (2014)
	France	22/70 (31 %)	Martin-Latil <i>et al.</i> (2016)
	Allemagne	11/50 (22 %)	Szabo <i>et al.</i> (2015)

	Italie	11/68 (16 %)	Di Bartolo <i>et al.</i> (2015)
	Suisse	12/102 (12%)	Giannini <i>et al.</i> (2018)
	Suisse	7/37 (19 %)	Moor <i>et al.</i> (2018)
	Pays-Bas	70/99 (71 %)	Boxman <i>et al.</i> (2019)
		saucisses de foie 62/90 (69 %)	
		pâté de foie	
Saucisses ou autres produits ne contenant pas de foie de porc	Allemagne	13/60 (22 %)	Szabo <i>et al.</i> (2015)
		salami	
	Suisse	0/18 (0 %)	Giannini <i>et al.</i> (2018)
	Pays-Bas	33/36 (92 %)	Boxman <i>et al.</i> (2017)
		produits sanguins liquides 7/24 (29 %)	
		produits sanguins en poudre	
	Pays-Bas	0/103 (0 %)	Boxman <i>et al.</i> (2019)
	saucisses		
	Chine	2/170 (1 %)	Geng <i>et al.</i> (2019b)
		sang	
	Etats-Unis	3/12 (25 %)	Cossaboom <i>et al.</i> (2016)
		andouillettes (<i>pork chitterlings</i>)	
Saucisses (non spécifié)	Canada	0/35 (0 %)	Mykytczuk <i>et al.</i> (2017)
	République Tchèque	0/92 (0 %)	Di Bartolo <i>et al.</i> (2012)
	Italie	0/128 (0 %)	Di Bartolo <i>et al.</i> (2012)
	Espagne	6/93 (6 %)	Di Bartolo <i>et al.</i> (2012)
	Royaume-Uni	6/63 (10 %)	Berto <i>et al.</i> (2012)

Les **charges génomiques virales** retrouvées dans les foies, muscles, ou produits à base de foie ou de viande de porc sont comprises entre 10 et 10⁷ copies d'ARN par gramme, selon les études et les produits (Pavio *et al.*, 2017). La **dose infectante** par voie orale chez l'homme n'est pas connue, mais elle a été estimée à 10⁵ copies d'ARN par gramme chez le porc (Andraud *et al.*, 2013) et serait supérieure à 10^{5.5} chez les primates non humains.¹³ Les charges virales présentes dans les produits constitueraient donc un risque d'infection chez l'homme. Néanmoins, la détection d'ARN viral dans un produit ne préjuge pas du **caractère infectieux du virus** ; ce point est délicat à évaluer, de par l'absence de modèles de culture *in vitro* validés et utilisables à grande échelle (*cf. supra*). Seules quelques études sont parvenues à démontrer la présence d'HEV infectieux dans des produits à base de porc (Bouwknegt *et al.*, 2007; Feagins *et al.*, 2007; 2008a; Takahashi *et al.*, 2012; Berto *et al.*, 2013b).

¹³ Avis de l'AFSSA n°2009-SA-0146 du 23 septembre 2009 relatif au virus de l'hépatite E : méthodes de détection, risques pour le consommateur et risque lié à l'environnement.

La détection du HEV dans un grand nombre de produits à base de foie de porc, et les questions émergent ces dernières années sur la possible présence du HEV dans la viande et les produits à base de viande de porc, conduisent à insister sur la **nécessité de consommer ces produits cuits à cœur**. Rappelons qu'un traitement thermique à 71°C pendant 20 minutes a prouvé son efficacité pour inactiver le virus dans des matrices complexes (Barnaud *et al.*, 2012). Les **tendances culinaires à risque**, même pour les produits ne contenant pas de foie de porc (rôti de porc à cuisson basse température, carpaccio de porc, tartare de porc, *etc.*, Figure 12), ne sont ainsi pas recommandées.



Figure 12 - Extrait d'une recherche internet sur les nouveaux modes de consommation de viande de porc (recherche Google du 12/10/2018)

En France, courant 2009, et suite à la parution d'un avis de l'Anses¹⁴ quant au risque d'hépatite E lié à la consommation de produits à base de foie de porc, la Direction Générale de l'Alimentation (DGAL) a recommandé à la Fédération française des industriels charcutiers, traiteurs et transformateurs de viandes (FICT) d'**inciter les fabricants à indiquer sur l'étiquetage des saucisses à base de foie cru de porc destinées à être consommées cuites, la mention « à consommer cuit à cœur »**.¹⁵ Dans la continuité de cette démarche, au cours des années suivantes, la DGAL a de nouveau sensibilisé la FICT quant au renforcement de la

¹⁴ Avis de l'AFSSA n°2009-SA-0101 du 30 avril 2009 relatif à une demande d'avis sur le risque de contamination humaine par le virus de l'hépatite E (VHE) après ingestion de figatelles (saucisses crues à base de foie de porc)

¹⁵ Lettre à diffusion limitée DGAL/MUS/SA/SDSSA/BETD N°0393 du 12 mai 2009 : Hépatite E dans certains produits à base de foie de porcs contaminés

vigilance sur l'apposition de la mention « à consommer cuit à cœur » sur l'étiquetage (lisibilité des mentions relatives aux conditions de consommation) et la **séparation physique dans les rayons des produits à consommer en l'état, des produits à cuire**. En réponse, la FICT a procédé en 2011 à une nouvelle information dans ce sens auprès des fabricants de produits crus contenant du foie de porc, des fabricants de produits crus contenant des viandes ou des abats de cerf et de sanglier ainsi que des entreprises utilisatrices de foies de porcs crus, de viande ou abats (foie, fressure) de sanglier ou de cerf.¹⁶ En 2011, les services d'inspection de la DGAI ont procédé à des vérifications de l'apposition de la mention « à consommer cuit à cœur » sur l'étiquetage des saucisses à base de foie cru de porc destinées à être consommées après cuisson. Au bilan, sur 185 inspections réalisées dans 56 départements entre juillet et septembre 2011, **le non-respect de ces recommandations a pu être largement constaté** (communication personnelle). Les non-conformités relevées concernaient des produits vendus en vrac sans aucun étiquetage ou des produits pré-emballés ne portant pas la recommandation de cuisson, ou étaient liées à un message incorrect, différent de la mention « à consommer cuit à cœur » ou au caractère illisible de la mention ; enfin, il a parfois été constaté l'absence de séparation des produits destinés à être consommés en l'état, des produits destinés à être consommés après cuisson. A l'occasion d'un cas groupé de trichinellose dans le Sud de la France, une étude a également mis en évidence la défaillance dans l'étiquetage des figatelles, notamment en ce qui concerne la taille de police de la mention « à consommer cuit à cœur » (Ruetsch *et al.*, 2016). **Les recommandations d'étiquetage des produits à risque ne semblent donc pas suffisantes pour réduire le risque d'exposition de l'homme au HEV**. Pour preuve, une recherche succincte sur des sites commercialisant ou promouvant les figatelles met en évidence des conseils de consommation contraires : « Le figatellu cru se mange comme un saucisson. On le coupe en tranches en lui ôtant sa peau. »¹⁷, « Si l'on a la patience d'attendre, il est également possible de le faire sécher, on peut le garder alors jusqu'au début de l'été sans problème, pour le consommer cru. »¹⁸, « Il peut être dégusté grillé à la braise, au four, dans une sauce avec des lentilles ou encore cru lorsqu'il est sec. »¹⁹. Rappelons qu'en l'absence de données sur l'effet

¹⁶ Note d'information de la FICT du 29 avril 2011 : Note d'information sur le virus de l'hépatite E dans les produits à base de foie de porc

¹⁷ <http://www.cuisinez-corse.com/charcuterie-corse-figatellu>, site consulté le 12/10/2018

¹⁸ <https://www.gietaravu.corsica/charcuterie-corse/les-figatelli>, site consulté le 12/10/2018

¹⁹ <http://www.figatelli.fr>, site consulté le 12/10/2018

du séchage et de la salaison sur le HEV, ces traitements ne peuvent pas être considérés comme efficaces pour l'inactivation du HEV dans les produits.²⁰

(ii) Les produits de gibier

Les **produits de gibier** sont également à risque vis-à-vis du HEV. Par exemple, deux cas d'hépatite E liés de manière certaine à la **consommation de viande de sanglier** ont été décrits dans la littérature, l'un en Espagne (Rivero-Juarez *et al.*, 2017) et l'autre au Japon (Li *et al.*, 2005) (Tableau I). De nombreuses études conduites en Europe et au Japon rapportent aussi des niveaux de contamination des **foies de sanglier** variant entre 2 et 38 %, avec des charges génomiques entre 40 et 10⁸ copies d'ARN par gramme (Adlhoch *et al.*, 2009; Forgach *et al.*, 2010; Kaba *et al.*, 2010; Rutjes *et al.*, 2010; Sato *et al.*, 2011; Kubankova *et al.*, 2015; Lhomme *et al.*, 2015; Montagnaro *et al.*, 2015; Serracca *et al.*, 2015; Motoya *et al.*, 2016; Anheyer-Behmenburg *et al.*, 2017; Thiry *et al.*, 2017). Concernant **la viande ou les produits à base de viande de sanglier**, entre 0 et 12 % des échantillons ont été détectés positifs au HEV, selon les études (Rutjes *et al.*, 2010; Schielke *et al.*, 2015; Anheyer-Behmenburg *et al.*, 2017).

Les autres études concernent plusieurs espèces de **cervidés**. Quelques cas liés à la consommation de produits à base de cerf ont été rapportés dans la littérature (Tableau I). Le HEV a aussi été détecté dans le **foie de cerf** (taux de détection de 2 à 10 %) et de **chevreuil** (taux de détection de 0 à 22 %) (Rutjes *et al.*, 2010; Lhomme *et al.*, 2015; Serracca *et al.*, 2015; Anheyer-Behmenburg *et al.*, 2017; Thiry *et al.*, 2017), tandis que les analyses réalisées sur des échantillons de foies de daims, cerfs Yezo et cerfs Sika se sont révélées négatives (Sonoda *et al.*, 2004; Ishida *et al.*, 2012; Anheyer-Behmenburg *et al.*, 2017). Le HEV a aussi été détecté dans 0 à 5 % d'échantillons de **viande de cerf** selon les études (Rutjes *et al.*, 2010; Anheyer-Behmenburg *et al.*, 2017).

(iii) Autres denrées alimentaires

D'autres denrées alimentaires d'origine animale sont suspectées d'être à l'origine d'infections humaines par le HEV. Par exemple, Lee *et al.* (2016) ont décrit le cas d'un patient

²⁰ Santé Publique France, 2014. Hépatite E : point sur les connaissances, disponible à : <http://invs.santepubliquefrance.fr/Dossiers-thematiques/Maladies-infectieuses/Hepatites-virales/Hepatite-E/Points-sur-les-connaissances>

infecté par une souche de HEV-7 et consommant régulièrement de la viande et du lait de **chameau**. Récemment, une infection expérimentale de macaques a été réalisée à partir de souches de HEV-8 isolées d'échantillons de fèces et de lait de chameau, ce qui soulève le risque potentiel d'une infection humaine par la viande et/ou le lait de chameau par ce génotype (Wang *et al.*, 2019). **Dans les pays occidentaux, le risque d'hépatite E lié à la consommation de lait de vache ou petits ruminants est controversé.** Chez la femme, le HEV a été isolé dans le lait dans une étude, montrant ainsi que la voie mammaire est une voie possible d'excrétion du HEV chez les mammifères (Rivero-Juarez *et al.*, 2016). Une publication chinoise a récemment mis en évidence une forte prévalence du HEV chez des **vaches laitières** et a rapporté la détection d'ARN infectieux du HEV-4 dans le **lait de vache** (Huang *et al.*, 2016). L'analyse des séquences montre une homologie de 99 % avec les souches de HEV humaines et porcines. De la même manière, Yan *et al.* (2016) ont détecté de l'ARN du HEV-4 chez huit bovins Jaunes du Sud²¹ sur 254 en Chine, avec 96,6 % d'homologie de séquence avec les souches humaines chinoises. Long *et al.* (2017) décrivent aussi une forte prévalence du HEV chez les chèvres et la présence d'ARN du HEV dans le **lait de chèvre**. En Turquie, de l'ARN du HEV a été détecté dans du lait cru de vache (29,16 % des échantillons), de chèvre (18,46 %), de brebis (12,3 %) et d'ânesse (24,5 %), de génotype 1, 4 et 3 (Demirci *et al.*, 2019). En Europe, l'étude de Sarchese *et al.* (2019) montre la présence du HEV dans la **population ovine italienne**, avec une séroprévalence de 21,3 % parmi 192 moutons analysés dans sept élevages, et de l'ARN du HEV-3 détecté dans 10,4 % et 1,6 % des échantillons de fèces et de sang, respectivement. Trois études conduites en Allemagne, en Chine et en Belgique ont quant à elles rapporté l'absence de détection du HEV dans le lait de vache (Baechlein et Becher, 2017; Geng *et al.*, 2018; Vercoouter *et al.*, 2018). Récemment, Yugo *et al.* (2018) ont mis en évidence que 20,4 % d'un échantillon de 983 vaches aux Etats-Unis présentait des IgG anti-HEV. Néanmoins, les auteurs ne sont pas parvenus à séquencer les souches isolées, qui ne correspondent donc pas à des souches de HEV, et ils suggèrent donc que la séroconversion observée chez bovins serait liée à un **agent antigéniquement proche du HEV** mais qui ne serait pas le HEV. **Le rôle des bovins et petits ruminants dans la transmission zoonotique du HEV doit donc être envisagé avec précaution.**

Le **lapin** est également **suspecté** d'être à l'origine de cas humains d'hépatite E mais la voie de transmission n'est pas connue. Une étude conduite en France a montré que, sur 919

²¹ race de bovins de type Zébu

patients infectés par le HEV durant la période 2015-2016, cinq d'entre eux étaient infectés par une souche de HEV cunicole (HEV-3ra). Aucun de ces patients n'a rapporté avoir été en contact direct avec des lapins, ce qui peut suggérer une infection d'origine alimentaire ou médiée par l'eau (Abravanel *et al.*, 2017a). Sahli *et al.* (2019) rapportent pour leur part trois cas d'infection par une souche cunicole de HEV chez des patients immunodéprimés n'ayant pas consommé de lapin ni ayant de contact avec des lapins.

Par ailleurs, des souches de HEV-3 et HEV-4 ont été détectées dans des **moules** en Espagne et en Ecosse (Crossan *et al.*, 2012; Mesquita *et al.*, 2016), dans des **huîtres** en Corée (Song *et al.*, 2010), dans des bivalves au Japon (Li *et al.*, 2007), et dans des mollusques en Chine et en Ecosse (huîtres et moules) (Gao *et al.*, 2015; O'Hara *et al.*, 2018). Une étude expérimentale a également montré la bioaccumulation du HEV dans les huîtres, les moules et les palourdes, principalement dans les tissus digestifs (Grodzki *et al.*, 2014). La consommation de mollusques a également été fortement suspectée d'être à l'origine d'un cas d'infection par le HEV-4 chez un patient japonais ayant voyagé au Vietnam (Koizumi *et al.*, 2004) et de cas groupés d'hépatite E sur un bateau de croisière (Said *et al.*, 2009). En France, l'étude de Mansuy *et al.* (2016) a aussi montré que la séropositivité HEV chez les donneurs de sang était associée à la consommation d'huîtres.

Enfin, de l'ARN du HEV a été retrouvé dans des **fraises** au Canada (Brassard *et al.*, 2012), des **framboises** surgelées vendues en Europe (Maunula *et al.*, 2013), et dans des **légumes verts** dans trois pays d'Europe (Kokkinos *et al.*, 2012). Une étude conduite en France rapporte également une contamination possible d'herbes et épices, à hauteur de 0,9 % (2/230) (Loisy-Hamon et Leturnier, 2015). Une étude italienne montre également la présence de HEV dans un échantillon de fruits et légumes sur 70 (1,4 %) (Purpari *et al.*, 2019).

1.4.c. La problématique liée à l'eau

La présence du HEV dans les mollusques, les fruits et les légumes est probablement liée à la contamination des eaux de surface et d'irrigation par des effluents d'élevage. En effet, l'ARN du HEV a été détecté dans des **effluents d'élevage porcins** dans de nombreuses études (Pina *et al.*, 2000; Kasorndorkbua *et al.*, 2005; Fernandez-Barredo *et al.*, 2006; McCreary *et al.*, 2008; Guillois *et al.*, 2016). Par inoculation expérimentale à des porcs, il a également pu être montré que **ces effluents contaminés pouvaient être infectieux** (Kasorndorkbua *et al.*,

2005). Plusieurs études ont aussi rapporté la présence de HEV dans les **eaux de surface à proximité d'élevages de porcs** (Steyer *et al.*, 2011; Gentry-Shields *et al.*, 2015), probablement contaminées par l'épandage du lisier comme engrais ou par le ruissellement des effluents d'élevage. Des souches de HEV ont également été retrouvées dans des **stations de traitement des eaux usées** (Ippagunta *et al.*, 2007; La Rosa *et al.*, 2010; Masclaux *et al.*, 2013; Baez *et al.*, 2017; Alfonsi *et al.*, 2018). De plus, dans plusieurs publications, des séquences de HEV proches de séquences retrouvées chez des patients atteints d'hépatite E ou chez des porcs ont été mises en évidence dans des rivières ou eaux de mer (Rutjes *et al.*, 2009; Ishida *et al.*, 2012; Iaconelli *et al.*, 2015). Une autre étude conduite en Ecosse a montré que des mollusques contenant du HEV étaient élevés près d'une usine de transformation de viande de porc, ce qui pose la question d'un lien éventuel avec les eaux usées des industries agro-alimentaires de la filière porcine (O'Hara *et al.*, 2018).

Des études sur les facteurs de risque de l'exposition de l'homme au HEV ont également mis en évidence le rôle de l'eau. Ainsi, la méta-analyse de Hartl *et al.* (2016) suggère que l'exposition environnementale est un facteur de risque vis-à-vis de la séropositivité HEV. L'enquête conduite en France par Mansuy *et al.* (2016) montre quant à elle que le fait de **boire de l'eau en bouteille est un facteur protecteur vis-à-vis de l'infection par le HEV**. Une autre étude réalisée dans le sud-ouest de la France suggère aussi qu'une transmission par l'eau pourrait expliquer des différences locales de séroprévalence (Mansuy *et al.*, 2015). Dans leur enquête conduite auprès de 36 patients atteints d'hépatite E aiguë en Grande-Bretagne, Hunter *et al.* (2016) émettent l'hypothèse du rôle des activités aquatiques de loisir dans l'infection par le HEV. Enfin, une étude chinoise a montré que des cas groupés d'hépatite E (HEV-4) dans un établissement de soins étaient probablement liés à l'utilisation d'eau du robinet contaminée (Chen *et al.*, 2016).

1.4.d. Autres voies d'exposition zoonotique

- (i) Le contact avec les animaux et l'exposition professionnelle

L'exposition au HEV *via* le **contact avec des porcs** a été mise en évidence par de nombreuses études sérologiques conduites auprès de personnes ayant une **exposition professionnelle** à ces animaux (**éleveurs de porcs, employés d'abattoir, bouchers,**

vétérinaires porcins), en comparaison à un groupe contrôle. Dans la plupart de ces études, les groupes exposés présentent une séroprévalence HEV plus élevée en comparaison avec les groupes non-exposés (Figure 13). Néanmoins, la comparaison directe de ces études entre elles n'est pas possible du fait des différences de performance entre les tests sérologiques utilisés. Une étude récente a également montré que le contact direct avec les porcs était associé à un risque d'hépatite E aiguë plus élevé (OR = 3,1 [1,3-7,3]) (Tulen *et al.*, 2019). D'autres études ont été conduites pour évaluer le risque de transmission du HEV par contact avec des animaux sauvages. Par exemple, deux études conduites en France et en Allemagne rapportent une séroprévalence plus élevée chez les **travailleurs forestiers** par rapport aux groupes contrôles (Dremsek *et al.*, 2012; Chaussade *et al.*, 2013). La séroprévalence chez les **chasseurs** est également apparue plus élevée que dans la population générale dans plusieurs études (Toyoda *et al.*, 2008; Schielke *et al.*, 2015). De plus, l'étude de Schielke *et al.* (2015) montre que les chasseurs utilisant des gants pour pratiquer l'éviscération des sangliers ont un risque de séropositivité HEV plus faible que ceux qui n'en utilisent pas ou que rarement, ce qui confirme la voie de transmission du HEV par contact.

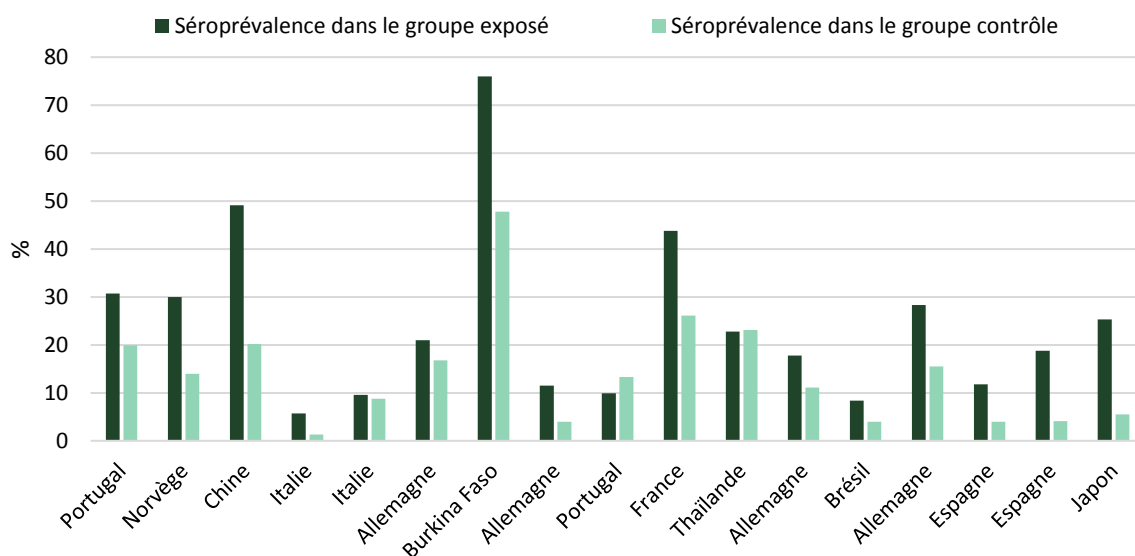


Figure 13 - Synthèse des études rapportant une différence de séroprévalence entre les personnes en contact professionnel avec les animaux à risque (porcs, animaux sauvages) et un groupe contrôle

Source : d'après Pavio *et al.* (2017)

En revanche, si plusieurs études décrivent la présence d'anticorps anti-HEV comme un indicateur d'une transmission du virus du porc ou du sanglier à l'homme, la transmission du

HEV par contact **semble rarement conduire à une hépatite clinique chez l'homme**. Un cas d'hépatite E a été rapporté chez un chirurgien s'entraînant à des pratiques de chirurgie chez le porc (Colson *et al.*, 2007). Perez-Gracia *et al.* (2007) ont également décrit un cas d'hépatite E aiguë chez un travailleur d'un abattoir de porcs. Dans ces deux cas, le contact avec les organes contaminés a été suspecté d'être à l'origine de l'infection. Un cas d'hépatite E aiguë a aussi été rapporté en France chez un patient ayant des contacts fréquents avec un cochon de compagnie (Renou *et al.*, 2007). La comparaison des séquences de HEV du patient et du cochon a montré une homologie de 92 à 98 % selon la région du génome. Même si les souches n'étaient pas identiques, elles étaient plus proches entre elles que de toutes les autres souches isolées dans la même région géographique. Les auteurs ont suspecté qu'une quasi-espèce du HEV ait été transmise du cochon à l'homme par contact direct ou contact avec ses fèces.

Le contact avec les lapins de compagnie est également suspecté d'être à l'origine d'infections par le HEV chez l'homme (Caruso *et al.*, 2015). De plus, une publication récente rapporte une séroprévalence HEV plus élevée chez des **travailleurs d'abattoirs de lapins** (IgG 46,1 %, IgM 6,7 %), en comparaison avec la population générale (IgG 10,8%, IgM 1,2 %) (Geng *et al.*, 2019a).

(ii) Le risque potentiel lié aux xénogreffes

Plusieurs publications ont également soulevé le **risque de transmission zoonotique du HEV par l'intermédiaire de xénogreffes** (Yoo et Giulivi, 2000; Meng, 2003; Denner, 2015; 2017). En effet, étant données les difficultés pour obtenir des transplants humains, le porc pourrait constituer un donneur prometteur, d'**organes** ou de cellules, notamment des **cellules d'îlots pancréatiques** utilisées comme traitement du diabète, mais ces organes ou cellules sont susceptibles de contenir du HEV. Plusieurs souches de porcs non-transgéniques et adaptées à la xénotransplantation existent. Les porcs de l'île d'Auckland (Living Cell Technologies (LCT), Nouvelle Zélande)) ont déjà été utilisés dans plusieurs essais cliniques, impliquant entre autres la transplantation de cellules d'îlots pancréatiques de porcs chez 14 patients diabétiques souffrant d'insensibilité à l'hypoglycémie (Wynyard *et al.*, 2014). Les porcs et les préparations cellulaires utilisés dans cet essai ont été testés vis-à-vis de 26 microorganismes et le HEV n'a pas été détecté. Dans un essai préclinique sur des PNH et les essais cliniques, les receveurs ont également tous été testés négatifs vis-à-vis du HEV à plusieurs dates jusqu'à un an après la transplantation (Wynyard *et al.*, 2014). Les mini-porcs Göttingen (Ellegaard, Danemark) sont

une race de porcs EOPS (Exempts d'Organismes Pathogènes Spécifiques) largement utilisés pour de nombreuses recherches biomédicales. Busby *et al.* (2013) ont détecté le HEV chez tous les porcs EOPS de leur étude (sept porcs sur sept). Morozov *et al.* (2015) rapportent également la présence du HEV chez trois porcs sur dix mini-porcs de moins d'un an et chez trois truies, ainsi que la détection d'anticorps anti-HEV. Dans une autre étude, les mêmes auteurs rapportent l'absence de détection du HEV chez des mini-porcs donneurs et dans des cellules d'îlots pancréatiques, mais des anticorps anti-HEV chez des singes receveurs (Morozov *et al.*, 2016). En conclusion de ces études, il apparaît que l'évaluation du risque lié aux xénogreffes s'avère délicate, notamment du fait de la difficulté à détecter le HEV lorsque les charges virales sont faibles, ce qui est le cas dans le sang et la plupart des organes. Les programmes d'assainissement des élevages de porcs à des fins de production de transplants devront donc inclure une phase de sélection des animaux négatifs HEV par des méthodes RT-PCR hautement sensibles.

(iii) Focus sur deux cas d'hépatite E liés à une souche murine

En fin d'année 2018, une **souche murine de HEV** appartenant à l'espèce *Orthohepevirus C*, a été isolée chez un patient présentant une hépatite clinique persistante et ayant reçu une greffe de foie, ce qui démontre ainsi la possibilité de transmission de cette espèce virale du rat à l'homme, alors que seule l'espèce *Orthohepevirus A* était jusqu'à présent considérée comme zoonotique (Siddharth *et al.*, 2018). La voie de contamination de ce patient n'a pas été clairement identifiée : parmi les hypothèses figurent celle d'une infection par consommation de denrées alimentaires souillées par des fèces de rat contenant des particules virales, et celle d'une transmission par la greffe de foie contenant du HEV. Au Canada, un autre cas d'hépatite causée par une souche murine de HEV a été décrit chez un patient immunocompétent, ayant probablement contracté l'infection au Gabon ou en République Démocratique du Congo (Andonov *et al.*, 2019). Aucun contact avec des rats ou des fèces de rat n'a pu être mis en évidence, et la source de cette infection est ainsi inconnue à ce jour.



Ce qu'il faut retenir

Dans les pays industrialisés, la transmission zoonotique des génotypes 3 et 4 du virus de l'hépatite E est prouvée. Elle survient majoritairement par voie alimentaire, avec le porc comme principal réservoir. En particulier, les produits contenant du foie de porc (figatelles, autres saucisses de foie, autres préparations non cuites à base de foie, *etc.*) présentent un risque pour le consommateur s'ils sont consommés crus ou insuffisamment cuits. La question d'un risque de transmission par consommation de viande et produits à base de viande de porc est également soulevée et en cours d'étude. Le réservoir porcin est suspecté d'être à l'origine de contaminations environnementales par le rejet d'effluents d'élevage contenant du HEV. L'exposition professionnelle au HEV de certains groupes en contact avec des porcs ou leurs produits a aussi été mise en évidence (Figure 14).

Ainsi, la suite du manuscrit se focalisera sur le porc uniquement.



Take home message

In industrialised countries, zoonotic transmission of HEV-3 and HEV-4 has been proved. It occurs mainly through food, with pigs as the main reservoir. In particular, products containing pork liver (figatelli, other liver sausages, other raw foodstuffs with liver, etc.) present a risk to the consumers if consumed raw or uncooked. The question of a potential risk of transmission through the consumption of pig meat and other meat products is also raised and under consideration. The pig reservoir is also suspected of causing environmental contamination through the release of livestock manure containing HEV. Occupational exposure to HEV of certain groups in contact with pigs or their products has also been highlighted (Figure 14).

Thus, the next sections of the manuscript will focus on pigs only.

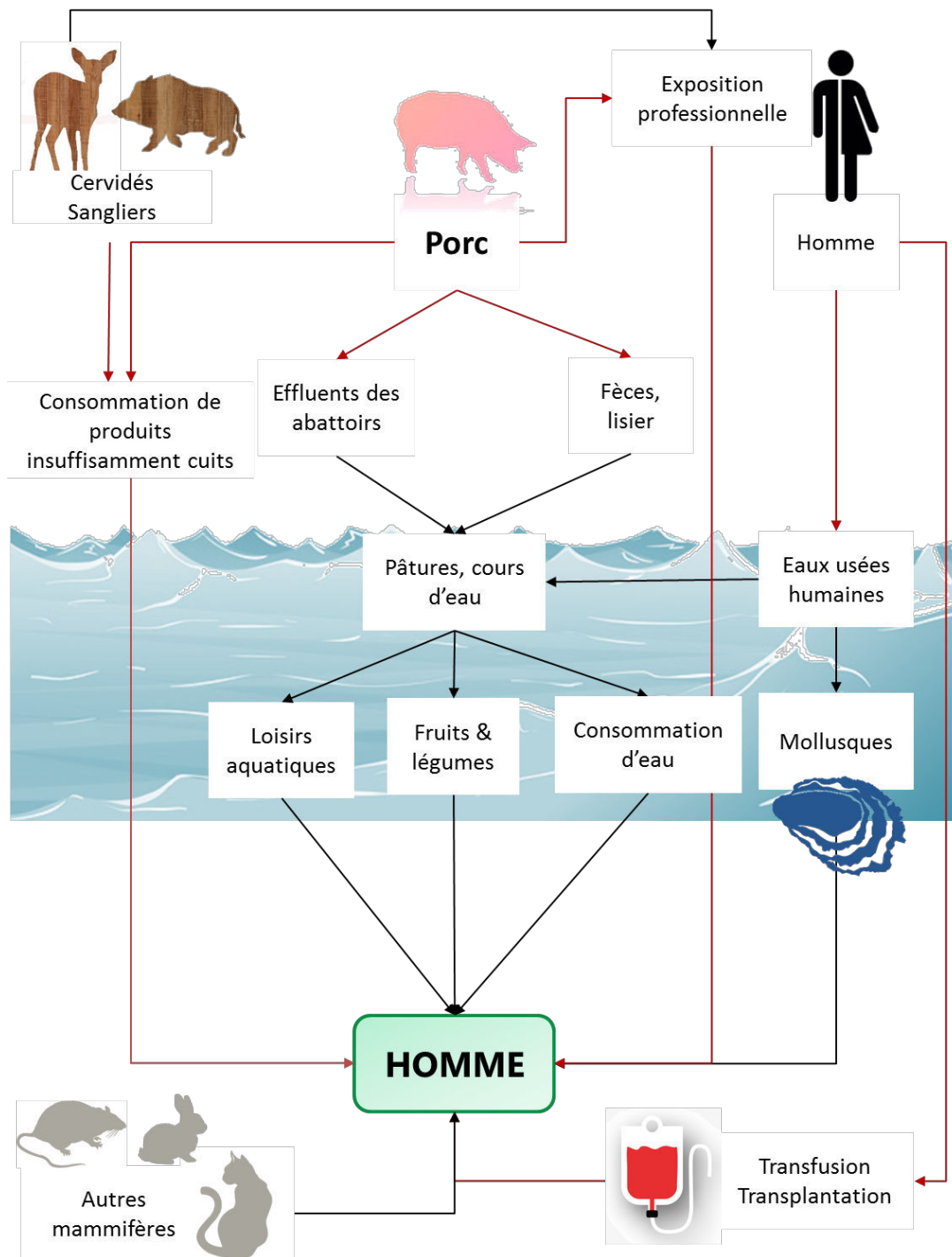


Figure 14 - Voies de transmission zoonotiques confirmées et majeures (rouge), suspectées ou mineures (noir) du virus de l'hépatite E dans les pays industrialisés

II. L'infection par le virus de l'hépatite E chez le porc : caractéristiques générales et épidémiologie dans la filière porcine

II.1. Aperçu général de l'infection par le HEV chez le porc

Chez le porc, l'infection naturelle ou expérimentale par le virus de l'hépatite E est **asymptomatique** (dos Santos *et al.*, 2009; Leblanc *et al.*, 2010; Meng, 2010). Comme chez l'homme, **la réplication virale a lieu principalement dans les hépatocytes et les particules virales sont excrétées dans les matières fécales**. L'infection est accompagnée d'une hépatite légère à modérée, révélée par des examens histopathologiques, mais aucune lésion macroscopique du foie n'est observée (de Deus *et al.*, 2008; dos Santos *et al.*, 2009). Un porc dit infectieux désigne un porc excréant des particules virales dans ses matières fécales. L'excrétion virale dans les fèces et la présence de virus dans le foie sont globalement concomitantes. **Le virus se transmet entre les porcs par voie féco-orale.**

Chez le porc, les méthodes de diagnostic de l'infection sont identiques à celles utilisées en médecine humaine, bien que les marqueurs virologiques et sérologiques suivent des cinétiques différentes. La recherche des IgA chez le porc serait plus efficace pour la détection de l'infection (Takahashi *et al.*, 2005). En plus de la détection des immunoglobulines dans le sérum des porcs, la recherche des anticorps à partir d'un exsudat musculaire est une méthode de diagnostic envisageable à l'abattoir (Casas *et al.*, 2011b).

II.2. Caractéristiques épidémiologiques de l'infection par le HEV dans la filière porcine

Cette partie a fait l'objet d'une revue bibliographique publiée dans le journal *Veterinary Research* en 2017 (Salines *et al.*, 2017a). Les objectifs et les thématiques de la revue sont présentés dans la Figure 15.

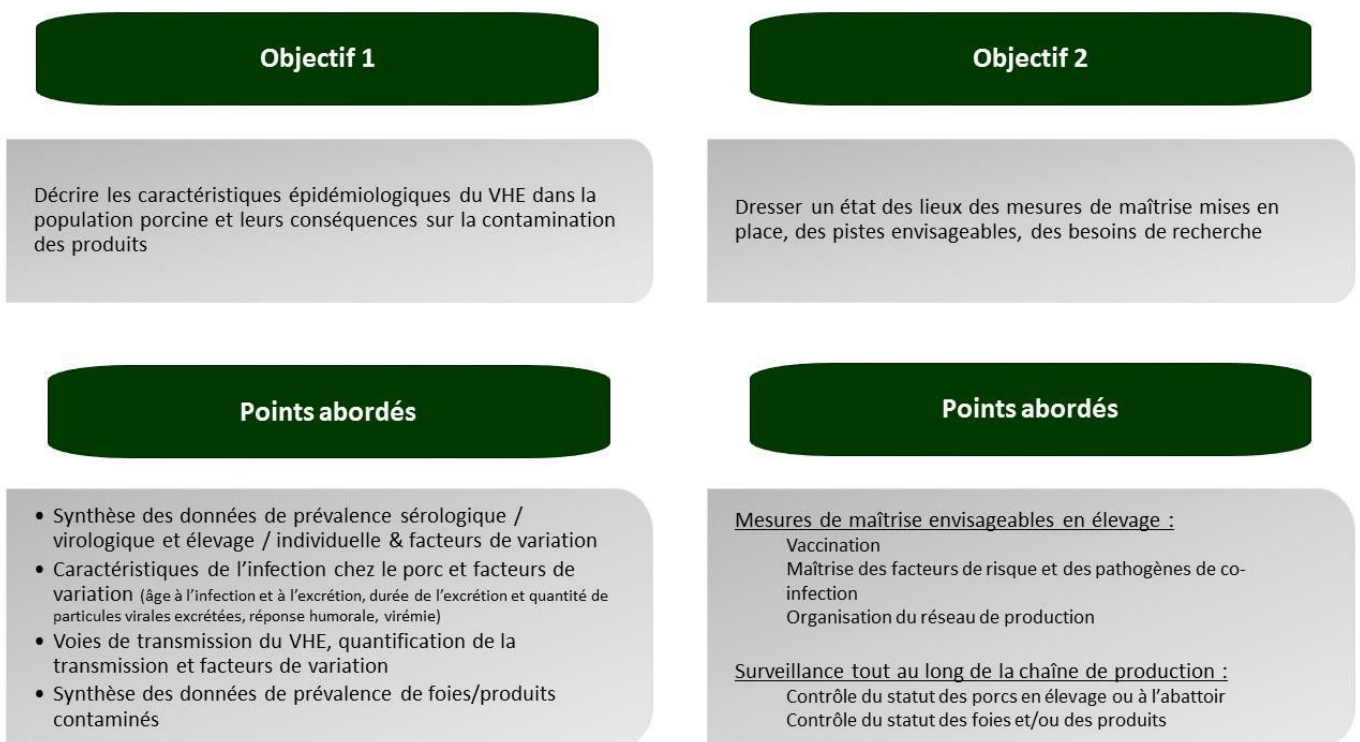


Figure 15 - Objectifs et thématiques abordés dans la revue de la littérature relative à l'épidémiologie du virus de l'hépatite E dans la filière porcine

Dans un souci de clarté, les paragraphes suivants s'attachent à présenter de manière synthétique et illustrée certaines des données détaillées dans la revue de la littérature ainsi que les nouvelles publications parues depuis. La revue réalisée présente les résultats de prévalence et séroprévalence du HEV chez le porc provenant de 86 études conduites dans des pays industrialisés. Ces études descriptives rassemblent des données de nature sérologique (IgG, IgM et IgA) ou de nature virologique (ARN viral dans les séras, les fèces, les lisiers et foies). Cette synthèse met en évidence une **très grande variabilité** dans les valeurs de prévalence et séroprévalence, tant à l'échelle individuelle qu'à l'échelle de l'élevage (Figure 16). Cette variabilité est observée à la fois **au sein d'une même étude et entre les études**. En France, une

enquête nationale a été réalisée afin d'estimer la prévalence et la séroprévalence du HEV dans les élevages de porcs (Rose *et al.*, 2011). Au total, 6 565 sera et 3 715 foies ont été collectés dans 35 abattoirs répartis sur l'ensemble du territoire français. Les résultats de cette étude montrent que **le HEV circule dans 65,3 % des élevages** et que **31 % des animaux présentent des anticorps anti-HEV à l'âge d'abattage** (*i.e.* vers 26 semaines d'âge). La séroprévalence intra-élevage du HEV varie de 5 à 90 % selon les élevages. A l'abattoir, les résultats suivants ont été obtenus : **24 % des élevages ont au moins un animal à foie positif au moment de l'abattage et au global, 4 % des foies sont infectés par le HEV.**

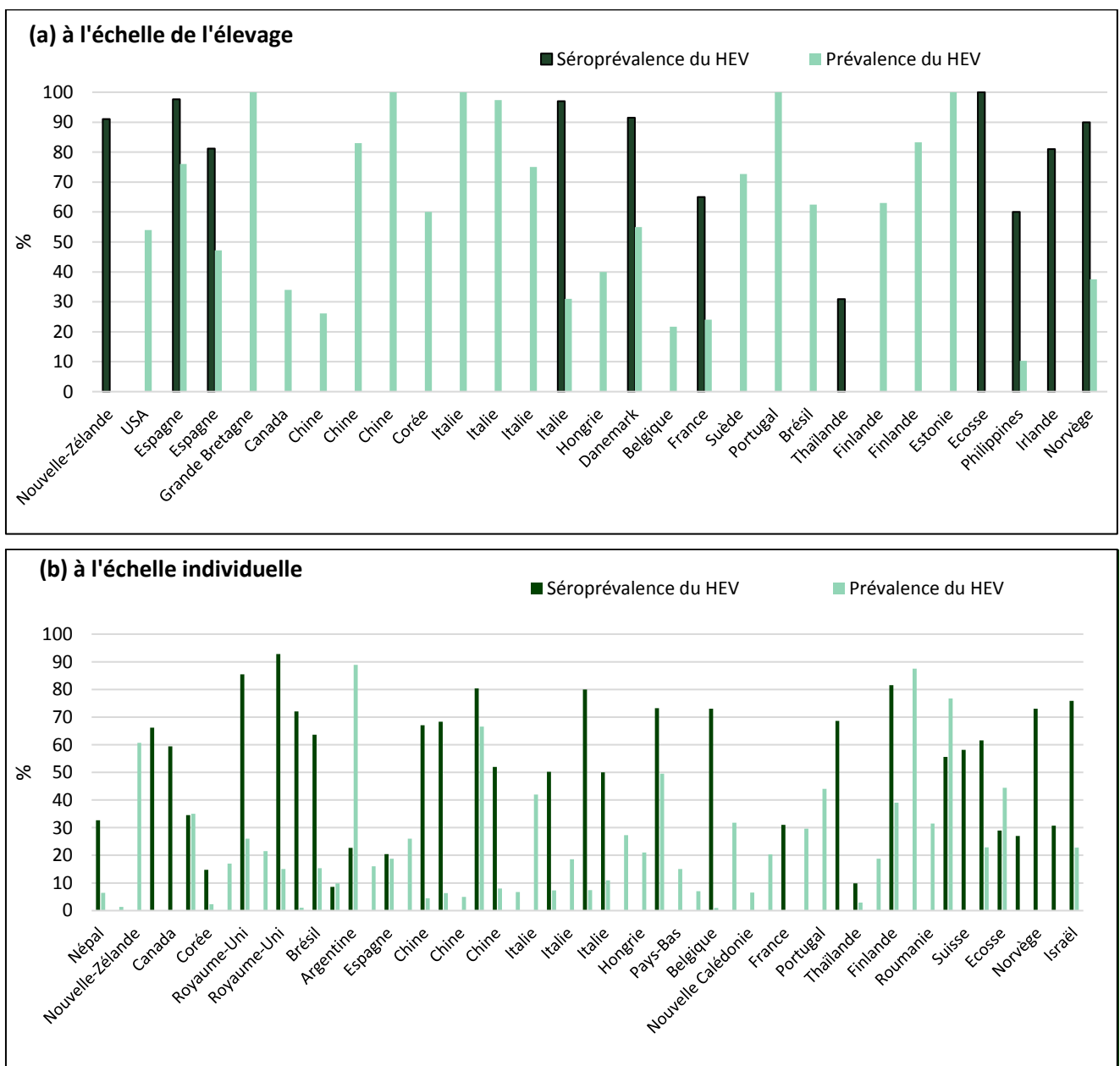


Figure 16 - Synthèse des données de séroprévalence et de prévalence du virus de l'hépatite E disponibles dans la littérature. (a) à l'échelle de l'élevage, (b) à l'échelle individuelle

La figure (a) représente la proportion d'élevages ayant au moins un animal séropositif HEV ou positif en RT-PCR dans le foie ou les fèces, tous âges/stades confondus ; la figure (b) représente la proportion de porcs séropositifs HEV ou positifs en RT-PCR dans le foie ou les fèces, tous âges/stades confondus.

Source : d'après Salines *et al.* (2017a)

Cette variabilité suggère l'existence de **facteurs de risque spécifiques** aux élevages. En plus des facteurs de risque présentés dans la revue ci-après et repris dans le Tableau III, Lopez-Lopez *et al.* (2018) ont récemment montré que les facteurs associés à une prévalence élevée du HEV étaient : un élevage extensif, l'absence de sas sanitaire et de période de quarantaine, et la présence de carnivores domestiques.

Tableau III - Facteurs de risque associés à une séroprévalence et/ou une prévalence HEV élevée dans les élevages de porcs

Facteurs liés...		
À la taille	Nombre de truies > 1000 (séroprévalence 54,2 % versus 18,9 %)	(Di Bartolo <i>et al.</i> , 2008;
	Elevages de taille moyenne versus élevages de grande taille (OR = 4,95)	Li <i>et al.</i> , 2009a; Jinshan <i>et al.</i> , 2010; Hinjoy <i>et al.</i> ,
	Nombre de porcs > 600 (séroprévalence comprise entre 78 % et 100 %, versus 0 % à 29 %)	2013; Walachowski <i>et al.</i> ,
	Petite taille versus grande taille (séroprévalence 90 % versus 76 %)	2014)
	Taille des cases en post-sevrage > 26 porcs par case (OR = 2,4)	
À la biosécurité	Durée du vide sanitaire en post-sevrage < 4 jours (OR = 1,7)	(Hinjoy <i>et al.</i> , 2013;
	Distance lisier-caillebotis en engraissement < 80 cm (OR = 1,9)	Walachowski <i>et al.</i> , 2014;
	Absence de sas sanitaire (OR = 3,6)	Lopez-Lopez <i>et al.</i> , 2018)
	Absence de période de quarantaine (OR = 2,7)	
	Présence de carnivores domestiques (chiens, chats) (OR = 3,9)	
	Présence de fèces d'oiseaux dans les bâtiments (OR = 2,9)	
Aux mélanges	Mélange d'animaux issus de salles différentes entre la maternité et le post-sevrage (OR = 1,8)	(Walachowski <i>et al.</i> , 2014)
Au type de production	Agriculture biologique versus conventionnel (séroprévalence 89 % versus 72 %)	(Rutjes <i>et al.</i> , 2014;
	Plein air versus conventionnel (séroprévalence 76 % versus 72 %)	Lopez-Lopez <i>et al.</i> , 2018)
	Elevage extensif versus intensif (OR = 2,2)	

La **dynamique de l'infection** par le HEV (âge à l'infection et à l'excrétion, durée de la période infectieuse et quantité de particules virales excrétées, réponse immunitaire humorale, virémie, transmission du virus) ainsi que ses **facteurs de variation** sont détaillés de la revue publiée en 2017. L'article présente également des **pistes pour la surveillance et le contrôle du HEV** dans la filière de production porcine.

Publication 1

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REVIEW

Open Access



From the epidemiology of hepatitis E virus (HEV) within the swine reservoir to public health risk mitigation strategies: a comprehensive review

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Abstract

Hepatitis E virus (HEV) is the causative agent of hepatitis E in humans, an emerging zoonosis mainly transmitted via food in developed countries and for which domestic pigs are recognised as the main reservoir. It therefore appears important to understand the features and drivers of HEV infection dynamics on pig farms in order to implement HEV surveillance programmes and to assess and manage public health risks. The authors have reviewed the international scientific literature on the epidemiological characteristics of HEV in swine populations. Although prevalence estimates differed greatly from one study to another, all consistently reported high variability between farms, suggesting the existence of multifactorial conditions related to infection and within-farm transmission of the virus. Longitudinal studies and experimental trials have provided estimates of epidemiological parameters governing the transmission process (e.g. age at infection, transmission parameters, shedding period duration or lag time before the onset of an immune response). Farming practices, passive immunity and co-infection with immunosuppressive agents were identified as the main factors influencing HEV infection dynamics, but further investigations are needed to clarify the different HEV infection patterns observed in pig herds as well as HEV transmission between farms. Relevant surveillance programmes and control measures from farm to fork also have to be fostered to reduce the prevalence of contaminated pork products entering the food chain.

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1 Introduction

Hepatitis E virus (HEV) is a non-enveloped single-stranded RNA virus. It is transmitted via the faecal-oral route and causes acute hepatitis in humans, clinical signs being similar to hepatitis A infection but usually more severe [1]. Chronic cases have also been described in immunocompromised patients [2]. There are four HEV genotypes: genotypes 1 and 2 are specific to humans and are currently circulating in Asia, Africa and Central America in epidemic waves linked to the consumption of contaminated water [3]. Genotypes 3 and 4 are responsible for sporadic autochthonous human cases in developed countries and are common to humans and other animal species [3, 4]. Genotype 3 in particular is highly prevalent in wild and domestic pigs, but the infection does not lead to a clinical disease [5]. Swine and human HEV strains are genetically very close, and cross-species transmission has been proved [6]. Moreover, a number of sporadic autochthonous cases have been related to the consumption of raw or undercooked pork products, especially liver-based products [7–9]. Thus, hepatitis E is considered to be an emerging zoonosis, domestic pigs being recognised as its main reservoir in industrialised countries [4, 10]. It is crucial to fully understand the conditions related to pig farm infection and HEV transmission dynamics within the swine population in order to limit the risk of introducing contaminated products into the food chain.

Several prevalence studies have been carried out in pig herds, either on a farm or individual scale. Prevalence

estimates derived from either virological or serological analyses have evidenced wide differences depending on the country and year of study. However, the available data are difficult to compare since the pigs' age and production stage vary according to studies, as do the HEV detection methods and biological matrix used for analyses. Moreover, the precision of the different estimates varies greatly between studies owing to huge differences in sample sizes. Even within the same study, the individual and farm-scale prevalences observed are also highly heterogeneous. This wide dispersion suggests the existence of various infection dynamics linked to farm-specific risk factors which have only been sporadically investigated to date. Observational studies mainly report the implication of farming practices in terms of hygiene, biosecurity and rearing conditions. Complementary to this approach, mathematical modelling studies, based either on experimental trials or longitudinal studies on infected farms have helped reveal new insights on HEV infection dynamics.

It is important to explore the epidemiological characteristics of HEV on pig farms for several purposes, e.g. to set up a surveillance programme, or identify control measures to manage the risk of HEV infection and transmission with the ultimate aim of reducing the prevalence of HEV-containing livers at the slaughterhouse. Information available from published papers has therefore been comprehensively gathered to identify key patterns of HEV infection as well as knowledge gaps and research needs. We have specifically focused our study on the epidemiological characteristics of the virus in domestic pigs and their products, since other aspects of this zoonosis have already been reviewed in various papers. The scope of this review thus covers prevalence, risk factors, transmission routes and infection dynamics on pig farms, surveillance and control strategies throughout the pork chain.

2 HEV prevalence in farmed pigs

It is crucial to know the prevalence of HEV on pig farms so as to be able to assess the health situation of the pig population and thus the risk to public health. We counted 86 studies (from 43 different countries) addressing HEV prevalence in farmed pigs. These studies are summarised in Additional file 1 [5, 11–21, 24, 47, 57, 66, 70–73, 79–139]. Various methods were used for data collection: samples were collected from slaughterhouses, randomly-selected or specifically-selected farms, or from serum/faeces/organ banks. Some studies were conducted at a given point in time, leading to an instantaneous prevalence estimate, whereas others were retrospective and estimated the prevalence from sera collected over a given period of time. The number of farms varied from 1 to 2 001; the number of samples from 40 to 6 565. Pigs

included in the studies differed in age and rearing conditions (family-scale or large-scale farms, organic or industrial production, for example). Little information was available on the swine breed or strain. Prevalence was investigated either on a farm or individual level. The virus was sought in several different biological materials, including various organs (e.g. the intestines or liver), serum, faeces, bile and caecal content. Individual or pooled samples were processed using RT-PCR, nested RT-PCR, real-time RT-PCR or antigen detection. The serological response to HEV infection was assessed by detection of anti-HEV antibodies (IgG, IgM or IgA) using ELISA tests with specificity ranging from 85 to 100% and sensitivity from 50 to 100%. The viral strains detected belonged either to genotype 3 or genotype 4.

2.1 Farm-scale prevalence

2.1.1 Farm-scale seroprevalence

Farm-scale seroprevalence reported in 15 studies ranged from 30 to 98% (Additional file 1). For instance, in a study conducted in France in 2011, 65% of the 186 randomly-selected farms were found to have at least one seropositive animal (95% confidence interval 57–74) [5]. The serological prevalence was even higher in a retrospective study conducted in Spain, 204 out of 208 farms (98%, 95% CI 96.1–99.9) having at least one anti-HEV IgG-positive pig [11]. Similarly, in a retrospective study recently carried out in Norway, anti-HEV IgG were detected in 90% (137/153) of the herds [12].

2.1.2 Farm-scale virological prevalence

Farm-scale virological prevalence reported in 25 studies ranged from 10 to 100% (Additional file 1). Widen et al. detected HEV-RNA in swine faeces from 17 out of 22 randomly-selected farms in Sweden (72.7%) [13]. Virological prevalence has also been estimated from HEV-RNA detection in sera: in 72 herds selected in Spain, at least one slaughtered pig tested positive for HEV-RNA in serum on 47.2% of farms [14]. Regarding the presence of HEV RNA in liver, 24% (95% CI 17–31) of 186 randomly-selected pig farms had at least one positive liver in the French national prevalence study conducted by Rose et al. in 2011 [5].

2.2 Individual prevalence

2.2.1 Individual seroprevalence

Individual seroprevalence ranged from 8 to 93% in the 45 studies analysed (Additional file 1). In France, 31% (95% CI 24–38) of the slaughter-aged pigs in 2011 were found HEV seropositive [5]. Similarly, Jinshan et al. detected 52% of sampled pigs positive for anti-HEV antibodies in Mongolia [15]. Crossan et al. separately tested the presence of the different types of anti-HEV antibodies in

Scotland and reported that, of 176 serum samples tested, 29% ($n = 51$) were anti-HEV IgG-positive, 36.9% ($n = 65$) anti-HEV IgA-positive and 29% ($n = 51$) anti-HEV IgM-positive. Overall seroprevalence (anti-HEV IgG+ and/or IgA+ and/or IgM+) was 61.4% ($n = 108$) [16]. In the same region and period, individual HEV seroprevalence was found by Grierson et al. to be even higher; they reported that 584 out of 629 pigs (92.8%) had anti-HEV antibodies at the time of slaughter [17].

2.2.2 Individual virological prevalence

Individual virological prevalence ranged from 1 to 89% in the 69 reported studies (Additional file 1). For instance, the HEV genome was detected in the faeces of 42% of 274 randomly-selected pigs from six different swine farms in northern Italy [18]. HEV RNA was also detected in serum: Crossan et al. reported a virological prevalence of 44.4% in serum (72/162) [16], whereas Grierson et al. detected HEV RNA in only 3% of plasma samples (22/629) in pigs at slaughter age [17]. In the same study, 15% of caecal contents (93/629) were found positive to HEV RNA [17]. Regarding the detection of HEV in liver, Rose et al. reported an individual prevalence of HEV RNA-positive livers of 4% (95% CI 2–6) at slaughter age [5].

Both at farm and individual levels, studies carried out in a given country at different times or retrospectively did not show any significant change in prevalence estimates over time, suggesting that HEV was constantly circulating in pig farms. The marked variability in individual prevalence estimates between farms is noteworthy: from 12.8 to 72.5% in Italy [18], from 4 to 58% in Argentina [19], and from 5 to 90% in France [5]. This may reflect different infection dynamics related to farm-specific risk factors.

2.3 Factors influencing HEV prevalence estimates

To date, few studies have reported the risk factors associated with high HEV prevalence on pig farms. We identified 12 studies addressing HEV risk factors, but only six of them quantified the impact of risk factors on HEV seroprevalence or on the prevalence of shedding pigs through odds ratio estimates (Table 1). The risk factors for a high HEV seroprevalence were mainly related to (1) farm characteristics and (2) farming practices. The farming scale (medium-size and family-scale farms, linked to the number of pigs and sows) was identified as a risk factor related to HEV seroprevalence [15, 18, 20, 21]. It was also shown that HEV seroprevalence was significantly higher in organic farms than in conventional ones [22]. Several high-risk rearing practices were reported, the main ones being late weaning, mingling practices at the nursery stage and poor hygiene [23]. Biosecurity measures such as requiring a shower upon entry were also found to be protective factors with respect to the

Table 1 Quantified risk factors associated with a high HEV seroprevalence in pig farms

Di Bartolo et al. [18]	Number of sows > 1000: HEV seroprevalence = 54.2 vs 18.9%
Li et al. [21]	HEV seroprevalence on family-scale farms = 90 vs 76% in large-scale farms ($p < 0.01$)
Jinshan et al. [15]	Number of pigs > 600: HEV seroprevalence ranged from 78 to 100%, vs 0 to 29%
Hinjoy et al. [20]	Medium-sized farms compared with large farms: OR 4.95 (1.79–13.70)
	Presence of bird faeces inside the pig house: OR 2.87 (1.07–7.71)
Walachowski et al. [23]	Duration of the nursery down period < 4 days: OR 1.7 (1.04–2.9)
	Distance between pit manure and slatted floor in fattening premises < 80 cm: OR 1.9 (1.1–3.5)
	Mingling of pigs from different premises between farrowing and nursery stages: OR 1.8 (1.1–2.9)
	Pen size in nursery rooms > 26 pigs/pen: OR 2.4 (1.2–4.8)
Rutjes et al. [22]	HEV seroprevalence on organic farms = 89 vs 72% on conventional farms ($p = 0.04$)
	HEV seroprevalence on free-range farms = 76 vs 72% on conventional farms ($p = 0.06$)

OR odds ratio.

prevalence of faecal HEV RNA shedding [24]. A seasonal influence on the prevalence of HEV RNA among swine was also reported, with a major peak in March–April followed by a smaller peak in September–October [25].

European wild boars are recognized as a potential reservoir of HEV [26, 27]. Moreover, some experimental studies evidenced that HEV strains could be transmitted from European wild boars (*Sus scrofa*) to domestic pigs [26, 28, 29]. Though no study directly related HEV prevalence in pig farms to contact with wild boars, they may play a potential role in the swine HEV epidemiology in free-ranged pig production units. The role of wild boars as HEV risk exposure for domestic pigs would deserve further investigation.

3 HEV infection characteristics and dynamics on pig farms

3.1 HEV infection features in pigs

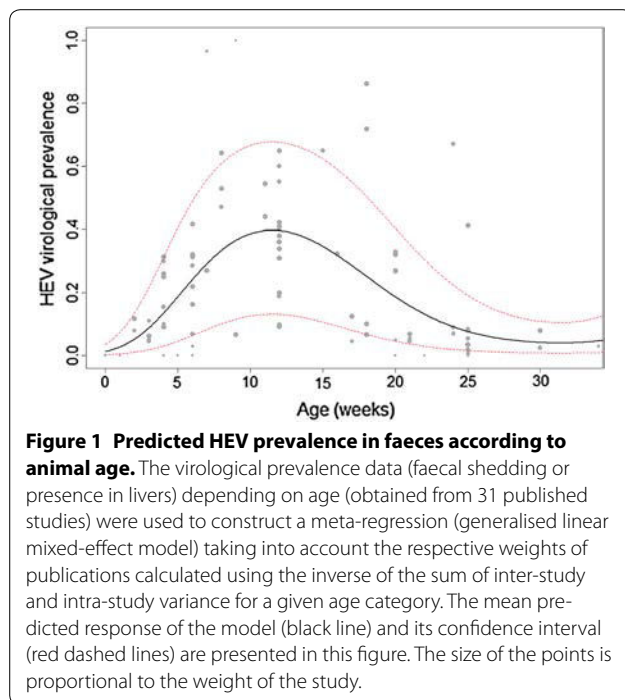
3.1.1 Age at HEV infection and shedding

The age at infection was only sporadically reported in the literature, with only three studies inferring from serological results the window within which infection took place. Almost all the studies conducted on pig farms only reported the age at shedding, and not the age at infection. Based on a large-scale seroprevalence survey conducted in Japan, the average age at infection was estimated to range from 59.0 to 67.3 days with more than 80% of infections occurring between the ages of 30 and 90 days [30]. The results of a longitudinal study on three French farms were quite different, most HEV infections occurring between 105 and 140 days of age [31]. Using Spanish data, Andraud et al. estimated the age at infection between 60.9 and 96.6 days [32]. Based on serological data from longitudinal studies in six pig herds, passive immunity was shown to delay early HEV infection of piglets by about 6 weeks in all but one farm on which the dynamics of infection were similar, whatever the animals' initial serological status. Although the protective role of passive immunity

cannot be denied, the latter case highlighted the strong interaction between farm-specific husbandry and hygiene practices and the HEV transmission process [32].

HEV infection dynamics have in the majority of studies been described through the monitoring of shedding pigs. These studies showed that the prevalence of HEV RNA in swine faeces and serum depend on the production stage, i.e. the pig's age (Additional file 2 [14, 15, 18, 21, 57, 66, 86–89, 91–93, 95, 96, 104, 108, 109, 113, 114, 118–120, 133, 135, 136, 140–150]). A broad shedding period from 1.5 to 5 months of age was globally reported at farm scale. In most cases, the faecal shedding peak was described in 3-month-old to 4-month-old pigs, and few animals had PCR-positive faecal samples after 6 months of age. We performed a meta-regression analysis using data from 31 studies published between 2002 and 2016 which reported the prevalence of faecal HEV shedding or presence of HEV in livers depending on pig age. A weighted generalised linear mixed-effect model with the publication as a random effect, using intra and inter-study variances for a given age category as weight for individual studies, was fitted to age-specific prevalence data. Despite marked variability between studies, the model showed that the probability of faecal shedding peaked around 90 days of age (Figure 1). The shedding prevalence estimate at 185 days (a common slaughter age) was 6.1% [1.2–15.4].

Few studies have explored the factors influencing variations in age at shedding. A longitudinal study conducted on three swine farms showed that HEV shedding in pigs which had been previously infected by Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) occurred later than in pigs that were PRRSV-negative or which had been infected by PRRSV after HEV infection (hazard ratio = 0.49, $p < 0.01$) [33]. Hence, the latency period (delay between infection and shedding) may be affected by different circumstances, modifying the age at shedding. When pigs were infected intravenously, the latency



period measured by Bouwknegt et al. was 3 days, compared to 6.9 days [5.82–7.9] when inoculated orally [34, 35]. According to the results of a co-infection trial, the HEV latency period was extended by a factor of 1.9 in the event of PRRSV co-infection (12.9 days [12.8–14.4]) [36]. In a recent longitudinal study on two Finnish farms identified as HEV-positive, piglets started to shed HEV between 7 and 12 weeks of age. Of these, 62.5% only began shedding HEV between the ages of 10 and 12 weeks [37]. The authors suspected that the difference in age of the onset of infection or shedding may have resulted from the quantity and/or quality of colostrum providing the piglets with maternal antibodies. The quantity of virus particles ingested may also modify the course of infection at the individual level. The minimal *per os* infectious dose is still not well known. However, it has been estimated that oral infection would require about 20 mg of faeces per day containing on average 10^8 genome equivalent (GE) per gram over three consecutive days to achieve a 50% probability of infection [38]. These results were then confirmed by testing different infection doses for inoculation by the oral route: a minimal viral load of 10^6 GE was necessary for pigs to be orally infected and to shed and transmit the virus [35]. Below that level, only sporadic shedding was detected, with no transmission to sentinel piglets.

3.1.2 Shedding duration and quantity of virus particles shed

Shedding duration is not easy to measure on farms as it requires an individual follow-up of pigs. The HEV

shedding period was estimated at around 27 days in two studies carried out on commercial pig farms in Europe [33, 39]. Data from three pig herds evidenced a huge variation in the infection dynamics according to the farms, with some batches exhibiting late and short-term infections, while others had early and long-term shedding periods [31]. When pigs were experimentally infected by the intravenous route, shedding lasted from 13 to 49 days depending on the viral dose inoculated [34], whereas an HEV infection trial described a shedding duration of 9.7 days [8.2–11.2] when pigs were orally infected [35]. This experimental estimate was lower than shedding durations observed on farms, suggesting the existence of factors influencing duration of the shedding period. One of them may be other pathogens co-infecting pigs. For instance, one trial showed that a PRRSV/HEV co-infection dramatically extended the shedding period by a factor of 5 to 48.6 vs 9.7 days [36].

Few data are available on the quantity of virus particles shed by infected animals. In field conditions, one study carried out in two pig herds in Japan reported an HEV load in faeces of between $10^{3.8}$ and 10^6 GE/g throughout the pigs' life [40]. Similarly, the quantity of HEV particles shed was evaluated between 10^4 and 10^6 GE/g of faeces in an experimental trial involving 18 pigs that had been orally infected [35]. When pigs were co-infected with PRRSV, the viral load shed increased to between 10^5 and 10^8 GE/g of faeces, and the accumulation of HEV in the environment was significantly higher too [36].

3.1.3 Humoral immune response

Fourteen studies investigated the humoral response of pigs following HEV infection (Additional file 2). In a longitudinal study carried out on six Spanish farms [41], IgM antibodies were first detected at 7 weeks of age in five farms and at 13 weeks of age in only one farm, whereas IgG antibodies were firstly observed at 13 weeks of age in four farms and at 18 weeks of age in the two other farms. At slaughter age (26 weeks), IgG antibodies were detected in 50 to 100% of pigs on five out of the six farms. In the study conducted by de Deus et al. [42], IgG antibodies were detected later (around 15 weeks), whereas IgA and IgM appeared at around 12 weeks. Similarly to the previous study, IgG antibodies were detected up to the slaughter age (22 weeks), whereas IgA and IgM only remained for 4–7 weeks.

Thanks to field data collected in Japan, Satou and Nishiura estimated the time required for seroconversion at 25 days (95% CI 20.9, 31.3) [30]. Similarly, the time to HEV seroconversion was estimated at 26.3 days in an experimental study and it was shown that co-infection with PRRSV delayed the time to seroconversion to 43.1 days, increasing the lag to seroconversion by a factor

of 1.6 [36]. The presence of maternal antibodies was also found to delay seroconversion in piglets [40, 43]. The time taken for maternally-derived antibodies to wane depends on the quantity of the initial intake of colostral antibodies [41], which is itself related to the sow's age [44] and HEV immune status. Passively acquired IgG remained detectable up to 9 weeks of age in piglets born to highly HEV-seropositive sows, compared to 1–3 weeks in piglets born to sows with weak anti-HEV immunity [42].

3.1.4 HEV viraemia

The natural course of infection in pigs involves infection at around 8–12 weeks of age coinciding with declining maternal antibodies, with a generally short viraemia lasting from 1–2 weeks followed by a more prolonged period of viral shedding in the faeces (Additional file 2). However, longer viraemia periods have also been reported, especially in the case of late HEV infections in pigs, possibly lasting up to slaughter age (Additional file 2). In an experimental infection study, Sanford et al. observed more prolonged periods of viraemia in some pigs, and one pig was continuously viraemic for 12 weeks post-infection [45]. A study in Scotland showed that 44.4% of pigs tested were viraemic at slaughter age [16], whereas another study conducted in the UK only reported 3% of viraemic pigs at slaughter age [17]. Maternally-derived antibodies were found to delay the onset of viraemia [40]. Furthermore, the amount of HEV RNA in the serum was found to be lower than that in the faeces, the highest serum HEV RNA titre being on day 90 in a pig from a litter with passive immunity ($10^{4.2}$ copies/mL) and on day 60 in a pig from a litter without passive immunity ($10^{5.6}$ copies/mL).

3.2 Routes of HEV transmission between pigs

It has been proved that the virus is mainly shed by the faecal route, leading to an accumulation of HEV in the pigs' environment at all production stages on infected farms, as well as in manure pits [46]. Depending on the type of floor (litter or slatted floor), the animals are constantly in contact (more or less direct) with the environmental HEV reservoir. The virus has also been detected in the urine of HEV-infected pigs [38, 47, 48], making urine a potential transmission route, especially given the considerable volume produced per day and the potentially longer viral shedding in this medium [48]. Given the urinary and faecal shedding routes, drinking water and/or feed may also be indirect vectors of HEV transmission, especially if feeding and drinking equipment can be easily contaminated by faeces and urine [46]. Finally, daily repeated contacts between pigs kept in the same pen and housed in a confined environment, as well as the mingling of pigs at different production stages may increase

the propagation of HEV on farms [23, 42, 49, 50]. These findings confirm that the faecal-oral route is the major transmission route of HEV in pigs [48, 49], even if several trials have highlighted the difficulty in inoculating pigs per os [51, 52]. Indirect transmission from one pen to another (without any pig mingling) was found to be low [35].

Three-month old and older pigs were recognised as the major shedding sources in farm conditions (Figure 1). Fernandez-Barredo et al. showed that weaning and early fattening stages were critical periods for HEV shedding with respectively 45 and 60% of shedding animals [46]. Breeding animals also play an important role in the spread and persistence of HEV within pig production units in two ways: (1) by providing maternally-derived antibodies that protect their piglets from early-life infection, (2) by possibly transmitting the virus via farrowing crates during lactation periods. Indeed, investigations into faecal shedding in sows around the farrowing period revealed prevalences ranging from 16 to 21% [41, 42, 46]. A high proportion of multiparous sows were found to shed the virus, as well as gilts and young sows but to a lesser extent [18]. A study recently carried out in China showed that farrowing sows had an approximately 2.5-fold higher risk of infection (OR 2.46, $p < 0.01$) than pre-farrowing sows [53]. Another study on Göttingen Minipigs in the context of xenotransplantation safety detected HEV in the sera of three sows 6 days after delivery and in their offspring [54]. Finally, in a longitudinal study on three pig farms, piglets from two farms shed the virus as early as the lactation phase in farrowing facilities [31]. Thus, horizontal transmission between sows and their piglets may occur in the early stage of a piglet's life. Moreover, sows may transmit the virus to the foetus by the transplacental route should viraemia occur during gestation, viral RNA having been detected in the livers of aborted foetuses [55]. However, these results are still controversial, since one experimental study did not show any vertical transmission after intravenous inoculation of HEV to pregnant gilts [56]. Nevertheless, it cannot be excluded that breeding animals may constitute an HEV reservoir on infected farms, periodically shedding the virus according to changes in their immune status due to physiological conditions (pregnancy, farrowing). Sows may thus maintain viral propagation in swine herds.

3.3 Quantitative data on HEV transmission

The persistence of a virus on farms is linked to (1) the intrinsic ability of the virus to remain in the animals' environment, (2) the possibility of regular reintroductions of the virus onto farms and (3) the ability of the virus to survive and spread in the population. This last criterion can be studied through the basic reproduction

number (R0) of the virus, which measures the number of secondary infections due to one infectious pig during its entire shedding period in a fully susceptible population. The higher the basic reproduction number, the easier it is for the virus to spread and the greater its ability to stay within the population. Using a large-scale seroprevalence survey dataset from Japanese pig farms, Satou and Nishiura estimated the HEV R0 between 4.02 and 5.17, meaning that one infectious animal could theoretically infect four to five other pigs during its infectious period [30]. Based on an experimental trial carried out in the Netherlands, this ratio was estimated at 8.8 [34]. However, this assessment relied on one-to-one HEV transmission experiments, accounting for horizontal transmission by direct contact only. The trial by Andraud et al. investigated the transmission of HEV from pigs inoculated by the oral route to pigs in direct contact (in the same pen) or indirect contact (in an adjacent pen) with the inoculated pigs, assuming both environmental and direct transmission routes [35]. Although much lower than previous estimates with a partial reproduction number of 1.41 [0.21–3.02], direct transmission alone could be considered as a factor fostering the infection's persistence within a population. The quantity of virus present in the environment was found to play a pivotal role in the transmission process, strongly influencing the probability of infection, with a within-pen transmission rate estimated at $2.10 \cdot 10^{-6}$ g/GE/day [$1.10 \cdot 10^{-7}$ – $7.10 \cdot 10^{-6}$]. Between-pen environmental transmission occurred to a lesser extent (transmission rate: $7.10 \cdot 10^{-8}$ g/GE/day [$5.10 \cdot 10^{-9}$ – $3.10 \cdot 10^{-7}$]) but could further generate a within-group infection process. The combination of these transmission routes could explain the persistence and high prevalence of HEV in pig populations. Moreover, the transmission of HEV was found even enhanced in the presence of co-infections. Indeed, based on a similar experimental design with pigs co-infected with PRRSV, the transmission of HEV by direct contact was estimated to be 4.7 times higher in pigs co-infected with PRRSV (direct transmission rate = 0.70 [$1.18 \cdot 10^{-3}$ –3.67]). Direct transmission therefore plays a more important role in HEV transmission when animals were co-infected and reflecting the increased quantity of virus particles shed [36]. The indirect transmission rate, considered to be the average number of animals that could be infected by a single genome equivalent present in the pen environment, was estimated at $6.59 \cdot 10^{-6}$ g/GE/day [$1.43 \cdot 10^{-10}$ – $1.27 \cdot 10^{-4}$], i.e. 3.3 times higher with co-infection than without. In other words, 3.3 times fewer virus particles were required to infect a co-infected animal than an HEV-only infected animal. The impact of maternally-derived antibodies on HEV transmission was also assessed by modelling field-based longitudinal data on HEV dynamics of infection [32]. In this study, HEV

transmission among piglets with passive immunity was estimated to be 13 times lower than in fully susceptible animals, with a relatively marked variability between herds (range: 5–21).

4 Consequences of HEV infection dynamics on the prevalence of contaminated livers and pork products

4.1 Prevalence of HEV-containing livers at the slaughterhouse

In the ten studies investigating the prevalence of HEV-containing livers in pigs of slaughter age (Table 2), all but one reported prevalences ranging between 0.8 and 10% of liver samples, but the prevalence reported in Italy was over 20% [57]. Two conditions are required for a high prevalence of HEV-containing livers at slaughter age: (1) the virus has to spread massively on farms; (2) the later the infection occurred, the higher the risk that pigs are still infectious at slaughter. One study on French pig farms reported several risk factors, such as the slaughter age, genetic background, lack of hygiene measures and origin of drinking water [23]. An experimental trial also showed that the co-infection of pigs with HEV and PRRSV increased the likelihood of HEV-containing livers at slaughter time [36]. Satou and Nishiura built a model from field data and using a sensitivity analysis, they showed that a decline in the force of infection would postpone the infectious process to a later age, which would in turn heighten the risk of pork-to-human transmission through the consumption of infected products [30].

4.2 Consequences on the safety of pork products entering the food chain

Nine prevalence studies were conducted on marketed pork products (Table 3). Different kinds of pork products were tested, such as raw livers, sausages, figatelli, pâté, etc. The prevalence of contaminated pork products varied from less than 1% to more than 50% depending on the country and the product. The highest prevalences were observed in products prepared with raw pork liver [7, 58]. No study was led on meat but, given the late viraemia at slaughter age that was observed in several studies (see above), there may be a potential risk to public health linked to the consumption of raw or undercooked pork meat.

The presence of HEV in food products consumed raw or undercooked raises the question of the thermal stability of HEV, which was addressed in three studies. The first one was based on heating faecal suspensions of HEV genotypes 1 and 2 to temperatures between 45 and 70 °C and inoculation in a cell culture permissive to HEV [59]. The second study used pigs inoculated with

Table 2 Prevalence of HEV RNA in livers collected at slaughterhouses reported in ten studies

References	Country	No. of samples	Prevalence of RNA-positive livers (%) [95% CI]
Bouwknegt et al. [51]	Netherlands	62	6.5 [1.8–15.7]
Rose et al. [5]	France	3 715	4 [2–6]
Di Bartolo et al. [57]	Italy	48	20.8
Di Bartolo et al. [68]	Spain	39	3
	Italy	33	6
	Czech Republic	40	5
Berto et al. [67]	UK	40	3
Gardinali et al. [70]	Brazil	118	1.7
de Souza et al. [71]	Brazil	453	1.3
Temmam et al. [72]	Madagascar	250	1.2
de Paula et al. [73]	Cameroon	345	0.8
Mykityczuk et al. [74]	Canada	19	10.5

Table 3 Prevalence of HEV-positive marketed pork products reported in nine studies

References	Country	No. of samples	Prevalence of RNA-positive pork products (%)
Yazaki et al. [75]	Japan	363	1.9% of livers sold in local grocery stores
Feagins et al. [76]	USA	127	11% of livers sold in local grocery stores
Colson et al. [7]	France	12	58% of marketed figatelli
Wenzel et al. [77]	Germany	200	4% of livers sold in butcher's shops and grocery stores
Berto et al. [67]	UK	63	10% of marketed sausages
Di Bartolo et al. [68]	Spain	93	6% of marketed sausages
	Czech Republic	92	0% of sausages
	Italy	128	0% of sausages
Pavio et al. [58]	France	394	30% of figatelli, 29% of liver sausages, 25% of quenelles, 3% of dried salted livers
Heldt et al. [78]	Brazil	50	36% of marketed pâté and blood sausages
Mykityczuk et al. [74]	Canada	111	47% of pork pâté, 0% of raw pork sausages

pork liver homogenates containing infectious genotype 3 HEV heated to 56 °C for 1 h, fried for 5 min (71 °C internal temperature) or boiled for 5 min [60]. Both studies showed that HEV was more likely to resist when heated to only 56 °C and was inactivated at temperatures higher than 71 °C. The third study was conducted on more complex foodstuffs prepared according to industrial recipes (liver pâté) and showed that it was necessary to heat the food to an internal temperature of 71 °C for 20 min to fully inactivate HEV [61]. To date, no information is available about the efficacy of drying on HEV persistence.

5 Improving HEV surveillance and control in the swine reservoir: from farm-targeted actions to pork product control

5.1 Options for control measures on pig farms

5.1.1 HEV vaccination on farms

No commercial vaccine is currently available against HEV in pigs. Some theoretical work has been carried out

to evaluate the benefits of vaccination against this zoonosis, which does not have any consequences on pig health or the economic performance of swine herds. Using a modelling approach, Backer et al. tested three effects of vaccination: a decrease in the virus transmission rate, in animal susceptibility to HEV infection, and in the duration of the infectious period [39]. As previously shown by Satou and Nishiura [30], a reduced transmission rate and susceptibility, which induces a decrease in the force of infection, led to an increase in the number of infectious animals at the slaughterhouse. When the vaccine affected the duration of the infectious period, the proportion of pigs still infectious at slaughter age was lower. Further work would be needed to evaluate the required efficacy for a vaccine to eradicate the infection and to develop the corresponding efficient vaccine, without forgetting considerations on interference with passive immunity, co-infecting pathogens and rearing practices. A cost-benefit analysis of vaccine development would also be necessary,

including public health consequences in the event of widespread consumer exposure to contaminated pork products, and the economic consequences linked to a potential loss of consumer confidence in pork safety.

5.1.2 Control of risk factors and co-infecting pathogens

As previously mentioned, a lack of hygiene measures and several farming practices (such as late weaning or mingling practices at the nursery stage) were reported as risky for HEV transmission and persistence. Biosecurity and farming practices should therefore be enhanced to reduce HEV risks [23].

As reported previously, co-infections with immunosuppressive swine viruses — frequently observed in pig herds — could lead to chronic HEV infection, which may dramatically increase the risk of pig livers containing HEV at slaughter time. For instance, a PRRSV/HEV co-infection or a PRRSV infection prior to HEV infection delayed HEV shedding and the onset of the anti-HEV humoral immune response, increased the quantity of virus particles shed and extended the shedding period, increased the direct transmission rate and HEV infection susceptibility, and increased the proportion of HEV-positive livers at slaughter time [33, 36]. Thus, controlling intercurrent swine diseases (e.g. through PRRSV vaccination programmes) could be a major lever in the control of hepatitis E. Further research is needed in this domain to better understand the interactions between HEV and immunosuppressive pathogens, including an evaluation of the effect of other immunosuppressive co-infections frequently encountered in the field as well as non-biotic components such as mycotoxins which are likely to interfere with the immune response.

5.1.3 Organisation of the pig production network

To prevent the spread of infectious agents, it is necessary to consider the pyramidal structure of the pig production sector and the way contacts between pig farms are organised [62]. Few data are available yet. A recent study reported the presence of HEV inside and outside farm buildings, on trucks and in the slaughterhouse yard, thus suggesting viral transmission between farms and throughout the production network [63]. However, further research is needed to (1) model the pig production network; (2) explain, assess and quantify the risk of HEV transmission between pig farms through animal introductions (replacement) or indirect vectors.

5.2 Surveillance throughout the pork chain

To our knowledge, no uninterrupted surveillance programme of the swine reservoir has ever been implemented in any country. Surveillance actions could be implemented at different steps: on pigs at the farming

stage or at the slaughterhouse, or on pig livers and pork products.

5.2.1 Monitoring of pigs on farms or at the slaughterhouse

Pig monitoring could be either serological or virological. (1) Serological monitoring could be a feasible large-scale approach. Data are available on the intrinsic features of the serological tests that could be used [64, 65], but further comparative analysis is still needed. Indeed, although a single HEV serotype exists, test performance varies depending on the HEV genotype [65]. However, more and more commercial ELISA tests are available and geared to HEV genotype 3, which is the main one circulating on pig farms in Europe and the US (e.g. HEV ELISA 4.0 V, MP Biomedicals). Moreover, some tests only detect IgM whereas others detect all immunoglobulin classes. Regarding the relevance of using serological tests, studies revealed a significant relationship between within-farm seroprevalence and the probability of detecting HEV-positive livers on that farm [5]. Indeed, Rose et al. observed that the probability of viral presence in the liver was significantly higher on farms where seroprevalence at the finishing stage was greater than 25%: OR 6.7 [2.1–21.6]. This result suggests that farms at risk are those in which the virus circulates intensely and spreads to more than 25% of fattening pigs [66]. However, at an individual level, some HEV RNA-positive pigs (detected in the liver) are seronegative because infection occurs late, not long before slaughter. This is why it appears that serological tests on fattening pigs from farrow-to-finish farms should be supplemented by tests on sows in order to clearly determine the HEV status of the farm. (2) The virus could also be detected in faeces as it appears that the virus' presence in the liver and viral shedding are well correlated [31]. This surveillance action could be performed on farms, e.g. for a pre-slaughter check by sampling several animals. It could also be done at the slaughterhouse, in ante mortem waiting areas.

5.2.2 Surveillance of pig livers and pork products

Many human cases in industrialised countries are related to the consumption of so-called “high-risk” products, i.e. pork products consumed raw or not well cooked and containing a high proportion of pork liver. Surveillance could therefore target those specific products (liver sausages, liver pâté, figatelli, etc.). To date, few detection tests have been developed [61, 67, 68] and only one method for HEV detection in food has been marketed (HepatitisE@CeeramTools™, quantitative RT-PCR Kits for food & environmental samples). The viral concentration in food is often low. Moreover, these complex matrices are composed of liver, fat, salt and spices that make detection difficult. The analysis of meat matrices

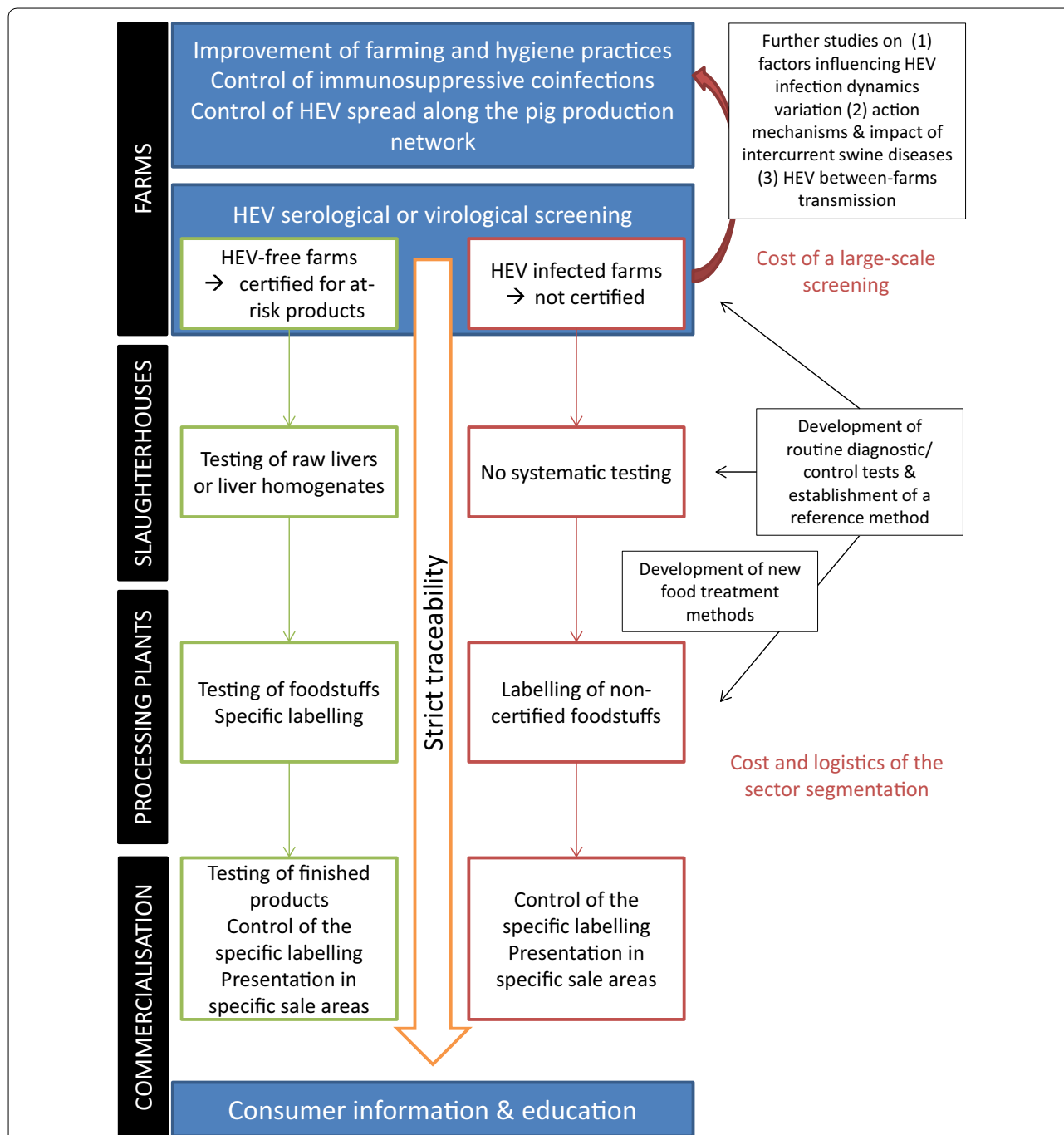


Figure 2 HEV surveillance and control of the swine reservoir: from farm-targeted actions to pork product control (adapted from [69]).

The left side of the diagram presents a number of measures to mitigate the risk of human exposure to swine HEV, with actions applying to both farms and foodstuffs. A certification process (green and red squares) could be implemented throughout the food chain to guarantee the absence of HEV in products derived from raw pork liver. The right side lists several knowledge gaps and research needs (black squares) in addition to the challenges involved in implementing these measures (in red).

requires a rotary mill that is not frequently employed in non-specialised laboratories. Fat removal is essential, but tedious and mostly manual. Analysing food products is more complex than analysing livers, so livers could be

tested after mixing and before adding other ingredients. As the transformation steps do not affect HEV stability (see above), the contamination of livers may be a relevant indicator of the risk of human exposure to HEV.

5.2.3 Applications and research needs

HEV monitoring activities in the pork production chain are needed for several purposes: (1) to acquire an uninterrupted series of prevalence data and monitor changes in prevalence and the virus itself (e.g. evolution of the prevalence of the different HEV subtypes and emergence of genotype 4, which is still only sporadically detected in Europe and the US); (2) to investigate more precisely HEV infection dynamics and factors influencing their variation; (3) to prevent contaminated livers from entering the food chain. For that purpose, the qualification of farms and/or animals and/or livers with regard to their HEV status could be considered. A French expert appraisal suggested three options that could be jointly implemented in order to prevent HEV-positive liver being used for the preparation of products containing raw liver [69]:

1. *Qualification of HEV-free farms* The farm could be qualified following serological testing on sampled animals (see above). This would enable the identification of farms eligible to market raw livers. However, this approach would be costly and would require constant testing, since the HEV status is unlikely to remain stable over time. Moreover, the logistics in slaughterhouses would then be complex, requiring an additional means of keeping HEV-free animals completely separate from HEV-positive ones.
2. *Real-time qualification of HEV-free batches at the slaughterhouse* Faecal samples could be taken from a determined number of pigs per batch, either in the ante mortem waiting area or the post mortem chain. The batches would be released after test results on a just-on-time basis. The HEV status of batches would be precisely known and only HEV-free batches would be used for the preparation of products containing raw liver. However, the logistics for the slaughterhouse would be both complicated and costly.
3. *Qualification of liver homogenates* RT-PCR could be performed on livers or liver homogenates to determine their HEV status. This approach would be less expensive yet would still enable an immediate risk management procedure to be followed depending on the result of the analysis. However, in the light of the HEV prevalence in livers, there would be a risk of detecting and rejecting many liver mixes.

These three options could lead to the creation of a separate sector dedicated to the fabrication of foodstuffs containing raw liver. Such a certification procedure requires regular food control capabilities relying on effective analytical tools for routine use, particularly on farms, at

processing facilities and points of sale. The effectiveness of the certification system also relies on the traceability of pork livers, and requires a reference on the product label for all items containing pork liver. The label should provide consumers with information on the possible hazards related to consumption of these products. The coexistence of these two sectors may pose problems both in terms of logistics for the slaughterhouses and processing plants, and a risk of confusion for the consumer between products with different food safety statuses.

Whatever the qualification method, further studies are needed to compare the current tests, develop a reference method and establish a sampling plan geared to the sector's situation. It is also necessary to investigate more precisely the risk linked to pork meat in order to assess the need for a meat surveillance and control plan. Figure 2 summarises the options for control measures throughout the food chain, and identifies knowledge gaps and challenges.

6 Conclusion

The epidemiology of HEV in the swine reservoir is far from being fully elucidated. Though many prevalence studies have been carried out in numerous countries in the last decade, there remain knowledge gaps that still have to be addressed. Research needs to focus on the factors that could explain the huge between-herd variation in infection dynamics, HEV transmission between farms and throughout the pig production network, and finally the mechanisms of action and impact of inter-current swine diseases. Further work also needs to be carried out to harmonise diagnostic tests and develop a standard reference method to detect HEV in complex foodstuffs. Surveillance plans and control programmes have to be carefully considered to mitigate the risk of human exposure to HEV through the consumption of pork products.

Additional files

Additional file 1. HEV serological and virological prevalence in the pig population at farm and individual levels. Farm-scale prevalence figures were reported in 34 studies. Farm-scale seroprevalence ranged from 30 to 98%, while farm-scale virological prevalence ranged from 10 to 100%. Individual prevalence figures were reported in 82 studies. Individual seroprevalence ranged from 8 to 93%, whereas individual virological prevalence ranged from 1 to 89%.

Additional file 2. Evolution of HEV RNA prevalence and anti-HEV antibody prevalence according to pig age. Thirty-seven studies explored the variation of HEV virological and serological prevalence with the pig age. m: months; w: weeks; d: days.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MS reviewed literature and drafted the manuscript. MA and NR supervised the project. All co-authors revised the manuscript. All authors read and approved the final manuscript.

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Additional file 1

Reference	Country	Year of study	Sampling methodology (type of farm, selection method, pig age)	Number of samples		Sample type (serum, liver, faeces)	Farm-scale prevalence		Individual prevalence	
				Number of farms	Number of pigs		Seroprevalence	Viral prevalence	Seroprevalence	Viral prevalence
Clayson et al., 1995 [79]	Nepal	-	Free-roaming pigs	-	55	Serum, faeces	-	-	32.7%	6.4%
Wu et al., 2000 [80]	Taiwan	-	Random sample collection from four different areas. Pig age: 191 were 2-5 months old; 44 were 6-7 months old	-	235	Serum	-	-	-	1.3%
Garkavenko et al., 2001 [81]	New Zealand	-	Pig age for serum collection: 24 sows, 23 1-week-old pigs, 25 20-week-old pigs, 66 3-year-old sows from a blood bank. Pig age for faeces collections: 17 7-week-old pigs, 7 12-week-old pigs, 21 10-week-old pigs	Serum collection: one 1 herd + blood blank from 22 herds Faeces collection: 2	183	Serum, faeces	91%	-	-	60.7%
Arankalle et al., 2001 [82]	India	1988 and 1993	Sample collection from archived blood samples	-	234	Serum	-	-	66.2%	-
Yoo et al., 2001 [83]	Canada	-	Sample collection in slaughterhouses. Pig age around 6 months	80	998	Serum	-	-	59.4%	-
Withers et al., 2002 [84]	USA	1999	Commercial and academic swine production sites. Pig age > 2 months old	4	84	Serum	-	-	34.5%	-
Huang et al., 2002 [85]	USA	-	Farms from six U.S. states + from Diagnostic Laboratories. Pig age: 2 to 4 months old	37	96	Serum, faeces	-	54%	-	35%
Choi et al., 2003 [86]	Korea	-	Random selection from the sera that had been submitted to the College of Veterinary Medicine, Seoul National University, for a routine examination of bacterial and viral diseases. Pig age for serological analyses: 1 to 4 months old. For serological analyses: 1 to 7 months old	23	128 serum samples for virological analyses. 264 serum samples for serological analyses	Serum	-	-	14.8%	2.3%
Banks et al., 2004 [47]	U.K.	-	-	2	42 faecal samples, 21 tissue samples, 256 sera	Serum, faeces, tissue	-	-	85.5%	Faeces: 26%; Tissue: 76%
Wibawa et al., 2004 [87]	Indonesia	2003	Eight swine farms on Bali Island	8	99	Serum	-	-	72%	1%
Vital et al., 2005 [88]	Brazil	-	Sample collection in four standard commercial herds. Pig age: from 1 to > 25 weeks old	4	357	Serum	-	-	63.6%	-
Munné et al., 2006 [19]	Argentina	-	Pig age for serological analyses > 6 months old. For virological analyses 1 to 2 months old	5	54 faeces samples for virological analyses. 97 serum samples for serological analyses	Serum, faeces	-	-	22.7%	88.9%
Fernández-Barredo et al., 2007 [89]	Spain	2002-2004	Four different production stages	21	131	Serum, faeces	-	76%	-	Serum: 14%, Faeces: 16%
Jung et al., 2007 [90]	Korea	1995-2004	Sample collection from archived hepatic tissues	-	388	Liver	-	-	-	10.8%
Kim et al., 2008 [91]	Korea	-	12 swine farms located in central and southern Korea	12	565	Faeces	-	-	100%	17.5%
Seminati et al., 2008 [92]	Spain	1998-2000	-	41	439	Serum	97.6% IgG and 82.9% IgM	-	41.9% IgG and 28.8% IgM	-

McCreary et al., 2008 [93]	U.K.	2007	Ten farms in the Yorkshire Humberside. Age groups: just weaned (3 to 5 weeks old), growers (10 to 12 weeks old), finishers (22 to 24 weeks old) and adult dry sows	10	200	Faeces	-	100%	-	21.5%
Ning et al., 2008 [94]	China	-	Sample collection from farms in 10 Shanghai suburban districts	37	426	Faeces	-	-	-	26%
Zhang et al., 2008 [95]	China	2004-2006	-	39	788	Serum	-	-	67%	4.5%
Li et al., 2008 [96]	China	-	-	16	904	Serum	-	-	68.3%	6.3%
Ward et al., 2008 [97]	Canada	2003-2004	Collection of faeces directly from the pen floor. Pig age: 2 to 4 months old	70	-	Faeces	-	34%	-	-
Yan et al., 2008 [98]	China	2007	Sample collection in Shanghai districts. Pig age: 2 to 4 months old	23	480	Faeces	-	26.1%	-	5%
Yu et al., 2008 [99]	Korea	2006-2007	Pig age: 2 to 3 months old	5	53	Faeces	-	60%	-	17%
di Bartolo et al., 2008 [18]	Italy	2006	Random selection of pigs on six farms. Pig age: from 3 to 9 months old, + sows	6	274	Faeces	-	100%	-	42%
Reuter et al., 2009 [100]	Hungary	2005-2006	Pig age between 3 weeks and 40 months	30	154	Faeces, liver, intestine	-	40%	-	27.3%
Shao et al., 2009 [101]	China	2007	Pig age: 4 to 6 months old	-	603	Bile	-	-	-	2%
Masia et al., 2009 [102]	Italy	2006-2007	Random sample collection. Pig age: 8 to 9 months old	6	95	Bile	-	-	-	6.3%
Sakano et al., 2009 [103]	Japan	2004	Sample collection during inspection at slaughterhouse. Pig age around 6 months old	17	169	Serum	-	-	74.6%	1.8%
Li et al., 2009 [21]	China	-	Serum collection in randomly-selected farms. Serum and liver collection in slaughterhouses	12	638 sera, 114 liver samples	Serum, liver	-	-	On farms: 79.4%. In slaughterhouses: 78.4%	-
Casas et al., 2009 [11]	Spain	1985-1997	Random selection in a serum bank	208	2.781	Serum	98%	-	48.4%	-
Forgach et al., 2010 [104]	Hungary	2005-2009	Four age groups	41	248 faecal samples, 45 livers	Faeces, liver	-	39%	-	Faeces: 21% ; Livers: 31%
Di Martino et al., 2010 [105]	Italy	2008-2009	Sample collection at one slaughterhouse. Pig age: 9 to 12 months old	-	150	Faeces	-	-	-	7.3%
Kaba et al., 2010 [106]	Democratic Republic of Congo	-	-	1	40	Faeces	-	-	-	2.5%
Geng et al., 2010 [107]	China	-	Sample collection from two stock farms	2	598 sera, 111 faecal samples	Serum, faeces	-	-	80.4%	66.6%
Breum et al., 2010 [108]	Denmark	2007-2008	Faecal sample collection from pigs in 26 herds (4-22 weeks of age) and from samples submitted to the National Veterinary Institute, Denmark, for routine diagnostic analyses. Sera collection from sows on 71 farms.	Pigs: 26 herds. Sows: 71 herds.	97 faecal samples from pigs + 213 serum samples from sows	Serum, faeces	91.5% (sows)	55% (pigs)	73.2% (sows)	49.5% (pigs)
Jinshan et al., 2010 [15]	China	2009	Pig age: 2 to 4 months old	14	356	Serum	-	-	52%	8%
Geng et al., 2010 [109]	China	2008	Most of the samples were collected from industrialised farms, but some were collected from small groups of animals raised by peasants.	-	1.967	Serum	-	-	82.2%	1.9%
Xia et al., 2010 [110]	China	2009	Sample collection in slaughterhouses	-	100	Bile	-	-	-	5.5%
Hakze-van der Honing et al., 2011 [111]	Netherlands	2008	Sample collection in slaughterhouses. Pig age: 5 to 6 months old	101	101	Faeces	-	-	-	15%
	Belgium			23	115		-	21.7%	-	7%

Dell'Amico et al., 2011 [112]	Bolivia	2006	Survey conducted in two rural communities. Pig age: 2 to 12 months old	-	121 (22 pools)	Faeces	-	-	-	31.8%
Kaba et al., 2011 [113]	New Caledonia	2009	Pig age: 4 to 26 weeks old	1	92	Faeces	-	-	-	6.5%
Steyer et al., 2011 [114]	Slovenia	2004-2005	Collection of individual and pooled samples. Three age groups.	6	85 individual samples + 51 pooled samples	Faeces	-	-	-	20.3%
dos Santos et al., 2011 [115]	Brazil	2008	Sample collection in three slaughterhouses. Pig age > 5 months old	-	115	Bile	-	-	-	9.6%
Vivek et al., 2011 [116]	India	-	Sample collection in slaughterhouses.	-	102	Serum	-	-	-	1.9%
Conlan et al., 2011 [117]	Lao People's Democratic Republic	2009	Study conducted in four provinces, one district per province.	95	181	Faeces	-	43.5%	-	11.6%
Rose et al., 2011 [5]	France	2008-2009	Sample collection in slaughterhouses. Herd sample through random selection of a list of slaughter dates and times	186	6,565 sera, 3,715 liver samples	Serum, liver	65%	24%	31%	4%
Widén et al., 2011 [13]	Sweden	-	Random selection of farms. Pig age: 2 to 4 months old	22	240	Faeces	-	72.7%	-	29.6%
Shen et al., 2011 [118]	China	2009	Random sample collection from medium- to large-scale pig farms.	6	209	Faeces	-	83%	-	6.7%
Martinelli et al., 2011 [119]	Italy	2008	10 farrow-to-finish farms, 17 farrow-to-weaning, 12 fattening. 10% of animals per farm.	39	1,422	Serum	97.4%	-	50.2%	-
di Bartolo et al., 2011 [57]	Italy	2008	Sample collection in a slaughterhouse. Pig age: from 3 to 10 months old	5	48	Serum, faeces, bile, liver	-	-	87%	64.6%
Jiménez de Oya et al., 2011 [14]	Spain	-	-	85	1,141	Serum	81.2%	47.2%	20.4%	18.8%
Berto et al., 2012 [120]	Portugal	2010-2011	Five farms from five different regions. 40 samples per farm, at four different stages of production (10 per stage)	5	200	Faeces	-	100%	-	44%
Wacheck et al., 2012 [121]	Germany	2009-2010	Sample collection in four Bavarian slaughterhouses	41	516 (516 sera, 198 meat juice samples)	Serum, meat juice	-	-	Serum samples: 68.6% IgG and 7% IgM; Meat juice samples: 67.6% IgG	-
Gardinali et al., 2012 [70]	Brazil	2010	Sample collection in one slaughterhouse. Pig age: > 25 weeks old	10	118	Liver, bile	-	-	-	Liver: 1.7%, Bile: 0.84%
de Souza et al., 2012 [71]	Brazil	2010	Sample collection in slaughterhouses. Pig age approximately 6 months old	-	151	Serum, faeces, liver	-	-	8.6% IgG	9.9%
Gardinali et al., 2012 [66]	Brazil	2009	Sample collection from five farrow-to-weaning farms (breeder sows and boars, suckling piglets, and weaned pigs up to 8 weeks of age); and from 9 grower-to-finish farms (9- to 24-week-old pigs)	14	170	Faeces	-	62.5%	-	15.3%
Hinjoy et al., 2013 [20]	Thailand	2009	Random farm selection.	94	879	Serum, faeces	30.9%	-	9.9%	2.9%
Lipej et al., 2013 [122]	Croatia	-	-	-	60	Serum, bile	-	-	91.7%	Serum: 13.3% ; Bile: 8.1%
de la Caridad Montalvo Villalba et al., 2013 [123]	Cuba	2007	Random selection of farms and pigs. Pig age: 1 to 102 weeks old	4	53	Faeces	-	-	-	18.8%
S de Paula et al., 2013 [73]	Cameroon	2012	Sample collection in slaughterhouses. Pig age: 6 months to 3 years	-	345	Liver	-	-	-	0.9%

Kantala et al., 2013 [124]	Finland	2007 and 2010	Study in a swine station (comparable to a fattening station) in 2007 and 2010. Individual follow-up of animals	2007: 11 farms. 2010: 8 farms	2007: 40 individual faecal samples + 37 individual blood samples. 2010: 36 individual faecal samples + 27 individual blood samples	Serum, faeces	-	2007: 64%, 2010: 63%	2007: 86.5%, 2010: 81.5%	2007: 35%, 2010: 39%
Temmam et al., 2013 [72]	Madagascar	2010-2011	Sample collection in slaughterhouses. Pig age > 6 months old	-	250	Serum, liver	-	-	71.2%	1.2%
Machnowska et al., 2014 [125]	Germany	2011	Three slaughterhouses in different regions of Germany	4	120	Faeces	-	-	-	2.5%
Thiry et al., 2014 [126]	Belgium	2010-2011	Sampling from the pig serum banks made by the regional animal health laboratories in Belgium. Farms were selected based on the availability of six sera from fattening pigs weighing less than 80 kg (for an age estimated between 2 and 6 months) and six sera from lactating sows (aged over one year).	70	Virological testing: 420 serum samples of fattening pigs aged less than 6 months. Serological testing: 420 serum samples of lactating sows	Serum	-	-	73%	0.9%
da Costa Lana et al., 2014 [127]	Brazil	-	Five large-scale farms, five family-scale farms. Pig age: 4 months old	10	50	Faeces, liver, gallbladder, intestines, bile	-	-	-	16%
Aniță et al., 2014 [128]	Romania	2009-2010	Pig age: 2 to 4 months old	5	19 pooled faecal samples	Faeces	-	-	-	31.5%
Owolodun et al., 2014 [129]	Nigeria	2009-2012	Serum sample collection at one slaughterhouse + archived serum samples. Pig age: 4 months to 3 years old. Faecal samples from 10 different regions. Pig age: 1 to 6 months old	-	286 sera, 90 faecal samples	Serum, faeces	-	-	55.6% IgG	76.7%
Wang et al., 2014 [130]	China	2011	Serum sample collection in 24 swine herds in nine counties. Bile sample collection at one slaughterhouse, pig age: 6 to 7 months old	24	980 sera, 106 bile samples	Serum, bile	100% IgG, 41.7 IgM	-	66.4% IgG, 1.6% IgM	30.2%
Shu et al., 2014 [131]	China	2011-2012	Faecal sample collection from piglets in markets and 3- to 6-month-old pigs on farms	-	256	Faeces	-	-	-	7.8%
Burri et al., 2014 [132]	Switzerland	2006 and 2011	Random selection from a serum bank. Pig age < 1 year old	2.001	2.001	Serum	-	-	58.1%	-
Liang et al., 2014 [53]	China	2011-2013	Pig age: from nursery to > 7 months old	34	561 sera, 288 bile samples	Serum, bile	-	-	64.7%	From 6.2% to 22.7% depending on the region
Monini et al., 2015 [133]	Italy	2012-2014	Eight swine farms located in northern, central and southern Italy. Pig age: 5 to 220 days old	8	242	Faeces	-	75%	-	18.6%
Ivanova et al., 2015 [134]	Estonia	-	-	-	380	Serum	100%	-	61.6%	22.9%
Kantala et al., 2015 [37]	Finland	2009	Individual samples: pig age = 1 day to 15 weeks. Pooled samples: pig age = 1 to 4 months old	6	273 (individual and pooled samples)	Faeces	-	83.3%	-	87.5%
Crossan et al., 2015 [16]	Scotland	2006	Pig age: slaughter age	23	176	Serum	100%	-	29% IgG, 36.9% IgA, 29% IgM. Overall prevalence: 61.4%	44.4%
Grierson et al., 2015 [17]	U.K.	2013	Sample collection as part of the 2013 Zoonoses in UK Pigs Abattoir Study, a cross-sectional study of pigs being slaughtered at 14 high-throughput slaughterhouses.	439	629	Serum, caecal content	-	-	92.8%	From caecal content: 15%. From serum: 3%. Both: 2%

Costanzo et al., 2015 [135]	Italy	2014	Sample collection from extensive breeding systems	8	216	Serum, faeces	-	-	80%	7.4%
Liu et al., 2015 [136]	Philippines	2010-2011	Household-raised pig population. Pig age: 2 to 24 months old	155	299	Serum, faeces	IgG: 60%. IgM: 33.5%	10.3%	IgG: 50.3%. IgM: 22.9%	7.4%
O'Connor et al., 2015 [137]	Ireland	2010-2011	Random selection from a serum bank.	16	330	Serum	81%	-	27%	-
Whilhelm et al., 2016 [24]	Canada	-	Farms were recruited using the Canadian Integrated Programme for Antimicrobial Resistance Surveillance (CIPARS) and FoodNet Canada on-farm sampling platforms.	88	528 pooled faecal samples	Faeces	-	34.1%	-	-
Caruso et al., 2016 [138]	Italy	-	Random selection of farms. Weaners and sows	42	504	Serum, faeces	97% IgG	31%	50% IgG	10.9%
Merino-Ramos et al., 2016 [139]	Mexico	2010-2013	Sample collection in nine states in the centre of the country. Pig age: 1 to 48 months old	109	683	Serum	-	-	30.7%	-
Lange et al., 2016 [12]	Norway	1994, 2009, 2010, 2013	Serum collection from a serum bank (national surveillance and control programme for specific viral infections in swine) + collection of faecal samples in eight herds representing different parts of Norway	Serum: 153. Faeces: 8	Serum: 663. Faeces: 118	Serum, faeces	90%	37.5%	73%	-

Additional file 2

Country	Number of pigs/samples		Pig age	HEV RNA prevalence (%)		Anti-HEV antibody prevalence (%)		Source
				Faeces	Serum	Bile		
Taiwan	11 sera	20 faeces	< 2 m	0	0	-	-	Wu et al., 2002 [140]
	67	22	2 m	40.9	4.5	-	-	
	255		3-4 m		1.2	-	-	
	112	12	5-6 m	66.6	1.8	-	-	
	76		> 7 m		0	-	-	
Korea	40		1 m	-	-	-	0	Choi et al., 2003 [86]
	50		2 m	-	-	-	6	
	50		3 m	-	-	-	10	
	50		4 m	-	-	-	36	
	40		5-7 m	-	-	-	25	
Indonesia	8		2 m	-	1	-	38	Wibawa et al., 2004 [87]
	28		3 m	-	0	-	46	
	17		4 m	-	0	-	88	
	35		5 m	-	0	-	89	
	11		6 m	-	0	-	82	
Brazil	9		1 w	-	-	-	100	Vital et al., 2005 [88]
	13		2 w	-	-	-	61.5	
	27		3-4 w	-	-	-	22.2	
	21		5-6 w	-	-	-	19.1	
	30		7-8 w	-	-	-	6.7	
	23		9-10 w	-	-	-	13.0	
	33		11-13 w	-	-	-	15.2	
	30		15-16 w	-	-	-	83.3	
	59		17-20 w	-	-	-	98.3	
	37		21-24 w	-	-	-	91.9	
75		>25 w	-	-	-	97.3		
Thailand	26		2-4 m	-	39	-	-	Cooper et al., 2005 [141]
	50		other	-	0	-	-	
Mexico	125 sera	92 faeces	2-4 m	31	6.4	-	-	
Japan	61		0 m	0	-	-	-	Nakai et al., 2006 [142]
	70		1 m	31.4	-	-	-	
	56		2 m	64.3	-	-	-	
	62		3 m	33.9	-	-	-	
	44		4 m	4.5	-	-	-	
	37		5 m	5.4	-	-	-	
	36		6 m	8.3	-	-	-	
Canada	51		2 w	11.8	0	-	-	Leblanc et al., 2007 [143]
	51		8 w	52.9	2	-	-	
	51		18 w	86.2	47.1	-	-	
	51		22-29 w	41.2	11.8	-	-	
Spain	-		< 1 m	-	0	-	-	de Deus et al., 2007 [144]
	23		1 m	-	30	-	-	
	17		2 m	-	47	-	-	
	20		3 m	-	55	-	-	
	-		> 3 m	-	0	-	-	
Spain	20		0-4 w	10	20	-	-	Fernandez-Barredo et al., 2007 [89]
	22		5-12 w	41	32	-	-	
	20		13-20 w	5	10	-	-	
	27		21-24 w	7	11	-	-	
China	133 (RNA) / 265 (Ig)		1-3 m	-	8.3	-	63.4	Zhang et al., 2008 [95]
	96 / 193		4-6 m	-	5.2	-	53.9	
	30 / 61	at slaughterhouse		-	6.7	-	55.7	
China	549		< 3 m	-	-	-	79.1	Li et al., 2008 [96]
	355		> 3 m	-	-	-	51.6	
Italy	64		< 120 d	42.2	-	-	-	di Bartolo et al., 2008 [18]
	37		> 120 d	27	-	-	-	
Korea	142		suckling	6.3	-	-	-	Kim et al., 2008 [91]
	141		post-weaning	16.3	-	-	-	
	142		growing	38.0	-	-	-	
Spain	19 sera	8 faeces	3-6 w	0	0	-	-	Seminati et al., 2008 [92]
	6	5	8-10 w	100	66.7	-	-	
	15	10	12-13 w	40	86.7	-	-	
	10	5	22 w	0	0	-	-	
U.K.	50		3-5 w	26	-	-	-	McCreary et al., 2008 [93]
	50		10-12 w	44	-	-	-	
	45		22-24 w	8.9	-	-	-	
China	167		< 10 w	6.6	-	-	-	Zhang et al., 2009 [145]
	143		10-15 w	9.8	-	-	-	
	135		16-20 w	6.7	-	-	-	
	109		>20 w	4.6	-	-	-	
China	219		1-3 m	-	-	-	71.2	Li et al., 2009 [21]
	213		3-6 m	-	-	-	85.9	
	44		> 6 m	-	-	-	88.6	

France	100	3 m	65	22	-	-	Kaba et al., 2009 [146]
	107	6 m	0	0	0	-	
China	101	2 m	-	7	-	-	Jinshan et al., 2010 [15]
	132	3 m	-	9	-	-	
	123	4 m	-	9	-	-	
China	178	< 3 m	-	-	-	62.9	Geng et al., 2010 [109]
	420	> 6 m	-	-	-	87.9	
Denmark	32	4-8 w	21.9	-	-	-	Breum et al., 2010 [108]
	33	9-12 w	54.5	-	-	-	
	32	13-22 w	71.9	-	-	-	
Hungary	204	1-4 w	9	-	-	-	Forgach et al., 2010 [104]
		5-10 w	27	-	-	-	
		11-16 w	36	-	-	-	
		> 17 w	10	-	-	-	
Italy	85	< 80 d	-	-	20	-	Martelli et al., 2010 [147]
	49	80-120 d	-	-	46.9	-	
China	32	0 d	-	0	-	IgG: 0, IgM: 0	Feng et al., 2011 [148]
	32	15 d	-	0	-	IgG: 100, IgM: 0	
	32	30 d	-	0	-	IgG: 90.6, IgM: 0	
	32	60 d	-	21.9	-	IgG: 9.4, IgM: 6.2	
	32	75 d	-	34.4	-	IgG: 21.9, IgM: 15.6	
	32	90 d	-	15.6	-	IgG: 56.3, IgM: 12.5	
	32	120 d	-	3.1	-	IgG: 100, IgM: 6.3	
China	26	< 1 m	15.4	-	-	-	Shen et al., 2011 [118]
	44	1-2 m	6.8	-	-	-	
	48	4 m	12.5	-	-	-	
Indonesia	25	1 m	-	-	-	44	Utsumi et al., 2011 [149]
	42	2 m	-	-	-	61.9	
	40	3 m	-	-	-	82.5	
	39	4 m	-	-	-	87.2	
	41	5 m	-	-	-	85.4	
	20	6 m	-	-	-	70	
Italy	19 sera	20 faeces / 19 bile	3-4 m	65	-	68.4	di Bartolo et al., 2011 [57]
	27 sera	28 / 26	9-10 m	10.7	-	38.5	
Italy	633	Sows	-	-	-	70.6	Martinelli et al., 2011 [119]
	58	< 2 m	-	-	-	12.1	
	133	2-3 m	-	-	-	30.8	
	325	4-6 m	-	-	-	41.5	
New Caledonia	20	4-8 w	0	-	-	-	Kaba et al., 2011 [113]
	32	9-16 w	18.8	-	-	-	
	32	17-24 w	0	-	-	-	
	8	> 24 w	0	-	-	-	
Slovenia	38	suckling	5.3	-	-	-	Steyer et al., 2011 [114]
	21	weaning	28.6	-	-	-	
	26	fattening	26.9	-	-	-	
Spain	130	3 w	-	-	-	6.9	Jiménez de Oya et al., 2011 [14]
	120	5 w	-	-	-	3.3	
	120	7 w	-	-	-	1.7	
	70	11 w	-	-	-	1.4	
	100	15 w	-	-	-	31	
	80	20 w	-	-	-	17.5	
	140	< 6 m	-	25.7	-	-	
201	> 6 m	-	13.9	-	-		
Belgium	420	< 6 m	-	4	-	-	Thiry et al., 2012 [150]
Brazil	25	1-4 w	8	-	-	-	Gardinali et al., 2012 [66]
	33	5-8 w	3	-	-	-	
	71	9-24 w	32.4	-	-	-	
Portugal	50	weaning	32	-	-	-	Berto et al., 2012 [120]
	50	growing	20	-	-	-	
	50	fattening	32	-	-	-	
Italy	117	< 6 m	6.8	-	-	75.2	Costanzo et al., 2015 [135]
	99	> 6 m	8.0	-	-	96.0	
Italy	67	suckling	4.5	-	-	-	Monini et al., 2015 [133]
	48	weaning	31.3	-	-	-	
	79	fattening	32.9	-	-	-	
Philippines	173	2-4 m	9.2	-	-	IgG: 37.6, IgM: 16.9	Liu et al., 2015 [136]
	93	5-7 m	5.4	-	-	IgG: 64.1, IgM: 27.2	
	33	8-24 m	3.0	-	-	IgG: 78.8, IgM: 42.4	



Ce qu'il faut retenir

Le virus de l'hépatite E est fortement prévalent dans la filière porcine, notamment en France où il circule dans 65 % des élevages et chez 31 % des animaux, ce qui conduit à une prévalence de foies positifs à l'abattoir de 4 %. La dynamique de l'infection par le HEV est variable, cette variabilité n'étant encore que partiellement expliquée. Entre autres, la taille de l'élevage, le type de production et certaines pratiques d'élevage et mesures de biosécurité apparaissent comme des facteurs de risque vis-à-vis du HEV. A l'échelle individuelle, les co-infections avec des pathogènes immunomodulateurs influencent aussi la dynamique de l'infection par le HEV.

Les programmes de lutte actuellement envisagés contre le HEV ne sont que théoriques. Ils incluraient des mesures au niveau de l'amont de la production (potentielle vaccination des porcs contre le HEV, contrôle des facteurs de risque et des pathogènes immunomodulateurs, maîtrise du risque lié aux mouvements d'animaux) et en aval (qualification des élevages indemnes de HEV pour dédier des lignes de production à la fabrication des produits contenant du foie, meilleurs traitements assainissants des produits, sensibilisation aux bonnes pratiques d'hygiène du consommateur). Il existe néanmoins d'importants trous de connaissance relatifs à l'épidémiologie du HEV qu'il est nécessaire d'investiguer afin de développer une stratégie de lutte plus ciblée et efficace contre le HEV.



Take home message

Hepatitis E virus is highly prevalent in the pig production sector, particularly in France where it circulates in 65% of pig farms and in 31% of pigs, leading to a 4% prevalence of positive livers at the slaughterhouse. HEV infection dynamics are variable and this variability is still partially explained. Among other things, the size of the farm, the type of production and certain farming practices and biosecurity measures appear to be risk factors for HEV. At the individual level, co-infections with immunomodulating pathogens may also affect HEV infection dynamics.

The control programmes currently being considered against HEV are only theoretical. They would include measures at the upstream level (potential vaccination of pigs against HEV, control of risk factors and immunomodulating pathogens, control of risk related to animal movements) and downstream (qualification of HEV-free farms to dedicate production lines to the manufacture of products containing liver, better treatments of products, higher consumer awareness). However, there are still significant knowledge gaps in HEV epidemiology that need to be investigated in order to develop a more targeted and effective strategy to control HEV.

PARTIE 1.2. De l'intérêt d'une complémentarité d'approches pour sécuriser la filière porcine vis-à-vis du risque lié au virus de l'hépatite E

I. Préambule sur la gestion des risques sanitaires : qu'est-ce qu'un plan de lutte ?

Dans le domaine de la santé, un plan de lutte peut être défini comme un **ensemble de mesures visant l'éradication ou la réduction de la prévalence d'un agent causal responsable d'une maladie à l'échelle d'un pays, d'une zone ou d'un compartiment**²². Le processus proposé par l'Organisation Mondiale de la Santé Animale (OIE) pour l'élaboration d'un plan de lutte recouvre plusieurs étapes : (i) **justification du programme** ; (ii) **finalité stratégique et objectifs** ; (iii) **élaboration du plan d'action** ; (iv) **mise en œuvre** ; (v) **suivi, évaluation et révision** (Figure 17).

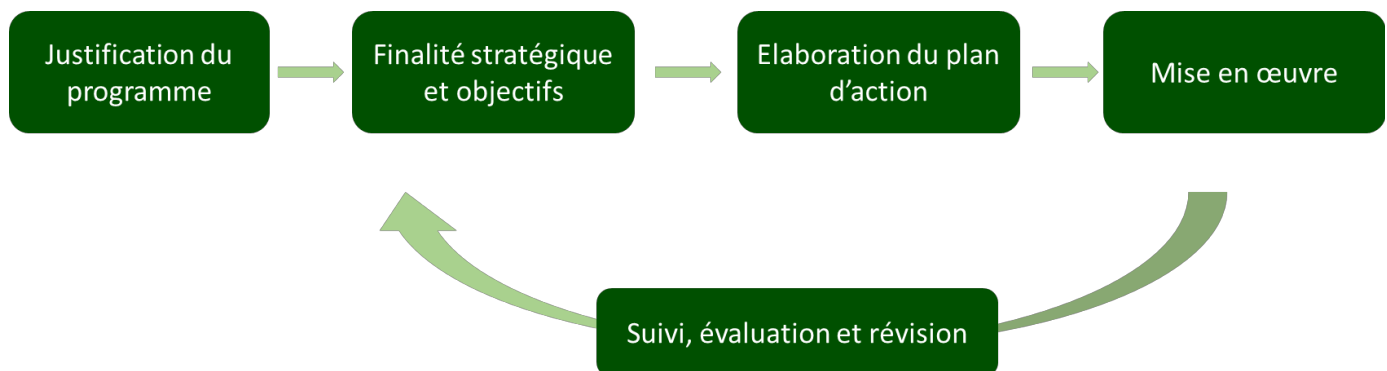


Figure 17 - Les étapes d'élaboration d'un programme de lutte contre une maladie²²

La justification du programme de lutte doit être fondée sur un **bilan de la situation épidémiologique du pays**. La finalité du programme de lutte doit être définie dès le début : elle

²² OIE, 2014, Lignes directrices pour la lutte contre les maladies animales. Disponible à : http://www.oie.int/fileadmin/Home/fr/Our_scientific_expertise/docs/pdf/F_Guidelines_for_Animal_Disease_Control_final.pdf

peut être **l'éradication du pathogène, ou son contrôle et la limitation de ses conséquences**. Les différentes solutions envisagées pour l'intervention doivent se fonder sur l'**efficacité**, sur la **faisabilité** et le **coût de mise en œuvre**, et sur les **bénéfices attendus** de la réussite du programme. Divers outils, par exemple l'analyse de la chaîne de valeur, peuvent permettre de mieux comprendre le rôle des différents intervenants du système de production, d'identifier les points de contrôle critiques pour le ciblage des mesures, et d'appréhender la faisabilité du programme et les incitations à y participer. La mise en œuvre du dispositif de maîtrise fait généralement entrer en jeu **plusieurs types d'acteurs**, *a minima* **l'autorité compétente et les professionnels de la filière**. Elle comprend généralement des mesures de **prévention**, de **surveillance** et de **contrôle**. Des **actions de révision** doivent être conduites afin d'évaluer l'efficacité des interventions au fur et à mesure de leur réalisation, d'identifier les lacunes à combler dans les connaissances et d'adapter les finalités, les objectifs et les méthodes ou activités du programme en fonction de l'évolution des besoins.

La phase d'élaboration d'un dispositif de maîtrise d'un pathogène nécessite la connaissance des *(i)* **facteurs biologiques** : espèces affectées, potentiel zoonotique, modes de transmission, transmissibilité à l'homme, *etc.* ; *(ii)* **outils techniques** disponibles : tests diagnostiques, vaccins, traitement, *etc.* ; *(iii)* **mesures de contrôle** possibles : contrôle des mouvements, abattage, désinfection, mesures sanitaires, *etc.* ; *(iv)* **considérations socio-économiques** : logistique et facilité de mise en œuvre, participation des parties prenantes, mesures incitatives et indemnités, répartition des rôles et des responsabilités, planification du budget et des ressources financières, *etc.*

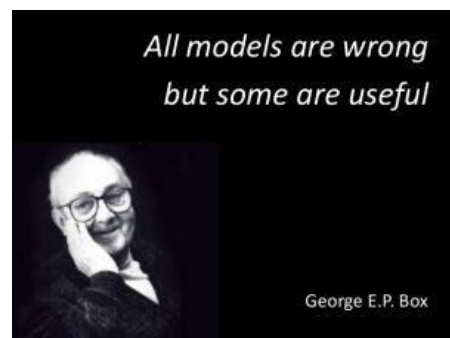
C'est dans ce contexte que notre projet de recherche, en combinant différentes approches méthodologiques, vise à **apporter de nouvelles connaissances nécessaires à la mise en place d'un dispositif de lutte contre le HEV dans la filière porcine**.

II. Utilité de la modélisation dans la compréhension et la maîtrise du risque lié au virus de l'hépatite E dans la filière porcine

Quelle(s) approche(s) ?

Le terme « modélisation » recouvre plusieurs approches. Dans l'objectif d'étudier la propagation et la persistance du HEV à deux échelles, celle de l'élevage et celle de la filière porcine, deux approches de modélisation ont été adoptées et sont présentées ci-après. La première relève de la **modélisation dynamique de la propagation d'un agent infectieux** dans une population, en prenant en compte le passage des individus par différents états de

santé. La seconde, relative à l'**analyse des réseaux sociaux**, décrit et/ou modélise les liens entre des individus ou groupe d'individus. Le couplage de ces deux types d'approches peut conduire à une modélisation multi-échelles.



I.1. La modélisation pour la compréhension et la maîtrise des phénomènes infectieux

I.1.a. Approches de modélisation des dynamiques infectieuses : bases théoriques

Un modèle peut être défini comme un **outil conceptuel qui décrit et explique le comportement d'un objet ou d'un système d'objets** (Keeling et Rohani, 2008). Un modèle mathématique utilise le langage des mathématiques pour produire une formalisation mathématique des connaissances spécifiques d'un système (Eykhoff, 1974). En épidémiologie, les modèles permettent par exemple de **décrire des comportements infectieux à différentes échelles ou d'extrapoler à partir d'un ensemble de conditions vers un autre**. Les modèles peuvent être **descriptifs et analytiques** ou **prédictifs**. Les modèles analytiques vont du réel vers le modèle, c'est-à-dire qu'ils sont utilisés pour **comprendre comment une maladie infectieuse se propage dans le « monde réel » et comment la dynamique d'infection varie**. Ainsi, des facteurs de risque peuvent être étudiés indépendamment les uns des autres. Les modèles prédictifs vont, eux, du modèle vers le réel. Ils sont par exemple utilisés pour **anticiper des événements ou des situations**, ou pour **prévoir l'efficacité de mesures de lutte** (Keeling et Rohani, 2008). L'approche analytique se base sur des données issues d'études de terrain ou expérimentales pour comprendre, affiner et valider certaines hypothèses. Les aspects prédictifs sont fondés sur des connaissances pré-établies pour évaluer l'évolution du comportement du système.

Les premiers modèles mathématiques en épidémiologie remontent à 1760, lorsque Bernoulli démontra, à partir d'un modèle, les bénéfices apportés par la variolisation lors d'une épidémie de variole (Bernoulli, 1760). A partir du début du XX^{ème} siècle, les modèles sont fondés sur le **principe de l'action de masse : le nombre de nouvelles infections est proportionnel aux nombres d'individus « Sensibles » (S) et « Infectieux » (I)** (Hamer, 1906). Kermack et McKendrick (1927) ajoutent ensuite un stade supplémentaire au processus infectieux, le stade **Retirés (R)**, qui contient les individus ne jouant plus aucun rôle dans le processus infectieux (immunisés, morts). Le modèle devient ainsi un modèle SIR (*Susceptible, Infectious and Removed*) (Figure 18) :

$$\left. \begin{aligned} \frac{dS}{dt} &= -\frac{\beta I}{N} S \\ \frac{dI}{dt} &= \frac{\beta I}{N} S - \gamma I \\ \frac{dR}{dt} &= \gamma I \end{aligned} \right\} (1)$$

où S , I et R représentent respectivement les nombres d'individus sensibles, infectieux et retirés dans une population de taille N . β est le **taux de transmission**, défini comme le **nombre moyen d'individus nouvellement infectés par un individu infectieux par unité de temps**. γ est le taux de guérison, équivalent à l'inverse de la durée moyenne de la période infectieuse.

Le modèle ci-dessus permet l'identification d'un paramètre seuil, appelé **nombre de reproduction de base** (*basic reproduction number*) et noté R_0 , permettant d'évaluer la **capacité d'invasion de l'agent infectieux au sein d'une population totalement sensible**. Il est défini comme étant le **nombre moyen d'individus infectés par un individu infectieux dans une population entièrement sensible au cours de son entière période infectieuse** (Diekmann et al., 1990; Diekmann et Heesterbeek, 2000). Dans le cas du système d'équations (1), l'épidémie ne peut se propager que lorsque $R_0 = \frac{\beta}{\gamma} > 1$.

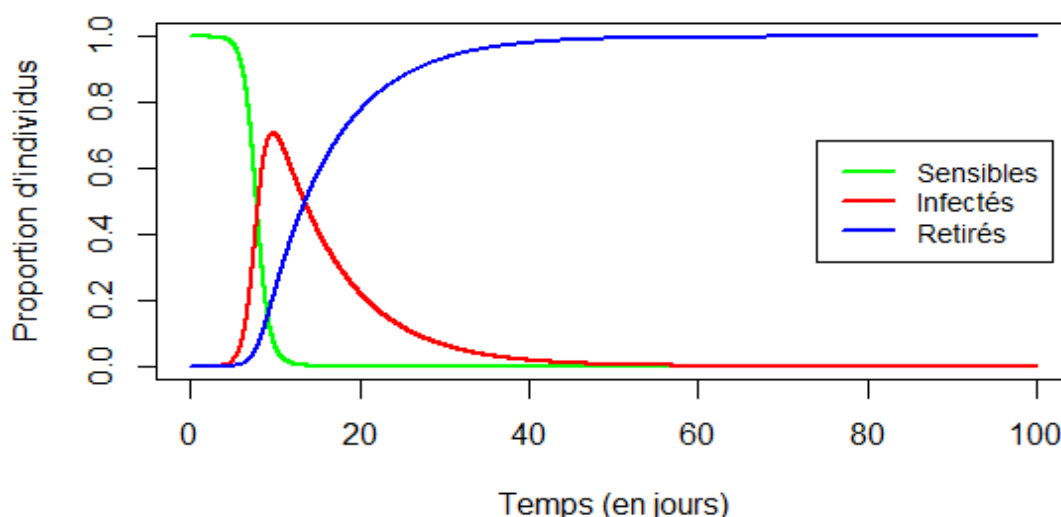


Figure 18 - Comportement d'un système SIR classique régi par le système d'équations (1), dans un système fermé sans démographie

Taux de transmission $\beta = 1,5 \text{ jours}^{-1}$; Taux de guérison $\gamma = 1/8 \text{ jours}^{-1}$; Nombre de reproduction de base $R_0 = 12$

Bien que le modèle SIR classique (1) soit le point de départ de toute problématique en modélisation épidémiologique, il s'avère inapproprié pour représenter certains processus infectieux. En effet, la présence d'une **phase de latence** (individus infectés mais pas encore infectieux (*i.e.* excréteurs)) ou l'acquisition d'une **immunité passive** peuvent jouer un rôle sur la dynamique de certaines infections et doivent par conséquent être représentées dans certains modèles (Hethcote, 1994). Les états *E* (Exposé) ou *M* (sous immunité maternelle) peuvent alors être ajoutés dans le modèle, ainsi que la **possibilité d'une réinfection** :

$$M \rightarrow S \rightarrow E \rightarrow I \rightarrow R \rightarrow S$$

Certains modèles sont construits de manière **déterministe** dont l'approche peut être discutée en fonction du problème étudié, notamment en ce qui concerne la taille de la population considérée. Un processus infectieux est, par nature, un processus **stochastique** : la présence d'un individu infecté dans une population provoquera l'infection de certains individus sensibles alors que d'autres échapperont à l'infection. L'approche déterministe est souvent utilisée dans le cas de grandes populations où elle donne une bonne approximation du comportement moyen du processus infectieux mais, **dans des populations de taille limitée, les modèles stochastiques sont, la plupart du temps, privilégiés** (Bailey, 1990; Daley et Gani, 1999; Diekmann et Heesterbeek, 2000). **Dans un modèle stochastique, les transitions entre les différents statuts infectieux et/ou physiologiques sont représentées par des lois de probabilités.** Ainsi, deux simulations avec le même jeu de paramètres conduisent à des résultats différents, représentant la variabilité dans le processus infectieux.

L'étude de la diffusion d'un agent pathogène au sein d'une population, qu'elle soit humaine ou animale, nécessite, dans la plupart des cas, la **prise en compte de la démographie et de la dynamique de population qui conditionnent la structure de contacts entre individus** : foyers familiaux, groupes scolaires, hétérogénéité spatiale, migrations, statuts physiologiques, regroupements par âge, *etc.* (Anderson et May, 1991; Xiao *et al.*, 2004; Viet *et al.*, 2006; Iwami *et al.*, 2007; Zhang, 2007; Lurette *et al.*, 2008). Le niveau de représentation de la population considérée est une question essentielle dans la construction d'un modèle. Selon la population, le pathogène et l'objectif de l'étude, deux types de modèles peuvent être développés : modèles individu-centrés ou agrégés. L'**approche individu-centrée (*individual-based model, IBM*)**, de plus en plus utilisée, se caractérise par le fait que chaque individu du système est explicitement représenté par un ensemble de variables (âge, sexe, statut

physiologique, localisation physique, *etc.*). Ainsi, les modèles individu-centrés permettent d'incorporer un haut degré d'hétérogénéité de la population et des interactions entre individus (Grimm, 1999; DeAngelis et Grimm, 2014; Willem *et al.*, 2017). Dans les **modèles agrégés**, la population est répartie en sous-groupes possédant des caractéristiques équivalentes (stade physiologique, âge, *etc.*) (Lesnoff *et al.*, 2004; Ezanno *et al.*, 2007; Lurette *et al.*, 2008).

Les transitions d'un état à un autre peuvent être régies par deux hypothèses différentes. L'**hypothèse markovienne** est la plus répandue dans la littérature : **la probabilité de transition d'un état à un autre ne dépend pas du temps passé dans l'état initial** (Lesnoff *et al.*, 2004; Viet *et al.*, 2004) contrairement à l'**hypothèse semi-markovienne** selon laquelle **la transition est dépendante du temps passé dans l'état initial** (Jacob et Magal, 2007). Certains modèles sont développés en **temps continu** avec une approche en événements discrets, prenant en compte les temps auxquels interviennent les événements pour chaque animal (Viet *et al.*, 2004) ; d'autres sont construits en **temps discret**, le pas de temps étant fixé en fonction des caractéristiques du système de production (Lurette *et al.*, 2008) et/ou de l'agent étudié (Ezanno *et al.*, 2007; Jacob et Magal, 2007).

1.1.b. L'analyse de réseaux : principe et utilisation en épidémiologie

L'analyse des réseaux sociaux (**Social Network Analysis, SNA**) est une discipline en constante expansion ces dernières années. Un réseau est composé de nœuds, qui sont les entités d'étude, et de liens, relations que l'on étudie. Les liens entre les nœuds peuvent être dirigés, c'est-à-dire qu'un lien entre deux points u et v relie soit u vers v , soit v vers u : dans ce cas, le réseau est dit **orienté** (*directed network*). Sinon, les liens sont symétriques et le graphe est alors dit **non-orienté** (*undirected network*). Un graphe peut avoir des liens multiples, c'est-à-dire que plusieurs liens différents relient la même paire de points. Un graphe est dit simple s'il n'a ni liens multiples ni boucles. Classiquement, on représente les données sous forme d'une matrice d'adjacence $n \times n$ pour un graphe à n sommets. Si les sommets sont comparables, le graphe est qualifié de **réseau à un mode** (*one-mode network*). Il peut également exister des réseaux représentés par des matrices de dimension $n \times e$, indiquant les interactions entre des types de sommets différents. Ce type de réseau est appelé **réseau à deux modes** (*two-mode network*).

L'**analyse structurale des réseaux sociaux** permet de caractériser les réseaux en calculant des indicateurs pour les nœuds, indiquant leur place et leur rôle dans le réseau. Des mesures simples du nombre de nœuds et de liens, de la densité des relations, du nombre de liens entre deux paires de nœuds, *etc.*, permettent une première description du réseau. Puis, en s'intéressant aux caractéristiques des nœuds, on peut **déduire des spécificités du réseau et évaluer l'influence de certains nœuds**. Plusieurs notions telles que la **centralité**, la **cohésion** ou la **connexion** sont utilisées, et caractérisées par des **indicateurs**. Ces indicateurs et leurs relations permettent de définir la structure du réseau et d'en déduire leurs propriétés. Ainsi, on distingue communément trois familles de réseaux : (i) les **réseaux aléatoires** (*random network*) : la position des nœuds et leurs relations sont aléatoires, il existe une homogénéité entre les nœuds du réseau ; (ii) les **réseaux en treillis** (*lattice network*) : les nœuds sont localisés de façon régulière sur une grille de points et uniquement les individus adjacents sont connectés entre eux ; (iii) les **réseaux sans échelle** (*scale-free network*) : il s'agit d'une combinaison entre les réseaux aléatoires et les réseaux en treillis (Figure 19). Dans une configuration sans échelle, une grande majorité de nœuds est très faiblement connectée, alors qu'un nombre réduit possède un degré très élevé : ces nœuds sont alors considérés comme des pivots (*hubs*) (Labatut, 2014).

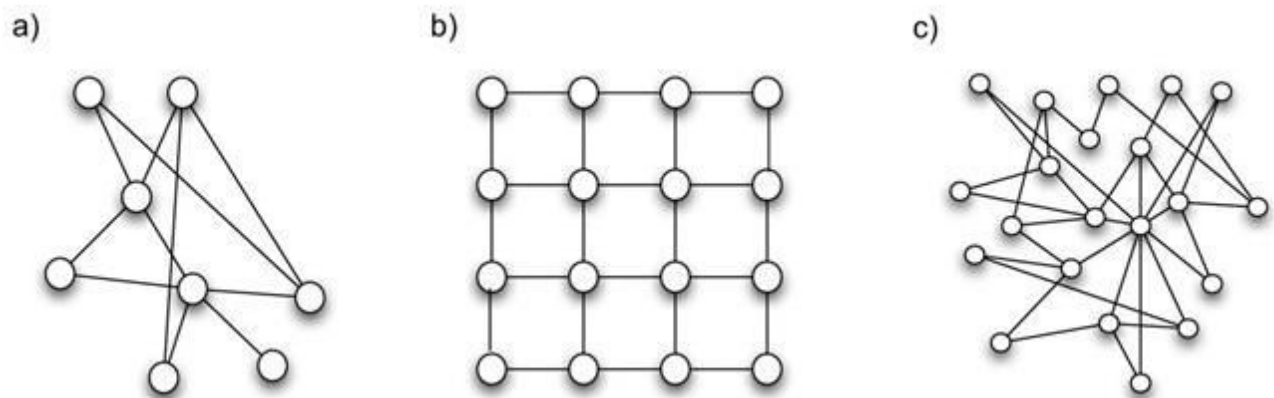


Figure 19 - Famille de réseaux classiquement décrites

(a) graphe aléatoire (b) graphe en treillis régulier (c) graphe sans échelle

Les pathogènes peuvent être transmis d'élevage en élevage par l'intermédiaire des mouvements d'animaux et la diffusion des maladies à l'échelle d'un territoire est étroitement liée aux caractéristiques du **réseau de mouvements d'animaux**. Comprendre la topologie des échanges d'animaux permet d'expliquer la dynamique d'infection et la propagation des

pathogènes, d'identifier des facteurs de risque liés aux caractéristiques du réseau et ainsi de développer des programmes de surveillance fondés sur le risque. C'est la raison pour laquelle les données de mouvements d'animaux sont de plus en plus étudiées par des méthodes d'**analyse de réseau**, fondées sur la **théorie des réseaux sociaux**. Ces méthodes analysent les relations et contacts entre acteurs pour pouvoir comprendre leur rôle dans la structure. En épidémiologie, cet outil d'analyse des réseaux sociaux est de plus en plus utilisé, notamment en médecine humaine pour des maladies telles que l'infection par le VIH ou la tuberculose qui impliquent des contacts étroits entre personnes. En médecine vétérinaire, l'utilisation de ces méthodes est plus récente (années 2010) ; dans ces applications, **les élevages sont considérés comme des nœuds et les mouvements d'animaux entre élevages comme des liens** (Wasserman et Faust, 1994; Bigras-Poulin *et al.*, 2006; Bigras-Poulin *et al.*, 2007; Natale *et al.*, 2009; Ribbens *et al.*, 2009; Nöremark *et al.*, 2011; Lindstrom *et al.*, 2012; Rautureau *et al.*, 2012; Buttner *et al.*, 2013; Guinat *et al.*, 2016a; Thakur *et al.*, 2016).

Bien que la plupart des publications présentant des analyses de réseau de productions animales soient motivées par l'étude des conséquences des échanges d'animaux sur l'épidémiologie des maladies, **le rôle spécifique des transports d'animaux dans la transmission des pathogènes et/ou l'exposition à une maladie n'a été que peu quantifié, particulièrement dans la filière porcine** (Keeling, 2005; Lloyd-Smith *et al.*, 2005; Ortiz-Pelaez *et al.*, 2006; Green *et al.*, 2008; Martin *et al.*, 2011; Porphyre *et al.*, 2011; Frössling *et al.*, 2012; Nicolas *et al.*, 2013; Beaunee *et al.*, 2015; Lee *et al.*, 2017; Sintayehu *et al.*, 2017). De plus, rares sont les publications ayant couplé la dynamique infectieuse d'un pathogène circulant dans un élevage avec les mouvements d'animaux, permettant ainsi **l'étude de la diffusion d'un pathogène à l'échelle d'un territoire ou d'une filière** (Beaunee *et al.*, 2015; Halasa *et al.*, 2016; Widgren *et al.*, 2016).

1.1.c. Complémentarité des approches de modélisation et des études de terrain et expérimentales

Les **enquêtes épidémiologiques réalisées sur le terrain**, en élevage ou en abattoir, permettent d'observer et de décrire les phénomènes infectieux en conditions réelles, d'étudier leur fréquence et leur répartition dans une ou des populations (*i.e.* **épidémiologie descriptive**) ainsi que d'identifier des facteurs de risque de la maladie (*i.e.* **épidémiologie analytique**). Des

mesures de maîtrise peuvent alors être mises en place dans les élevages pour limiter les troubles associés à l'agent pathogène étudié ou sa prévalence. Ce type d'enquête est généralement **lourd**, à la fois en termes de temps de travail et de budget (déplacements, analyses de laboratoire, *etc.*). De plus, l'observation en conditions réelles peut compliquer l'identification de facteurs de risque, du fait des **interactions avec des facteurs de confusion difficilement maîtrisables sur le terrain**. En effet, les élevages présentent des **différences de structure, de pratiques et d'environnement** (infectieux ou non) qui influent largement la dynamique de l'agent infectieux considéré. Les résultats peuvent aussi être dépendants des caractéristiques spécifiques de l'échantillon enquêté, ce qui rend leur **extrapolation à la population générale difficile**. L'**approche expérimentale** peut compléter utilement les enquêtes de terrain, tous les facteurs étant contrôlés. Mais, pour des raisons logistiques, éthiques et financières, l'expérimentation ne peut être réalisée que sur un **faible effectif d'animaux**, ce qui réduit la puissance statistique des analyses de la dynamique infectieuse, et seul un **petit nombre de facteurs** peut être évalué simultanément.

Pour toutes ces raisons, les **approches complémentaires de modélisation** sont de plus en plus couramment utilisées en épidémiologie. La modélisation est devenue **un outil essentiel et puissant à la compréhension de l'émergence et de la propagation de maladies infectieuses**, car elle permet d'**intégrer les multiples composantes d'un système** (liées au pathogène lui-même, à l'individu, ou aux caractéristiques de la population). La modélisation permet également de représenter toutes les combinaisons possibles y compris celles qui n'existent pas encore dans les élevages. Cependant, **la modélisation ne peut se détacher du terrain**. Par exemple, les hypothèses structurelles émises lors de la construction du modèle proviennent de données récoltées lors d'étude de terrain. De plus, la **validation du modèle** ne peut être faite que par comparaison avec des données existantes. Mais les données de terrain déjà acquises peuvent se révéler insuffisantes et la mise en place d'**essais expérimentaux** peut s'avérer nécessaire à l'**estimation de paramètres** clés du modèle : paramètres de transmission, durée d'excrétion, R_0 . Enfin, si les modèles permettent d'évaluer l'efficacité théorique de mesures de maîtrise d'un pathogène, **ces stratégies de contrôle demandent à être testées en conditions réelles**, et leur faisabilité technique, sociale et économique doit être validée conjointement avec les différentes parties prenantes de la filière, sous peine de ne rester qu'à l'état de théorie. Ainsi, **la modélisation nourrit et se nourrit des études de terrain**, qu'elles soient épidémiologiques (en élevage ou en conditions expérimentales) ou socio-économiques

(Figure 20) et c'est cette **complémentarité d'approches** qui fait toute la richesse et la pertinence des projets de recherche transversaux sur les maladies animales infectieuses.

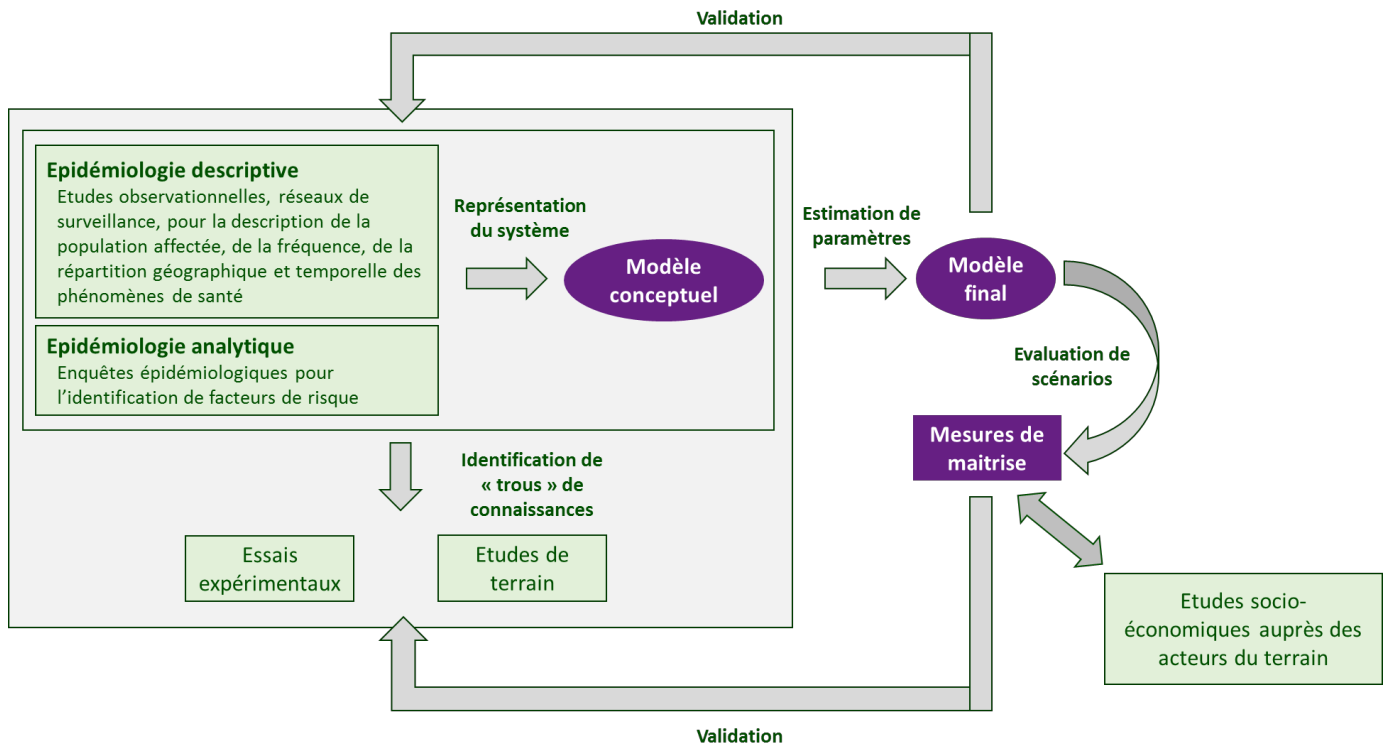


Figure 20 - Complémentarité des approches de modélisation et des enquêtes de terrain

I.2. Premières approches de quantification de la dynamique du virus de l'hépatite E chez le porc

Les études présentées ci-dessous ne sont pas de réelles études de modélisation permettant de proposer un système de dynamique du HEV dans une population mais elles utilisent le cadre des modèles infectieux pour des approches plus statistiques d'estimation de paramètres, pour décrire et quantifier la dynamique de l'infection par le HEV chez les porcs. Par exemple, Satou et Nishiura (2007) ont construit un modèle à partir de données sérologiques dans des élevages de porcs au Japon : ils ont calculé l'âge à l'infection en prenant en compte la distribution du temps entre l'infection et la séroconversion et ont estimé un **R0 compris entre 4,02 et 5,17**. Ensuite, Backer *et al.* (2012) ont obtenu des valeurs de R0 similaires en utilisant une approche bayésienne pour analyser la prévalence de porcs excréteurs par groupe d'âge, à partir de données du Royaume-Uni. En utilisant un cadre de modélisation simple, ils ont évalué l'efficacité de mesures de lutte, notamment une **potentielle vaccination des porcs contre le HEV**. En 2009, Bouwknegt *et al.* (2009) ont utilisé le principe d'un modèle SIR pour quantifier la transmission du HEV entre les porcs. A partir d'essais de transmission un à un après inoculation par voie intra-veineuse d'un porc, ils ont obtenu un **R0 plus élevé de 8,8** [4,4-19]. Ensuite, la même équipe a construit un modèle dose-réponse pour évaluer la contribution des fèces comme source de la transmission du HEV entre les porcs (Bouwknegt *et al.*, 2011). Elle a alors montré que la voie de **transmission féco-orale** était probable mais pas suffisante pour expliquer la transmission observée, et en a conclu que d'autres routes de transmission entraient probablement en jeu. L'**hypothèse d'une transmission environnementale** a ensuite été confirmée par Andraud *et al.* (2013). A partir d'un essai expérimental de transmission, ils ont exploré plusieurs voies de transmission du HEV : la transmission directe entre des porcs d'une même case, la transmission environnementale au sein d'une case, et la transmission environnementale entre deux cases, représentant le possible transfert de matières fécales entre deux cases adjacentes. Ils ont entre autres montré que les deux premières modalités pouvaient être considérées comme les voies de transmission majeures du HEV, et que **l'accumulation et la persistance du HEV dans l'environnement du fait de l'excrétion fécale jouaient un rôle majeur dans la transmission virale entre les porcs**.

Bien que ces études aient apporté des éléments cruciaux pour la compréhension des patterns de transmission du HEV, elles ne proposent pas un modèle conceptuel de propagation

dans une population dynamique, divisée en groupes d'animaux avec des structures de contact très hétérogènes, et affectée par de nombreux facteurs externes liés, entre autres, au type de conduite en bandes et aux pratiques d'élevage. **A ce jour, aucun modèle dynamique du HEV, intégrant la dynamique de population et la circulation virale dans l'élevage, n'a été développé.**



Ce qu'il faut retenir

Les approches de modélisation sont des outils complémentaires aux études menées sur le vivant. Conjointement avec des enquêtes en élevage et des essais expérimentaux, elles permettent de représenter et d'expliquer la diffusion et la persistance d'un pathogène dans une population dynamique, et d'évaluer un risque et/ou l'efficacité de mesures de maîtrise. A ce titre, la modélisation s'avèrerait être une approche pertinente pour la compréhension de la circulation du virus de l'hépatite E dans la filière porcine. Actuellement, il n'existe pas de modèle dynamique représentant la diffusion du virus de l'hépatite E, ni au sein d'un élevage, ni entre des élevages de porcs.

La modélisation permettrait également de développer des stratégies de maîtrise du risque HEV. La mise en place de mesures de lutte contre le HEV est susceptible de nécessiter des modifications de pratiques des différentes parties prenantes de la filière. Il apparaît ainsi opportun d'étudier les freins et les motivations à ces changements de pratiques.



Take home message

Modelling approaches are complementary tools to studies conducted on living organisms. In conjunction with farm surveys and experimental trials, they can represent and explain the spread and persistence of a pathogen in a dynamic population, and assess a risk and/or the effectiveness of control measures. As such, modelling would prove to be a relevant approach for understanding the circulation of the hepatitis E virus in the pig sector. Currently, there is no dynamic model representing the spread of the hepatitis E virus, either within a farm or between pig farms.

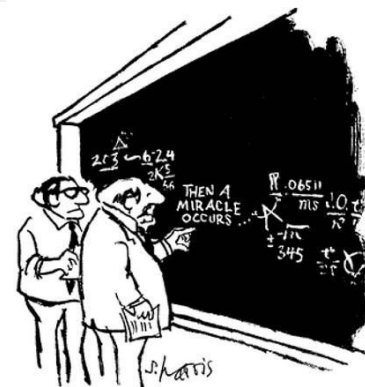
Modelling would also allow the development of strategies to control HEV risk.

The implementation of such measures is likely to require changes in the practices of the various stakeholders in the sector. It therefore seems appropriate to study the barriers and motivations to these changes in practices.

III. Apport des sciences humaines et sociales pour la maîtrise des risques dans les filières de production animale

Quelle(s) approche(s) ?

Les sciences humaines et sociales offrent un large éventail de disciplines permettant d'étudier des comportements et d'évaluer les déterminants de la motivation des acteurs à changer un comportement ou adopter de nouvelles pratiques. Parmi elles, la psychologie sociale se définit comme « l'étude des relations réelles ou imaginées de personne à personne dans un contexte social donné, en tant qu'elles affectent les personnes impliquées dans cette situation » (Allport, 1924). En d'autres termes, c'est un domaine de la psychologie qui



"I think you should be more explicit here in step two."

propose des concepts, des théories et des méthodes pour analyser différents phénomènes et aspects relationnels de la vie sociale. Plusieurs concepts, présentés ci-après, ont émergé dans le champ de la psychologie sociale pour comprendre et/ou prédire des comportements de personnes ou groupes de personnes, et ainsi susciter puis accompagner des changements de pratiques. La sociologie des organisations, quant à elle, est une branche de la sociologie qui étudie comment les acteurs construisent et coordonnent des activités organisées.

Nous avons choisi de présenter quelques théories et modèles issus de ces deux disciplines et traitant des comportements et des changements, à l'échelle individuelle (*psychologie*) et/ou collective (*sociologie*). Pour information, les abréviations PS (pour psychologie sociale) ou SO (pour sociologie des organisations) sont accolées aux modèles présentés selon qu'ils relèvent de l'une ou l'autre des disciplines.

II.1. Susciter et accompagner le changement : cadres théoriques

II.1.a. Comprendre les décisions des acteurs : étude du lien attitude-comportement

(i) L'attitude

Dans le langage ordinaire, l'attitude correspond, au sens propre, à une position du corps, à une manière de se tenir, et au sens figuré, à une conduite tenue dans certaines circonstances. En psychologie sociale, l'attitude désigne « un **état mental et neuropsychologique** de préparation à l'action, organisé par l'expérience du sujet et exerçant une influence directrice ou dynamique sur sa réponse à tous les objets et à toutes les situations s'y rapportant » (Allport, 1935). Autrement dit, l'attitude est considérée comme une **variable intermédiaire** qui prépare l'individu à agir d'une certaine manière à l'égard d'un objet donné. L'attitude est une **construction hypothétique**, elle ne s'observe pas comme un comportement peut l'être ; elle est déduite à partir des déclarations ou réponses des individus. L'idée essentielle de la définition d'Allport est que l'attitude est ce qui est supposé être derrière le comportement. Sous cet axe, **l'attitude serait une cause du comportement**. Plusieurs théories de l'attitude ont été développées. En particulier, le **modèle tripartite de Rosenberg et Hovland** attribue à l'attitude trois composantes : (i) la **composante affective**, qui concerne les émotions positives ou négatives que l'individu a à l'égard de l'objet attitudinal, la prédisposition à évaluer cet objet comme étant bon ou mauvais, intéressant ou inintéressant, *etc.* ; (ii) la **composante cognitive**, qui fait référence aux connaissances et croyances présentes et passées que l'individu a concernant cet objet ainsi qu'à la crédibilité que l'individu accorde à ces informations ; (iii) la **composante conative**, relative aux comportements passés et présents de l'individu face à cet objet et à ses intentions comportementales (Rosenberg, 1960).

(ii) L'intention comportementale : TRA et TCP (PS)

Deux modèles successifs, la **Théorie de l'Action Raisonnée** (TAR, *theory of reasoned action*) et la **Théorie du Comportement Planifié** (TCP, *theory of planned behaviour*), se sont attachés à l'étude du lien attitude-comportement, en plaçant l'intention comportementale au cœur de ce lien (Fishbein, 1967; Ajzen, 1991) (Figure 21). Le postulat de départ de la TAR est que **l'intention dérive de l'attitude**, elle-même liée aux croyances et à l'information disponible

quant aux conséquences du comportement, et de **normes subjectives**, relatives elles-mêmes aux croyances et à la motivation de l'individu à se soumettre. La principale critique apportée à la TAR consiste à dire que seules les conduites les plus simples sont sous le contrôle de la volonté et peuvent donc être modélisées selon cette théorie. Le modèle de la TAR évolue alors, pour inclure le **contrôle comportemental perçu** : c'est le modèle de la TCP. Le contrôle comportemental perçu est défini ainsi : il s'agit de la facilité ou de la difficulté perçue à réaliser un comportement, aussi appelée auto-efficacité. La notion de contrôle comportemental perçu suggère que l'individu se serve de son expérience antérieure pour anticiper et donc éviter les difficultés à venir. Par conséquent, s'il décide que la réalisation de son comportement ne sera pas gênée par des obstacles majeurs et si son attitude, ainsi que la norme subjective, sont favorables à cette réalisation, alors son intention comportementale augmentera davantage.

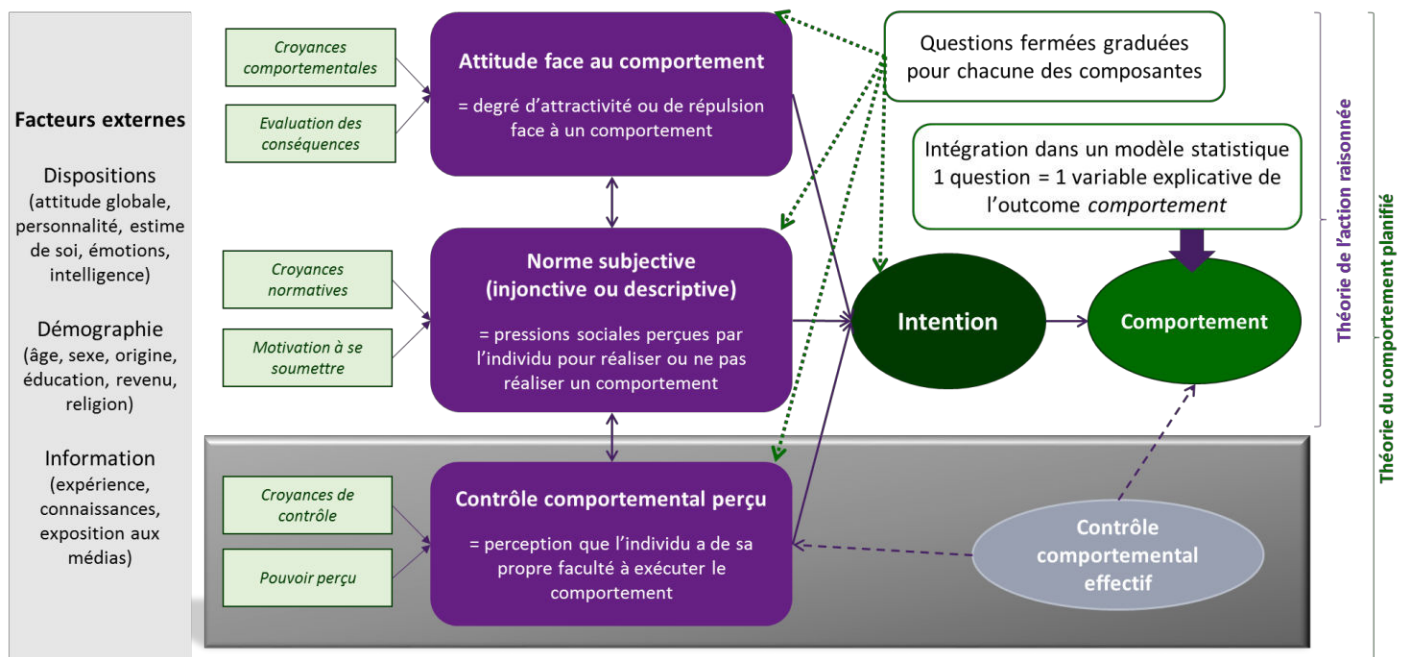


Figure 21 - Théorie de l'Action Raisonnée (TAR) et Théorie du Comportement Planifié (TCP) : concept et utilisation pratique possible

Le cadre gris foncé correspond à l'apport de la TCP par rapport à la TAR.

D'un point de vue pratique, la TCP peut être utilisée afin d'étudier l'intention d'un individu à adopter un comportement, en évaluant, par des questions fermées graduées, chacune des composantes déterminant l'intention, et en intégrant ces variables dans un modèle statistique dont la variable à expliquer est le comportement.

II.1.b. Conduire le changement : modèles et théories du changement

- (i) La théorie du champ de forces et le modèle du changement de Lewin (PS)

La **théorie du champ de forces** postule que l'activité des individus est influencée par les forces présentes dans leur environnement (Bamberg, 2013; Autissier *et al.*, 2018) (Figure 22). Ces forces peuvent être soit des propulseurs soit des freins du changement. Par défaut, l'individu est dans un état quasi stationnaire ; un changement peut avoir lieu soit en augmentant le nombre et/ou l'intensité des **forces propulsives**, soit en diminuant le nombre et/ou l'intensité des **forces restrictives**. Selon Lewin, **il est plus efficace de diminuer les forces restrictives** que d'augmenter les forces propulsives pour obtenir un changement.

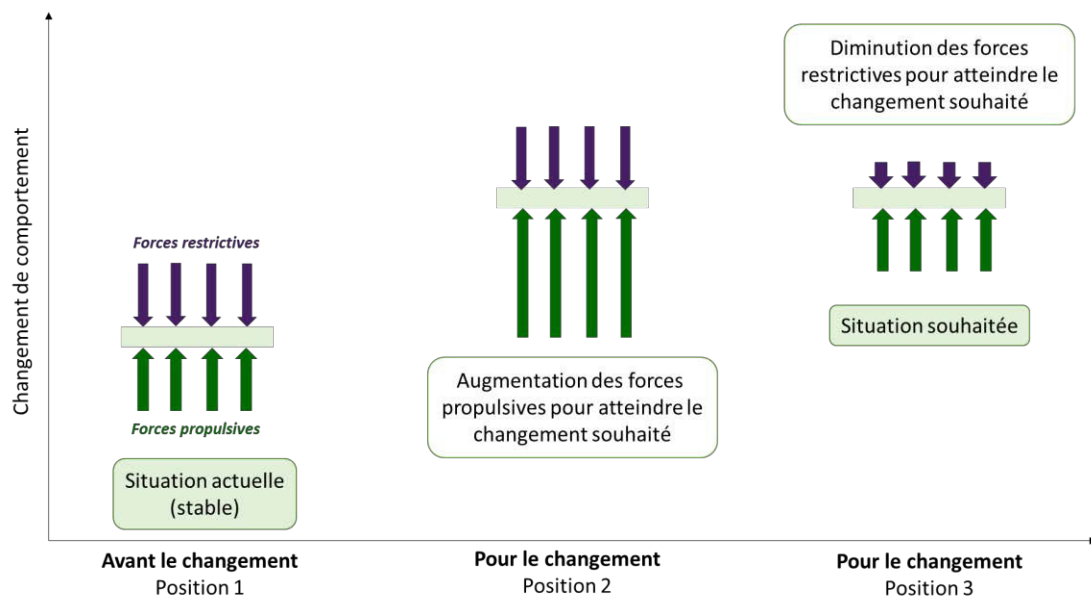


Figure 22 - La théorie du champ de forces selon le modèle de Lewin

d'après Bamberg (2013)

Lewin donne aussi une place aux **normes sociales** qui agissent comme des résistances au changement dans le champ de forces. Pour favoriser le changement, il est ainsi nécessaire soit de réduire l'attachement des individus à la norme, soit de modifier la norme. De plus, d'après Lewin, les résistances au changement proviennent davantage de facteurs collectifs et affectifs que de facteurs individuels et rationnels. Ainsi, il serait **plus aisé de provoquer le changement d'un groupe d'individus plutôt que d'individus pris isolément**. A partir de ce postulat, Lewin a développé un modèle de changement composé de trois étapes :

- La **phase de décrystallisation**, pendant laquelle le groupe prend conscience qu'il n'est pas satisfait de sa situation actuelle et que les objectifs qui sont importants pour lui ne sont pas atteints. Les normes du groupe sont remises en question : ce déséquilibre facilite l'ouverture pour apprendre et adopter de nouveaux comportements.
- La **phase de déplacement**, au cours de laquelle on observe un changement de comportement du groupe, rendu possible par la réduction des forces de résistance créées par l'attachement aux anciennes normes. Le groupe commence à mettre en pratique des nouvelles conduites.
- La **phase de cristallisation**, au cours de laquelle le nouveau comportement reste stable grâce aux nouvelles normes qui empêchent le retour à l'état précédent.

(ii) Le modèle transthéorique de changement de Prochaska et Di Clemente (PS)

Le **modèle transthéorique** est lui aussi un modèle séquentiel, qui comporte sept étapes et huit modes d'accompagnement du changement (Prochaska et Di Clemente, 1982) (Figure 23). Le passage par ces sept stades est graduel et plus l'individu avance, plus il est prêt au changement.

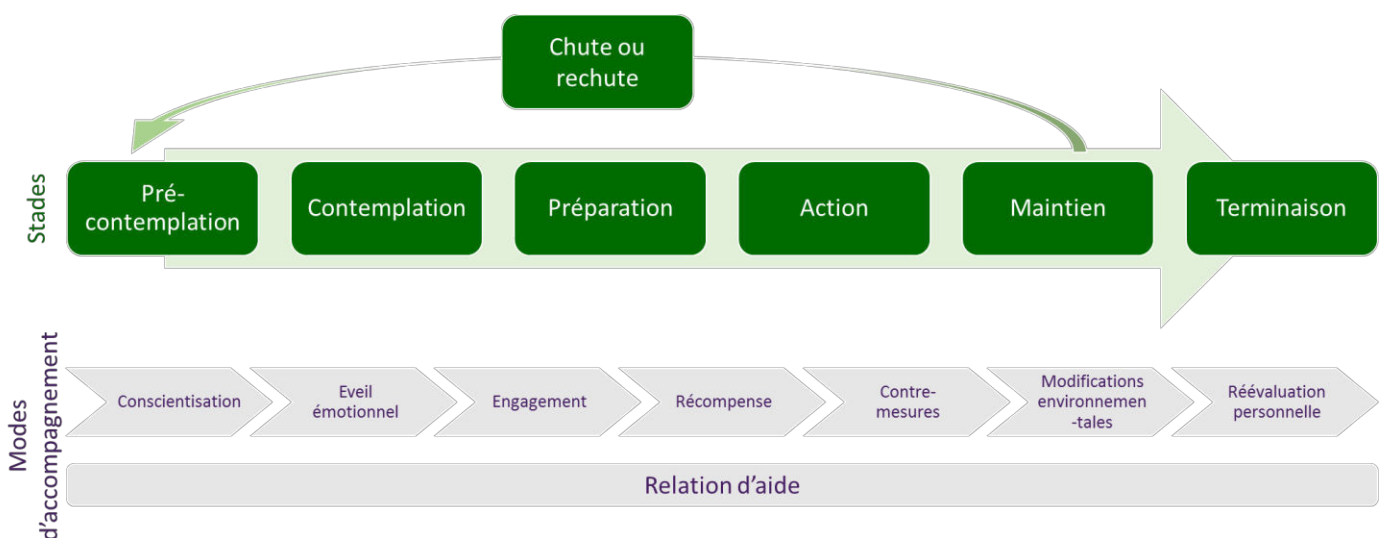


Figure 23 - Modèle transthéorique du changement : stades et modes d'accompagnement du changement

d'après Bamberg (2013)

Ce modèle est particulièrement utilisé dans le cadre de la désaccoutumance à des addictions, que nous prendrons comme exemple ci-après. Dans la **phase de pré-**

contemplation, l'individu n'envisage pas de changer de comportement (« *Je ne vois pas quel est le problème* »). Dans la **phase de contemplation**, la personne est consciente du fait qu'elle doit changer de comportement et a envie de le faire (« *Oui ce serait bien que j'arrête de fumer* »). Au **stade de préparation**, l'individu a l'intention de changer et projette des actions à court terme (« *Je voudrais aller voir un tabacologue* »). Ensuite, le **stade de l'action** est la phase où la personne a déjà réalisé des actions concrètes, elle évalue les avantages et les désavantages du nouveau comportement et essaye différentes alternatives de comportements plus favorables ; cette phase est la plus instable (« *J'ai réduit ma consommation mais je sens que je vais craquer* »). Dans la **phase de maintien**, qui commence généralement six mois après l'atteinte de l'objectif, la personne essaie de ne pas retourner au comportement antérieur et a davantage confiance dans sa capacité à maintenir le changement (« *Ca y est, j'ai trouvé un nouvel équilibre* »). Des **rechutes** sont possibles, avant d'atteindre le **stade de terminaison**, où la personne a intégré le nouveau comportement dans son quotidien.

A chaque étape, l'individu peut être confronté à des résistances au changement et il a donc besoin d'un **accompagnement** spécifique. La **conscientisation** permet d'apporter les informations pertinentes sur les risques ou les conséquences de continuer le comportement inadapté par rapport aux avantages d'adopter le nouveau comportement. L'**éveil émotionnel** consiste à pousser la personne à exprimer ses peurs et ses représentations liées au changement. L'**engagement** consiste à encourager la personne à avoir confiance dans sa capacité à changer et à s'engager à le faire, par exemple au sein d'un groupe. L'obtention de **récompenses** favorise le maintien du comportement : meilleure estime de soi, valorisation de l'effort par d'autres personnes, économies, gain de temps, etc. Les **contre-mesures** visent à aider l'individu à éviter les situations ou stimuli susceptibles de le faire revenir à l'ancien comportement. Les **modifications environnementales** visent à fournir à l'individu toutes les alternatives possibles à l'ancien comportement dans son environnement. Enfin, avec la **réévaluation personnelle**, il s'agit d'inciter la personne à se former sa propre opinion sur l'intérêt de changer.

(iii) La diffusion des innovations (PS/SO)

Dans les années 1960, E. Rogers a théorisé la **diffusion des innovations** en distinguant : (i) les **phases d'innovation et de décollage**, au cours de laquelle des organisations ou **individus pionniers ou innovateurs** adoptent l'innovation considérée ; (ii) suivies de phases de croissance/développement et maturité, au cours desquelles la majorité (précoce ou

tardive) adopte l'innovation ; (iii) puis les **retardataires**, ou **suiveurs**, finissent par adopter l'innovation au cours de la phase de déclin (Rogers, 2003) (Figure 24). Citons comme exemple de ce modèle une étude menée par des étudiants de l'Ecole supérieure d'agriculture d'Angers auprès d'adhérents de plusieurs coopératives sur l'agriculture écologiquement intensive²³. A l'issue de 61 entretiens, une typologie d'agriculteurs a émergé, articulant attitude face au risque, modalités d'accomodement avec les nouvelles techniques, et canaux d'information. Les innovateurs (6/61) introduisent les nouveaux systèmes à l'échelle de leur exploitation, créent ou innovent localement, et font souvent partie de réseaux d'informations marginaux. Ceux qui sont en cours d'appropriation des innovations (8/61) accomodent le risque dans le cadre d'essais à petite échelle (bandes, micro-parcelles), puis sur une partie croissante de l'exploitation quand les résultats sont bons. Les agriculteurs « en phase de changement » (27/61) mettent en œuvre des changement déjà déployés dans leur environnement proche et dont l'efficacité est avérée. Ils sont dans le schéma de la « vulgarisation par-dessus la haie », c'est-à-dire qu'ils attendent de voir ce que la pratique en question donne chez leurs voisins, avant de les imiter, ce qui constitue un effet « tache d'huile » (Boisseau, 1974). Enfin, les autres agriculteurs (20/61) ne se placent pas dans une dynamique de changement, ils sont d'abord en recherche de stabilité.

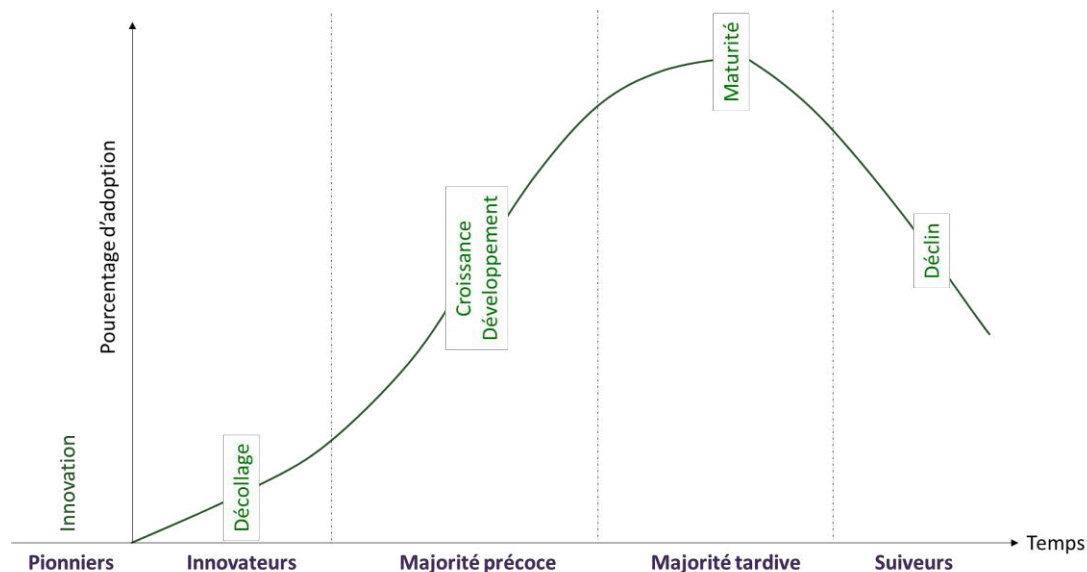


Figure 24 - Courbe de diffusion des innovations

d'après Rogers (2003)

²³ Groupe ESA, 2013, *Les agriculteurs et l'Agriculture Ecologiquement Intensive : dynamiques d'innovation et préoccupations*.

(iv) Le management des transitions : comment amorcer un déverrouillage ? (PS/SO)

La notion de **verrouillage** fait référence à un ensemble de mécanismes par lesquels le développement et la diffusion d'alternatives ou d'innovations sont inhibés et exclus (Vanloqueren et Baret, 2009). Elle s'applique à des situations où il existe au moins une technologie alternative considérée comme plus efficace que celle qui fait office de standard (Fares *et al.*, 2012). Différents mécanismes sont imbriqués dans des cycles d'auto-renforcement :

- Les **rendements croissants d'adoption** : ce mécanisme est relatif au fait que plus une innovation est répandue, plus les individus auront intérêt à l'adopter, même si, individuellement, ils sont convaincus par d'autres solutions.
- La **compatibilité avec les standards** : l'adhésion à des innovations sera d'autant plus grande qu'elles sont compatibles avec la situation standard.
- La **mobilitation et l'état des connaissances** : au niveau individuel, l'indisponibilité des connaissances, ou l'immaturation des pratiques alternatives, conforte les comportements d'aversion au risque d'innovation. Des biais informationnels, liés à la mauvaise organisation du conseil ou au choix erroné des indicateurs de performance ou d'efficacité, perpétuent alors une situation, même sous-optimale.
- Les **effets d'irréversibilité** : certaines pratiques peuvent s'auto-entretenir, avec une très forte inertie et une quasi-irréversibilité, alors même qu'elles ne sont pas durables.

Le diagnostic d'une situation de verrouillage est un préalable important pour susciter le changement (Bidaud, 2013). Plusieurs auteurs ont proposé des **stratégies de déverrouillage**, ou management des transitions. Par exemple, Geels et Schot (2007) expliquent que **les innovations sont essentiellement incrémentales**, et que les réorientations potentielles d'une trajectoire à l'échelle globale se font d'abord à partir d'**expérimentations dans des niches** où l'innovation a pu se développer. Bidaud (2013) décrit lui une intervention publique à plusieurs niveaux : (i) des actions à un niveau global sur le **paysage socio-technique** (modifications réglementaires, fiscales, politiques, *etc.*) ; (ii) le soutien et l'accompagnement des **niches d'innovation** ; (iii) la **favorisation d'interfaces** visant à combiner ces innovations locales avec le régime dominant ; (iv) la **remise en question de la conception traditionnelle verticale de l'innovation**, dans laquelle les individus impactés appliquent les pratiques recommandées par des organismes de recherche ou les pouvoirs publics.

(v) Le changement organisationnel (SO)

Le **changement organisationnel** se définit comme toute modification durable dans un sous-système de l'organisation, pourvu que cette modification soit observable par ses membres ou les individus qui sont en relation avec ce sous-système (Collerette *et al.*, 2005). Le processus de changement s'opère selon un processus de déconstruction - reconstruction :

- L'**éveil** : en présence d'une pression pour un changement, l'éveil consiste à s'interroger sur l'utilité de prêter attention à cette pression.
- La **désintégration** : elle consiste à déterminer quels sont les aspects jugés non-adaptés dans le système de représentation et les pratiques actuelles.
- La **reconstruction** : suite à la désintégration de certains éléments de son univers de pertinence, le système se met à la recherche de significations nouvelles dans sa façon de percevoir le réel qui l'aideront à réagir de façon satisfaisante aux situations qui se présentent. La reconstruction s'opère en même temps entre les membres du système, c'est alors une sorte de renégociation des significations partagées.
- L'**intégration** : lorsque les nouvelles significations et les comportements qui en découlent se stabilisent, la phase d'intégration s'enclenche graduellement. Il s'agit de l'atteinte d'un état d'équilibre permettant alors à de nouveaux changements de s'insérer.

Pour Crozier et Friedberg (1977), le changement est le **résultat d'un processus collectif** à travers lequel sont mobilisées voire créées les ressources des participants dont la mise en œuvre libre permet au système de s'orienter ou de se réorienter. Ainsi, les personnes ne changent que si : elles sont **associées** ; elles ont un **intérêt personnel** dans le changement ; elles **s'approprient concrètement** le changement et en perçoivent les enjeux ; le changement est **réaliste** et à leur portée ; le système des **priorités** est clair et cohérent ; elles sont **encouragées** et stimulées par leur hiérarchie ; la **hiérarchie** s'applique elle-même le changement.

(vi) La résistance au changement dans les organisations (SO)

La **résistance au changement** est définie comme « l'expression implicite ou explicite de réactions de défense à l'endroit de l'intention du changement » (Collerette *et al.*, 2005). La résistance au changement est aussi une volonté des individus d'exercer leur liberté dans le système (Crozier et Friedberg, 1977). Selon Kotter et Schlesinger (1979), la résistance au

changement trouve son origine dans quatre phénomènes : (i) l'**esprit de clocher** : l'individu se concentre sur son propre intérêt et non pas sur l'intérêt de l'organisation ; (ii) le manque de confiance et la **méconnaissance des intérêts du changement** ; (iii) la **peur** de ne pas être capable de développer les compétences et les comportements nouveaux ; (iv) une **évaluation du processus de changement différente** de celle des managers.

II.1.c. Quelles méthodes pour évaluer la capacité des individus et/ou d'un système à changer ?

- (i) Le questionnaire

Le **questionnaire** est une **série de questions standardisées posées à un individu**, qui permet des inférences statistiques (Blanchet *et al.*, 2013). Il permet par exemple d'estimer la fréquence d'une pratique, de la décrire et de l'expliquer. De nombreux types de questions existent : question ouverte *versus* question fermée, question dichotomique *versus* question à choix multiple *versus* question graduée (échelle de Lickert par exemple, de 1 à 5 ou de 1 à 7), question courte *versus* question longue, question directe *versus* question indirecte, question guidée, question piège, *etc.* Le questionnaire permet d'obtenir un grand nombre de réponses avec des moyens réduits, et de limiter les biais liés à un intervieweur.

- (ii) L'observation

L'**observation** est une méthode d'enquête par laquelle **on observe directement, par la présence sur le terrain, les phénomènes sociaux qu'on cherche à étudier**²⁴. Dans une enquête par observation, les séances d'observation alternent avec des moments de réflexion et d'écriture sur ce qui a été observé (consignation des données collectées, analyse méthodologique, pistes d'analyse sociologique). L'observation peut être libre ou méthodique (*i.e.* selon un plan raisonné et contrôlé d'observation), directe ou indirecte (*i.e.* via des documents d'enregistrement, *e.g.* recensement, cadastre, registre d'état civil). L'**observation participante**, quant à elle, suppose que le sociologue ne se contente pas d'observer son terrain

²⁴ Revillard, A., 2015, Méthodes qualitatives – Observation directe et enquête de terrain, Master 1 Sociologie 2015-2016, Sciences Po Paris, 28p. Disponible à : <https://annerevillard.files.wordpress.com/2013/12/syllabus-observation2015-2016.pdf>

en restant dans une posture d'extériorité, mais qu'il participe aux activités en cours en adoptant un rôle déjà existant dans la situation étudiée.

(iii) L'entretien

L'**entretien** est une méthode sociologique d'investigation qui permet « l'analyse du sens que les acteurs donnent à leurs pratiques et aux événements auxquels ils sont confrontés : leurs systèmes de valeurs, leurs repères normatifs, leurs interprétations de situations conflictuelles ou non, leurs lectures de leurs propres expériences » (Van Campenhout et Quivy, 2011). On distingue trois types d'entretiens (Blanchet *et al.*, 2013):

- L'**entretien non-directif** : dans ce type d'entretien, l'enquêteur annonce le thème de l'entretien mais ne pose pas de questions directes. Il donne à l'enquêté la liberté d'organiser son discours comme il le souhaite, au risque de ne pas recueillir certaines informations.
- L'**entretien directif** : cette forme d'entretien se rapproche du questionnaire, car l'enquêteur pose une série de questions pré-établies, permettant d'obtenir des réponses à toutes les problématiques envisagées par l'intervieweur mais limitant la collecte d'informations qui ne rentrent pas dans le champ des hypothèses de départ.
- L'**entretien semi-directif** : ce type d'entretien, intermédiaire entre les deux premiers, se caractérise par le fait qu'il laisse à l'interviewé un large espace pour donner son point de vue, tout en restant centré sur les thématiques prédéfinies. L'enquêteur pose des questions, le plus souvent ouvertes, et laisse l'enquêté développer ses réponses, en l'encourageant à apporter davantage d'informations.

(iv) Les études CAP

L'enquête de type **Connaissances – Attitudes – Pratiques** (CAP, ou KAP pour Knowledge – Attitudes - Practices) est une **méthode quantitative** spécifique qui a pour objectif d'évaluer et analyser les connaissances, attitudes et pratiques d'un groupe de personnes²⁵. Le recueil des données se fait par un **questionnaire standardisé** permettant de recueillir des données qualitatives et/ou quantitatives. Cette méthode est très largement employée dans le domaine de la **santé humaine**, notamment dans les pays en voie de développement, comme

²⁵ Médecins du Monde. Data collection, quantitative methods: the KAP survey model. Disponible à : <https://www.medecinsdumonde.org/en/actualites/publications/2012/02/20/kap-survey-model-knowledge-attitude-and-practices>

instrument de planification et d'évaluation stratégiques pour identifier le besoin éducationnel d'une cible spécifique.

(v) Les approches participatives

Une approche participative est une méthode incluant une **implication active du public dans un processus décisionnel**. Le public concerné dépend du sujet abordé : citoyens lambda, parties prenantes d'un projet ou d'une politique en particulier, experts, membres du gouvernement ou entreprises privées. Les processus décisionnels se déroulent généralement selon un cycle en trois étapes comprenant la **planification**, la **mise en œuvre** et l'**évaluation** : l'approche participative peut être utilisée dans toutes ces étapes ou pour certaines d'entre elles. On distingue différents niveaux de participation selon que l'objectif est : *(i)* la **transmission d'informations** (unidirectionnelle) ; *(ii)* la **consultation** (bidirectionnelle, mais la partie consultante délimite la question) ; *(iii)* la **participation active** : toutes les parties impliquées peuvent alors délimiter la question dans une plus ou moins grande mesure. De nombreuses méthodes participatives peuvent être utilisées (Sloccum, 2003). Le choix de la méthode dépend des objectifs, de la thématique considérée, des participants, du temps et des ressources disponibles. Parmi ces méthodes, peuvent être cités :

- La **Charrette**, qui est un processus conçu pour permettre aux personnes issues de divers groupes de la société de parvenir à un consensus dans un court laps de temps. La phase préparatoire subdivise la question principale en plusieurs composantes auxquelles sont affectés des sous-groupes de personnes. Ces sous-groupes soumettent périodiquement des rapports à l'ensemble du groupe qui sont ensuite débattus lors du cycle de discussion suivant. Cette séquence se répète jusqu'à obtenir un consensus à une date finale fixée.
- Le **focus groupe**, qui est une discussion structurée d'un groupe interactif de 4 à 12 personnes généralement, animée par un modérateur dans un cadre non contraignant et détendu. Il permet d'obtenir des informations sur les préférences et les valeurs de diverses personnes concernant un sujet donné, ainsi que sur les raisons qui les sous-tendent.
- Le **World Café**, qui est un processus créatif visant à faciliter le dialogue et le partage de connaissances et d'idées, en vue de créer un réseau d'échanges et d'actions. Dans ce processus, les participants débattent d'une question ou d'un sujet en petits groupes autour de tables. Les participants changent de table à intervalles réguliers ; un modérateur reste à la table et résume la conversation précédente aux nouveaux arrivés. Les conversations en cours

sont alors nourries des idées issues des conversations précédentes avec les autres participants. Au terme du processus, les principales idées sont résumées au cours d'une assemblée plénière et les possibilités de suivi sont soumises à discussion.

A ces méthodes sont associés de nombreux outils. Parmi eux :

- La **séance de brainstorming** correspond à une période de libre réflexion qui sert à articuler les idées, suivie d'un débat organisé sur ces idées. L'objectif est de réduire les inhibitions des participants, de stimuler la créativité et la réflexion spontanées, ainsi que de permettre la prise en considération de points de vue dissidents ou marginaux. Le brainstorming est ainsi utile pour recueillir une grande quantité d'idées avant les analyses de scénarios, la résolution des problèmes, la prise de décision ou la planification.
- Les **diagrammes d'arborescence** sont des outils visuels qui permettent de préciser et de déterminer le degré de priorité des problèmes, des objectifs ou des décisions. Les informations sont structurées dans un diagramme semblable à un arbre. La question principale est représentée par le tronc de l'arbre, tandis que les facteurs, influences et résultats pertinents s'apparentent à des systèmes de racines et de branches.
- La **roue du futur** est une méthode qui permet de structurer les réflexions et les questions sur l'avenir. Le nom d'une tendance ou d'un événement est inscrit au centre d'une feuille de papier, puis des petits rayons sont dessinés en partant du centre. Les impacts ou conséquences primaires sont inscrits à l'extrémité de chaque rayon. Les impacts secondaires de chaque impact primaire forment ensuite le deuxième anneau de la roue. Cet effet de vague se poursuit jusqu'à l'obtention d'une image complète des implications de l'événement.

II.2. Application à des problématiques de gestion des risques dans les filières de production animale

Ces théories, modèles et outils sont applicables et appliqués dans le domaine des filières de production animale. En particulier, plusieurs études ont utilisé le cadre de **la TAR et/ou de la TCP** pour **explorer les comportements des éleveurs et leur propension à changer leurs pratiques**, par exemple la manière dont les éleveurs de porcs cherchent de l'information et prennent leurs décisions relatives à la gestion des maladies animales (Alarcon *et al.*, 2014), la façon dont les éleveurs de bovins mettent en œuvre des programmes de maîtrise des pathogènes zoonotiques dans leur élevage (Ellis-Iversen *et al.*, 2010) ou encore les contraintes perçues par les éleveurs et les vétérinaires en ce qui concerne les mesures de biosécurité (Gunn *et al.*, 2008). Les outils utilisés par les auteurs sont variables et vont du **questionnaire** (Bahnson *et al.*, 2001; Marvin *et al.*, 2010; Young *et al.*, 2010a; Simon-Grife *et al.*, 2013; Laanen *et al.*, 2014; Guinat *et al.*, 2016b) à l'**observation** (Racicot *et al.*, 2012b) en passant par l'**entretien semi-directif** (Marier *et al.*, 2016; Poizat *et al.*, 2017) et des **approches d'épidémiologie participative** (Calba *et al.*, 2015; Chenais *et al.*, 2017).

La littérature rapporte plusieurs facteurs qui influencent la prise de décision des éleveurs, en particulier en matière de santé animale ou publique. D'abord, des **caractéristiques individuelles** telles que l'âge, le sexe, l'éducation, la personnalité, *etc.* peuvent influencer les opinions (Racicot *et al.*, 2012a; Wilson *et al.*, 2015; Frössling et Nöremark, 2016). Les éleveurs doivent également avoir une **connaissance** suffisante de la maladie et des stratégies de gestion pour apporter des changements efficaces. Ainsi, le **manque de sensibilisation** et l'**accès difficile aux résultats de la recherche scientifique** peuvent expliquer des résistances aux changements (Benjamin *et al.*, 2010; Ellis-Iversen *et al.*, 2010; Racicot *et al.*, 2012a; Ritter *et al.*, 2017). Concernant les pathogènes zoonotiques, ce manque de connaissance est encore plus flagrant (Bahnson *et al.*, 2001; Ellis-Iversen *et al.*, 2010; Young *et al.*, 2010a; Young *et al.*, 2010b). L'**impact de la maladie** sur la santé animale et / ou les performances économiques de l'exploitation peuvent également inciter les agriculteurs à prendre des mesures pour lutter contre des pathogènes (Alarcon *et al.*, 2014). Le HEV circule de manière asymptomatique dans les élevages de porcs, et sans causer de baisse de performances ni de pertes économiques, ce qui signifie que cette problématique est susceptible d'être méconnue par les éleveurs. Un autre point intéressant est que le seuil auquel un problème devient un problème réel dépend du **cadre**

de référence des éleveurs, souvent lui-même influencé par leurs expériences antérieures (Jansen *et al.*, 2010; Jansen *et al.*, 2016). Il a également été démontré que l'évaluation d'un problème par les éleveurs est effectuée par rapport à d'autres problèmes qui nécessitent également leurs efforts (Leach *et al.*, 2010a; Bruijn *et al.*, 2013; Horseman *et al.*, 2014). Le **contexte agricole** est donc un facteur important à prendre en compte (lois et réglementations, prix du marché, demandes des consommateurs, signaux de rappel, *etc.*) (Ritter *et al.*, 2017). La **perception par les éleveurs de leur propre responsabilité** face au problème a été démontrée comme un facteur clé de leur motivation également, en particulier lorsqu'il s'agit d'un problème de santé du consommateur (Toma *et al.*, 2001; Sorge *et al.*, 2010; Nielsen, 2011). Les autres incitations internes peuvent inclure la satisfaction professionnelle, la réputation, la reconnaissance de la famille, *etc.* (Leach *et al.*, 2010b; Bruijn *et al.*, 2013; Alarcon *et al.*, 2014; Roche *et al.*, 2015). En ce qui concerne l'**environnement professionnel direct des éleveurs**, plusieurs études ont montré que les **vétérinaires** et les **techniciens** jouent un rôle important dans la diffusion d'informations en tant que **courroie de transmission** et peuvent inciter les éleveurs à adopter les meilleures pratiques de gestion (Ellis-Iversen *et al.*, 2010; Alarcon *et al.*, 2014). Si les freins et motivations des acteurs d'une filière de production animale reposent particulièrement sur la personnalité et les caractéristiques de l'éleveur, le **rôle de l'Etat, des structures de conseil et des accompagnants** est central (Ducrot *et al.*, 2019). Ainsi, l'Etat, dont les actions dérivent d'une stratégie globale de gouvernance de la santé (animale ou publique), peut influencer sur les décisions des éleveurs *via* des **actions réglementaires ou incitatives** (par exemple financières). Les organisations de production, quant à elles, ont un **rôle structurant, de coordination, de réflexion stratégique sur les orientations de la filière**, qui peuvent également passer par des **incitations financières**. L'une des thématiques majeures d'application des sciences sociales en santé publique concerne la réduction de l'usage des antibiotiques. La revue de littérature de Ducrot *et al.* (2019) dresse une synthèse des facteurs sociaux et psychologiques qui jouent un rôle dans la décision des éleveurs de réduire leur usage d'antibiotiques. Nous en proposons une représentation graphique en Figure 25.

Au bilan, l'un des intérêts de ces études psycho-sociales conduites auprès d'un ou plusieurs acteurs d'une filière animale est d'expliquer pourquoi certains programmes de maîtrise de pathogènes, bien que leur efficacité biologique théorique ait été prouvée, ne sont pas correctement mis en place sur le terrain ou, au contraire, **d'explorer en amont, et donc de garantir, la possibilité d'une mise en œuvre correcte et efficace d'un plan de lutte dans les élevages**. C'est dans ce contexte que les sciences sociales ont été utilisées dans notre projet.

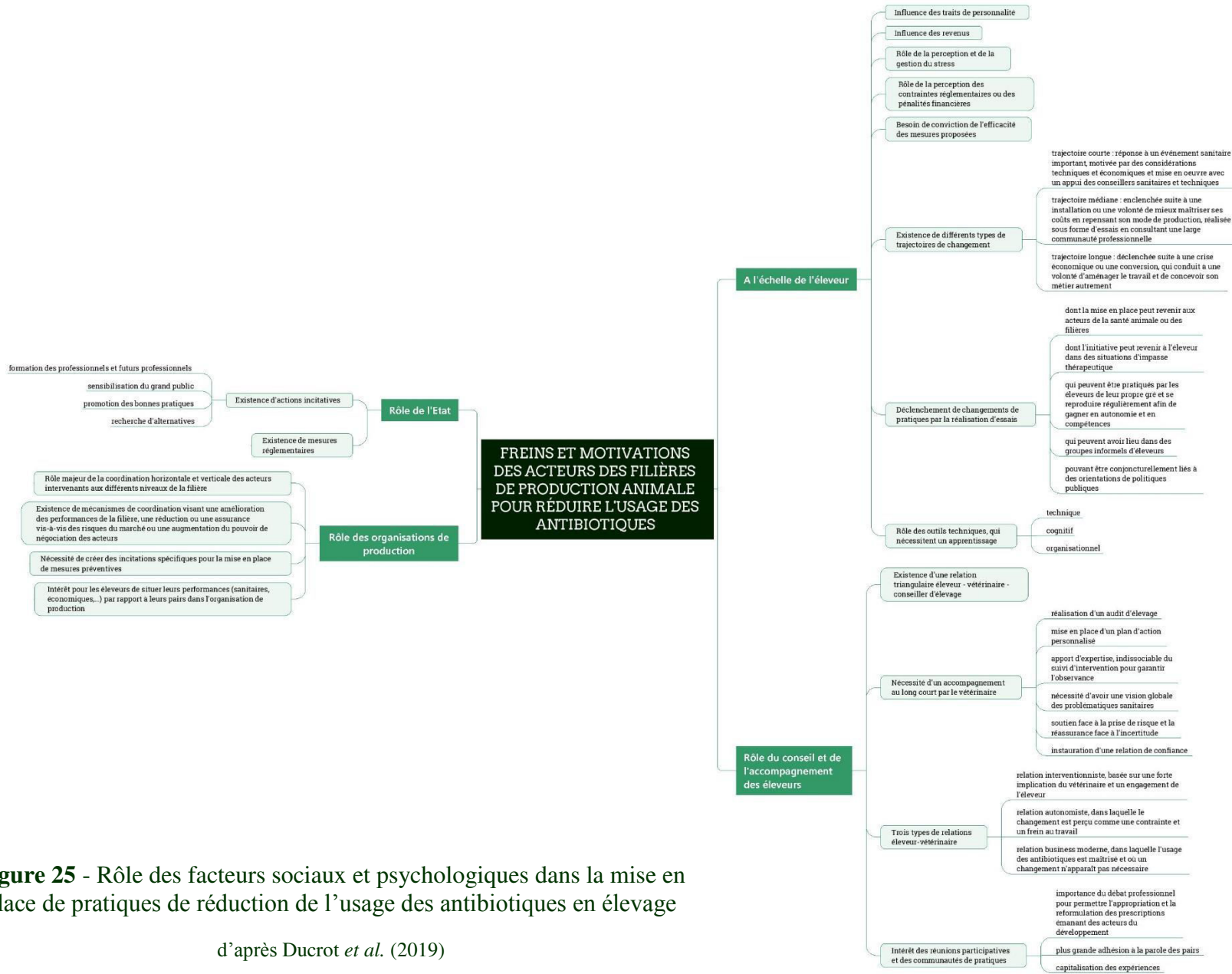


Figure 25 - Rôle des facteurs sociaux et psychologiques dans la mise en place de pratiques de réduction de l'usage des antibiotiques en élevage

d'après Ducrot *et al.* (2019)



Ce qu'il faut retenir

Différentes approches et méthodes peuvent être employées pour explorer la connaissance que les acteurs d'une filière de production animale ont d'une problématique, ainsi que leur capacité et leur volonté de faire évoluer leurs comportements et leurs pratiques pour mettre en place des mesures de maîtrise d'un pathogène dans leur filière.

La lutte contre le HEV étant susceptible de nécessiter des changements de pratiques des différentes parties prenantes de la filière, il apparaît pertinent de conduire de telles études explorant les freins et les motivations des acteurs à adopter de nouvelles pratiques afin de garantir l'élaboration d'un plan de lutte applicable et appliqué sur le terrain.



Take home message

Different approaches and methods can be used to explore the knowledge that actors in an animal production chain have of a problem, as well as their ability and willingness to change their behaviour and practices to implement measures to control a pathogen in their production sector.

As HEV control is likely to require changes in the practices of the various stakeholders in the sector, it seems appropriate to conduct such studies exploring the obstacles and motivations of the actors to adopt new practices in order to ensure the development of a control plan that would be implementable and implemented on the field.

PARTIE 1.3. Stratégie d'approche du projet de recherche

I. Questions de recherche

La présente thèse s'inscrit dans la continuité des précédents travaux de l'Anses sur la thématique du HEV. La question de recherche principale de ce travail peut être formulée de la manière suivante : « **Comment réduire l'exposition humaine au virus de l'hépatite E via la consommation de produits à base de porc ?** ».

Si les étapes « aval » de la chaîne de production sont intégrées à la réflexion générale, la thèse se focalise préférentiellement et de manière quasi-exclusive sur la **phase d'élevage des porcs jusqu'à leur abattage**. Le travail s'ancre ainsi dans l'approche classiquement adoptée en hygiène alimentaire en Europe qui considère la sécurité sanitaire des aliments comme un continuum « de la fourche à la fourchette » : l'objectif est alors de limiter la présence de contaminations (microbiologiques, chimiques, physiques) des animaux vivants pour garantir la production de denrées alimentaires saines.

La question de recherche principale, au caractère finaliste, se décline ainsi en sous-questions plus fondamentales centrées sur l'élevage : **Comment le virus de l'hépatite E se propage-t-il et persiste-t-il dans et entre des élevages de porcs ? Quelles mesures permettent de lutter efficacement contre le virus de l'hépatite E dans la filière porcine ? Sont-elles réalisables sur le terrain ?**

II. Objectifs et volets de la thèse

De ces questions de recherche dérivent des objectifs scientifiques et opérationnels. L'**objectif scientifique général** du travail est ainsi de comprendre les modalités de propagation et de persistance du virus de l'hépatite E **à deux échelles** : d'une part au sein-même d'un élevage de porcs naisseur-engraisseur, d'autre part à l'échelle plus globale de la filière de

production porcine. L'**objectif opérationnel** du projet est de fournir au gestionnaire du risque des éléments d'**aide à la décision pour l'élaboration et la mise en place effective d'un plan de lutte contre le virus de l'hépatite E dans la filière porcine.**

C'est ainsi tout naturellement que la thèse se compose de **trois volets** complémentaires et correspondant aux trois chapitres successifs du présent manuscrit :

Comment expliquer la propagation et la persistance du HEV à l'échelle d'un troupeau ?

Comment expliquer la propagation et la persistance du HEV à l'échelle de la filière porcine ?

Comment sécuriser la filière porcine de manière efficiente ?

Volet 1 : Comment expliquer la propagation et la persistance du HEV à l'échelle d'un troupeau ?

L'objectif de ce bloc de travail est de **comprendre les modalités de la propagation et la persistance du HEV dans un élevage de porcs naisseur-engraisseur et d'identifier les facteurs de variation.** Pour ce faire, plusieurs types d'étude ont été conduits.

D'une part, des **données de terrain** provenant d'un suivi longitudinal de trois élevages de porcs naisseurs-engraisseurs - réalisé en 2011 préalablement à la thèse - ont été analysées.

D'autre part, des **essais expérimentaux** de la transmission du HEV en présence de co-infection avec des virus immunomodulateurs ont été réalisés. Un essai de transmission du HEV lors de co-infection avec le virus du syndrome dysgénésique et respiratoire porcin (SDRP) a été mené en 2014 lors d'un stage de Master 2 préalable à la thèse. Un essai de transmission du HEV lors de co-infection avec le circovirus porcin de type 2 (PCV2) a ensuite été conduit dans le cadre de la thèse.

Enfin, une **approche par modélisation multi-pathogènes** a été développée pour comprendre les conditions de propagation et de persistance du HEV dans un élevage naisseur-engraisseur dont les porcs peuvent être co-infectés par un pathogène intercurrent (SDRP ou PCV2 par exemple). Le **modèle stochastique individu-centré** construit dans ce but couple un

modèle de dynamique de population, permettant de prendre en compte le type de conduite d'élevage, avec un modèle épidémiologique multi-pathogènes décrivant la transmission du HEV en présence du pathogène intercurrent. Les paramètres de ce modèle sont fondés sur les données expérimentales et de terrain préalablement obtenues.

Ce module fait l'objet du chapitre II du présent manuscrit.

Volet 2 : Comment expliquer la propagation et la persistance du HEV à l'échelle de la filière porcine ?

L'objectif est ici de **comprendre les modalités de la propagation et la persistance du HEV dans la filière de production porcine.**

Dans un premier temps, l'ensemble des **mouvements de porcs en France** - entre les élevages et vers les abattoirs - sur la période 2012-2014 a été analysé et modélisé selon deux méthodes : le premier type de modèle est adapté aux pathogènes transmis uniquement par l'introduction d'animaux infectés dans un élevage (en particulier au HEV), tandis que le second réseau correspond aux pathogènes transmis également par voie indirecte lors du passage des camions dans les élevages même sans déchargement d'animaux. Ces deux réseaux ont été étudiés par des méthodes d'**analyse de réseaux sociaux** (statistiques descriptives, recherches de composants connectés et de communautés, analyse temporelle).

Ensuite, des **applications exploratoires au cas du HEV** ont été entreprises. Ainsi, des données épidémiologiques ont été couplées avec les données de mouvement précitées afin d'évaluer l'impact des mouvements de porcs sur la prévalence du HEV ainsi que le risque d'exposition des départements français au HEV en fonction de l'importance et de la provenance de leurs échanges.

Enfin, le modèle intra-troupeau développé dans le volet 1 a été couplé avec les données de mouvements préalablement analysées dans le but de construire un **modèle inter-troupeau** représentant la persistance et la propagation du HEV à l'échelle de la filière porcine. Cette partie a été réalisée lors d'un **séjour de travail à l'étranger** (*Swedish National Veterinary Institute - SVA, Uppsala, Suède*).

Ce volet fait l'objet du chapitre III du présent manuscrit.

Volet 3 : Comment sécuriser la filière porcine de manière efficiente ?

L'objectif de ce dernier axe est de **proposer un programme de lutte** pour réduire la prévalence du HEV en élevage porcine et le risque de mise sur le marché de produits contaminés.

La première étape a consisté en la **synthèse des mesures de lutte** proposées à partir des modèles développés dans les volets 1 et 2, notamment en ce qui concerne les pratiques d'élevage et la gestion des pathogènes intercurrents. A ce stade, un **rapport d'aide à la décision** a été rédigé à l'attention des gestionnaires du risque pour la sécurisation de la filière vis-à-vis du HEV.

La seconde étape avait pour but d'étudier la **faisabilité de ce programme de lutte** contre le HEV en termes de mise en place pratique sur le terrain et d'acceptabilité par les acteurs de la filière. Pour ce faire, après une **enquête préliminaire** permettant de dresser un état des lieux des connaissances que les acteurs de la filière avaient du HEV, une **enquête qualitative** a été conduite auprès de ces mêmes acteurs. Cette deuxième enquête a donné lieu à l'**encadrement d'un stage de Master 2**. Une **réunion de concertation** des organisations publiques et privées en charge de la gestion du risque HEV a également été organisée.

Ce volet fait l'objet du chapitre IV du présent manuscrit.

Au global, la stratégie d'approche de la question de recherche est **multi-pathogènes, multi-échelles, et multidisciplinaire**. Le contexte, les questions de recherche et les volets de ce travail de recherche sont synthétisés de manière schématique dans la Figure 26 et la Figure 27.

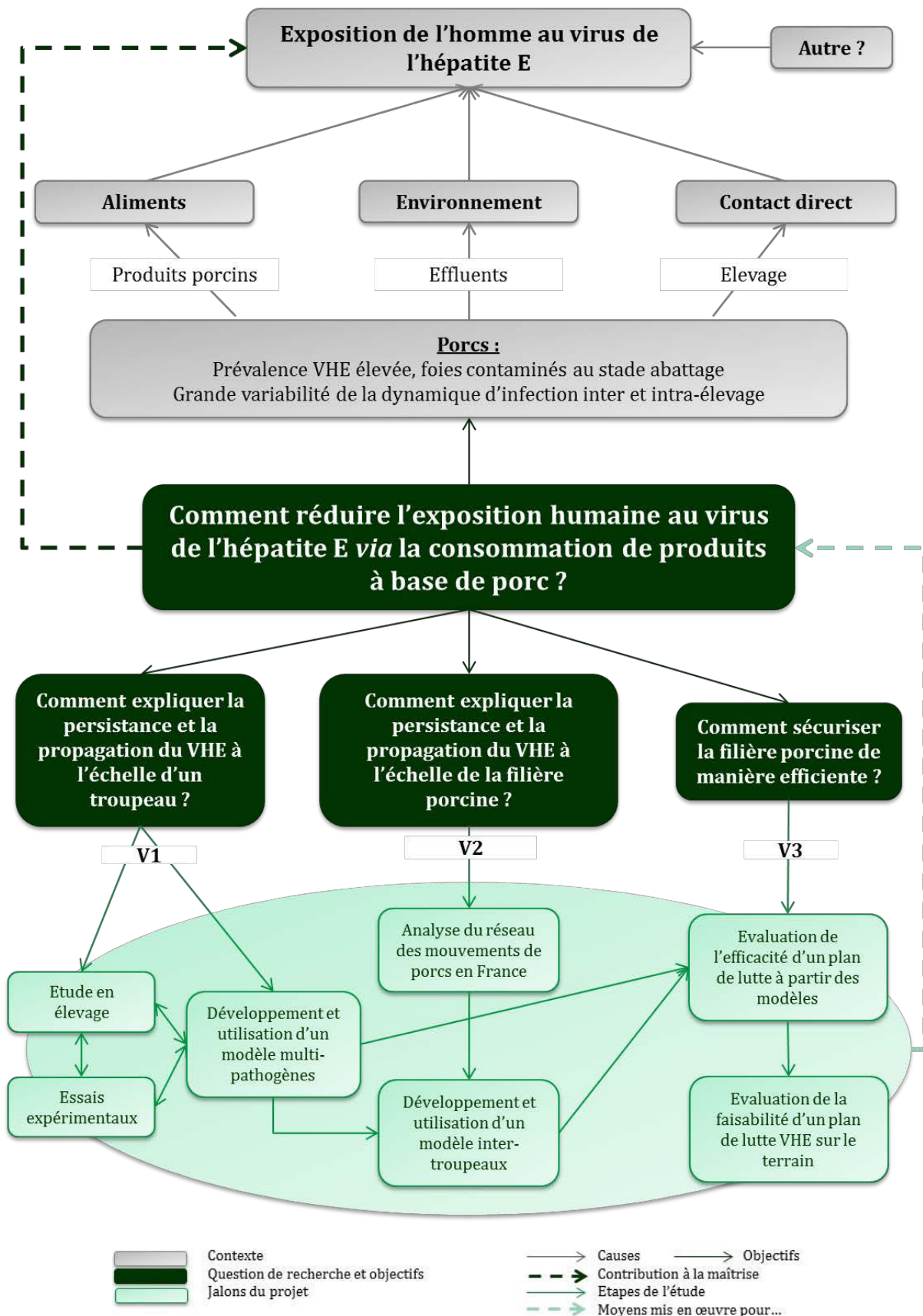


Figure 26 - Contexte, questions de recherche et volets de la thèse

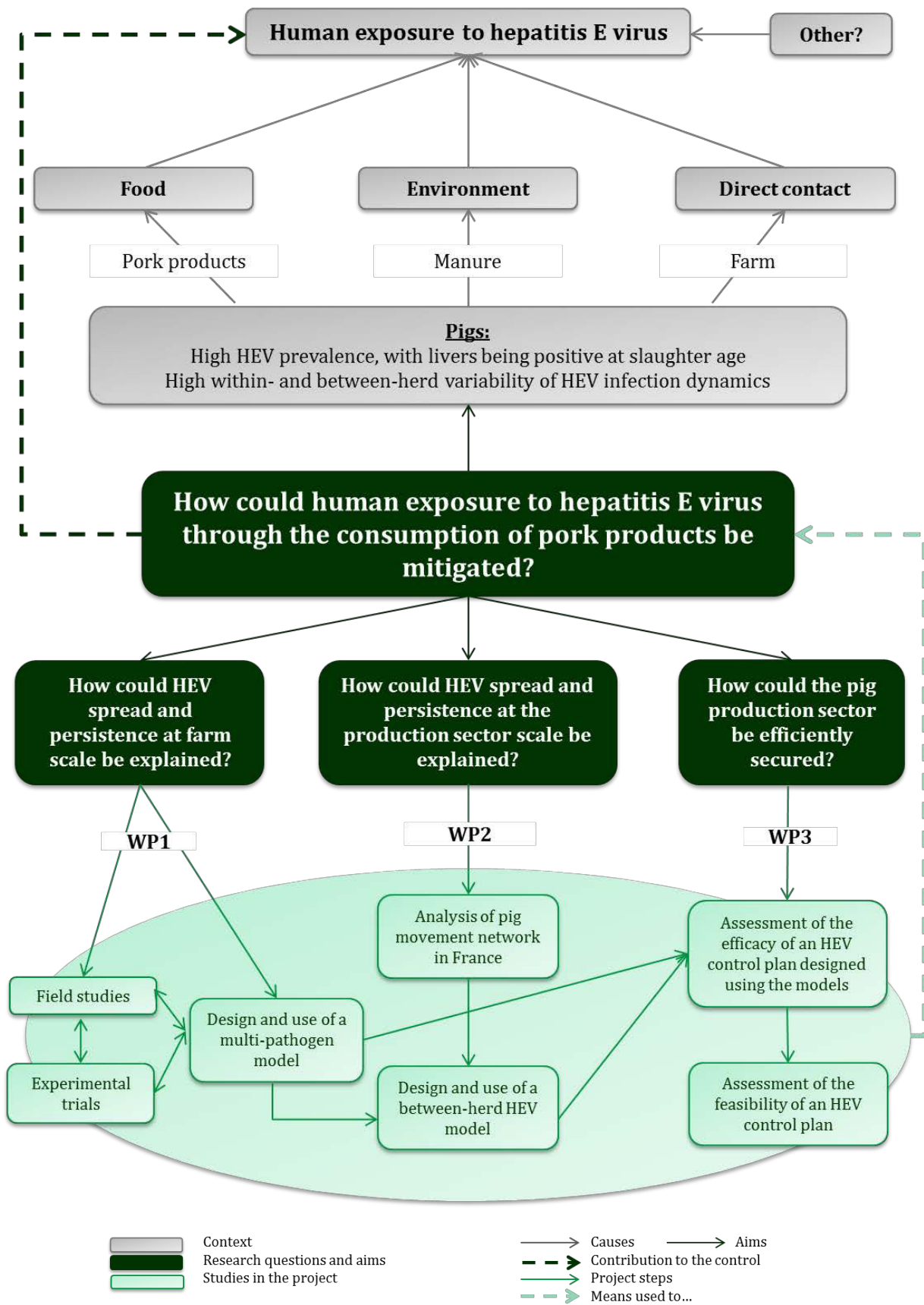


Figure 27 - Context, research questions and work packages of the thesis

The image features a solid green background. In the upper right quadrant, there is a white geometric shape that resembles a 3D rectangular prism or a cutaway view of a structure. This shape is composed of several parallel white lines that create a sense of depth and perspective. The lines are arranged in a way that suggests a three-dimensional volume, with some lines receding into the distance. The overall composition is clean and modern, with a focus on geometric forms and color contrast.

Chapitre II

**Etude de la propagation et de la
persistance du virus de l'hépatite E dans
un élevage de porcs naisseur-engraisseur**

PARTIE 2.1. Le terrain comme support de la modélisation : d'une étude observationnelle en élevage aux essais expérimentaux

I. Etude de la dynamique de l'infection par le virus de l'hépatite E chez le porc et de ses facteurs de variation en conditions naturelles

La dynamique de l'infection par le HEV chez le porc conditionne directement la probabilité que le foie contienne des particules virales à l'abattage, c'est-à-dire qu'il présente un risque pour la santé publique. Une **grande variabilité de la dynamique infectieuse** est décrite dans la littérature (Salines *et al.*, 2017a) et n'est que **partiellement expliquée** à ce jour. En effet, si des facteurs de risque ont été identifiés à l'échelle de l'élevage, notamment en ce qui concerne la structure de l'élevage et les pratiques d'élevage, d'hygiène et de biosécurité (Di Bartolo *et al.*, 2008; Li *et al.*, 2009a; Jinshan *et al.*, 2010; Hinjoy *et al.*, 2013; Rutjes *et al.*, 2014; Walachowski *et al.*, 2014; Lopez-Lopez *et al.*, 2018), peu d'études ont décrit les variations des profils individuels d'infection par le HEV (de Deus *et al.*, 2008; Casas *et al.*, 2011a; Feng *et al.*, 2011), et encore moins se sont intéressées aux facteurs pouvant expliquer ces variations (Andraud *et al.*, 2014). De plus, à l'instar des hépatites E chroniques décrites chez des patients humains immunodéprimés, il est possible que des **pathogènes immunomodulateurs porcins**, comme le virus du syndrome dysgénésique et respiratoire porcin (SDRP) ou le circovirus porcin de type 2 (PCV2) – qui sont fortement prévalents dans la filière de production porcine et affectent à la fois la réponse immunitaire innée et adaptative du porc – influencent la dynamique de l'infection par le HEV chez le porc.

C'est dans ce contexte qu'un **suivi longitudinal de trois élevages porcins naisseurs-engraisseurs** a été réalisé. Le premier objectif de cette étude observationnelle était de **décrire, à partir de données individuelles, les profils d'infection par le HEV**. En parallèle, l'étude a permis d'évaluer (*i*) l'influence de **caractéristiques individuelles des porcelets** ou de

spécificités liées aux portées, (ii) le rôle de l'immunité anti-HEV, (iii) ainsi que l'impact de co-infections avec le virus du SDRP et/ou le PCV2 sur la dynamique de l'infection par le HEV chez le porc. Cette étude a été publiée dans le journal *Transboundary and Emerging Diseases* (Salines *et al.*, 2019c).

Publication 2

Salines M., Dumarest M., Andraud M., Mahé S., Barnaud E., Cineux M., Eveno E., Eono F., Dorenlor V., Grasland B., Bourry O., Pavio N., Rose N., 2019. Natural viral co-infections in pig herds affect hepatitis E virus (HEV) infection dynamics and increase the risk of contaminated livers at slaughter. *Transboundary and Emerging Diseases*, doi.org/10.1111/tbed.13224.

Natural viral co-infections in pig herds affect hepatitis E virus (HEV) infection dynamics and increase the risk of contaminated livers at slaughter

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Abstract

Hepatitis E virus (HEV) is a zoonotic pathogen, in particular genotype 3 HEV is mainly transmitted to humans through the consumption of contaminated pork products. This study aimed at describing HEV infection patterns in pig farms and at assessing the impact of immunomodulating co-infections namely Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Porcine Circovirus Type 2 (PCV2), as well as other individual factors such as piglets' immunity and litters' characteristics on HEV dynamics. A longitudinal follow-up was conducted in three farrow-to-finish farms known to be HEV infected. Overall, 360 piglets were individually monitored from birth to slaughter with regular blood and faecal sampling as well as blood and liver samples collected at slaughterhouse. Virological and serological analyses were performed to detect HEV, PCV2 and PRRSV genome and antibodies. The links between 12 explanatory variables and four outcomes describing HEV dynamics were assessed using cox-proportional hazard models and logistic regression. HEV infection dynamics was found highly variable between farms and in a lower magnitude between batches. HEV positive livers were more likely related to short time-intervals between HEV infection and slaughter time (<40 days, OR = 4.1 [3.7–4.5]). In addition to an influence of piglets' sex and sows' parity, the sequence of co-infections was strongly associated with different HEV dynamics: a PRRSV or PCV2/PRRSV pre- or co-infection was associated with a higher age at HEV shedding (Hazard Ratio = 0.3 [0.2–0.5]), as well as a higher age at HEV seroconversion (HR = 0.5 [0.3–0.9] and HR = 0.4 [0.2–0.7] respectively). A PCV2/PRRSV pre- or co-infection was associated with a longer duration of shedding (HR = 0.5 [0.3–0.8]). Consequently, a PRRSV or PCV2/PRRSV pre- or co-infection was strongly associated with a higher risk of having positive livers at slaughter (OR = 4.1 [1.9–8.9] and OR = 6.5 [3.2–13.2] respectively). In conclusion, co-infections with immunomodulating viruses were found to affect HEV dynamics in the farrow-to-finish pig farms that were followed in this study.

KEYWORDS

hepatitis E virus, infection dynamics, PCV2, PRRSV, public health, risk factors

1 | INTRODUCTION

Hepatitis E virus (HEV) is a non-enveloped single-stranded RNA virus causing acute and occasionally chronic hepatitis in humans (Emerson & Purcell, 2003; Kamar et al., 2011). In industrialized countries, hepatitis E cases are mainly related to genotype 3 and 4 (HEV-3 and HEV-4) which are shared between humans and other animal species (Dalton, Bendall, Ijaz, & Banks, 2008; Purcell & Emerson, 2008). In Europe, HEV-3 is particularly prevalent in the pig population (Rose et al., 2011), with swine and human HEV strains being genetically very close (Bouquet et al., 2011; Meng et al., 1998). Moreover, some autochthonous cases in industrialized countries have been related to the consumption of raw or undercooked pork products, especially those containing liver (Colson et al., 2010; Moal, Gerolami, & Colson, 2012; Motte et al., 2012). Thus, hepatitis E is now considered as a foodborne zoonosis with domestic pigs recognized as one of the main reservoirs in developed countries (Dalton et al., 2008; Pavio, Meng, & Renou, 2010). The epidemiology of HEV in the pig-farming sector is far from being fully elucidated (Salines, Andraud, & Rose, 2017). Comparing outcomes of prevalence and seroprevalence studies evidences great variability between countries (Salines et al., 2017). Within a same study in a given country, the individual and farm-scale prevalence figures may also vary greatly (Rose et al., 2011). Within-farm and between-farm variability has been explored in several studies. For instance, de Deus et al. (2008), Feng et al. (2011) and Casas et al. (2011) individually followed a 45, 32 and 120 piglet sample from one Spanish, one Chinese and six Spanish farrow-to-finish farms respectively. They highlighted a great individual variability in ages at HEV shedding and immunological profiles. This heterogeneity may reflect a wide range of infection dynamics related to farm- or individual-specific risk factors which have only been sporadically explored to date. Farm-level observational studies have highlighted husbandry practices in terms of hygiene, biosecurity and rearing conditions as pivotal factors favouring HEV spread on farms (e.g. farm size, mingling practices, origin of drinking water, presence of a hygiene lock) (Hinjoy et al., 2013; Jinshan, Manglai, Takahashi, Nagashima, & Okamoto, 2010; Li et al., 2009; Walachowski et al., 2014). Between-farm pig movements and the contact network topology have also been found to influence the epidemiological HEV situation of farms (Salines, Andraud, & Rose, 2018). However, individual risk factors related to piglets' specific characteristics (e.g. gender) or inherited from their dam (e.g. litter characteristics such as number of mummified, live-born or weaned piglets, parity rank of the dam, maternal immunity) have not been investigated to date. Using mathematical modelling based either on experimental trials or on field studies revealed new insights on HEV infection dynamics (Andraud, Casas, Pavio, & Rose, 2014; Andraud et al., 2013; Salines et al., 2015). As such, a partial protection conferred by maternally derived antibodies (MDAs) was shown to delay HEV infection in growing pigs (Andraud et al., 2014). Immunomodulating swine pathogens, that are widespread in the pig population, may also affect HEV infection dynamics. PRRSV

(Porcine Reproductive and Respiratory Syndrome Virus) was previously demonstrated to have a suppressive effect on the antiviral innate immunity by inhibiting the IFN- α response (Albina, Carrat, & Charley, 1998; Van Reeth, Labarque, Nauwynck, & Pensaert, 1999). Besides, as suggested by Loving, Osorio, Murtaugh, and Zuckermann (2015), this decreased IFN- α response could be involved in the delayed and low specific immune response characterizing PRRSV infection. Nonetheless, the immunosuppressive potential of PRRSV and its facilitating role for other viral/bacterial co-infection is still debated (Rahe & Murtaugh, 2017). Some compelling studies have shown yet that PRRSV infection could alter the immune response to viral infection or vaccination (Van Reeth, Nauwynck, & Pensaert, 2001; Suradhat et al., 2006). More specifically, PRRSV co-infection is likely to lead to chronic HEV infection (Salines et al., 2015), with apparently extended latency and infectious period, increased HEV faecal shedding and impaired humoral immune response. Another swine virus, the porcine circovirus of type 2 (PCV2), is known to modulate the immune response as well. PCV2 can cause PCV2- systemic disease also named post-weaning multisystemic wasting syndrome, which leads to severe B and T lymphocyte depletion in blood and lymphoid tissues (Kekarainen & Segales, 2015). PCV2 DNA genome is able to inhibit the production of IFN- α by stimulated plasmacytoid dendritic cells (Vincent et al., 2007). Some CpG motifs in the PCV2 genome have been shown to also inhibit the production of IFN- α by porcine peripheral blood mononuclear cells in vitro (Wikstrom, Fossum, Fuxler, Kruse, & Lovgren, 2011; Wikstrom et al., 2007). PCV2 also modulates the expression of another cytokine, the immunosuppressive interleukine 10 (IL-10) by increasing its production in vitro and in vivo (Darwich et al., 2003, 2008; Fort et al., 2010; Kekarainen, Montoya, Mateu, & Segales, 2008). This IL-10 under-expression may be due to the interaction between the capsid protein of PCV2 and gC1qR protein (also named p32, HABP, C1qBP) that is a membrane receptor of the C1q component of the complement system. This has been demonstrated in lung alveolar macrophages after PCV2 infection (Du et al., 2016). Thus, as PCV2 induces the production of IL-10 that is a cytokine affecting innate and adaptive immune response, PCV2 infection in pigs may affect HEV infection. However, to date, only few data report on HEV/PCV2 co-infection (Jackel et al., 2018; Martin et al., 2007; Savic et al., 2010). In these studies, PCV2 and HEV were simultaneously detected in pigs but no direct correlation between the two infections could be evidenced.

Given the risk HEV represents to public health, it is necessary to fully understand the conditions related to HEV transmission dynamics within an infected pig farm in order to mitigate the risk of introducing contaminated products into the pork chain. A longitudinal follow-up was therefore conducted in three French pig farms known to be HEV infected so as to describe the within herd HEV infection patterns at the individual pig level and to assess the impact of co-infections with PRRSV and/or PCV2, anti-HEV immunity and litters' and individual piglets' characteristics on HEV infection dynamics.

2 | MATERIAL AND METHODS

2.1 | Study design

2.1.1 | Ethical statement

This study was carried out in strict accordance with the guidelines of the Good Experimental Practices (GEP) standard dictated by the European Union. The study was conducted in accordance with the recommended procedure of the Anses/ENVA/UPEC (French Agency for Food, Environmental and Occupational Health and Safety/Ecole Nationale Vétérinaire d'Alfort/Université Paris Est Créteil) ethical committee (agreement #16 to the National committee for ethics in animal experimentation). ANSES-Ploufragan is approved for animal experimentation and is registered under certification number C-22-745-1 delivered by the official French veterinary services.

2.1.2 | Cohort study

A longitudinal follow-up study was conducted in three farrow-to-finish pig farms located in Brittany region (North-Western part of France) and followed over the 2011–2012 period. These farms were selected to be farrow-to-finish pig farms with a majority of growing pigs reared on site, and were previously identified as HEV infected in a national prevalence and seroprevalence survey (Rose et al., 2011). Before starting the study and to ensure that HEV has been circulating since the prevalence study, the HEV status of three farms was checked at the slaughterhouse by randomly sampling 20 pigs per batch at the slaughter line (blood and liver) with three repetitions for each farm. The serological positive results confirmed the HEV positivity of the farms (File S1). The three farms were also known to be PRRSV and PCV2 positive, but a PCV2 vaccination programme was implemented in growing pigs from Farm 2 using an inactivated vaccine based on a PCV2 strain belonging to PCV2a genogroup. Sows were vaccinated against PRRSV in the three farms using a modified live vaccine, the vaccination schedule being the same in the three farms (booster vaccination 20 days post-farrowing on average). No PRRSV vaccination was implemented in growing pigs from any farm under study. In these three farms, three successive pig batches were followed, a batch being defined as a group of contemporary piglets in the same physiological stage. Farm 1 had 310 sows, conducted in a 5-week management system (leading to 900 piglet batches, approximately), farm 2 had 230 sows conducted in a 3-week management system (leading to 500 piglet batches) and farm 3 had 218 sows conducted in a 3-week management system (leading to 300 piglet batches). In each batch, a representative sample of 10 sows was randomly selected stratifying on parity (gilts, parities 1–2, 3–4 and 5 or more). At farrowing, all the piglets from the selected sows were identified and four piglets per litter were randomly selected to be ear-tagged and tattooed, leading to a cohort of 40 piglets per batch. This sample size per batch enabled the detection of the infection at each sampling time at a prevalence threshold of 7% with

95% confidence. This selection process resulted in a sample of 120 piglets monitored per farm and overall 360 pigs were individually followed from birth to slaughter. Selected piglets could not be cross-fostered and remained with their native dam until weaning; this ensured they received only colostrum from their dam. Cross-fostering was allowed for the other littermates. The monitored piglets were reared with other piglets in the batch and subjected to the same practices as other piglets in the farm after weaning. Individual blood and rectal faecal swab samples were taken at 1, 6, 10, 14, 18 and 22 weeks of age. Faecal swab samples were kept frozen (–80°C) until use. Blood samples were also taken from the related dams one week after farrowing to assess the transfer of maternal antibodies to the piglets through colostrum. Blood samples were collected by jugular vein puncture, using evacuated tubes (Vacuette, Dutscher SAS) without additive. Serum was obtained by centrifugation of blood samples for 10 min at 3,500 g and stored at –20°C until subsequent analysis. At slaughterhouse, blood and liver samples were collected on these same pigs.

2.1.3 | Virological and serological analyses

HEV RNA extraction and quantification were performed on faeces and liver using real-time quantitative RT-PCR as described in Barnaud, Rogée, Garry, Rose, and Pavio (2012). Results were expressed in terms of Cycle threshold (Ct). The detection of anti-HEV antibodies in serum was performed using the HEV ELISA 4.0v kit (MP Diagnostics) according to the manufacturer's instructions. This ELISA test detects all classes of anti-HEV antibodies including IgG and IgM with a specificity of 98.8% (Hu et al., 2008). Samples were positive when the optical density (OD) at 450 nm wavelength obtained for the sample was higher than the threshold defined as the mean for negative controls +0.3.

The detection of anti-N-PRRSV antibodies was performed using PRRS X3 Ab ELISA tests (IDEXX Laboratories) according to the manufacturer's instructions. Results were expressed as sample to positive control (S/P) optical density ratios. A sample was considered positive when the S/P ratio was equal or higher than 0.4.

PCV2 DNA extraction and quantification were performed on serum using real-time PCR based on TaqMan technology as described in (Grasland et al., 2005). Results were expressed in genomic copy number per millilitre of serum (ge/mL).

2.2 | Statistical analyses

2.2.1 | Outcome definitions

For each of the 360 followed pigs, four outcome variables were defined:

- The estimated age at HEV shedding, calculated as the age at first positive faecal sample minus 7 days, to take sampling intervals into account.

- The estimated duration of HEV shedding period, calculated as the interval between the first and the last positive faecal samples, plus 14 days to take sampling intervals into account.
- The estimated age at HEV seroconversion of pigs having shed HEV, derived from the individual antibodies' kinetics fitted with cubic splines (Green & Silverman, 1994). The age at seroconversion was calculated as the age when the cubic spline crossed the threshold on the upward slope. For the particular cases of animals being seropositive all over the study period, the age at seroconversion was defined as the age at lowest OD.
- The HEV status of the liver (positive or negative) at time of slaughter.

2.2.2 | Explanatory variables

Thirteen explanatory variables were considered to be compared with the outcomes:

Individual piglet's and litter's characteristics

(i) piglet's sex (one should note that pigs were not housed separately depending on their gender, and that male were castrated); (ii) sow's parity (note that a sow that had never delivered piglets at the time of inclusion in the study was attributed a parity of 0); (iii) number of

stillborn piglets in the litter; (iv) number of mummified piglets in the litter; (v) number of live-born piglets in the litter; (vi) number of ingoing piglets into the litter; (vii) number of outgoing piglets from the litter; (viii) number of weaned piglets in the litter; (ix) age at slaughter and time interval between infection and slaughter. Continuous variables were categorized according to their distributions (mean, median or other quantiles depending on the shape of the distribution), making sure that categories contained at least 10% of the whole sample.

Anti-HEV serological status

(i) anti-HEV piglet's antibody status at first week of age, categorized as absent (OD < threshold) or present (OD > threshold); (ii) anti-HEV sow's immunity one week after farrowing, categorized as absent (OD < threshold) or present (OD > threshold).

Co-infections with viruses

- Exposure to co-infecting pathogens: pig's status regarding PCV2 and PRRSV was recorded and pigs were categorized as PCV2 infected, PRRSV infected, or PCV2 and PRRSV infected, whatever the order of the infections.
- Sequence of co-infections: First, ages at HEV/PCV2/PRRSV infection were calculated as followed: (1) pig's age at HEV infection was calculated as the age at HEV shedding minus 14 days,

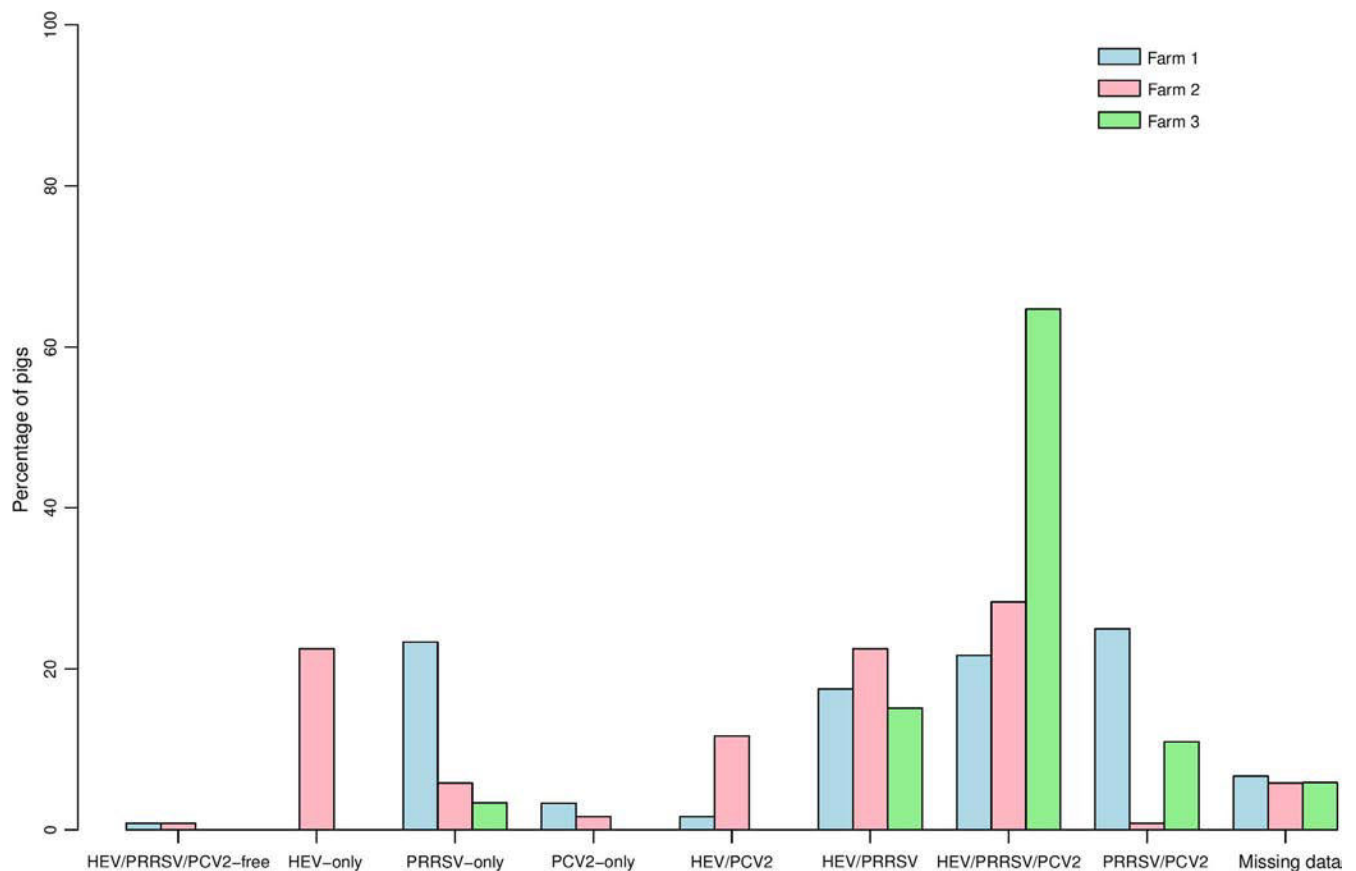


FIGURE 1 Cumulative incidence of HEV, PRRSV and/or PCV2 infections in the three French farrow-to-finish pig farms (3 batches per farm, $n = 360$ pigs)

corresponding to HEV latency period as described in Salines et al. (2015); (2) pig's age at PCV2 infection was calculated as the age at first PCV2 positive PCR minus 7 days, as described in literature (Andraud et al., 2008, 2009); (3) similarly to the calculation of the age at HEV seroconversion, the age at PRRSV seroconversion was derived from the individual antibodies' kinetics fitted with cubic splines; then, pig's age at PRRSV infection was calculated as the age at PRRSV seroconversion minus 7 days, as described in literature (Diaz, Darwich, Pappaterra, Pujols, & Mateu, 2005). Then, the sequence of infections was computed and four possible statuses were attributed to piglets: (i) infected by HEV first; (ii) infected by PCV2 first (i.e. PCV2 pre-infection) or during the HEV infection (considered as a PCV2 co-infection); (iii) infected by PRRSV first (i.e. PRRSV pre-infection) or during the HEV infection (considered as a PRRSV co-infection); (iv) pre-co-infected both by PCV2 and PRRSV, whatever the order.

2.2.3 | Statistical models

Observed data of the age at HEV shedding, the shedding duration and the age at HEV seroconversion were fitted to different distributions (Weibull, lognormal and exponential) using the function 'fitdistcens' of the R package 'fitdistrplus'; the quality of fit was then evaluated using the Akaike Information Criterion (AIC) as described in Delignette-Muller and Dutang (2015). Finally, the average age at

HEV shedding, shedding duration and age at HEV seroconversion were estimated using parametric survival regression with the previously selected distribution.

Cox-proportional hazard models were built to explore the link between (i) the explanatory variables and the age at HEV shedding; (ii) the explanatory variables and the age at HEV seroconversion; (iii) the explanatory variables and the duration of HEV shedding period. For this third model, the age at HEV shedding was included as a supplementary explanatory variable to account for the possible confounding effect of the age at HEV infection on the duration of HEV shedding. The influence of 'farm' and 'batch' variables on these three outcomes was also tested in cox-proportional hazard models and it appeared that 'farm' influenced the outcomes more than 'batch' (p -value < 0.0001 vs. >0.01 respectively). Thus, the 'farm' variable was included in the three models as a frailty effect (Proc PHREG in SAS (2014)) to account for non-independence of piglets within farms. The proportional hazard assumption of the Cox model were checked by (i) plotting the survival curves (Kaplan Meier estimate) and checking that they were not crossing; (ii) plotting the Log(-log SDF) versus time to check graphically and (iii) computing the Schoenfeld residuals to be plotted versus time.

A generalized estimating equation (GEE) logistic regression was performed to assess the link between the explanatory variables and the probability of livers being HEV positive at slaughterhouse. Again, the variability related to the 'farm' variable was greater than for the 'batch' variable. The 'farm' variable was therefore included

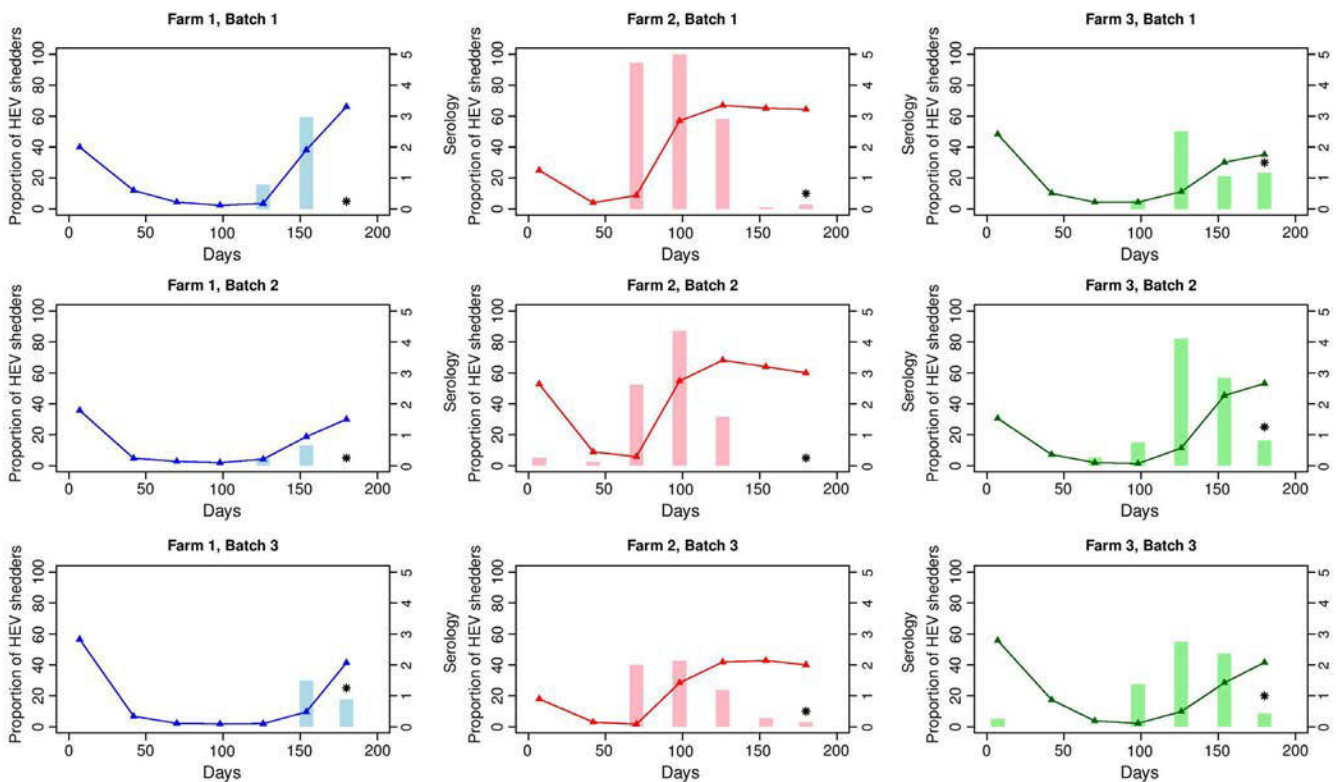


FIGURE 2 HEV course of infection in the three French farrow-to-finish pig farms (3 batches per farm, $n = 360$ pigs). Proportion of HEV shedders (bars) and HEV ELISA average optical density (lines) at 7, 42, 70, 98, 126, 154 and 180 days of age. The bar and star at 180 days stand for the proportion of HEV positive livers at slaughterhouse

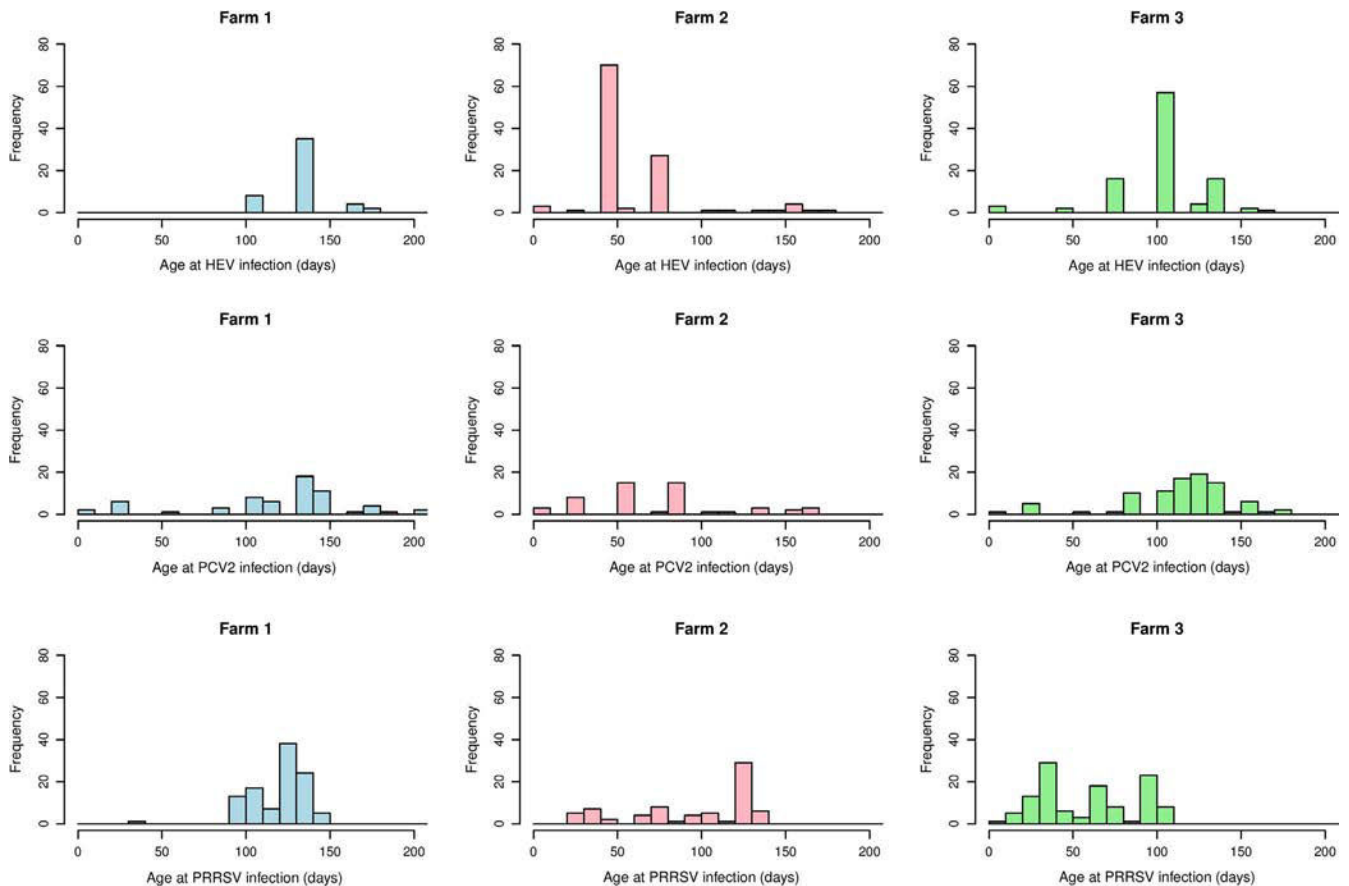


FIGURE 3 Distribution of the ages at HEV, PRRSV and PCV2 infection derived from a longitudinal follow-up in three French farrow-to-finish pig farms (3 batches per farm, $n = 360$ pigs)

as repeated statement (Proc GENMOD in SAS). These four models were all built as followed: first, a univariate analysis was conducted. All variables having a significant effect at univariate step ($p < 0.20$) were selected for a bivariate analysis aiming to remove too highly correlated variables. If variables did not show strong collinearity ($p > 0.05$), they were included in a multivariate model. A backward procedure was then applied until all remaining variables in the final model were significantly related to the outcome ($p < 0.05$).

3 | RESULTS

3.1 | HEV, PCV2 and PRRSV infection profile of the three farms

3.1.1 | Exposure to and sequence of infections

In the nine followed batches, 69.4% [95% Confidence Interval 64.4–74.2], 60.4% [55.0–65.4] and 84.1% [80.0–87.8] of pigs were found infected by HEV, PCV2 and PRRSV respectively (cumulative incidence of each virus), based on virological (HEV, PCV2) and serological (PRRSV) data (Figure 1). Co-infections were frequent, with variable proportions of co-infection cases depending on the farm. For instance HEV-only infection was only found in Farm 2

(representing 8.6% [5.9–12.0] of the 360 followed pigs and 22.5% [15.4–31.0] of the 120 piglets in Farm 2). Triple infections (i.e. detection of the three viruses or antibodies over a pig's life) were the most frequently encountered situation (53.7% [48.3–58.9] of the 360 pigs), especially in Farm 3 (64.7% [54.9–72.7] vs. 21.7% [14.7–30.1] and 28.3% [20.5–37.3] in Farms 1 and 2 respectively). Double infection cases were mainly PRRSV/HEV co-infections (e.g. 22.5% [15.4–31.0] of pigs in Farm 2). More precisely, 15.9% [12.2–20.0] of pigs were first infected by HEV, whereas 11.8% [8.5–15.4], 32.5% [27.7–37.6] and 39.8% [34.6–45.0] had pre- or co-infections with PCV2, PRRSV or both PCV2 and PRRSV respectively.

3.1.2 | HEV infection dynamics

The three studied farms exhibited variable HEV infection profiles (Figure 2). There were also differences between batches within a farm but to a less extent with more consistent patterns. HEV faecal shedding profiles differed greatly between farms i.e. late shedding in Farm 1 versus early shedding in Farm 2, with shedders as early as lactating period. Within-batch spread was also variable depending on farms, leading to heterogeneous prevalence figures: for instance up to 100% of pigs shed HEV in Farm 2, versus 60% in Farm 1 at most (Figure 2). The highest proportion of positive livers at slaughter was reached in Farm

TABLE 1 Effect of co-infections, immunity and litter characteristics on the age at HEV shedding (3 farrow-to-finish pigs farms, 3 batches per farm, $n = 360$ pigs)

Variable	Category	n	Univariate model		Multivariate model	
			Hazard Ratio [95%CI]	p-value	Hazard Ratio [95%CI]	p-value
Exposure to co-infecting pathogens			Likelihood-ratio Chi ² = 8.59	0.035**		
	None	29	-	-		
	PCV2	22	0.63 [0.34–1.17]	0.14		
	PRRSV	105	0.68 [0.42–1.10]	0.11		
	PRRSV and PCV2	181	1.03 [0.64–1.68]	0.89		
Temporal order of co-infections			Chi ² = 43.44	<0.01***	Chi ² = 25.52	<0.01***
	HEV first	54	-	-	-	-
	PCV2 pre- or co-infection	40	0.56 [0.35–0.87]	0.011	0.64 [0.41–1.01]	0.056
	PRRSV pre- or co-infection	110	0.21 [0.13–0.34]	<0.01	0.28 [0.17–0.47]	<0.01
	PRRSV and PCV2 pre- or co-infection	135	0.20 [0.12–0.33]	<0.01	0.26 [0.15–0.46]	<0.01
Piglet's HEV serology (1 week of age)			Chi ² = 0.01	0.92		
	Negative	114	-	-		
	Positive	243	0.99 [0.75–1.29]	0.92		
Sow's HEV serology one week after farrowing			Chi ² = 0.022	0.88		
	Negative	108	-	-		
	Positive	251	1.02 [0.78–1.34]	0.88		
Sex			Chi ² = 1.93	0.16*		
	Female	172	-	-		
	Male	187	1.20 [0.93–1.54]	0.16		
Sow's parity			Chi ² = 32.52	<0.01***	Chi ² = 21.85	<0.01***
	0–1	104	-	-	-	-
	2–3	88	1.22 [0.88–1.68]	0.23	1.36 [0.99–1.88]	0.059
	>4	167	0.51 [0.37–0.70]	<0.01	0.62 [0.44–0.85]	<0.01
Cross-fostering: number of ingoing piglets into the litter			Chi ² = 2.42	0.30		
	0–1	207	-	-		
	2–5	84	1.32 [0.93–1.87]	0.12		
	6–12	68	1.22 [0.77–1.94]	0.39		
Cross-fostering: number of outgoing piglets from the litter			Chi ² = 3.22	0.20*		
	0–4	260	-	-		
	5–7	67	0.71 [0.46–1.07]	0.10		
	8–12	32	0.66 [0.33–1.33]	0.25		
Number of weaned piglets in the litter			Chi ² = 4.34	0.11*		
	7–10	83	-	-		
	11–12	200	1.06 [0.77–1.44]	0.73		
	13–14	76	1.49 [0.98–2.25]	0.06		
Number of liveborn piglets in the litter			Chi ² = 10.72	<0.01***		
	0–11	68	-	-		
	12–14	112	0.67 [0.48–0.95]	0.03		
	15–18	179	0.58 [0.41–0.80]	0.001		

(Continues)

TABLE 1 (Continued)

Variable	Category	n	Univariate model		Multivariate model	
			Hazard Ratio [95%CI]	p-value	Hazard Ratio [95%CI]	p-value
Number of stillborn piglets in the litter	0-1	267	-	-		
	2-6	92	0.91 [0.68-1.21]	0.50		
Number of mummified piglets in the litter	0	247	-	-		
	1-2	112	0.77 [0.59-1.02]	0.069		

Note: Summary statistics as obtained thanks to a cox-proportional hazard model with the 'farm' effect being included as a frailty effect. Shaded areas represent variables that were not retained in the multivariate model.

*** $p < 0.01$.

** $p < 0.05$.

* $p < 0.20$.

3 with more than 24% [0.11-0.42] of positive livers in the first batch (Figure 2), consistently with what was observed at the selection phase (File S1). This was associated with a late shedding peak, reached after 120 days of age, and a high proportion of HEV shedding pigs (Figure 2).

More precisely, the average age at HEV shedding was estimated to 124.5 days [95% CI 106.4-144.3], 92.9 days [84.4-102.3] and 137.0 days [130.3-146.1] in Farm 1, 2 and 3, respectively, with Weibull distributions (File S2). The average duration of HEV shedding at the pig level was estimated to 16.2 days [95% CI 14.6-17.9], 35.5 days [31.2-40.5] and 25.9 [22.4-30.1] in Farm 1, 2 and 3, respectively, with lognormal distributions (File S2).

3.1.3 | Ages at PCV2 and PRRSV infection and comparison with HEV dynamics

The infection profiles regarding the two co-infecting pathogens also differed greatly (Figure 3, Files S3 and S4). In addition to different ages at infection, the cumulated prevalence of PCV2 viraemic pigs was found higher in Farm 3 than in Farms 1 and 2 (up to 60% vs. 40% and 35% respectively), the lowest being observed in Farm 2. The distributions of the ages at PRRSV infection were more narrowly spread within farms but between-farm variability was also found, with PRRSV infections occurring much earlier in Farm 3 than in Farm 1. The comparison of the distributions of the ages at HEV, PRRSV and PCV2 infection also highlighted different profiles in the sequences of infections depending on farms, for example PRRSV infection occurred much earlier than HEV infection in Farm 3 for every batch.

3.2 | Factors affecting HEV infection features

3.2.1 | Age at HEV shedding

The univariate analysis showed that both exposure to and temporal order of co-infections were associated with the age at HEV shedding, as well as six out of the eight variables related to piglet's

and litter's characteristics (Table 1). The multivariate model evidenced that a PRRSV or PCV2/PRRSV pre- or co-infection was associated with a higher age at HEV shedding similarly (Hazard Ratio = 0.28 [0.17-0.47] and 0.26 [0.15-0.46] respectively). Sow's parity was also associated with the age at HEV shedding, with piglets from oldest sows (parity higher than 4) exhibiting later HEV shedding (HR = 0.62 [0.44-0.85]) (Table 1). Other individual or litter characteristics such as number of live-born and mummified piglets, cross-fostering or sex did not remain in the multivariate model.

3.2.2 | Age at HEV seroconversion

The univariate analysis showed an impact of the exposure to and the sequence of co-infections and of six variables reflecting piglet's and litter's characteristics (Table 2). According to the results of the multivariate model, males exhibited HEV seroconversion later than females (HR = 0.70 [0.53-0.91]) and HEV seroconversion was delayed in piglets from oldest sows (HR = 0.39 [0.27-0.55]) for sows of parity higher than 4 vs. parity less than 1). A PRRSV or PCV2/PRRSV pre- or co-infection was also associated with a higher age at HEV seroconversion (HR = 0.53 [0.30-0.91] and HR = 0.41 [0.24-0.69] respectively) (Table 2).

3.2.3 | Duration of the HEV shedding period

The model evidenced a strong impact of the sequence of co-infections on the duration of the HEV shedding period, with a PCV2/PRRSV pre- or co-infection lengthening the shedding period (HR = 0.50 [0.32-0.79]). This variable was the only one related to this outcome (Table 3).

3.2.4 | HEV status of livers

From the results of the univariate analysis, both exposure to and sequence of co-infections were found to affect the probability of

TABLE 2 Effect of co-infections, immunity and litter characteristics on the age at HEV seroconversion of HEV shedders (3 farrow-to-finish pigs farms, 3 batches per farm, $n = 249$ pigs)

Variable	Category	n	Univariate model		Multivariate model	
			Hazard Ratio [95%CI]	p-value	Hazard Ratio [95%CI]	p-value
Exposure to co-infecting pathogens	None	27	-	-		
	PCV2	16	0.26 [0.13-0.51]	<0.01		
	PRRSV	66	0.72 [0.43-1.20]	0.21		
	PRRSV and PCV2	136	0.59 [0.36-0.97]	0.04		
Temporal order of co-infections			Chi ² = 20.21	<0.01***	Chi ² = 12.69	<0.01***
	HEV first	54	-	-	-	-
	PCV2 pre- or co-infection	34	1.03 [0.66-1.60]	0.90	0.98 [0.62-1.53]	0.91
	PRRSV pre- or co-infection	71	0.45 [0.26-0.76]	<0.01	0.53 [0.30-0.91]	0.02
Piglet's HEV serology (1 week of age)			Chi ² = 1.52	0.22		
	Negative	79	-	-		
Sow's HEV serology one week after farrowing			Chi ² = 1.24	0.27		
	Positive	169	0.84 [0.63-1.10]	0.22		
Sex			Chi ² = 6.38	0.01**	Chi ² = 7.03	<0.01***
	Female	119	-	-	-	-
Sow's parity			Chi ² = 34.37	<0.01***	Chi ² = 32.99	<0.01***
	0-1	74	-	-	-	-
Cross-fostering: number of ingoing piglets into the litter	2-3	76	1.01 [0.73-1.40]	0.95	0.93 [0.67-1.30]	0.67
	>4	94	0.40 [0.28-0.57]	<0.01	0.39 [0.27-0.55]	<0.01
	6-12	33	1.27 [0.80-2.01]	0.94		
Cross-fostering: number of outgoing piglets from the litter			Chi ² = 5.36	0.07*		
	0-4	165	-	-		
	5-7	51	1.50 [1.06-2.13]	0.37		
Number of weaned piglets in the litter			Chi ² = 4.14	0.13*		
	8-12	31	1.52 [0.99-2.33]	0.05		
	13-14	11	1.58 [0.73-3.42]	0.25		
Number of liveborn piglets in the litter			Chi ² = 0.08	0.96		
	7-10	67	-	-		
	11-12	136	1.00 [0.72-1.40]	0.98		
Number of liveborn piglets in the litter			Chi ² = 14.79	<0.01***		
	12-14	46	1.05 [0.69-1.61]	0.81		
	15-18	114	0.55 [0.39-0.78]	<0.01		

(Continues)

TABLE 2 (Continued)

Variable	Category	n	Univariate model		Multivariate model	
			Hazard Ratio [95%CI]	p-value	Hazard Ratio [95%CI]	p-value
Number of stillborn piglets in the litter	0-1	183	-	-		
	2-6	66	1.07 [0.79-1.44]	0.67		
Number of mummified piglets in the litter	0	171	-	-		
	1-2	78	1.21 [0.92-1.60]	0.17		

Note: Summary statistics as obtained thanks to a cox-proportional hazard model with the 'farm' effect being included as a frailty effect. Shaded areas represent variables that were not retained in the multivariate model.

*** $p < 0.01$.

** $p < 0.05$.

* $p < 0.20$.

liver being HEV-positive at slaughter, as well as six variables linked to piglet's and litter's characteristics. A short time-period between HEV infection and slaughter (<40 days) also increased the odds of a liver being HEV positive at slaughter (Odds Ratio = 4.07 [3.72-4.45]). The multivariate model evidenced that a PRRSV pre- or co-infection increased the risk of having positive livers at slaughter (OR = 4.10 [1.87-8.97]), particularly when combined with a PCV2 pre- or co-infection (OR = 6.49 [3.18-13.23]) (Table 4).

4 | DISCUSSION

Understanding the features and drivers of HEV infection dynamics on pig farms is crucial in order to implement HEV surveillance programmes and to assess and manage public health risks. Quite a few studies have investigated the dynamics of HEV infection at individual and collective levels in pig population in recent years (Berto, Mesquita, Hakze-van der Honing, Nascimento, & Poel, 2012; Casas et al., 2011; de Deus et al., 2008; Feng et al., 2011; Gardinali et al., 2012). The primary interest of our results lies in both describing and explaining within- and between-farm variability of HEV infection dynamics. To the best of our knowledge, this is the first individual follow-up conducted in several pig farms and monitoring HEV dynamics along with other co-infecting pathogens simultaneously. Though previous cohort studies exploring HEV infections have been already conducted in Spain and China, they only included a small number of animals, raised in a single batch from several farms (Casas et al., 2011) or in a single farm (de Deus et al., 2008; Feng et al., 2011); this limits the investigation of variation factors at farm, batch or individual levels. Other studies aiming to describe HEV course of infection consisted of biological samples taken at successive ages or production stages but from different pigs (Berto et al., 2012; Gardinali et al., 2012). In that sense, the monthly individual follow-up proposed here offered a unique opportunity to describe HEV course of infection while accounting for within and between-farm variability. A preliminary

check of the status of the farms to be selected revealed that they were still contaminated by HEV 2 years after a large prevalence survey (Rose et al., 2011). Moreover, the infection profile in terms of prevalence of HEV-containing livers at slaughter time was consistent with the results obtained thereafter in the follow up study. It suggests a strong ability of HEV to maintain in farrow-to-finish farms and a good stability in terms of dynamics of infection. In addition to a descriptive analysis of HEV patterns, statistical models were built to explain features of HEV infection dynamics.

Several outcomes were considered to describe HEV dynamics: the age at HEV shedding, the age at HEV seroconversion, the duration of HEV shedding and the HEV virological status of the liver at slaughter time. These four parameters were chosen in order to accurately describe the infection pattern at an individual scale in terms of shedding and immune response; they were also relevant to inform on the related public health risk. In that sense, they offer a full view of HEV characteristics on pig farms. The degree of uncertainty due to the sampling design and to the calculation method of these parameters was taken into account. Indeed, samples were taken every month and the dates of events occurring in these time intervals were therefore uncertain. For instance if piglets were found shedder at one sample only, it was very unlikely that they shed the virus for only one day. To address this, it was considered that the age at HEV shedding was the age at first positive faecal sample minus 7 days and the duration of HEV shedding period was calculated as the interval between the first and the last positive faecal samples plus 14 days. By doing so, the individual shedding period was at least 14 days, which is consistent with literature data (Salines et al., 2015). The age at HEV infection was inferred using a fixed latency value, which may affect the results. However, choosing 14 days as latency duration is a careful choice: indeed, experimental trials have shown that latency may vary between 7 and 14 days (Andraud et al., 2013; Salines et al., 2015), choosing 14 days can thus lead to underestimating the number of co-infected pigs. It therefore confirms the above results regarding the effect of immunomodulating viruses on HEV infection dynamics. A

TABLE 3 Effect of co-infections, immunity and litter characteristics on the duration of HEV infectious period (3 farrow-to-finish pigs farms, 3 batches per farm, $n = 249$ pigs)

Variable	Category	<i>n</i>	Univariate model		Multivariate model	
			Hazard ratio [95%CI]	<i>p</i> -value	Hazard ratio [95%CI]	<i>p</i> -value
Exposure to co-infecting pathogens			Likelihood-ratio Chi ² = 0.37	0.94		
	None	27	-	-		
	PCV2	16	1.08 [0.57–2.03]	0.82		
	PRRSV	66	1.02 [0.62–1.66]	0.95		
	PRRSV and PCV2	136	0.93 [0.58–1.51]	0.78		
Temporal order of co-infections			Chi ² = 10.05	0.018*	Chi ² = 10.05	0.018*
	HEV first	54	-	-	-	-
	PCV2 pre- or co-infection	34	0.92 [0.59–1.44]	0.72	0.92 [0.59–1.44]	0.72
	PRRSV pre- or co-infection	71	0.70 [0.44–1.11]	0.13	0.70 [0.44–1.11]	0.13
	PRRSV and PCV2 pre- or co-infection	90	0.50 [0.32–0.79]	<0.01	0.50 [0.32–0.79]	<0.01
Piglet's HEV serology (1 week of age)			Chi ² = 0.014	0.90		
	Negative	79	-	-		
	Positive	169	1.02 [0.76–1.37]	0.90		
Sow's HEV serology one week after farrowing			Chi ² = 0.37	0.54		
	Negative	74	-	-		
	Positive	175	0.91 [0.68–1.23]	0.54		
Sex			Chi ² = 0.13	0.72		
	Female	119	-	-		
	Male	130	0.95 [0.72–1.26]	0.72		
Sow's parity			Chi ² = 2.94	0.23		
	0–1	79	-	-		
	2–3	76	0.77 [0.54–1.10]	0.89		
	>4	94	1.03 [0.73–1.43]	0.12		
Cross-fostering: number of ingoing piglets into the litter			Chi ² = 0.20	0.90		
	0–1	165	-	-		
	2–5	51	0.96 [0.67–1.37]	0.81		
	6–12	33	0.90 [0.57–1.43]	0.66		
Cross-fostering: number of outgoing piglets from the litter			Chi ² = 2.83	0.23		
	0–4	207	-	-		
	5–7	31	0.70 [0.44–1.09]	0.12		
	8–12	11	1.11 [0.54–2.31]	0.77		
Number of weaned piglets in the litter			Chi ² = 0.54	0.76		
	7–10	67	-	-		
	11–12	136	0.95 [0.68–1.33]	0.77		
	13–14	46	1.09 [0.71–1.67]	0.69		
Number of liveborn piglets in the litter			Chi ² = 2.76	0.25		
	0–11	55	-	-		
	12–14	80	1.26 [0.86–1.85]	0.24		
	15–18	114	0.97 [0.67–1.40]	0.88		

(Continues)

TABLE 3 (Continued)

Variable	Category	n	Univariate model		Multivariate model	
			Hazard ratio [95%CI]	p-value	Hazard ratio [95%CI]	p-value
Number of stillborn piglets in the litter	0-1	183	-	-		
	2-6	66	0.98 [0.72-1.35]	0.92		
Number of mummified piglets in the litter	0	171	-	-		
	1-2	78	1.15 [0.86-1.53]	0.35		

Note: Summary statistics as obtained thanks to a cox-proportional hazard model with the 'farm' effect being included as a frailty effect and the age at HEV shedding being included as an explanatory variable. Shaded areas represent variables that were not retained in the multivariate model.

* $p < 0.05$.

sensitivity analysis would make it possible to consolidate the validity of our conservative method; it may show an even higher impact of PRRSV and/or PCV2 on HEV infection dynamics. The ages at seroconversion (HEV) or at infection (PRRSV) based on serological data were derived from modelling the antibodies kinetic curve by cubic splines to infer from the discrete sampling scheme the most likely seroconversion time. PRRSV serological data at day 180 were missing for Farms 2 and Farms 3. However, this did not affect the results: indeed, if pigs were PRRSV infected before HEV infection, they produced antibodies earlier than 180 days of age, therefore they have been detected at previous sampling points. Otherwise, they have been considered as HEV infected at first.

High between-farm variability of the HEV infection dynamics was evidenced, in contrast to more stable within-farm HEV pattern. Other studies also pointed a number of farm-specific factors that influence HEV infection features, for example farm size, genetic background, lack of hygiene measures, origin of drinking water, frequency of pig exchanges, etc. (Di Bartolo et al., 2008; Hinjoy et al., 2013; Jinshan et al., 2010; Li et al., 2009; Salines et al., 2018; Walachowski et al., 2014). This is the reason why the farm variable was included as a repeated and frailty effect in the GEE and survival models respectively. By doing so, it was possible to investigate the proper effect of other factors measured at the individual pig level and to extend our conclusions beyond the farm specificities. Several possible factors likely to explain features of HEV infection dynamics were considered: factors related to piglets' and litters' characteristics that had not been investigated to date; factors linked to anti-HEV immunity (in particular the effect of maternally derived antibodies) that had only been partially explored (Andraud et al., 2014); factors concerning immunomodulating pathogens that had only been studied in experimental conditions (Salines et al., 2015).

Pigs in Farm 1 got infected late and shed the virus for a short time period with a limited spread at the batch level, whereas pigs in Farm 2 got infected early and were shedders for a long time with a huge spread, and pigs in Farm 3 were infected late and shed the virus for a long time period with an important spread at the batch level as well. Some piglets in Farms 2 and 3 were found to shed HEV as early as lactating phase, suggesting the possible HEV transmission from sows

to piglets at this stage. Interestingly, the proportion of shedding sows found at farrowing or one week later was 0, 4.8 and 4.8% in farms 1, 2 and 3 respectively (data not shown). In our study and on all three farms, 7.2% of pigs (26/360) had HEV positive liver at slaughterhouse versus 6.2% (6/96) in Casas et al. (2011). The prevalence of HEV positive livers varied between farms, with a high proportion of positive livers in Farm 3 (up to 24% in the first batch). Our model evidenced that the time period between HEV infection and slaughter affected the HEV liver status, with a time-interval lower than 40 days increasing the probability of livers being HEV-positive at slaughterhouse by a factor of 4 in the univariate model. The variability in the prevalence of positive livers is therefore consistent with the different HEV infection patterns depending on farms, with a high proportion of contaminated livers in Farm 3 where infection occurred late in association with long shedding and high spread among pigs.

Our study showed that males exhibited HEV seroconversion later than females and were also more likely having HEV-containing livers at slaughter. To the best of our knowledge, the association between sex and HEV infection characteristics has never been reported in pigs. However, this is consistent with epidemiological studies in human population showing a high male/female sex ratio, probably associated with host factors that are still unknown (Borgen et al., 2008; Ijaz et al., 2005; Lewis, Morgan, Ijaz, & Boxall, 2006; Mansuy et al., 2009; Said et al., 2009). Our models also evidenced that HEV shedding and seroconversion were delayed for piglets from a high parity sow. This may be related to the sow's immunological status as regard HEV, old sows having a better immunity than young ones (e.g. in terms of IgG quantity and/or affinity, or other non-specific antiviral factors), hence delivering a stronger maternal immunity to their piglets. In addition, among the 17 sows out of 90 found HEV positive in faeces at any sampling time, six were of parity higher than four (out of 38 sows of parity higher than four) four were of parity between one and four (out of 21 sows in this parity category) and seven were gilts (out of 21 gilts in total) (data not shown). There is a trend of more frequent HEV shedding in gilts compared to multiparous sows (OR = 2.9 [0.8-10.3], p -value = 0.06). It suggests a higher susceptibility of gilts to infection, favouring transmission to their

TABLE 4 Effect of co-infections, immunity and litter characteristics on the probability of liver being HEV-positive at slaughter (3 farrow-to-finish pigs farms, 3 batches per farm, $n = 360$ pigs)

Variable	Category	<i>n</i>	Univariate model		Multivariate model	
			Odds ratio [95%CI]	<i>p</i> -value	Odds ratio [95%CI]	<i>p</i> -value
Exposure to co-infecting pathogens			Likelihood-ratio Chi ² = 5.49	<0.01***		
	None	29	-	-		
	PCV2	22	0.99 [0.67–1.44]	0.94		
	PRRSV	105	1.11 [0.24–5.30]	0.89		
	PRRSV and PCV2	181	0.98 [0.24–3.92]	0.97		
Temporal order of co-infections			Chi ² = 48.63	<0.01***	Chi ² = 34.09	<0.01***
	HEV first	54	-	-	-	-
	PCV2 pre- or co-infection	40	1.45 [0.19–11.10]	0.72	1.49 [0.22–10.35]	0.69
	PRRSV pre- or co-infection	110	4.06 [2.36–6.99]	<0.01	4.10 [1.87–8.97]	<0.01
	PRRSV and PCV2 pre- or co-infection	135	6.39 [3.70–11.03]	<0.01	6.49 [3.18–13.23]	<0.01
Piglet's HEV serology (1 week of age)			Chi ² = 0.28	0.59		
	Negative	114	-	-		
	Positive	243	0.74 [0.25–2.23]	0.59		
Sow's HEV serology one week after farrowing			Chi ² = 0.08	0.78		
	Negative	108	-	-		
	Positive	251	0.84 [0.24–2.93]	0.78		
Sex			Chi ² = 12.99	<0.01***	Chi ² = 136.91	<0.01***
	Female	172	-	-	-	-
	Male	187	1.29 [1.12–1.49]	<0.01	1.39 [1.32–1.47]	<0.01
Sow's parity			Chi ² = 197.17	<0.01***	Chi ² = 66.75	<0.01***
	0–1	104	-	-	-	-
	2–3	88	1.91 [1.38–2.65]	<0.01	2.49 [1.29–4.80]	<0.01
	>4	167	1.25 [0.65–2.39]	0.50	1.32 [0.49–3.57]	0.58
Cross-fostering: number of ingoing piglets into the litter			Chi ² = 0.80	0.67		
	0–1	207	-	-		
	2–5	84	0.48 [0.054–4.19]	0.50		
	6–12	68	1.04 [0.32–3.41]	0.95		
Cross-fostering: number of outgoing piglets from the litter			Chi ² = 22.50	<0.01***		
	0–4	260	-	-		
	5–7	67	1.29 [0.17–9.61]	0.80		
	8–12	32	0.82 [0.32–2.12]	0.69		
Number of weaned piglets in the litter			Chi ² = 1.02	0.60		
	7–10	83	-	-		
	11–12	200	0.97 [0.63–1.49]	0.89		
	13–14	76	0.46 [0.08–2.63]	0.38		
Number of liveborn piglets in the litter			Chi ² = 34.94	<0.01***		
	0–11	68	-	-		
	12–14	112	0.94 [0.21–4.12]	0.93		
	15–18	179	1.37 [0.23–8.01]	0.73		

(Continues)

TABLE 4 (Continued)

Variable	Category	n	Univariate model		Multivariate model	
			Odds ratio [95%CI]	p-value	Odds ratio [95%CI]	p-value
Number of stillborn piglets in the litter	0-1	267	-	-		
	2-6	92	0.64 [0.45-0.91]	0.014		
Number of mummified piglets in the litter	0	247	-	-		
	1-2	112	0.49 [0.37-0.65]	<0.01		
Time period between HEV infection and slaughter	>40 days	187	-	-		
	≤40 days	59	4.07 [3.72-4.45]	<0.01		

Note: Summary statistics as obtained thanks to a generalized estimating equation (GEE) logistic regression model with the 'farm' effect being included as a repeated statement.

Shaded areas represent variables that were not retained in the multivariate model.ssss

*** $p < 0.01$.

** $p < 0.05$.

piglets at an early stage. However, the two variables linked to anti-HEV immunity (serological status of the dam and piglets at one week post-farrowing) were not statistically associated with HEV infection features. Based on serological data from longitudinal studies in six pig herds, Andraud et al. (2014) showed that passive immunity delayed HEV infection of piglets by about six weeks in all but one farm on which the dynamics of infection were similar, whatever the animals' initial serological status. It suggests that beyond maternally derived passive immunity, other factors depending on host characteristics, farm-specific husbandry and hygiene practices have an impact on HEV transmission process.

Our models also showed a strong impact of a pre- or co-infection with PRRSV, alone or associated with PCV2 on all outcomes. This is in accordance with a previous experiment in which PRRSV was found to delay the age at HEV shedding with an increased latency period by a factor of 1.9, to delay the age at HEV seroconversion by a factor of 1.6, to lengthen HEV shedding period by a factor of 5 and to increase the probability of livers being HEV positive at 49 days post-infection (Andraud et al., 2013; Salines et al., 2015). The delayed age at HEV shedding evidenced in our study may be due either to a lengthened latency period or to a reduced sensitivity to infection. However, the extended latency period seems more likely, as the experimental trial conducted by Salines et al. (2015) showed that PRRSV co-infection extended the HEV latency period but also increased the susceptibility to HEV infection of pigs exposed to infectious particles in the environment and enhanced transmission of the virus between pigs. Our results suggest that PCV2 alone did not affect HEV infection dynamics as PRRSV. This may be related to specific characteristics of the infection dynamics observed in the farms under study. Hence, the average age at infection for PCV2 and HEV was very similar in almost the three farms under study whereas it was more different between PRRSV and HEV. This might be specific to these three farms and does not preclude a similar behaviour in all HEV/PCV2/PRRSV

co-infected farms. Investigations in a larger sample of herds would be required to evaluate this assumption. As regards potential immune mechanisms specific to co-infecting viruses, further work would be needed as PCV2 and PRRSV both have a suppressive effect on the innate immunity but their specific impact on HEV infection is still unknown (Butler et al., 2014; Darwich & Mateu, 2012).

In conclusion, these results show that co-infections with viruses affecting pig immune response, mainly PRRSV – alone or associated with PCV2 – have a major impact on HEV dynamics. These intercurrent pathogens may lead to extended HEV shedding and chronic HEV infection, increasing the risk of having HEV contaminated livers at slaughter age. At the batch level, the sequence of infection both influence the extent of HEV spread between pigs and the average age at infection. Taken together all these individual- and population-based characteristics directly influence the prevalence of HEV-containing livers at slaughter time. Controlling these pig-specific pathogens may therefore be a major lever to mitigate public health risk related to HEV.

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CONFLICT OF INTEREST

The authors declare that no competing interests exist.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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II. Etude de la dynamique de l'infection par le virus de l'hépatite E chez des porcs co-infectés par un virus immunomodulateur en conditions expérimentales

Le suivi longitudinal de trois élevages de porcs naisseurs-engraisseurs a mis en évidence que **la co-infection naturelle de porcs par le virus du SDRP, seule ou couplée à une co-infection par le PCV2, est associée à une excrétion plus tardive du HEV dans les fèces, une durée d'excrétion fécale du HEV plus longue, une séroconversion vis-à-vis du HEV retardée, et une augmentation du risque de positivité du foie à l'abattoir.** Néanmoins, et malgré la prise en compte statistique de facteurs de confusion, cette étude en conditions naturelles ne suffit pas à conclure de manière certaine quant à la **relation de causalité** pouvant exister entre ces variables associées. C'est la raison pour laquelle des **essais expérimentaux** ont été réalisés chez des porcs EOPS (Exempts d'Organismes Pathogènes Spécifiques) afin **d'étudier de manière spécifique, en conditions contrôlées, l'effet de la co-infection par le virus du SDRP et par le PCV2 – séparément – sur la dynamique de l'infection par le HEV.**

Ces essais ont donné lieu à trois publications internationales : une avant la thèse dans *Veterinary Research* (Salines *et al.*, 2015b), et deux dans le cadre de la thèse dans *International Journal of Food Microbiology* (Salines *et al.*, 2018d) et *Veterinary Microbiology* (Salines *et al.*, 2019a). Si les articles dans *Veterinary Research* et *Veterinary Microbiology* s'intéressent à **l'influence des co-infections sur les paramètres généraux de la dynamique infectieuse (période de latence, période infectieuse, paramètres de transmission, statut du foie à l'abattage)**, la publication dans *International Journal of Food Microbiology* traite plus spécifiquement de l'impact de la co-infection par le SDRP sur le **risque de présence du HEV dans le sang et les muscles des porcs co-infectés.** Deux articles ont aussi été publiés dans des revues nationales (Annexe 2 et Annexe 3) (Salines *et al.*, 2015a; Rose *et al.*, 2017).

Publication 3

Salines M., Barnaud E., Andraud M., Eono F., Renson P., Bourry O., Pavio N., Rose N., 2015. Hepatitis E virus chronic infection of pigs co-infected with Porcine Reproductive and Respiratory Syndrome Virus. *Veterinary Research*, 46:55.

RESEARCH ARTICLE

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Hepatitis E virus chronic infection of swine co-infected with Porcine Reproductive and Respiratory Syndrome Virus

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Abstract

In developed countries, most of hepatitis E human cases are of zoonotic origin. Swine is a major hepatitis E virus (HEV) reservoir and foodborne transmissions after pork product consumption have been described. The risk for HEV-containing pig livers at slaughter time is related to the age at infection and to the virus shedding duration. Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is a virus that impairs the immune response; it is highly prevalent in pig production areas and suspected to influence HEV infection dynamics. The impact of PRRSV on the features of HEV infections was studied through an experimental HEV/PRRSV co-infection of specific-pathogen-free (SPF) pigs. The follow-up of the co-infected animals showed that HEV shedding was delayed by a factor of 1.9 in co-infected pigs compared to HEV-only infected pigs and specific immune response was delayed by a factor of 1.6. HEV shedding was significantly increased with co-infection and dramatically extended (48.6 versus 9.7 days for HEV only). The long-term HEV shedding was significantly correlated with the delayed humoral response in co-infected pigs. Direct transmission rate was estimated to be 4.7 times higher in case of co-infection than in HEV only infected pigs (0.70 and 0.15 per day respectively). HEV infection susceptibility was increased by a factor of 3.3, showing the major impact of PRRSV infection on HEV dynamics. Finally, HEV/PRRSV co-infection – frequently observed in pig herds – may lead to chronic HEV infection which may dramatically increase the risk of pig livers containing HEV at slaughter time.

Introduction

Hepatitis E virus is a non-enveloped single-stranded RNA virus causing an acute hepatitis in humans. It is mainly transmitted by the oro-fecal route and is responsible for clinical signs similar to hepatitis A virus infection [1]. Chronic cases have been described, mainly in immunocompromised patients [2,3]. Four HEV genotypes have been described. Genotypes 1 and 2 infect only humans and circulate in Asia, Africa and Central America in epidemic waves linked to the consumption of contaminated water [4–6]. Genotypes 3 and 4 are shared between humans and other animal species and are responsible for autochthonous sporadic cases in industrialized countries. In particular, the number of hepatitis E cases linked to genotype 3 has considerably increased in the last decade

[6,7], in relation to better diagnosis. This genotype is highly prevalent in the swine population [8]. Some studies have shown that swine and human HEV strains are genetically very close [9] and HEV cross-species transmission has been proven [10,11]. Moreover, a number of autochthonous cases have been related to the consumption of undercooked pork meat, especially liver products [12–16]. Thus, hepatitis E is now recognized as a foodborne zoonosis for which domestic pigs are considered as the main reservoir in developed countries [7,17,18]. Understanding factors influencing the transmission dynamics of HEV in pig herds is crucial to limit the risk of an introduction of contaminated products in the food chain. Several studies have described experimental HEV infection trials via oral or intravenous route [19–24] but few studies were aimed at quantifying HEV transmission [20,25]. The results of these studies on HEV transmission were different than those observed in pig farms on the field, with the latent and infectious period estimates being generally longer

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than in experimental trials [26–28]. Moreover, a high variability of HEV infection dynamics is observed on pig farms and has not yet been fully explained [29]. Some factors affecting swine immune response may also influence the course of HEV infection. Porcine Respiratory and Reproductive Syndrome Virus (PRRSV) is a highly prevalent virus that impairs the immune response. It has been detected together with HEV in several studies but no evidence of a causal relationship has been shown to date [30–32]. Since chronic cases in humans are generally linked to immunosuppressive conditions [33–36], PRRSV might be suspected as a frequent co-factor affecting the features of HEV infection in pigs.

The impact of a PRRSV infection on HEV infection dynamics (in terms of viral shedding duration and quantity, transmission and humoral immune response) has therefore been studied through a transmission experiment involving HEV/PRRSV co-infection of specific-pathogen-free (SPF) pigs compared to an infection trial with HEV only that was previously led in our facilities, under the same conditions [25].

Materials and methods

HEV-only infection experiment

A transmission trial with HEV only has been carried out before the co-infection experiment [25]. The experiment was conducted in Anses air-filtered level-3 biosecurity facilities. Briefly, sixty-eight SPF Large-White piglets were used for the experiment. Eight pigs were kept as negative controls and the others were allocated to six rooms containing two pens per room. Rooms 1 to 3 were used to evaluate direct and environmental transmission, whereas Rooms 4 to 6 were used to examine between-pen transmission. The inoculated pigs received orally 10^8 ge (genome equivalent) under a volume of 10 mL of a genotype 3 HEV suspension (strain FR-SHEV3e, Genbank access number JQ953665). Individual fecal samples were collected four days before inoculation and three times per week from 0 to 39 days post-infection (dpi) when the pigs were killed for necropsy. Blood samples were collected twice a week during the same period and clinical signs and rectal temperature were monitored on a daily basis.

HEV/PRRSV co-infection experiment

Animal housing conditions and inoculation

The experiment was conducted in the same Anses air-filtered level-3 biosecurity facilities. Twenty five-week-old SPF Large-White piglets were included in the study; they were HEV and PRRSV free and they did not have any maternal antibodies against these two viruses. Pigs were housed in metallic flat decks with a punched floor for feces and urine evacuation. As in the field situation, fecal material could accumulate in the corners and was not removed during the trial. Three rooms were used: two negative control

pigs were housed in Room 1 whereas the 18 remaining piglets were randomly allocated to 3 independent pens distributed in Room 2 and Room 3 (6 piglets per pen) stratifying on gender (3 males and 3 females per pen), weight and the litter they came from. Room 2 contained 2 pens separated by a solid partition to prevent contamination of a pen by the other one (Figure 1). The average weights at weaning (sd) were 9.5 kg (2.7), 9.3 kg (1.6), 9.3 kg (2.3) and 9.3 kg (1.4) for Controls and groups #1, #2 and #3 respectively. In each pen, 3 piglets were inoculated with both HEV and PRRSV at day 0. For inoculation, piglets to be inoculated were grouped in a pen and they were put in contact with their corresponding pen-mates at day 1. The 3 inoculated piglets received the following: (i) orally 10^8 ge under a volume of 10 mL of a genotype 3 HEV suspension (strain FR-SHEV3e, Genbank access number JQ953665) prepared according to the protocol previously described in Andraud et al. [25] (ii) and by nasal route 2.5 mL per nostril of a PRRSV suspension (strain PRRS-2005-29-24-1 “Finistere”, genotype 1, subtype 1) titrating 10^5 TCID₅₀/mL. The experiment was performed in accordance with EU and French regulations on animal welfare in experiments. The protocol was approved by the Anses/ENVA/UPEC ethical committee (agreement #16 with the National committee for Ethics in animal experimentation).

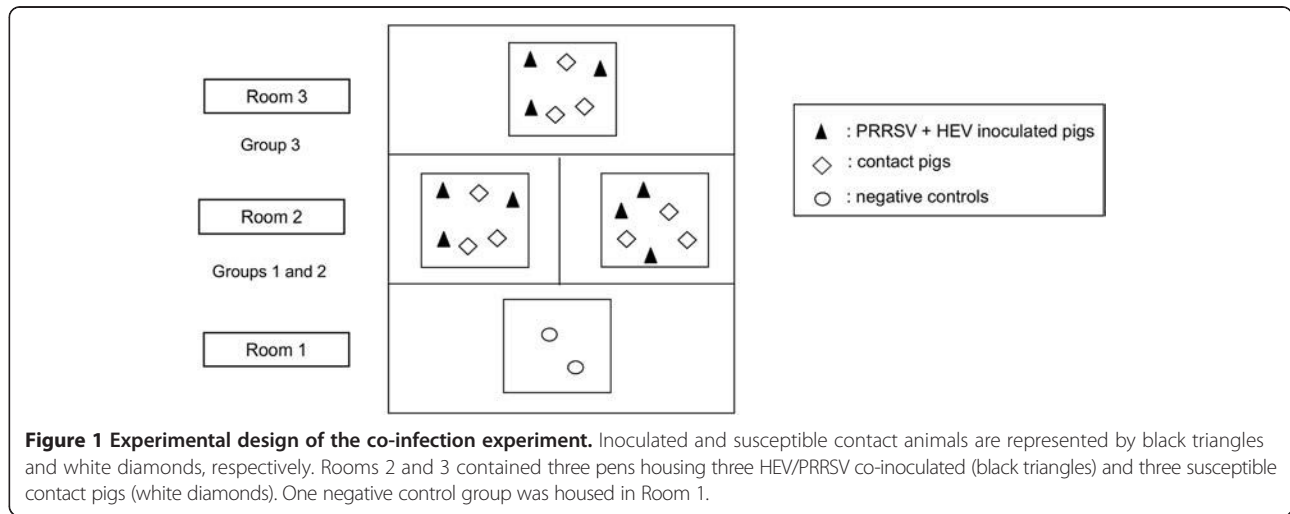
Data collection

Individual fecal samples were collected three days before inoculation and three times a week until the end of the experiment (49 dpi). Blood samples were collected before inoculation and once a week until the end of the experiment. Clinical examination was also performed (clinical signs, rectal temperature, feces consistence, weight, food consumption and trough cleanliness were recorded daily). Euthanasia was carried out by intravenous injection of 1 g/50 kg live weight of Nesdonal® (thiopental-sodium, Merial, Lyon, France) followed by exsanguination. Necropsy was performed and liver samples were taken.

Because HEV is a zoonotic agent, strict biosecurity measures were applied to prevent any transmission from pigs to animal technicians.

Virology and serology analyses

HEV RNA quantification in fecal and liver samples was performed, after manual total RNA extraction, using real-time quantitative RT-PCR as described in Barnaud et al. [37] and Andraud et al. [25]. The results were expressed in terms of Cycle threshold (Ct). Standard quantification curves were produced by plotting the Ct values against the logarithm of the input copy numbers of standard RNA. Standard RNA was obtained after *in vitro* transcription of a plasmid pCDNA 3.1 ORF 2–3 HEV, as described in Barnaud et al. [37]. The results are expressed in genomic copy number per gram of feces (ge/g).



The detection of anti-HEV antibodies was performed using the HEV ELISA 4.0v kit (MP Diagnostics, Illkirch, France) according to the manufacturer's instructions, except the serum quantity used (10 μ L instead of 20 μ L). This sandwich ELISA allows the detection of all antibody classes (IgG, IgM and IgA) and uses a recombinant antigen that is present in all HEV strains. Samples were positive when the optical density at 450 nm wavelength obtained for the sample was higher than the threshold defined as the mean for negative controls + 0.3.

PRRSV RNA detection in sera was performed using a real-time RT-PCR as described in Charpin et al. [38]. Briefly, RNA extraction was performed using the NucleoSpin[®] 8 virus kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. RNA detection was then performed using the mix GoTaq 1-Step RT-qPCR System (Promega) supplemented with probes and specific primers of the target gene (ORF7 pan-PRRSV) and of the internal reference gene (swine Beta-Actin). The RT-PCR was performed on a Bio-Rad Chromo4 real-time PCR detection system (Bio-Rad) according to the following program: 50 °C for 30 min, 94 °C for 2 min followed by 45 cycles of 94 °C for 15 s and 60 °C for 30 s. The results are expressed in Ct.

Models

Estimation of durations related to HEV infection dynamics

The latent and infectious period durations and the duration of the period required to produce anti-HEV antibodies were estimated using survival data analyses. For each inoculated animal, the latent period was determined as the time elapsed between the inoculation day and the date of the first positive fecal sample for HEV RNA. The latent period after inoculation was fitted to a gamma distribution, from which the shape and scale parameters were estimated by the maximum log-likelihood

method. A nonparametric bootstrap procedure was used to determine the 95% confidence interval of the parameter estimates.

A parametric model for survival data was built to estimate the duration of the infectious period, using the RT-PCR performed on livers after euthanasia as the last observation date. Two parametric models were tested (log-normal and Weibull distributions of survival times) and compared using the Akaike Information Criterion (AIC).

The impact of PRRSV co-infection on the time to HEV seroconversion was also studied with a parametric survival model applied to the data from the co-infection trial and the only HEV infection experiment [25]. The link between the earliness of the HEV antibody response and the duration of the infectious period was studied with a Cox model. The immune response was considered as absent or late if the delay before seroconversion was longer than 25 dpi, and as early if it was shorter than 25 dpi [39].

All analyses were performed using the R software (survreg and coxph functions) [40].

Quantification of HEV shedding, environmental accumulation and transmission

The distributions of HEV shed viral loads with time (with and without co-infection) are represented with box plot series. A linear mixed model (proc Mixed, SAS 9.3, [41]) which took into account repeated measurements with time was built to study the difference in the quantity of HEV shed particles between co-infected and non co-infected pigs.

The environmental load corresponds to the accumulation of viral particles in the environment through fecal shedding by infected animals, which is partially compensated by the clearance rate hereafter denoted δ . The clearance rate takes into account feces elimination

through the metallic flat deck and HEV intrinsic mortality in the environment. As described in Andraud et al. [25], for each pen (k) and every sampling time (t_i), the average quantity of genome equivalent shed in the environment per gram of feces was calculated with:

$$V_k(t_i) = \sum_j V_k^j(t_i)/N_k$$

where $V_k^j(t_i)$ represents the quantity of virus shed per gram of feces in pen k by pig j at time t_i and N_k the total number of animals in pen k . Thus the cumulated viral load in the environment of pen k between two sampling times t_i and t_{i+1} is given by the equation:

$$\begin{aligned} E_{ki} &= E_k(t_{i+1}) \\ &= \left(E_k(t_i) + \int_0^{\Delta t} V_k(t_i + u) e^{\delta u} du \right) e^{-\delta \Delta t}, \text{ with } \Delta t \\ &= t_{i+1} - t_i. \end{aligned}$$

Two HEV transmission routes were investigated in this study: (i) transmission due to direct contact between infected and naïve pigs; (ii) indirect transmission via an environmental reservoir of the virus in the pen. A Bayesian model similar to the one described in Andraud et al. [25] was used. Briefly, on each sampling interval $D_i = [t_i, t_{i+1}]$ of duration d_i , the probability for a susceptible pig j housed in pen k to escape infection is given by:

$$p_i^{(k)} = \exp\left(-d_i \left(\beta_w \pi_i^{(k)} + \beta_E^{(w)} \frac{E_{ki}^{(w)}}{N} \right)\right),$$

where $\pi_i^{(k)}$ represents the proportion of shedding pigs in the time interval D_i located in pen k , $E_{ki}^{(w)}$ is the environmental pool of viral particles in time interval D_i in the pen, β_w is the within-pen transmission rate by direct contact and $\beta_E^{(w)}$ is the within-pen environmental transmission rate. For each pig j , the time interval in which the infection occurred was determined by estimating the latent period λ_j . Let $D_{I_j} = [t_{I_j}, t_{I_j+1}]$ denote the time interval during which the first positive fecal sample was detected in pig j . The contribution of contact animal j in pen k to the likelihood model, i.e. the probability for its first positive fecal sample to stand in the interval $D_{I_j} = [t_{I_j}, t_{I_j+1}]$ is:

$$L^{(j)}(D_I, \pi_w, E | \beta_w, \beta_E^{(w)}, \lambda, \delta) = \left\{ \prod_{l=1}^{I_j} p_{l-1}^{(k)} (1 - p_{l-1}^{(k)}) \right\} \times f_{Lat}(\lambda_j, \alpha, s),$$

The probability of infection (given by the first term of the equation aforementioned) is weighted by the probability that the estimated latent period λ_j is consistent with the data observed in inoculated animals. f_{Lat} represents the prior distribution of the latent period based on

the estimation of the latent period in inoculated animals. The global likelihood is given by:

$$L(D_I, \pi_w, E | \beta_w, \beta_E^{(w)}, \lambda, \delta) = \prod_{j=1}^{N_c} L^{(j)}(D_I, \pi_w, E | \beta_w, \beta_E^{(w)}, \lambda, \delta),$$

where N_c is the total number of contact pigs.

The direct and indirect transmission rates β_w and $\beta_E^{(w)}$ respectively, the latent period λ_j for each contact animal and the HEV clearance rate were estimated by Bayesian inference using Monte Carlo Markov Chain. An informative prior distribution based on Andraud et al. [25] was used for the environmental clearance rate δ , which was assumed to be normally distributed with mean 0.3 and standard deviation 0.075. The prior distributions of transmission parameters were based on the results obtained by Andraud et al. [25]; they were constructed such that the expected value is equal to the posterior mean and 33% of the prior mass covers the 95% confidence interval for parameters derived from data obtained by Andraud et al. [25,42] (normal distribution $(-2,3)$ and $(-13.5,5)$ for β_w and $\beta_E^{(w)}$ respectively). The prior distribution of the latent period in contact pigs was based on the distribution of the latent period in inoculated pigs (gamma distribution $\Gamma(26,2)$).

Parameter updating was performed sequentially by the Metropolis-Hastings algorithm. Three chains were run with random initial conditions, 110 000 steps per chain, a burnin of 10 000 steps and thinning parameter of 10. Convergence was assessed by visual inspection and diagnostic tests (auto-correlation, Heidelberger, Gelman-Rubin diagnostics).

The whole model was performed using the R software [40].

Results

HEV-only infection experiment

In this trial, the average HEV latent period in inoculated animals lasted 6.9 days (5.8; 7.9) and average infectious period lasted 9.7 days (8.2; 11.2) (Table 1) [25]. Direct transmission rate was estimated at 0.15 (0.03; 0.31) pigs per day and indirect transmission rate was estimated at $2 \cdot 10^{-6}$ g/ge/day ($1 \cdot 10^{-7}$; $7 \cdot 10^{-6}$) (Table 1) [25]. HEV serology results on individual blood samples for HEV-only infected pigs are presented in Additional file 1 [25].

HEV shedding and seroconversion in the context of HEV/PRRSV co-infection

HEV infection data are presented in Figures 2 and 3 for quantitative RT-PCR on fecal samples and serological results respectively. In our trial, all inoculated animals were infected by HEV. None of the 2 negative-control pigs excreted HEV from day 3 to day 49. Inoculated and contact animals started to shed HEV between 9 and 18 dpi and between 25 and 32 dpi respectively. All exposed individuals shed HEV until the end of the trial (49 dpi) (Figure 2). At

Table 1 Summary of the infectious dynamics parameters and comparison with data from the HEV-only infection experiment [25]

	HEV + PRRSV	HEV alone [25]
Latent period (days)	13.4 (8.6; 17.1)	7.1 (3.2; 12.3)
Infectious period (days)	48.6 (27.9; 84.6)	9.7 (8.2; 11.2)
Seroconversion period (days)	43.1 (35.7; 52.2)	26.3 (23.5; 29.5)
Direct transmission (days ⁻¹) β_w	0.70 (1.2·10 ⁻³ ; 3.67)	0.15 (0.03; 0.31)
Indirect transmission (g/ge/d) β_E^w	6.6·10 ⁻⁶ (1.4·10 ⁻¹⁰ ; 1.3·10 ⁻⁴)	2.0·10 ⁻⁶ (1.1·10 ⁻⁷ ; 7.0·10 ⁻⁶)

β_w is the direct transmission rate, defined as the mean number of newly infected pigs generated by a single infectious individual in a fully susceptible population per day. β_E^w represents the within-pen transmission rates related to the environmental component, defined as the mean number of newly infected pigs per HEV genome equivalent per gram of feces in the environment (see text for more details). Numbers in brackets are the upper and lower limits of the 95% credibility interval.

the necropsy stage, 14 livers out of 18 were positive, the 4 negative livers being from contact pigs (Figure 2).

The detection of anti-HEV antibodies was performed on all groups of animals until 49 dpi (Figure 3). None of the negative controls showed anti-HEV antibody response. Only 4 inoculated animals out of 9 produced anti-HEV antibodies between 35 and 49 dpi, 3 in group 2 and one in group 3; none of the inoculated animals from group 1 seroconverted. Seven contact individuals out of 9 seroconverted between 42 and 49 dpi, two from groups 1 and 2 and all three contact animals from group 3 (Figure 3).

PRRSV infection and seroconversion in the context of HEV/PRRSV co-infection

All animals inoculated with PRRSV were viremic from the first sampling time (7 dpi). The viremia of contact animals started between 7 and 42 dpi. One contact individual did not show any detectable PRRSV viremia during the experiment (Figure 4). Finally, all animals except 2 contact individuals were viremic for PRRSV before HEV shedding was detected.

Regarding clinical data (data not shown), inoculated and contact animals showed hyperthermia (rectal temperature >40 °C) between 1 and 14 dpi and 14 and 28 dpi, respectively. Co-infected pigs necropsied at 49 dpi did not show any macroscopic lesion possibly linked to hepatitis.

Quantification of HEV infection dynamics parameters in the context of HEV/PRRSV co-infection

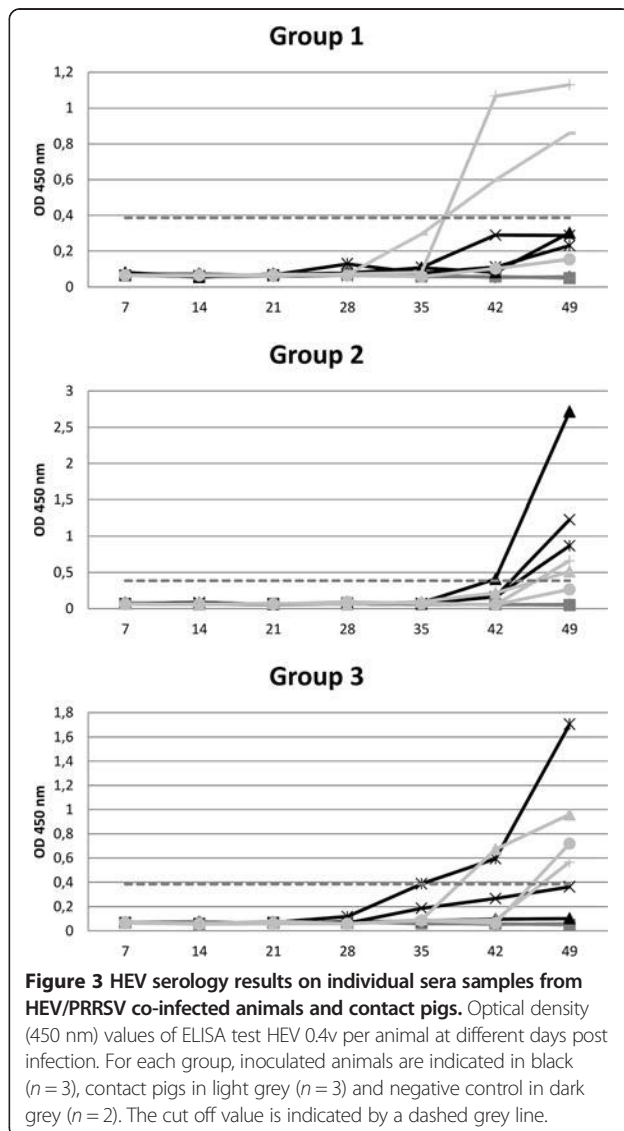
Convergence of MCMC was assessed through visual inspection and conventional diagnostic tests. Heidelberg and Geweke diagnostics failed to reject the convergence hypothesis, which was also supported by the Gelman-Rubin test based on three independent chains with a potential scale reduction factor (PSRF) close to 1.0 (≤1.02) (Additional file 2).

HEV latent and infectious periods

The duration of the latent period in pigs inoculated with HEV and PRRSV was fitted to a gamma distribution with shape parameter $\alpha = 25.7$ (11.6; 180.4) and scale parameter $s = 0.5$ (0.08; 1.1) leading to an estimated mean duration of the latent period of 12.9 days (12.8; 14.4). In contact animals, individual distributions of latent periods (Additional

	dpi	2	4	7	9	11	14	16	18	21	23	25	28	32	35	39	42	44	46	49	Necropsy (liver)	
Control	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group 1	Inoculated	0	0	0	1.08·10 ⁵	0*	2.23·10 ⁴	2.23·10 ⁵	1.24·10 ⁶	2.62·10 ⁶	1.38·10 ⁷	1.35·10 ⁷	5.96·10 ⁶	8.27·10 ⁶	7.51·10 ⁶	2.95·10 ⁷	4.43·10 ⁷	8.02·10 ⁶	8.76·10 ⁶	1.49·10 ⁷	1.46·10 ⁶	
	Inoculated	0	0	0	0	0	2.23·10 ⁴	1.72·10 ⁵	1.91·10 ⁶	6.80·10 ⁶	2.24·10 ⁷	3.56·10 ⁷	7.66·10 ⁷	1.32·10 ⁸	9.91·10 ⁷	6.76·10 ⁷	1.51·10 ⁸	1.09·10 ⁸	4.01·10 ⁷	5.85·10 ⁷	1.87·10 ⁶	
	Inoculated	0	0	0	0	0	0	0	1.50·10 ⁵	1.53·10 ⁶	5.85·10 ⁶	1.14·10 ⁷	1.30·10 ⁷	4.06·10 ⁷	3.99·10 ⁷	9.27·10 ⁷	4.28·10 ⁸	5.02·10 ⁸	7.20·10 ⁷	6.40·10 ⁷	1.02·10 ⁶	
	Contact	0	0	0	0	0	0	0	0	0	0	2.67·10 ⁵	1.16·10 ⁶	1.79·10 ⁷	1.54·10 ⁷	3.75·10 ⁷	1.92·10 ⁸	1.42·10 ⁸	5.31·10 ⁷	4.87·10 ⁷	8.72·10 ⁵	
	Contact	0	0	0	0	0	0	0	0	0	0	0	2.91·10 ⁵	3.68·10 ⁶	3.51·10 ⁶	6.24·10 ⁶	1.53·10 ⁷	4.91·10 ⁷	1.17·10 ⁸	1.09·10 ⁸	3.49·10 ⁷	0
	Contact	0	0	0	0	0	0	0	0	0	0	1.78·10 ⁵	8.84·10 ⁵	1.43·10 ⁶	3.04·10 ⁶	5.43·10 ⁶	1.20·10 ⁷	4.29·10 ⁷	1.91·10 ⁸	1.20·10 ⁸	1.20·10 ⁶	0
Group 2	Inoculated	0	0	0	0	0	1.56·10 ⁵	1.09·10 ⁶	1.57·10 ⁶	2.81·10 ⁶	1.67·10 ⁷	2.69·10 ⁷	8.71·10 ⁷	6.33·10 ⁷	4.44·10 ⁸	3.92·10 ⁸	1.64·10 ⁸	9.92·10 ⁷	1.45·10 ⁸	1.56·10 ³		
	Inoculated	0	0	0	0	0	0	2.19·10 ⁵	5.44·10 ⁵	6.85·10 ⁶	1.86·10 ⁷	1.51·10 ⁷	2.31·10 ⁷	1.55·10 ⁷	1.79·10 ⁷	2.57·10 ⁷	7.88·10 ⁶	1.37·10 ⁸	1.97·10 ⁸	6.80·10 ⁷	2.42·10 ⁶	
	Inoculated	0	0	0	0	0	1.03·10 ⁵	6.97·10 ⁵	5.05·10 ⁶	3.85·10 ⁷	4.20·10 ⁷	3.96·10 ⁷	6.72·10 ⁷	1.25·10 ⁸	1.39·10 ⁸	5.93·10 ⁷	8.10·10 ⁷	1.32·10 ⁸	1.13·10 ⁸	4.86·10 ⁶	3.59·10 ⁵	
	Contact	0	0	0	0	0	0	0	0	0	0	1.65·10 ⁶	0*	1.45·10 ⁶	4.12·10 ⁶	4.94·10 ⁶	abs	1.18·10 ⁷	2.51·10 ⁷	5.55·10 ⁷	1.12·10 ⁶	
	Contact	0	0	0	0	0	0	0	0	0	0	0	0	1.82·10 ⁶	5.38·10 ⁶	8.40·10 ⁶	4.43·10 ⁶	8.37·10 ⁷	1.43·10 ⁸	5.24·10 ⁶	1.69·10 ⁶	
	Contact	0	0	0	0	0	0	0	0	0	0	0	0	1.47·10 ⁶	7.26·10 ⁶	1.97·10 ⁷	3.03·10 ⁷	4.37·10 ⁷	2.49·10 ⁸	3.07·10 ⁷	3.44·10 ⁶	
Group 3	Inoculated	0	0	0	0	0	2.37·10 ⁵	3.12·10 ⁵	3.88·10 ⁶	6.75·10 ⁶	7.98·10 ⁶	1.05·10 ⁷	4.21·10 ⁷	2.64·10 ⁷	4.26·10 ⁷	1.51·10 ⁷	2.90·10 ⁷	1.60·10 ⁸	2.08·10 ⁸	3.24·10 ⁶		
	Inoculated	0	0	0	0	0	0	6.06·10 ⁵	9.54·10 ⁵	4.08·10 ⁶	3.22·10 ⁶	1.55·10 ⁷	3.02·10 ⁷	1.76·10 ⁷	1.80·10 ⁷	1.88·10 ⁷	8.52·10 ⁶	1.85·10 ⁷	5.75·10 ⁷	2.04·10 ⁴		
	Inoculated	0	0	0	0	0	8.04·10 ⁴	4.35·10 ⁵	2.26·10 ⁶	1.14·10 ⁷	2.25·10 ⁷	1.73·10 ⁷	2.37·10 ⁷	3.66·10 ⁷	3.17·10 ⁷	1.04·10 ⁷	4.39·10 ⁷	4.09·10 ⁷	1.43·10 ⁷	5.61·10 ⁷	9.63·10 ⁵	
	Contact	0	0	0	0	0	0	0	0	0	0	0	0	8.80·10 ⁴	2.04·10 ⁵	2.34·10 ⁶	3.40·10 ⁶	4.04·10 ⁶	5.02·10 ⁶	7.39·10 ⁵	0	
	Contact	0	0	0	0	0	0	0	0	0	0	0	0	8.26·10 ⁶	1.50·10 ⁷	8.47·10 ⁷	0*	1.45·10 ⁷	8.67·10 ⁷	1.03·10 ⁸	5.87·10 ⁵	
	Contact	0	0	0	0	0	0	0	0	0	0	0	0	1.31·10 ⁶	8.42·10 ⁶	1.12·10 ⁶	0*	4.41·10 ⁶	7.78·10 ⁶	8.29·10 ⁵	0	

Figure 2 HEV RNA quantification in fecal and liver samples from HEV/PRRSV co-infected animals and contact pigs. Quantitative HEV RT-PCR results on individual fecal samples (HEV copies/g of feces) at each sampling time and from liver samples at necropsy. Shaded zones correspond to periods in which infected individuals were considered infectious, corresponding to the time between the first and last HEV positive fecal samples for each animal. dpi: day post infection; *tested in duplicate; abs: missing.



file 2) were merged to obtain a global distribution of the latent period, leading to a mean latent period duration of 13.4 days (8.6; 17.1) (Table 1).

The duration of the infectious period was fitted to a log-normal distribution, leading to an estimated mean duration of the infectious period of 48.6 days (27.9; 84.6) (Table 1).

Estimation of time to HEV seroconversion

Time-to HEV seroconversion was fitted to log-normal distribution, with means 43.1 days (35.7; 52.2) with PRRSV co-infection and 26.3 days (23.5; 29.5) with only HEV infection (Table 1). The duration of the infectious period was significantly associated with the earliness of the humoral immune response. An absent or late immune response was related to a lengthening of the infectious period duration showed by a delay in time-to end of shedding (Hazard Ratio HR = 0.35 (0.19; 0.64)) (Figure 5).

HEV shedding and accumulation in the environment

The distribution of the HEV shed viral load with time (with and without co-infection) is shown in Figure 6. PRRSV infection was found to be significantly associated with the increase of the quantity of HEV particles shed by inoculated animals ($P = 0.05$) from the linear mixed model accounting for repeated measurements. The interaction between time and PRRSV infection was also significant and positive, i.e. the impact of the PRRSV infection increased with time ($P = 0.04$). However, the effect of the PRRSV infection was not found to be statistically significant in contact animals ($P > 0.05$).

The viral load accumulated in the environment was modeled for each experimental pen (Figure 7). The environment was HEV-free until 15 to 20 dpi; then the environmental load increased and reached $1.0 \cdot 10^8$ to $1.5 \cdot 10^8$ ge/g of feces until the end of the trial.

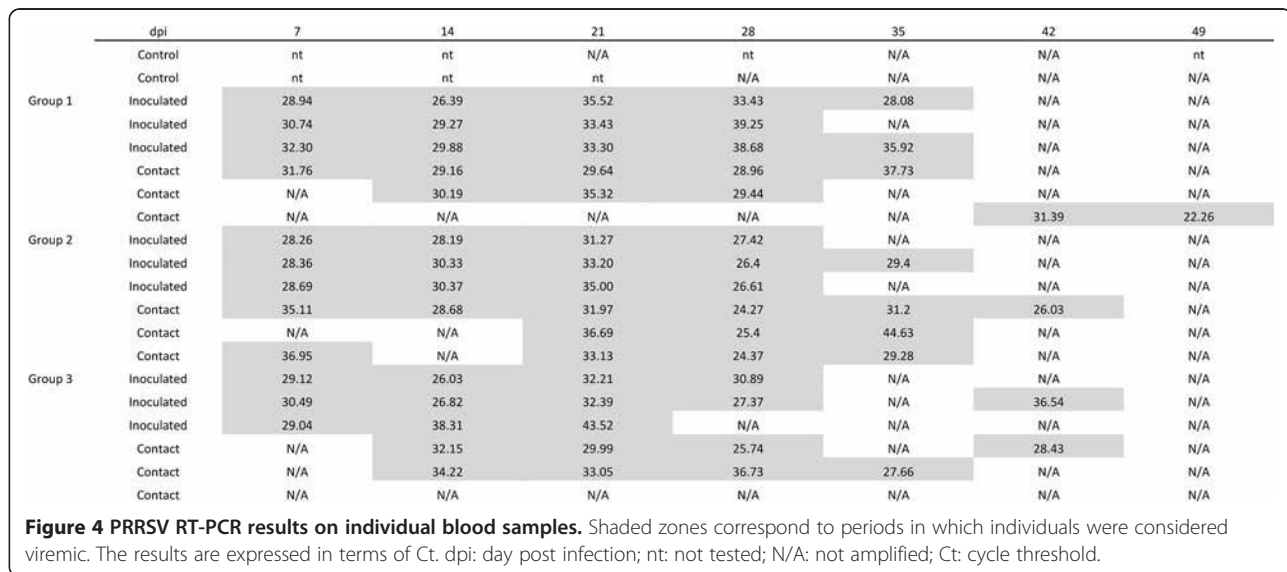
HEV transmission parameters

The results show that, in experimental conditions, one infectious pig was able to infect 0.70 pig per day by direct contact ($\beta_w = 0.70$ ($1.18 \cdot 10^{-3}$; 3.67)) (Table 1). The indirect transmission rate can be considered as the average number of animals that can be infected by a single genome equivalent present in the pen environment ($\beta_E^{(w)} = 6.59 \cdot 10^{-6}$ g/ge/day ($1.43 \cdot 10^{-10}$; $1.27 \cdot 10^{-4}$)). In other words, the inverse of $\beta_E^{(w)}$ corresponds to the average number of viral copy number of genome per gram of feces in the environmental pool required to infect one animal in one day, i.e. $1.51 \cdot 10^5$ ge/g/day ($7.86 \cdot 10^3$; $7.00 \cdot 10^9$) (Table 1).

Discussion

Several studies suggested a possible link between HEV and PRRSV infections [30–32]. Our study was aimed at evaluating the impact of PRRSV infection on hepatitis E dynamics of infection through an experimental HEV/PRRSV co-infection trial. As shown in Table 1, the comparison of the results with those derived from a previous infection trial with HEV alone [25] evidenced a modification of hepatitis E infection dynamics in the presence of PRRSV. Although the two trials were not carried out simultaneously, they were conducted under the same experimental conditions making the comparison of the results fully relevant (same experimental facilities, same handlers, pigs from the same SPF herd and genetically similar, same age of the animals, same sex ratio, same HEV strain, same dose, same inoculation protocol and same contact structure).

HEV shedding was delayed in case of PRRSV co-infection, with a latent period estimated to 13.4 days, against 7.1 days with HEV alone [25], i.e. an increase by a factor of 1.9. In the Bouwknegt et al. trial, the latent period was estimated at only 3 days in intravenously inoculated animals [20], confirming that the route of inoculation



modifies viral fate. The infectious period was longer with PRRSV co-infection: 48.6 days, against 9.7 days with HEV alone, i.e. an increase by a factor of 5 ($p < 0.01$). These results were therefore closer to estimates obtained from field data (27 days (20; 39)) than experimental results obtained with HEV only [26]). In the trial described by Bouwknecht et al., the infectious period was estimated between 13 and 49 days according to replications, showing a high inter-individual variability [20]. Moreover, the origin of the animals included in this study and their status regarding PRRSV were not mentioned.

HEV shedding in inoculated individuals was also significantly increased with PRRSV/HEV co-infection. However, the effect of PRRSV infection on the quantity of shed viral particles was not significant in contact animals. This could

be explained by the low number of animals included – especially since one contact animals was lately infected by PRRSV and another did not show any PRRSV viremia during the experiment – and by a large inter-individual variability in contact animals. As a consequence of the longer shedding period and the higher quantity of viral particles shed in feces of co-infected animals, the viral load accumulated in the environment was higher with PRRSV co-infection with more than 10^8 HEV ge/g of feces estimated in the environment, which causes a higher and longer infection pressure on susceptible animals. The direct transmission rate when animals were co-infected was increased by a factor of 4.7 (0.70 versus 0.15 per day with HEV infection only [25]). Thus the direct transmission route played a more important role in HEV transmission when animals

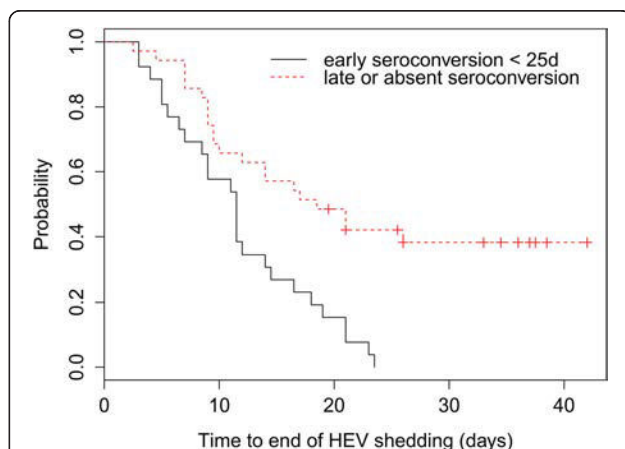


Figure 5 Survival curves of time-to-end of HEV shedding according to early or late HEV seroconversion. The black and red survival curves correspond to the duration of the infectious period in pigs having an early seroconversion (less than 25 dpi) or a late or absent seroconversion (more than 25 dpi) respectively.

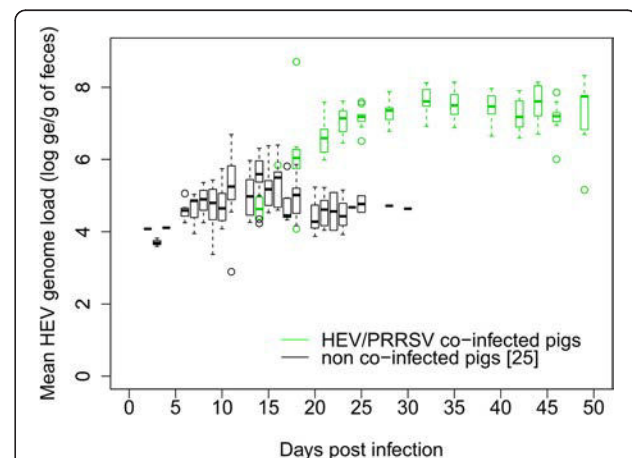
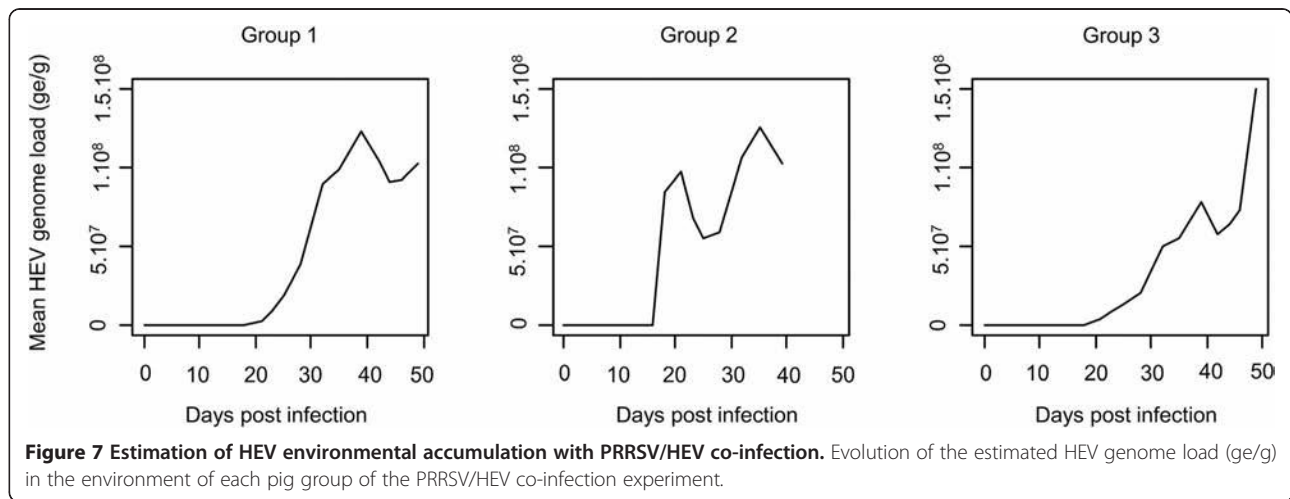


Figure 6 Distribution of the number of HEV genome equivalent (log ge/g feces) shed by individual pigs with time in inoculated animals with or without PRRSV co-infection. Co-infected animals are indicated in green ($n = 9$), only-HEV infected animals [25] are in black ($n = 18$).



were co-infected which was consistent with the larger amount of HEV particles shed individually than in HEV infected pigs only. The indirect transmission rate was 3.3 times higher with co-infection ($6.6 \cdot 10^{-6}$ and $2.0 \cdot 10^{-6}$ g/ge/day respectively [25]). Otherwise stated, 3.3 times less viral particles were required to infect a co-infected animal ($1.5 \cdot 10^5$ versus $5.0 \cdot 10^5$ ge/g for HEV only infected piglets [25]). Because inoculated and contact animals (except two contact pigs) were infected by PRRSV before HEV shedding, these data suggest a higher HEV susceptibility in PRRSV co-infected pigs. In a model built from an experimental HEV infection by intravenous route, Bouwknegt et al. showed that the HEV oral dose for which the infection probability was equal to 50% would be $1.4 \cdot 10^6$ ge/g [22], which was 10 times more than the dose required to infect a PRRSV co-infected pig in our study. These data are consistent with the hypothesis of a higher HEV infection susceptibility in PRRSV co-infected pigs.

The time-to HEV seroconversion was 1.6 times longer in PRRSV co-infected pigs than in HEV only infected pigs (43.1 and 26.3 days respectively [25]). This impaired immune response was significantly associated with a lengthening of the infectious period duration and could thus explain the presence of viral particles in livers when pigs were euthanized more than 49 days post infection for the inoculated ones. However, this study did not aim at investigating the mechanisms leading to a possible immune failure linked to PRRSV infection and the mechanisms causing a chronic HEV infection. In humans, immunopathogenic mechanisms leading to chronic hepatitis E are poorly known. The role of cellular immunity in chronic hepatitis E control has been shown [3,35,36]. A study was led on patients suffering from HIV and chronically infected with HEV [34]. One of them had a low anti-HEV lymphocyte T CD4+ rate, a persistent viremia (longer than 24 months) and a delayed anti-HEV seroconversion. Thus, though immune mechanisms still need to be clarified,

literature data suggest that an impaired innate and adaptive immune response could lead to chronic HEV infection in humans. In pigs, the immunopathogenic mechanisms linked to PRRSV infection are not fully understood yet, but PRRSV infection clearly results in a late adaptive immune response [43,44]. Thus the delayed anti-HEV seroconversion and the lengthening of the infectious period duration that we observed in PRRSV co-infected pigs seem consistent with the immunopathogenic mechanisms of chronic hepatitis E that have been described in humans (impaired cellular and humoral immune response) and could be explained by a specific orientation of the immune response linked to PRRSV infection. The increase of the duration of the latent period might be explained by the activation of the innate immune response linked to the PRRSV infection, delaying HEV shedding but this would require further work to assess the underlying mechanisms.

To our knowledge, this work is the first study focusing on the impact of HEV/PRRSV co-infection on hepatitis E epidemiology in pigs. These results show that PRRSV has a major impact on HEV infection dynamics and that HEV/PRRSV co-infection could lead to extended HEV shedding and maybe chronic infection. This chronicity may dramatically increase the risk of pig livers containing HEV at slaughter age. Immunopathogenic mechanisms leading to a chronic HEV infection have to be further investigated. This study shows an important interaction between an animal health concern - PRRSV, which dramatically affects the competitiveness of pig farms, and a zoonotic pathogen - HEV, which has a major impact in human health. These data emphasize the necessity to manage human and animal health globally and the importance of PRRSV eradication programs, which could be a major lever in the control of hepatitis E.

Additional files

Additional file 1: HEV serology results on individual sera samples for only-HEV infected pigs [25]. Optical density (450 nm) values of ELISA test HEV 0.4v per animal at different day post infection. Shaded zones correspond to the period in which individuals were considered HEV seropositive. dpi: days post infection, abs: missing.

Additional file 2: Estimation of transmission parameters by Bayesian inference (MCMC estimation, 3 chains, 110 000 iterations, 10 000 burnin iterations, thinning interval = 10). β_w is the direct transmission rate, defined as the mean number of newly infected pigs generated by a single infectious individual in a fully susceptible population per day. β_E^w represents the within-pen transmission rates related to the environmental component, defined as the mean number of newly infected pigs per viral particle per gram of feces in the environment. δ is the HEV clearance rate, taking into account feces elimination through the metallic flat deck and HEV destruction in the environment. λ_1 to λ_9 are latent periods for contact animals (see text for more details).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MS analyzed the data, participated to the sample analyses and drafted the manuscript, EB analyzed the HEV samples and interpreted the results, MA developed the mathematical model and participated to the data analyses, FE participated in the animal experiment, monitored and pre-treated the samples and participated to the analysis of PRRSV samples, PR and OB analyzed and interpreted the results from PRRSV samples, NP supervised the HEV-related laboratory work and participated in the coordination of the study, NR conceived, coordinated the study and participated in the animal experiment and the data analyses. All co-authors revised the manuscript and approved the final submitted version.

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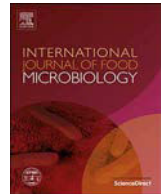
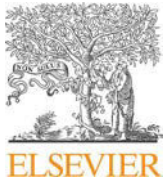
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Publication 4

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Short communication

Persistent viremia and presence of hepatitis E virus RNA in pig muscle meat after experimental co-infection with porcine reproductive and respiratory syndrome virus

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ABSTRACT

Although hepatitis E virus (HEV) transmission has been demonstrated after consumption of products containing infected pig liver, human cases can be also associated with other pig meat products, such as sausages. Data on HEV viremia and dissemination in muscle meat of infected animals are still sparse, especially during long-term infection. Previously, we have shown that experimental co-infection of pigs with HEV and porcine reproductive and respiratory syndrome virus (PRRSV) lengthens HEV infection up to 49 days and increases the likelihood of the presence of HEV RNA in the liver of the pig at a later stage of infection. In the present study, we show that during experimental HEV-PRRSV co-infection, prolonged HEV viremia, up to 49 days post-inoculation (dpi), is detected. The long-term viremia observed was statistically associated with the absence of HEV seroconversion. HEV RNA was also frequently detected, at a late stage of infection (49 dpi), in the three different types of muscle tested: femoral biceps, psoas major or diaphragm pillar. The HEV RNA load could reach up to $1 \cdot 10^6$ genome copies per gram of muscle. Detection of HEV in muscle meat was statistically associated with high HEV loads in corresponding liver and fecal samples. The presence of HEV in pig blood, femoral biceps and major psoas, corresponding to ham and tenderloin muscles respectively, is of concern for the food industry. Hence, these results indicate new potential risks for consumers and public health regarding pork products.

1. Introduction

Hepatitis E virus (HEV) is responsible for acute and occasionally chronic hepatitis in humans after enteric transmission. In developed countries, it is mainly of zoonotic origin, with pigs being the major reservoir (Pavio et al., 2017). Confirmed cases of zoonotic transmission have been associated with the consumption of raw or undercooked food products containing infected pig liver (e.g. pig liver sausages) (Colson et al., 2010; Guillois et al., 2016; Renou et al., 2014). More generally, case-control studies have identified the consumption of pig meat products as a major factor associated with HEV infections. Said and colleagues (2017) demonstrated, using epidemiological data collected

from confirmed cases, that consuming ham and/or sausages from a given British supermarket brand was statistically associated with a higher risk of having an HEV infection (Said et al., 2017). Faber et al. (2018) collected exposure data from notified hepatitis E cases in Germany, with individually matched population controls, using a semi-standardized telephone interview. They identified ready-to-eat pork products (e.g. raw ham, frankfurter, spreadable sausages made of raw meat, liver sausage or liver pâté) as major sources for autochthonous hepatitis E (Faber et al., 2018). Data on the prevalence of HEV in pork products, other than in pig livers, are still very sparse and few publications report on the detection of HEV RNA in different categories of pig meat (e.g. sausages) (Berto et al., 2012; Di Bartolo et al., 2012;

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Intharasongkroh et al., 2017; Szabo et al., 2015). One study found up to 20% of raw sausages (salami, without liver added) to be positive for HEV RNA (Szabo et al., 2015).

HEV RNA has been frequently detected in liver, bile or fecal samples from slaughtered pigs, (Salines et al., 2017), but until now, few studies have examined the presence of HEV RNA in other organs or tissues of naturally infected pigs at slaughterhouse time. In one report, HEV RNA was amplified in several organs and tissues, such as the bladder (10/43) or tonsils (3/43) of slaughtered pigs ($n = 43$) (Leblanc et al., 2010). In this study, none of the loin samples tested were HEV-positive (Leblanc et al., 2010). In a second report, HEV RNA was present along the different stages of the pork production chain, from the carcass dissection to liver removal steps and in pig lingual muscle, with an estimated prevalence of 2.7% ($n = 112$) (Di Bartolo et al., 2012).

HEV replicates in the liver but HEV RNA can be amplified in other pig organs and tissues after experimental infections (Bouwknegt et al., 2009; Williams et al., 2001). The detection of HEV-negative strands (replication intermediate) suggests that HEV can replicate in extra-hepatic sites, such as the small intestine, lymph nodes, and colon (Williams et al., 2001). In another study, positive HEV RNA hybridization signals were also detected in the liver, small and large intestine, tonsil, spleen, and kidney (Choi and Chae, 2003), supporting the presence of HEV in organs other than liver. In the study described by Bouwknegt et al. (2009), where the course of HEV infection was determined in pigs after intravenous inoculation and contact-infection, HEV RNA was detected in the longissimus, biceps femoris and iliopsoas, of both animal categories (Bouwknegt et al., 2009). The authors could not determine whether this was due to intrinsic and/or extrinsic contamination (i.e. cross-contamination with blood during necropsy).

HEV infection in pigs is usually acute, asymptomatic and self-resolving within 3 weeks (Salines et al., 2017). However, like in humans, where chronic cases are observed in solid organ transplant recipients under immunosuppressive treatment (Kamar et al., 2017), experimental HEV infection of pigs under active immune suppression led to chronic HEV shedding, lasting up to 13 weeks (Cao et al., 2017). In natural rearing conditions, pig immune responses can be modulated by frequent intercurrent infection with immune-modulating porcine viruses (e.g. porcine reproductive and respiratory syndrome virus) (Rahe and Murtaugh, 2017). Long-term HEV infection may influence the within-host course and HEV dissemination in organs. No study has addressed the question of the presence of HEV in pig organs during chronic infection, which is important regarding the risk of HEV presence in pig blood or meat at slaughter time. We have previously shown that co-infection with HEV and porcine reproductive and respiratory syndrome virus (PRRSV) affects the HEV time course. PRRSV co-infection with HEV extended HEV fecal shedding by a factor of 5, and increased the frequency of HEV RNA detection in pig livers at late stages of infection (49 days) (Andraud et al., 2013; Salines et al., 2015). These results suggest that HEV pathogenesis and dissemination could be affected by PRRSV co-infection. Thus, the aim of the present study was to assess the presence of HEV in serum and muscle meat of pigs in the context of PRRSV co-infection, after a natural route of inoculation.

2. Materials and methods

2.1. HEV/PRRSV co-infection experiment

Experimental HEV/PRRSV co-infection of Specific-Pathogen-Free (SPF) pigs was previously described (Salines et al., 2015). Briefly, 18 Large-White piglets were randomly allocated to 3 independent pens (3 inoculated and 3 contact piglets per pen). In each pen, the inoculated piglets received: (i) orally 10^8 HEV RNA copies in a volume of 10 mL of a genotype 3 HEV suspension (strain FR-SHEV3e, Genbank accession number JQ953665) prepared according to the protocol described in Andraud et al. (2013); and (ii) 2.5 mL per nostril of a PRRSV suspension (strain PRRS-FR-2005-29-24-1 “Finistere”, genotype 1, subtype 1,

Genbank accession number KY366411) titrating 10^5 TCID₅₀/mL. Two negative control pigs were included in a separate room. The protocol was approved by the Anses/ENVA/UPEC Ethics Committee (Approval No. 16 with the French National Committee for Ethics in animal experimentation). Since HEV is a zoonotic agent, biosecurity measures were applied to prevent any transmission from pigs to animal care handlers.

2.2. Sample collection

Blood samples were collected once a week until the end of the study (49 dpi). For euthanasia, anesthesia was carried out with intravenous injection of 1 g/50 kg live weight of Nesdonal® (thiopental-sodium, Merial, Lyon, France). This anesthesia is highly reproducible and has no impact on the quality of bleeding thereafter. Exsanguination was then performed by cutting deeply with a sharp blade into the carotid artery on both sides, with the anesthetized pigs hung by the legs. The carcasses were processed 20 min after exsanguination to ensure the absence of remaining blood flow. Necropsy was performed and liver and muscles samples (femoral biceps, psoas major and diaphragm pillar) were collected and kept frozen until used. To avoid cross-contamination, each muscle sample was handled using single use sterile materials (gloves, clamps, blades, and tips).

2.3. RNA extraction

RNA extractions from serum, fecal or muscle juice samples were performed manually using the QIAamp Viral RNA extraction Mini kit (QIAGEN, Illkirch, France), according to the manufacturer's instructions, except that sample size was 200 μ L. Fecal samples were solubilized in a 10% phosphate buffered saline suspension. Muscle juices were recovered after one cycle of freeze and thaw at -20°C , from 20 g of each muscle (Feurer et al., 2018). Comparison of HEV recovery rate from muscle juice or from muscle homogenate (Fast-prep 24, MP Biomedicals, Illkirch, France), was performed after spiking with a viral suspension of HEV3 (Genbank accession number EF494700), and showed similar results (data not shown).

2.4. HEV RNA quantification

HEV RNA quantification in serum, liver and muscles samples was performed, after RNA extraction, using real-time quantitative RT-PCR targeting HEV ORF3 (Jothikumar et al., 2006). Standard quantification curves were produced by plotting the quantification cycle (Cq) values against the logarithm of the input copy numbers of a standard RNA. Standard RNA was obtained after *in vitro* transcription of a plasmid pCDNA 3.1 ORF 2–3 HEV, as previously described (Barnaud et al., 2012). Results were expressed in HEV RNA copy number per gram of feces or muscle or per milliliter of serum (RNA copies/g or RNA copies/mL).

2.5. HEV serology

HEV serology was previously determined, and is presented in Fig. 3 of the publication on HEV/PRRSV co-infection of pigs (Salines et al., 2015). Briefly, anti-HEV antibodies were detected using the HEV ELISA 4.0v kit (MP Diagnostics, Illkirch, France), according to the manufacturer's instructions, except the serum quantity used (10 μ L instead of 20 μ L). Samples were positive when the optical density at 450 nm wavelength was higher than the threshold defined as the mean for negative controls + 0.3.

2.6. Statistical analysis

Time to viremia onset, viremia duration and period between shedding and viremia were estimated using a parametric survival model.

Table 1

HEV RNA quantification in serum, muscle, feces and liver samples from HEV/PRRSV co-infected animals and contact pigs (n = 20).

	dpi	Viremia kinetic (RNA copies/mL serum)						HEV RNA in muscle at 49 dpi (RNA copies/g)			HEV RNA in feces at 49 dpi (RNA copies/g)	HEV RNA in liver at 49 dpi (RNA copies/g)	
		7	14	21	28	35	42	49	FB	PM			DP
Group 1	Control	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Control	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Inoculated	nd	nd	nd	3.56 · 10 ³	1.43 · 10 ⁴	1.91 · 10 ⁴	1.70 · 10 ³	nd	nd	nd	1.49 · 10 ⁷	1.46 · 10 ⁶
	Inoculated	nd	nd	4.22 · 10 ⁴	3.85 · 10 ⁴	7.70 · 10 ⁴	2.14 · 10 ⁴	4.02 · 10 ³	nd	nd	nd	5.85 · 10 ⁷	1.87 · 10 ⁶
	Inoculated	nd	nd	1.33 · 10 ⁴	2.92 · 10 ⁴	7.49 · 10 ⁴	5.21 · 10 ⁴	2.15 · 10 ⁴	nd	nd	1.43 · 10 ⁴	6.40 · 10 ⁷	1.02 · 10 ⁶
	Contact	nd	nd	nd	nd	2.12 · 10 ⁴	2.80 · 10 ⁴	9.44 · 10 ³	nd	nd	nd	4.87 · 10 ⁷	8.72 · 10 ⁵
Group 2	Control	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Control	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Inoculated	nd	nd	4.51 · 10 ³	2.07 · 10 ⁴	2.24 · 10 ⁴	nd	nd	4.33 · 10 ³	nd	6.23 · 10 ³	1.45 · 10 ⁵	1.56 · 10 ³
	Inoculated	nd	nd	2.16 · 10 ⁴	2.43 · 10 ⁴	2.05 · 10 ⁴	1.24 · 10 ⁴	nd	nd	2.18 · 10 ⁴	2.70 · 10 ³	6.80 · 10 ⁶	2.42 · 10 ⁶
	Inoculated	nd	nd	6.31 · 10 ⁴	1.69 · 10 ⁴	5.55 · 10 ⁴	2.80 · 10 ⁴	nd	8.14 · 10 ³	3.55 · 10 ³	5.40 · 10 ³	4.86 · 10 ⁶	3.59 · 10 ⁵
	Contact	nd	nd	nd	nd	nd	2.64 · 10 ⁴	7.33 · 10 ³	5.91 · 10 ⁴	6.75 · 10 ⁵	6.42 · 10 ³	5.55 · 10 ⁷	1.12 · 10 ⁶
Group 3	Control	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Control	nd	nd	nd	nd	1.76 · 10 ⁴	6.64 · 10 ⁴	2.94 · 10 ³	6.92 · 10 ³	nd	nd	3.07 · 10 ⁸	3.44 · 10 ⁶
	Inoculated	nd	nd	nd	4.40 · 10 ³	2.08 · 10 ⁴	2.11 · 10 ⁴	1.22 · 10 ⁴	nd	nd	nd	2.08 · 10 ⁸	3.24 · 10 ⁶
	Inoculated	nd	nd	nd	nd	8.68 · 10 ³	1.72 · 10 ⁴	1.92 · 10 ⁴	nd	1.59 · 10 ⁴	6.17 · 10 ³	5.75 · 10 ⁷	2.04 · 10 ⁴
	Inoculated	nd	nd	4.10 · 10 ⁴	1.95 · 10 ⁴	1.46 · 10 ⁴	1.00 · 10 ⁴	1.15 · 10 ³	nd	1.09 · 10 ⁶	1.62 · 10 ⁴	5.61 · 10 ⁷	9.63 · 10 ⁵
	Contact	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	7.39 · 10 ⁵	nd
Contact	nd	nd	nd	nd	nd	nd	nd	5.32 · 10 ³	nd	nd	1.03 · 10 ⁸	5.87 · 10 ⁵	
Contact	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	8.29 · 10 ⁵	nd	

Quantitative RT-PCR results on individual serum samples (HEV RNA copies/mL of serum) at each sampling time and from fecal, liver and muscle samples at necropsy (HEV RNA copies/g). Shaded zones correspond to periods in which infected individuals were viremic and to HEV-positive fecal, liver and muscle samples. dpi: days post infection; nd: not detected; FB: femoral biceps; PM: psoas major; DP: diaphragm pillar.

Two parametric models were tested (lognormal and Weibull distributions of survival times) and compared using Akaike Information Criterion (AIC). Statistical associations between viral RNA quantities in the different matrices were evaluated using Pearson correlation tests. The links between HEV quantities in serum and muscles and the seroconversion as regards HEV (as binary variable) were assessed using Kruskal–Wallis tests. Statistics were analyzed using R software (Ihaka and Gentleman, 1996).

3. Results

The results for HEV RNA quantification in serum, muscle, liver and fecal samples are presented in Table 1. HEV RNA was detected in the serum samples of all inoculated animals at 35 dpi and in 45% of contact pigs at 42 dpi (Table 1, Fig. 1). HEV RNA yields in the sera ranged from $1.1 \cdot 10^3$ to $7.7 \cdot 10^4$ RNA copies/mL. Time-to viremia onset, viremia duration and period between HEV shedding and viremia were fitted to lognormal distributions. On average, HEV viremia, in both inoculated and contact pigs, started at 23.4 dpi [95% confidence interval 21.2–25.7] and lasted 28.8 days [95% CI 18.6–44.8]. The delay between HEV fecal excretion and viremia was on average 7.9 days [95% CI 5.8–11.0], in both inoculated and contact infected pigs. At 49 dpi, HEV RNA quantities in the serum and feces of inoculated pigs were statistically correlated (correlation coefficient $CC = 0.83$, p -value < 0.01). In contact pigs, significant associations were found between HEV RNA levels in serum and liver ($CC = 0.82$, p -value < 0.01).

Results on HEV seroconversion of infected pigs have been already published (Salines et al., 2015). Briefly, 4 out of 9 inoculated animals produced anti-HEV antibodies between 35 and 49 dpi, and 7 out of 9 contact individuals seroconverted between 42 and 49 dpi. Statistical analysis indicated that at 49 dpi, in both inoculated and contact pigs, high viral load in serum was significantly associated with the absence of seroconversion during the study period (p -value < 0.01 and p -value < 0.05, respectively).

HEV RNA was detected in the three types of muscles tested: femoral biceps, psoas major and diaphragm pillar (Fig. 2), with quantities ranging from $2.3 \cdot 10^3$ to $1.1 \cdot 10^6$ RNA copies/g. No significant

differences in the proportions of positive samples, nor in the mean viral RNA levels, were found between the three types of muscles (p -value > 0.1).

No statistical associations were found between the different parameters tested: HEV RNA yields in serum and in muscle samples at 49 dpi; viremia duration and HEV presence in muscle; time to viremia onset and HEV presence in muscles; HEV presence in muscle and seroconversion; HEV quantities in muscle and seroconversion (p -value > 0.1). In contrast, in contact infected pigs, statistical associations were found between HEV RNA levels in muscle and liver ($CC = 0.79$, p -value < 0.01), and HEV RNA levels in muscle and feces ($CC = 0.68$, p -value < 0.05).

PRRSV viremia was detected in all inoculated animals and in all contact infected animals, except one that did not show any detectable PRRSV viremia (results shown in Salines et al., 2015).

4. Discussion and conclusions

Motivated by studies suggesting the presence of HEV in pig blood, muscle or meat products without pig liver (Berto et al., 2012; Di Bartolo et al., 2012; Grierson et al., 2015; Szabo et al., 2015) and by a previous study showing unusually long lasting HEV excretion after PRRSV co-infection (Salines et al., 2015), the presence of HEV RNA was investigated in serum and muscle meat of experimentally HEV/PRRSV co-infected pigs. We found that HEV RNA was frequently detected in both serum and muscles of co-infected pigs. Viremia started 7.9 days after initial fecal shedding and lasted 28.8 days. In a previous study by Bouwknegt et al. (2009), (where HEV transmission and dissemination were studied using a different setting, with different pigs, HEV strains, methods of detection and after intravenous inoculation), HEV contact infected pigs exhibited viremia starting after 13 days of fecal excretion and lasting 11 days (Bouwknegt et al., 2009). In the present study, earlier and longer viremia was observed in the HEV/PRRSV co-infected pigs, which may suggest that PRRSV co-infection modulates HEV physiopathology and length of viremia. The presence of HEV RNA in pig serum has been described in several studies performed at the slaughterhouse (for review Salines et al., 2017); hence the present data

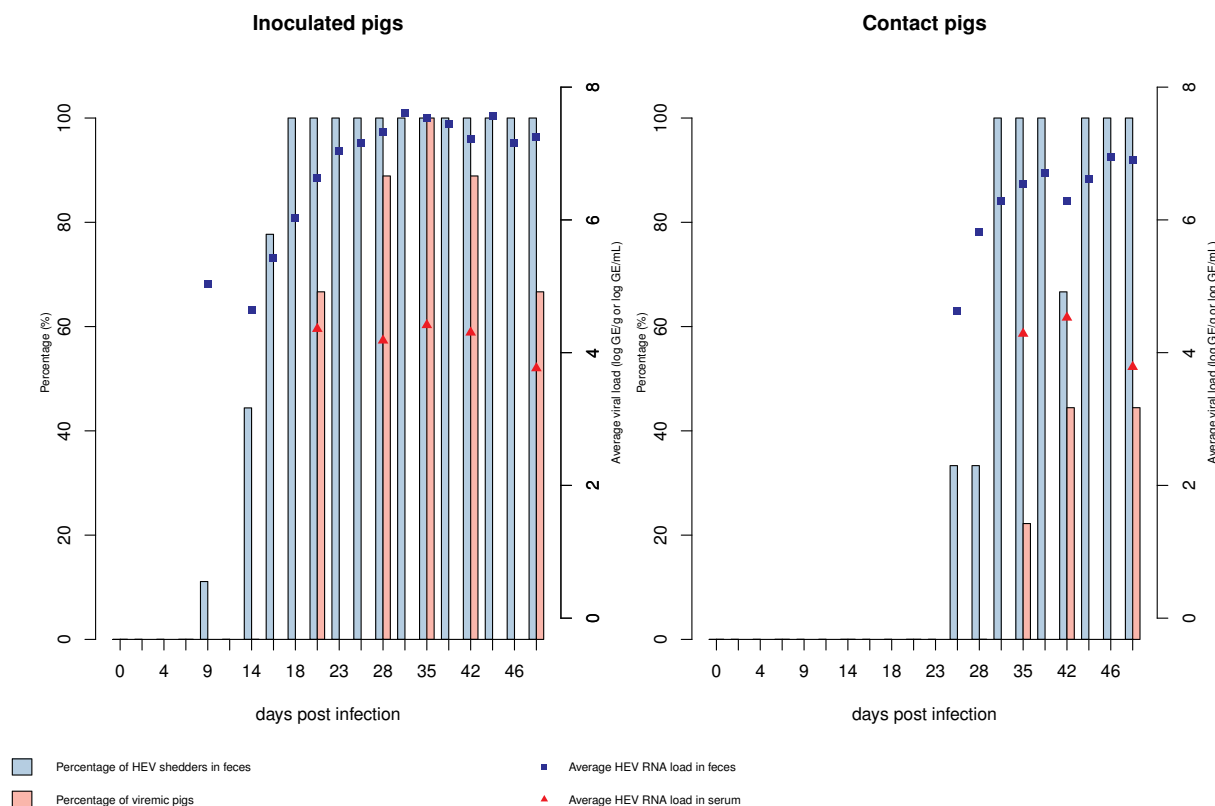


Fig. 1. Percentage of HEV shedding and viremic pigs and average HEV RNA copy numbers in feces and serum (log RNA copies/g or log RNA copies/mL) of HEV/PRRSV co-infected pigs (n = 18).

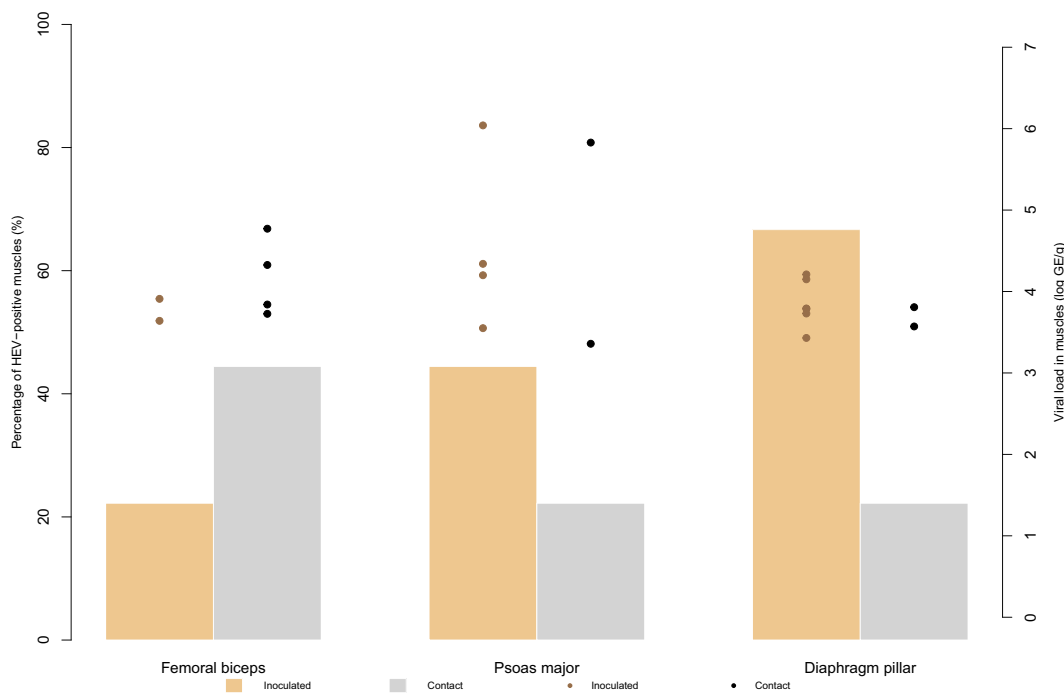


Fig. 2. Percentage of HEV-positive muscle (bars) and HEV RNA copies (log RNA copies/g) in positive muscle (dots) of HEV/PRRSV co-infected pigs (n = 18).

support the possible risk of HEV exposure through any pig blood-derived products insufficiently heated, used in the food industry.

In the present study as well, muscles of both infected and contact pigs were HEV-positive at 49 dpi. Bouwknegt et al. (2009) reported that only a few animals of the HEV-infected group were found HEV-positive in muscle up to 32 days after fecal shedding (Bouwknegt et al., 2009).

These findings therefore suggest that muscle from HEV/PRRSV co-infected pigs would be more likely to contain HEV at a later stage than during HEV-only infection.

In our previous results, we have shown that HEV-PRRSV co-infection was associated with delays in HEV seroconversion (Salines et al., 2015). PRRSV infection has an impact on innate immunity that also

affects the development of an effective adaptive immune response, such as production of neutralizing antibodies (Rahe and Murtaugh, 2017). Here, we observed prolonged and high-level viremia in the absence of seroconversion (Salines et al., 2015), in agreement with a lack of virus neutralization. Furthermore, for the 3 inoculated pigs of group 2, HEV RNA was amplified at high levels in muscle, but not detected in serum at 49 days post inoculation (Table 1), which is a rather unique finding. It can be hypothesized that, in some circumstances, induced by the co-infection with PRRSV, HEV may replicate in muscle cells, in spite of the absence of apparent HEV replication in muscle, as shown in one experimental infection (no negative-strand of HEV RNA detected pig muscle) (Williams et al., 2001). Another possible explanation would be that long-term viremia favors the interaction of HEV particles with heparan sulfate expressed at the surface of muscle cells. Indeed, it has been shown that heparan sulfate proteoglycans (HSPGs) are required for cellular binding of the hepatitis E virus ORF2 capsid protein and for viral infection (Kalia et al., 2009). In skeletal muscle, HSPGs are the major proteoglycans (PGs) in the basal lamina and on the cellular surface of myocytes (Sanes et al., 1986). This class of polysaccharides is highly expressed and plays a major role in the functional integrity of skeletal muscle (Jenniskens et al., 2006). In humans, two cases of HEV-associated severe myositis have been described (Del Bello et al., 2012; Mengel et al., 2016). One of them reports on a liver transplant recipient with acute hepatitis E, associated with Guillain-Barre syndrome (Del Bello et al., 2012). The patient developed severe muscle weakness and his condition worsened. HEV viremia was found by RT-PCR, but HEV RNA was undetectable in cerebrospinal fluid. A biopsy of the left biceps showed myopathic changes, with a significant percentage of necrotic muscle fibers (10%), and signs of inflammation. The presence of HEV RNA in the muscle was not investigated in the biopsy. It would be of interest to test for the accumulation of HEV particles in muscle in humans, in cases of severe myositis. The presence of HEV in pig muscle, as observed during the present study, may also have an impact on the understanding of HEV physiopathology.

Although the oral infectious dose of HEV in humans is unknown, in pigs it is estimated to be 10^5 HEV RNA copies (Andraud et al., 2013). Here, up to $6 \cdot 10^5$ HEV RNA copies/g of muscle (psoas major) were quantified. It is therefore possible that these HEV quantities are sufficient to induce an infection in case of consumption of infected raw or undercooked meat. To prevent such exposure of consumers, in the absence of surveillance of HEV in pig meat, consumers should be advised to cook pork products very well.

The present findings highlight that pig meat products such as ham and tenderloin may contain HEV, under specific circumstances. Studies in natural conditions of pig breeding, with multi-pathogen exposure, would provide new insights into HEV dissemination in pigs.

A study conducted on 1034 pig muscles collected in French slaughterhouses did not show any HEV-positive sample, not even in pigs with HEV-positive liver (Feurer et al., 2018). Comparison with the present study is limited since the parts of muscle collected were different (gluteus medius or semi-membranosus), and no indication was provided regarding the pigs' PRRSV status. Hence, based on our study, investigation on the presence of HEV-positive muscles at the slaughterhouse should be conducted with a larger sample, stratified on the farm PRRSV status, and collecting femoral biceps and psoas major muscles.

Ham and tenderloin muscle can be consumed dried or undercooked (rare), respectively. HEV infectivity has not been directly assessed in drying conditions, but HEV remains infectious after 28 days at room temperature (Johne et al., 2016). Sufficient cooking, 20 min at 71 °C, inactivates HEV in food products contaminated artificially (Barnaud et al., 2012).

From our observations, co-infections with swine pathogens impairing the immune response against HEV may increase the risk of contaminated pig meat and products entering the food chain. Further studies are required to investigate whether other intercurrent infections

(porcine circovirus-2), exposure to immunomodulatory molecules (toxins), or stress conditions would have an impact on the HEV infection course.

In conclusion, HEV contamination of pig meat and, not only of pig livers, has to be considered when assessing the HEV risk related to the consumption of pork products from a public health perspective, and surveillance plans should be implemented in the pork chain. We found that the presence of HEV in muscle might be predictable from the fecal viral genome load, which would be of great interest for easier detection of infected animals at the slaughterhouse. Testing fecal samples could therefore make it possible to identify pigs at risk of introducing infected meat into the food chain.

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Authors' contributions

MS analyzed the data and drafted the manuscript. AD and GS analyzed the HEV samples and interpreted the results. PR and OB analyzed and interpreted the results from PRRSV samples. MA participated in data analysis. NR designed and coordinated the study, and participated in animal experiments and data analysis. NP supervised the HEV laboratory work and contributed to the coordination of the study and manuscript writing. All co-authors revised the manuscript and approved the final submitted version.

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Publication 5

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Short communication

Impact of porcine circovirus type 2 (PCV2) infection on hepatitis E virus (HEV) infection and transmission under experimental conditions



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ABSTRACT

Hepatitis E virus is a zoonotic pathogen for which pigs have been identified as the main reservoir in industrialised countries. HEV infection dynamics in pig herds and pigs are influenced by several factors, including herd practices and possibly co-infection with immunomodulating viruses. This study therefore investigates the impact of porcine circovirus type 2 (PCV2) on HEV infection and transmission through experimental HEV/PCV2 co-infection of specific-pathogen-free pigs. No statistical difference between HEV-only and HEV/PCV2-infected animals was found for either the infectious period or the quantity of HEV shed in faeces. The HEV latency period was shorter for HEV/PCV2 co-infected pigs than for HEV-only infected pigs (11.6 versus 12.3 days). Its direct transmission rate was three times higher in cases of HEV/PCV2 co-infection than in cases of HEV-only infection (0.12 versus 0.04). On the other hand, the HEV transmission rate through environmental accumulation was lower in cases of HEV/PCV2 co-infection ($4.3 \cdot 10^{-6}$ versus $1.5 \cdot 10^{-5}$ g/RNA copies/day for HEV-only infected pigs). The time prior to HEV seroconversion was 1.9 times longer in HEV/PCV2 co-infected pigs (49.4 versus 25.6 days for HEV-only infected pigs). In conclusion, our study shows that PCV2 affects HEV infection and transmission in pigs under experimental conditions.

1. Introduction

Hepatitis E virus is a non-enveloped single-stranded RNA virus that can cause acute hepatitis in humans. Chronic cases have also been described, mainly in immunocompromised patients (Lhomme et al., 2016). Genotypes 3 and 4 affect both humans and other animal species, and are responsible for sporadic autochthonous cases of hepatitis in humans in industrialised countries (Doceul et al., 2016). In particular, genotype 3 is widespread in pig populations (Salines et al., 2017) and a number of autochthonous cases have been linked to the consumption of undercooked pork meat, especially liver products (Colson et al., 2012; Guillois et al., 2016). In order to limit the risk of contaminated products entering the food chain, it is crucial to understand the factors influencing HEV transmission and persistence in pig herds. High variability in HEV infection dynamics has previously been described (Salines et al.,

2017) and may be related to husbandry practices in terms of hygiene, biosecurity and rearing conditions (Walachowski et al., 2014; Lopez-Lopez et al., 2018) or to individual characteristics such as protection conferred by maternally-derived antibodies (Andraud et al., 2014). Various factors affecting swine immune response may also influence the course of HEV infection. Notably, in a previous study, we have shown that pigs experimentally co-infected with porcine reproductive and respiratory syndrome virus (PRRSV) exhibited chronic HEV infection with extended latency and infectious periods, increased faecal shedding and transmission, as well as an increased risk of HEV-positive livers at slaughter (Salines et al., 2015). Porcine circovirus type 2 (PCV2) also has immunomodulating characteristics for instance by inhibiting IFN- α production and by increasing the expression of IL-10, an anti-inflammatory cytokine (Darwich et Mateu, 2012). PCV2 may therefore impact HEV infection dynamics. Moreover, as the primary causative

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agent of post-weaning multisystemic wasting syndrome (PMWS) and other porcine circovirus-associated diseases (PCVADs), it can sometimes induce hepatitis in pigs (Rosell et al., 2000). However, to date, only few data report on HEV/PCV2 co-infection (Martin et al., 2007; Hosmillo et al., 2010; Savic et al., 2010; Yang et al., 2015; Jackel et al., 2018). In these studies, PCV2 and HEV were simultaneously detected in pigs but the impact of co-infections on HEV dynamics was not investigated.

Given the lack of data on this specific issue, the present study was designed to investigate how PCV2 infection impacts HEV infection dynamics (in terms of viral shedding duration and quantity, transmission and humoral immune response). A transmission experiment was therefore carried out, with specific-pathogen-free (SPF) pigs infected with HEV or co-infected with HEV and PCV2 at the same time.

2. Material and methods

2.1. Experimental design

The trial was conducted at ANSES's air-filtered level-3 biosecurity facilities. The 44 five-week-old SPF Large White piglets included in the study were HEV- and PCV2-free and with no maternal antibodies against these two viruses at the beginning of the study. These piglets were randomly allocated into eight groups, housed in six rooms (Fig. 1). Two negative control pigs were housed in Room 1. The four piglets housed in Room 2 were only orally inoculated with a PCV2-b genogroup suspension (GenBank accession number AF201311), titrating 10^5 TCID₅₀/mL in a volume of 5 mL. In Rooms 5 and 6 (groups 4, 5, 6), three piglets per group were orally inoculated with 10^7 HEV RNA copies of a genotype 3 HEV suspension (strain FR-SHEV3f, GenBank accession number JQ953666) in a volume of 10 mL. In Rooms 3 and 4 (groups 1, 2, 3), three piglets per group were orally inoculated with both HEV and PCV2, following the same inoculation protocols as for the other groups. In each of the six groups (HEV-only and HEV/PCV2), the three inoculated piglets were in contact with three pen mates (contact piglets) from day 1. Individual faecal samples were collected three days

before inoculation and three times a week until the end of the experiment at 49 days post inoculation (dpi). Blood samples were collected before inoculation and once a week until the end of the experiment. Clinical examination was also performed (clinical signs, rectal temperature, faeces consistence, weight, food consumption and trough cleanliness were recorded daily). After euthanasia, necropsies were performed and organ and fluid samples collected, among them liver and bile samples. The experiment was performed in accordance with EU and French regulations on animal welfare in experiments. The protocol was approved (referral 17-022) by the ANSES/ENVA/UPEC ethical committee registered under number #16.

2.2. Sample analyses

After performing manual total RNA extraction, HEV RNA in faecal samples was quantified using real-time quantitative RT-PCR as described in Barnaud et al. (2012). Results were expressed in HEV RNA copy number per gram of faeces (RNA copies/g). Since HEV shedding in faeces and presence in serum have been shown to be correlated (Salines et al., 2018), HEV RT-PCR was performed on serum samples of 49 day-old pigs only if their faeces were positive at 46 and/or 49 dpi. Similarly, and as bile is considered as a relevant proxy of liver status (de Deus et al., 2008; Bouwknegt et al., 2009), bile samples of 49-day old pigs having positive faecal samples at 46 and/or 49 dpi were analysed. Anti-HEV antibodies were detected using the HEV ELISA 4.0 V kit (MP Diagnostics, Illkirch, France) according to the manufacturer's instructions, apart from the serum quantity used (10 μ L instead of the recommended 20 μ L). Samples were considered to be positive when their optical density (OD) at a wavelength of 450 nm was higher than the threshold, which was defined as the mean optical density of negative control pig samples + 0.3. PCV2 DNA was extracted and quantified from the serum using real-time PCR based on TaqMan technology as described in Grasland et al. (2005). Results were expressed in genomic equivalent DNA copies/mL of serum. PCV2-antibodies were detected by PCV2 specific ELISA as already described with a positive cut-off for OD ratios higher than 1.5 (Fablet et al., 2017).

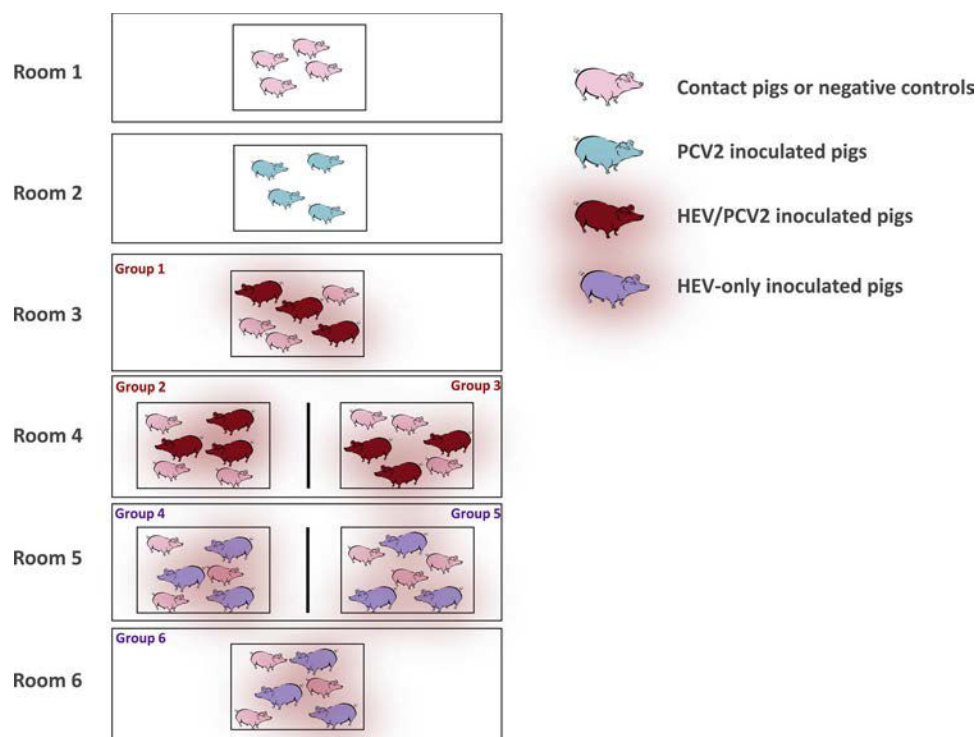


Fig. 1. Experimental design of the HEV/PCV2 co-infection trial.

2.3. Statistical analyses

The infectious period and time prior to HEV seroconversion were estimated using survival analyses. Two parametric models were tested (lognormal and Weibull survival time distributions) and compared using the Akaike Information Criterion (AIC). Cox-proportional hazard models were used to assess the effect of PCV2 co-infection on the lengths of the infectious period and the time prior to HEV seroconversion. The distributions of individual HEV viral loads in faeces were analysed according to time since inoculation (with and without co-infection). A linear mixed model taking into account repeated measurements over time was used for this investigation in order to assess the different quantities of HEV particles shed by co-infected as opposed to HEV-only infected pigs.

The HEV infection dynamics in each group were modelled using a SEIR (Susceptible – Exposed – Infectious – Recovered) model as per the estimation process described in Gallien et al. (2018). Briefly, pigs were considered as “susceptible” during the time window from exposure (day 0 = day of inoculation) to the point at which they actually became infected (t_{inf}), progressing to the “exposed” state. The time at which individuals were considered to be “infectious” (i.e. began shedding), denoted t_{sh} , was considered to lie between the times of the last HEV-negative PCR sample (t_{neg}) and the first HEV-positive PCR faecal sample (t_{pos}) for each animal ($t_{neg} < t_{sh} < t_{pos}$). The latency period δ_E therefore corresponds to the delay between infection and shedding ($\delta_E = t_{sh} - t_{inf}$). Pigs were considered “recovered” as soon as they no longer produced HEV-positive PCR samples. Two transmission routes were considered to be involved in this infection process: transmission by direct contact between pen mates and oro-faecal transmission via the environmental compartment. Environmental viral load E_t represents the accumulation of viral particles in the environment through faecal shedding by infected animals. E_t is partially offset by its clearance rate ($\delta = 0.3 \text{ day}^{-1}$) and was calculated as described in Andraud et al. (2013) and Salines et al. (2015). Let β_{DC} and β_{Env} denote direct contact and environmental transmission rates, respectively. The force of infection exerted on a typical susceptible individual i located in pen k at time t is defined by:

$$\lambda_k(t) = \beta_{DC} \frac{I_k(t)}{n-1} + \beta_{Env} \frac{E_k(t)}{n},$$

where I and E respectively represent the number of infectious animals and the viral load in pen k at time t , n being the total number of pigs in each pen. With these notations, the probability p_i of individual i getting infected at time $T_{inf}^{(i)}$ is given by

$$p_i = 1 - \exp(-\lambda_k(T_{inf}^{(i)}))$$

while the probability of having escaped infection in time interval $[0, t_{inf}^{(i)}]$ is given by

$$q_i = \exp\left(-\int_0^{T_{inf}^{(i)}} \lambda_k(\tau) d\tau\right)$$

An informative gamma prior was used to analyse the duration of the latency period δ_E . Its parameters were fixed using data from previous experiments and from observations of inoculated pigs ($\alpha = 4$, $\kappa = 3$). Very wide normal distributions were initially used as prior for the log-transformed transmission rates ($\log(\beta_{DC}) \sim N(-2, 4)$ and $\log(\beta_{Env}) \sim N(-8, 4)$). The global likelihood can be written as:

$$\begin{aligned} & L(T_{Neg}, T_{Pos}, I, E | \beta_{DC}, \beta_{Env}, \delta_E, T_{inf}, \alpha, \kappa) \\ &= \prod_{i \in \text{contact-infected}} e^{-\int_0^{T_{inf}^{(i)}} \lambda(\tau) d\tau} \times \left(1 - e^{-\lambda(T_{inf}^{(i)})}\right) \times \gamma(\delta_E^{(i)}, \alpha, \kappa) \\ & \quad \times \prod_{\substack{i \in \text{contact-} \\ \text{non infected}}} e^{-\int_0^{\max(t_{obs})} \lambda(t) dt} \\ & \quad \times \prod_{i \in \text{inoculated}} \gamma(\delta_E^{(i)}, \alpha, \kappa) \end{aligned}$$

The first term of the likelihood denotes the probability of detected infections occurring for an individual i at time $T_{inf}^{(i)}$; the second term represents the probability of observed infection failure whenever some individual would remain susceptible throughout the experiment; and the third term gives the distribution of the latency period in seeder pigs. Bayesian inference was performed using the Metropolis-Hastings algorithm: ten independent chains of 50,000 iterations were run with a burn-in period of 10%. Initial values were randomly drawn from prior distributions. Convergence was assessed by inspecting parameter outputs visually as well as through conventional diagnostic tests (Heidelberger, Geweke and Gelman-Rubin diagnostics). The impact of PCV2 infection on the HEV latency period and the transmission parameters' distribution were then assessed using a Kruskal-Wallis test. All the analyses were performed using R software (R 3.5.1).

3. Results

3.1. Infection data

No clinical sign related to PCV2 or HEV infection was observed in any infected pig. All PCV2 inoculated pigs and pigs in contact were seropositive at 28 dpi except one that was found seropositive at 45 dpi (Supplementary File 1). Control pigs and HEV-only inoculated pigs remained PCV2 seronegative throughout the study. PCV2 viraemia in contact pigs started between 10 and 28 dpi and lasted until 28–49 dpi. Viral loads ranged between 1.10^3 and 8.10^6 genomic equivalent DNA copies/mL of serum with a viraemia peak around 17 days post-inoculation (Supplementary File 1). HEV infection data are presented in Figs. 2 and 3 for quantitative RT-PCR on faecal samples and serological results respectively. All but two animals (one HEV/PCV2-inoculated pig and one HEV contact pig) shed HEV during the experiment. Inoculated animals started to shed HEV between 11 and 25 dpi, and contact animals between 23 and 46 dpi. Sporadic or intermittent shedding was observed in a few animals (Fig. 2). Of the 36 pigs, 20 produced anti-HEV antibodies: 14 of the 18 HEV-only infected pigs versus just six of the 18 HEV/PCV2 co-infected pigs. Seroconversion occurred between 24 and 49 dpi for inoculated animals, and between 38 and 45 dpi for contact animals (Fig. 3). At the end of the experiment, four out of the 17 analysed pigs (23%) had HEV RNA in their bile and one of them was viraemic (6%), with a viral load of $4.7.10^3$ RNA copies/mL (Fig. 2). These positive pigs were HEV/PCV2 co-infected (both inoculated and contact pigs).

3.2. Estimated durations related to HEV infection dynamics

Latency periods were estimated at 12.3 days [4.4–25.5] in HEV-only pigs and 11.6 days [2–21.6] in HEV/PCV2 co-infected pigs. The latency period was significantly shorter in HEV/PCV2 co-infected pigs than in HEV-only infected pigs ($p < 0.05$).

Survival analysis of the infectious period (lognormal distribution) gave a mean duration of 11.8 days [8.3–16.7] for HEV-only infected animals and 16.6 days [10.7–25.9] for HEV/PCV2 co-infected animals. No statistical difference was found between HEV-only and HEV/PCV2-infected pigs (HR = 0.6 [0.3–1.4], $p > 0.05$).

Survival analysis of the time prior to HEV seroconversion (using the Weibull distribution) gave a mean duration of 25.6 days [19.3–33.8] for HEV-only infection and 49.4 days [40.4–60.4] for HEV/PCV2 co-infection. The time prior to HEV seroconversion was statistically longer in HEV/PCV2- than in HEV-only infected pigs (HR = 0.3 [0.1–0.8], $p < 0.05$).

3.3. HEV shedding and environmental accumulation

The distribution of the shed HEV viral load against time (with and without co-infection) is shown in Fig. 4. The linear mixed model accounting for repeated measurements did not show the PCV2 infection to

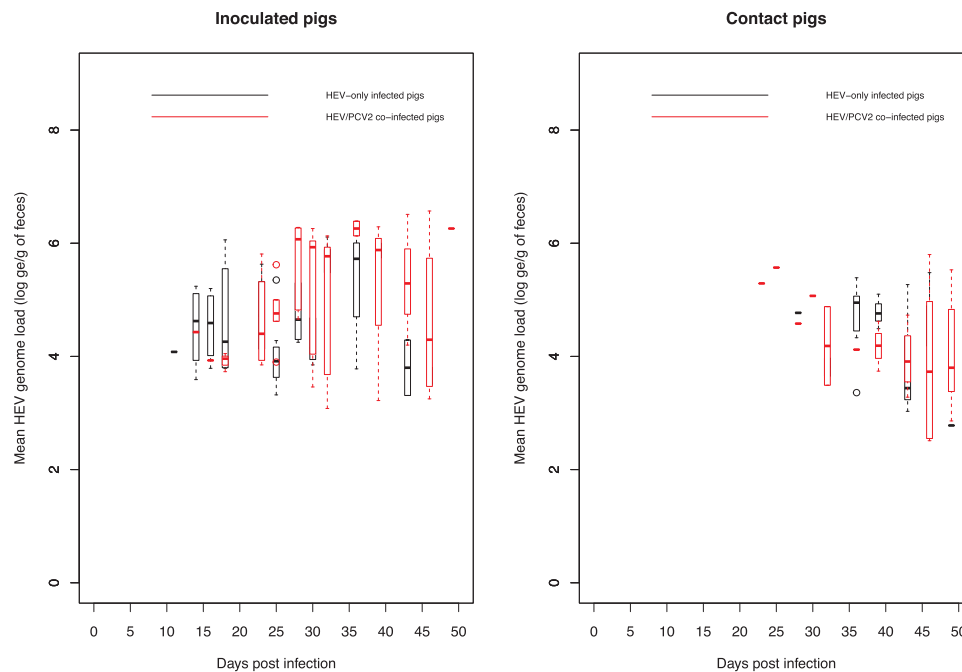


Fig. 4. Distribution of the number of HEV genome equivalents (log RNA copies/g faeces) shed by individual pigs, versus time, in HEV inoculated and contact animals with or without PCV2 co-infection ($n = 36$).

published studies on the topic, especially in cases of natural infection by the oral route (Bouwknegt et al., 2009; Andraud et al., 2013; Salines et al., 2015). This variability was taken into account for the parameter estimation by taking uninformative or little informative prior distributions; algorithm convergence therefore allows to gain confidence in the obtained results. For the HEV-only infected group, the infection kinetics slightly vary from those described in Andraud et al. (2013), who reported a latency period of 6.9 days [5.8–7.9] and an infectious period of 9.7 days [8.2–11.2]. This gap may be related to the different HEV strain used for inoculation (strain FR-SHEV3e in Andraud et al. (2013), versus strain FR-SHEV3f in the present trial) as well as to the lower inoculation dose (10^7 genomic equivalent in the present experiment versus 10^8 in the HEV/PRRSV experiment). In the trial described by Bouwknegt et al. (2009), the infectious period was estimated at between 13 and 49 days, depending on the replicate block, but their pigs were intravenously inoculated (versus oral inoculation in the present experiment).

From our analyses, no statistical difference was found between HEV-only and HEV/PCV2 groups, either in the infectious period, or in the quantity of HEV shed in faeces. The latency period was found to be less than one day shorter in HEV/PCV2 co-infected pigs than in HEV-only infected pigs which, although statistically significant, is likely to have a limited biological impact on HEV infection dynamics. The direct transmission rate of HEV was found to be three times higher in cases of HEV/PCV2 co-infection than in cases of HEV-only infection (0.12 versus 0.04), meaning that one co-infected pig is likely to infect three times more pigs than a pig infected only with HEV. The environmental transmission rate of HEV was found to be lower in cases of HEV/PCV2 co-infection ($4.3 \cdot 10^{-6}$ versus $1.5 \cdot 10^{-5}$ g/RNA copies/day for HEV-only infected pigs), meaning that three times more HEV particles in the environment are needed in order to infect a pig already carrying PCV2. The lower environmental force of infection in cases of PCV2 infection may delay HEV infections. Short time to slaughter after HEV infection seems to be a key point of liver contamination. Thus, delaying HEV infection is likely to increase the risk of pig livers containing HEV at slaughter time. Regarding immune response, fewer HEV/PCV2-infected pigs than HEV-only infected pigs presented a humoral immune response (6/18 versus 14/18 pigs, respectively). Moreover, the time prior to HEV

seroconversion was 1.9 times longer in HEV/PCV2 co-infected pigs than in HEV-only infected pigs (49.4 versus 25.6 days). This could be especially problematic if pig HEV status is screened using serological method: this long time prior to HEV seroconversion would lead to many false negative animals. Although PCV2 did not affect HEV infection dynamics as much as PRRSV did in the trial that we previously conducted (Salines et al., 2015), it cannot be excluded that in combination with other factors, as for PMWS, it may influence HEV infection. This is consistent with the immunomodulating effect of both PCV2 and PRRSV described in literature, where innate immunity is somewhat suppressed due to a reduction in the $IFN\alpha$ response, delaying the onset of the adaptive response (Darwich et Mateu, 2012; Butler et al., 2014). Four out of the 17 tested pigs had HEV RNA in the bile at the end of the experiment, which can be considered as a reliable proxy of the liver contamination. This late-stage positivity illustrates the increased risk of having HEV positive livers entering the food chain when animals were co-infected. Moreover, the detection of one HEV/PCV2 co-infected pig being HEV viraemic at the end of the experiment also raises the question of a potential risk linked to other pork products that is still debated in the literature (Salines et al., 2018). Further analyses would be necessary to assess the level of contamination of pig muscles in cases of PCV2 infection, especially as correlations between HEV RNA levels in muscles, liver and faeces have been shown (Salines et al., 2018). Such analyses could inform on the risk for public health linked to the consumption of undercooked or raw pig meat or other pork products that do not contain liver. Our present results could also be used to feed dynamic models representing HEV spread and persistence on farms in which PCV2 may circulate. Our data, obtained under controlled conditions, can also add supplementary explanations to the previously published field studies in which HEV and PCV2 were detected simultaneously in pigs and in which causal relationship was suspected but not demonstrated (Martin et al., 2007; Hosmillo et al., 2010; Savić et al., 2010; Yang et al., 2015; Jackel et al., 2018). Further work is needed to investigate whether there are other underlying immune mechanisms specific to co-infecting viruses. Moreover, it should be noted that the pigs in the present experiment were simultaneously inoculated with HEV and PCV2; the same kind of study could be reproduced with different inoculation time sequences (e.g. pigs

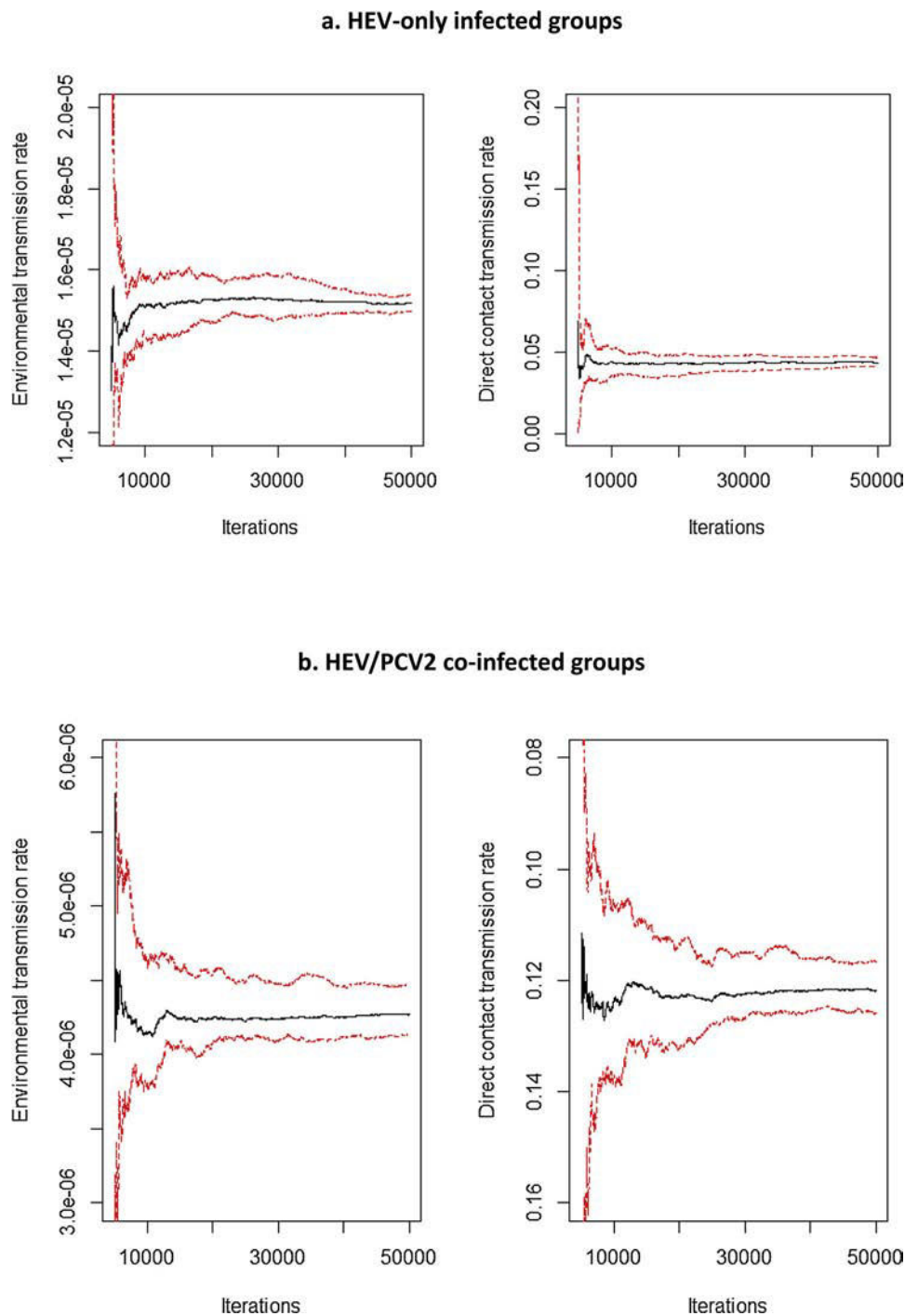


Fig. 5. Running average of transmission parameter estimates from ten independent Monte-Carlo Markov chains for (a) HEV-only and (b) HEV/PCV2-infected groups.

inoculated with PCV2 a week before HEV) and probably with more pigs included to reduce the impact of inter-individual variability in infection dynamics.

To our knowledge, this study is the first to focus on the impact of HEV/PCV2 experimental co-infection on HEV infection and transmission in pigs. Our results show that, in experimental settings, PCV2 co-infection increases the direct transmission of HEV and impairs the humoral immune response towards it. The effect observed in this PCV2/HEV co-infection trial was less marked than previously observed when PRRSV was involved, however, and failed to explain the long-term HEV shedding that has been observed in the field at an individual level. A combination of PCV2 co-infection with other factors may lead to

chronic HEV infection. Additional studies (e.g. on-farm intervention studies, other co-infection trials, dynamic modelling approaches) should therefore be conducted to explore the potential synergistic effects of multiple co-infections and devise effective control strategies that would include measures targeting intercurrent pathogens (vaccination, eradication programme).

Authors' contributions

MS and MA developed the mathematical model, analysed the data and drafted the manuscript. MP and CB analysed the HEV and PCV2 samples respectively, and interpreted the results. NP and BG supervised

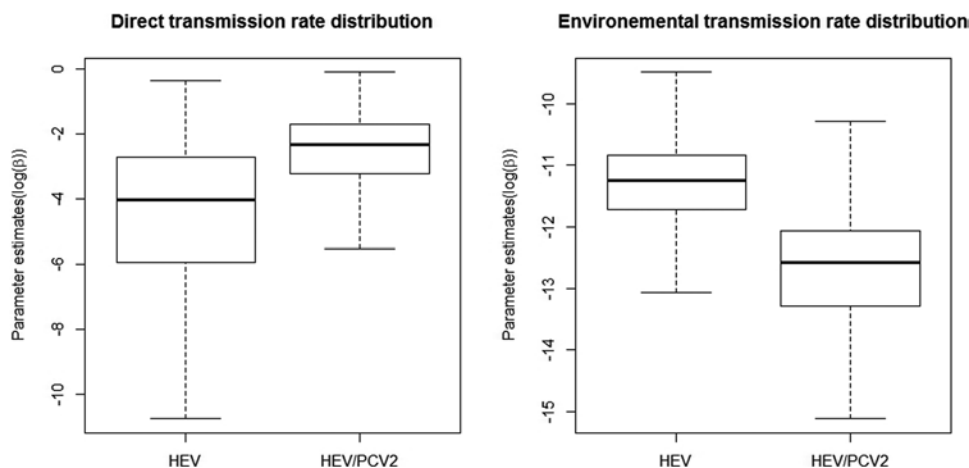


Fig. 6. Distribution of direct and environmental HEV transmission parameters estimated from ten independent Monte-Carlo Markov chains.

the HEV- and PCV2-related laboratory work respectively, and helped coordinate the study. NR conceived and coordinated the study, in addition to participating in the animal experiment and data analyses. All the co-authors revised the manuscript and approved the final submitted version.

Competing interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.vetmic.2019.05.010>.

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Ce qu'il faut retenir

Les études réalisées en conditions naturelles et expérimentales ont permis de mettre en évidence et de quantifier le rôle central des co-infections immunomodulatrices dans la dynamique de l'infection par le HEV chez le porc. Ces infections intercurrentes, notamment celle par le virus du SDRP, conduisent à une infection chronique par le HEV, augmentant ainsi significativement le risque de présence du HEV dans le foie lors de l'abattage des porcs. De plus, en situation expérimentale, il a été montré que des porcs co-infectés par le virus du SDRP présentent une virémie persistante et de l'ARN du HEV dans plusieurs de leurs muscles. Les co-infections immunomodulatrices ont ainsi un impact majeur sur le risque pour la santé publique lié au HEV.



Take home message

The studies conducted both under natural and experimental conditions have highlighted and quantified the central role of immunomodulating co-infections in the dynamics of HEV infection in pigs. These intercurrent infections, particularly that caused by PRRSV, lead to chronic HEV infection thus significantly increasing the risk of livers being HEV-positive at slaughter. Moreover, under experimental conditions, it has been shown that PRRSV/HEV co-infected pigs have persistent viraemia as well as HEV RNA in several muscles. Immunomodulating co-infections have therefore a major impact on the public health risk associated with HEV.

PARTIE 2.2. Une approche de modélisation multi-pathogènes pour comprendre la propagation et la persistance du virus de l'hépatite E dans un élevage de porcs naisseur-engraisseur

Les études de terrain précédemment présentées ont permis de mettre en évidence l'**impact majeur des co-infections immunomodulatrices** des porcs sur la dynamique de l'infection par le HEV, tant en conditions naturelles qu'expérimentales. Toujours à l'échelle individuelle, plusieurs études ont montré le **rôle de l'immunité maternelle** anti-HEV dans les profils d'infection et la transmission du HEV (Andraud *et al.*, 2014; Krog *et al.*, 2019). Au niveau de l'élevage, la **structure de l'élevage, certaines pratiques d'élevage, d'hygiène et de biosécurité** sont reconnues comme ayant aussi une influence sur la dynamique de l'infection par le HEV (Tableau III) (Di Bartolo *et al.*, 2008; Li *et al.*, 2009a; Jinshan *et al.*, 2010; Hinjoy *et al.*, 2013; Rutjes *et al.*, 2014; Walachowski *et al.*, 2014; Lopez-Lopez *et al.*, 2018).

Ainsi, il est essentiel de **prendre en compte tous ces facteurs explicatifs, de manière globale et intégrée**, pour comprendre les modalités de propagation et de persistance du HEV dans un élevage de porcs. Les **approches de modélisation dynamique** apparaissent alors tout à fait pertinentes pour intégrer la dimension liée à la population de porcs et celle relative aux caractéristiques épidémiologiques de l'infection par le HEV chez le porc. Si plusieurs études se sont attachées à décrire et quantifier la transmission du HEV entre les porcs, notamment en conditions expérimentales (Satou et Nishiura, 2007; Bouwknegt *et al.*, 2009; Bouwknegt *et al.*, 2011; Backer *et al.*, 2012; Andraud *et al.*, 2013), elles ne sont pas aisément transposables sur le terrain aux conditions réelles d'élevage, qui associent une population animale dynamique divisée en groupes d'animaux ayant une structure de contact hétérogène à de nombreux facteurs de variation liés à la conduite et aux pratiques d'élevage. **A ce jour, il n'existe pas de modèle prenant en compte la population dynamique d'un élevage et la circulation virale au sein de cet élevage**, seule assurance d'explorer des hypothèses de déterminisme de la propagation et de la persistance du HEV extrapolables à la situation réelle. Dans ce contexte, l'objectif de

l'étude présentée ci-après a été de développer une **approche de modélisation multi-pathogènes** afin de décrire et d'expliquer les conditions de la diffusion et de la persistance du HEV dans un élevage naisseur-engraisseur dans lesquels les porcs sont susceptibles d'être co-infectés par un pathogène intercurrent. Pour ce faire, un **modèle stochastique individu-centré** a été construit en couplant un **modèle de dynamique de population** avec un **modèle épidémiologique multi-pathogènes** représentant la diffusion conjointe et les interactions du HEV et d'un virus immunomodulateur (virus du SDRP, PCV2). Les paramètres du modèle sont principalement dérivés des études expérimentales préalablement conduites (*cf. supra*). Ce modèle a aussi été utilisé pour évaluer l'influence de la structure et de la conduite de l'élevage sur la dynamique du HEV dans l'élevage, ainsi que l'efficacité de stratégies de maîtrise du HEV.

Les résultats de ce travail de modélisation ont été soumis dans le journal *Epidemics* (Salines *et al.*, 2019d) et publiés dans les *Journées Recherche Porcine* (Annexe 4) (Salines *et al.*, 2019e). A l'issue de ce travail, et à la demande du Groupement Technique Vétérinaire (GTV) de Bretagne, un point d'actualité sur le HEV en général et les travaux de l'Anses en particulier a été publié dans un article associé à une communication orale lors de la Journée Vétérinaire Bretonne et dans le Bulletin des GTV (Annexe 5) (Salines *et al.*, 2019b).

Publication 6 (Submitted)

Salines M., Rose N., Andraud M., 2019. Tackling hepatitis E virus spread and persistence on farrow-to-finish pig farms: insights from a stochastic individual-based multi-pathogen model. *Epidemics*.
Submitted paper.

Tackling hepatitis E virus spread and persistence on farrow-to-finish pig farms: insights from a stochastic individual-based multi-pathogen model

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Abstract

Hepatitis E virus (HEV) is a zoonotic agent of which domestic pigs have been recognised as the main reservoir in industrialised countries. The great variability in HEV infection dynamics described on different pig farms may be related to the influence of other pathogens, and in particular viruses affecting pigs' immune response. The objective of this study was to develop a multi-pathogen modelling approach to understand the conditions under which HEV spreads and persists on a farrow-to-finish pig farm taking into account the fact that pigs may be co-infected with an intercurrent pathogen. A stochastic individual-based model was therefore designed that combines a population dynamics model, which enables us to take different batch rearing systems into account, with a multi-pathogen model representing at the same time the dynamics of both HEV and the intercurrent pathogen. Based on experimental and field data, the epidemiological parameters of the HEV model varied according to the pig's immunomodulating virus status. HEV spread and persistence was found to be very difficult to control on a farm with a 20-batch rearing system. Housing sows in smaller groups and eradicating immunomodulating pathogens would dramatically reduce the prevalence of HEV-positive livers at slaughter, which would drop from 3.3% to 1% and 0.2% respectively (p-value < 0.01). It would also decrease the probability of HEV on-farm persistence from 0.6 to 0 and 0.34 respectively (p-value < 0.01) on farms with a 7 batch rearing system. A number of

33 farming practices, such as limiting cross-fostering, reducing the size of weaning pens and
34 vaccinating pigs against immunomodulating viruses, were also shown to be pivotal factors for
35 decreasing HEV spread and persistence.

36

37 **Keywords**

38 Hepatitis E virus, farm-level risk factors, multi-pathogen model

39

40 **1. Introduction**

41

42 Hepatitis E virus (HEV) is a non-enveloped single-stranded RNA virus usually leading to
43 asymptomatic infections in humans, but which can also cause acute or chronic hepatitis
44 depending, inter alia, on the patient's immunity context (Emerson and Purcell, 2003; Kamar
45 et al., 2011). If genotypes 1 and 2 are exclusively human viruses mainly prevalent in
46 developing countries, genotypes 3 and 4 are shared by humans and other animal species, and
47 are responsible for sporadic human cases in industrialised countries (Dalton et al., 2008;
48 Purcell and Emerson, 2008). HEV-3 is particularly widespread in the swine population
49 (Salines et al., 2017) and a number of autochthonous cases have been linked to the
50 consumption of raw or undercooked pork products, especially those containing a high
51 proportion of liver (Colson et al., 2012; Guillois et al., 2016; Moal et al., 2012; Motte et al.,
52 2012). Hepatitis E is thus recognised as a foodborne zoonosis with domestic pigs being the
53 major reservoir in developed countries (Pavio et al., 2017). The risk of contaminated products
54 entering the food chain is intrinsically related to HEV dynamics in pig herds. However, the
55 epidemiology of HEV in the pig production sector is far from being fully understood. Indeed,
56 prevalence figures from the literature show a high between- and within-survey variability that
57 is only partially explained to date (Salines et al., 2017). This heterogeneity may indicate a
58 broad spectrum of infection dynamics related to farm-specific risk factors. For instance, farm-
59 level observational studies have evidenced that husbandry practices (in terms of hygiene,
60 biosecurity and rearing conditions) may favour HEV spread on farms (Walachowski et al.,
61 2014). Individual risk factors related to piglets' specific characteristics or inherited from their
62 dam have also been sporadically investigated using experimental trials or field studies. The
63 piglet's sex and sow's parity have thus been shown to influence HEV infection dynamics

64 (Salines et al., 2019b). Andraud et al. (2014) also evidenced that the partial protection
65 conferred by maternally-derived antibodies (MDAs) delayed HEV infection in growing pigs.
66 More recently, Crotta et al. (2018) developed a baseline Quantitative Risk Assessment (QRA)
67 model reproducing the dynamics of HEV infection in a closed population of naturally-
68 infected pigs in a farrow-to-finish pig farm in order to assess the risk of occurrence of
69 viraemic pigs at slaughter. Their model predicted 13.8% of viraemic pigs at slaughter. They
70 also highlighted that a reduction in the maternal immunity coverage would lead to a decrease
71 in the prevalence of viraemic pigs at slaughter (dropping to 12.5%), whereas a 100% passive
72 immunity cover would greatly increase the risk of viraemic pigs (19.8%).

73
74 Several studies have been conducted in order to describe and quantify HEV transmission
75 between pigs. For instance, Satou and Nishiura (2007) built a model that took the distribution
76 of time between infection and seroconversion into account to calculate age at infection. They
77 then estimated the basic reproduction ratio from serological data pertaining to Japanese pig
78 farms ($R_0 = 4.02-5.17$). Backer et al. (2012) obtained similar R_0 values using a Bayesian
79 framework to analyse the prevalence of HEV shedding according to age group from UK data.
80 They also assessed the effectiveness of control measures, including any potential vaccination
81 of pigs against HEV to come, which they found to be more effective when done later rather
82 than earlier in the pig's life. In 2009, Bouwknegt et al. (2009) estimated a higher R_0 of 8.8
83 [4.4-19] through the analysis of serial one-to-one transmission experiments with intravenous
84 inoculation of the initial seeder pig. The same team then developed a dose-response model to
85 assess the contribution of faeces as a source of HEV transmission among pigs (Bouwknegt et
86 al., 2011). They proved that the faecal-oral route of infection was likely but not sufficient to
87 explain the observed transmission, and concluded that other transmission routes may come
88 into play. The hypothesis of environmental transmission was further confirmed by Andraud et
89 al. (2013). An experimental trial was used to investigate HEV transmission factoring in
90 several routes: direct transmission between pen mates, within-pen environmental
91 transmission, and between-pen environmental transmission representing the transfer of faecal
92 material between adjacent pens. They highlighted that the first two modalities were the major
93 routes for HEV transmission and that HEV persistence and accumulation in the environment
94 due to faecal shedding played a major role in viral transmission among pigs.

95
96 Immunomodulating swine pathogens such as porcine reproductive and respiratory syndrome
97 virus (PRRSV) or porcine circovirus type 2 (PCV2) are highly prevalent in the pig production

98 sector, and are known to affect both innate and adaptive pig immune response (Butler et al.,
99 2014; Darwich and Mateu, 2012). Like the chronic hepatitis E cases described in
100 immunocompromised patients (Kamar et al., 2013), they may thus influence HEV infection
101 dynamics. For instance, HEV/PRRSV co-infection has been found to lead to chronic HEV
102 infection both under experimental and natural conditions (Salines et al., 2015; Salines et al.,
103 2019b). Indeed, the authors revealed that PRRSV co-infection delayed, extended and
104 increased HEV shedding, increased HEV transmission among pigs, and increased the risk of
105 HEV-positive livers at slaughter. Co-infection with PCV2 has also been shown to increase
106 direct HEV transmission and delay the time to HEV seroconversion under experimental
107 conditions (Salines et al., 2019a).

108
109 Although all these studies have helped disentangle HEV transmission patterns, they did not
110 combine HEV dynamics and population dynamics — the population being split into animal
111 groups with an extremely heterogeneous contact structure — with numerous external factors
112 linked to the batch rearing system (BRS) and various farming practices. Until now, there was
113 no model integrating both the dynamic population of a farm and HEV circulation on this same
114 farm. To fill this gap, the authors built a stochastic individual-based model to clarify the
115 conditions under which HEV spreads and persists in a farrow-to-finish herd in which pigs
116 may be co-infected with an intercurrent pathogen. This model couples the population
117 dynamics of a farrow-to-finish pig herd, including breeding and growing pigs, with a multi-
118 pathogen model. The latter combines two epidemiological models: the first one represents the
119 dynamics of an immunomodulating virus (hereafter noted IMV, e.g. PRRSV, PCV2) in a
120 simplified way, whereas the second one takes into account detailed epidemiological features
121 of HEV such as passive immunity, environmental compartments and co-infections with the
122 IMV. This kind of model may be used to monitor a wide range of output variables among
123 which the most relevant were selected to summarise the on-farm spread and persistence of
124 HEV and to evaluate the risk of HEV entering the food chain. The impact of the farm's
125 structure and potential control strategies (based on the modification of husbandry practices
126 and/or prophylactic measures targeting the intercurrent IMV) on viral spread and persistence
127 at herd level was also assessed. The aims of this study were therefore (1) to decipher HEV
128 infection dynamics on farrow-to-finish pig farms; (2) to evidence control strategies that could
129 be implemented on farrow-to-finish pig farms to reduce HEV spread and persistence in the
130 pig production sector. The overall goal of this project was to support risk management
131 decisions regarding HEV.

132 **2. Material and methods**

134 **2.1. Population dynamics model**

135
136 The population model represents the population dynamics on a typical farrow-to-finish pig
137 farm managed according to a specific batch rearing system (BRS) (Cador et al., 2016). As
138 such, three main hierarchical levels were considered: individual, population and facilities
139 (Andraud et al., 2009b).

141 **2.1.1. Individuals**

142
143 Individuals are characterised by an identity number, their age, sex, physiological stage and
144 their location on the farm (room and pen numbers). The individual physiological stage defines
145 the subpopulation the animal belongs to: growing pigs or breeding sows. Additional state
146 variables describe the sow's reproduction cycle: parity rank, time to next oestrus, time to next
147 parturition, and time to next artificial insemination (AI).

149 **2.1.2. Population**

150
151 The farm is managed according to a BRS, meaning that the herd population is divided into
152 batches. The reproductive cycle of sows in a given batch are synchronised so that all breeding
153 events (i.e. AI, farrowing and weaning) occur at the same time. Consequently, a given batch
154 of sows gives birth to piglets simultaneously, these contemporary piglets forming a group of
155 growing pigs also constituting a batch.

157 **2.1.3. Facilities**

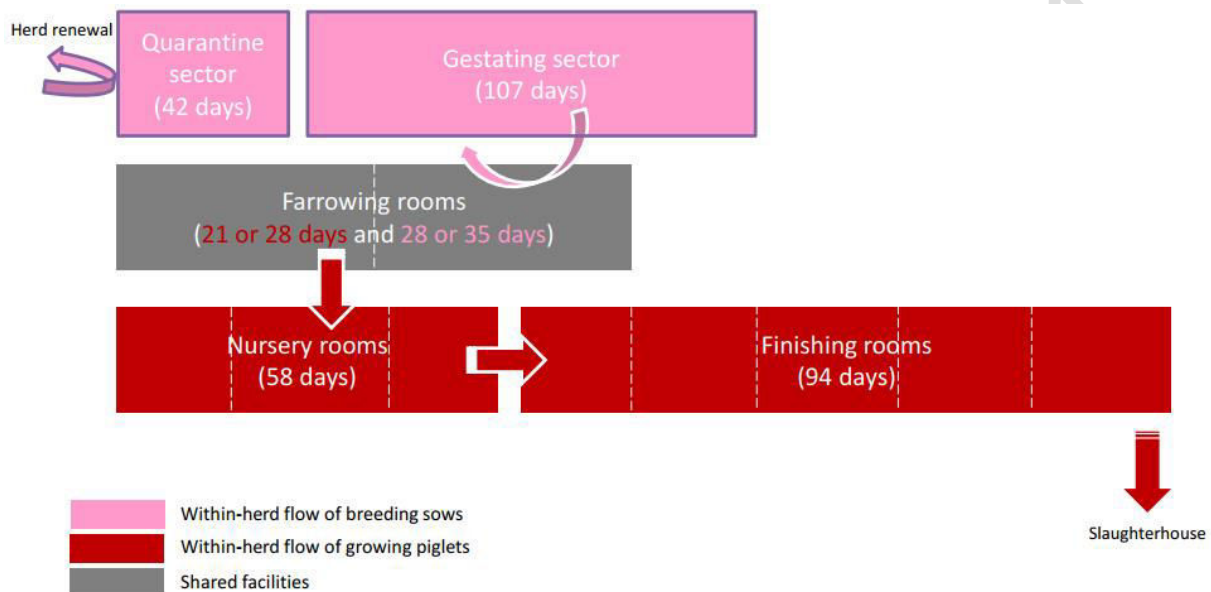
158
159 According to their physiological stage, animals evolve through five types of facilities: the
160 quarantine, gestating and farrowing facilities for breeding sows; the farrowing, nursery (i.e.
161 weaning) and finishing facilities for growing pigs (Figure 1). Farrowing, nursery and finishing
162 facilities are divided into several rooms, managed in line with an all-in-all-out principle, i.e.
163 all animals from the same batch leave the facility at the same time and immediately enter an
164 empty room. Each batch is therefore managed independently, with limited relationships

165 through environmental components. The quarantine sector is composed of a single room used
 166 for replacement gilts to become used to the herd's microbiota. The two subpopulations
 167 (breeding sows and growing pigs) physically interact only in farrowing rooms.

168

169 **Figure 1. Facilities modelled in the farrow-to-finish pig farm and duration of stay in**
 170 **each compartment.** Adapted from Cadore et al., 2016

171



172

173

174

175 **2.1.4. Processes related to population dynamics**

176

177 The parameters governing population dynamics are summarised in Table 1. More details on
 178 the population dynamics model are given in Supplementary File 1.

179 The breeding sow cycle: the sow's reproductive cycle lasts 145 days. Gilts are placed in the
 180 quarantine room for 42 days, whatever the BRS. After quarantine or weaning, both gilts and
 181 sows are moved to the gestation sector, where they are inseminated five days later. They
 182 remain in this sector until they reach 107 days of gestation. In the event of AI failure or
 183 abortion, the affected sows are transferred to the following batch.

184 Lactating stage: seven days before farrowing, sows enter the farrowing sector, where they
 185 give birth to a batch of piglets. Dams remain with their litter for three or four weeks until
 186 weaning, depending on the BRS. Cross-fostering practices are considered after colostrum

187 intake. At the end of the lactation period, sows are moved back to the service room to begin a
 188 new reproductive cycle, while piglets are moved to an empty nursery room.

189 The growing pig cycle: piglets stay in the nursery sector until 86 days of age, when they are
 190 moved to a finishing room. When they weigh over 115 kg or when they are older than 180
 191 days of age, they are sent to the slaughterhouse.

192
 193 All population events (death, litter size, culling and reproductive failures) are governed by
 194 probabilities related to the age of the animals or the time spent in each specific physiological
 195 state (Supplementary File 1). Only the movement between rooms and sectors is set
 196 deterministically with respect to the batch rearing system being considered (Table 1).

197
 198 **Table 1. Parameters governing the population dynamics model in 4-, 7- and 20-batch**
 199 **rearing systems.**
 200

Parameter description (unit)	Value / Distribution		
Type of batch rearing system	4 batches	7 batches	20 batches
Duration of a sow's reproductive cycle (days)	135	142	135
- Days in the gestating sector	107		
- Days in the farrowing room	28	35	28
Days in the quarantine sector	42		
Duration of a growing pig's cycle (days)	180		
- Days in the farrowing room	21	28	21
- Days in the nursery room	58		
- Days in the finishing room	94		
Interval between two successive batches (days)	35	21	7
Probability of success for artificial insemination	0.95		
Average number of piglets per litter	N (13 ; 3.6), min=1, max=22		
Total number of sows	200	196	1000
Number of sows per batch	50	28	50
Average number of piglets per batch	650	364	650

201
 202 **2.2. Multi-pathogen epidemiological model**

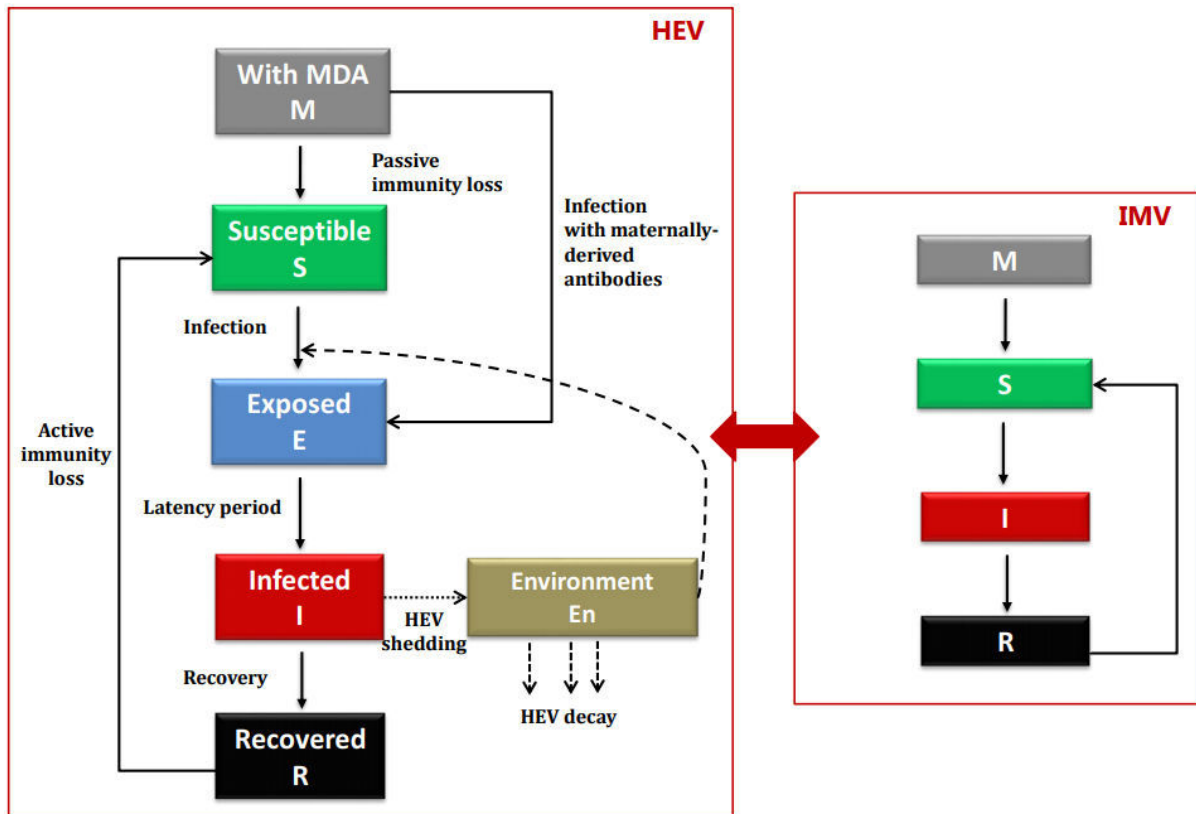
203
 204 **2.2.1. Epidemiological processes**

205 The epidemiological model is a multi-pathogen model combining two epidemiological
 206 models representing the interacting dynamics of HEV and an IMV (Figure 2).

207

208 **Figure 2. HEV and IMV infection states for breeding sows and growing pigs.**

209



210

211 HEV model: both the environment and maternally-derived antibodies (MDAs) have been
 212 shown to influence HEV infection dynamics. Therefore, an MSEIR – Maternally Immune
 213 (M), Susceptible (S), Exposed (E), Infectious (I) and Recovered (R) – model was considered
 214 to describe HEV infection dynamics taking those factors into account. Basically, newborn
 215 piglets born to immune sows acquire anti-HEV MDAs by colostrum intake (health state M),
 216 providing partial and temporary protection from infection. HEV transmission occurs through
 217 the faecal-oral route, either by direct contact with an infectious pig or by ingestion of viable
 218 virus in the contaminated environment: the pen or its vicinity (Bouwknegt et al., 2008;
 219 Bouwknegt et al., 2011). Susceptible (S) or partially protected pigs (M) can be infected,
 220 entering the exposed (E) state. After the latency period, the infectious animal (I) sheds HEV
 221 in the environment, where the virus can continue to be viable, feeding the environmental viral
 222 pool. Thus, the overall virus load in a pen’s environment corresponds to the accumulation of

223 viral particles shed by all infectious individuals, partially compensated by faeces removal
224 through the slatted floor, the natural decay of the virus and the cleaning/disinfecting
225 operations on empty pens which are carried out whenever the room is emptied (Andraud et
226 al., 2013). Assuming a gamma distribution for antibody waning, recovered pigs (R) lose their
227 immunity over time, and eventually revert to full susceptibility (S).

228

229 *IMV model:* to describe the spread of an IMV on a pig farm, a generic MSIRS model
230 accounting for partial protection conferred by MDAs was developed. We assumed the IMV is
231 transmitted by the oral-nasal route, either by direct contact between pen mates or through
232 airborne transmission at room and sector levels.

233

234 Several transmission pathways have been considered for HEV and the IMV, given their
235 different biological characteristics (see-below). Given its oro-faecal transmission route,
236 within- and between-adjacent-pen transmission have been taken into account for HEV. For
237 the IMV, both direct and airborne transmission routes have been considered, hence broader
238 transmission possibilities have been included: within-pen, between-adjacent-pen, within-room
239 and within-herd transmission routes.

240

241 Transitions between epidemiological statuses occur stochastically. At each time step and for
242 each individual, Monte Carlo procedures are used to assess the occurrence of all stochastic
243 events.

244

245 **2.2.2. Forces of HEV infection and HEV transmission probability**

246

247 Each day, the force of HEV infection is calculated taking into account two components
248 (Supplementary File 2):

249

250 *Within-pen force of infection:* one HEV infectious animal can infect its pen mates by direct
251 contact or indirectly through its contaminated faeces, accumulated in the environment:

$$252 \lambda_{p,r}^{\text{HEV,wp}}(t) = \frac{\beta_{\text{HEV}} \times I_{p,r}^{\text{HEV}}(t) + \beta_E^{\text{wp}} \times Q_{p,r} \times Q_{\text{ing}}}{N_{p,r}(t)}, \quad (1)$$

253 where $N_{p,r}(t)$ and $I_{p,r}$ correspond to the total number of animals and the number of infected
254 animals in pen p of room r at time t , respectively. β_{HEV} denotes the individual HEV
255 transmission rate. The second term of the right-hand side corresponds to the environmental

256 contribution to the force of infection. β_E^{wp} is the HEV environmental transmission rate within
 257 a pen, corresponding to the average number of animals that can be infected by a single
 258 genome equivalent present in the pen environment, i.e. to the inverse of the average number
 259 of viral particles in the environment that is needed in the environment to infect one pig
 260 (Andraud et al., 2013; Salines et al., 2015). Q_{ing} is the quantity of faeces ingested by a pig per
 261 day (Bouwknegt et al., 2011).

262 $Q_{p,r}$ is the HEV quantity accumulated in pen p , calculated as follows:

$$263 \quad Q_{p,r}(t) = Q_{p,r}(t-1) \times (1 - \varepsilon_1)(1 - \varepsilon_2) + \sum_{i=1}^{N_{p,r}(t)} \frac{w_{HEV}^i \times Q_{shed}^i}{\sum Q_{shed}^i}, \quad (2)$$

264 where w_{HEV}^i is the quantity of HEV particles shed in the environment by an infectious pig per
 265 gram of faeces, following a symmetric bell shape function calibrated on experimental data
 266 (data not shown) (Andraud et al., 2013; Salines et al., 2015) depending on the number of days
 267 post-infection, and Q_{shed}^i is the quantity of faeces it sheds per day. ε_1 and ε_2 are respectively
 268 the daily proportion of faeces passing through the slatted floor and the daily HEV mortality
 269 rate. A third decay rate, ε_3 , corresponding to the proportion of faeces eliminated through
 270 cleaning operations, is sporadically applied when the room is emptied and the batch is
 271 transferred to the next sector.

272

273 Between-adjacent-pens force of infection: contaminated faeces shed by pigs in a given pen
 274 can be transferred to an adjacent pen and are therefore likely to infect a susceptible animal in
 275 that pen. Thus, the between-adjacent-pens force of infection of a pen p is equal to the sum of
 276 the weighted force of infection of its two neighbours.

$$277 \quad \lambda_{p,r}^{HEV,bap} = Q_{ing} \times \beta_E^{bap} \times \left(\frac{Q_{p-1,r} + Q_{p+1,r}}{N_{p,r}} \right), \quad (3)$$

278 where β_E^{bap} is the HEV indirect environmental transmission rate between pens (Andraud et
 279 al., 2013).

280

281 Transmission probability: the HEV transmission probability at time t in pen p of room r is
 282 thus equal to:

$$283 \quad \pi_{p,r}^{HEV}(t) = 1 - \exp\left(-(\lambda_{p,r}^{HEV,wp}(t) \times \Delta t + \lambda_{p,r}^{HEV,bap}(t) \times \Delta t)\right), \quad (4) \text{ where } \Delta t \text{ is the time step}$$

284 $(\Delta t = 1)$.

285

286

2.2.3. Forces of IMV infection and IMV transmission probability

For the IMV, airborne transmission is assumed within and between all rooms, leading to four components for the force of IMV infection (Supplementary File 2):

Within-pen force of infection: the within-pen force of infection is:

$$\lambda_{p,r}^{IMV,wp}(t) = \beta_{IMV} \times \frac{I_{p,r}^{IMV}(t)}{N_{p,r}(t)}, \quad (5)$$

where β_{IMV} is the individual IMV transmission rate and $I_{p,r}^{IMV}(t)$ is the number of IMV infected animals in pen p of room r .

Between-adjacent-pens force of infection: keeping the same notations, the between-adjacent-pens force of infection is the sum of the forces of infection of the two neighbouring pens weighted by a coefficient C_{IMV}^{bap} :

$$\lambda_{p,r}^{IMV,bap}(t) = \beta_{IMV} \times C_{IMV}^{bap} \left(\frac{I_{p-1,r}^{IMV}(t)}{N_{p-1,r}(t)} + \frac{I_{p+1,r}^{IMV}(t)}{N_{p+1,r}(t)} \right), \quad (6)$$

Within-room force of infection: a within-room force of infection is also defined to account for airborne transmission at room level. It is assumed to be proportional to the within-room prevalence weighted by coefficient C_{IMV}^{wr} :

$$\lambda_r^{IMV,wr}(t) = \beta_{IMV} \times C_{IMV}^{wr} \left(\frac{I_r^{IMV}(t)}{N_r(t)} \right), \quad (7)$$

where I_r^{IMV} is the number of infected animals in room r .

In farrowing rooms, a specific coefficient $C_{IMV}^{wr,fa} > C_{IMV}^{wr}$ is applied to take into account the numerous operations occurring in this sector (castration, piglet health care, etc.) with farmers entering pens and possibly transferring the virus from one pen to another through contaminated material, etc.

Between-rooms force of infection: based on the same assumptions, a between-rooms transmission possibility is represented to allow for potential viral transfer between the different farm sectors through air flow, material transportation, farmer movements, etc.:

$$\lambda^{IMV,br}(t) = \beta_{IMV} \times C_{IMV}^{br} \frac{I^{IMV}(t)}{N(t)}, \quad (8)$$

where I^{IMV} is the total number infected animals on the farm and $C_{IMV}^{br} < C_{IMV}^{wr}$ is a between-rooms coefficient.

318 Transmission probability: the IMV transmission probability at time t is thus equal to:

$$319 \pi_{p,r}^{IMV}(t) = 1 - \exp\left(-(\lambda_{p,r}^{IMV,wp}(t) \times \Delta t + \lambda_{p,r}^{IMV,bap}(t) \times \Delta t + \lambda_r^{IMV,wr}(t) \times \Delta t + \lambda^{IMV,br}(t) \times \right. \\ 320 \left. \Delta t)\right), \quad (9)$$

321 with $\times \Delta t = 1$

322
323

324 2.2.4. Epidemiological parameters

325

326 The two epidemiological models run simultaneously in the population (Figure 2). The piglet's
327 individual characteristics with respect to HEV dynamics vary depending on its state of health
328 regarding the IMV (latency period, individual transmission rate, quantity of HEV shed). All
329 the parameters involved in the infectious process are fully described in Table 2, along with
330 their definition and the origin of input values. HEV parameters were derived from
331 transmission experiments and other data in the literature. The values of the IMV model
332 parameters were consensually chosen to represent the transmission of a typical airborne virus
333 such as PRRSV or PCV2.

334

335 **Table 2. Parameters governing the two models of viral infection dynamics.**

336 HEV: hepatitis E virus, IMV: immunomodulating virus, ge: genome equivalent, MDAs:
337 maternally-derived antibodies

Notation	Parameter description (unit)	Value / Distribution		Reference
		HEV-only	HEV/IMV co-infected	
Parameters of the HEV model				
D_{HEV}^M	Days of maternal immunity	$\Gamma(7.9 ; 5.8)$		Andraud et al. (2014)
p_{HEV}^{MDA}	Infection probability with MDAs	0.08		Andraud et al. (2014)
D_{HEV}^E	Latency (days)	$\Gamma(5.2 ; 1.3)$	$\Gamma(25.7 ; 0.5)$	
β_{HEV}	Direct transmission rate (pigs/day)	0.15	0.69	
β_E^{wp}	Within-pen environmental transmission rate (g/ge/day)	6.10^{-6}		
β_E^{bap}	Between-adjacent-pens environmental transmission rate (g/ge/day)	7.10^{-8}		Andraud et al. (2013) Salines et al. (2015)
D_{HEV}^I	Infectious period (days)	9.7	48.6	
w	Quantity of HEV particles shed in faeces depending on the post-infection time, weighted by maximum shed quantity Qmax (ge/g/day)	N (5 ; 1) Qmax = 10^6	N (25 ; 5) Qmax = 10^8	
Q_{shed}	Average amount of faeces shed by a pig	100 for piglets		Murai et al. (2018)

	(g/day)	1000 for finishing pigs 2000 for sows	
Q_{ing}	Average quantity of faeces ingested by a pig (g/day)	25	Bouwknegt et al. (2011)
ε_1	Faeces elimination rate through slatted floor (/day)	0.70	Best guess
ε_2	HEV decay rate in the environment (/day)	0.08	Johne et al. (2016)
ε_3	Faeces removal rate by cleaning	0.98	Best guess
D_{HEV}^R	Days of active immunity	$\Gamma(6.3 ; 29.4)$	Best guess
Parameters of the IMV model			
D_{IMV}^M	Days of maternal immunity	N (45 ; 8)	
p_{IMV}^{MDA}	Infection probability with MDAs	0.3	
β_{IMV}	Direct transmission rate (pigs/day)	0.13	Consensual parameters representing the transmission of a typical airborne virus, such as PRRSV or PCV2 (Andraud et al., 2009a; Andraud et al., 2008; Rose et al., 2015)
D_{IMV}^R	Days of active immunity (days)	$\Gamma(6.3 ; 29.4)$	
C_{IMV}^{bap}	Transmission coefficient between adjacent pens	0.1	
C_{IMV}^{wr}	Within-room transmission coefficient	0.05	
$C_{IMV}^{wr,fa}$	Within-room transmission coefficient in farrowing room	0.1	
C_{IMV}^{br}	Between-rooms transmission coefficient	0.01	

338

339

340 2.3. Initialisation and simulations

341

342 2.3.1. Stochasticity

343

344 The model has been developed in a C++ language (Visual Studio IDE). It is a discrete-time
345 model and is implemented on a daily basis during which the individuals are subjected to two
346 types of processes run sequentially. First, the demographic processes are considered with a
347 biologically relevant and logical order: ageing and mortality for all individuals; reproduction
348 processes for breeding animals along with birth of offspring, culling and replacement of sows.
349 If time-relevant, batches are transferred into the sector and room corresponding to their
350 physiological state, the individuals being distributed among the pens. The epidemiological
351 process is then implemented both for the IMV and HEV.

352

353 At the beginning of a simulation, the herd is composed only of sows. The initial number of
354 sows is equal to the number of batches multiplied by the number of pens in the farrowing
355 room. Sows are 100 days old, of parity rank 0 and placed in the gestation room. The eleventh

356 year, when the herd is assumed to be demographically stable, a single IMV infectious gilt is
357 introduced once in the quarantine sector to initiate the IMV infectious process. In the fifteenth
358 year, a single HEV-exposed gilt is then introduced in the quarantine sector to initiate the HEV
359 infectious process. We assume no subsequent introduction of IMV- or HEV-infected animals.
360 The model is initialised in the same way for every simulation.

361

362 Two-hundred simulations were run for each tested scenario. Following visual inspection for
363 model stability, this number of simulations was deemed sufficient to obtain stable outcomes
364 in terms of means and variances (Supplementary File 3). The number of animals in each
365 epidemiological state in every pen of every room was recorded daily. Furthermore, this
366 individual-based model allowed the age at which each growing pig is infected to be recorded.
367 Daily snapshots of the population were also recorded as model outputs to monitor the
368 demographic process throughout the simulations.

369

370

371 **2.4. Assessment of characteristics related to HEV on-farm spread** 372 **and persistence and implementation of control strategies**

373

374 **2.4.1. Outcomes**

375 Specific outcomes were selected to analyse on-farm spread and persistence of HEV and to
376 assess the risk of its introduction into the food chain: (i) the age at HEV infection of growing
377 pigs; (ii) the proportion of batches having HEV-infected animals at slaughter time (170-day-
378 old pigs); (iii) the HEV prevalence in slaughter-aged growing pigs (170-day-old pigs); (iv) the
379 probability of HEV on-farm persistence five years post-introduction.

380

381 **2.4.2. Evaluation of different scenarios**

382 The influence of several farm characteristics on these outcomes was evaluated (Table 3):

- 383 - The type of BRS (4, 7, or 20 batches, corresponding to 5, 3 and 1 week between-batch
384 intervals respectively);
- 385 - The type of housing for gestating sows (large groups (i.e. collective pen), medium
386 groups (i.e. one pen per batch), or small groups (i.e. six sows per pen));

387 - The farm’s sanitary status regarding the IMV (IMV-free or IMV-infected).

388 The impact of several control measures was then assessed (Table 3). First, different farming
 389 practices were tested: (i) cross-fostering practices: high cross-fostering rate (i.e. higher than
 390 15%), medium cross-fostering rate (i.e. less than 15%) or no cross-fostering; (ii) mingling
 391 practices at weaning: nursery pen size (small pens, i.e. less than 50 pigs per pen, or large pens,
 392 i.e. more than 50 pigs per pen) and type of mingling (by litter or randomly). An IMV control
 393 measure was also tested by vaccinating sows against IMVs at each reproductive cycle two
 394 years after the IMV was introduced (sows being thus transferred to status R as regards the
 395 IMV).

396

397 **Table 3. Description of control scenarios tested in the HEV multi-pathogen model.**

398 Scenario 1 can be considered as the reference scenario. Scenario 8 represents the “worst-case
 399 scenario” whereas scenario 11 represents the “best-case scenario”.

400

Scenario	Type of housing for gestating sows	Cross-fostering practices			Modalities for mingling at weaning			Control of the IMV		
	Large groups	No	Medium rate (15 %)	High rate (> 15 %)	Small pens (< 50)	Large pens (> 50)	By litter	Randomly	No vaccination	Anti-IMV vaccination of sows
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										

401

402 **2.4.3. Statistical analyses**

403 Cox-proportional hazard models were built to assess the influence of the different scenarios
 404 on age at HEV infection. The impact of the different explanatory variables on the proportion
 405 of batches having HEV-positive animals at slaughter time was assessed using a logistic

406 regression. A generalised estimating equation (GEE) logistic regression was used to evaluate
407 the impact of the explanatory variables on HEV prevalence in slaughter-aged pigs, the
408 simulation being included as a repeated statement to account for the non-independence of the
409 proportions of positive pigs for the different batches in a given simulation. The impact of the
410 different measures on HEV persistence probability was evaluated using non-parametric
411 survival analyses (log rank test). These analyses were performed using R and SAS software
412 (Ihaka and Gentleman, 1996; SAS, 2014).

413

414 The IMV's prevalence in growing pigs was also computed under the different scenarios, the
415 descriptive results being included as supplementary material.

416

417

418 **3. Results**

419

420 Statistical analyses were performed to assess the relative impact of herd management and
421 control measures on the dynamics of HEV infection. The results from univariate analyses are
422 provided in Supplementary File 4 and in Figures 3 to 8.

423

424 **3.1. Description of simulations after HEV introduction on an IMV-** 425 **positive farm (baseline scenario) and model validation**

426

427 As shown in Supplementary File 5, the IMV spread enzootically both in the reproductive and
428 growing pig herds, without fading out in any simulation.

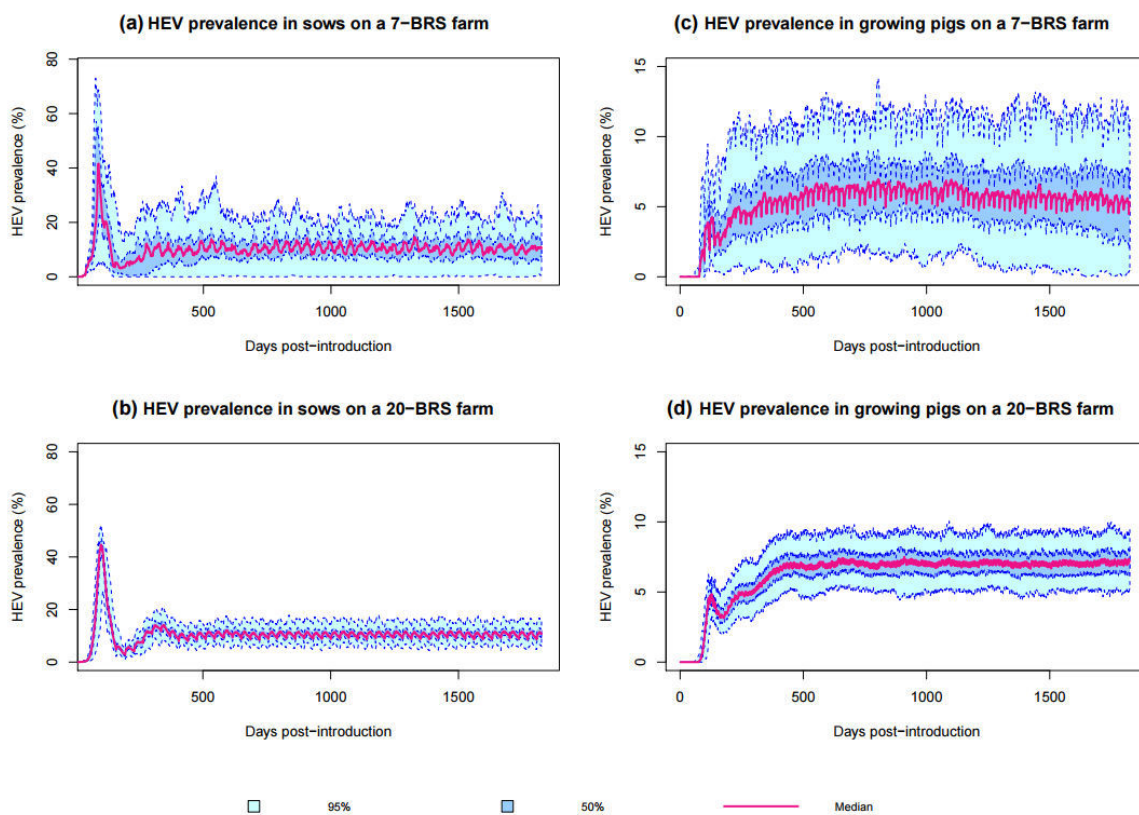
429 After the introduction of an HEV-infected gilt in the quarantine sector, an epidemic peak was
430 first observed in the breeding part of the herd due to massive infections of a large pool of
431 naive animals (Figures 3a and 3b). Infected sows entering the farrowing sector then initiated
432 the infectious process in growing pigs by infecting suckling piglets. The latter spread the
433 infection in the nursery and finishing sectors. In this baseline scenario (scenario 1), pigs
434 contracted HEV on average between 88 and 91 days of age, depending on the BRS. Without
435 any subsequent HEV reintroduction, HEV persisted enzootically in most of the simulations up
436 to five years post-introduction (between 60% and 100%, depending on the BRS, cf. infra),
437 HEV extinction occurring first in the sow herd before fading out in the growing pigs
438 (Supplementary File 6). The average HEV prevalence in slaughter-aged growing pigs ranged

439 between 2.8 and 4.6% on average, depending on the BRS. The average environmental viral
440 load did not exceed 7 log genome equivalents per gram of faeces and ranged between 2 and 4
441 log (data not shown).

442

443 **Figure 3. HEV prevalence in sows and growing pigs (median, 50% and 95%) on 7- and**
444 **20-batch rearing system farrow-to-finish pig farms if there is no fade-out (88 and 195**
445 **out of 200 simulations for sows on 7- and 20-BRS farms respectively, 119 and 195 out of**
446 **200 simulations for growing pigs on 7- and 20-BRS farms respectively).**

447



448

449

450 The baseline scenario (scenario 1) shows that pigs become infected when they are 88 days old
451 on average, which is consistent with the field study of Salines and colleagues who described a
452 mean age at infection of 91 days (Salines et al., 2019b). Moreover, the simulations led to a
453 mean prevalence of infectious pigs at slaughter age ranging between 2.8% and 4.6%, in line
454 with a nationwide French study conducted by Rose et al. (2011) that reported 4% [2-6] of
455 HEV-positive livers at the slaughterhouse. The HEV loads accumulated in the environment
456 were consistent with viral loads found in the liquid manure of pig farms investigated in
457 previous studies. For instance, Guillois et al. (2016) estimated the viral load in the liquid

458 manure of a chronically HEV-infected pig farm at between 3.10^4 and 5.10^6 copies of HEV
459 RNA/g, depending on the type of room that was sampled.

460

461

462 **3.2. Impact of farm characteristics on HEV infection dynamics**

463

464 **3.2.1. Batch rearing system**

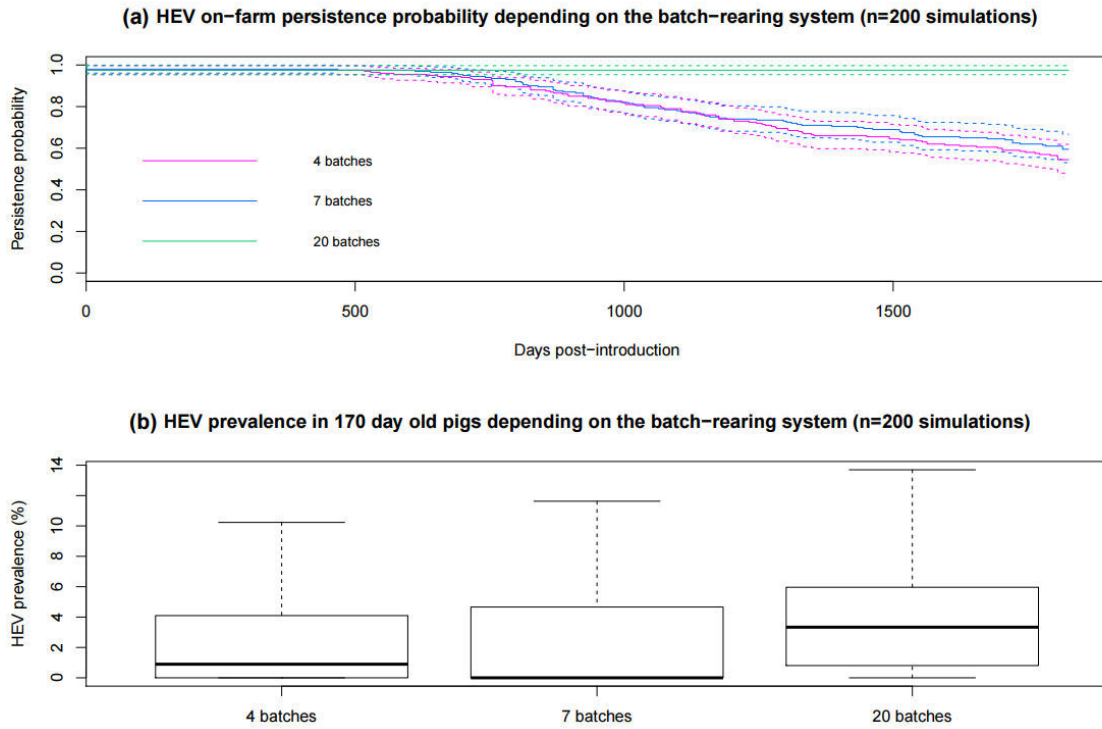
465

466 HEV prevalence appeared globally higher on 20-BRS farms than on 7-BRS ones throughout
467 the simulation period, with lower variability (Figure 3). The HEV infection of growing pigs
468 occurred significantly earlier on a 20-BRS farrow-to-finish pig farm (on average 84 days of
469 age) than on 7-BRS farms (87 days; Supplementary File 4, Table a). The proportion of
470 batches being HEV-positive at slaughter time was significantly associated with the BRS,
471 reaching 80% [79-81] of batches for the most intensive system (20-BRS; Supplementary file
472 4, Table b). Although lower, the difference obtained between the 4- and 7-BRS was also
473 found significant, with on average 56% [54-58] and 45% [44-46] of positive batches
474 respectively (Supplementary file 4, Table b). Moreover, the HEV prevalence in slaughter-
475 aged growing pigs was higher on a 20-BRS farm than on a 7-BRS farm (on average 4.5%
476 [3.7-5.1] versus 3.3% [3.1-3.5], p-value < 0.01; Supplementary file 4, Table c, Figure 4b).
477 Finally, a quasi-systematic persistence was observed up to five years post-introduction in
478 herds managed according to the 20-BRS (Figure 4a). The behaviour was significantly
479 different for the other two BRS farms, where the virus was found in only 55 and 60% of the
480 herds for the 4- and 7-BRS farms respectively five years post-introduction (p-value < 0.01,
481 Figure 4a). Since infection dynamics on 7- and 20-BRS farms were the most significantly
482 different and 4- and 7-BRS farm patterns were highly similar, the following control measures
483 were evaluated on 7- and 20-BRS farms only.

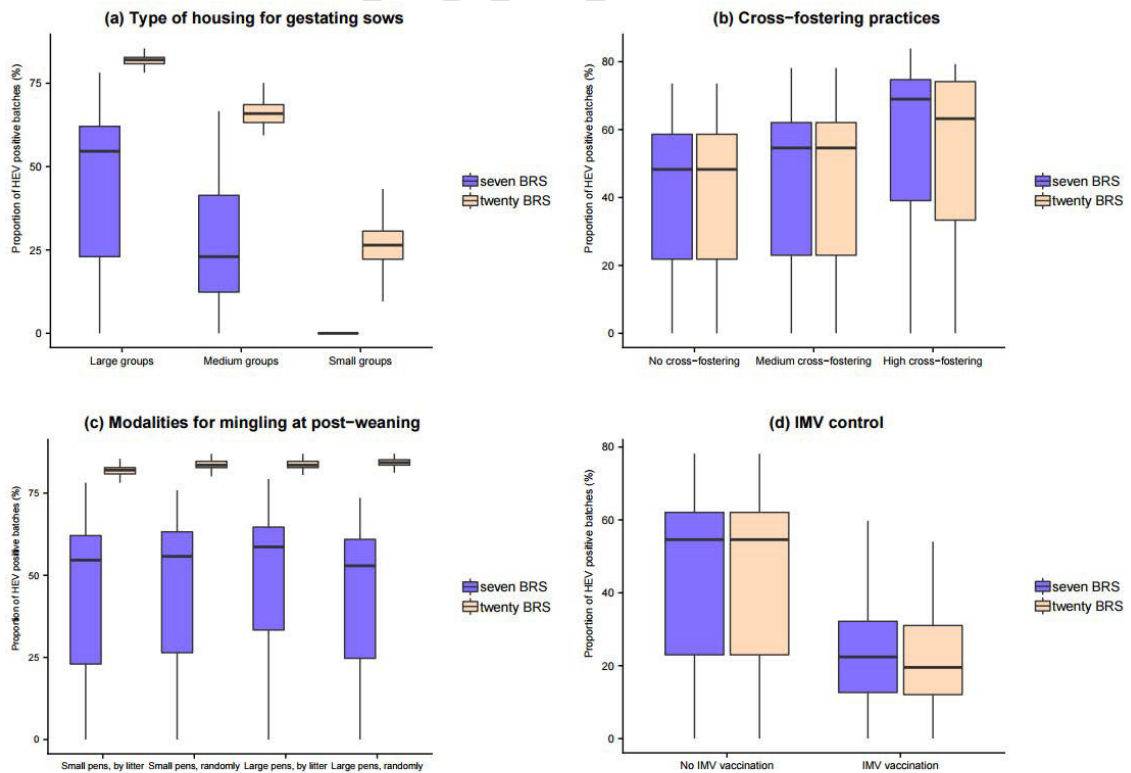
484

485 **Figure 4. HEV persistence probability (a) and HEV prevalence in slaughter-aged**
486 **growing pigs (b) on a farrow-to-finish pig farm depending on the type of batch rearing**
487 **system (n = 200 simulations).**

488

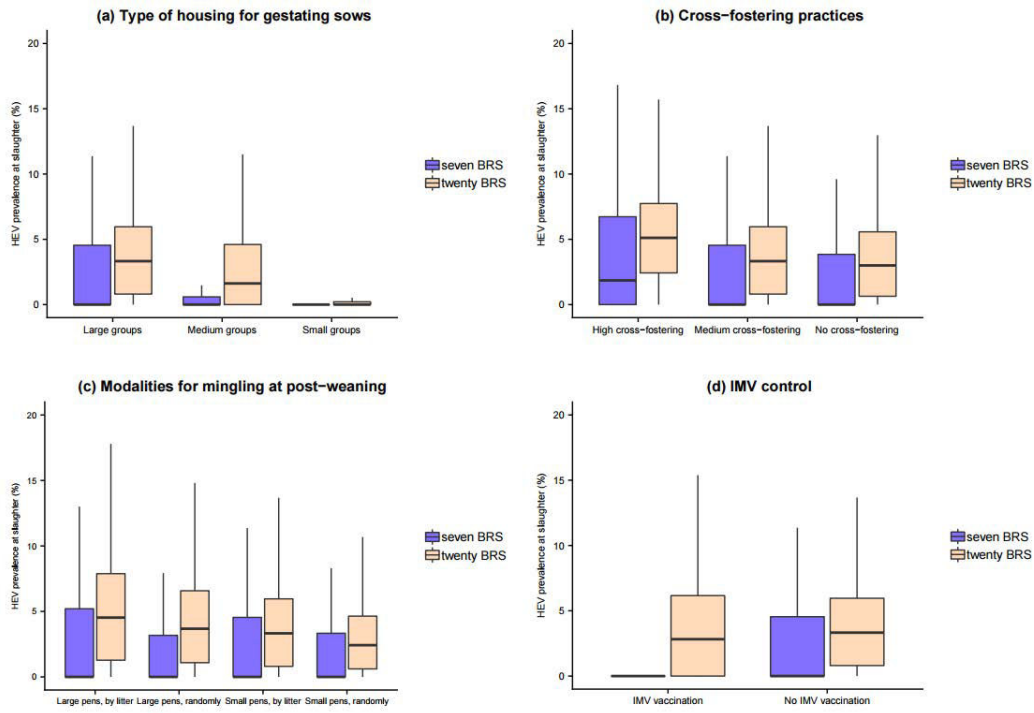


489
 490 **Figure 5. Proportion of batches having HEV-infected pigs at slaughter time on a 7- or**
 491 **20-batch rearing system farrow-to-finish pig farm depending on farming practices and**
 492 **health management measures (n = 200 simulations).**
 493

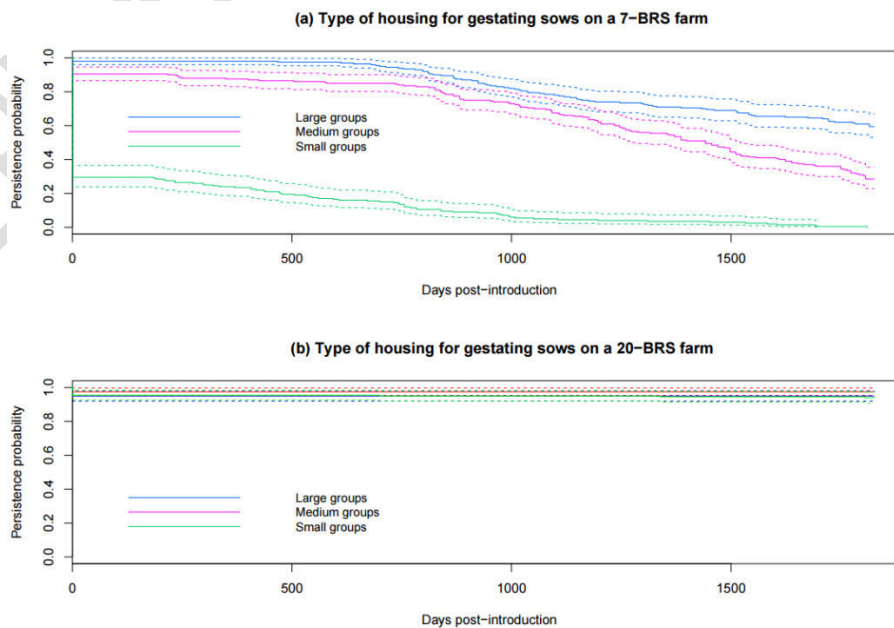


494

495 **Figure 6. HEV prevalence in slaughter-aged growing pigs on a 7- or 20-batch rearing**
 496 **system farrow-to-finish pig farm depending on farming practices and health**
 497 **management measures (n = 200 simulations).**
 498

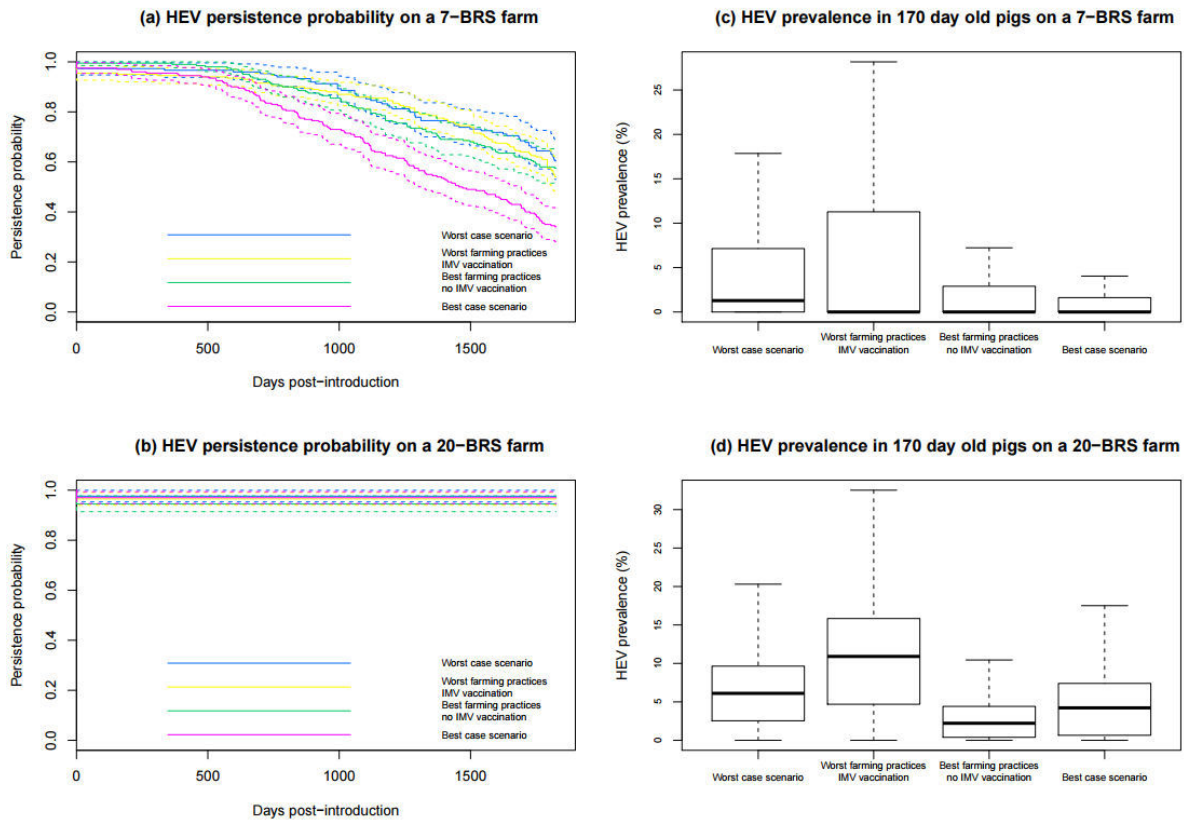


499
 500
 501 **Figure 7. HEV persistence probability on a 7- (a) or 20- (b) batch rearing system farrow-**
 502 **to-finish pig farm depending on the type of housing for gestating sows (n = 200**
 503 **simulations).**



506 **Figure 8. HEV persistence probability and prevalence in slaughter-aged pigs on 7- and**
 507 **20-batch rearing system farms in combined HEV control scenarios (n = 200**
 508 **simulations).**

509



510

511

512 3.2.2. Type of housing for gestating sows

513

514 Both on a 7- and 20-BRS farm, when sows were housed in medium or small groups, pigs
 515 were infected later than when they were housed in large gestation pens (on average 90, 103
 516 and 87 days respectively on a 7-BRS farm; and 87, 102 and 84 respectively on a 20-BRS
 517 farm) (Supplementary File 4, Table a). The proportion of batches with HEV-positive livers at
 518 slaughter time was significantly lower when sows were housed in medium or small groups
 519 rather than large groups, both on a 7- and a 20-BRS farm (Supplementary file 4, Table b,
 520 Figure 5a), dropping to 1% of batches when sows were managed in small groups on a 7-BRS
 521 farm. However, the results obtained for the 20-BRS farms were more contrasted, with up to
 522 25% of batches found HEV-positive in the presence of small groups of sows. Moreover, sow
 523 housing management had a similar impact on HEV prevalence in growing pigs at slaughter,
 524 which was found to fall below 1% for both BRS farms when sows were kept in small pens

525 (0.1% [0.06-0.2] and 1% [0.9-1.1] for 7- and 20-BRS farms respectively; Supplementary file
526 4, Table c, Figure 6a). Moreover, the size of sow groups in the gestating stage was
527 significantly associated with the persistence probability on 7-BRS farms (p-value < 0.01,
528 Figure 7a). Indeed, disease extinction was systematically observed when sows were kept in
529 small groups, and the probability of persistence dropped to 29% [23-35] when sows were
530 housed in medium groups. Interestingly, these results were not transposable to 20-BRS farms,
531 for which sow housing modalities did not have any significant impact on the probability of
532 HEV persistence (p-value > 0.05, Figure 7b). As HEV did not persist at all on a 7-BRS farm
533 with small gestation pens, the effectiveness of the following control measures was evaluated
534 only on farms housing sows in large groups.

535

536 **3.2.3. Farms' sanitary status**

537

538 On a 7-BRS farm, pigs contracted HEV 40 days earlier on average when the herd was IMV-
539 free compared to an IMV-infected farm leading to infections in the nursery stage (55 days of
540 age), whereas the average age of infection in IMV-infected farms corresponded to the
541 fattening stage (95 days of age; Supplementary file 4, Table a). The absence of IMV led to a
542 decrease in positive batches (11% [10-12]) and positive pigs (0.2% [0.1-0.2]) at slaughter age
543 (Tables 5 and 6). Furthermore, the persistence probability dropped to 0.34 [0.28-0.41] after
544 five years post-introduction in an IMV-free herd (p-value < 0.01).

545 A more contrasted effect was observed on a 20-BRS IMV-free farm in which infections were
546 slightly — but significantly — postponed (90 days of age) compared to the infection process
547 in an IMV-infected farm (Supplementary file 4, Table a). The proportion of batches having
548 HEV-infected animals at slaughter time was only decreased by 2.5% on average [1.9-3.1]
549 (Supplementary file 4, Table b) and no significant impact of the farm's IMV status on HEV
550 prevalence in growing pigs at slaughter age was observed on a 20-BRS farm (Supplementary
551 file 4, Table c). HEV persistence was not affected by the farm's IMV status when managed
552 according to the 20-BRS (p-value > 0.05).

553

554

555 **3.3. Assessment of the effectiveness of control measures**

556

557 **3.3.1. Impact of farming practices**

558 Cross-fostering practices: all outputs were found to be significantly influenced by cross-
559 fostering practices (Tables 4, 5, 6). More precisely, the higher the cross-fostering rate, the
560 sooner the infection was contracted in growing pigs. Intensive cross-fostering led to infections
561 on average one week earlier than the two alternative strategies (on average 89 days of age;
562 Supplementary file 4, Table a). On a 7-BRS farm, the proportion of HEV-positive batches at
563 slaughter time was significantly lower when there was no adoption (41% [40-43.5]) compared
564 to a medium cross-fostering rate, whereas high cross-fostering rate increased the probability
565 of HEV-positive batches at slaughter (59% [56-59.5]; Supplementary file 4, Table b, Figure
566 5b). Similar results were obtained concerning HEV prevalence at slaughter age, with
567 proportions varying with the level of cross-fostering from 2.6% to 3.9% on a 7-BRS farm and
568 from 3.6 to 5% on a 20-BRS farm (Supplementary file 4, Table c, Figure 6b). On a 7-BRS
569 farm, cross-fostering practices were associated with the HEV persistence probability (p-value
570 < 0.01), with an average persistence probability equal to 0.55 [0.49-0.62] when no adoption
571 was allowed, compared to 0.61 [0.67-0.80] in the event of a high cross-fostering rate
572 (Supplementary File 7). Cross-fostering practices did not affect HEV persistence probability
573 on a 20-BRS farm (p-value > 0.05, Supplementary File 7).

574

575 Modalities for mingling in the nursery: HEV infection occurred on average one week later
576 when pigs were housed in large rather than small nursery pens (Supplementary file 4, Table
577 a). Keeping piglets with their litter mates was also found to postpone average age at infection
578 by 4 days. Infections occurred earlier when pigs were randomly mixed compared to by-litter
579 mingling (on a 7-BRS farm: on average 82 versus 87 days in small pens, 87 versus 92 days in
580 large pens; on a 20-BRS farm: 78 versus 84 days in small pens, 84 versus 90 days in large
581 pens; Supplementary file 4, Table a). The proportion of positive batches at slaughter was
582 increased by 5% when pigs were housed in large rather than small nursery pens, and
583 increasing to up to 50% of batches. A random mixing of pigs was found to reduce the
584 proportion of positive batches at slaughter when pigs were housed in large pens on a 7-BRS
585 farm (44% [42.3-45.8]) while the opposite results were obtained in all other cases when
586 random mixing was practised (Supplementary file 4, Table b, Figure 5c). The HEV
587 prevalence in growing pigs at slaughter age was higher when pigs were housed in large
588 weaning pens compared to small pens, rising from 3.2% [3.0-3.5] to 4.0% [3.4-4.7] on a 7-
589 BRS farm (from 4.4% [4.3-4.6] to 4.9% [4.7-5.3] on a 20-BRS farm). Random mixing
590 lowered this proportion compared to by-litter mingling, particularly on a 20-BRS farm with
591 small pens in the weaning facilities (3.5% [3.3-3.7]) (Supplementary file 4, Table c, Figure

592 6c). Modalities for mingling in the nursery did not affect HEV persistence probability
593 significantly either on a 7-BRS farm or on a 20-BRS farm (p-value > 0.05, Supplementary
594 File 7).

595

596 **3.3.2. Impact of IMV control through vaccination of sows**

597

598 Anti-IMV sow vaccination decreased the IMV spread in growing pigs both on a 7- and a 20-
599 BRS farm (data not shown).

600 Vaccinating sows against IMV postponed HEV infection in growing pigs by about one week,
601 with an average age at infection of 93 days irrespective of the BRS (Supplementary File 4,
602 Table a). The proportion of positive batches at slaughter was significantly reduced for both
603 BRS farms, with a higher impact on 7-BRS farms where only 22% [21-24] of batches were
604 found positive (Supplementary file 4, Table b, Figure 5d). This result was also reflected in
605 HEV prevalence among growing pigs with 2% ([1.6-2.4]) of positive animals at slaughter age
606 for the 7-BRS farm, whereas no significant impact was observed in a herd managed according
607 to the 20-BRS (Supplementary file 4, Table c, Figure 6d). Five years after introduction, the
608 probability of HEV persistence was also lower when sows were vaccinated against the IMV
609 on 7-BRS farms only (0.34 [0.28-0.41] versus 0.60 [0.53-0.67], p-value < 0.01,
610 Supplementary File 7).

611

612 **3.3.3. Results from combined scenarios**

613

614 Four scenarios, hereinafter denoted scenarios 13 to 16, and a combination of improving
615 management practices and vaccination campaigns against the IMV, were considered. For
616 statistical comparison, the worst scenario in terms of management practices (i.e. presenting
617 high levels of mingling at all production stages; scenario 13) was taken as a reference. In this
618 context, the vaccination of sows against the IMV without improving farming practices
619 (scenario 14) led to later HEV infections in growing pigs, which occurred on average at 109
620 days of age versus 90 days on a 7-BRS farm (103 versus 85 days of age on a 20-BRS farm;
621 Supplementary file 4, Table a). This strategy also led to a significant decrease in the
622 proportion of positive batches at slaughter time; from 53.6% to 46.1% on 7-BRS farms (from
623 83.6 to 81.6% on 20-BRS farms). However, IMV vaccination of sows was related to an
624 increased HEV prevalence in slaughter-aged growing pigs when farming practices were not
625 improved. Indeed, a 2% increase in the proportion of positive pigs at slaughter age was

626 observed, reaching 7% [5.8-8.4] on 7-BRS farms. This tendency was even clearer on 20-BRS
627 farms, reaching an average 12.2% [11.4-13.0] of slaughter-aged piglets (Tables 5 and 6,
628 Figure 8d). Combining all the best farming practices, even without vaccinating sows against
629 the IMV (scenario 15), led to an earlier age at HEV infection of growing pigs compared to the
630 worst-case scenario (82 days on a 7-BRS farm; 78.2 days on a 20-BRS; Supplementary file 4,
631 Table a). In this case, the proportion of HEV-positive batches at slaughter decreased both on
632 7- and 20-BRS farms (on average 43.5 [41.6-45.5] and 78.2% [77.2-79.2] respectively). HEV
633 prevalence among slaughter-aged growing pigs also fell to 2.3% [1.8-2.8] on 7-BRS farms
634 (3.3% [3.1-3.5] on 20-BRS farms; Tables 5 and 6, Figures 8c and 10d). In the best-case
635 scenario (scenario 16), which combined best farming practices and IMV vaccination, growing
636 pigs contracted HEV later than in the worst-case (reference) scenario (94.3 days of age on a 7-
637 BRS farm, 87 days of age on a 20-BRS farm; Supplementary file 4, Table a). IMV
638 vaccination did not impact the model outcomes at slaughter age, with a similar proportion of
639 positive batches and positive animals as scenario 15, when the herd was managed according
640 to the 7-BRS. In contrast, vaccination practised in a 20-BRS herd was found counter-effective
641 when optimal farming management was implemented, with a higher proportion of positive
642 pigs at slaughter age than with scenario 15 (5.4% [5.1; 5.7]; Tables 5 and 6, Figure 8d). On a
643 7-BRS farm, the HEV persistence probability was reduced in the best-case scenario compared
644 to the worst-case one, dropping from 0.60 [0.53-0.69] to 0.34 [0.28-0.41] (p-value < 0.01). No
645 significant impact of the combined scenarios on the HEV persistence probability was
646 observed on a 20-BRS farm (p-value > 0.05, Figure 8b). In this 16th scenario, IMV prevalence
647 in growing pigs was also much lower than for the worst-case scenario (Supplementary File 8).

650 **4. Discussion**

651
652 Although understanding HEV infection dynamics in pig populations is clearly pivotal to
653 managing the risk of human exposure to the virus, there are still substantial knowledge gaps
654 on HEV infection at pig farm level (Van der Poel et al., 2018). Mathematical models
655 incorporating the epidemiological characteristics of pathogens appear to be relevant tools for
656 an in-depth understanding of infection dynamics through the identification of influential
657 factors. We therefore developed a model representing within-herd HEV infection dynamics.
658 The model combines population dynamics at a farm level with the on-farm viral spread at an

659 individual level. Interactions are of primary importance regarding the spread of infectious
660 diseases within a population. In the present case, individuals interact at different levels
661 depending on the process considered. Indeed, the population is made up of two
662 distinguishable sub-populations, sows and growing pigs, which physically interact only in the
663 farrowing sector during lactation. However, even during this period, contacts are restricted to
664 sows and their respective (possibly fostered) newborns. These interactions may allow not only
665 the transfer of maternally-derived antibodies to piglets but also the transmission of infectious
666 agents from sows to their litter. Batch rearing management systems generate batches of
667 animals at specific locations in the herd depending on their physiological status. These groups
668 are in turn distributed among several pens generating multiple sub-populations inside the
669 rooms. Pen mates are in direct contact and share the same environment; neighbouring pens are
670 also in close interaction either through airborne contact (for the IMV) or the environmental
671 route (for HEV). An airborne transmission route was also considered for IMV at room and
672 global herd levels, taking the relative prevalence of infectious individual as a proxy for viral
673 load in the air. Finally, although the batches of animals are managed according to an all-in-all-
674 out strategy, with cleaning and disinfection procedures, the animals may be exposed to any
675 viral particles remaining in the environment when settled in a new room.

676 The specificity and originality of our model lies in the multi-pathogen modelling framework:
677 the model integrates the epidemiological interactions between HEV and a generic
678 immunomodulating pathogen on an individual scale. These interactions have been proven to
679 dramatically affect HEV dynamics both under experimental and natural conditions (Salines et
680 al., 2019a; Salines et al., 2015; Salines et al., 2019b). Factoring an environmental
681 compartment into the HEV model design is also of particular importance, since the key role of
682 viral environmental accumulation in HEV dynamics has already been demonstrated: indeed,
683 despite frequent cleaning and disinfection procedures in pig herds, the accumulation of viral
684 particles in the pigs' environment can explain HEV persistence on farms (Andraud et al.,
685 2013). Most of the epidemiological parameter values were derived from published data when
686 available. In particular, the model uses different parameters for HEV dynamics depending on
687 the pig's status regarding an IMV; these parameters were obtained from several experimental
688 trials. The IMV parameters were chosen to represent the typical behaviour of an airborne
689 immunomodulating virus; they were not selected to specifically represent the dynamics of
690 PRRSV and/or PCV2 but the chosen R_0 was consistent with the ones reported for PRRSV
691 and PCV2 in the literature (5.4 and 5.9, respectively) (Andraud et al., 2009a; Rose et al.,

692 2015). Following animals on an individual and daily basis grants a detailed and subtle
693 understanding of HEV dynamics, especially in the situation of individual co-infections.

694

695 Complementary outputs were selected to assess HEV on-farm spread and persistence both
696 comprehensively and as precisely as possible. Firstly, the age at HEV infection reflects the
697 speed of HEV transmission and the force of HEV infection on the herd. The proportion of
698 HEV-positive batches at slaughter time and HEV prevalence at slaughter age provide direct
699 information on the risk of HEV-positive livers entering the food chain, and are therefore a key
700 indicator of the risk of human exposure to the virus. These two outcomes are also particularly
701 relevant from a risk management point of view: for instance, they can be used to design liver
702 testing programmes at slaughterhouses with an appropriate sampling size both as regards the
703 number of batches and number of livers to be selected. Finally, HEV on-farm persistence
704 probability five years post-introduction expresses the ability of the virus to remain on the farm
705 and thus gives an indication of the risk for public health as well. It also reflects the probability
706 of the infection spreading from one farm to another: the longer the farm hosts the virus, the
707 more likely the virus can be transmitted to another farm. It should be noted that these
708 indicators should be interpreted all together. For instance, a late HEV infection could be
709 considered risky because pigs are more likely to be still hosting the virus at slaughter age, but
710 if it is combined with a more limited viral spread, the risk for public health would end up to
711 be lower. Moreover, the statistical significance highlighted by tests may sometimes be of
712 limited practical importance. Indeed, the outcomes of such models represent a tremendous
713 quantity of data which induces a very high statistical power. Therefore, the effect of the
714 sample size should be considered in order not to give too much importance to insignificant
715 (but statistically significant) results. For instance, even when it is statistically significant, a
716 difference of only a few days in the age at HEV infection may have a limited practical impact,
717 unlike differences in HEV prevalence at slaughter.

718

719 Comparison with field data has shown that all outcomes of the baseline scenario were
720 consistent with field data: age at HEV infection (88 versus 91 days of age), HEV prevalence
721 in slaughter-aged pigs (2.8-4.6% versus 2-6%), HEV persistence on farms (64% 5 years after
722 introduction versus 2 years in 80% of tested farms), HEV loads accumulated in the
723 environment. Indeed, the baseline scenario (scenario 1) shows that pigs become infected when
724 they are 88 days old on average, which is consistent with the field study of Salines and
725 colleagues who described a mean age at infection of 91 days (Salines et al., 2019b). The age

726 at infection is known to be strongly related to the basic reproduction number and the host
727 lifespan ($R_0 \approx \frac{L}{A}$) (Anderson and May, 1991). Owing to this relationship and the numerical
728 results obtained in our study (assuming an average lifespan of 180 days for growing pigs), the
729 basic reproduction number for hepatitis E would vary between 1.6 and 2.3. These values
730 appear relatively low in regard to the estimates of Bouwknegt et al. (2008) or Satou and
731 Nishiura (2007). However, in the context of batch rearing systems, the animals are housed in
732 relatively small groups with limited (but real) contact between groups. Based on these
733 considerations, the estimates provided here could be considered as resulting from several
734 locally clustered transmission processes, as was the case in Backer et al. (2012), who
735 estimated similar reproduction numbers from field data. Furthermore, the protection conferred
736 by maternally-derived antibodies was also considered in the model structure and may be
737 responsible for delaying the infectious process and consequently reducing the reproduction
738 ratio (estimated at population level). The simulations led to a mean prevalence of infectious
739 pigs at slaughter age ranging between 2.8% and 4.6%, in line with a nationwide French study
740 conducted by Rose et al. (2011) that reported 4% [2-6] of HEV-positive livers at the
741 slaughterhouse. It is also consistent with the meta-analysis conducted by Salines et al. (2017)
742 using 31 international studies, which resulted in a figure of 6.1% [1.2-15.4] of pigs being
743 infectious at slaughter age. In around 60% of simulations, our baseline scenario evidenced
744 that HEV could persist five years after HEV introduction without any subsequent viral
745 reintroduction. This is a conservative scenario, as HEV is likely to be reintroduced on farms,
746 especially through herd renewal practices. To the best of our knowledge, no study specifically
747 designed to assess HEV on-farm persistence duration is available in the published literature,
748 but a few cases of natural HEV fade-out have been reported on some farms (ANSES, personal
749 communication). Wang et al. (2019) also reported that an HEV strain can persist on a farm for
750 at least two years in four out of five cases. For all these reasons, one can reasonably consider
751 these results (baseline scenario) as trustful. The predictions of the other scenarios cannot be
752 validated since no field data have been published yet.

753

754 From our results, it appears that farms using a 20-BRS have a particularly high risk of HEV
755 spread and persistence. Indeed, all other things being equal, HEV prevalence at slaughter age
756 was on average 1.3 times higher and HEV persistence five years post-introduction was 1.6
757 times more likely on a 20-BRS farm than on a 7-BRS farm. The large population and short
758 between-batch intervals probably play a major role in the differences observed between the

759 two BRSs, viral spread being less easy to manage in a large population. Moreover, the higher
760 environmental load linked to the greater number of infected pigs on the farm (data not shown)
761 may also be responsible for a greater HEV on-farm spread. To our knowledge, no data is
762 available yet on HEV dynamics depending on the type of BRS, but this same difference
763 between BRSs has already been observed for other viral pig diseases, e.g. influenza viruses
764 (Cador et al., 2016). The type of housing for gestating sows, another characteristic of farm
765 structures, has been found to play a pivotal role in HEV infection dynamics: housing gestating
766 sows in small groups drastically reduced HEV prevalence at slaughter age (dropping from 2.9
767 to 0.1%) and HEV on-farm persistence (dropping from 0.60 to 0.29), particularly on a 7-BRS
768 farm. This may be related to limited viral spread in the reproductive herd linked to the fact
769 that the simulated infection was introduced through a gilt, and to particularly marked
770 segregation between sows, and consequently in the growing pig population. Thus, though pigs
771 were on average infected later, the more confined viral spread eventually reduced the HEV
772 risk for public health. The farm's status regarding the IMV was also shown to greatly
773 influence HEV infection dynamics, especially on a 7-BRS farm, with HEV prevalence in
774 slaughter-age pigs being 17 times lower on an IMV-free farm than on an IMV-positive one,
775 and HEV persistence probability being divided by more than two. These outcomes confirm
776 the major impact of IMV infection on HEV dynamics previously evidenced under
777 experimental and natural conditions, thus the interest of implementing IMVs' eradication
778 programmes on pig farms. Interestingly, pigs were found to contract HEV much earlier ($HR =$
779 $1.70 [1.69-1.70]$) when the herd was IMV-free, which was related to low HEV infection
780 levels of sows in this context, leading to a limited number of passively immune piglets that
781 could contract HEV at an early age. This result clearly shows the impact of the protection
782 conferred by MDAs.

783
784 The model has also made it possible to evaluate the effectiveness of three farming practices
785 on reducing the risk of HEV. Firstly, the model has revealed that a lower cross-fostering rate
786 would decrease the risk of HEV spread and persistence. Indeed, HEV prevalence in slaughter-
787 age growing pigs was 1.5 times lower when no cross-fostering was allowed, and HEV on-
788 farm persistence was 1.1 times lower in this case also. This is consistent with the results of the
789 field study conducted by Walachowski et al. (2014). Drastically reducing cross-fostering is
790 likely to confine HEV spread to fewer litters, which limits the overall on-farm dissemination
791 and persistence. Our results have also shown that HEV prevalence at slaughter age would be
792 lower when weaning pen groups are smaller, which is also consistent with the study of

793 Walachowski et al. (2014). Surprisingly, mixing pigs randomly when moving them from the
794 farrowing sector to small nursery rooms reduced HEV prevalence at slaughter age compared
795 to by-litter mixing. On a 20-BRS farrow-to-finish pig farm, the impact of these farming
796 practices on HEV prevalence at slaughter age was lower than on a 7-BRS, and there was no
797 impact at all on HEV on-farm persistence probability. Again, the large population and short
798 between-batch intervals probably make virus control particularly difficult on this kind of
799 farm. From a health management point of view, a key finding of this study is that
800 implementing anti-IMV vaccination of sows at each reproduction cycle would positively
801 affect HEV infection dynamics — if farming practices are satisfactory — with HEV
802 prevalence at slaughter being 1.7 times lower and HEV persistence 1.8 times less frequent on
803 a 7-BRS farm on which sows are IMV-vaccinated (assuming 100% efficacy of the IMV
804 vaccine represented in the model). Health management measures for IMVs on pig farms may
805 therefore be a potential lever with which to mitigate the HEV risk indirectly, at least on 7-
806 BRS farms. This would be a valuable strategy for controlling both HEV, which is a public
807 health issue, and immunomodulating pathogens that can lead to serious animal health
808 disorders and economic losses for farmers. Besides, while no HEV vaccine is available for
809 pigs, there are vaccines against some immunomodulating pathogens such as PRRSV and
810 PCV2. However, the vaccine's efficacy in controlling the IMV needs to be considered. For
811 instance, PRRSV vaccines are all modified live vaccines, and the interactions between HEV
812 and the PRRSV strains used in vaccines are difficult to predict. Further studies, e.g.
813 experimental co-infection of pigs with HEV and PRRSV vaccine strains, would help shed
814 light on this issue.

815

816 Combining all the effective farming practices appeared helpful in reducing HEV risk,
817 especially on a 7-BRS farm. The effect was even higher when adding sow vaccination against
818 the IMV on a 7-BRS farm. These synergetic measures had both direct and indirect impacts as
819 they affected HEV infection dynamics as well as the IMV prevalence level — when sows are
820 vaccinated — and thus HEV indirectly. However, in the event of unsatisfactory husbandry
821 practices, IMV vaccination even had an adverse effect by increasing the risk of HEV entering
822 the food chain. One hypothesis for this would be that vaccinating sows against IMV leads to a
823 later IMV infection of pigs, once they have lost their maternal immunity; in that case, and in
824 combination with bad farming practices, HEV/IMV co-infections occur less frequently but
825 later, which increases the risk of still having HEV-infected pigs at slaughter time. The priority
826 should therefore be given to the improvement of farming practices and, if health measures are

827 planned to be implemented, they should be considered in synergy with good farming
828 practices.

829

830

831 **5. Conclusion**

832

833 In conclusion, our model revealed difficulties in containing HEV spread once the virus was
834 introduced on a 20-BRS farm, with a low fade-out probability. On a 7-BRS farm, housing
835 gestating sows in smaller groups and controlling intercurrent pathogens could be major levers
836 with which to mitigate the risk of HEV for public health. These results bring to light the
837 relevance of using indirect ways to control HEV and of considering animal and public health
838 in an integrated manner. In the case of more intensive BRSs such as 20-BRS farms, for which
839 few control measures have shown their efficacy in the present study, other control strategies
840 could be evaluated in the future using this model. These could include stricter biosecurity
841 practices (e.g. increasing the efficacy of cleaning and disinfection operations), different herd
842 renewal modalities, a lower mingling rate in the finishing sector and comprehensive
843 eradication plans for intercurrent pathogens. HEV infection dynamics on farms using other
844 BRSs could also be explored. Having more field data (e.g. data on the duration of the active
845 immunity, the possible HEV re-infection of recovered animals) would also be valuable for a
846 more accurate validation of the model. From a more operational perspective, it would be
847 worthwhile to test all these control measures on the field as well by carrying out an
848 intervention study on pig farms. The first step to carry out this kind of study would be to
849 select relevant farms (i.e. having risky farm practices and/or bad health situation, and where
850 HEV circulated) and where farmers would be voluntary to adopt other farming practices.
851 Interventions that could be studied would include cross-fostering reduction, decrease in the
852 size of nursery pens and PRRSV and/or PCV2 eradication programme, depending on the
853 health status of the farm. Further investigations should also focus on studying HEV spread
854 and persistence all along the pig production chain, from farms to slaughterhouses and
855 processed products. Fostering research efforts in this way would lead to a better
856 understanding of HEV risk at each step of the food chain. Taken together, modelling and field
857 data would make it possible to design a comprehensive HEV control plan and support public
858 health policies on this issue.

859

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862

863 **Competing interests**

864 The authors declare that they have no competing interests.

865

866 **Authors' contributions**

867 MS and MA developed the mathematical model and drafted the manuscript. NR coordinated
868 the study. All the authors participated in data analysis and interpretation, and revised the
869 manuscript. All the authors read and approved the final manuscript.

870

871

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872 **Supplementary Files**

873

874 **Supplementary file 1. Further details on the population dynamics model.**

875

876 Mortality: the probability p_m that an animal dies depends on its age. The daily mortality rate
877 m_r is equiprobable in the time interval Δt and follows the equation:

878
$$m_r = 1 - \exp\left(\frac{\log(1 - p_m)}{\Delta t}\right)$$

879 When entering a new room, pigs are stressed and the probability they die a few days after the
880 change is higher. Mortality probabilities and associated age limits are presented in Table 3a.

881 Abortion: the probability p_a that a sow has aborted in a time interval Δt depends on the
882 number of days before farrowing. The daily abortion rate a_r follows the equation:

883
$$a_r = 1 - \exp\left(\frac{\log(1 - p_a)}{\Delta t}\right)$$

884 Abortion probabilities associated to the number of days before farrowing are presented in
885 Table 3b.

886 Culling: if the sow is satisfying one of the following conditions, it may be culled:

- 887 - Parity rank: if its parity rank is higher than 7, the sow is culled.
888 - Litter size: if the sow has just left farrowing room and its litter size is less than 8, it has a
889 0.50 probability to be culled.
890 - Failed AI: if there has been one failed AI since the last time the sow farrowed, the culling
891 probability is 0.50. If the second AI fails too, the sow is culled.
892 - Abortion: if the sow has aborted twice, it is culled. If it has aborted once and the following
893 AI has also failed, the probability it is culled is 0.70. If it has aborted once and the two
894 following AIs have also failed, it is culled.
895 - Specific parameters for gilts: if the gilt is aged between 260 and 290 days, the culling
896 probability is 0.50. If it is older than 290 days, it is culled.

897 **Supplementary table 1. Parameters used to calculate daily mortality and abortion rates**
898 **in the population dynamics model**

899

900 *Supplementary table 1a. Mortality probabilities associated with age limits*

Age limit (days)	Associated mortality probability (p_m)
3	0.088
Age at weaning	0.052

Age at weaning +2	0.006
Age at the end of post-weaning	0.0023
Age at the end of post-weaning + 5	0.0025
180	0.04
200	0.02
355	0.01
700	0.02
1,400	0.02
2,000	0.02

901

902 *Supplementary table 1b. Abortion probabilities associated with the number of days before*

903 *farrowing*

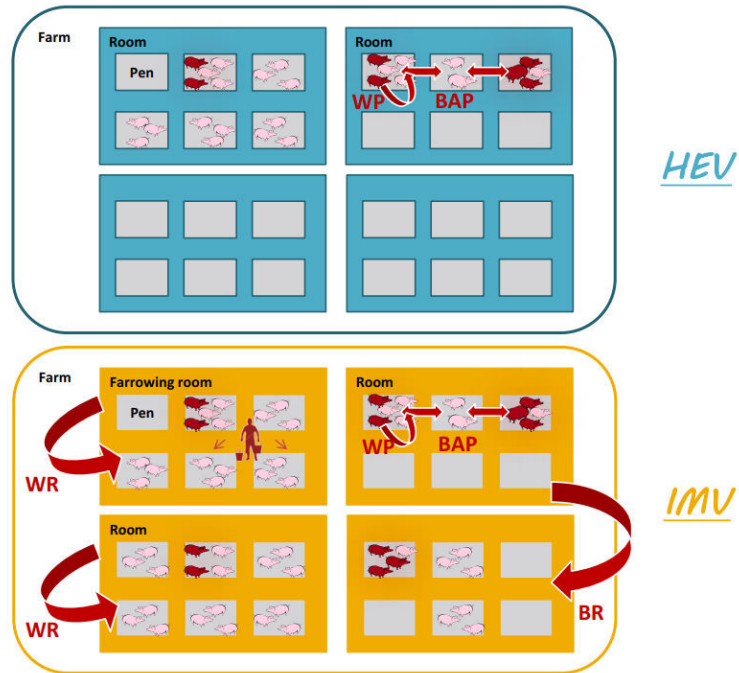
Number of days before farrowing	Associated abortion probability (p_a)
11	0
55	0.005
94	0.01
113	0.03
115	0

904

905

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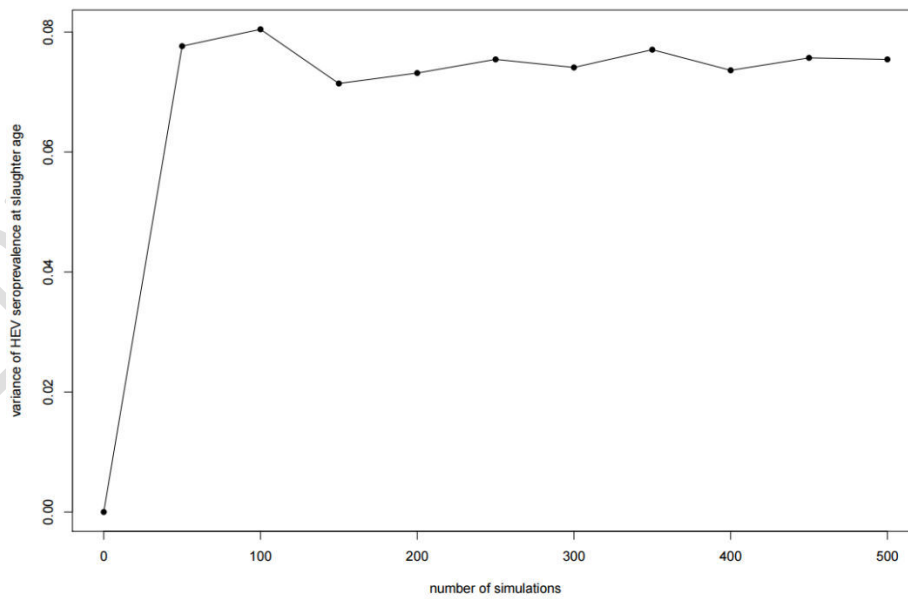
907 **Supplementary file 2. HEV and IMV transmission routes and associated forces of**
908 **infection.** WP: within-pen; BAP: between-adjacent-pens; WR: within-room; BR: between-
909 rooms



910

911

912 **Supplementary file 3. Variance of HEV seroprevalence at slaughter age depending on**
913 **the number of simulations.**



914

915

916 **Supplementary file 4. Relative impact of herd management and control measures on the dynamics of HEV infection (results from**
 917 **univariate analyses)**

918

919 **Table a. Influence of the farm's structure, farming and health practices on the age at which growing pigs contract HEV**

920 Survival analysis of the age at which growing pigs contract HEV using Cox proportional hazard models. Scenarios are detailed in Table 3.

921

Scenario	Variable	Modality	Age at which growing pigs contract HEV			
			Hazard Ratio [95% CI]		p-value	
1	Type of batch rearing system	7 batches	-			
2		4 batches	0.97 [0.96-0.97]		p < 0.01	
3		20 batches	1.03 [1.03-1.03]		p < 0.01	
			7-batch rearing system		20-batch rearing system	
			Hazard Ratio [95% CI]		p-value	
			Hazard Ratio [95% CI]		p-value	
1	Type of housing for gestating sows	Large groups	-		-	
4		Medium groups	0.93 [0.92-0.93]		p < 0.01	
5		Small groups	0.77 [0.76-0.78]		p < 0.01	
1	IMV status	IMV-positive	-		-	
6		IMV-free	1.70 [1.69-1.70]		p < 0.01	
1	Cross-fostering practices	Medium rate	-		-	
7		No adoption	0.98 [0.98-0.98]		p < 0.01	
8		High rate	1.09 [1.09-1.10]		p < 0.01	
1	Modalities for mingling at weaning	Small pens, by litter	-		-	
9		Small pens, randomly	1.20 [1.20-1.20]		p < 0.01	
10		Large pens, by litter	0.93 [0.92-0.93]		p < 0.01	
11		Large pens, randomly	1.06 [1.06-1.06]		p < 0.01	
1	Control of the IMV by vaccinating sows	No	-		-	
12		Yes	0.86 [0.86-0.87]		p < 0.01	
13	Worst-case scenario	-		-		
14	Worst farming practices, IMV vaccination	0.73 [0.72-0.73]		p < 0.01		
15	Best farming practices, no IMV vaccination	1.26 [1.25-1.26]		p < 0.01		
16	Best-case scenario	0.94 [0.93-0.94]		p < 0.01		

922

923 **Table b. Influence of the farm's structure, farming and health practices on the proportion of batches having HEV-infected animals at**
 924 **slaughter time**

925 Logistic regression was used to evaluate the impact of explanatory variables on the proportion of batches having HEV-positive animals at
 926 slaughter time.

Scenario	Variable	Modality	Proportion of batches having HEV-infected animals at slaughter time			
			Odds Ratio [95% CI]		p-value	
1	Type of batch rearing system	7 batches	-		p < 0.01	
2		4 batches	1.54 [1.47-1.62]		p < 0.01	
3		20 batches	4.79 [4.62-4.97]		p < 0.01	
			7-batch rearing system		20-batch rearing system	
			Odds Ratio [95% CI]	p-value	Odds Ratio [95% CI]	p-value
1	Type of housing for gestating sows	Large groups	-	p < 0.01	-	p < 0.01
4		Medium groups	0.43 [0.41-0.45]	p < 0.01	0.43 [0.42-0.44]	p < 0.01
5		Small groups	0.020 [0.018-0.022]	p < 0.01	0.086 [0.083-0.088]	p < 0.01
1	IMV status	IMV- positive	-		-	
6		IMV-free	0.15 [0.14-0.15]	p < 0.01	0.86 [0.84-0.89]	p < 0.01
1	Cross-fostering practices	Medium rate	-	p < 0.01	-	p < 0.01
7		No adoption	0.86 [0.83-0.90]	p < 0.01	0.97 [0.94-1.01]	p > 0.05
8		High rate	1.66 [1.59-1.74]	p < 0.01	1.49 [1.40-1.58]	p < 0.01
1	Modalities for mingling after weaning	Small pens, by litter	-	p < 0.01	-	p < 0.01
9		Small pens, randomly	1.06 [1.01-1.10]	p < 0.05	1.04 [1.01-1.07]	p < 0.01
10		Large pens, by litter	1.23 [1.18-1.28]	p < 0.01	1.07 [1.04-1.10]	p < 0.01
11		Large pens, randomly	0.95 [0.91-0.99]	p < 0.05	1.13 [1.09-1.16]	p < 0.01
1	Control of the IMV by vaccinating sows	No	-		-	
12		Yes	0.36 [0.34-0.37]	p < 0.01	0.54 [0.52-0.56]	p < 0.01
13	Worst-case scenario		-	p < 0.01	-	p < 0.01
14	Worst farming practices, IMV vaccination		0.74 [0.71-0.77]	p < 0.01	0.87 [0.84-0.90]	p < 0.01
15	Best farming practices, no IMV vaccination		0.67 [0.64-0.70]	p < 0.01	0.70 [0.68-0.73]	p < 0.01
16	Best-case scenario		0.34 [0.32-0.35]	p < 0.01	0.68 [0.66-0.70]	p < 0.01

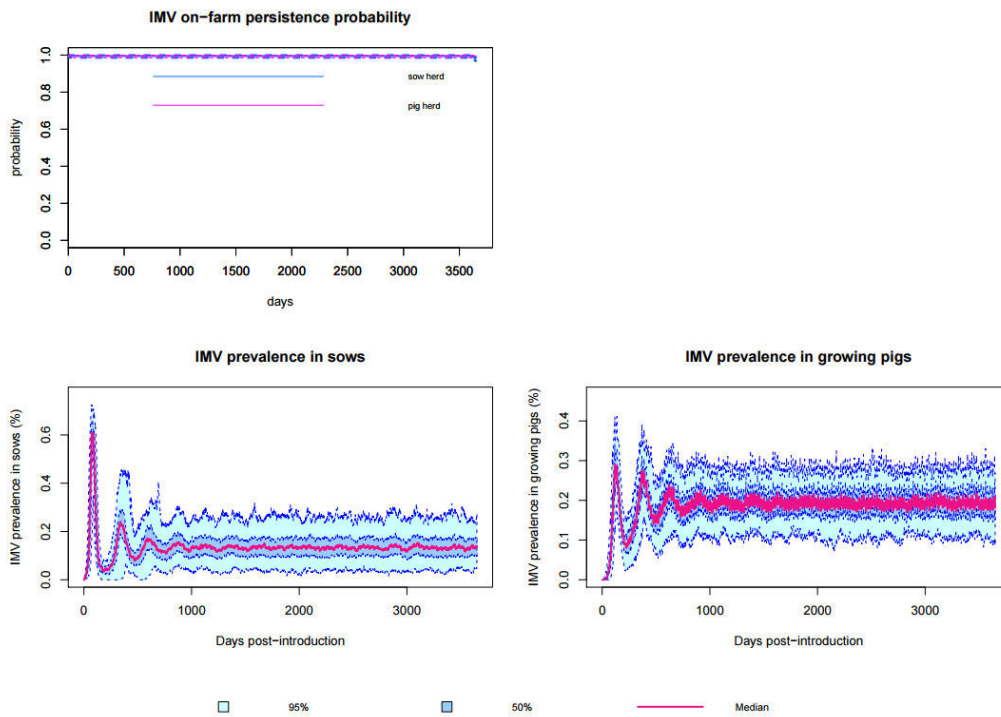
927

928 **Table c. Influence of the farm's structure, farming and health practices on HEV prevalence in growing pigs at slaughter time**
 929 Generalised estimating equation (GEE) logistic regression was used to evaluate the impact of explanatory variables on HEV prevalence in
 930 slaughter-age pigs.
 931

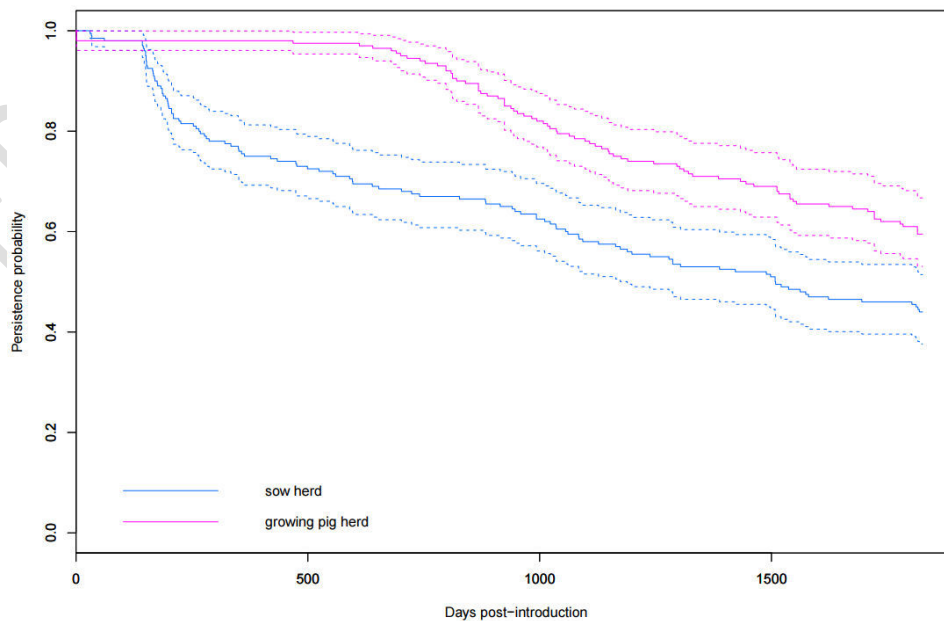
Scenario	Variable	Modality	HEV prevalence in slaughter-aged growing pigs			
			Odds Ratio [95% CI]		p-value	
1	Type of batch rearing system	7 batches	-		p < 0.01	
2		4 batches	0.84 [0.75-0.93]		p < 0.01	
3		20 batches	1.37 [1.27-1.49]		p < 0.01	
			7-batch rearing system		20-batch rearing system	
			Odds Ratio [95% CI]	p-value	Odds Ratio [95% CI]	p-value
1	Type of housing for gestating sows	Large groups	-	p < 0.01	-	p < 0.01
4		Medium groups	0.61 [0.53-0.69]	p < 0.01	0.71 [0.68-0.74]	p < 0.01
5		Small groups	0.033 [0.021-0.051]	p < 0.01	0.22 [0.21-0.24]	p < 0.01
1	IMV status	IMV- positive	-		-	
6		IMV-free	0.057 [0.051-0.063]	p < 0.01	0.97 [0.93-1.02]	p > 0.05
1	Cross-fostering practices	Medium rate	-	p < 0.01	-	p < 0.01
7		No adoption	0.91 [0.82-1.01]	p > 0.05	0.93 [0.90-0.96]	p < 0.01
8		High rate	1.45 [1.31-1.60]	p < 0.01	1.40 [1.35-1.46]	p < 0.01
1	Modalities for mingling after weaning	Small pens, by litter	-	p < 0.01	-	p < 0.01
9		Small pens, randomly	0.78 [0.71-0.86]	p < 0.01	0.78 [0.75-0.81]	p < 0.01
10		Large pens, by litter	1.23 [1.11-1.36]	p < 0.01	1.36 [1.31-1.41]	p < 0.01
11		Large pens, randomly	0.84 [0.73-0.96]	p < 0.01	1.23 [1.09-1.17]	p < 0.01
1	Control of the IMV by vaccinating sows	No	-		-	
12		Yes	0.60 [0.53-0.67]	p < 0.01	0.98 [0.92-1.04]	p > 0.05
13	Worst-case scenario		-	p < 0.01	-	p < 0.01
14	Worst farming practices, IMV vaccination		1.42 [1.26-1.59]	p < 0.01	1.73 [1.65-1.81]	p < 0.01
15	Best farming practices, no IMV vaccination		0.44 [0.39-0.50]	p < 0.01	0.42 [0.41-0.44]	p < 0.01
16	Best-case scenario		0.45 [0.39-0.51]	p < 0.01	0.71 [0.69-0.74]	p < 0.01

932

933 **Supplementary file 5. IMV persistence and prevalence in sows and growing pigs**
 934 **(median, 50%, 95%) on a 7-batch rearing system farrow-to-finish pig farm.**

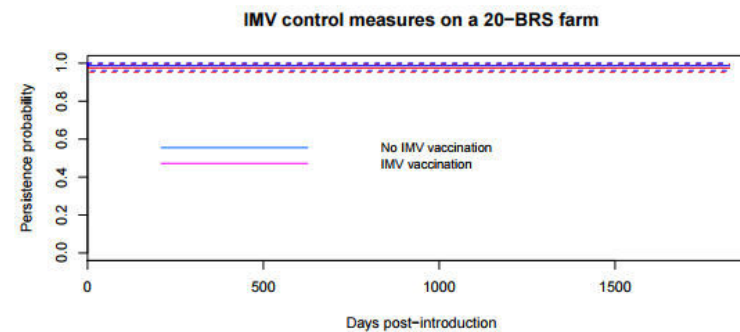
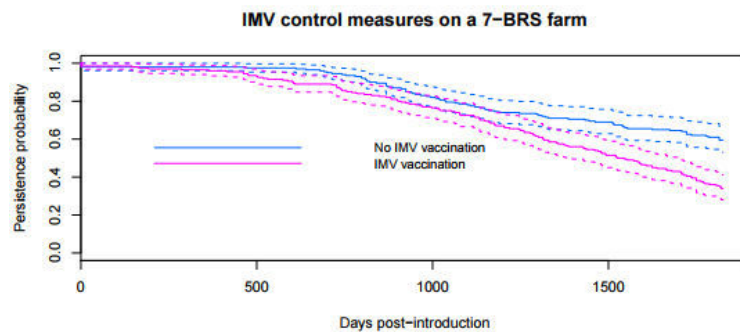
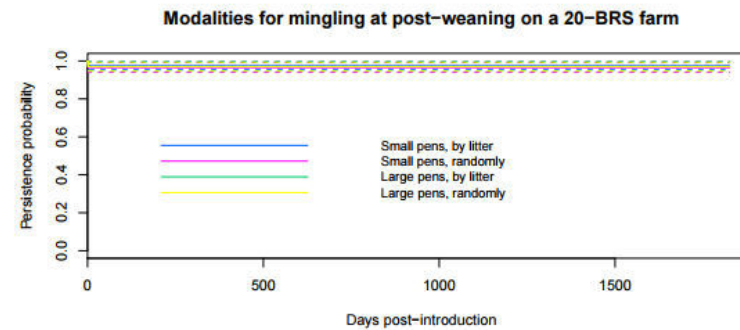
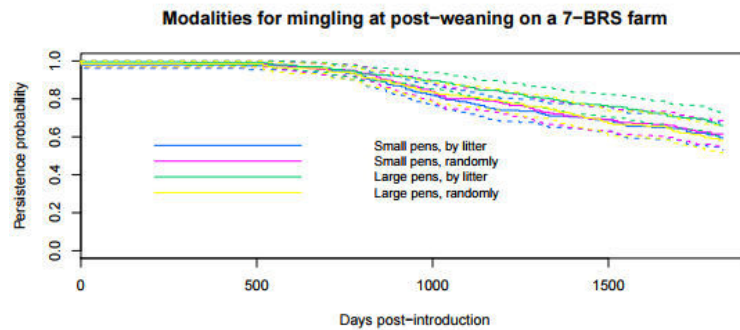
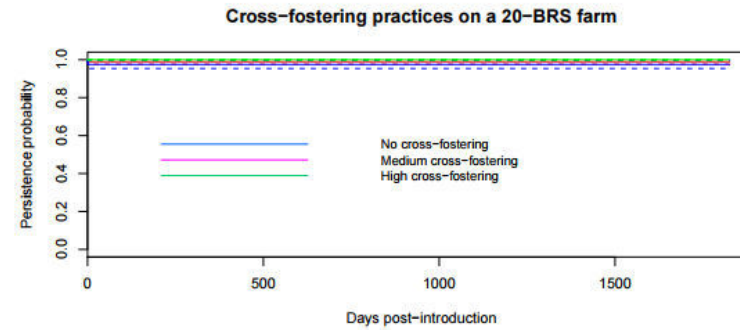
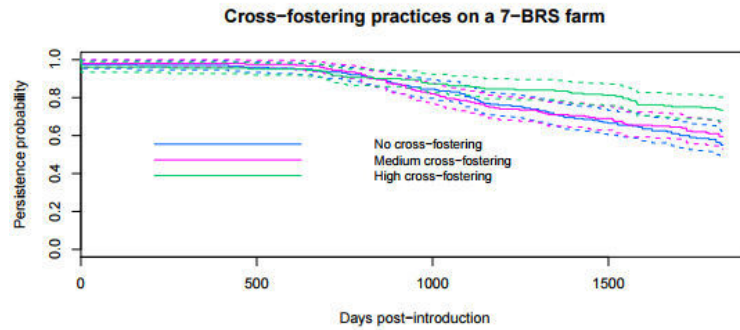


935
 936
 937 **Supplementary file 6. HEV on-farm persistence five years post-introduction in the sow**
 938 **herd and growing pigs, on a 7-batch rearing system farrow-to-finish pig farm (n = 200**
 939 **simulations).**
 940



941

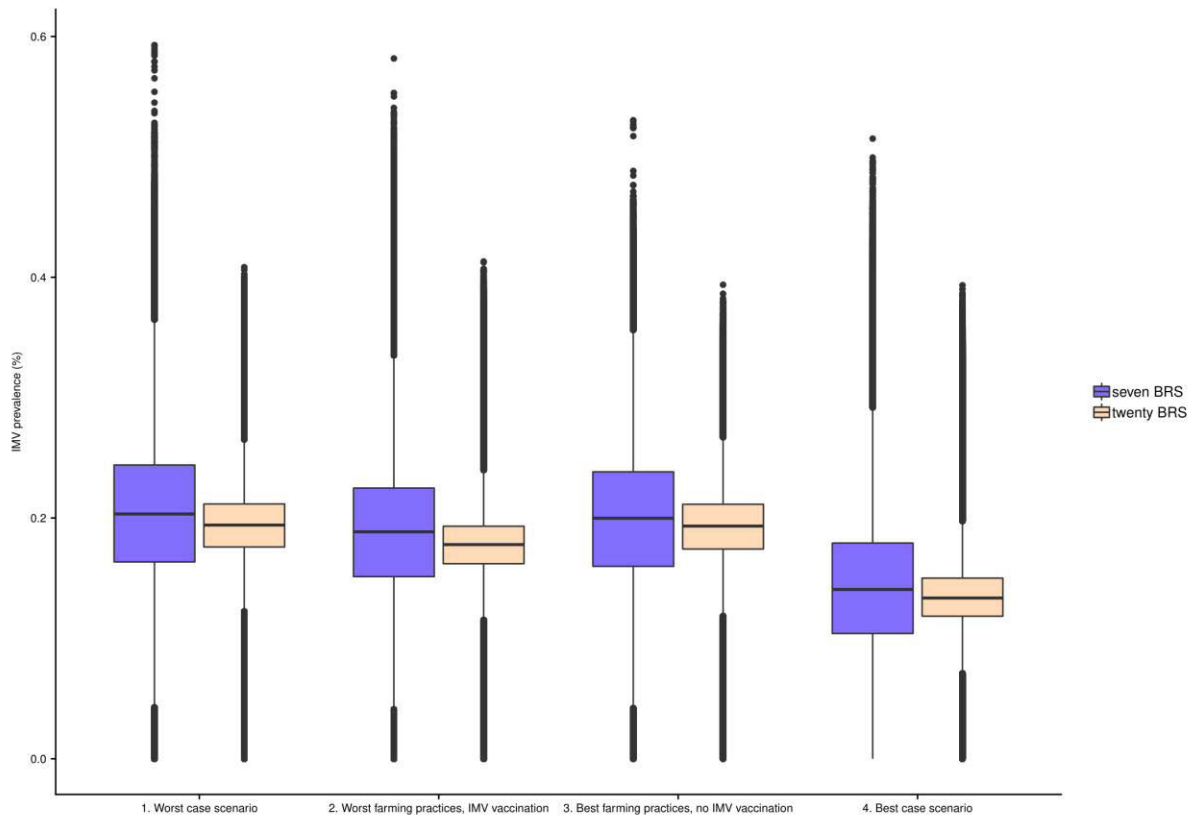
942 **Supplementary file 7. HEV persistence probability on a 7- or 20-batch rearing system farrow-to-finish pig farm depending on farming**
943 **practices and health management measures (n = 200 simulations).**



944

945 **Supplementary file 8. Immunomodulating virus (IMV) prevalence in growing pigs on a**
946 **7- or 20-batch rearing system farrow-to-finish pig farm in combined HEV control**
947 **scenarios (n = 200 simulations).**

948



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Ce qu'il faut retenir

A partir d'une approche innovante de modélisation multi-pathogènes, le modèle développé a apporté de nouveaux éléments dans la compréhension de la dynamique de l'infection par le HEV dans un élevage de porcs naisseur-engraisseur. Il a permis de mettre en évidence l'influence majeure de la structure de l'élevage (type de conduite en bandes, système de logement des truies gestantes) ainsi que de certaines pratiques d'élevage (modalités d'adoption, taille des cases en post-sevrage, modalités de mélange au sevrage) et sanitaires (vaccination des truies contre les pathogènes intercurrents). En particulier, ce dernier point souligne la pertinence d'utiliser des moyens indirects pour cibler le HEV et de considérer la santé animale et la santé publique de manière intégrée.

Ce travail contribue à une meilleure connaissance des facteurs expliquant la propagation et la persistance du HEV au sein d'un élevage de porcs. Il apparaît également nécessaire de comprendre les voies de diffusion préférentielle du HEV entre les élevages et ainsi la persistance du virus dans la filière de production porcine. Pour ce faire, une approche de modélisation multi-échelles a été développée dans la suite du projet de recherche, tenant compte des échanges de porcs entre élevages pour la construction d'un modèle inter-troupeaux de la dynamique du HEV.



Take home message

Based on an innovative multi-pathogen modelling approach, the model we have developed has given insights for the understanding of HEV infection dynamics on a farrow-to-finish pig farm. It made it possible to evidence the major role of the farm's structure (type of batch management system, type of housing facilities for gestating sows) as well as of some farming practices (cross-fostering practices, size of the nursery pens, modalities for mingling weaned piglets) and health control measures (sow vaccination against immunomodulating pathogens). In particular, the latter point underlines the relevance of using indirect ways to target HEV and of considering animal and public health in an integrated manner.

This work contributes to a better understanding of the factors explaining HEV spread and persistence on a pig farm. It also appears necessary to understand the preferential distribution pathways of HEV between farms and thus the persistence of the virus in the pig production chain. To do this, a multi-scale modelling approach has been developed in the next steps of the research project. It integrates between-farm pig trade to build a between-herd model of HEV dynamics.

An abstract graphic design featuring a solid green background. A white geometric shape, resembling a stylized 'L' or a corner, is positioned in the upper right quadrant. This shape is composed of a solid white area and a section with a fine, repeating pattern of small white dots. The overall composition is clean and modern.

Chapitre III

**Analyse de la propagation et de la
persistance du virus de l'hépatite E dans
la filière de production porcine**

PARTIE 3.1. Les mouvements de porcs comme point d'intérêt pour l'étude du risque lié au virus de l'hépatite E dans la filière de production porcine

I. Etude des caractéristiques du réseau des mouvements de porcs en France

Avant d'initier la construction d'un modèle inter-troupeaux représentant la diffusion du HEV entre les élevages de porcs par l'intermédiaire des échanges d'animaux, il est apparu opportun de commencer par une **analyse descriptive du réseau des mouvements de porcs en France**. Ainsi, à partir des échanges de porcs enregistrés dans la base de données BDporc sur la période 2012-2014, **deux types de réseaux** ont été construits **selon les caractéristiques épidémiologiques du pathogène considéré** : le premier réseau est adapté aux pathogènes transmis uniquement par l'introduction d'animaux infectés dans un élevage (*Animal Introduction Model* - AIM) ; l'autre réseau correspond à des pathogènes transmis également par voie indirecte lors du passage des camions dans les élevages sans déchargement d'animaux (*Transit Model* - TM). Ces deux réseaux ont été étudiés par des méthodes de *Social Network Analysis* (statistiques descriptives, recherche de composants connectés et de communautés, analyse temporelle).

Ce travail a été publié dans le journal *PLoS One* (Salines *et al.*, 2017b). Pour faciliter la compréhension de cet article, un tableau définissant et illustrant les principaux indicateurs utilisés pour l'analyse du réseau est présenté en Annexe 6.

Publication 7

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RESEARCH ARTICLE

Pig movements in France: Designing network models fitting the transmission route of pathogens

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Abstract

Pathogen spread between farms results from interaction between the epidemiological characteristics of infectious agents, such as transmission route, and the contact structure between holdings. The objective of our study was to design network models of pig movements matching with epidemiological features of pathogens. Our first model represents the transmission of infectious diseases between farms only through the introduction of animals to holdings (Animal Introduction Model AIM), whereas the second one also accounts for pathogen spread through intermediate transit of trucks through farms even without any animal unloading (i.e. indirect transmission—Transit Model TM). To take the pyramidal organisation of pig production into consideration, these networks were studied at three different scales: the whole network and two subnetworks containing only breeding or production farms. The two models were applied to pig movement data recorded in France from June 2012 to December 2014. For each type of model, we calculated network descriptive statistics, looked for weakly/strongly connected components (WCCs/SCCs) and communities, and analysed temporal patterns. Whatever the model, the network exhibited scale-free and small-world topologies. Differences in centrality values between the two models showed that nucleus, multiplication and post-weaning farms played a key role in the spread of diseases transmitted exclusively by the introduction of infected animals, whereas farrowing and farrow-to-finish herds appeared more vulnerable to the introduction of infectious diseases through indirect contacts. The second network was less fragmented than the first one, a giant SCC being detected. The topology of network communities also varied with modelling assumptions: in the first approach, a huge geographically dispersed community was found, whereas the second model highlighted several small geographically clustered communities. These results underline the relevance of developing network models corresponding to pathogen features (e.g. their transmission route), and the need to target specific types of holdings/areas for surveillance depending on the epidemiological context.

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Data Availability Statement: Movement data are third-party data and are part of the National Swine Identification Database owned by BDporc. The authors did not collect the data and the authors do not claim ownership of the data. The owner of the data can be contacted at: BDPorc, 43, rue Sedaine, CS 91115, 75538 PARIS cedex 11 – FRANCE. The authors did not have any special access privileges that others would not have.

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1. Introduction

Swine infectious diseases have economic consequences for the pig industry and can affect public health. They can be transmitted from farm to farm through animal trade, either because of the introduction of infected animals, or only because of transit movements of contaminated trucks acting as mechanical vectors [1]. Disease spread is closely linked to the movement network topology [1, 2]; gaining insights into spatial and contact patterns of pig trade could therefore be a major lever to control the spread of swine infectious diseases. To do so, animal movement data are increasingly modelled into networks and studied using social network analysis (SNA) methods [2–22]. Animal trade networks are composed of nodes, which are either farms or slaughterhouses, markets, trade operators, etc., and of links, which are shipments of animals between these units. These networks are directed: animal movements along the network links are considered directed paths for the spread of a disease from one farm to another. Cattle, sheep, pig and poultry markets have already been modelled in several countries [2, 4–22], using either movements reported by farmers through questionnaires, or movements systematically recorded in a harmonised database. Unlike cattle movements, a special feature of swine trade data is that pig movements are reported at a batch scale, without the possibility of tracking animals individually. Moreover, the pig production sector is organised in a pyramidal way, with movements going from the nucleus and multiplying farms at the top, to the production farms at the bottom (from farrowers to finishers). This particular structure affects the network topology and has to be accounted for [6]. Pig movements can exhibit intricate patterns, for instance when trucks collect pigs at several farms before unloading all of them at a single site (e.g. a slaughterhouse). To our knowledge, most of swine trade networks published in the literature have simplified these complex trajectories going through several farms by representing only direct operations from the loading locations to the unloading sites [2, 7–10, 19, 23]. By doing so, intermediate transit movements of trucks in farms without any animal unloading have been neglected. Yet these movements can contribute to the spread of diseases for which indirect transmission through mechanical vectors occurs (e.g. African Swine Fever—ASF, Porcine Epidemic Diarrhoea—PED, Foot and Mouth Disease—FMD, Porcine Reproductive and Respiratory Syndrome Virus—PRRSV) [24–26]. To fit as closely as possible with the pathogens' epidemiological features, network models should take their various transmission routes into account. To explore the role of trucks in indirect disease spread, some research teams developed two-mode networks with trucks or rounds being considered as a second class of nodes in addition to holdings [6, 21]. This method makes it possible to obtain relevant data regarding the functioning of rounds, such as the number of rounds concerning a given farm, or the number of holdings connected in a round. However, two-mode networks are not easy to analyse: centrality measures cannot all be computed, contact chains are not calculated, and communities and connected components are usually not looked for [6, 21]. Two-mode networks are thus often altered in a one-mode network to be more deeply analysed [6].

The objective of our study was therefore to design two one-mode network models matching with the transmission route of pathogens, and to analyse empirical data of French pig trade. We focused our model analysis on the different levels of the pyramidal structure inherent to the pig production system.

2. Materials and methods

2.1. Data

2.1.1. Database description. Since 2010, pig movements in France have been recorded and stored in the National Swine Identification Database (BDporc). This database is managed

by swine industry professionals and is recognised by the French Ministry for Agriculture. For the present study, we analysed the data from June 2012 to December 2014. Two levels of information were gathered in the dataset: the characteristics of swine production units and the details of the animal movements between the different production sites. The main features of all swine holdings in mainland France are included in the database: identification number, type of holding (farm, slaughterhouse, rendering company, market, assembly centre, trading company), type of farming activity (boar station *BS*, nucleus *SEL*, multiplication *MU*, farrowing *FA*, farrowing-to-finishing *FF*, finishing *FI*, farrowing-post-weaning *FPW*, post-weaning *PW*, post-weaning-finishing *PWF*, small producers *SP*), type of production (free-range or not), and location (post code and GPS coordinates). Movements of pigs were reported at a batch level: groups of animals were sent off the production sites (loadings, further denoted L) and dispatched to either alternative production units or slaughterhouses (unloadings, further denoted U). A single truck could load and unload animals at several production sites: one round corresponds to a series of movements of a truck, from the first loading operation to the last unloading event making the truck empty. Each loading and unloading operation was individually reported for each round with several pieces of information: the farm and the round IDs, the chronological sequence of the operations during the round, the batch size and the animal category (breeding animals, piglets, and growing pigs).

2.1.2 Data cleaning and pre-processing. Data included both movements occurring within France and movements from/to foreign countries. However, imports and exports of animals were recorded at the country level, with a lower data resolution than movements occurring within France. Therefore, movements from/to foreign countries were considered separately to have a global overview of international trade movements, when a thorough analysis of within-France data was performed.

A series of cleaning processes were performed on the dataset, discarding records for which the principal pieces of information were unavailable (e.g. round or herd identification numbers, animal category). Farms were categorised into 11 groups according to their major activity; markets, assembly centres and trading companies were gathered into the single “trade operators” category. Direct movements to slaughterhouses and rendering plants were excluded from the analysis as they do not play a major role in pathogen spread. When these movements were part of longer rounds collecting pigs from several herds before going to the slaughterhouse/rendering plant, only the last movement (from the last farm to the slaughterhouse) was excluded. Considering the absence of any seasonality in pig trade shown in previous studies [2, 7, 23, 27, 28], movement data were aggregated on a six-month basis.

2.2. Model design

One-mode directed networks were built: holdings were considered as nodes, movements between two nodes were considered as links. All movements between two given holdings during the time period were aggregated into a single link. We designed two types of network to model a round (Fig 1A) in two different ways depending on the route of transmission of the considered pathogen. (i) In the first network model, called hereafter the Animal Introduction Model (AIM) (Fig 1B), links between holdings represented movements of animals being unloaded at farms. In-between movements forming a round were replaced by direct movements between holdings, i.e. intermediate transit movements of a truck through a farm without unloading any animal were excluded. All sites corresponding to unloading operations were assumed to be linked to all prior loading sites of the same round. For example, assuming successive loadings at sites L1 and L2 followed by an unloading operation at site U4, then holding U4 was linked to L1 and L2. This model is relevant for pathogens that spread between

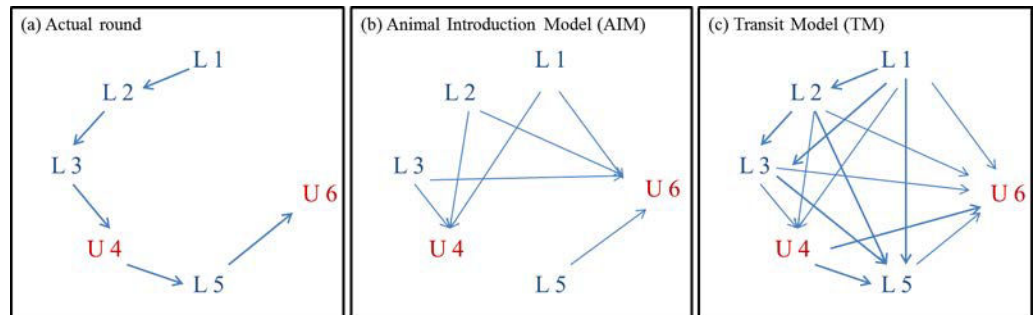


Fig 1. Types of network models built to represent pig movements. Nodes L and U correspond to holdings where loading and unloading operations occurred, respectively. The number corresponds to the chronology of animal collection by a truck in one round. Fig 1.a describes the actual round of a given truck, whereas Fig 1.b and Fig 1.c describes how the links between holdings were modelled, depending on the transmission route of the pathogen considered. In the Animal Introduction Model—AIM (Fig 1.b), movements forming a round were replaced with direct movements between holdings, i.e. intermediate transit movements of a truck through a farm without unloading any animal were neglected. This network accounts for the transmission of a disease only through the introduction of animals into farms. In the Transit Model—TM (Fig 1.c), each holding was assumed to be linked to every other upstream and downstream farm in a given round through incoming and outgoing links, respectively. This type of network can be used to explore the spread of a pathogen both through the introduction of animals to farms and through the indirect route.

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holdings only through the introduction of animals to farms (i.e. diseases that spread via physical contact and for which the indirect transmission route is negligible). (ii) In the second network model, further denoted Transit Model (TM) (Fig 1C.), links between holdings represented both movements of animals and truck transit through a farm without any animal unloading. In a given round, each holding was therefore linked to all upstream and downstream farms (incoming and outgoing links, respectively). In other words, each round was modelled as a full graph. This model could be used for pathogens that spread not only because of the introduction of animals to farms but also through the transit of trucks through farms even without any animal introduction (i.e. diseases for which indirect transmission occurs, with trucks acting as mechanical vectors).

2.3. Network analysis

Considering the pyramidal structure of the pig production sector, all analyses presented below were performed at three different scales: the whole network, the breeding farm subnetwork (boar stations, nucleus/multiplication farms) and the production farm subnetwork. Network analysis was performed on within-France movements only.

2.3.1. Network descriptive indicators. Several descriptive statistics of the network characteristics were calculated for each network model and for each semester to analyse changes in network properties over the study period. The first semester was running from January 1st to June 30th, the second one from July 1st to December 31st. The classical metrics that were computed were: the *size* (number of active nodes and links), the *average degree* (mean of the total number of ingoing and outgoing links for each node), the *average path length* (the average number of links along the shortest paths—or geodesics—between all pairs of nodes), the *diameter* (the longest geodesic), and the *density* (ratio of the number of links and the number of possible links for active nodes). We also calculated the *clustering coefficient* (proportion of neighbours of a node that are linked to each other), the *Jaccard similarity coefficient* (the JSC of two nodes being the number of common neighbours divided by the number of neighbours of each of the two nodes considered), the *assortativity degree* (Pearson correlation coefficient between the degrees of linked nodes), and the *reciprocity ratio* (proportion of mutual connections, in a directed

graph). The distributions of the four main centrality measurements were computed for each holding type: *degree*, *in-degree* (number of different holdings from which a holding receives animals), *out-degree* (number of links going from a node), *closeness* (number of steps required to access every other node from a given node) and *betweenness* centralities (number of geodesics going through a node). For each network model, a power-law distribution defined as $p(x) \sim x^{-\alpha}$ was fitted to the observed degree distribution. We used a maximum-likelihood estimator to estimate scaling parameter (α) and the Kolmogorov–Smirnov (KS) goodness-of-fit statistic to test power law fit of the data as described by Clauset et al. [29].

2.3.2. Detection of connected components and communities. Connected components.

Weakly connected components (WCCs) are sections of the network where every holding can be reached from every other holding whatever the link direction. Based on this definition, no connection exists between two WCCs and they can be considered as independent subnetworks. *Strongly connected components* (SCCs) are subgraphs in which every node can be reached from every other node via one or several directed paths. The number of WCCs and SCCs and the size of the largest WCCs and SCCs were determined with the two network models AIM and TM, and for the whole population as well as separately for the breeding farm and production farm subpopulations.

Communities. Detection of *network communities*, defined as subsets of nodes in which there are significantly more links than expected by chance, i.e. groups of highly connected farms, was performed using the Infomap algorithm [30]. Briefly, the hierarchical map equation measures the per-step average code length necessary to describe a random walker's movement on a network, given a hierarchical network partition, and looks for the community structure that minimises the expected description length of the random walker trajectory. In the core algorithm, each node is first assigned to its own module. Then, in random sequential order, each node is moved to the neighbouring module that results in the largest decrease of the map equation. When adding movements does not result in a decrease of the map equation, the node stays in its original module. This procedure is repeated, each time in a new random sequential order, until no move generates a decrease of the map equation. The network is then rebuilt, with the modules of the last level forming the nodes at this level, and, exactly as at the previous level, the nodes are joined into modules. This hierarchical rebuilding of the network is repeated until the map equation cannot be reduced further. The Infomap algorithm is the only one that can be applied on directed networks and it is considered to have the best performance [31]. We ran the algorithm with 1,000 trials, on the two network models AIM and TM. Like for the connected component detection, we looked for communities in the whole graph and in the two subgraphs (breeding/production farms). We also calculated the percentages of links connecting two different communities (i.e. bridges, or crossing links).

2.3.3. Temporal network analysis. Link and node preservation. We counted the number of nodes remaining active from one semester to another, as well as the number of links being preserved from one semester to another.

Node loyalty. In order to explore the nodes' tendency to re-establish connections with the same herds or to change trade partners over time, the *node loyalty* was computed for each kind of model. The loyalty measures the fraction of preserved links of a node for a pair of two consecutive network configurations in time, the time window in our case being a semester. It involves values between 0 and 1, a loyalty value of zero indicating that all connections were different between the two time windows, a loyalty of one indicating that exactly the same set of links was preserved. We computed the loyalty on the incoming contacts of nodes, thus quantifying the tendency of a farmer to purchase animals from the same sellers.

Outgoing and ingoing contact chains. The *outgoing and ingoing contact chains* (OCC and ICC, respectively) were computed for each type of holding over a one-month period. These

measures capture the sequence of contacts through direct and indirect movements, taking into account the order in which movements happen during a fixed time-period. The OCC is the number of nodes in contact with a certain node, the root, through movements of animals leaving the root. In other words, the *set of influence* of the root corresponds to the set of nodes that can be reached from the root through time-respecting paths within the observation window. Similar to the OCC, the ICC is the number of nodes in contact with the root holding through movements reaching the root. The *source set* of the root is defined as the set of nodes that can reach the root through time-respecting paths within the observation window. These two measures reflect the potential epidemic size of a disease in the network [32].

Network analyses were performed using the Igraph package in R software [33].

3. Results

3.1. Swine trade description

3.1.1. Within-France movements. A total of 21,446 sites were recorded in the BDporc database, among them 97.9% were farms, 1.5% slaughterhouses and rendering plants, and 0.6% trade operators (Table 1). The number of farms decreased by 2.9% between June 2012 and December 2014.

The database contained 2,382,510 movement records, from which 9% were discarded after the cleaning process (16, 44, and 40% due to missing or incomplete round, foreign movements or missing herd identification numbers, and animal mortality or missing animal category, respectively). A total of 838,777 rounds occurred between June 2012 and December 2014. They were composed of several loading and unloading operations: rounds between farms implied on average 2.5 holdings (range: 2–32), whereas rounds going to slaughterhouses were on average composed of a single movement. The leading destination of movements was slaughterhouses/rendering plants (75.2% of unloading operations), followed by farms (22.8%) and trade operators (2.0%). Growing pigs were the main animal category involved in movements (67% of unloaded animals), followed by piglets (31%) and breeding pigs (2%). The average number of animals transported in a given round varied with the destination site: in the second half of 2014, a round going to farms transported on average 188 animals, whereas those going to slaughterhouses and trade operators transported on average 84 and 25 pigs, respectively. The number of animals transported in a single round increased by 4%, 1.6% and 24.8% over the study period for rounds going to farms, slaughterhouses and trade operators, respectively. The number of rounds decreased by 4% over the same period, leading to an overall decrease of 0.6% in the total number of unloaded animals. The decline in exchanges mainly affected breeding pigs and trade operators. These data are detailed in S1 Table.

The distribution of distances travelled by pigs in a round varied with the animal category. Excluding movements to slaughterhouses, rendering plants and trade operators from distance calculations, breeding pigs travelled on average 270 km (median: 200, range: 0–1,000), whereas growing pigs travelled on average 74 km (median: 42, range: 0–999).

3.1.2. Movements from/to foreign countries. A total of 12,065 rounds came from or went abroad over the study period, corresponding to 1.4% of the total number of rounds recorded in the whole database. Animals sent abroad were mostly growing pigs (59.4% of animals unloaded abroad), culled sows and boars (28.7%) and breeding pigs (9.6%). Outgoing shipments mainly went to Belgium and Germany (48.6% and 32.1%, respectively—mainly pigs and culled sows/boars to slaughterhouse), Italy (7.0%—mainly pigs to slaughterhouses) and Spain (7.2%—mainly pigs to slaughterhouses and breeding pigs). Animals imported from abroad were growing pigs, piglets and breeding pigs (43.6%, 38.0% and 18.1%, respectively). Incoming shipments came primarily from Spain (47.3%—mainly pigs to slaughterhouses),

Table 1. Number and proportion of sites categorised according to their major activity.

	Abbreviation	Type	Number	Percentage
Breeding farms	BS	Boar Station	73	0.35
	SEL	Nucleus	117	0.56
	MU	Multiplier	343	1.63
Production farms	PW	Post-weaning	162	0.77
	PWF	Post-weaning—Finishing	2,273	10.83
	FA	Farrowing	465	2.21
	FF	Farrowing-to-Finishing	5,064	24.12
	FPW	Farrowing—Post-weaning	288	1.37
	FI	Finishing	4,414	21.02
	SP*	Small Production	7,457	35.51
	WB	Wild-boar	342	1.63
	Total no. of farms			20,998
	TR	Trade operators	117	
	SR	Slaughterhouses / Rendering plants	331	

As expected given the pyramidal structure inherent to the pig production system, PWF, FF, FPW, FI and SP are the most represented farm types in France. * Small Production farms were defined as farms rearing fewer than 80 animals.

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Belgium (33.3%—mainly piglets) and Denmark (11.5%—mainly breeding pigs). Shipments to and from non-EU countries represented only 0.5% and 0.4% of foreign movements, respectively.

3.2. Network description

3.2.1. Network mapping. The density of active holdings and movements varied with regions, e.g. the network in north-western France was much denser than in south-eastern France (Fig 2.1). Breeding farms were mostly located in the upper left diagonal part (Fig 2.2). The network appeared denser using the TM than the AIM. Node degree was higher in the TM approach than in the AIM, especially for farrowing and farrow-to-finish farms, and particularly in the centre of France (Fig 2.3.B). Network maps were similar over the five semesters (data not shown).

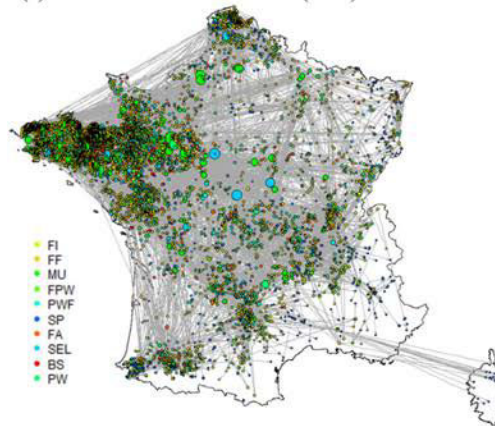
3.2.2. Network descriptive indicators. Whole network. Network descriptive statistics are summarised in Table 2.

In the second half of 2014 for example, the network contained 11,013 and 13,784 active holdings when using the AIM and the TM, respectively. The number of links per semester was around six times higher in the TM than in the AIM (132,677 and 21,691 links, respectively). Regarding link multiplicity, 51% of links between two holdings happened only once per semester in the AIM versus 68% in the TM. A holding exchanged animals on average with four different farms in the AIM, while a holding was in contact with 19 other farms on average in the TM (average degree). Fig 3 shows the degree distributions of holdings on a log–log scale for the AIM and the TM. Whatever the model, the distribution appeared similar in the five semesters (data not shown) and showed power-law-like behaviour (power-law exponent *alpha* values being equal to 2.78 and 5.82 with p-values of the KS test being 0.29 and 0.78 for the AIM and the TM, respectively), suggesting a scale-free structure of the network.

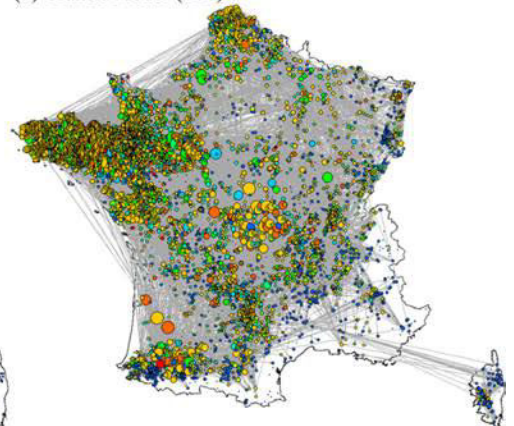
Distance indicators varied with the model used: a given pair of connected nodes was separated by approximately two animal movements in the AIM versus six movements in the TM (average path length). The average path length was shorter in the AIM and similar in the TM

1. Whole network

(a) Animal Introduction Model (AIM)

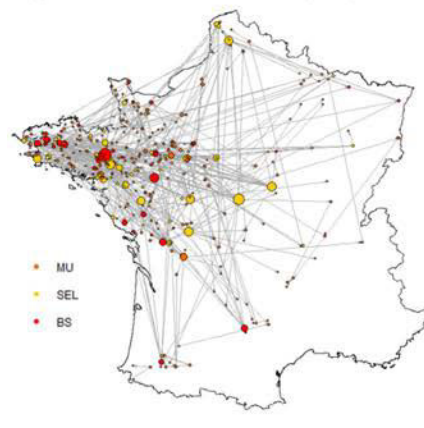


(b) Transit Model (TM)

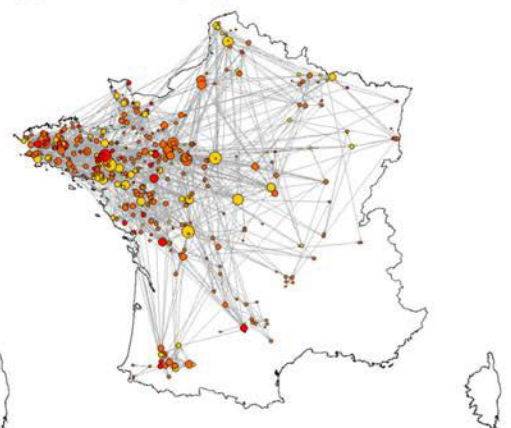


2. Breeding farm subnetwork

(a) Animal Introduction Model (AIM)

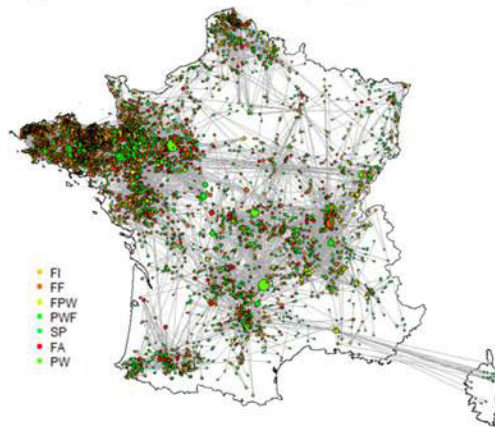


(b) Transit Model (TM)



3. Production farm subnetwork

(a) Animal Introduction Model (AIM)



(b) Transit Model (TM)

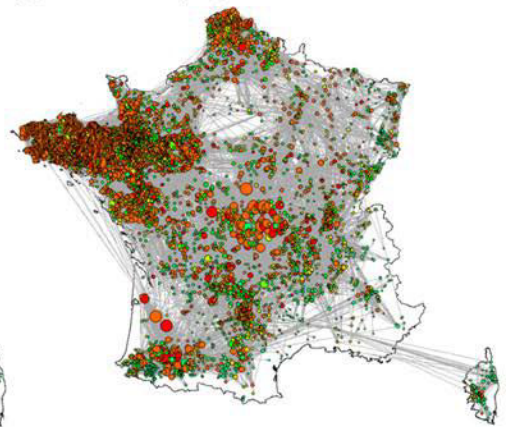


Fig 2. Mapping of the pig movement network in France (second half of 2014) applying the two different models (Animal Introduction Model [AIM] and Transit Model [TM]) to the whole network, the breeding farm subnetwork and the production farm subnetwork. The points are active holdings only (i.e. farms having had at least one movement over the semester). Their size is proportional to their degree. Direct

movements to slaughterhouses are excluded. BS: boar station, SEL: nucleus, MU: multiplication, FA: farrowing, FF: farrowing-to-finishing, FI: finishing, FPW: farrowing-post-weaning, PW: post-weaning, PWF: post-weaning-finishing, SP: small producers.

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to in a random graph of the same size. The diameter also increased from 10 links in the AIM to 20 links in the TM. The network modelled with the TM was four times denser than the AIM one. The clustering coefficients of the network were low, but ten times higher in the TM than in the AIM, suggesting that nodes tended to gather when considering the TM. Moreover, the clustering coefficient was higher in the AIM and the TM than in a random graph of the same size. Whatever the model, the Jaccard similarity coefficient was equal to zero for almost all pairs of nodes, showing the dissimilarity of nodes. The assortativity of the AIM network was negative (i.e. the network was disassortative). On the contrary, the assortativity degree of the TM network was positive, indicating that nodes were more often linked to nodes with similar degrees. Whatever the model, the reciprocity ratio was low, reflecting that links were rarely bidirectional. All these indicators were globally stable over time, at a semester scale.

Specificities of breeding/production farms. The modelling approach was found to affect more the indicators of the production farm subnetwork than the ones of the breeding farm subnetwork (Table 2). For example, comparing the TM and AIM approaches, the number of links in the production farm subnetwork was increased by a factor of eight, while it was only three-times higher in the breeding farm subnetwork. Centrality values within farm type were highly heterogeneous (Fig 4): for example, degree centrality ranged from 1 to 121 (median: 17) for multiplication farms in the AIM. For the two types of models, there were significant differences in the centrality values (degree, closeness and betweenness) between types of pig farms (Kruskal-Wallis test: p -value < 0.0001). In the AIM, nucleus, multiplication and post-weaning farms had higher values for degree and betweenness centrality, whereas farrowing and farrow-to-finish herds presented higher values for in-degree centrality in the TM (Fig 4).

3.2.3 Detection of connected components and communities. Connected components. In both models, few weakly connected components (WCCs) were detected, the largest one gathering around 90% of holdings (Table 3). In the whole network, the number of WCCs increased by four times between the AIM and the TM, whereas it decreased by a factor of 1.5 in the breeding farm subnetwork, and increased by a factor of 14 in the production farm subnetwork. In the AIM, a high number of strongly connected components (SCCs) was found, the largest one containing less than 1% of farms. On the contrary, the TM network was less fragmented, with a lower number of SCCs and the detection of a giant SCC (GSCC) containing more than 70% of pig herds. The TM production farm network was more cohesive than the TM breeding farm one. Removing all farrow-to-finish herds from the production farm network led to a decrease in the size of the GSCC from 70% to 30% of the nodes contained in the GSCC. All connected components were globally stable over time, at a semester scale.

Communities. The topology of network communities varied with the modelling assumptions. In the AIM approach, a huge geographically dispersed community was found in the whole network, whereas the TM highlighted several small geographically clustered communities (Fig 5).

In the breeding farm subnetwork, a similar number of communities was detected using the two different models, but breeding pig communities were geographically more dispersed and contained approximately four times more holdings in the AIM than in the TM (Table 4). In the production farm subnetwork, more communities were detected in the AIM than in the TM, and they gathered twice more farms. Communities were found to be permeable, since at

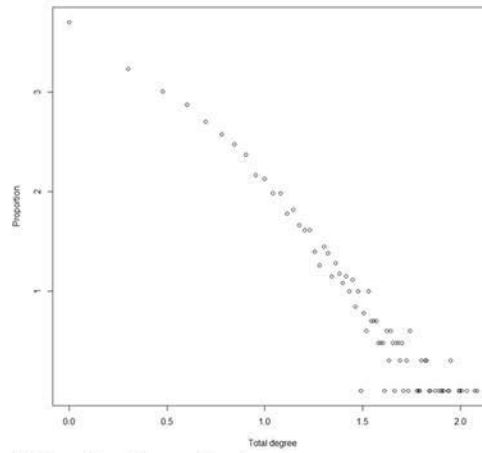
Table 2. Descriptive indicators of the pig movement network in France (2012–2014) using the two different network models (Animal Introduction Model [AIM] and Transit Model [TM]) and for three different considered populations (whole network, breeding farm subnetwork, production farm subnetwork).

Semester	Whole network																				
	No. of active nodes		No. of links		Average degree		Average path length		Diameter		Density (x10 ⁻⁴)		Clustering coefficient		% of pairs of nodes with Jaccard similarity coefficient = 0		Assortativity degree		Reciprocity ratio		
	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	
2012–2	11,558	14,166	23,477	137,348	4.06	19.39	2.55	5.78	10	20	1.76	6.84	0.015	0.27	99.59	98.11	-0.079	0.19	0.0045	0.090	
2013–1	11,419	14,161	22,969	134,901	4.02	19.05	2.28	5.78	10	20	1.76	6.73	0.015	0.26	99.60	98.12	-0.097	0.17	0.0050	0.090	
2013–2	11,223	13,817	22,367	136,235	3.99	19.72	2.36	5.75	11	18	1.77	7.14	0.015	0.27	99.59	98.05	-0.11	0.18	0.0051	0.097	
2014–1	11,013	13,784	21,691	132,677	3.94	19.25	2.46	5.88	9	20	1.79	6.98	0.014	0.27	99.60	98.07	-0.085	0.18	0.0061	0.093	
2014–2	10,872	13,360	20,842	129,155	3.83	19.33	2.22	5.82	11	21	1.76	7.24	0.013	0.27	99.63	98.00	-0.11	0.20	0.0055	0.094	
Breeding farm subnetwork																					
Semester	No. of active nodes		No. of links		Average degree		Average path length		Diameter		Density		Clustering coefficient		% of pairs of nodes with Jaccard similarity coefficient = 0		Assortativity degree		Reciprocity ratio		
	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	
	2012–2	404	453	703	1,826	3.48	8.06	1.78	6.16	5	20	4.31	8.92	0.035	0.30	94.97	89.64	-0.040	0.15	0.010	0.13
2013–1	396	446	716	1,879	3.62	8.43	1.71	5.92	4	16	4.58	9.47	0.035	0.30	94.12	88.67	-0.038	0.15	0.0042	0.12	
2013–2	395	452	648	1,796	3.28	7.95	1.73	7.18	4	21	4.16	8.81	0.031	0.33	95.33	90.86	-0.029	0.18	0.014	0.13	
2014–1	401	454	654	1,806	3.26	7.96	1.78	7.56	5	23	4.08	8.78	0.029	0.32	95.25	90.61	-0.047	0.09	0.011	0.13	
2014–2	392	445	616	1,753	3.14	7.88	1.73	6.99	5	22	4.02	8.87	0.034	0.35	95.52	90.70	-0.039	0.17	0.0065	0.12	
Production farm subnetwork																					
Semester	No. of active nodes		No. of links		Average degree		Average path length		Diameter		Density		Clustering coefficient		% of pairs of nodes with Jaccard similarity coefficient = 0		Assortativity degree		Reciprocity ratio		
	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	AIM	TM	
	2012–2	9,730	12,653	14,243	119,657	2.93	18.91	2.01	6.21	8	23	1.50	7.47	0.019	0.28	99.82	98.18	-0.058	0.27	0.0068	0.10
2013–1	9,561	12,559	13,742	116,842	2.87	18.61	1.64	6.20	7	21	1.50	7.41	0.021	0.27	99.83	98.19	-0.056	0.26	0.0078	0.10	
2013–2	9,340	12,193	13,538	118,333	2.90	19.41	1.64	6.09	7	20	1.55	7.96	0.020	0.28	99.83	98.08	-0.017	0.26	0.0074	0.11	
2014–1	9,130	12,053	12,895	115,194	2.82	19.11	1.60	6.27	7	22	1.55	7.93	0.017	0.28	99.83	98.06	-0.00074	0.26	0.0089	0.10	
2014–2	8,955	11,820	12,675	111,939	2.83	18.94	1.64	6.22	10	21	1.58	8.01	0.016	0.28	99.82	98.01	-0.019	0.28	0.0083	0.10	

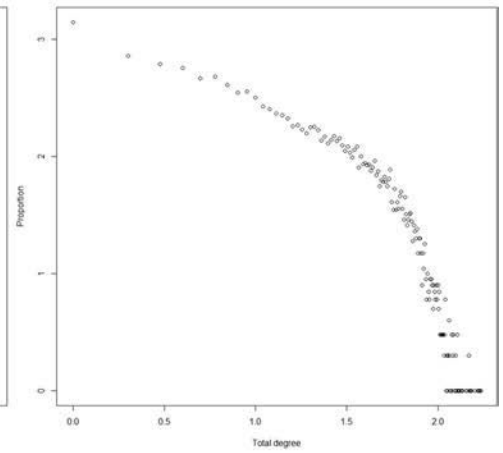
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1. Whole network

(a) Animal Introduction Model (AIM)

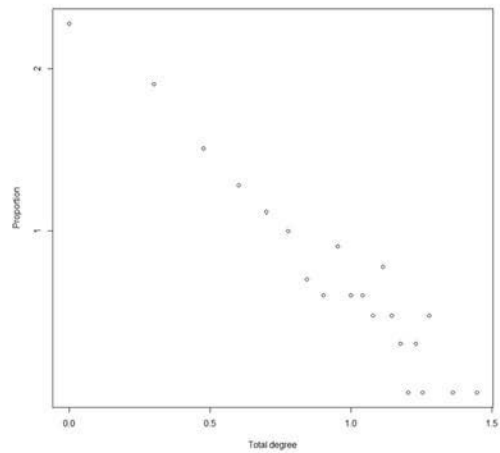


(b) Transit Model (TM)

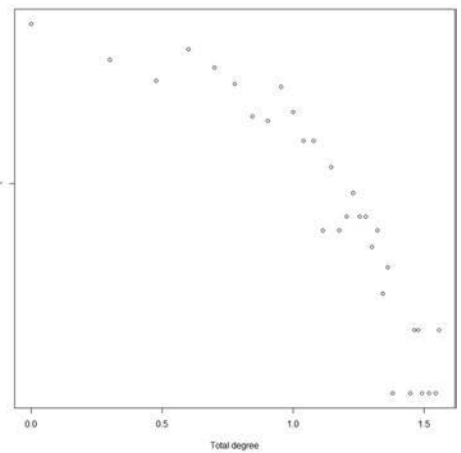


2. Breeding farm subnetwork

(a) Animal Introduction Model (AIM)

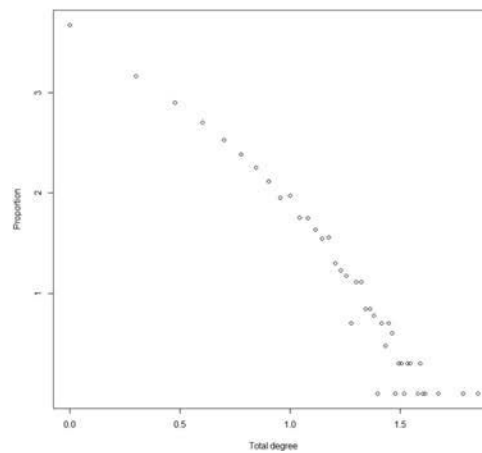


(b) Transit Model (TM)



3. Production farm subnetwork

(a) Animal Introduction Model (AIM)



(b) Transit Model (TM)

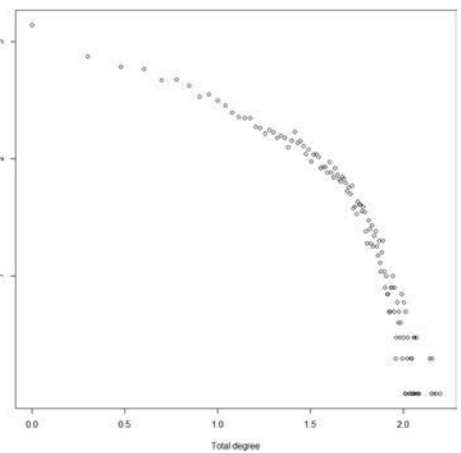
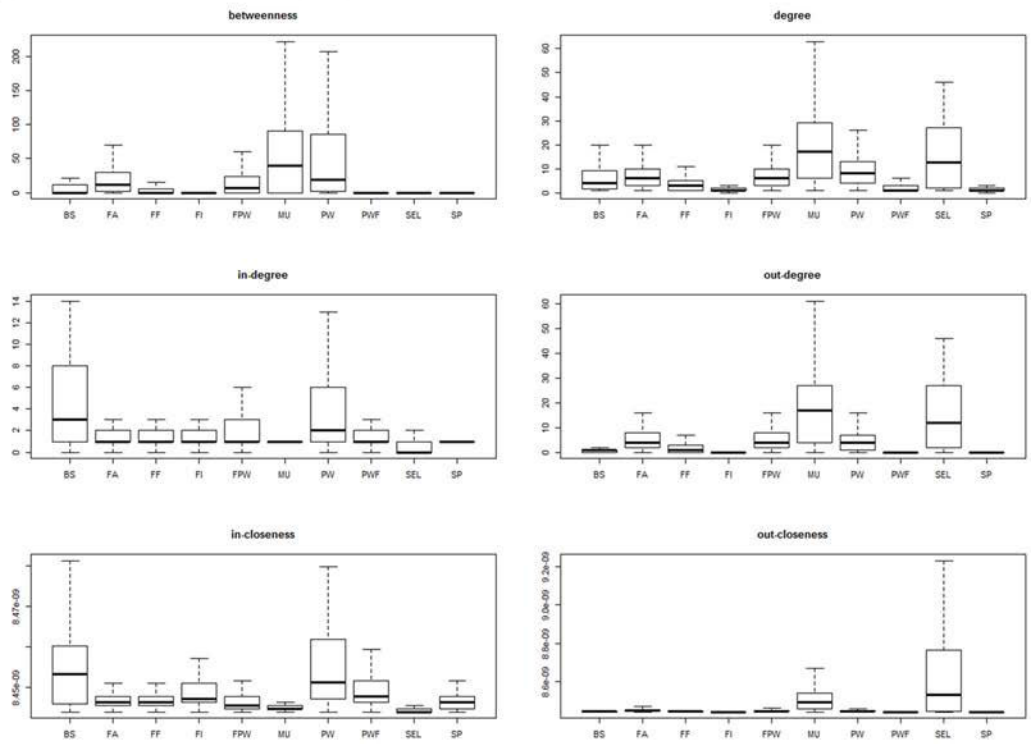


Fig 3. Distribution of pig farm degrees (log scale) using the two different network models (Animal Introduction Model [AIM] and Transit Model [TM]) and in three different considered populations (whole network, breeding farm subnetwork, production farm subnetwork) (second half of 2014).

<https://doi.org/10.1371/journal.pone.0185858.g003>

(a) Animal Introduction Model (AIM)



(b) Transit Model (TM)

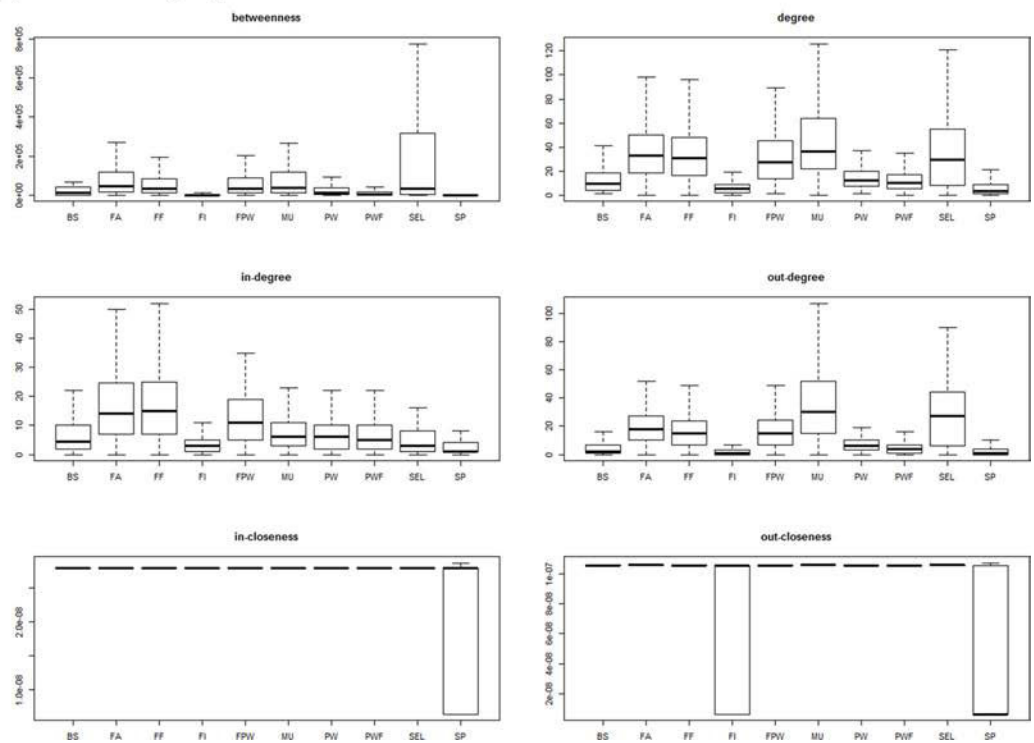


Fig 4. Distribution of degree, betweenness and closeness centralities of pig holdings in France according to different farm categories (second half of 2014) using the two different network models (Animal Introduction Model [AIM] and Transit Model [TM]). BS: boar station, SEL: nucleus, MU: multiplication, FA: farrowing, FF: farrowing-to-finishing, FI: finishing, FPW: farrowing-post-weaning, PW: post-weaning, PWF: post-weaning-finishing, SP: small producers.

<https://doi.org/10.1371/journal.pone.0185858.g004>

Table 3. Connected components in the pig movement network in France (2012–2014) using the two different network models (Animal Introduction Model [AIM] and Transit Model [TM]) and in three different considered populations (whole network, breeding farm subnetwork, production farm subnetwork).

Whole network								
Semester	Weakly connected components (WCCs)				Strongly connected components (SCCs)			
	No. of WCCs		Size of largest WCC (% of active nodes)		No. of SCCs		Size of largest SCC (% of active nodes)	
	AIM	TM	AIM	TM	AIM	TM	AIM	TM
2012–2	226	995	10,885 (94.2%)	13,063 (92.2%)	11,436	4,006	18 (0.2%)	10,075 (71.1%)
2013–1	227	1,091	10,703 (93.7%)	12,970 (91.6%)	11,290	4,087	19 (0.2%)	9,954 (70.3%)
2013–2	211	1,113	10,510 (93.7%)	12,629 (91.4%)	11,089	3,990	24 (0.2%)	9,700 (70.2%)
2014–1	232	1,207	10,261 (93.2%)	12,511 (90.8%)	10,871	4,092	17 (0.2%)	9,542 (69.2%)
2014–2	220	1,045	10,156 (93.4%)	12,182 (91.2%)	10,746	3,851	22 (0.2%)	9,381 (70.2%)
Breeding farm subnetwork								
Semester	Weakly connected components (WCCs)				Strongly connected components (SCCs)			
	No. of WCCs		Size of largest WCC (% of active nodes)		No. of SCCs		Size of largest SCC (% of active nodes)	
	AIM	TM	AIM	TM	AIM	TM	AIM	TM
2012–2	9	6	387 (95.8%)	439 (96.9%)	396	170	3 (0.7%)	254 (56.1%)
2013–1	7	3	382 (96.5%)	441 (98.9%)	393	197	2 (0.5%)	223 (50.0%)
2013–2	14	5	360 (91.14%)	444 (98.2%)	385	174	6 (1.5%)	255 (56.4%)
2014–1	12	5	375 (93.5%)	445 (98.0%)	394	174	3 (0.7%)	242 (53.3%)
2014–2	20	6	321 (81.9%)	435 (97.8%)	388	216	2 (0.5%)	178 (40.0%)
Production farm subnetwork								
Semester	Weakly connected components (WCCs)				Strongly connected components (SCCs)			
	No. of WCCs		Size of largest WCC (% of active nodes)		No. of SCCs		Size of largest SCC (% of active nodes)	
	AIM	TM	AIM	TM	AIM	TM	AIM	TM
2012–2	810	59	7,222 (74.2%)	12,450 (98.4%)	9,623	3,086	18 (0.2%)	9,475 (74.9%)
2013–1	817	65	6,888 (72.0%)	12,385 (98.6%)	9,443	3,040	19 (0.2%)	9,398 (74.8%)
2013–2	844	60	6,546 (70.1%)	12,046 (98.8%)	9,224	2,930	24 (0.3%)	9,130 (74.9%)
2014–1	861	61	6,199 (67.9%)	11,912 (98.8%)	9,008	2,902	17 (0.2%)	9,001 (74.7%)
2014–2	839	80	6,120 (68.3%)	11,593 (98.1%)	8,838	2,869	22 (0.2%)	8,826 (74.7%)

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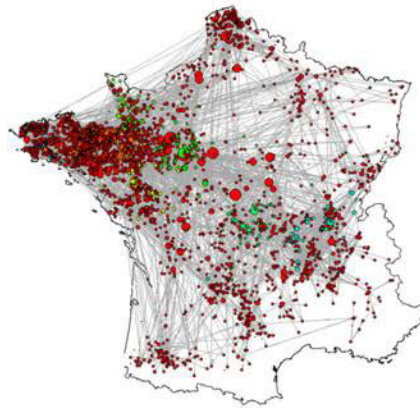
least 25% of links connected two communities (Table 4). Communities were also found to be stable over the five semesters (maps not shown).

3.2.4. Temporal network analysis. Link and node preservation. More than 98% and 77% of nodes remained active during two consecutive semesters in the AIM and in the TM, respectively. Most holdings that were not active from one semester to another were small producers. Only 51% and 36% of links were preserved from one semester to another in the AIM and in the TM, respectively.

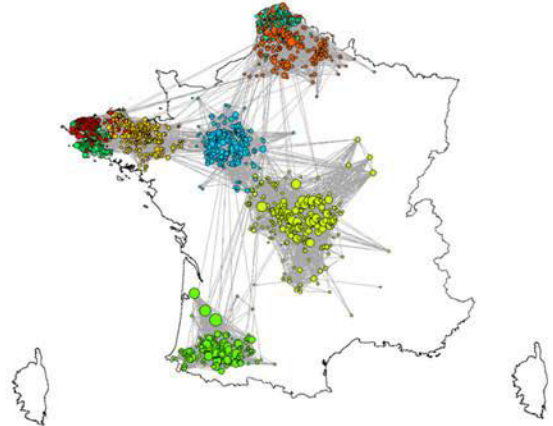
Node loyalty. The distribution of loyalty values computed in the AIM showed two peaks in 0 and 1, whereas the TM loyalty distribution was skewed to the right (Fig 6). In both cases, the distributions reflected a diverse range of patterns between establishing new connections versus repeating existing ones. The distributions of loyalty values did not exhibit variation moving along consecutive time windows (data not shown). The 0 and 1 loyalty values corresponded to low degree nodes for which few loyalty values are available, given the loyalty definition. Node degree and node loyalty were found to be correlated in both network models (Pearson correlation coefficient p -value < 0.001).

1. Whole network

(a) Animal Introduction Model (AIM)

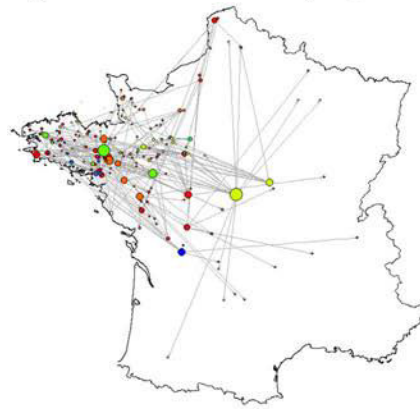


(b) Transit Model (TM)

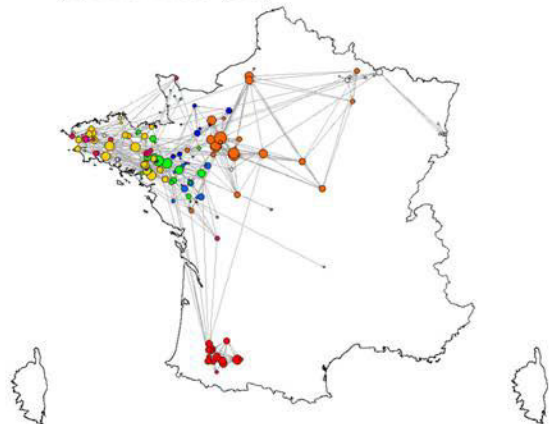


2. Breeding farm subnetwork

(a) Animal Introduction Model (AIM)

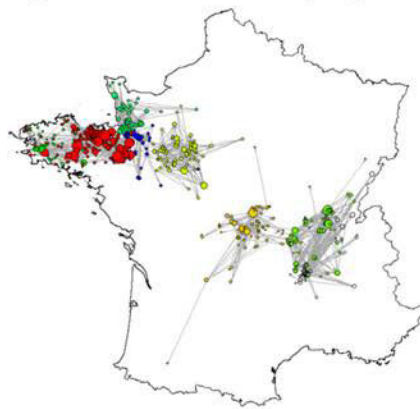


(b) Transit Model (TM)



3. Production farm subnetwork

(a) Animal Introduction Model (AIM)



(b) Transit Model (TM)

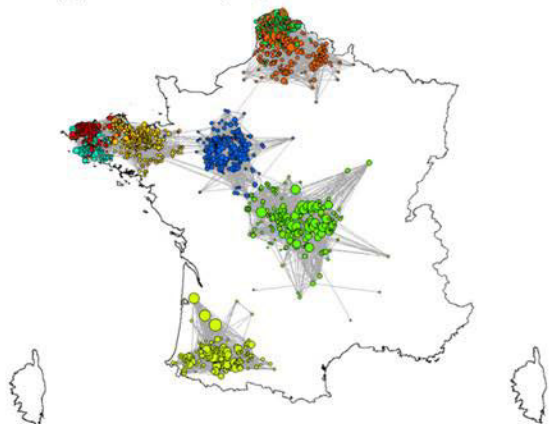


Fig 5. Mapping of the eight largest communities in the pig movement network in France (second half of 2014) using the two different network models (Animal Introduction Model [AIM] and Transit Model [TM]) and in three different considered populations (whole network, breeding farm subnetwork, production farm subnetwork).

<https://doi.org/10.1371/journal.pone.0185858.g005>

Table 4. Communities in the pig movement network in France (2012–2014) using the two different network models (Animal Introduction Model [AIM] and Transit Model [TM]) and in three different concerned populations (whole network, breeding farm subnetwork, production farm subnetwork).

Whole network						
Semester	No. of communities		Size of largest community (% of active nodes)		No. of crossing links (% of total no. of links)	
	AIM	TM	AIM	TM	AIM	TM
2012–2	1,673	1,816	3,079 (26.6%)	417 (2.9%)	9,541 (40.6%)	47,143 (34.3%)
2013–1	1,653	1,937	3,283 (28.8%)	384 (2.7%)	9,249 (40.3%)	45,980 (34.1%)
2013–2	1,573	1,957	3,344 (29.8%)	393 (2.8%)	8,758 (39.2%)	45,241 (33.2%)
2014–1	1,553	2,073	3,326 (30.2%)	363 (2.6%)	8,511 (39.2%)	43,628 (32.9%)
2014–2	1,523	1,874	3,338 (30.7%)	351 (2.6%)	8,013 (38.4%)	43,289 (33.5%)
Breeding farm subnetwork						
Semester	No. of communities		Size of largest community (% of active nodes)		No. of crossing links (% of total no. of links)	
	AIM	TM	AIM	TM	AIM	TM
2012–2	73	70	81 (20.0%)	21 (4.6%)	303 (43.1%)	857 (46.9%)
2013–1	60	72	162 (40.9%)	32 (7.2%)	311 (43.4%)	831 (44.2%)
2013–2	66	71	152 (38.5%)	37 (8.2%)	236 (36.4%)	682 (38.0%)
2014–1	66	71	174 (43.4%)	21 (4.6%)	239 (36.5%)	739 (40.9%)
2014–2	75	66	66 (16.8%)	31 (7.0%)	254 (41.2%)	645 (36.8%)
Production farm subnetwork						
Semester	No. of communities		Size of largest community (% of active nodes)		No. of crossing links (% of total no. of links)	
	AIM	TM	AIM	TM	AIM	TM
2012–2	1,802	825	123 (1.3%)	407 (3.2%)	3,999 (28.1%)	38,452 (32.1%)
2013–1	1,787	863	178 (1.9%)	388 (3.1%)	3,655 (26.6%)	37,007 (31.7%)
2013–2	1,705	848	175 (1.9%)	337 (2.8%)	3,420 (25.3%)	37,181 (31.4%)
2014–1	1,684	872	136 (1.5%)	351 (2.9%)	3,335 (25.9%)	35,625 (30.9%)
2014–2	1,653	874	181 (2.0%)	335 (2.8%)	3,217 (25.4%)	34,996 (31.3%)

<https://doi.org/10.1371/journal.pone.0185858.t004>

Ingoing and outgoing contact chains. Ingoing and outgoing contact chains computed over a one-month period exhibited different distributions depending on the network model and the farm type (Fig 7). The TM contact chain figures were much higher than the AIM ones. In the AIM and in the TM, nucleus and multiplication farms showed a larger OCC than other farm types. In the TM, the ICC was found to be higher for production farms than for the other holding types. The contact chain distributions computed over one-month periods were stable over time (data not shown).

4. Discussion

Exploring the topology of animal movements provides insights into disease epidemiology and gives the opportunity to implement targeted surveillance strategies and control measures. The primary interest of our study lies in building pig movement network models adapted to the epidemiological features of pathogens, in particular to their transmission route. To our knowledge, most studies in the literature only took into account direct movements of animal introduction or built two-mode networks that cannot be explored as deeply as one-mode ones [2, 6, 8, 9, 21, 27]. Only a few studies mentioned the role of trucks, material, visitors or staff as potential indirect vectors, or explored the issue of shared trucks [19, 21]. Truck transit movements may nevertheless play a central role in the transmission of highly contagious diseases such as

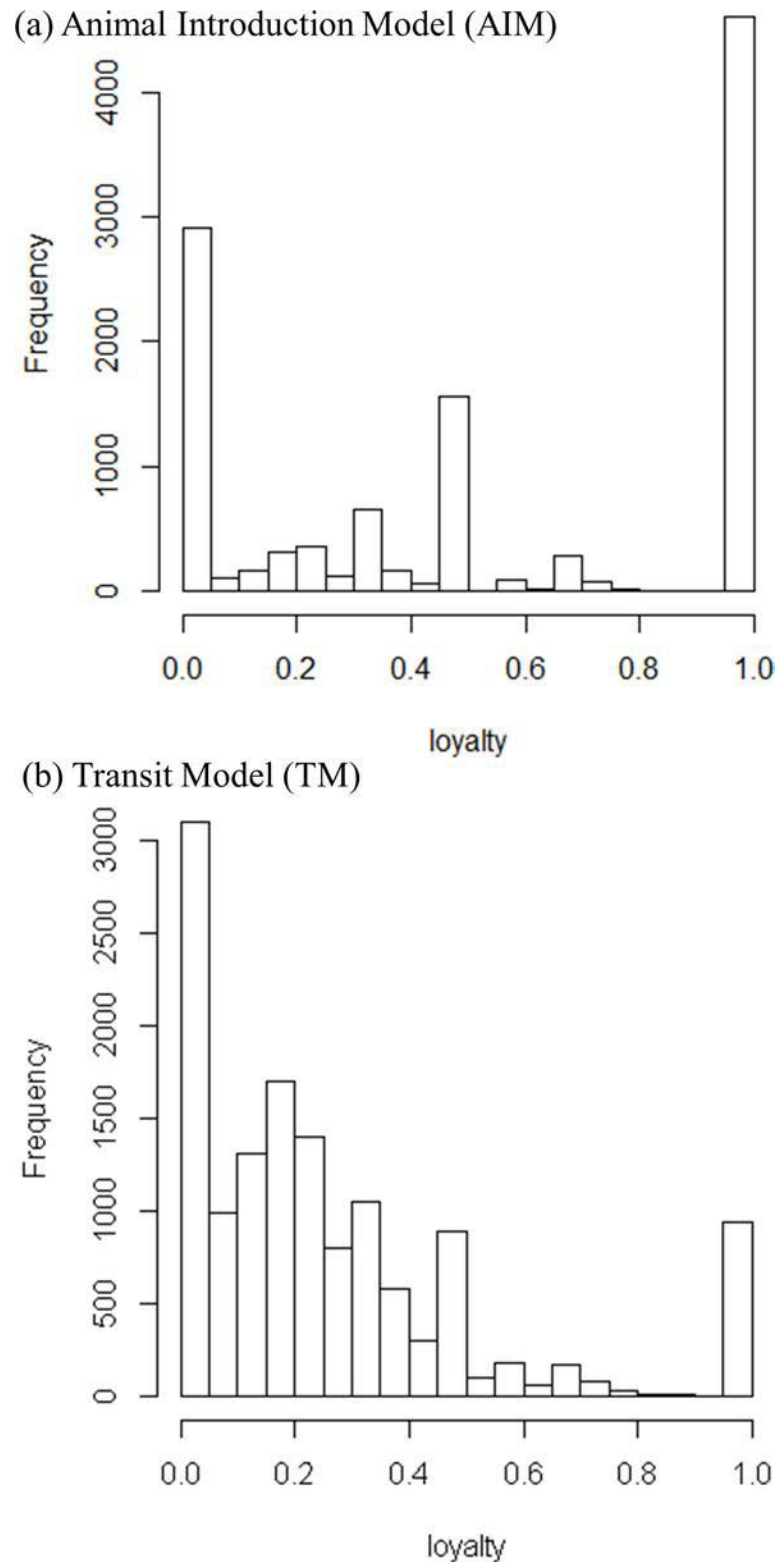
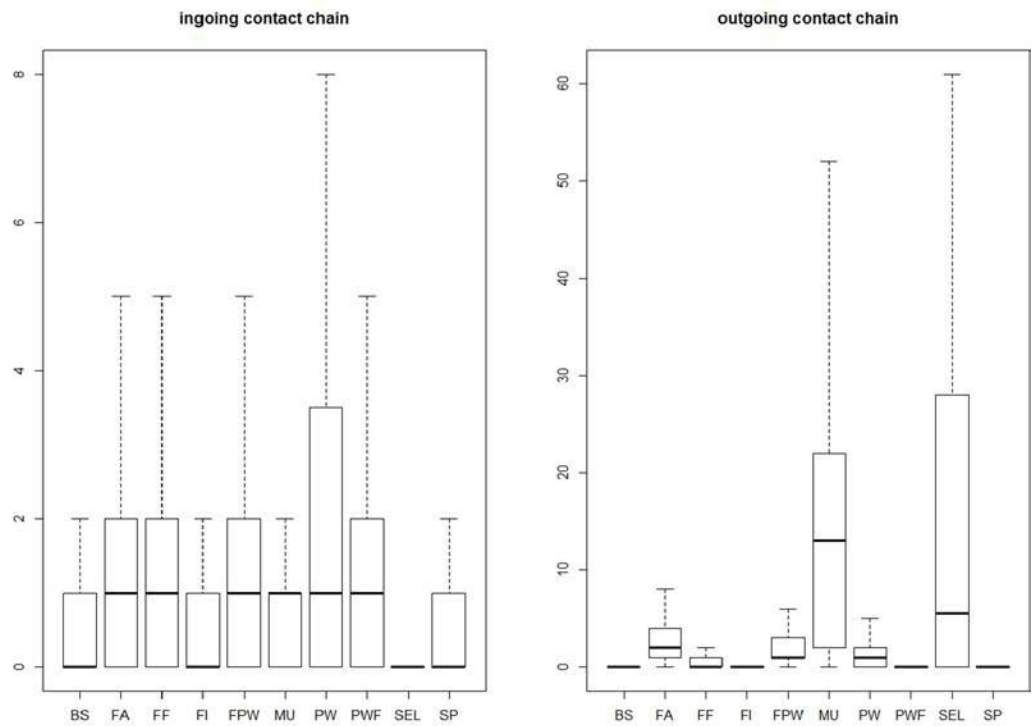


Fig 6. Node loyalty distributions in the pig movement network in France (second half of 2012 / first half of 2013) using the two different network models (Animal Introduction Model [AIM] and Transit Model [TM]).

<https://doi.org/10.1371/journal.pone.0185858.g006>

(a) Animal Introduction Model (AIM)



(b) Transit Model (TM)

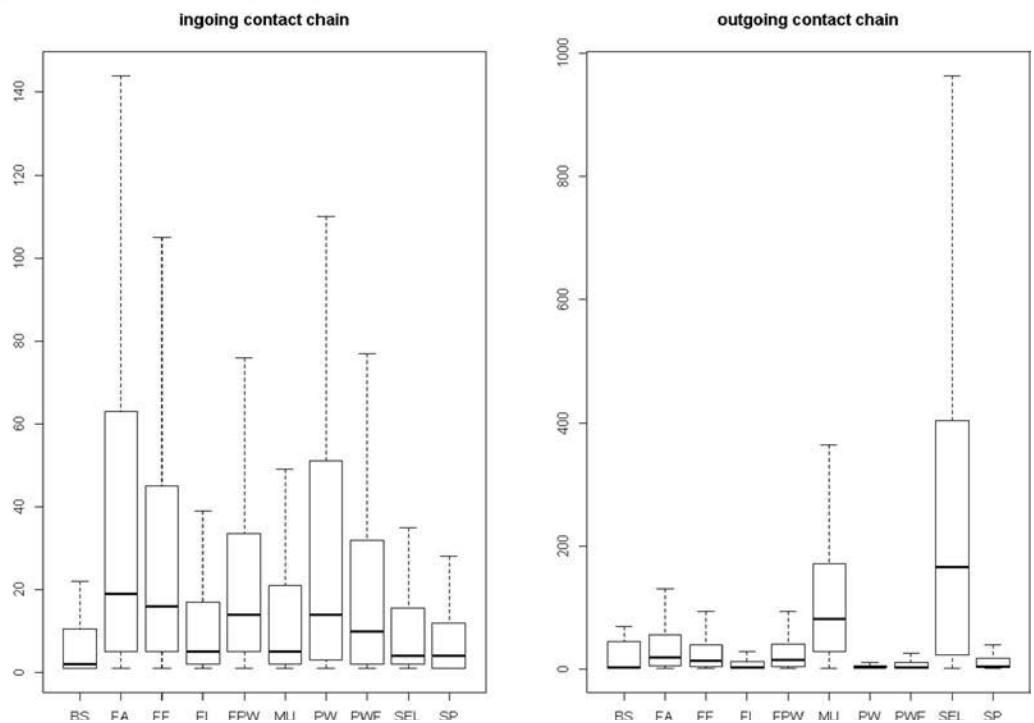


Fig 7. Distribution of ingoing and outgoing contact chains of pig holdings in France according to different farm categories (1 to 31 December 2014) using the two different network models (Animal Introduction Model [AIM] and Transit Model [TM]). BS: boar station, SEL: nucleus, MU: multiplication, FA: farrowing, FF: farrowing-to-finishing, FI: finishing, FPW: farrowing-post-weaning, PW: post-weaning, PWF: post-weaning-finishing, SP: small producers.

<https://doi.org/10.1371/journal.pone.0185858.g007>

ASF, PED, and FMD. The pig production sector is organised in a pyramidal way: at the top, nucleus farms provide purebred sows and boars to multiplication farms, which produce cross-bred pigs and gilts to supply production farms, producing pigs for slaughter. Assuming that this specific structure leads to a particular topology of the movement network, we performed a multi-scale analysis suiting the pyramidal organisation: we analysed both the whole network and two subnetworks containing (1) only the breeding farms (nucleus, multipliers, and boar stations); and (2) only the production farms (from farrowers to finishers). Our network analysis did not account for movements to slaughterhouses, as they are considered as an epidemiological dead-end. Because several studies have proven that trade in the pig production sector does not show any seasonal pattern in France [2, 7, 23, 27, 28], we analysed the network at a semester scale. This temporal scale was considered appropriate to reflect the global trade behaviour of farms while making it possible to observe evolutions over the study period. Our two models were applied to all movement data recorded in France from June 2012 to December 2014 in the National Swine Identification Database (BDporc). The information provided by this database is managed by swine industry professionals, is recognised by the French Ministry for Agriculture, and can therefore be considered trustworthy. Moreover, a thorough cleaning stage was carried out to manage incorrect or incomplete data. This kind of electronic data is also more accurate than movements reported in questionnaires [34]. An even more accurate alternative would be to use GPS (Global Positioning System) to geographically locate trucks and precisely track their movements, but this would require the approval of transportation operators to share this kind of data, as well as advanced analytical methods to manage such data. In contrast with other studies that were limited to a single region or a sample of voluntary farms or to a short period of time [6, 9, 21], we used recent data from the whole country and covering a long period of time. Finally, the quality of data—in terms of accuracy, reliability, and comprehensiveness—guarantees the robustness of our results.

The analysis of movements over the study period showed a decline in the number of rounds, while the number of animals moved per round increased, leading to an overall slight decrease in animal trade movements, which was also reported in other European studies [8]. This is consistent with the intensification of the pig production industry (that is to say a decrease in the number of pig farms balanced by an increase in the number of animals reared), resulting in the observation of fewer movements involving larger pig batches. The level of round complexity was highly heterogeneous, the average number of holdings implied in a round being 2.5 but reaching 32. This is consistent with the distance travelled by pigs in a round (excluding foreign movements), ranging from 0 to 1,000 km. The distances reported in our study are longer than in other European countries such as Belgium or England/Wales [8, 19], in accordance with the results of the comparative study conducted by Relun et al. [23]. The longest and most complex rounds implied culled boar/sows and breeding pigs. They were mainly located in central and south-western France where the production is less intensive and rounds are thus composed of several movements of small batches. Movements from/to foreign countries represent a small fraction of the pig trade in France and are linked to specific markets, but they are nevertheless important to take into consideration in order to prevent the introduction of a disease that is absent from France (e.g. FMD, ASF, PED).

Whatever the modelling approach, network structure properties exhibited overall stability over the study period: (i) at a semester scale, active nodes globally remained the same from one semester to another, except for small production farms; (ii) network metrics were similar from one semester to another; and (iii) connected components and communities were also stable over the study period. This stability of the pig production network has already been described in several papers [8, 23, 27] and enables us to generalise the findings of our study to the current swine trade network. However, loyalty distributions showed relative volatility of farms' trade

partners, indicating that future links may be difficult to predict. The same trend has already been described in a cattle movement network [35].

Our two network models exhibited two classical patterns of connectivity described in other studies [2, 6, 8, 9, 21, 23, 28], known as (i) small-world, and (ii) scale-free topologies. (i) Whatever the model, our networks had higher clustering coefficients and shorter or similar average path length than random graphs of the same size (corresponding to a small-world topology) [36, 37]. This means that most nodes are not directly connected to each other but can be reached through a small number of connections. This allows diseases to spread quickly within clusters but also to reach other clusters in the network by crossing a few links. This topology facilitates persistent infection in the pig population but the size of an epidemic in a small-world network tends to be smaller when compared to a random network. (ii) The holdings' degree in both networks showed power-law-like behaviour (heavy tailed distribution), meaning that many of the nodes had few connections while a few nodes had many connections (corresponding to a scale-free structure) [38]. This indicates the presence of highly connected nodes, i.e. of hubs, that are of central importance with regard to disease spread (also called super-spreaders). Epidemics can therefore spread faster in scale-free networks than in random ones. Scale-free networks can withstand random attacks but are highly vulnerable to targeted attacks towards the hubs [11, 39, 40].

Size, degree and distance metrics (average path length, diameter, density) observed in the AIM are consistent with the literature data, especially for the pig movement networks in France [23, 41]. As expected, given the model assumptions, these values increased when switching from the AIM to the TM. The differential modelling approach affected more production farms than breeding farms, suggesting that production farms may play a key role in the spread of indirectly transmitted diseases. The assortativity degree of the AIM was negative, in accordance with the results of previous studies [6, 7, 10]. However, the TM network was found to be assortative. According to [42], disassortative networks are particularly sensitive to the removal of high-degree farms since they are dispersed over the whole network. Thus, fewer holdings have to be removed to destroy the largest component compared to a network with positive assortativity degree. Like in Thakur et al. [21], the reciprocity ratio was very low, reflecting the pyramidal structure of the pig production sector with unidirectional links going from the top breeding farms to the bottom production farms. Similarly, the Jaccard similarity coefficient was zero for almost all pairs of nodes, showing that movements occurred mainly between different farm types.

Centrality values within a farm type were highly heterogeneous (except for closeness centrality, see below). In the AIM network, the high out-degree distributions observed for breeding farms compared with production farms is in accordance with previously published papers [6, 10, 21] and with the pyramidal structure of pig production. It shows their potential key role in disease spread to the whole network in case of introduction of the disease to this kind of farm. Their high betweenness score also proves that disease surveillance should be primarily directed towards these units. Indeed, holdings with a high betweenness centrality could build so-called bridges between different network components. Removing these specific holdings would fragment the network. In the TM network, farrow and farrow-to-finish farms exhibited high in-degree distribution, whereas post-weaners had the highest in-degree values in the AIM. This results in a similar total degree for farrow, farrow-to-finish, nucleus and multiplication farms in the TM. This could be explained by the fact that farrow and farrow-to-finish farms were part of more complex rounds involving more truck transit movements. It shows that farrow and farrow-to-finish farms are more vulnerable to the introduction of diseases for which indirect transmission can occur, and that surveillance measures specific to these diseases should target these farm categories. In the AIM, post-weaning and post-weaning—finishing

farms exhibited the highest median ingoing closeness, which is consistent with the literature [10]. A high value for ingoing closeness centrality implies that the trade partners of a specific holding can reach the node in only a few movements. In the AIM, nucleus and multiplication farms had the highest median outgoing closeness [10]. High outgoing closeness means that a seller reaches its client in only a few steps. Thus, holdings with high outgoing closeness centrality can spread a pathogen in the production network faster. The distributions of the ingoing and outgoing closeness centralities were not highly informative in the TM because their range was too small. As explained in [43], the small range of closeness values implies that slight changes in the network structure greatly affect the ranking of farms according to the closeness centrality. Being used as additional information to the more powerful centrality parameters (see above) [10], closeness centrality is therefore not considered as the most appropriate measure for the detection of central holdings in a trade network, especially in terms of animal disease control and risk-based surveillance.

In both models, few WCCs were observed, the largest one containing around 90% of farms. This is consistent with the literature [6, 21, 27]. Like in previously published papers [6, 28], the AIM exhibited a high number of small SCCs, the largest one containing only 1% of farms. On the contrary, the TM network was less fragmented, with a low number of SCCs and the presence of a giant SCC joining 70% of farms. This is consistent with the clustering coefficient being ten times higher in the TM than in the AIM, reflecting a gathering trend. The GSCC disappeared when removing farrow and farrow-to-finish farms, showing their central role in TM network cohesion.

Community structures in networks are densely connected subgroups of nodes. Identification of communities in a trade network shows which holdings are preferentially linked. We looked for communities in both models of the swine trade network thanks to the Infomap algorithm. To our knowledge, this method has never been used in previous papers studying animal movements, although it is the only one applicable to directed networks and considered one of the best in terms of performance [30, 31]. The topology of the detected communities varied with the modelling approach: in the AIM, we detected one huge geographically dispersed community, while the TM exhibited several small geographically clustered communities. The topology of communities detected in the AIM is rather consistent with the literature, reporting communities forming spatial clusters and tending to cover quite large areas [6, 8, 23]. When considering the two subnetworks, the AIM breeding farm subnetwork presented larger communities than the TM one, whereas the AIM production farm subnetwork contained smaller communities than the TM one. Although these communities are permeable and crossing links can act as potential bridges for disease spread from one community to another, community borders could be used to define geographical compartments. Compartmentalisation can be an effective strategy for controlling disease epidemics while minimising disruption to trade business [8, 23]. Stopping disease spread within a community would reduce the probability of pathogen transfer to a connected community. Our results show that geographical compartmentalisation would be easier to limit the introduction of a disease transmitted through the indirect route than for a disease transmitted through animal introduction.

Timely movement tracking is of major interest to understand the origin of the pathogen introduction and the potential spread through downstream contacts. This is the reason why ingoing and outgoing contact chains were computed. The choice of a one-month duration period reflects the time needed to detect the occurrence of a disease and has been discussed in several papers [21, 27]. As expected, the ICC and OCC values were much higher in the TM than in the AIM, showing that the potential epidemic size would be larger for an indirectly transmitted disease than for a directly transmitted pathogen. Moreover, the AIM OCC was higher for breeding farms than for production ones, in line with their key role in the spread of

a directly transmitted disease. In the TM, the ICC was higher for production farms, showing their vulnerability to indirectly transmitted disease. These results are in accordance with the other centrality measures (see above) and, for the AIM, with previously published papers [21, 27].

5. Conclusion

The primary interest of our study lies in developing, analysing and comparing two one-mode pig trade network models matching the transmission route of pathogens. From a modelling point of view, our data could be used to parametrise other models, such as exponential random graph models (ERGMs) aiming at explaining network structure [23, 44]. Our network models could also be coupled with epidemiological models of pathogen transmission within herds, this combination resulting in a between-herd epidemiological model. This kind of model would be particularly useful to understand or to assess the persistence and/or spread of a disease in a production sector. From a more operational perspective, our network models have produced useful outputs that can help to design risk-based disease surveillance and control programmes adapted to disease characteristics. They bring to light the relevance of accounting for transit movements to understand the indirect transmission of diseases. Depending on the epidemiological context, the potential epidemic size and the pathogen spread pattern would differ, as do the type of farming units that have to be targeted and the scale at which control measures should be implemented.

Author contributions

MS performed network analysis and drafted the manuscript. MA cleaned the database, designed the network models and supervised network analysis. NR initiated and coordinated the project. All co-authors revised the manuscript and approved the final submitted version.

Supporting information

S1 Table. Pig movements within France from June 2012 to December 2014 at a semester scale.

(DOCX)

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II. Combiner l'analyse de réseau et des données épidémiologiques pour définir des pistes de surveillance basée sur le risque

La structure particulière du réseau des mouvements de porcs analysé dans la publication ci-dessus montre la capacité des échanges de porcs à permettre la diffusion des pathogènes sur le territoire français. C'est dans ce contexte qu'une **méthode quantitative combinant analyse de réseau et données épidémiologiques** a été développée. Cette approche a permis de quantifier le rôle des mouvements d'animaux sur le risque lié au HEV à deux échelles : tout d'abord en mesurant l'**impact des mouvements d'animaux sur la prévalence du HEV dans les élevages**, puis en évaluant le **risque pour les départements français d'être exposés au HEV du fait de mouvements en provenance de départements infectés**. Outre les informations qu'elle apporte sur le risque HEV lié aux mouvements d'animaux, cette méthode a comme avantage d'être générique et ainsi **transposable à tout autre pathogène d'intérêt** pour la filière de production porcine.

Ce travail a donné lieu à une publication dans le journal *Preventive Veterinary Medicine* (Salines *et al.*, 2018a) ainsi qu'à un article associé à une communication orale aux *Journées Recherche Porcine (Annexe 7)* (Salines *et al.*, 2018b).

Publication 8

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Combining network analysis with epidemiological data to inform risk-based surveillance: Application to hepatitis E virus (HEV) in pigs



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ABSTRACT

Animal movements between farms are a major route of pathogen spread in the pig production sector. This study aimed to pair network analysis and epidemiological data in order to evaluate the impact of animal movements on pathogen prevalence in farms and assess the risk of local areas being exposed to diseases due to incoming movements. Our methodology was applied to hepatitis E virus (HEV), an emerging foodborne zoonotic agent of concern that is highly prevalent in pig farms. Firstly, the pig movement network in France (data recorded in 2013) and the results of a nation-wide seroprevalence study (data collected in 178 farms in 2009) were modelled and analysed. The link between network centrality measures of farms and HEV seroprevalence levels was explored using a generalised linear model. The in-degree and ingoing closeness of farms were found to be statistically associated with high HEV within-farm seroprevalence ($p < 0.05$). Secondly, the risk of a French *département* (i.e. French local administrative areas) being exposed to HEV was calculated by combining the distribution of farm-level HEV prevalence in source *départements* with the number of movements coming from those same *départements*. By doing so, the risk of exposure for *départements* was mapped, highlighting differences between geographical patterns of HEV prevalence and the risk of exposure to HEV. These results suggest that not only highly prevalent areas but also those having at-risk movements from infected areas should be monitored. Pathogen management and surveillance options in the pig production sector should therefore take animal movements into consideration, paving the way for the development of targeted and risk-based disease surveillance strategies.

1. Introduction

Developing risk-based surveillance programmes for animal diseases is essential to support both strategic and operational decision-making in the field of animal and veterinary public health (Reist et al., 2012). Indeed, mobilising resources towards targeted high-risk populations improves the sensitivity and cost-effectiveness of surveillance systems (Stärk et al., 2006). The sub-populations to be targeted are usually chosen based on epidemiological studies assessing the probability of occurrence of the hazard in the sub-population (e.g. farms with specific risk factors) and/or the consequences of the disease potentially being introduced in this sub-population (e.g. economic effects, spread to other herds or countries) (Stärk et al., 2006). However, most current pathogen surveillance programmes do not quantitatively include the risk related to animal movements, even though these are a major transmission route between farms. The exposure of farms or areas to pathogens is therefore closely related to the movement network's features. As such, animal movement data have been increasingly studied using

social network analysis (SNA) methods, with farms being considered as nodes, and animal movements between farms as links (Wasserman and Faust, 1994; Bigras-Poulin et al., 2006; Bigras-Poulin et al., 2007; Martínez-López et al., 2009; Natale et al., 2009; Ribbens et al., 2009; Nøremark et al., 2011; Lindstrom et al., 2012; Rautureau et al., 2012; Buttner et al., 2013; Dorjee et al., 2013; Guinat et al., 2016; Thakur et al., 2016). Although in most studies network analyses have been motivated by the consequences of animal trade on the epidemiology of animal diseases (Keeling, 2005; Lloyd-Smith et al., 2005; Bigras-Poulin et al., 2007; Martínez-López et al., 2009; Rautureau et al., 2012; Buttner et al., 2013), the specific role of animal shipments in pathogen transmission and/or exposure has only scarcely been documented and rarely quantified, especially in the swine sector (Ortiz-Pelaez et al., 2006; Green et al., 2008; Martin et al., 2011; Porphyre et al., 2011; Frössling et al., 2012; Nicolas et al., 2013; Beaune et al., 2015; Lee et al., 2017; Salines et al., 2017b; Sintayehu et al., 2017). Analysing contact patterns related to pig trade could provide new insight into infection dynamics, pathogen spread and risk factors, helping to design risk-based

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surveillance programmes.

Hepatitis E is an emerging foodborne zoonosis of concern for which pigs have been recognised as a major reservoir in industrialised countries (Dalton et al., 2008; Pavio et al., 2010; Adlhoch et al., 2016; EFSA et al., 2017). Indeed, several human hepatitis E cases have been related to the consumption of raw or undercooked products containing pig liver (Colson et al., 2010; Moal et al., 2012; Motte et al., 2012). HEV is highly prevalent in pig farms and is likely to spread between farms through the introduction of infected pigs, especially due to the pyramidal structure of the pig production sector (Salines et al., 2017a). To date, no continuing HEV surveillance programmes have ever been implemented in industrialised countries (Salines et al., 2017a).

The aim of our study was therefore to combine network analysis with disease epidemiology and propose methods to quantify the epidemiological role of animal movements on two different scales: firstly by measuring the impact of animal movements on pathogen prevalence at the farm level; and secondly by assessing the risk of French *départements*¹ being exposed to diseases due to incoming movements from infected areas. Our methodology was applied to hepatitis E virus (HEV) in the pig production sector.

2. Materials and methods

2.1. Data

2.1.1. Movement data

2.1.1.1. Pig movement database. As described by Salines et al. (2017b), pig movement data were obtained from the National Swine Identification Database (BDporc), managed by swine industry professionals and recognised by the French Ministry for Agriculture. All pig movements between farms and to slaughterhouses, rendering plants and trade operators are systematically recorded in this database. Movements of pigs are reported at the batch level: groups of animals are sent off production sites (loadings, further denoted L) and dispatched either to other production units or to slaughterhouses (unloadings, further denoted U). A single truck can load and unload animals at several production sites: one round corresponds to a series of movements by a truck, from the first loading operation to the last unloading event leaving the truck empty.

2.1.1.2. Design of the movement network (Fig. 1). Movement data recorded from January to December 2013 were modelled into a one-mode directed network aggregated on a one-year basis: holdings were considered as nodes, and movements between two nodes were considered as directed links. All movements between two given holdings during the time period were aggregated into a single link. In-between movements forming a round were replaced with direct movements between holdings, meaning that intermediate transit movements by a truck through a farm without any animal unloading were excluded. All sites corresponding to unloading operations were assumed to be linked to all prior loading sites for the same round. For example, assuming successive loadings at sites L1 and L2 followed by an unloading operation at site U1, then holding U1 was linked to L1 and L2.

2.2. Prevalence data

As described by Rose et al. (2011), a nation-wide study was undertaken in 2009 to collect representative HEV prevalence data accounting for the production level diversity throughout the country. In short, previous data had indicated a farm-level prevalence close to 70% (Rose et al., 2010); the number of herds required to estimate 70% with

10% relative precision and 95% confidence, was 165. This number was increased to 186 to anticipate uncontrolled events. The herds to be sampled were determined by random selection of a list of slaughter dates and times from a database table. The observed minimum within-herd prevalence in this same preliminary study was close to 10% (Rose et al., 2010) and this value was retained as the minimum within-herd target prevalence to be detected. Given the sensitivity and specificity of the commercial serological tests (Rose et al., 2010), this led to sampling of 30 pigs in batches with less than 50 pigs, 40 pigs in batches of 50–100 pigs and 50 pigs in batches with more than 100 pigs. Finally, 6565 sera and 3715 livers were randomly sampled from 186 pig farms located in 49 different French *départements*, corresponding to between 26 and 42 individual serum samples per farm and between 16 and 20 liver samples per farm collected at the slaughterhouse. Serum samples were tested with the anti-HEV total immunoglobulin for human diagnosis, EIAGEN HEV Ab Kit[®] by Adaltis (Ingen, France) adapted to pig serum.

2.3. Statistical analyses

2.3.1. Farm centrality indicators and within-farm HEV seroprevalence

2.3.1.1. Farm centrality indicators. Only 178 farms out of the 186 sampled in the prevalence study were recorded in the movement database. Using the pig movement network, several centrality measures were calculated for each of the 178 farms: the *in-degree*, i.e. the number of different holdings from which a holding receives animals; the *out-degree*, i.e. the number of different holdings to which a holding sends animals; the *ingoing and outgoing closeness*, which focus on how close a farm is to all the others in the network through incoming or outgoing links; the *betweenness*, i.e. the number of geodesics going through a node; the average monthly *ingoing contact chain* (ICC), i.e. the number of holdings in contact with a given holding (called the root) through time-respecting paths reaching the root within a month; the average monthly *outgoing contact chain* (OCC), i.e. the number of holdings in contact with a root through time-respecting movements of animals leaving the root within a month; and the *node loyalty*, measuring the fraction of preserved links of a node for a pair of two consecutive network configurations over time, with the time window in our case being a half-year. All continuous variables were categorised according to the form of their distribution, with categories containing at least 10% of the sample size.

2.3.1.2. Within-farm HEV seroprevalence. The HEV seroprevalence of each of the 178 farms was defined as the number of HEV-seropositive pigs in relation to the total number of pigs sampled in the farm. The individual sensitivity and specificity of the test (Rose et al., 2010) were used to correct the apparent seroprevalence estimates (Rogan and Gladen, 1978).

2.3.1.3. Statistical model. A univariable analysis was conducted to assess the statistical link between each explanatory variable (i.e. the farms' centrality metrics) and the outcome (i.e. the unbiased within-farm HEV seroprevalence). To do so, a generalised estimating equation (GEE) logistic regression was performed using Proc GENMOD in SAS 9.4. with the "farm" effect being included as a repeated statement (SAS, 2014). Factors associated with the outcome ($p < 0.20$) were then subjected to bivariable analysis. The objective was to identify strong correlations between each explanatory variable to prevent multicollinearity. If variables did not show strong collinearity ($p > 0.05$), they were included in a multivariable model. We also investigated the role of farm type as a potential confounding factor, by testing the link between farm type and the explanatory variables and the outcome with chi-squared tests and logistic regression, respectively.

¹ In France, *départements* are local administrative areas corresponding to NUTS level 3 (Nomenclature of Territorial Units for Statistics).

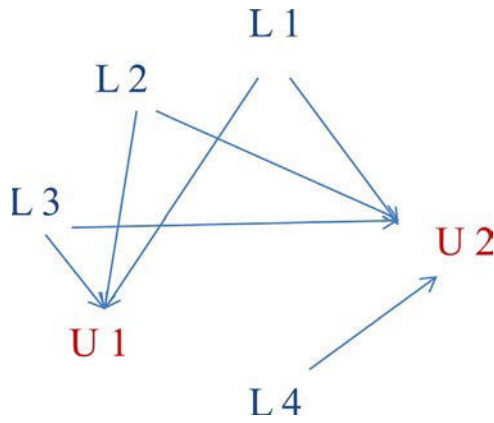


Fig. 1. Design of the network model representing pig movements in France in 2013. Nodes L and U correspond to holdings where loading and unloading operations occurred, respectively. The number corresponds to the chronology of animal collection by a truck in one round. Movements forming a round were replaced with direct movements between holdings, meaning that intermediate transit movements by a truck through a farm without unloading any animals were excluded.

2.4. Indicator of risk of exposure to HEV of French départements

2.4.1. Pig movements at département level

For each département, the number of pig shipments coming from each of the other départements in 2013 was calculated.

2.4.1.1. Departmental farm-level HEV seroprevalence (Fig. 2). HEV prevalence was defined at the département level as the number of farms having at least one HEV-seropositive pig out of the total number of farms sampled in the département. The standard deviation for farm-level HEV prevalence was calculated thanks to an exact binomial test and weighted with a correction factor reflecting the sampling rate (i.e. the proportion of sampled farms among the total number of farms in the département). For each of the 49 départements where data were available, uncertainty regarding the farm-level HEV prevalence estimate was represented by a beta distribution using the estimate and the confidence interval to define the parameters of the distribution θ .

2.4.1.2. Estimation of the risk of exposure at departmental level. An

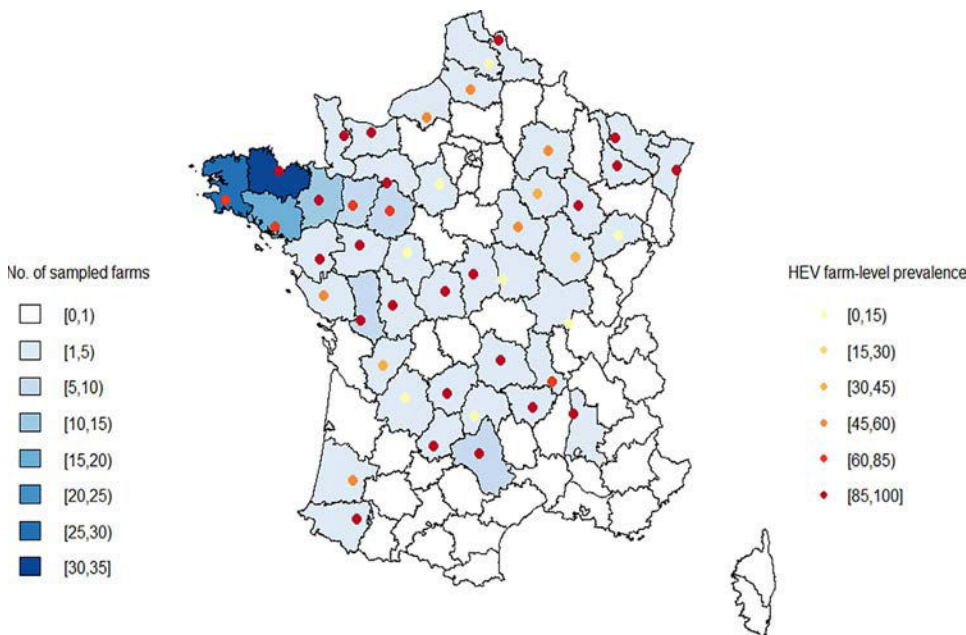


Fig. 2. Number of farms sampled per département in the 2009 nation-wide HEV survey and observed farm-level HEV prevalence by département. Farm-level HEV prevalence was defined as the number of farms having at least one HEV-seropositive pig among the total number of tested farms in the département.

indicator of the risk of a département being exposed to HEV was computed as follows: first, for each département, an HEV farm-level prevalence value was randomly sampled from the beta distribution; the corresponding number of HEV-positive farms in the département was then derived from this selected prevalence value and the individual status of the herds was randomly assigned. Source herds were then randomly selected according to the actual number of movements leaving the source département, leading to a number of infected outgoing movements. Lastly, the indicator of the risk of a département being exposed to HEV was calculated as the number of positive movements it had received from source départements divided by its total number of external incoming movements. To stabilise the outputs of the procedure, the whole calculation was repeated 10,000 times, resulting in a risk distribution of HEV exposure for each département. The exposure risk model was implemented in R (Ihaka, 1996).

3. Results

3.1. Farm centrality indicators and within-farm HEV seroprevalence

The farms' mean in- and out-degrees were 2.46 (range: 0–22) and 5.14 (range: 0–134), respectively. Mean ingoing and outgoing closeness were $2.17 \cdot 10^{-9}$ and $2.18 \cdot 10^{-9}$, respectively, with little variability. Mean betweenness was 27.06 (range: 0–1439). Mean monthly ingoing and outgoing contact chains were 0.98 (range: 0–5) and 1.15 (range: 0–29), respectively. Mean node loyalty was 0.65 (range: 0–1). In the 178 studied farms, HEV unbiased seroprevalence ranged from 0% to 100% HEV-seropositive pigs (mean: 29%, median: 17%).

The univariable analysis showed that two of the eight analysed centrality indicators were statistically associated with the outcome (Table 1): high in-degree and ingoing closeness for farms were significantly and positively associated with high within-farm HEV seroprevalence. Since in-degree and ingoing closeness were correlated (chi-squared test, $p < 0.01$), they were not included in a multivariable model. Farm type was associated with all explanatory variables ($p < 0.05$) but not with within-farm HEV seroprevalence ($p > 0.1$).

3.2. Indicator of risk of exposure to HEV of French départements

3.2.1. Departmental farm-level HEV prevalence and related uncertainty

Departmental farm-level HEV prevalence distributions were plotted

Table 1
Statistical relationships between farms' network centrality indicators and within-farm HEV seroprevalence.

Centrality measures Category	Definition	Estimate	Standard Error	Odds Ratio [95% Confidence Interval]	p-value
In-degree	Number of different holdings from which a holding receives animals				
≤ 4		–	–	–	–
> 4		0.57	0.31	1.78 [0.97–3.26]	0.06*
Out-degree	Number of different holdings to which a holding sends animals				
≤ 1		–	–	–	–
> 1		0.21	0.25	1.23 [0.76–1.99]	0.4
Ingoing closeness	Focuses on how close a farm is to all the others in the network through incoming links				
≤ 2.176.10 ⁻⁹		–	–	–	–
> 2.176.10 ⁻⁹		0.65	0.29	1.91 [1.08–3.38]	0.02*
Outgoing closeness	Focuses on how close a farm is to all the others in the network through outgoing links				
≤ 2.175.10 ⁻⁹		–	–	–	–
> 2.175.10 ⁻⁹		0.038	0.35	1.04 [0.52–2.06]	0.9
Betweenness	Number of geodesics (shortest paths) going through a vertex				
= 0		–	–	–	–
> 0		–0.0009	0.001	0.999 [0.997–1.001]	0.4
Average monthly ingoing contact chain	Number of holdings in contact with a given holding (called the root) through time-respecting paths reaching the root within a month				
≤ 1		–	–	–	–
> 1		0.14	0.25	1.15 [0.71–1.87]	0.6
Average monthly outgoing contact chain	Number of holdings in contact with a root through time-respecting movements of animals leaving the root within a month				
= 0		–	–	–	–
> 0		–0.028	0.24	0.97 [0.61–1.56]	0.9
Node loyalty	Fraction of preserved links of a node for a pair of two consecutive network configurations over time, with the time window in our case being a half-year				
≤ 0.65		–	–	–	–
> 0.65		–0.26	0.26	0.77 [0.46–1.30]	0.3

Summary statistics as obtained thanks to a generalised estimating equation (GEE) univariable logistic regression with the “farm” effect being included as a repeated statement. *statistically significant effect.

(see examples in Supplementary File 1, figure a). Due to the varying number of sampled farms depending on the *département* (Fig. 2), quite a few *départements* exhibited large farm-level prevalence distributions (e.g. *département* A in Supplementary File 1, Fig. a).

3.2.2. Estimated risk indicator of HEV exposure of *départements* through pig movements

Distributions of the risk indicator of French *départements* being exposed to HEV were plotted (see examples in Supplementary File 1, Fig. b). The median risk of exposure for each *département* was mapped (Fig. 3). Geographical patterns of HEV prevalence and HEV exposure risk showed major differences (Figs. 2 and Fig. 3).

4. Discussion

Understanding the features of movement networks is crucial to analyse infection dynamics, pathogen occurrence and risk factors and to support risk-based surveillance strategies.

Although network studies have often been motivated by the outcome of animal movements on pathogen epidemiology (Keeling, 2005; Rautureau et al., 2012; Buttner et al., 2015; Thakur et al., 2015), the specific role of animal shipments in pathogen transmission and/or exposure has rarely been quantified, especially in the swine sector. The primary advantage of our study lies in combining epidemiology and network analysis to quantify both the impact of animal movements on pathogen prevalence within farms and the risk of areas being exposed to diseases due to between-area movements. HEV was chosen as a pathogen for implementation. Indeed, pig movements are likely to play a

pivotal role in HEV epidemiology (Salines et al., 2017a), although they have only scarcely been explored to date (Nantel-Fortier et al., 2016). We assessed the role of pig shipments in relation to within-farm HEV seroprevalence level and to the risk of exposure of French *départements* to HEV.

Pig movement data originated from the French National Swine Identification Database (BDporc), in which all pig shipments are systematically recorded. The information provided by this database is recognised by the French Ministry for Agriculture and can therefore be considered trustworthy. Moreover, a thorough cleaning stage was carried out to manage incorrect or incomplete data. The quality of data in terms of accuracy, reliability, and comprehensiveness guaranteed the robustness of our results (Salines et al., 2017b). The random selection process for tested farms and for individual pigs tested from each farm (Rose et al., 2011) ensured reliable estimates for the seroprevalence values used in our study. Moreover, the within-farm apparent seroprevalence estimates were corrected for serological test characteristics, providing true seroprevalence estimates. Eight production sites surveyed in 2009 ceased activity before 2013, limiting movement data availability to only 178 out of the 186 farms. This is consistent with the observed overall decrease in the number of pig farms in France (Salines et al., 2017b). On the *département* scale, the model involving the random sampling of farm-level HEV prevalence from beta distributions – with a weighted confidence interval – made it possible to take into account the low precision of some prevalence figures in quite a few *départements* where a low number of farms had been sampled. Temporal variability of both pig movements and HEV seroprevalence was a limitation of our study. Indeed, one should note that movement and

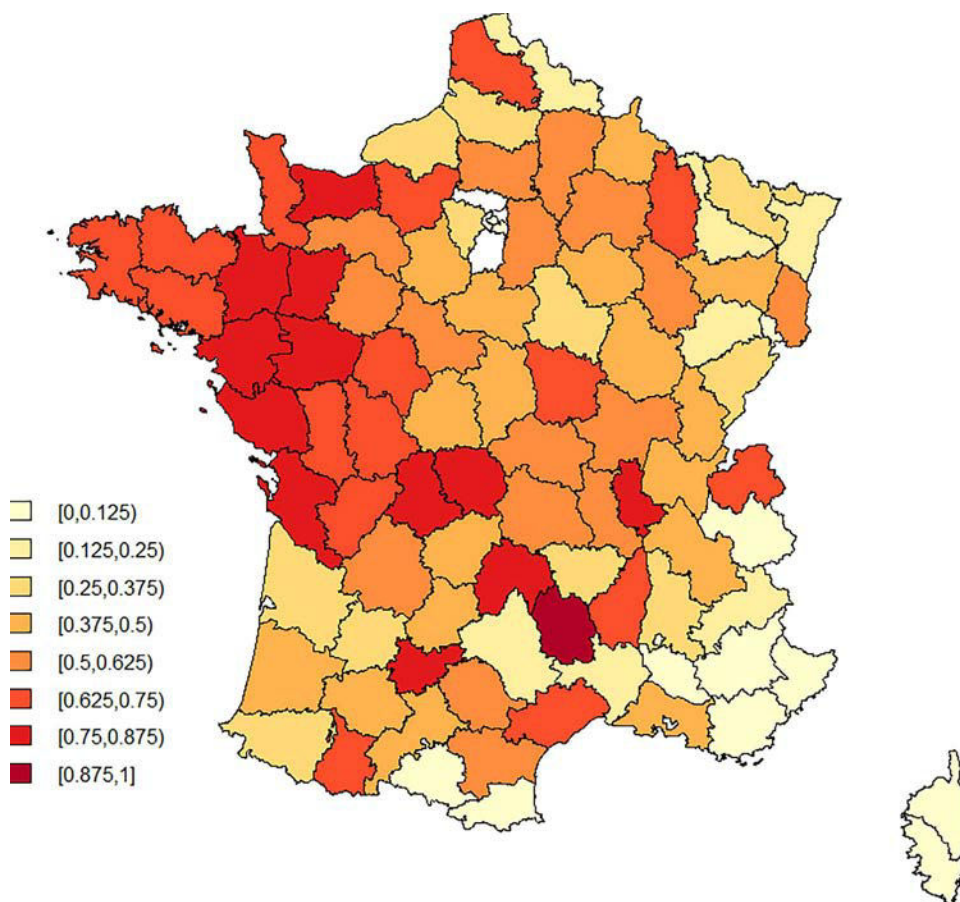


Fig. 3. Median risk of French départements being exposed to HEV through external incoming pig movements (10,000 simulations). An indicator of the risk of a French département being exposed to HEV was calculated as the number of infected movements it had received from source départements divided by its total number of external incoming movements.

prevalence data were not simultaneously collected. However, the French pig movement network has been found to be stable over time (Salines et al., 2017b), so we can assume that combining the 2009 prevalence data with the 2013 pig movement data is still consistent. Moreover, 70% of the 178 farms included in our study showed a loyalty equal to 1 (i.e. they exchanged animals with the same suppliers/buyers over the year), reflecting the stability of their movements. Regarding HEV prevalence, our data were dated (2009) and HEV prevalence is likely to vary over time. However, a more recent study also conducted in France reported similar prevalence figures (59% seroprevalence in Feuerer et al. (2017) vs 65% in Rose et al. (2011)). Aggregating movement data on a yearly basis also appeared to be relevant due to the absence of seasonality in the French pig network (Relun et al., 2016; Salines et al., 2017b) and provided indicators representing the overall activity of farms over a year. A possible improvement to the network model may involve weighting links depending on the number of animals exchanged.

In the recent literature, several farm connectivity indicators were identified as risk factors for disease occurrence and spread (Martin et al., 2011; Frössling et al., 2012; Lee et al., 2017; Sintayehu et al., 2017). Our study found that the farms' in-degree was positively associated with high within-farm HEV seroprevalence. This is consistent with several studies conducted in livestock production sectors showing that farms having a high in-degree were more likely to be infected with a pathogen (Martin et al., 2011; Frössling et al., 2012; Lee et al., 2017; Sintayehu et al., 2017). Since repeated animal shipments to a farm from the same supplier were aggregated into a single link, the association between HEV seroprevalence and in-degree not only indicates that the HEV seroprevalence of farms increases with the number of incoming shipments, but it also proves that buying animals from several suppliers is linked to higher HEV seroprevalence. Our results also showed that the greater the ingoing closeness of a pig farm, the higher its HEV

seroprevalence. A high value for the ingoing closeness centrality of a given farm indicates that the farm can be reached by its trade partners in only a few movements. Farm centrality in the network therefore appears to be a factor in vulnerability to HEV. This is consistent with the findings of previously published papers (Lee et al., 2017; Sintayehu et al., 2017). As Lee et al. (2017) demonstrated for PRRSV, we found that the odds of having higher within-herd HEV seroprevalence was increased more by ingoing closeness than by in-degree, meaning that the level of connectivity with all other holdings in the network is a better predictor of HEV infection than the number of directly connected farms. Unlike for other pathogens (Lee et al., 2017), no significant association was found between HEV within-farm seroprevalence and out-degree or outgoing closeness. The absence of an effect for these centrality indices was expected since HEV is mainly transmitted by infected pigs introduced into a naïve population. Introduction into a farm due to the sole transit of a possibly contaminated truck loading pigs in the farm for an outgoing shipment is therefore extremely unlikely. Unlike Sintayehu et al. (2017) regarding bovine tuberculosis, our statistical model did not show any significant effect of a herd's betweenness on within-herd HEV seroprevalence. Production units with high betweenness centrality play a key role in the spread of disease throughout the network since they can build so-called bridges between distinct network components. Since we explored the role of centrality metrics in HEV occurrence in farms, and not in their ability to transmit HEV to other farms, the lack of an effect for betweenness was also expected. Ingoing and outgoing contact chain values were not found to have a significant effect on HEV seroprevalence either. Again, as we did not investigate a farm's potential for spreading HEV, the lack of a link between OCC and HEV seroprevalence is coherent. An association between ICC and HEV seroprevalence could have been expected. This kind of association has indeed been demonstrated in other studies, but Frössling et al. (2012) showed that this link was pathogen-dependent: indeed, high ICC was

found to be a risk factor in the occurrence of bovine coronavirus but not for bovine respiratory syncytial virus.

To the best of our knowledge, the exposure of a geographical area to a pathogen due to animal movements has never been quantified. The choice of the departmental level for our study was policy-oriented; indeed, French *départements* are local administrative areas and surveillance programmes are often designed and implemented on this scale. Due to the low precision of HEV farm-level prevalence data in quite a few *départements*, the distribution of the risk of exposure was large in these *départements* and the results in these *départements* therefore lack precision. Nevertheless, the outputs of the procedure used to assess the risk of HEV exposure were stabilised thanks to a high number of simulations. Given the form of the risk distribution, the median appeared the most appropriate metric for the risk of exposure. High variability in the median risk of exposure to HEV was observed depending on the French *département*, confirming the relevance of designing targeted and differentiated surveillance strategies based on the area's risk level. Moreover, the discrepancy between the departmental observed prevalence figures and the departmental risk levels provides justification for monitoring not only highly prevalent areas but also those having at-risk movements coming from infected areas.

Confounding factors may bias our results. Indeed, we had limited data regarding farm and *département* characteristics. For instance, no detailed data was available regarding farm size, pig density or farm management practices, but we checked that farm type (breeding, farrowing-to-finishing, etc.) was not a confounding factor. Several research teams have recently developed farm-level risk scores based on animal movements. For instance, Schärer et al. (2015) introduced a cumulative score taking several parameters into account, including the ICC, the number of animals per incoming movement, the type of pasture and the number of weeks per year with movements. Another study proposed a method for calculating a disease-specific relative ratio for the increased probability of infection due to the introduction of animals (Frössling et al., 2014). Ribeiro-Lima et al. (2015) also identified farms with a higher risk of bovine tuberculosis infection using a model based on a risk score at movement level. A further stage in our study could be to build a farm-level risk score including both risk factors linked to pig movements and other farm-specific risk factors for HEV that have previously been identified (Walachowski et al., 2014). Such a score would make it possible to target only high-risk farms for more effective surveillance.

5. Conclusion

Combining network analysis with epidemiological data demonstrated that direct network connectivity and farm centrality in the network are related to the within-herd HEV seroprevalence level and that some areas are more at risk for HEV due to their pig movements. More generally, the methods we proposed prove that farm- or area-level parameters derived from animal movements can support the risk-based selection of farms for surveillance programmes or the implementation of differentiated surveillance strategies depending on the area's movement characteristics. Therefore, risk-based epidemiological approaches benefiting from network analysis should be promoted.

Author contributions

MS and MA performed the statistical analyses. MS drafted the manuscript. NR initiated and supervised the project. All the co-authors revised the manuscript and approved the final submitted version.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.prevetmed.2017.11.015>.

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Ce qu'il faut retenir

Le couplage de méthodes d'analyse de réseau avec des données épidémiologiques a montré que la connectivité directe des élevages et leur centralité de proximité dans le réseau sont associées à la séroprévalence du HEV intra-élevage et que certaines aires géographiques sont plus à risque vis-à-vis du HEV du fait de leur approvisionnement depuis des zones infectées. Si cette approche a mis en évidence des associations statistiques entre mouvements et prévalence HEV, elle ne permet pas de décrire de manière fine et dynamique la diffusion du HEV à l'échelle nationale, d'expliquer les facteurs de la propagation et la persistance virale dans la filière de production porcine, ni de tester des mesures de lutte sur un territoire. C'est dans cet objectif qu'un modèle dynamique inter-troupeaux, couplant la dynamique infectieuse du HEV circulant dans un élevage avec les échanges de porcs, a été développé.



Take home message

Combining network analysis methods with epidemiological data has shown that farms' direct connections as well as their closeness centrality in the network are associated with high HEV on-farm seroprevalence, and that some areas are at greater risk regarding HEV due to their supply from infected regions. While this approach has highlighted statistical associations between pig movements and HEV prevalence, it does not allow for a detailed and dynamic description of the spread of HEV at the national level, or an explanation of the factors of spread and viral persistence in the pig production chain, or the testing of control measures in a territory. It is with this objective in mind that a between-herd dynamic model, coupling the infectious dynamics of HEV circulating on a farm with pig exchanges, has been developed.

PARTIE 3.2. Un modèle inter-troupeaux pour comprendre la propagation et la persistance du virus de l'hépatite E dans la filière porcine

L'article précédemment présenté, ainsi que l'étude réalisée par Nantel-Fortier *et al.* (2016), suggèrent un **probable rôle des mouvements d'animaux** dans la diffusion du HEV entre des élevages de porcs mais, à notre connaissance, aucune équipe de recherche n'a à ce jour développé de modèle permettant d'évaluer et de comprendre la circulation du HEV entre des élevages en prenant en compte la dynamique virale intra-élevage et la dynamique démographique liée aux échanges d'animaux vivants. Dans ce contexte, l'objectif de l'étude présentée ci-après a été de développer une **approche de modélisation multi-échelles** afin de décrire et d'expliquer les conditions de la diffusion et de la persistance du HEV dans une communauté d'élevages français et d'évaluer la prévalence d'animaux positifs à l'abattoir sous différentes conditions. Pour ce faire, un modèle stochastique a été développé en couplant le modèle intra-élevage décrit dans le chapitre II (Salines *et al.*, 2019d) avec les données de mouvements de porcs sur la période 2012-2015 présentées au début du présent chapitre III (Salines *et al.*, 2017b). Ce modèle a aussi été utilisé pour évaluer différents **scenarii d'introduction** du HEV dans la communauté ainsi que l'**effet d'un assainissement de la population** vis-à-vis des pathogènes intercurrents (virus du SDRP, PCV2 par exemple).

Ce travail a donné lieu à une collaboration avec l'équipe d'épidémiologie du *Swedish Veterinary Institute* (Dr Stefan Widgren, SVA, Uppsala, Suède) et à une mission de trois mois sur place.

Les résultats de ce travail de modélisation sont présentés dans le projet de publication ci-dessous qui sera soumis dans une revue internationale à comité de lecture.

Publication 9 (Draft)

Salines M., Andraud M., Rose N., Widgren S. A between-herd data-driven stochastic model to explore the spatio-temporal spread of hepatitis E virus in the French pig production network. In prep.

A between-herd data-driven stochastic model to explore the spatio-temporal spread of hepatitis E virus in the French pig production network

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Abstract: Hepatitis E virus is a zoonotic pathogen for which pigs are recognized as the major reservoir in industrialised countries. A multiscale model was developed to assess the HEV transmission and persistence pattern in the pig production sector through an integrative approach taking into account within-farm dynamics and animal movements based on actual data. Within-farm dynamics included both demographic and epidemiological processes. Direct contact and environmental transmission routes were considered along with the possible co-infection with immunomodulating viruses (IMVs) known to modify HEV infection dynamics. Movements were limited to 3,017 herds forming the largest community on the swine commercial network in France and data from the national pig movement database were used to build the contact matrix. Between-herd transmission was modelled by coupling within-herd and network dynamics using the SimInf package. Different introduction scenarios were tested as well as a decrease in the prevalence of IMV-infected farms. After introduction of a single infected gilt, the model showed that the transmission pathway as well as the prevalence of HEV-infected pigs at slaughter age were affected by the type of the index farm, the health status of the population and the type of the infected farms. These outcomes could help design HEV control strategies at a territorial scale based on the assessment of the farms' and network's risk.

Keywords: between-herd model; hepatitis E virus; interactions; multi-scale modelling; public health

32 **1. Introduction**

33

34 Hepatitis E virus (HEV) is a non-enveloped single-stranded RNA virus frequently leading to
35 asymptomatic infections in humans, but also causing acute or chronic hepatitis - depending,
36 inter alia, on the patient's immune status (Emerson and Purcell, 2003; Kamar et al., 2011). If
37 genotypes 1 and 2 are exclusively human viruses mainly present in developing countries,
38 genotypes 3 and 4 are shared by humans and other animal species and are responsible for
39 sporadic human cases in industrialised countries (Dalton et al., 2008; Purcell and Emerson,
40 2008). In particular, HEV-3 is highly prevalent in European swine populations (Salines et al.,
41 2017a), e.g. in the French pig production sector, where around 65% of farms have been found
42 to host at least one HEV seropositive pig (Rose et al., 2011). A number of locally acquired cases
43 have been linked to the consumption of raw or undercooked pork products, especially those
44 containing liver in high proportion (Yazaki et al., 2003; Holub et al., 2009; Colson et al., 2012;
45 Moal et al., 2012; Motte et al., 2012; Trmal et al., 2012; Chalupa et al., 2014; Garbuglia et al.,
46 2015; Riveiro-Barciela et al., 2015; Guillois et al., 2016). In that way, hepatitis E is recognised
47 as a foodborne zoonosis with domestic pigs being the major reservoir in Western countries
48 (Pavio et al., 2017).

49

50 The risk of slaughtering HEV-positive pigs, and thus to enter contaminated products into the
51 food chain, is strongly related to HEV dynamics in pig herds. Observational and experimental
52 studies have evidenced several risk factors affecting HEV behaviour on pig farms, such as
53 husbandry practices in terms of hygiene, biosecurity and rearing conditions (Walachowski et
54 al., 2014), piglet's sex and sow's parity (Salines et al., 2019b). The protection conferred by
55 maternally-derived antibodies (MDAs) was also shown to impact HEV dynamics (Andraud et
56 al., 2014; Crotta et al., 2018). Moreover, pigs exhibited chronic hepatitis when co-infected with
57 immunomodulating viruses (IMVs), e.g. porcine reproductive and respiratory syndrome virus
58 (PRRSV) or porcine circovirus type 2 (PCV2) (Salines et al., 2015; Salines et al., 2019a; Salines
59 et al., 2019b). Recently, we have developed a stochastic individual-based model representing
60 HEV spread and persistence on a farrow-to-finish pig farm in which pigs may be co-infected
61 with IMVs (Salines et al., 2019c). This model gave insights on HEV spread and persistence and
62 evidenced or confirmed several risk factors, e.g. the type of housing for gestating sows, cross-
63 fostering and mingling practices and health status regarding the IMVs. However, this model
64 only explored HEV dynamics in a single and isolated farrow-to-finish herd, without taking into

65 consideration animal trade with other holdings, although pig movements are likely to play a
66 pivotal role in HEV dynamics in the pig production sector . For instance, Nantel-Fortier et al.
67 (2016) reported the presence of HEV inside and outside farm buildings, on trucks and in
68 slaughterhouse yards, thus suggesting viral transmission between farms and throughout the
69 production network. Recently, we have also shown, by combining French network indicators
70 with epidemiological data, that the in-degree and ingoing closeness of farms were associated
71 with high HEV within-farm seroprevalence (Salines et al., 2018).

72

73 To represent infection spread at a regional or national scale, multi-scale models can be designed
74 by coupling infection dynamics within herds together with interactions between interconnected
75 herds. Such approaches have already been developed, particularly to explore the transmission
76 of bacterial diseases between cattle farms (Brooks-Pollock et al., 2014; Beaunee et al., 2015;
77 Widgren et al., 2016b; Widgren et al., 2018) or pig herds (Schulz et al., 2018). Several
78 approaches have been recently used to implement such models that may be computationally
79 challenging (Bui et al., 2016; Widgren et al., 2016a; Picault et al., 2017). In particular, the
80 SimInf package developed in R software is recognized as an efficient and flexible modelling
81 framework for fast event-based epidemiological simulations of infectious disease spread
82 (Widgren et al., 2016a). It makes it possible to integrate within-herd infection dynamics as a
83 continuous-time Markov process and demographic data as scheduled events. Thus, using the
84 SimInf framework, the aims of our study were: (i) to model the spatio-temporal spread of HEV
85 in a cluster of highly connected French pig farms, real pig movement data and HEV within-
86 herd epidemiological dynamics being incorporated; (ii) to investigate different introduction and
87 control scenarios.

88

89

90 **2. Materials and methods**

91

92 **2.1. Population dynamics model**

93

94 ***2.1.1. Farms' structure: type, facilities, populations, management system***

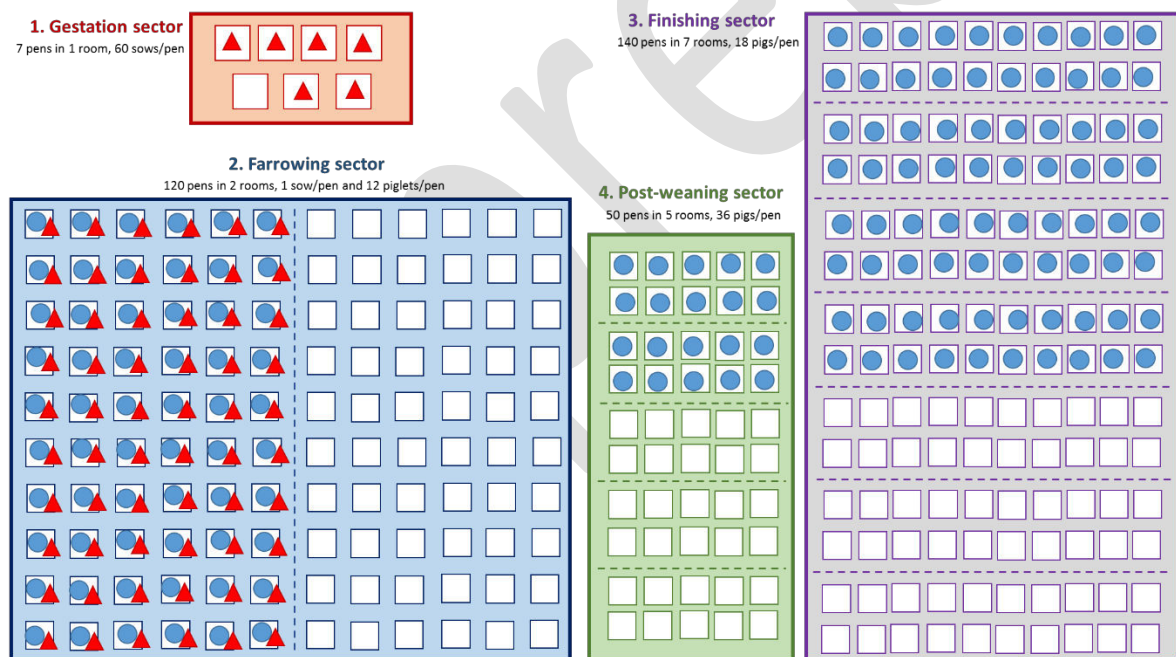
95

96 Eight farm types are considered: nucleus (*SEL*), multiplication (*MU*), farrow-to-finish (*FF*),
97 farrowing (*FA*), farrowing post-weaning (*FPW*), post-weaning (*PW*), post-weaning finishing

98 (PWF) and finishing (FI) farms. All farms (within each type) were assumed to have the same
 99 structure and size(Figure 1), accounting for one to four sectors, depending on their type (Table
 100 1): gestation, farrowing, post-weaning (i.e. nursery) and finishing sectors. Each of the sectors
 101 is divided into rooms, including themselves several pens. Two populations are considered:
 102 breeding sows and growing pigs. Depending on its type, a farm can host one or both populations
 103 (Table 1).

104
 105 **Figure 1. Farm structure, facilities and populations considered.**

106 Farms can be composed of one to four sectors depending on their type: gestation, farrowing, post-
 107 weaning and finishing sectors (coloured squares). Each sector is divided into rooms (dashed lines),
 108 that are composed of pens (white squares). Two populations are considered: breeding sows (red
 109 triangles) and growing pigs (blue dots).



113 **Table 1. Types of sectors, animal populations and events per farm depending on the**
 114 **farm type**

115 Farms are composed of one to four sectors, depending on their type: nucleus (*SEL*), multiplication
 116 (*MU*), farrow-to-finish (*FF*), farrowing (*FA*), farrowing post-weaning (*FPW*), post-weaning (*PW*),
 117 post-weaning finishing (*PWF*) and finishing (*FI*) farms. They can rear one or two populations
 118 (breeding sows, growing pigs). Six types of events can occur depending on the farm type: movement
 119 of sows from gestation to farrowing sector (*ges-fa*); piglet birth (*birth*); movement of sows from
 120 farrowing back to gestation sector (*fa-ges*); movement of piglets from farrowing to post-weaning

121 sector (*fa-pw*); movement of growing pigs from post-weaning to finishing sector (*pw-fi*); movement of
 122 growing pigs leaving the finishing sector (*fi*).
 123

		Farm type							
		SEL	MU	FF	FA	FPW	PW	PWF	FI
Sectors	Gestation	X	X	X	X	X			
	Farrowing	X	X	X	X	X			
	Post-weaning	X	X	X		X	X	X	
	Finishing	X	X	X				X	X
Animal populations	Breeding sows	X	X	X	X				
	Growing pigs	X	X	X	X	X	X	X	X
Events	<i>ges-fa</i>	X	X	X	X	X			
	<i>birth</i>	X	X	X	X	X			
	<i>fa-ges</i>	X	X	X	X	X			
	<i>fa-pw</i>	X	X	X	X	X			
	<i>pw-fi</i>	X	X	X		X	X	X	
	<i>fi</i>	X	X	X				X	X

124
 125 Animals evolve in a sequential way through the above-mentioned facilities: the breeding sows
 126 in the gestation and farrowing sectors; the growing pigs in the farrowing, post-weaning and
 127 finishing sectors. Thus, the two populations physically interact in the farrowing sector only.
 128 The farms are managed according to a batch-rearing system (BRS), meaning that the herd
 129 population is divided into sets of individuals from the same physiological stage, called batches.
 130 For instance, for farms rearing sows, the reproductive cycles of sows belonging to a given batch
 131 are synchronised so that all breeding events occur at the same time for all sows. Consequently,
 132 a given batch of sows gives birth to piglets simultaneously, these contemporary piglets forming
 133 a group of growing pigs also constituting a batch. The batches are managed with an all-in-all-
 134 out strategy, i.e. all animals from a batch leave a facility simultaneously and enter an empty
 135 room at once. In the model, all farms are considered to be managed with a 7-batch rearing
 136 system (i.e. a 3-week interval management system), with parameters being detailed in Table 2.

137
 138 **Table 2. Parameters governing the population dynamics model in a 7-batch rearing**
 139 **system.**

140 *FA*: farrowing farms, *FPW*: farrowing post-weaning farms, *SEL*: nucleus farms, *MU*: multiplication
 141 farms, *FF*: farrow-to-finish farms
 142

Parameter description (unit)	Value
Duration of a sow reproductive cycle (days)	142
- Duration in gestating room (days)	107
- Duration in farrowing room (days)	35

Duration of a growing pig cycle (days)	180	
- Duration in farrowing room (days)	28	
- Duration in post-weaning room (days)	86	
- Duration in finishing room (days)	94	
Interval between two successive batches (days)	21	
Annual renewal rate of sow herds (%)	40	
Number of animals:	<i>In FA and FPW</i>	<i>In SEL, MU and FF</i>
- Total number of sows	420	210
- Number of sows per batch	60	30
- Number of piglets per litter	12	
- Number of piglets per batch	720	360

143

144 2.1.2. Population dynamics processes

145

146 Life cycle of breeding sows and growing pigs. After 107 days in the gestation sector (i.e. seven
147 days before farrowing), sows from a batch are transferred into the farrowing sector (one sow
148 per pen) where they give birth to 12 piglets each (Table 2). Dams remain with their litter for
149 four weeks until weaning. At the end of the lactation period, sows are moved back to the
150 gestation sector to begin a new reproductive cycle, when piglets are moved to an empty nursery
151 room (36 pigs per pen, three litters being gathered in one pen). Piglets stay in the nursery sector
152 until 86 days of age when they are moved to a finishing room (18 pigs per pen, i.e. 1.5 litter per
153 pen). When they are 180 day old (i.e. after 94 days in the finishing sector), they are sent to the
154 slaughterhouse. Every 21 days, five replacement gilts are introduced in herds rearing sows and
155 five sows are culled.

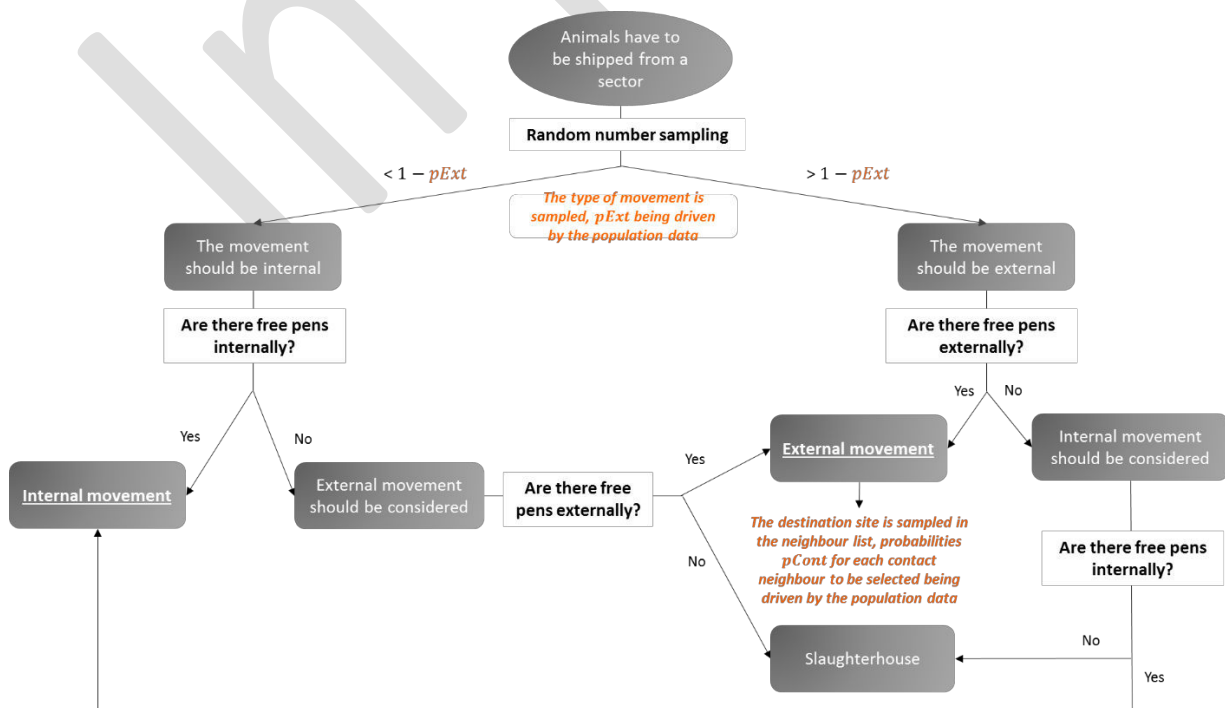
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157 Implementation of population events. Six types of events can occur in the population depending
158 on the farm type (Table 1): movement of sows from gestation to farrowing sector (*ges-fa*);
159 piglet birth (*birth*); movement of sows from farrowing back to gestation sector (*fa-ges*);
160 simultaneous movement of piglets from farrowing to post-weaning sector (*fa-pw*); movement
161 of growing pigs from post-weaning to finishing sector (*pw-fi*); movement of growing pigs
162 leaving the finishing sector (*fi*). Event times are determined deterministically by the different
163 cycle durations as explained above. The number of animals to be moved are also fixed by the
164 production system, as described above (Table 2, Figure 1). The three first types of events
165 (corresponding to the sow reproductive cycle: *ges-fa*, *birth*, *fa-ges*) are always internal (i.e. the
166 animals remain in the same farm), when the three others (corresponding to movements of
167 growing pigs: *fa-pw*, *pw-fi*, *fi*) can be either internal or external (i.e. the animals are shipped to
168 another site). Selecting the pens of destination is a two-step process detailed in Figure 2. First,
169 the type of movement (internal or external) is selected with probability p_{Ext} that the animals

170 are shipped to another farm, derived from real movement data (section 2.1.3). In case of external
 171 movement, the destination site is sampled among the set of possible destination farm from the
 172 movement database (see below). When leaving the finishing sector (f_i event), two possible
 173 pathways were considered for growing pigs: (i) animals leaving FF , PWF and FI farms are sent
 174 to the slaughterhouse; (ii) a fraction of females is used for the renewal of the sow population
 175 either on the same farm (i.e. self-renewal, in SEL farms) or on another farm (in cases of animals
 176 reared in SEL and MU farms), and the others are sent to the slaughterhouse. Again, the choice
 177 of the destination of finishing events is driven by the population data presented in the following
 178 section.

180 **Figure 2. Selection process of the movements' destinations.**

181 Each time animals have to be shipped from a sector, as defined by the production cycle, the
 182 type of event (i.e. internal versus external) is determined according to the probability p_{Ext}
 183 that is the probability that animals are shipped externally, as defined by the population data. In
 184 cases of no free pens found internally (resp. externally), external (resp. internal) movement is
 185 considered. If all pens (internally and in contact farms) are full, animals are sent to
 186 slaughterhouse. If animals are shipped externally, the destination site is sampled in the contact
 187 neighbours of the farm of origin, the probability p_{Cont} of a destination farm to be sampled
 188 being defined in the population data.



190

191 **2.1.3. Data on animal movements between farms**

192

193 Dataset. French pig movement data recorded during the period 1st June 2012 to 31st December
194 2014 were used to drive the population demographics in the model. The data originated from
195 the National Swine Identification Database (BDporc). The dataset, described in detail in Salines
196 et al. (2017b), contained 21,446 farms and 2,382,510 between-farm movement records. Briefly,
197 the main features of all swine holdings in mainland France (continental France and Corsica)
198 were included in the database: identification number (ID), type of holding, type of farming
199 activity, farm size and location. Movements of pigs were reported at the batch level with the
200 following information: farm IDs where animals were loaded or unloaded, round number and
201 chronological sequence of the operations forming the round, batch size and animal category.
202 First, as described in Salines et al. (2017b), a one-mode directed network was built, with
203 holdings being considered as nodes, and movements between two nodes as links. In this
204 network, called Animal Introduction Model in Salines et al. (2017b), in-between movements
205 forming a round were replaced by direct movements between holdings, i.e. intermediate transit
206 movements of a truck through a farm without unloading any animal were neglected. The
207 analysis of the network revealed the existence of communities, defined as subsets of nodes in
208 which there are significantly more links than expected by chance - i.e. groups of highly
209 connected farms (Infomap algorithm (Rosvall et al., 2009)). This approach evidenced a large
210 community including 3,017 farms (Figure 3), among them 55 *SEL*, 210 *MU*, 1,375 *FF*, 86 *FA*,
211 62 *FPW*, 8 *PW*, 546 *PWF* and 675 *FI* farms. In this community, around 78,000 movements
212 occurred over the study period. Data derived from this community were used to feed SimInf
213 population dynamics sub-model. To achieve this task, we first defined a standard herd size,
214 structure and batch-rearing system to all herds, corresponding to the average characteristics
215 over all the community. Within-farm movements were scheduled following the evolution of the
216 animals through their life- or reproductive-cycles. Who-to-Whom (site-to-site) contact
217 probabilities were then evaluated over the study period to represent the external movements,
218 with a rescaling step to take into account the difference between the standard and the actual
219 herd sizes.

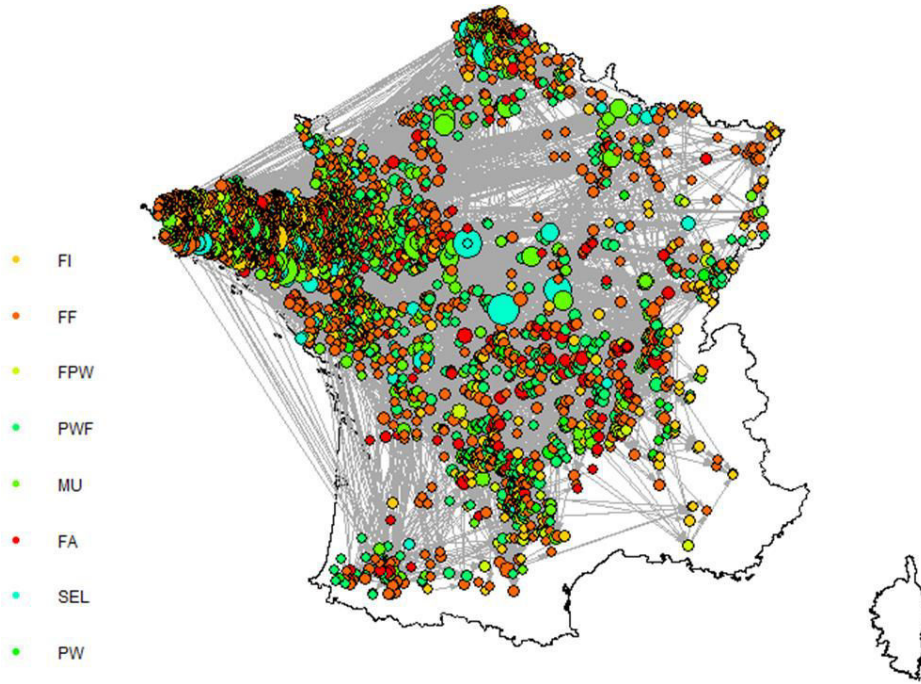
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221 **Figure 3. Largest community in the pig movement network in France (2012-2014),** 222 **derived from Salines et al. (2017b).**

223 Using Infomap algorithm, a large community including 3,017 farm was identified in the
224 French pig movement network (data from 2012 to 2014). Farm and movement data from this

225 community was used as input population data in the present model. The size of the dots is
 226 proportional to the total degree of the holding, the colours are related to the farm type. *FI*:
 227 finishing farm, *FF*: farrow-to-finish farm, *FPW*: farrowing post-weaning farm, *PWF*: post-
 228 weaning finishing farm, *MU*: multiplication farm, *FA*: farrowing farm, *SEL*: nucleus farm,
 229 *PW*: post-weaning farm.

230



231

232

233 Calculation of the probability for a movement to be external. For each farm i in the community,
 234 the probabilities $pExt_i^{fa-pw}$, $pExt_i^{pw-fi}$ and $pExt_i^{fi}$ that the corresponding possibly external
 235 movements ($fa-pw$, $pw-fi$ and fi , respectively) are actually external have been calculated. For
 236 *FA* farms, $fa-pw$ movements are always external, so that:

237
$$pExt_i^{fa-pw} = 1$$

238 Similarly, $pw-fi$ movements are always external for *FPW* and *PW* farms, leading to:

239
$$pExt_i^{pw-fi} = 1$$

240 for these two farm types.

241 For the other farm types, one may assume that, for an average-sized farm as designed in the
 242 population model, the total number of animals shipped over the study period from a sector a to
 243 a sector b is:

244
$$n_{average}^{a,b} = \frac{n_{days}}{BBI} \times n_{batch}^{pigs}$$

245 where n_{days} is the total number of days over the study period, BBI the number of days between
 246 two successive batches (i.e. between-batch interval) and n_{batch}^{pigs} the average number of pigs per
 247 batch.

248 Denoting $R_{average}^i$ the ratio between the actual size of a farm i as recorded in the population
 249 data and the average size of the farm i as designed in the population model, the expected number
 250 of animals shipped by the farm i from a sector a to a sector b over the study period can be
 251 expressed as:

$$252 \quad nExp_i^{a,b} = n_{average}^{a,b} \times R_{average}^i$$

253 Let $nObs_i^{a,b}$ denote the observed number of animals shipped externally by a farm i from a
 254 sector a to the sector b of another farm (as recorded in the population data). Then, the
 255 probability that the movement from a sector a of a farm i to a sector b is external is:

$$256 \quad pExt_i^{a,b} = \frac{nObs_i^{a,b}}{nExp_i^{a,b}}$$

257
 258 Calculation of the contact probability associated to each neighbour. For each external
 259 movement from a sector a of a farm i to an external sector b , the probability that the movement
 260 is directed to a contact farm j is calculated by:

$$261 \quad pCont_{i,j}^{a,b} = \frac{n_{i,j}^{a,b}}{n_i^a},$$

262 where $n_{i,j}^{a,b}$ is the number of animals shipped from the sector a of the farm i to the sector b of
 263 the contact farm j over the study period, as observed in the population data, and n_i^a is the total
 264 number of animals shipped externally from the sector a of the farm i over the study period,
 265 again as observed in the population data.

266
 267 - Final structure of input data. Finally, 11 variables were used to describe each of the 3,017
 268 farms and to drive the population dynamics: farm ID, farm type, and nine variables
 269 corresponding to the contact matrix with contact probabilities associated to each sector of
 270 each farm.

271 272 **2.2. Epidemiological model**

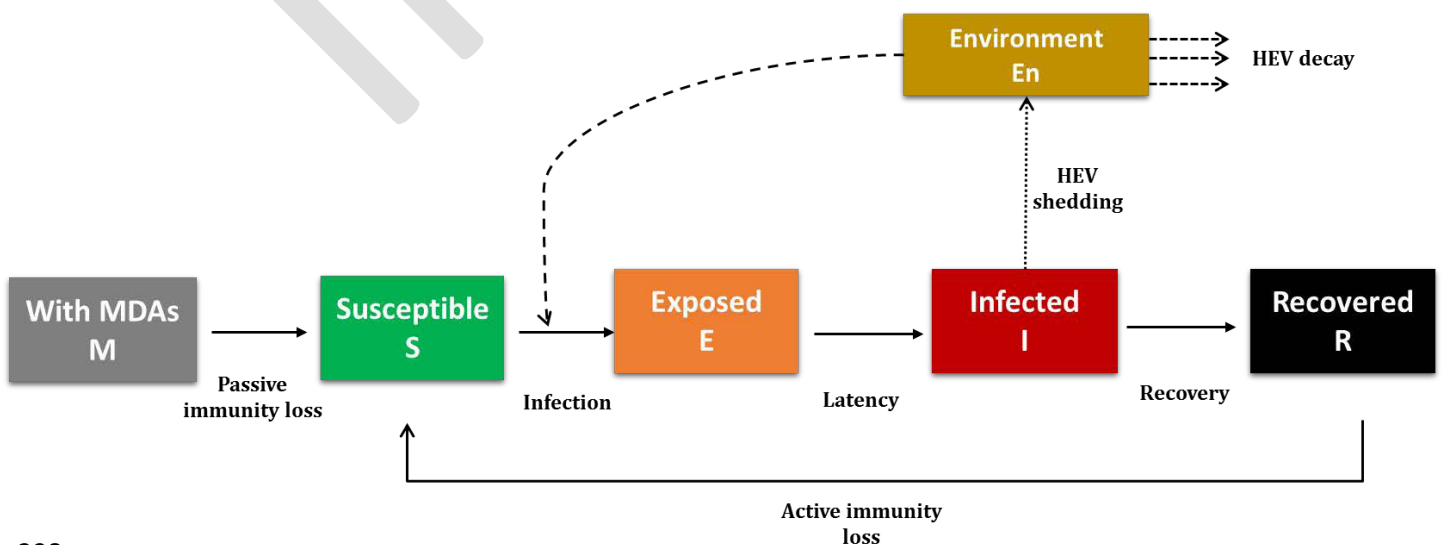
273 274 **2.2.1. Epidemiological process**

275 As described in Salines et al. (2019c), an MSEIR – Maternally Immune (M), Susceptible (S),
 276 Exposed (E), Infectious (I) and Recovered (R) – model including an environmental
 277 compartment was considered to describe HEV infection dynamics taking those factors into
 278 account (Figure 4). Briefly, new-born piglets born from immune sows acquire anti-HEV
 279 maternally-derived antibodies by colostrum intake (health state M), providing complete but
 280 temporary protection towards infection. Susceptible (S) pigs can then be infected, entering the
 281 exposed (E) state. HEV transmission occurs through faecal-oral route, either by direct contact
 282 with an infectious pig or by ingestion of viable virus in the contaminated environment in the
 283 pen or the neighbourhood (Bouwknegt et al., 2008; Bouwknegt et al., 2011). After the latency
 284 period, the infectious animal (I) shed HEV in the environment, where the virus can continue to
 285 be viable, feeding the environmental viral pool. Thus, the overall virus load in a pen’s
 286 environment corresponds to the accumulation of viral particles shed by all infectious
 287 individuals, partially compensated by faeces removal through the slatted floor, the natural decay
 288 of the virus and the cleaning/disinfecting operations of empty pens (Andraud et al., 2013).
 289 Recovered pigs (R) lose their immunity over time, assuming a gamma-distribution for antibody
 290 waning, and eventually revert to full susceptibility (S). Transitions between epidemiological
 291 statuses occur stochastically.

293 **Figure 4. HEV infection process as represented with a MSEIRS model.**

294 The epidemiological model has been built as a MSEIR – Maternally Immune (M), Susceptible (S),
 295 Exposed (E), Infectious (I) and Recovered (R) – model including an environmental compartment.

296 MDAs: maternally-derived antibodies.



298

2.2.2. Forces of HEV infection and HEV infection process

As described in Salines et al. (2019c), HEV force of infection takes two components into account: a within-pen and a between-pen force of infection. Briefly, one infectious pig can infect its pen mates by direct contact or indirectly through its contaminated faeces accumulated in the environment, leading to the following within-pen force of infection:

$$\lambda_p^{HEV,wp}(t) = \frac{\beta_{HEV} \times I_p^{HEV}(t) + \beta_E^{wp} \times Q_p \times Q_{ing}}{N_p(t)}, \quad (1)$$

where $N_p(t)$ and I_p correspond to the total number of animals and the number of infected animals in the pen p at the time t , respectively. β_{HEV} denotes the individual HEV transmission rate. β_E^{wp} is the HEV environmental transmission rate within a pen, corresponding to the average number of animals that can be infected by a single genome equivalent present in the pen environment (Andraud et al., 2013; Salines et al., 2015). Q_{ing} is the quantity of faeces ingested by a pig per day (Bouwknegt et al., 2011). Q_p is the HEV quantity accumulated in the pen p , calculated as follows:

$$Q_p(t) = Q_p(t-1) \times (1 - \varepsilon_1) \times (1 - \varepsilon_2) + \frac{w_{HEV} \times I_p^{HEV}(t)}{N_p(t)}, \quad (2)$$

where w_{HEV} is the quantity of HEV particles shed in the environment by an infectious pig per gram of faeces. ε_1 and ε_2 are the daily proportion of faeces passing through the slatted floor and the daily HEV mortality rate, respectively. A third decay rate, ε_3 , corresponding to the proportion of faeces eliminated through cleaning operations, is sporadically applied when the room is emptied, and the batch is transferred to the next sector.

Moreover, contaminated faeces shed by pigs in a given pen can be transferred to an adjacent pen and are therefore likely to infect a susceptible animal in the adjacent pen. Thus, the between-adjacent-pen force of infection of a pen p is equal to the sum of the weighted force of infection of its two neighbours.

$$\lambda_p^{HEV,bap} = Q_{ing} \times \beta_E^{bap} \times \left(\frac{Q_{p-1} + Q_{p+1}}{N_p} \right), \quad (3)$$

where β_E^{bap} is the HEV indirect environmental transmission rate between pens (Andraud et al., 2013).

Finally, the infection process is event-driven owing to Gillespie algorithm with transition rates as described in Table 3.

330 **Table 3. Transition rates for each health state transition as illustrated in Figure 4.**

331 λ is the global force of infection as described in equations (1) and (3), ρ is the latency rate for exposed
 332 animals E, γ is the recovery rate for infectious animals I, σ and μ denote the maternal and active
 333 immunity waning respectively.

334

Health state transition		Transition rate
Passive immunity waning	$M \rightarrow S$	$\sigma \times M$
Infection	$S \rightarrow E$	$(\lambda_p^{\text{HEV,wp}} + \lambda_p^{\text{HEV,bap}}) \times S$
Latency	$E \rightarrow I$	$\rho \times E$
Recovery	$I \rightarrow R$	$\gamma \times I$
Active immunity waning	$R \rightarrow S$	$\mu \times R$

335

336 **2.2.3. Epidemiological parameters**

337

338 All parameters involved in the infectious process are fully described in Table 4 along with their
 339 definition and the origin of the input values. Since HEV dynamics has been shown to be strongly
 340 affected by co-infections with immunomodulating viruses such as PRRSV or PCV2 (Salines et
 341 al., 2015; Salines et al., 2019a; Salines et al., 2019c), some epidemiological parameters of the
 342 model depend on the farm's status regarding IMVs.

343

344 **Table 4. Epidemiological parameters governing the HEV infection dynamics in cases of**
 345 **IMV-free or IMV-positive farms.**

346 IMV: immunomodulating virus

347

Notation	Parameter description (unit)	Value		Reference
		IMV-free farms	IMV-positive farms	
D_{HEV}^M	Duration of maternal immunity (days)		45	Andraud et al. (2014)
D_{HEV}^E	Latency duration (days)	7.4	13.1	
β_{HEV}	Direct transmission rate (pigs/day)	0.15	0.70	
β_E^{wp}	Within-pen environmental transmission rate (g/ge/day)	2.10^{-6}	$6.6.10^{-6}$	Andraud et al. (2013)
β_E^{bap}	Between adjacent pen environmental transmission rate (g/ge/day)	2.10^{-8}	$6.6.10^{-8}$	Salines et al. (2015)
w	Quantity of HEV particles shed in faeces (ge/g/day)	10^4	10^6	
Q_{ing}	Average quantity of faeces ingested by a pig (g/day)		25	Bouwknegt et al. (2011)
ε_1	Faeces elimination rate through slatted floor (/day)		0.70	Expert opinion

ε_2	HEV decay rate in the environment (/day)	0.08	Johne et al. (2016)
ε_3	Faeces removal rate by cleaning	0.98	Expert opinion
D_{HEV}^I	Infectious period (days)	9.7	48.6 Andraud et al. (2013) Salines et al. (2015)
D_{HEV}^R	Duration of active immunity (days)	185	Expert opinion

348

349

350

2.3. Initialisation and simulations

351

352 At the beginning of a simulation, all herds rearing sows (i.e. *SEL*, *MU*, *FF*, *FA* and *FPW*) were
 353 composed of seven batches of sows, all being in the susceptible health state; the other farms
 354 were empty. At the end of the first year, i.e. after a period of population's initialisation, one
 355 HEV exposed gilt was introduced in a farm when a replacement event happens. The index farm
 356 was sampled according to different criteria depending on the scenario tested (see below). We
 357 assumed no subsequent introduction of HEV infected animals on the index farm. Simulations
 358 were run for five years after HEV introduction. One hundred simulations were run for each
 359 tested scenario. The number of animals in each epidemiological state in every pen of every farm
 360 was recorded four times a year.

361

2.4. Assessment of characteristics related to HEV spread in the network and evaluation of potential scenarios

364

2.4.1. Outcomes

366

367 Within-farm HEV dynamics was described by reporting within-herd HEV prevalence in sows
 368 and growing pigs on the index farm and HEV on-farm persistence five years post-introduction.
 369 Three outcomes were then selected to assess HEV spread in the network and evaluate the risk
 370 of HEV introduction into the food chain: (i) the proportion of HEV positive farms over the
 371 study period, i.e. the proportion of farms having at least one HEV-infected animal; (ii) the time
 372 at which farms got infected; (iii) the proportion of HEV-positive pigs sent to the slaughterhouse
 373 over the study period.

374

375

376 **2.4.2. Scenarios**

377

378 Eight different scenarios were run, as described in Table 5 to explore the impact of the type of
 379 the farm of introduction (*SEL*, *MU*, *FF* or *FA*) and of decreasing IMV prevalence in the
 380 community (going from 100% to 60% of IMV-positive FF farms) on the outcomes.

381

382 **Table 5. Description of the different scenarios (S) of the HEV between-herd model.**

383 IMV: immunomodulating virus, *SEL*: nucleus farm, *MU*: multiplication farm, *FF*: farrow-to-finish
 384 farm, *FA*: farrowing farm.

385

Proportion of IMV-free FF farms	Type of the index farm			
	<i>SEL</i> with $pExt_i^{fi} > 0.1$	<i>MU</i> with $pExt_i^{fi} > 0.1$	<i>FF</i> with more than 5 different contacts	<i>FA</i> with more than 5 different contacts
0	S1	S2	S3	S4
0.4	S5	S6	S7	S8

386

387 **2.4.3. Statistical models**

388

389 Three statistical models were built:

- 390 • A logistic regression was performed to compare the proportion of HEV-infected farms in
 391 the community depending on the type of the index farm and on the proportion of IMV-free
 392 FF farms in the community.
- 393 • A cox-proportional hazard model was used to assess the influence of four variables on
 394 farms' HEV positivity, with the simulation being included as a frailty effect. The four
 395 explanatory variables were: (i) at the population scale: the type of the index farm and the
 396 proportion of IMV-free FF farms; (ii) at the individual farm scale: the farm type and the
 397 IMV-status (positive or negative). The effect of the interaction between the farm type and
 398 the farm IMV-status was also evaluated.
- 399 • A generalised estimating equation (GEE) logistic regression was used to compare HEV
 400 prevalence in pigs slaughtered in the community depending on the type of the index farm
 401 and on the proportion of IMV-free FF farms in the community. The simulation was
 402 included as a repeated statement in the model to take into account the non-independence
 403 of the proportions of positive pigs for the different farms in a given simulation.

404 Statistics were performed using SAS 9.1. software (functions *proc logistic*, *proc genmod* and
 405 *proc phreg*).

406 **3. Results**

407

408 **3.1. Descriptive results of the population and epidemiological**
409 **dynamics**

410

411 **3.1.1. Demographics**

412

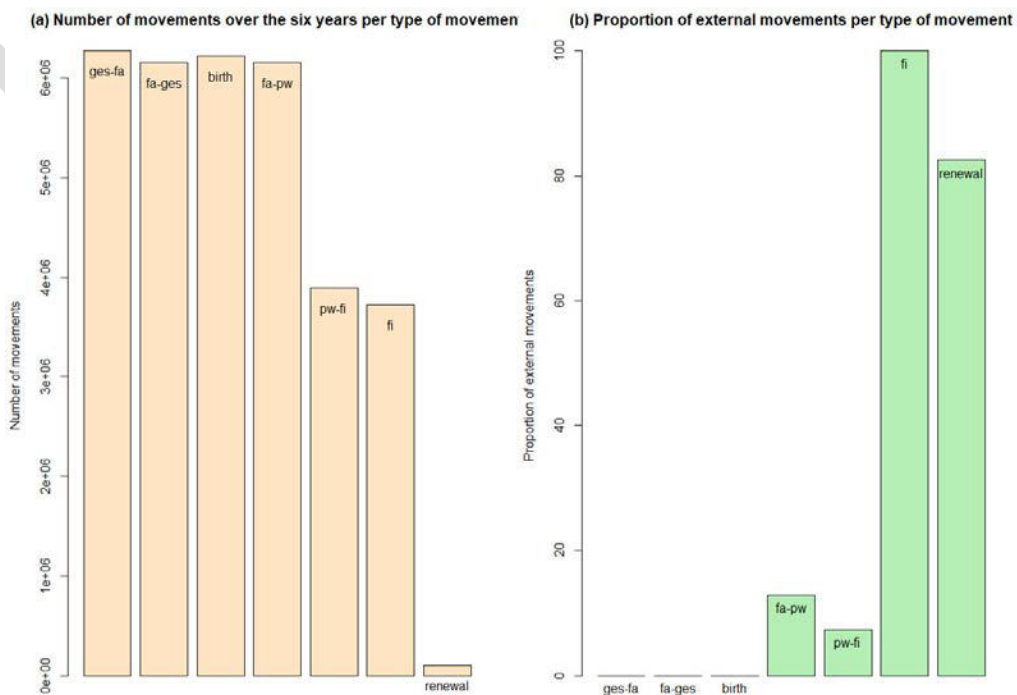
413 At the end of the study period, an average of 406,560 sows and 5,456,799 pigs were present in
414 the community which is consistent with the expected number of pigs on 3,017 farms. A total of
415 32,629,140 movements occurred over the six years (Supplementary File 1). Among them,
416 15.3% were between-farm movements when the others were within-herd (i.e. between-sector).
417 More precisely, 12.9%, 7.4% of *fa-pw* and *pw-fi* movements were external, respectively.

418

419 **Supplementary File 1. Simulated network description: number of movements (a) and**
420 **proportion of external movements (b) per type of movement**

421 *ges-fa*: movements from the gestation to the farrowing sector; *fa-ges*: movements from the farrowing
422 to the gestation sector; *fa-pw*: movements from the farrowing to the post-weaning sector; *fi*:
423 movements from the finishing sector to the slaughterhouse.

424



425

426

427 **3.1.2. HEV dynamics on the index farm**

428

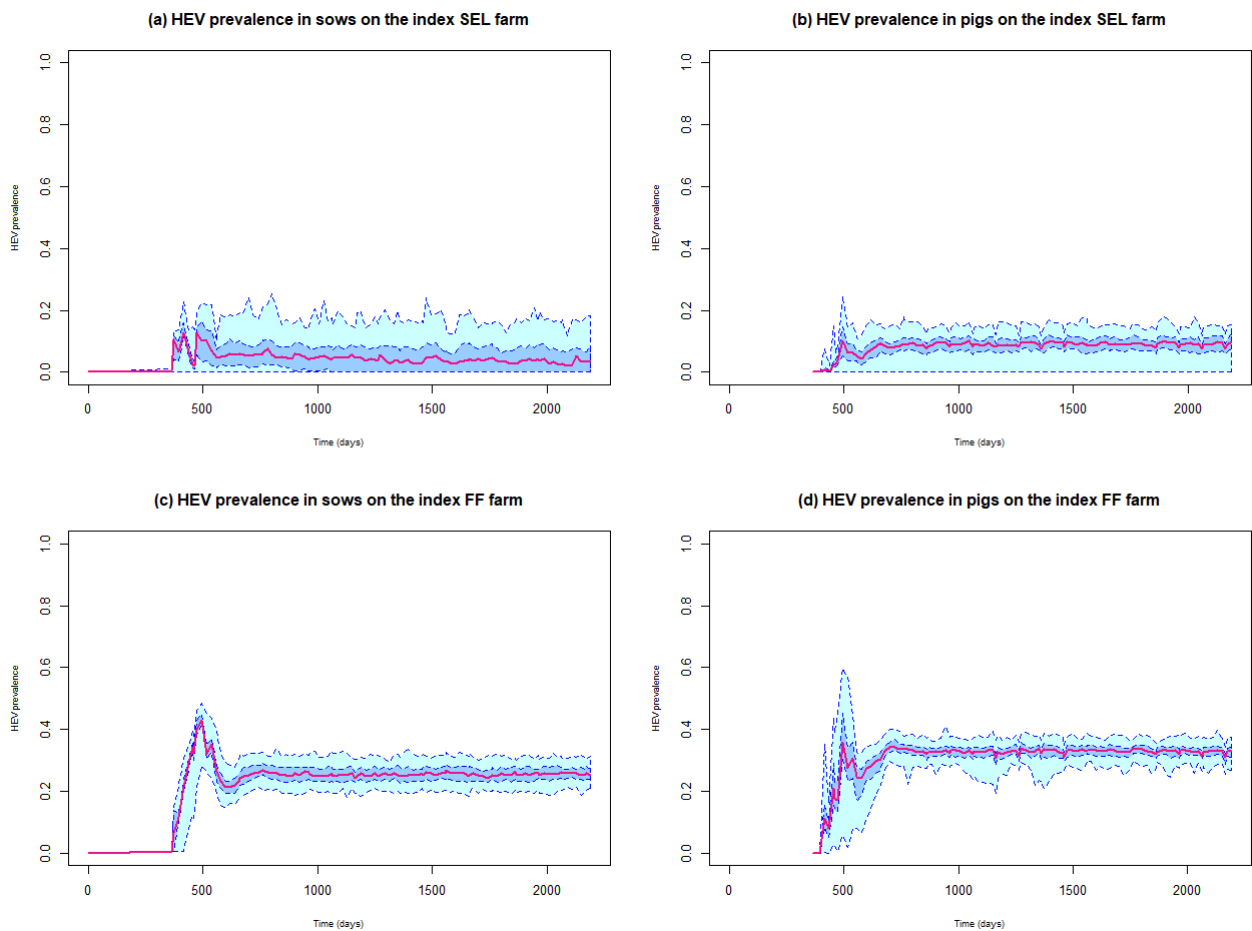
429 After the introduction of an HEV-infected gilt in the gestation sector, an epidemic peak was
430 first observed in the breeding part of the herd due to massive infections of a large pool of naive
431 animals (Supplementary File 2). Infected sows entering the farrowing sector then initiated the
432 infectious process in growing pigs by infecting suckling piglets. The latter spread the infection
433 in the nursery and finishing sectors. HEV prevalence levels were lower on *SEL* and *MU* farms
434 than on *FF* and *FA* farms (Supplementary File 2).

435

436 **Supplementary File 2. HEV prevalence in sows and growing pigs (median, 50% and**
437 **95%) on the index farm in case of HEV introduction on a nucleus (a and b) or farrow-**
438 **to-finish (c and d) farm (Scenarios S1 and S3).**

439 Pink line: median; dark blue area: 50%; light blue area: 95%; SEL: nucleus farm; FF: farrow-to-finish
440 farm

441



442

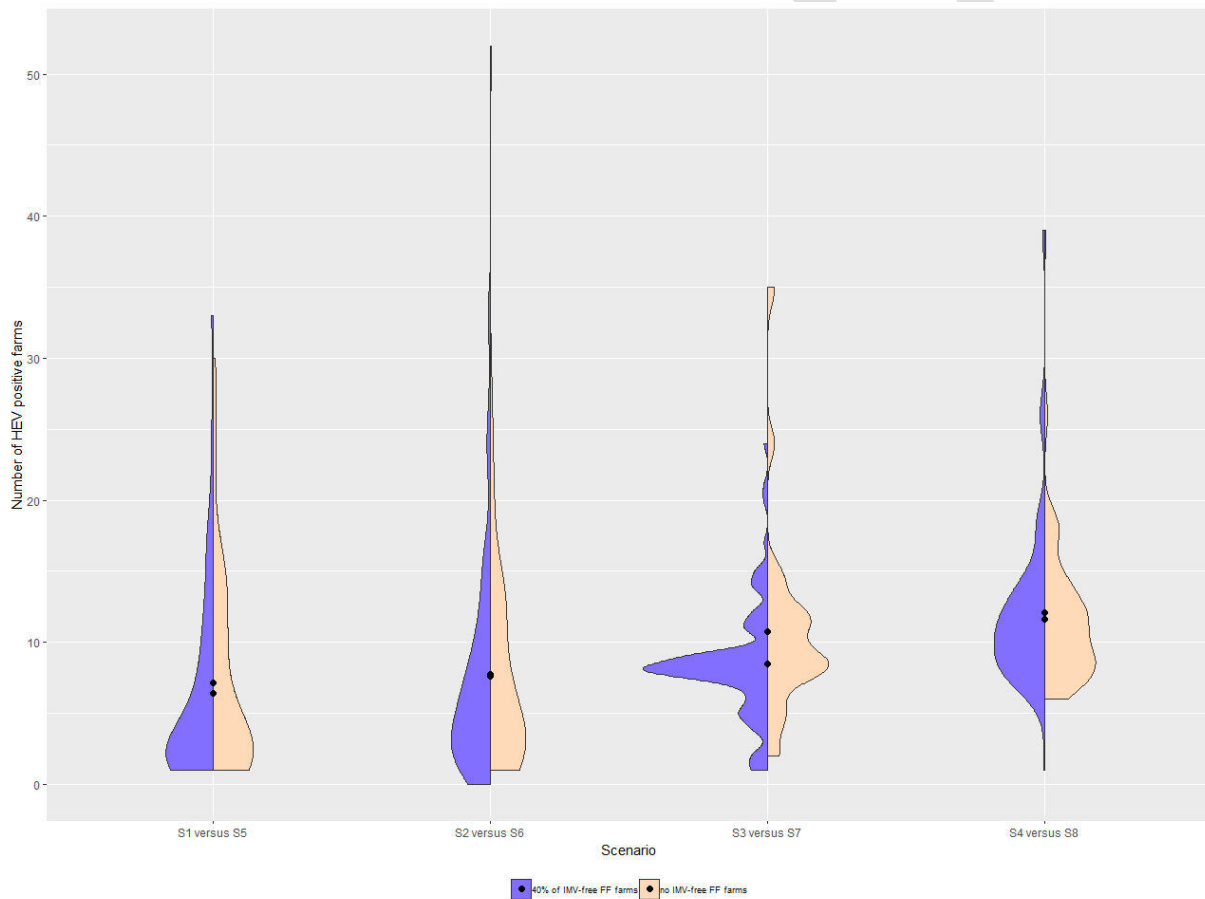
443

3.2. Factors affecting HEV spread in the community

The distribution of the number of HEV positive farms in the eight tested scenarios is presented in Figure 5. The maximum number of positive farms was 52, with on average nine farms getting infected. In case of *FA* index farm, at least six farms were infected when all *FF* farms were IMV-positive. The minimal number of infected farms fell to one when the proportion of IMV-positive herds was reduced to 60%.

Figure 5. Distribution of the number of HEV positive farms depending on the scenario

S: scenario; FF: farrow-to-finish pig farm.



As shown in Table 6, the proportion of HEV-positive farms over the study period was affected both by the type of the index farm, with a higher proportion of infected farms in case of HEV introduction on a *MU*, *FF*, *FA* farm compared to on a *SEL* farm (Odds Ratio = 1.14 [1.06-1.23], OR = 1.42 [1.33-1.52] and OR = 1.76 [1.65-1.88], respectively), and by the proportion of IMV-

461 free *FF* farms in the community (OR = 0.93 [0.89-0.97] when the prevalence of IMV-positive
 462 farms was 60% compared to 100%).

463

464 **Table 6. Effect of the index farm and of the IMV situation in the community on the**
 465 **farm-level prevalence over the study period**

466 Summary statistics obtained thanks to a multivariate logistic regression.

467

Variable	Modality	Results of the multivariate model	
		Odds Ratio [95% CI]	p-value
Type of the index farm	SEL	Chi ² = 335.58 -	p < 0.01 -
	MU	1.14 [1.06-1.23]	p < 0.01
	FF	1.42 [1.33-1.52]	p < 0.01
	FA	1.76 [1.65-1.88]	p < 0.01
Proportion of IMV-free FF farms	0	Chi ² = 10.11 -	p < 0.01 -
	0.4	0.93 [0.89-0.97]	p < 0.01

468

469 As shown in Table 7, farms got infected earlier in case of HEV introduction on a *FF* or *FA* farm
 470 (Hazard Ratio = 1.49 [1.30-1.71] and HR = 1.75 [1.53-2.00], respectively) compared to an
 471 introduction on a *SEL* farm. The farm type was also associated with the time to HEV infection
 472 with earlier infection of *PWF* farms compared to the other farm types (HR = 1.25 [1.08-1.45]).
 473 The proportion of IMV free farms did not significantly influence the time to infection.

474

475 **Table 7. Effect of population and farm features on the farms' time to HEV infection**

476 Summary statistics obtained thanks to a cox-proportional hazard model with the simulation being
 477 included as a frailty effect.

478

	Variable	Modality	Results of the multivariate model	
			Hazard Ratio [95% CI]	p-value
Population features	Type of the index farm	SEL	Chi ² = 93.41 -	p < 0.01 -
		MU	1.05 [0.91-1.21]	p > 0.20
		FF	1.49 [1.30-1.71]	p < 0.01
		FA	1.75 [1.53-2.00]	p < 0.01
	Proportion of IMV-free FF farms	0	Chi ² = 0.39 -	p > 0.10 -
		0.4	0.97 [0.88-1.07]	p > 0.10
Farm features	Farm type	SEL	Chi ² = 2544.42 -	p < 0.01 -
		MU	0.60 [0.51-0.70]	p < 0.01
		FF	0.22 [0.19-0.25]	p < 0.01
		FA	0.83 [0.69-0.99]	p < 0.05

	FPW	0.27 [0.21-0.36]	p < 0.01
	PW	1.20 [0.85-1.70]	p > 0.20
	PWF	1.25 [1.08-1.45]	p < 0.01
	FI	0.77 [0.66-0.89]	p < 0.01
Farm's IMV status		Chi ² = 0.15	p > 0.20
	positive	-	-
	negative	1.02 [0.92-1.13]	p > 0.20

479

480 **3.3. Factors affecting the risk of slaughtering HEV-positive pigs**

481

482 The type of the index farm was associated with the proportion of HEV-positive pigs slaughtered
 483 (p < 0.01). HEV introduction in a *MU*, *FF* or *FA* farm led to a higher risk of having HEV-
 484 positive livers entering the food chain compared to the HEV introduction on a nucleus farm
 485 (OR = 2.07 [1.69-2.55], OR = 2.23 [1.85-2.70] and OR = 4.41 [3.79-5.28], respectively; Table
 486 8). Reducing the prevalence of IMV-infected *FF* farms was associated with a lower risk of
 487 slaughtering HEV-positive pigs (OR = 0.88 [0.79-0.98], Table 8).

488

489 **Table 8. Effect of the type of the index farm and of the IMV situation in the community**
 490 **on the proportion of HEV-positive pigs sent to the slaughterhouse**

491 Summary statistics obtained thanks to a generalised estimating equation (GEE) logistic regression
 492 model with the simulation being included as a repeated statement.

493

Variable	Modality	Results of the multivariate model	
		Odds Ratio [95% CI]	p-value
Type of the index farm	SEL	Chi ² = 375.80	p < 0.01
	MU	-	-
	FF	2.07 [1.69-2.55]	p < 0.01
	FA	2.23 [1.85-2.70]	p < 0.01
Proportion of IMV-free FF farms		4.47 [3.79-5.28]	p < 0.01
	0	Chi ² = 5.53	p < 0.05
	0.4	-	-
		0.88 [0.79-0.98]	p < 0.05

494

495

496 **4. Discussion and conclusions**

497

498 Though previous studies have shown the potential role of pig trade in the spread of HEV
 499 (Nantel-Fortier et al., 2016; Salines et al., 2018), they did not make it possible to describe HEV
 500 diffusion at the territory scale in a dynamic and precise way, or to explain the reasons for HEV

501 spread and persistence in the pig production sector, or to assess the efficacy of HEV control
502 measures in the country. This is the reason why the present study reports on the design of a
503 between-herd HEV model that combines HEV within-farm dynamics with pig trade network.
504 For this model, the chosen level of representation was the pen. Indeed, it made it possible to
505 mimic HEV within-farm dynamics consistently with HEV behaviour described in Salines et al.
506 (2019c). Moreover, the pen scale appeared as the most relevant one to represent the within-pen
507 environmental accumulation and transmission of HEV, that has been previously evidenced as
508 a pivotal transmission pathway (Andraud et al., 2013). HEV epidemiological parameters were
509 estimated from several experimental trials (Andraud et al., 2013; Andraud et al., 2014; Salines
510 et al., 2015). The majority of them differed according to the animal's health status regarding
511 the IMV: expanded latency and infectious periods, higher transmission rates for IMV-positive
512 animals than for IMV-negative ones. Nucleus and multiplication farms were considered free
513 from immunomodulating viruses consistently with health situations of these farm types in
514 France (as stated in the health charter of pig producers, available online¹). All or part of
515 production farms were considered IMV-positive, depending on the scenarios tested. In the case
516 of an IMV-infected farm, the HEV epidemiological parameters were the same for all animals,
517 meaning that all HEV infected animals were considered co-infected with the IMV. By doing
518 so, the frequency of co-infection was over-estimated, as well as all HEV outcomes.

519 Regarding the population structure, the 3,017 represented farms corresponded to French farms
520 belonging to a single community as described in the analysis of the French network of pig
521 movements (Salines et al., 2017b). These farms have therefore preferential trade relationships
522 likely to favour spread of pathogens. All farms were composed of a given number of pens,
523 grouped into rooms, themselves grouped into sectors. The farm size was standardized for all
524 farms within a farm type, which is one of the limitations of the model since the size seems to
525 be a risk factor as regards HEV (Di Bartolo et al., 2008; Li et al., 2009; Jinshan et al., 2010;
526 Hinjoy et al., 2013; Walachowski et al., 2014); this point would require future improvements
527 to fit real data better. The within-farm demographics was deterministically driven by the time
528 pigs should stay in each sector, related to the batch-management system. Again, the batch-
529 management system was the same for all farms (seven batches, i.e. three weeks interval) which
530 could be upgraded in the future to make it possible to explore the effect of the batch-
531 management system, which was shown to affect HEV on-farm persistence (Salines et al.,

¹ <https://www.ifip.asso.fr/fr/content/eqs-naissance-d%E2%80%99une-charte-sanitaire-dans-la-fili%C3%A8re-g%C3%A9n%C3%A9tique-fran%C3%A7aise>

532 2019c). The between-farm demographics was derived from real data recorded in the national
533 pig movement database from 2012 to 2015. These data were incorporated in the model in the
534 form of a contact matrix with probabilities (*i*) for internal or external transfer (*ii*) and, in the
535 latter case, for transfer to a given neighbour. By doing so, possible temporal evolutions of the
536 pig movement network were not taken into account, but the descriptive analysis we had
537 previously performed showed a stable structure of the network over the study period (Salines
538 et al., 2017b).

539

540 When introduced on an IMV-positive *FF* farm, HEV spread in an enzootic way, first in the
541 reproductive herd before affecting piglets and growing pigs. Though the prevalence levels
542 observed in this model were higher than in the within-herd model previously built (Salines et
543 al., 2019c) probably in relation with the co-infection of all animals, the overall HEV behaviour
544 was consistent with the published data (Salines et al., 2019b). HEV prevalence was lower on
545 *SEL* and *MU* farms compared to *FF* farms, which could be explained by their IMV-free status
546 as described in Salines et al. (2019c). Our analysis showed that the number of contaminated
547 farms in the community over the study period was affected by the type of the index farm, with
548 an introduction on a *MU*, *FF* and *FA* farm being more risky than on a *SEL* farm, with an
549 increasing number of positive farms from *MU* to *FA* index farms. This could be explained (*i*)
550 by the different contact patterns between these four farm types, with *FA* farms sending pigs
551 regularly and at age at which they are likely to be HEV-positive; (*ii*) by their different health
552 status regarding the IMV, with *SEL* and *MU* farms being IMV-free when *FF* and *FA* farms
553 were IMV-positive, thus having a higher HEV prevalence and long-lasting persistence. The
554 influence of IMVs was confirmed by the fact that improving the population health status (i.e.
555 decreasing the prevalence of IMV-positive *FF* farms) led to a reduced number of HEV-positive
556 farms over the study period, which highlights again the role of intercurrent pathogens in the
557 HEV dynamics. An interesting outcome is that the dynamics of HEV spread was affected by
558 the farm type (both the type of the index farm and the type of the infected farm) but not by the
559 IMV-related variables. Indeed, the introduction on a *FF* or on a *FA* farm led to a quicker
560 contamination of other farms, which could again be explained by the riskier contact patterns of
561 these farms. Moreover, all farm types were likely to be infected later, except *PWF* farms which
562 got HEV infected earlier because they are frequent receivers of pigs at a risky age of infection.
563 The non-significant results for *PW* farms was probably related to the lack of statistical power
564 given the low number of *PW* farms in the community (only eight). In addition, if *SEL* farms
565 send animals frequently, they send less animals than *FA*, *PW* and *PWF* farms and at a less risky

566 age regarding HEV, the prevalence being low at late fattening stage. Considered together, these
567 results show that at an individual scale, the farm's susceptibility to HEV infection was more
568 related to its frequency of animals' introduction than to its own health situation but that on a
569 collective scale, HEV spread on a breeding community was linked both to the population health
570 status and to the contact patterns. Finally, our analyses evidenced that the risk of slaughtering
571 HEV-positive pigs was related to the type of the index farm, with a 4-times higher risk in the
572 case of introduction on a *FA* farm, and to the population health status, with a lower risk when
573 the prevalence of IMV-positive *FF* farms was decreased.

574

575 This model developed at a territory scale, has revealed differences in HEV spatial diffusion
576 patterns related to the introduction pathway, the health status of the pig population, and the type
577 of the exposed farms. If *SEL* and *MU* farms are often considered as the riskiest herds in the pig
578 production sector due to large contact chains, the HEV case highlights that contact patterns
579 have to be considered together with farms' health status regarding immunomodulating
580 pathogens. It appears therefore essential that *SEL* and *MU* farms preserve their IMV-free status,
581 when production farms implement eradication or control programmes of IMVs. Our model can
582 be viewed as an experimental one, with theoretical results that cannot be directly extrapolated
583 to the natural conditions. However, if not relevant from an absolute point of view, they make it
584 possible to compare different scenarios and to identify the riskiest elements. As such, these
585 outcomes can support surveillance strategies by helping target farms having a dense contact
586 network and poor health situation. Our study also gives insight on the HEV diffusion pathway
587 in a HEV-free farming community, which could be structured to provide processing companies
588 with safe livers for the production of raw pork products. Further developments of the model
589 would also make it possible to modify the network structure while simulations are running. This
590 could be particularly useful to simulate trade restriction measures or trade reorganisation, which
591 could occur in the case of the introduction of a regulated disease, an epidemic peak or a
592 modification of the producers' supply network. Incorporating intermediate loading operations
593 could also make it possible to take into account a possible environmental transmission with
594 trucks acting as mechanical vector. These results could also be used as inputs in other studies,
595 e.g. in a quantitative microbiological risk assessment aiming at assessing the risk of consumers
596 to be exposed to HEV. Finally, designing multi-scale models combining complex within-farm
597 dynamics with animal demographics appears particularly relevant to deal with such
598 multifaceted public health issues. Thus, this kind of research approach should be fostered in the

599 future to have a comprehensive and detailed view of pathogen dynamics on a territory scale and
600 support decision-making.

601

602

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607

608 **Authors' contributions**

609 MS and SW designed the mathematical model, implemented the model in the SimInf R package
610 and performed the simulations. MS drafted the manuscript. All authors participated in data
611 analysis and interpretation and in the manuscript writing. All authors read and approved the
612 final manuscript.

613

614 **Conflict of interest statement**

615 The authors declare that they have no competing interests.

616

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Ce qu'il faut retenir

A partir d'une approche innovante de modélisation multi-échelles, le modèle développé a apporté de nouveaux éléments dans la compréhension de la dynamique de l'infection par le HEV dans une communauté d'élevages. Il a permis de mettre en évidence l'influence du type d'élevage d'introduction, du type d'élevage exposé et du statut sanitaire de la population vis-à-vis des pathogènes intercurrents. Ce travail pourrait ainsi contribuer au développement d'une stratégie de surveillance et de maîtrise du risque HEV dans la filière porcine fondée sur le risque que présentent les élevages, en combinant les éléments apportés par les deux approches de modélisation intra- et inter-troupeaux présentés dans les chapitres II et III.



Take home message

Based on an innovative multi-scale modelling approach, the model developed has brought new elements to the understanding of the dynamics of HEV infection in a pig farming community. It highlighted the influence of the type of introduction farm, the type of exposed farm and the health status of the population with respect to intercurrent pathogens. This work could thus contribute to the development of a strategy for monitoring and controlling HEV risk in the pig sector based on the risk posed by pig herds, by combining the factors provided by the two approaches of within- and between-herd modelling presented in Chapters II and III.



Chapitre IV

Elaboration d'un plan de sécurisation de la filière porcine vis-à-vis du risque lié au virus de l'hépatite E

PARTIE 4.1. Synthèse des mesures de maîtrise du virus de l'hépatite E envisageables dans la filière porcine

A partir des données récentes disponibles dans la littérature concernant le risque présenté par les produits porcins et l'épidémiologie du HEV dans les élevages de porcs, et des résultats obtenus au cours de la thèse, notamment en ce qui concerne l'influence des co-infections immunomodulatrices ainsi que la diffusion du virus dans la filière porcine, des **pistes d'action** ont été identifiées. Elles ont été **formulées sous forme de propositions**, soumises aux organisations publiques et privées gestionnaires du risque. L'ensemble du rapport est disponible en **Annexe 8**, seules les pistes d'action sont reprises ci-après :

Dix pistes d'action pour la maîtrise du risque HEV dans la filière porcine

AXE I - Lutte en élevage

Piste 1 : Accompagner les élevages (particulièrement ceux de grande taille ayant un mode de production intensif) vers des pratiques de biosécurité externe (sas sanitaire, quarantaine) et interne (limitation des adoptions et des mélanges, gestion des effluents) plus sûres.

Piste 2 : Soutenir la mise en place de programmes d'éradication des pathogènes immunomodulateurs, notamment du virus du SDRP.

Piste 3 : En collaboration avec les vétérinaires sanitaires, réaliser des dépistages HEV dans les élevages souhaitant s'engager dans un programme de maîtrise du HEV et assurer le suivi de leur situation sanitaire.

AXE II - Organisation de la filière

Piste 4 : Envisager la structuration d'une filière spécifique permettant aux élevages reconnus indemnes de HEV de fournir des foies sains pour la fabrication des produits à risque.

AXE III - Surveillance

Piste 5 : Inclure le HEV dans les prochains plans de surveillance et de contrôle annuels sur carcasses, abats et produits transformés.

AXE IV - Communication

Piste 6 : Réaliser une campagne d'information pour sensibiliser les acteurs de la filière porcine, notamment à l'échelon de l'élevage, à la problématique du HEV.

AXE V - Recherche

Piste 7 : Evaluer la faisabilité technique et l'acceptabilité sociale d'un plan de lutte par les différents acteurs de la filière.

Piste 8 : Réaliser une étude d'intervention en élevage permettant d'évaluer l'efficacité en conditions réelles des mesures de lutttes proposées.

Piste 9 : Evaluer la prévalence de carcasses contaminées à l'abattage à partir d'un échantillonnage ciblé sur les facteurs de risque identifiés en élevage.

Piste 10 : Evaluer, à partir de viande de porcs infectés en conditions expérimentales, l'efficacité des process de séchage et de salaison utilisés dans les IAA sur la diminution de la charge virale dans les produits de charcuterie et salaison.



Ce qu'il faut retenir

Des mesures de maîtrise du HEV ont été identifiées, à l'échelle de l'élevage (structuration des élevages, mesures de biosécurité externe et interne, maîtrise de la situation sanitaire vis-à-vis des pathogènes intercurrents) et de la filière (organisation des échanges et de l'approvisionnement en matières premières pour les produits à risque). La mise en place d'un tel programme de lutte nécessiterait des changements de pratiques de la part des différents acteurs de la filière. Pour garantir l'application de ces mesures sur le terrain, il apparaît alors nécessaire d'étudier leur faisabilité technique et leur acceptabilité, c'est-à-dire les freins et motivations des acteurs à adopter de nouveaux comportements. C'est dans cet objectif que les sciences sociales ont été mobilisées dans la suite du projet.



Take home message

Measures to control HEV have been identified, at the farm level (farm structure, external and internal biosecurity measures, health management as regards intercurrent pathogens) and the production sector level (organisation of trade and of the supply in raw material for at-risk foodstuffs). The implementation of such a control programme would require changes in practices by the various actors in the sector. To ensure that these measures are applied in the field, it is then necessary to study their technical feasibility and acceptability, i.e. the obstacles and motivations of the actors to adopt new behaviours. It is with this objective in mind that the social sciences were mobilized in the rest of the project.

PARTIE 4.2. Retour vers le terrain : évaluation de la faisabilité d'un plan de lutte contre le virus de l'hépatite E dans la filière de production porcine

I. Enquête préliminaire : quelle connaissance les acteurs de la filière porcine ont-ils du virus de l'hépatite E ?

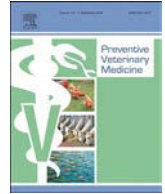
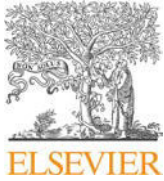
L'implication des acteurs de la filière dès la phase d'élaboration d'un programme de lutte contre un pathogène à l'échelle de l'élevage est la première garantie d'un plan de maîtrise effectivement mis en place sur le terrain. Entre autres choses, **l'engagement des parties prenantes dépend de la connaissance qu'elles ont du pathogène en question et de leur sensibilisation à l'importance de la problématique pour la filière.** L'importance de l'hépatite E, en tant qu'infection zoonotique émergente pour laquelle les cas sont difficiles à relier avec certitude avec la consommation de denrées alimentaires contaminées, est mésestimée, même parmi le monde médical et scientifique. En outre, le HEV circule dans les élevages de porcs sans causer aucun signe clinique ni perte de production. Pour toutes ces raisons, **le HEV est susceptible d'être méconnu des acteurs de la filière porcine**, notamment les éleveurs et les vétérinaires. Dans ce contexte, une **enquête préliminaire** a été conduite auprès des éleveurs de porcs et des vétérinaires du secteur porcin afin d'**évaluer leur niveau de connaissance du HEV et de déterminer les éléments nécessitant de renforcer les efforts de communication et de sensibilisation.**

Les questionnaires diffusés sont disponibles en ligne²⁶. Ce travail a donné lieu à une publication dans le journal *Preventive Veterinary Medicine* (Salines *et al.*, 2018c) et à la création d'une brochure d'information à destination des éleveurs et vétérinaires (Annexe 9).

²⁶ Questionnaire à destination des éleveurs de porcs : <https://forms.gle/qXMg6jxKZq4gVA8C8>
Questionnaire à destination des vétérinaires en production porcine : <https://forms.gle/pEKSFMTRg8wH2Eq47>

Publication 10

Salines M., Andraud M., F. Terrade, Rose N., 2018. Are French pig farmers and veterinarians knowledgeable about emerging foodborne pathogens? The case of hepatitis E virus. *Preventive Veterinary Medicine* 156, 1-7.



Are French pig farmers and veterinarians knowledgeable about emerging foodborne pathogens? The case of hepatitis E virus

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ABSTRACT

Hepatitis E virus (HEV) is an emerging zoonotic pathogen mainly transmitted via food in developed countries, and for which domestic pigs are recognised as the main reservoir. To implement an efficient HEV surveillance and control plan in the pig production sector, it is first necessary to assess the level of knowledge of pig-farming main actors about this pathogen. To this aim, an online survey was conducted between September and October 2017 to evaluate pig farmers' and veterinarians' knowledge about HEV epidemiology and its zoonotic potential. The questionnaire was filled in by 383 pig farmers and 46 pig veterinarians. Of this population, 77.8% of farmers and 8.7% of veterinarians had never heard of HEV. Our results highlight knowledge gaps among responding farmers, especially regarding the clinical and epidemiological features of HEV, while veterinarians appear to be well-informed about this pathogen. These findings indicate significant room for further improvement and the need for more information aimed at French pig farmers, with veterinarians acting as a priority channel through which information may be transferred from scientists to farmers. These educational efforts will facilitate farmers' involvement in future HEV surveillance and control plans.

1. Introduction

Food safety management used to be downstream-oriented, with a specific focus on the examination of food-processing operations and the control of finished products. However, in the last decades a significant turning point has been observed in the vision of food hygiene, with growing awareness of the importance of an integrated approach considering the whole food chain (“from farm to fork”). In this perspective, prevention and control measures have to be implemented at each production/processing/distribution stage, involving all stakeholders (Anonymus, 2002). In particular, for food of both animal and plant origins, special attention has been given to farming steps in order to avoid the introduction of foodborne pathogens on farms or limit their prevalence at the primary production level (European Food Safety, 2007, 2008). However, no surveillance or control programme at farm level can be effectively implemented without stakeholders' involvement, notably that of farmers and veterinarians. Among other things, their involvement primarily depends on their knowledge of the pathogen and their understanding of the importance of the issue. Several studies have evidenced major knowledge gaps among farmers regarding food safety topics, reducing their ability and/or their willingness to

implement control programmes (Bahnsen et al., 2001; Ellis-Iversen et al., 2010; Young et al., 2010a; Young et al., 2010b). For instance, a study led in the US showed that pork producers recognise their key role in pork food safety and express their willingness to participate, but also revealed that they need additional information and education about pathogens and control measures (Bahnsen et al., 2001). Ellis-Iversen et al. (2010) also explored motivational factors for the implementation of zoonotic disease control programmes among English and Welsh cattle farmers; they showed that some farmers do not implement control programmes because of external barriers, including lack of knowledge. Fewer studies have investigated veterinarians' knowledge regarding food safety issues in industrialised countries. Marvin et al. (2010), for example, reported that veterinarians were more familiar with food safety issues than were other professional groups. Moreover, a number of publications noted that farmers considered private veterinarians as the most knowledgeable and trustworthy regarding animal diseases, zoonoses and antimicrobial use (Alarcon et al., 2014; Laanen et al., 2014; Marier et al., 2016; Mahon et al., 2017; Poizat et al., 2017). If sufficiently informed, veterinarians may therefore be an efficient channel through which to pass food safety fundamentals on to farmers.

The case of hepatitis E virus (HEV) is a challenging issue. Hepatitis E

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is a worrying zoonosis mainly transmitted via food in developed countries and for which domestic pigs are recognised as the main reservoir (Dalton et al., 2008; Pavo et al., 2010; EFSA et al., 2017). Although the majority of human cases are asymptomatic, HEV can also cause acute, fulminant or chronic hepatitis that may be difficult to treat (Emerson and Purcell, 2003; Kamar et al., 2011). However, hepatitis E is a little-known disease even among medical doctors and scientists. It is considered as an emerging zoonosis in the sense that the number of reported cases has been constantly increasing these last few years in European countries (e.g. in France, only nine cases were reported in 2002, versus 2292 in 2016), but this is due more to a higher number of diagnostic tests than to an actual increase in the number of cases (CNR, 2017; EFSA et al., 2017). Although foodborne transmission from pork products is proven, few cases have actually been tied to the consumption of an HEV-contaminated pork product. For all these reasons, the knowledge of stakeholders in the pig farming sector about HEV may be imperfect. Moreover, HEV circulates on pig farms without causing any clinical signs in pigs or leading to financial losses. As Alarcon et al. (2014) showed that drivers for disease control by pig farmers were ‘pig mortality’, ‘feeling of entering in an economically critical situation’ and ‘animal welfare’, raising awareness about HEV may be problematic. To date, no HEV surveillance and control plan has ever been implemented in Europe (Salines et al., 2017a). However, articles on the risk of HEV related to the consumption of contaminated pork products have recently been published in the mass media after new scientific findings (for example following the paper by Said et al. (2017)). Thus, pig producer organisations are becoming increasingly aware of the risk of a crisis of confidence in the pork sector and are willing to control HEV on pig farms.

In this context, the aim of our study was to assess the level of knowledge of French pig farmers and veterinarians concerning HEV in order to raise actors’ awareness of this issue and to involve them in future risk management strategies.

2. Material and methods

2.1. Survey design

Two similar questionnaires were developed to investigate pig farmers’ and veterinarians’ level of knowledge concerning HEV (see Supplementary File 1). Both were composed of 19 questions and the time needed to fill them in was estimated at 10 min. Most of the questions were closed to increase the comparability of respondents’ answers. A short introduction briefly explained the context of the study, without going into too much detail to avoid helping respondents answer the questionnaire. Emphasis was placed on the anonymity of the answers and the shortness of the survey. The first question aimed to find out whether the responding farmers/veterinarians had ever heard of HEV. The main features of HEV were then addressed in the following questions, in particular regarding the overall situation of HEV in the French pig farming sector, clinical and epidemiological characteristics in pigs (clinical signs, treatment, vaccine, transmission routes between pigs and control measures) as well as HEV zoonotic transmission routes from pigs to humans. Veterinarians were also asked to rank the importance of the HEV issue at three levels: public health, economy of the pig production sector and risk of negative media exposure. Finally, the last part of the questionnaire was designed to collect general data on the respondent’s characteristics: gender, age, type of farm or of veterinary practice. The final question was open-ended to allow respondents to express their comments, questions and concerns.

2.2. Data collection and analysis

The target populations were all pig farmers and pig veterinarians in metropolitan France. The questionnaires were developed as an online survey using Google Forms software (<https://docs.google.com/forms>).

Table 1
Main characteristics of questionnaire respondents (n = 383 farmers and 46 veterinarians).

Variable	Category	Farmer sample (n = 383) Number (percentage)	Veterinarian sample (n = 46) Number (percentage)
Gender	Male	307 (80.2%)	31 (67.4%)
	Female	76 (19.8%)	15 (32.6%)
Age	< 30 y/o	16 (4.2%)	2 (4.3%)
	30–39 y/o	78 (20.4%)	18 (39.1%)
	40–49 y/o	107 (27.9%)	15 (32.7%)
	50–59 y/o	155 (40.5%)	7 (15.2%)
	≥ 60 y/o	27 (7.0%)	4 (8.7%)
Type of farm	Nucleus	7 (1.8%)	–
	Multiplication	15 (3.9%)	–
	Farrowing	9 (2.4%)	–
	Farrowing Post-weaning	4 (1.1%)	–
	Farrowing-to-finishing	228 (59.5%)	–
	Post-weaning	2 (0.5%)	–
	Post-weaning	56 (14.6%)	–
	Finishing	–	–
	Finishing	40 (10.4%)	–
	Other ^a	22 (5.8%)	–
Type of veterinary practice ^b	Independent practitioner	–	22 (47.8%)
	Salaried practitioner	–	20 (43.4%)
	Academic, researcher, teacher	–	1 (2.2%)
	Pharmaceutical industrial	–	1 (2.2%)
	No answer	–	2 (4.4%)
	Pig farms as only clients	–	32 (69.5%)
Level of veterinarians’ specialisation ^b	Pig farms as major clients	–	7 (15.2%)
	Pig farms as occasional clients	–	2 (4.4%)
	No answer / NA	–	5 (10.9%)

^a The category “other” includes small farms (< 10 sows or < 10 finishing places) or owners of pet pigs.

^b For practitioners only. NA: not applicable.

The survey was e-mailed to farmers by grading, weighing and marking bodies upon the request of the French Interprofessional Pork Council (INAPORC); and to veterinarians by the French Association for Pig Veterinary Medicine (AFMVP). Data were collected between 1st September and 15th October 2017.

Respondents’ characteristics and knowledge concerning HEV were quantitatively described. For ease of reading, most of the descriptive results in the following section are presented as colour-coded charts with the correct answers highlighted in green. A Chi-square test was performed to investigate any association between respondents’ features (age, farm type) and their HEV knowledge.

3. Results

3.1. Study sample

The questionnaire was sent to 8075 pig farmers and 150 veterinarians having a pig practice. A total of 383 farmers and 46 veterinarians filled in the questionnaire, which corresponds to a response rate of approximately 4.7% and 30.7% respectively. The characteristics of respondents and farms/practices are summarised in Table 1. Most of the farmers had farrow-to-finish herds; most of the veterinarians were practitioners specialised in pigs.

3.2. Basic knowledge about HEV

Of the 383 farmers and 46 veterinarians, 77.8% and 8.7% respectively had never heard of HEV. For farmers, having heard of HEV was neither related to the type of farm nor to the respondents' age (p -value > 0.1). In the following sections, only the answers of the 85 farmers (22.2%) and of the 42 veterinarians (91.3%) having heard of HEV were analysed, considering that the others answered the questions randomly. Most farmers had heard about HEV through professional media or their veterinarian (as mentioned by 31 and 13 farmers respectively). Veterinarians had heard about HEV through veterinary schools or research institutes (as cited by 21 veterinarians), professional media (19), professional associations (18) or other veterinarians (14).

3.3. Situation of the French pig production sector regarding HEV

More than 68% of farmers and 92% of veterinarians were aware that HEV was present in France; 76% and 97.6% of them respectively knew that it can infect pigs. Only one out of the 85 farmers and five out of the 42 veterinarians knew that HEV was present on 61%–80% of farms (Rose et al., 2011). The majority of the respondents said that they did not know HEV prevalence in the French pig production sector or they underestimated it (Fig. 1).

3.4. Clinical and epidemiological features of HEV in pigs

3.4.1. Clinical signs, treatment and vaccination

More than 68% of the 85 farmers versus 14% of the 42 veterinarians wrongly thought that HEV caused clinical signs in pigs (Fig. 2). The following symptoms were cited: digestive disorders (27 farmers versus 1 veterinarian), production losses (20 vs. 0), mortality (13 farmers), neurological disorders (7 farmers), reproductive disorders (7 farmers) and respiratory disorders (6 farmers). Around 14% of the 85 farmers mistakenly thought that there was a treatment against HEV, most of them mentioning antibiotics (Fig. 2). More than 11% of farmers incorrectly thought that a vaccine against HEV existed (Fig. 2). Veterinarians knew that there was neither a treatment nor a vaccine against HEV (Fig. 2).

3.4.2. Transmission routes between pigs and control measures on pig farms

Direct and environmental within- and between-pen HEV transmission routes were rightly mentioned by 31, 43 and 39 farmers and by 23, 32 and 39 veterinarians respectively, whereas airborne transmission was wrongly mentioned by 3 farmers and 5 veterinarians (Fig. 3) (Kasornrorkbua et al., 2004; Bouwknegt et al., 2008; Andraud et al., 2013). The most frequently mentioned HEV control measures were the reinforcement of cleaning-disinfection protocols and an extension of fallowing periods (53 farmers and 35 veterinarians), strengthening of internal biosecurity measures (47 and 36), reduction of pig mingling (43 and 2), and checking of water quality (17 and 25) (Fig. 4) (Walachowski et al., 2014).

3.5. HEV as an issue

3.5.1. HEV's zoonotic potential

Among the 85 farmers and 42 veterinarians having heard of HEV, 80% and 100% of them respectively rightly thought that HEV can infect humans. These percentages dropped to 57% and 98% respectively when asking whether HEV transmission from pigs to humans was possible. Only 34%, 35% and 49% of the 85 farmers knew that HEV can be transmitted to humans through contact with soiled pigs, an accidental injection/cut with soiled equipment or the consumption of contaminated pork products, in that order (Fig. 5). These figures were higher for veterinarians: respectively 55%, 64% and 81% for the three aforementioned transmission routes, the major one being recognised as the consumption of contaminated pork products (Fig. 5) (Chaussade et al., 2013; Dalton and Izopet, 2018).

3.5.2. Concerns about HEV

A number of farmers expressed concern in the open comments section of the questionnaire and asked for a report on the answers to the survey. Of the 429 respondents, 292 farmers (76.2%) and 42 veterinarians (91.3%) wished to be provided with more information. Similarly, 227 farmers (59.3%) and 43 veterinarians (93.5%) said they were willing to participate in another survey on HEV. When asked about the importance of HEV in the pig production sector, veterinarians agreed that it is an important issue because of the risk of negative media exposure (87%), in terms of public health (74%) and from an economic

According to you, what is the percentage of HEV infected farms in France?

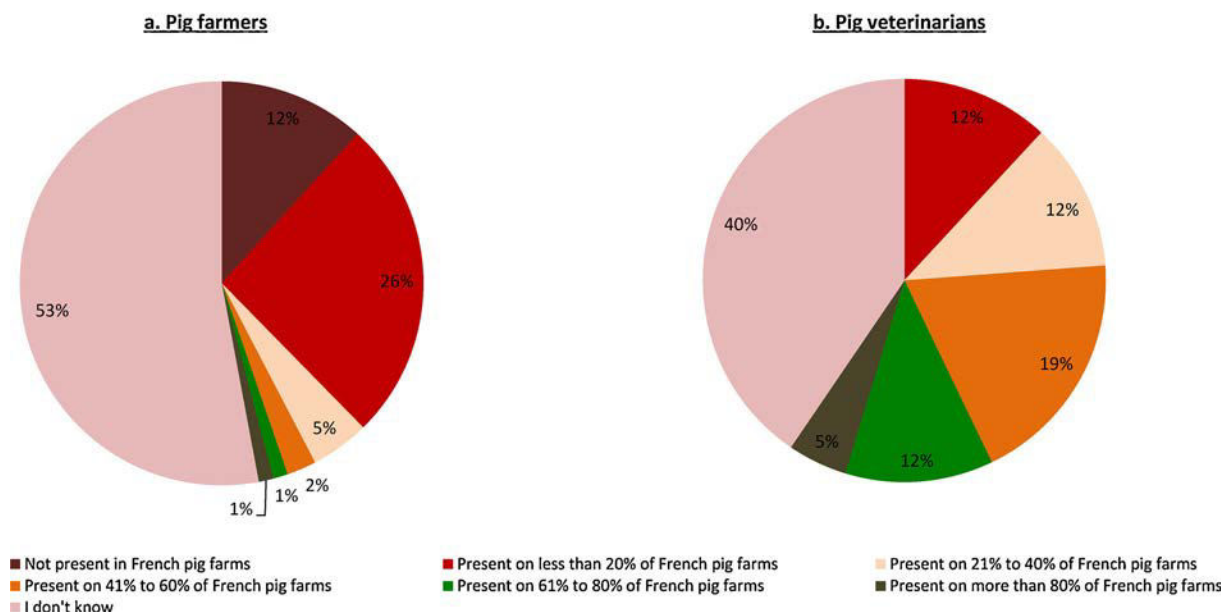


Fig. 1. Pig farmers' (a) and veterinarians' (b) opinion regarding HEV prevalence in the French pig production sector (85 farmers, 46 veterinarians).

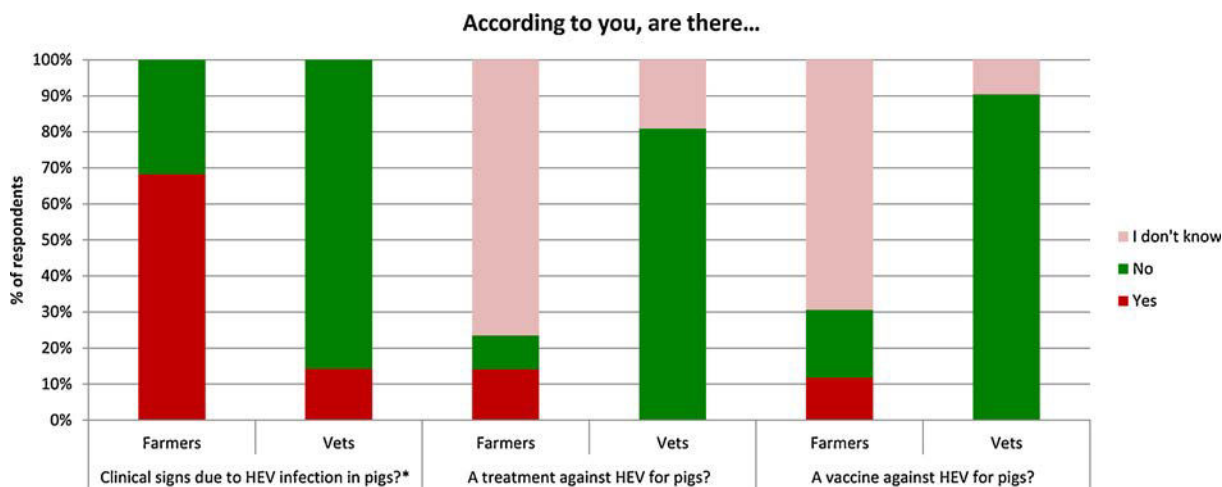


Fig. 2. Pig farmers' and veterinarians' opinion regarding clinical signs of HEV, treatment and vaccination (85 farmers, 46 veterinarians). * the "I don't know" option was not proposed for this question.

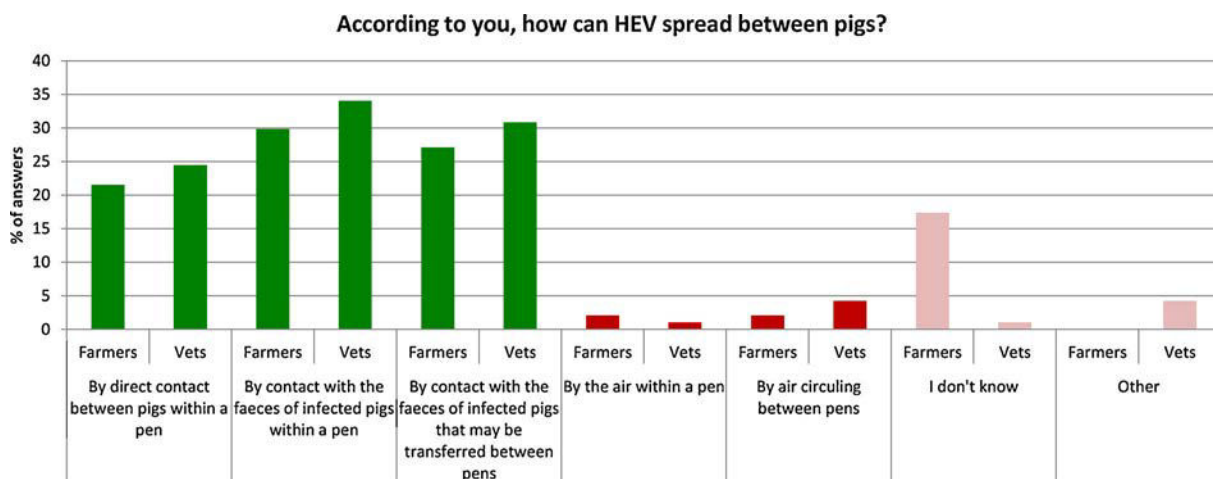


Fig. 3. Pig farmers' and veterinarians' opinion regarding HEV transmission routes between pigs (85 farmers, 144 answers from farmers; 46 veterinarians, 94 answers from veterinarians).

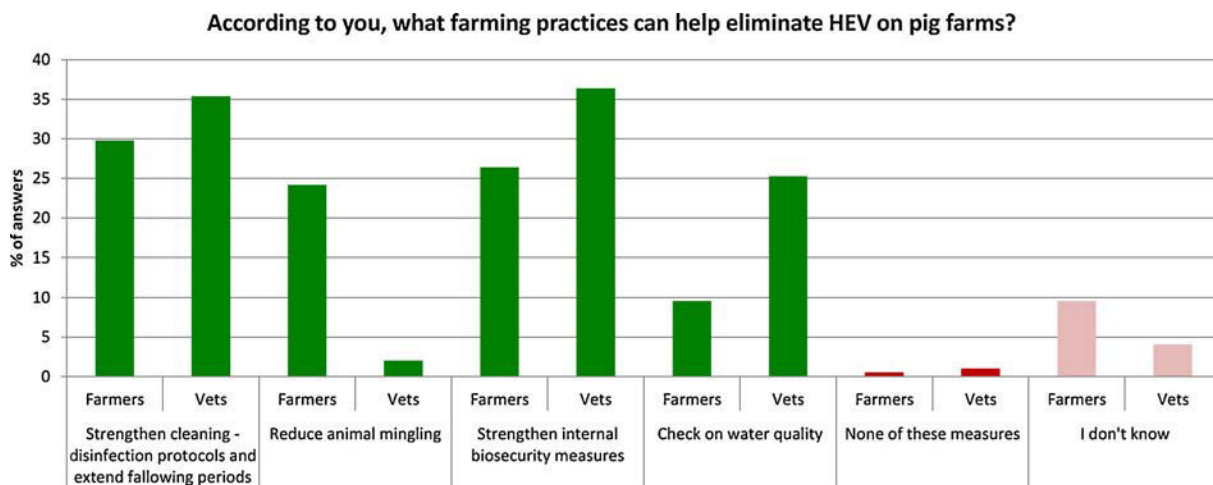


Fig. 4. Pig farmers' and veterinarians' opinion regarding possible HEV control measures (85 farmers, 178 answers from farmers; 46 veterinarians, 99 answers from veterinarians).

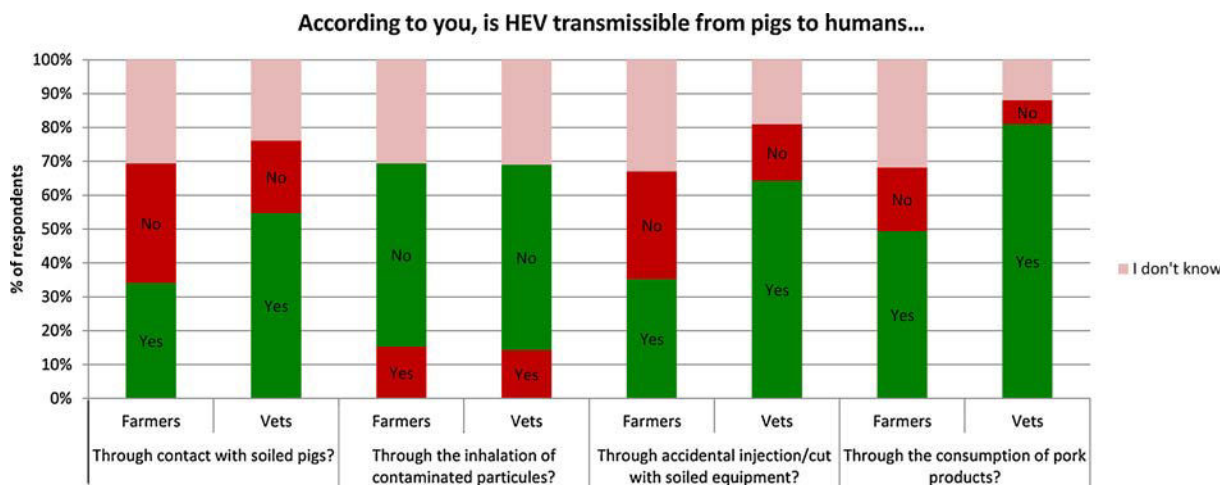


Fig. 5. Pig farmers’ and veterinarians’ opinion regarding zoonotic transmission routes of HEV from pigs to humans (85 farmers, 46 veterinarians).

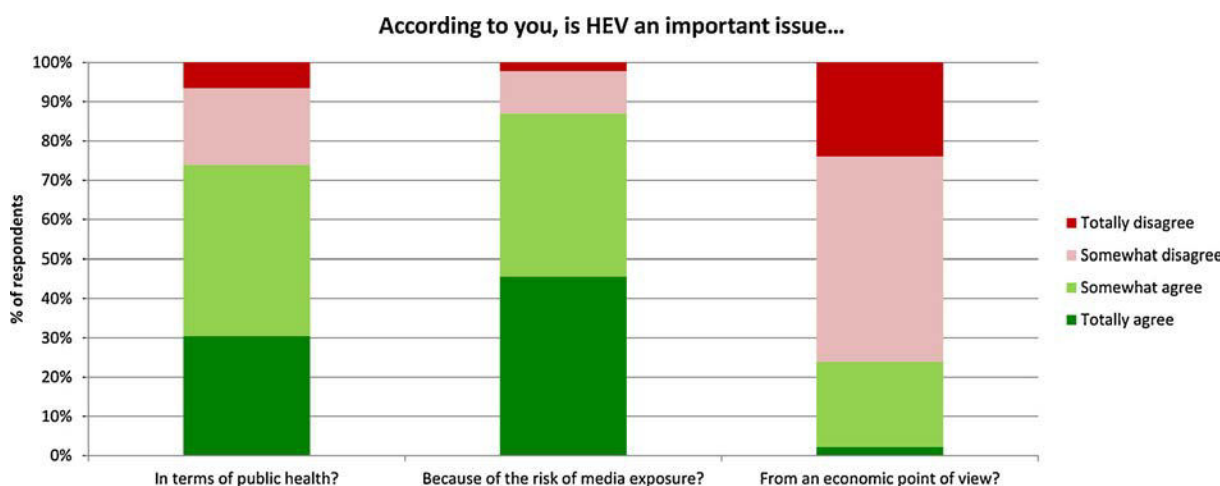


Fig. 6. Pig veterinarians’ opinion regarding the importance of the HEV issue in the pig production sector (n = 46).

point of view (24%) (Fig. 6).

4. Discussion

The very first step in raising the awareness of stakeholders and involving them in a disease control plan is to ensure that they are well-informed about the pathogen in question. The primary interest of our study lies in providing baseline data about pig farmers’ and veterinarians’ knowledge concerning HEV. As far as we are aware, this is the first study on this topic. Many studies have investigated farmers’ level of knowledge about non-zoonotic animal diseases (Jansen et al., 2010; Guinat et al., 2016; Jones et al., 2016). Other studies have addressed farmers’ and/or veterinarians’ knowledge concerning on-farm biosecurity measures (Kristensen and Jakobsen, 2011; Racicot et al., 2012; Simon-Grife et al., 2013; Laanen et al., 2014) or resistance to antimicrobials (Jones et al., 2015). However, fewer studies have addressed the particular case of farmers’ and veterinarians’ knowledge about zoonotic diseases, and most of these have been oriented towards zoonotic risks for professionals (Dowd et al., 2013; Robin et al., 2017). Moreover, these publications have generally reported data on stakeholders’ attitudes towards and perceptions of a disease, with no specific focus on their knowledge.

Assuming that pig farmers and veterinarians would be the two major actors involved in the implementation of a future on-farm HEV surveillance and control plan, our study was designed as a two-level survey targeting farmers and veterinarians similarly and

simultaneously. E-mail contact was chosen as a way to reach the largest population. However, the response rates observed in published publications having used the same communication channel are quite low (Guinat et al., 2016). In comparison, our survey has satisfactory response rates, especially as regards veterinarians. For veterinarians, the absolute number of respondents also makes sense, since the total population of swine practitioners is small. Because of the low response rate in the farmer population, the results of this survey have to be interpreted with caution. Our study sample shows great diversity in terms of type of farming activity and of veterinary practice. The distribution between the different types of farming (breeders, farrowers-to-finishers, finishers, etc.) from our survey is close to the results of the last official French agricultural census (Agreste, 2013), but comparison with more recent data (Salines et al., 2017b) evidences an over-representation of farrow-to-finish pig farms. No published data have been found to assess the representativeness of the veterinary sample. It is likely that the responding farmers and veterinarians are a biased sample, as they may be the most involved in their work or the most interested in this issue. Farrow-to-finish farmers, for instance, may be more interested in the safety of the end product they are marketing than other types of farmer.

Despite these biases, the results from this survey led to a number of noteworthy conclusions as regards the level of knowledge of pig farming stakeholders concerning HEV. Our study revealed HEV knowledge gaps among pig farmers. Only a minority of them had ever heard of HEV, but of those who had, a majority was aware of possible HEV transmission from pigs to humans, half of them knowing that the

virus could be transmitted through the consumption of pork products. However, their knowledge on the clinical and epidemiological aspects of HEV in pigs was poor. Several papers have already shown that farmers' knowledge of zoonoses is low. For instance, Mahon et al. (2017) recently reported that two-thirds of Irish farmers were unaware that a zoonosis is a disease a person gets from an animal, and that 90% did not know that apparently healthy animals may be a source of infection for humans. Bahnson et al. (2001) also evidenced knowledge gaps among American pork producers, e.g. regarding the zoonotic potential of *Salmonella*, *Trichinella* and *Campylobacter* to pass from pigs to humans. Canadian dairy producers also showed knowledge gaps regarding zoonotic risks linked to *Brucella* and *Cryptosporidium* (Young et al., 2010a). Similarly to what Marvin et al. (2010) reported regarding knowledge of zoonoses in the American pig production sector, the veterinarians in our survey were better-informed about HEV than farmers. Such an outcome was expected, mainly because veterinarians have better access to continuous training programmes and updated scientific information. Still, around 15% of veterinarians wrongly thought that HEV causes clinical signs. On a similar point, Marvin et al. (2010) also interestingly reported that *Yersinia enterocolitica* — a pathogen that does not have any clinical impact on pig health — was the least familiar hazard to all the respondents to their survey, including veterinarians, only one third of respondents being concerned about the transmission of *Y. enterocolitica* from pigs or pork to people. Thus, one of the critical points limiting farmers' and veterinarians' knowledge concerning HEV may be the absence of clinical signs of the disease in pigs. Moreover, scientific knowledge about HEV is constantly evolving and some HEV features are still poorly documented, e.g. the efficiency of control measures (Salines et al., 2017a). Informing stakeholders about these points may therefore be challenging.

A few studies have concluded that producers recognise the need to control foodborne pathogens, that they are aware of their role in food safety and that they are willing to adopt better practices to improve it (Bahnson et al., 2001; Ellis-Iversen et al., 2010; Marier et al., 2016). However, our findings suggest that pig farmers' knowledge related to HEV may reduce their ability to participate in future HEV control and surveillance plans. The possibility of improving production practices to decrease HEV prevalence as needed will depend in part on farmers having a solid knowledge base. Thus, additional farmer education on the clinical and epidemiological features of HEV is needed. Since veterinarians have been shown to be the best placed for passing food safety knowledge on to farmers (Bahnson et al., 2001; Marvin et al., 2010), and as they seem to have satisfactory knowledge about HEV, they could act as a knowledge transfer channel between researchers and farmers. However, veterinarians and farmers are generally federated within specifically clustered groups and their respective representatives therefore need to consider how to formalise information transfer between these groups. In France, for example, there are specific continuous training organisations for veterinarians, but they address questions very specific to veterinarians, and knowledge transfer to farmers is not a priority. Farmer organisations also have their own objectives, with a strong emphasis on the economic resilience of the system. Further improvements should be considered in the future to facilitate communication and knowledge transfer between veterinary and farmer organisations and prevent conflicts of interest between both parties. Because HEV is still little-known and research projects are currently underway to better understand its spread and persistence in the pig production sector, farmers and veterinarians should also be regularly provided with updated information from the scientific field. To this aim, an initial factsheet on HEV features has been designed and will be sent to the 8075 pig farmers that were contacted for this survey. Moreover, stakeholders' behaviour towards a given pathogen does not only depend on their knowledge but also on various factors that could be classified into three categories: (i) attitude towards the behaviour (i.e. the individual's degree of attractiveness or repulsion towards the particular behaviour), (ii) subjective norm (i.e. an individual's

perception about the particular behavior, which is influenced by the judgment of significant others), and (iii) perceived behavioural control (i.e. an individual's perceived ease or difficulty of performing the particular behaviour) (Ajzen, 1991). These key factors themselves depend on external features such as demographical factors (age, gender, religion, origin, etc.), global dispositions (personality, general attitude, self-esteem, emotions, etc.) and education (experience, knowledge, access to media, etc.). Further studies would therefore be required to determinate whether farmers and veterinarians are willing and able to implement control and surveillance plans of HEV in the pig production sector.

5. Conclusions

Our baseline study highlighted HEV knowledge gaps among pig farming stakeholders that have to be filled. Targeted educational efforts need to be made in an attempt to raise the awareness of farmers and veterinarians concerning HEV. Before initiating an HEV risk mitigation plan, further studies are needed to investigate the barriers to controlling the pathogen as perceived by farmers, as well as their preferred motivators. This kind of data would help risk managers facilitate surveillance and control implementation by steering efforts to remove specific obstacles and thereby create favourable conditions for HEV control on pig farms.

Author contributions

MS, FT, MA and NR designed the questionnaires. MS analysed the data and drafted the manuscript. NR initiated and coordinated the project. All the co-authors revised the manuscript and approved the final submitted version.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.prevetmed.2018.04.015>.

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II. Evaluation de la faisabilité technique et de l'acceptabilité d'un plan de maîtrise du virus de l'hépatite E dans la filière porcine

Le bon fonctionnement de la chaîne de l'innovation suppose que des chercheurs, enseignants et étudiants de tous niveaux connaissent intimement la pratique, ses conditions, ses contraintes et ses besoins. Faute de quoi, beaucoup de nouveautés sont inadéquates et rejetées [...]. En définitive, la science et la technique « proposent », mais ce sont la pratique et l'économie qui « disposent ». [...] Ce sont les agriculteurs eux-mêmes [...] qui mettent au point les systèmes de production les plus avantageux, en fonction de leurs conditions de milieu et de prix, et en fonction des contraintes de superficie, de main-d'œuvre et de financement de leurs exploitations.

Mazoyer M., Roudart L., 2002. *Histoire des agricultures du monde : Du néolithique à la crise contemporaine*. Ed. Seuil, 705 p.

L'étude menée auprès des éleveurs et des vétérinaires a révélé des trous de connaissance et une faible sensibilisation des éleveurs à la problématique du HEV, mais aussi le potentiel pour les vétérinaires d'agir comme courroie de transmission d'informations et comme accompagnateurs des éleveurs. C'est à partir de ce constat qu'il a été choisi d'**évaluer la faisabilité technique et l'acceptabilité de mesures de maîtrise du HEV auprès des éleveurs et de leur environnement professionnel direct : vétérinaires et conseillers d'élevage**. Une enquête visant à étudier les **freins et motivations à d'éventuels changements de pratiques** en élevage de porcs a ainsi été conduite sous la forme d'**entretiens semi-directifs** auprès de ces trois catégories d'acteurs. Cette étude donnera lieu à une publication dont le projet est présenté ci-après et un résumé a été accepté pour un poster aux *Journées Recherche Porcine 2020*.

En parallèle, une **réunion de concertation des organisations publiques et privées** potentiellement impliquées dans la gestion du risque lié au HEV a été organisée. Les objectifs étaient d'une part de fournir un **point d'information** sur les données et travaux récents sur le HEV dans la filière porcine, d'autre part de **susciter réflexions et échanges** sur les stratégies pouvant être mises en place. Les participants ont reçu en amont un document de synthèse (Annexe 8) et un compte-rendu, inclus ci-après, a été rédigé à l'issue de cette réunion.

Publication 11 (Draft)

Teixeira-Costa C., Andraud M., Rose N., **Salines M.** Controlling hepatitis E virus in the pig production sector: assessment of the technical and behavioural feasibility of on-farm risk mitigation strategies. In prep.

1 **Controlling hepatitis E virus in the pig production sector:** 2 **assessment of the technical and behavioural feasibility of** 3 **on-farm risk mitigation strategies**

4
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10
11
12 **Abstract:** Hepatitis E virus is a zoonotic agent whose main reservoir in industrialised
13 countries is pigs. Recent studies conducted on pig farms, in experimental situations, or through
14 modelling approaches have led to a better understanding of the spread of HEV on pig farms and
15 to a set of measures to reduce its prevalence and the risk of marketing contaminated products.
16 The objective of this study was to assess the feasibility of a set of HEV control strategies on pig
17 farms. Individual semi-structured interviews were conducted with farmers, veterinarians and
18 farming advisors to collect general data, their level of knowledge of HEV, their opinion on the
19 technical feasibility of some changes in practices, their perception of the respective
20 responsibilities of the different actors and their feelings about the importance of the issue,
21 following the framework of the theory of planned behaviour. The interviews made it possible
22 to highlight potential barriers (lack of knowledge, scientific gaps, perceived inability to control
23 HEV, low perception of the importance of the issue) and preferred motivators (professional
24 satisfaction, family recognition, opportunity to achieve higher quality standards) for the
25 implementation of on-farm risk mitigation strategies. Three clusters of stakeholders were also
26 evidenced, with a group of leaders who could help unlock reluctance and disseminate
27 innovations. This kind of behavioural approach appeared useful to help risk managers facilitate
28 zoonotic control on pig farms.

29
30 **Keywords:** decision-making process; disease control; foodborne zoonosis; hepatitis E
31 virus; pig production sector; theory of planned behaviour

32 **Highlights**

- 33 • An interview-based study was led to assess the feasibility of HEV on-farm control.
- 34 • Farmers, advisors and veterinarians were all willing to participate in HEV control.
- 35 • Lack of knowledge, scientific gaps, inability to control HEV would be barriers.
- 36 • Family recognition, opportunity for higher quality standards would be motivators.
- 37 • A cluster of potential leaders would help engage stakeholders in such a programme.

38
39

40 **1. Introduction**

41

42 Hepatitis E virus (HEV) is a non-enveloped single-stranded RNA virus that can cause acute or
43 chronic hepatitis (Emerson and Purcell, 2003; Kamar et al., 2011; Choi et al., 2018). In many
44 industrialised countries, a number of locally acquired cases have been linked to the consumption
45 of raw or undercooked pork products, especially those containing liver in high proportion (Moal
46 et al., 2012; Renou et al., 2014; Guillois et al., 2016; Pavio et al., 2017). Several risk factors of
47 HEV presence in the liver of slaughtered pigs have been evidenced at the individual or the farm
48 scale through field studies, experimental trials or modelling approaches. They are related to the
49 farms' size, the type of production (e.g. free-ranged or organic versus conventional farming),
50 the batch management system (e.g. one week versus three week between-batch interval),
51 biosecurity measures (e.g. absence of an hygiene lock, no quarantine sector), farming practices
52 (e.g. cross-fostering and mingling practices), farms' health status regarding intercurrent
53 pathogens affecting pigs' immunity (e.g. porcine circovirus type 2 (PCV2) and porcine
54 respiratory and reproductive syndrome virus (PRRSV)) (Li et al., 2009; Jinshan et al., 2010;
55 Hinjoy et al., 2013; Walachowski et al., 2014; Lopez-Lopez et al., 2018; Salines et al., 2019a;
56 Salines et al., 2019b; Salines et al., 2019c). To our knowledge, no HEV systematic control or
57 surveillance programme is implemented in the European pig production sector yet (Salines et
58 al., 2017a). Potential control measures could be drawn from these recent findings in order to
59 design a risk mitigation plan limiting HEV on-farm spread and persistence and thus HEV
60 presence in foodstuffs. However, the effective implementation of these upstream measures
61 would rely on the stakeholders' involvement, primarily the one of farmers, but also of their
62 direct professional environment, i.e. farming advisors and veterinarians. Their commitment
63 would depend on a combination of several external and internal factors that is crucial to
64 understand for motivating them to change. Literature extensively reports on factors influencing

65 farmers' decision-making, particularly about animal or public health issues and that are not only
66 based on policies, economics or rational judgments (Edwards-Jones, 2006; Ritter et al., 2017).
67 First, individual characteristics such as age, sex, education, personality, previous experiences,
68 routines, family influences etc. can affect farmers' opinions on animal health, prevention and
69 control strategies and their decision-making (Racicot et al., 2012; Wilson et al., 2015; Frössling
70 and Nöremark, 2016). Farmers also need to have sufficient knowledge about the disease and
71 management strategies to make effective changes (Benjamin et al., 2010; Ellis-Iversen et al.,
72 2010; Racicot et al., 2012; Ritter et al., 2015). In the specific case of HEV, farmers' knowledge
73 has been shown to be quite low according to the results of our previous survey (Salines et al.,
74 2018). The impact of the disease on animal health and/or on the farm's economic performances
75 can also motivate farmers to take steps toward disease control and prevention (Alarcon et al.,
76 2014). HEV spreads on pig farms without leading to any clinical signs in pigs or causing
77 financial losses, meaning that the problem awareness among farmers may be low. Another
78 interesting point is that the threshold at which an issue becomes an actual problem depends on
79 the farmers' frame of reference, itself often influenced by farmers' descriptive and injunctive
80 norms and previous experiences (Jansen et al., 2010; Jansen et al., 2016). It has also been shown
81 that the farmers' evaluation of a problem is not performed according to an absolute scale but in
82 relation to other issues that also require their efforts (Leach et al., 2010a; Bruijnis et al., 2013;
83 Horseman et al., 2014). Global farming context is therefore an important factor to consider
84 (laws and regulations, market prices, consumer demands, cues and nudges, etc.) (Ritter et al.,
85 2017). Farmers' perception of their own responsibility in dealing with the problem has been
86 evidenced as a key factor in their motivation as well, especially for zoonotic pathogens raising
87 concerns about consumer health or consumer perception of the production sector quality (Sorge
88 et al., 2010; Nielsen, 2011; Toma et al., 2015). Other internal incentives can include
89 professional satisfaction, reputation, family recognition, etc. (Leach et al., 2010b; Bruijnis et
90 al., 2013; Alarcon et al., 2014; Roche et al., 2015). The efficacy and the cost-effectiveness of
91 recommended strategies, as well as their feasibility and practicality, are also known to be strong
92 drivers for farmers to adopt recommended disease prevention and control measures (Gunn et
93 al., 2008; Valeeva et al., 2011; Garforth et al., 2013; Alarcon et al., 2014; Toma et al., 2015).
94 Regarding the farmers' professional environment, several studies have shown that veterinarians
95 and farming advisors play a significant role in spreading information and motivating farmers to
96 adopt best management practices (Alarcon et al., 2014; Laanen et al., 2014; Marier et al., 2016;
97 Mahon et al., 2017; Poizat et al., 2017). However, their own mindset, opinion (e.g. on the
98 effectiveness of control and prevention measures) and self-efficacy (i.e. their belief to their

99 ability to perform a behaviour and obtain a desired outcome (Bandura, 1977)) have only been
100 scarcely explored yet (Ritter et al., 2017; Hidano et al., 2018).

101

102 In this context, and with the goal of providing risk managers with tangible and pragmatic
103 elements for decision-making, the aim of our study was to evaluate the technical and
104 behavioural feasibility of on-farm HEV control measures from the perspective of pig farmers,
105 advisors and veterinarians through semi-directed interviews.

106

107

108 **2. Materials and methods**

109

110 **2.1. Survey design**

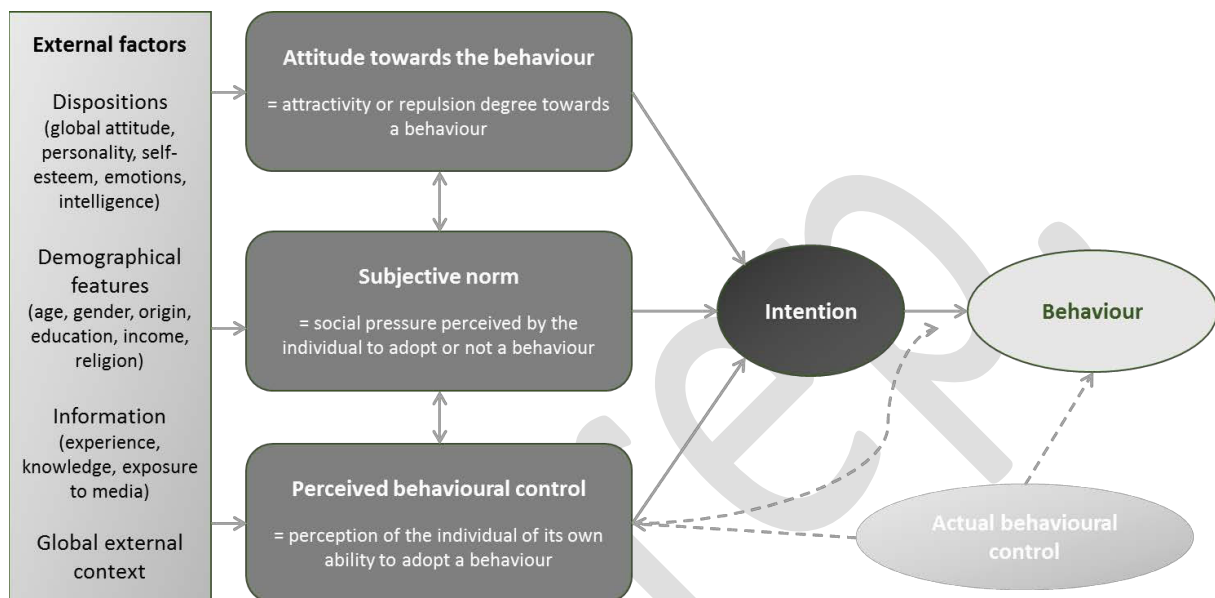
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112 *2.1.1. Survey methodology*

113

114 The three categories of stakeholders (farmers, veterinarians and advisors) were interviewed
115 using semi-structured questionnaires designed with a similar framework. First, several
116 questions were asked to gather general data and, for farmers, a Mindmap was used as a support
117 to collect the farm's characteristics. Then, the conversation was directed to address three key
118 points. The two first ones were the level of knowledge of the interviewees regarding HEV and
119 their practices and their possible modifications. For the latter, the interviewees were first asked
120 to describe the structure and the management of their farm or of their clients' farms, then if it
121 would be possible to change some of their practices and why/why not. Several practices'
122 modifications were assessed: type of housing facilities for gestating sows, cross-fostering
123 practices at farrowing, mingling practices at weaning, management of intercurrent pathogens
124 (PRRSV, PCV2), HEV screening of the herd and of slaughtered pigs, potential HEV
125 vaccination in case one was available. The third key point was their attitude towards HEV issue
126 in the pig production sector: their opinion in terms of control measures, their willingness to pay
127 for them and their perception about the responsibility of the stakeholders in addressing the
128 problem. The interview was concluded with general questions about the individual's
129 characteristics (e.g. age, sex, education, personality, previous experiences etc.). Throughout the
130 interview, open-ended questions alternated with several types of closed-ended questions (binary
131 questions, graduated questions with Lickert scale, multiple-choice questions) according to a

132 logical and consistent process. Eight questions were part of the framework of the Theory of
 133 Planned Behaviour, which stated that intention to adopt a behaviour depends on the perceived
 134 behavioural control, the norms and the attitude (Ajzen, 1991) (Figure 1). Moreover, brief
 135 information on HEV was also provided to enable interviewees to answer the questions in an
 136 informed way.
 137



138
 139 **Figure 1: Framework of the Theory of Planned Behaviour (Ajzen, 1991)**
 140

141 2.1.2. Sample selection

142
 143 Stakeholders were sampled as follows: (i) First, producers' organisations and veterinarians
 144 were asked to provide a list of farmers representing different types of farms (e.g. multiplication
 145 farms, nucleus, farrow-to-finish farms, etc.) and following several kind of quality charts. All
 146 sampled farmers were located in the Western part of France, corresponding to main major pig
 147 production area. (ii) Then, farming advisors were selected in the main producers' groups in
 148 Western France. (iii) Finally, veterinarians specialised in pig health and who practiced as liberal
 149 practitioners or employees of different companies were sampled. Finally, 59 farmers, 12
 150 farming advisors and 26 veterinarians were included in the contact list.
 151

152 2.1.3. On-site interviews

153
 154 Interviews were held from April to June 2019. They were grouped, as far as possible, by
 155 geographical area. The appointments were made by email or by phone. The interviews were

156 preferably led face to face, but some were carried out by phone for practical reasons. The
157 questionnaire was not sent to the participants prior to the interview and all professionals were
158 investigated in the same way. With the participants' agreement, the conversations were
159 recorded while notes were taken. All the interviews were conducted by the same interviewer
160 which allowed answers to be compared and avoided information bias.

161

162

163 **2.2. Data analysis**

164

165 The interviews were transcribed in order to carry out a qualitative analysis of the interviewees'
166 comments and to include verbatim in the results. The quantitative data from the interviews were
167 recorded in an Access database. The distribution of the responses to graduated questions was
168 represented by boxplots. As part of the application of the theory of planned behaviour, the effect
169 of seven explanatory variables on the outcome variable 'behavioural intention' (question:
170 "Would you be willing to participate in an HEV control programme?") was analysed by
171 Spearman correlation tests (univariate analysis) and a principal component analysis (PCA,
172 multivariate analysis) followed by hierarchical clustering (HC). The seven explanatory
173 variables were divided into three groups: (1) variables representing attitude towards the
174 behaviour ("Would controlling HEV be satisfactory?", "Do you feel directly concerned by this
175 issue?", How do you perceive that better managing pig health would mitigate the risks for
176 human health?"), (2) those describing the effect of subjective norms ("Would your relatives
177 want you to participate in an HEV control programme?", "Is hepatitis E an important issue for
178 human health?", "Is hepatitis E an important issue for the pig production sector?"), (3) and
179 those related to perceived behavioural control ("Do you feel able to control HEV?"). The
180 statistical analyses were carried out using the R software (R 3.5.1).

181

182

183 **3. Results**

184

185 **3.1. Features of the study sample**

186

187 A total of 11 veterinarians, 10 farming advisors and nine farmers agreed to participate in the
188 study (Table 1). Of the 30 interviews, five were conducted by phone. The majority of the

189 interviewees were men, with large age range. All interviewees were specialised in pig
 190 production only. The respondents were mainly located in the North-Western France, except
 191 three veterinarians working in the North and South-West regions of the country. The interviews
 192 lasted on average an hour with a maximum to 2h24.

193

194

Table 1. Interviewees' and interviews' characteristics

	Farmers	Farming advisors	Veterinarians
Number of interviews (of which phone interviews)	9 (1)	10 (0)	11 (4)
Average age [range]	47 [29-57]	41 [26-55]	47 [36-56]
Sex ratio (men/women)	8/1	8/2	10/1
Average duration (min) [range]	62 [45-90]	60 [45-75]	81 [45-144]

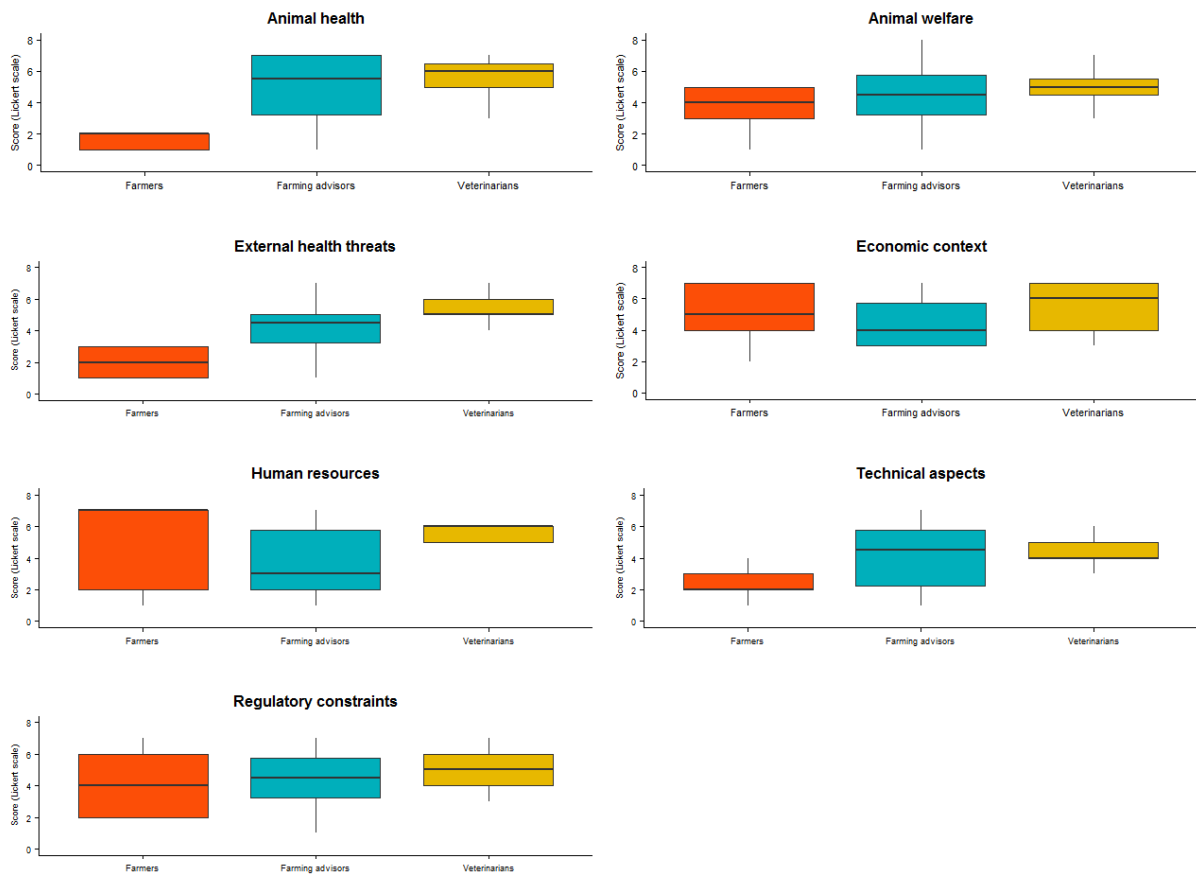
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196 The farmer sample included two multiplication farms, four production farrow-to-finish farms,
 197 two farrowing farms and one post-weaning farrowing farm. These farms were managed
 198 according to a 4-, 5-, 7- or 10- batch management system. The number of sows ranged from 85
 199 to 600 (mean: 283) and the number of fattening pigs ranged from 560 to 5,000 (mean: 2,350).

200

201 When asked about several potentially worrying aspects of pig farming, farmers gave a particular
 202 importance to human resources, explaining that they experienced difficulties in recruiting
 203 skilled employees, which was confirmed by veterinarians (Figure 2). Farming advisors and
 204 veterinarians attributed high score to animal health and external health threats.

205



206

207 **Figure 2. Distributions of the interviewees' scores attributed to potentially worrying**
 208 **aspects of pig farming**

209

210 Regarding the interviewees' knowledge of HEV, a high within- and between-group diversity
 211 was observed. All surveyed veterinarians, half of the interviewed farming advisors and one
 212 farmer had ever heard of HEV but their knowledge about it was variable.

213

214

215 **3.2. Would it be feasible to...**

216

217 **3.2.1. ... house gestating sows in smaller groups?**

218

219 Three of the nine surveyed farmers housed their gestating sows in large pens (more than 15
 220 sows per pen) and stated that changing this housing system to a more segregated one would be
 221 impossible. Indeed, it would require significant structural changes that would be too costly. A
 222 veterinarian also explained: *“There are many of them and in particular on the largest farms,*
 223 *where the size of the groups is much larger and reviewing the management of these farms by*

224 *moving from large groups to small groups is probably totally unthinkable given the constraints*
225 *of the buildings”*. Moreover, some farmers explained that they recently changed this structure
226 to meet welfare requirements. Veterinarians and farming advisors had various opinions
227 regarding welfare criteria for gestating sows: for some of them, housing sows in smaller groups
228 would help reduce competition thanks to a more quickly established hierarchy and would
229 improve food and health monitoring. For the others, large pens would reduce locomotor
230 disorders and decrease competition thanks to increased escape possibilities.

231

232 ***3.2.2. ... have safer mingling practices?***

233

234 All the veterinarians and farming advisors interviewed stated that they already recommend
235 farmers to reduce cross-fostering and mingling of weaned piglets for the beneficial effect of
236 these practices on other diseases. They therefore believed that improving these practices to
237 reduce the risk of HEV would be feasible in the medium to long term. However, they explained
238 that limiting cross-fostering could be sometimes delicate given the genetic evolution towards
239 increasingly prolific sows: *“Five to six years ago, we were weaning between 11.5 and 12*
240 *piglets; today, I see farms with 15 or more weaned pigs. At some point, with this level of*
241 *prolificacy, they have to homogenate the litter sizes”* said one veterinarian. Farmers, for their
242 part, claimed to limit these practices already, even if a 10% cross-fostering threshold would be
243 difficult to meet because of the need to maintain the technical and economic performances of
244 the farm. Regarding mingling practices of weaned pigs, the farmers interviewed housed on
245 average 28 [14-34] pigs per nursery pen; four of them housed more than 30 pigs per pen. When
246 these farmers were asked whether it would be possible for them to make smaller nursery pens,
247 half would agree to do so.

248

249 ***3.2.3. ... improve management of intercurrent pathogens?***

250

251 Only one veterinarian believed that the fact that co-infection with PRRSV and/or PCV2
252 increases the risk of HEV would encourage farmers to take action to better manage these
253 pathogens: *“Farmers feel responsible. They want to feed people safely, so it’s an argument that*
254 *could be presented to them, it would only increase their motivation”*, explained this veterinarian.
255 Half of the farming advisors also thought that this could be an additional argument to convince
256 farmers to take action against PRRSV and/or PCV2. One of them said: *“Yes, it can be another*
257 *argument to convince them to take action if they have not already done so, but it is up to us to*

258 *communicate on this too*". The other veterinarians and farming advisors did not consider it
259 necessary to specifically increase the management efforts already undertaken for intercurrent
260 pathogens for the sole reason of HEV control. According to them, controlling HEV would not
261 be sufficient incentive to motivate farmers: if farmers take measures to better manage
262 PRRSV/PCV2, it would be for their direct technical and economic consequences, not for their
263 impact on HEV dynamics.

264

265 **3.2.4. ... look for HEV on the farms and on slaughtered pigs?**

266

267 If there were a readily available routine test, six out of 11 veterinarians and seven out of 10
268 farming advisors would be interested in accessing it. Five of the interested veterinarians and all
269 advisors would encourage farmers to test their animals. However, opinions regarding the type
270 of farms which should be tested in priority differed. One of the veterinarians explained that "*the*
271 *most sensitive part will be the part that is directly related to human consumption, so it is the*
272 *fattened pigs, meaning we should test farrow-to-finish farms or finishing farms or post-weaning*
273 *finishing farms, as long as they sell finished pigs*". Nevertheless, the majority of them stressed
274 the importance of starting at the top of the pyramid, i.e. of testing nucleus farms and multipliers:
275 "*I would start by cleaning up the top of the pyramid, you see, nucleus, multipliers, if we want*
276 *to try to limit the introduction of shedders, [...] because it is true that they are the most at-risk*
277 *of disseminating HEV*". Seven out of nine farmers would be interested in testing their farm for
278 HEV in order to know their status. Nevertheless, all of them said they would like this test to be
279 free of charge. The two farmers who did not wish to know the status of their farm mentioned
280 the fear of diagnosing a new pathology on their animals they could not treat: "*By searching, we*
281 *always end up finding*", highlighted one of them.

282 The majority of the interviewees did not support screening of animals entering a farm for
283 various reasons: (i) the objectives of such screening were still unclear for them, as one advisors
284 stated: "*if the farm is positive, it may not change much and since it is a healthy carrying all*
285 *farms are equally likely to be positive*", (ii) the cost may be charged on farmers instead of on
286 slaughterhouses, (iii) and these screenings could only be considered in the case of a collective
287 approach, otherwise some farmers would not be able to sell their positive animals.

288 Seven out of 11 veterinarians and eight out of 10 farming advisors would recommend screening
289 livers at slaughter: "*According to me, an important control point would be to screen for the*
290 *presence of the virus on livers that are intended for human consumption*", said one veterinarian.

291 Four out of nine farmers also highlighted the fact that it would probably be interesting to test

292 the livers and sort them before processing, which would limit the constraints for farmers. The
293 other farmers considered that they are not directly concerned by this question since it is related
294 to the downstream part of the chain; they even explained that they do not know what the future
295 of the livers of their animals is.

296

297 **3.2.5. ... vaccinate pigs against HEV?**

298

299 If a vaccine against HEV were available for pigs, four out of 11 veterinarians and eight out of
300 10 farming advisors think that farmers might be ready to vaccinate their animals because it is a
301 human health issue: *“the interest is for the pork sector and for public health, so [...] they would*
302 *be ready to vaccinate if they are told to vaccinate”* explained a veterinarian. Developing a
303 multivalent vaccine would also facilitate vaccination implementation, as well as a financial
304 support for the vaccine. According to these respondents, vaccination should also be part of a
305 *“collective approach”*, with for example the development of a sub-sector providing HEV-free
306 livers for liver-based products, and better payment for the farmers involved in this kind of
307 production. On the contrary, the others considered vaccination unthinkable, particularly
308 because of the asymptomatic nature of the infection in pigs: *“Honestly, I think [farmers] will*
309 *only do so if it becomes compulsory, if it is part of a public health or other approach”*, said one
310 farming advisor. Nevertheless, four out of nine farmers said they would be willing to vaccinate
311 despite the fact that there are no symptoms in pigs because this is a human health issue. For the
312 other five, vaccination against HEV would not be feasible given the cost of vaccines, the
313 additional workload involved and the unseen consequences of the infection on animals.

314

315 **3.2.6. ... create a specific chain dedicated to the production of liver-based products?**

316

317 Unanimously, the veterinarians were in favour of organising such a sub-sector, provided that
318 farmers derive added value from it: *“It could probably be another type of outlet [...], it is true*
319 *that today the marketing of livers is null or almost null [...], it would certainly be an economic*
320 *plus”*, said a veterinarian. The opinion of advisors was similar, only one seemed reluctant to
321 this idea because, according to him, it would not be of interest to the farmer: *“it would be more*
322 *the responsibility of the slaughterhouse to sort the livers and to notify them as HEV-free”*. All
323 veterinarians and farmers stressed that it would be necessary to better pay farmers who would
324 move towards this free status, otherwise they would not be interested in. All the interviewed
325 farmers were interested in this HEV-free qualification for various reasons: high interest in

326 taking part in HEV control, new outlet, market diversification, professional development. All
327 but one confirmed, however, that better remuneration would be necessary. One farming advisor
328 explained that producing HEV-free pigs would be a relevant marketing differentiation factor
329 for the French market opposed to other big producers. However, five farmers out of nine feared
330 competitive distortion in the case of new norms or regulatory constraints forcing them to adopt
331 more expensive farming practices.

332
333

334 **3.3. Assessment of factors affecting interviewees' willingness to participate in** 335 **an HEV control programme**

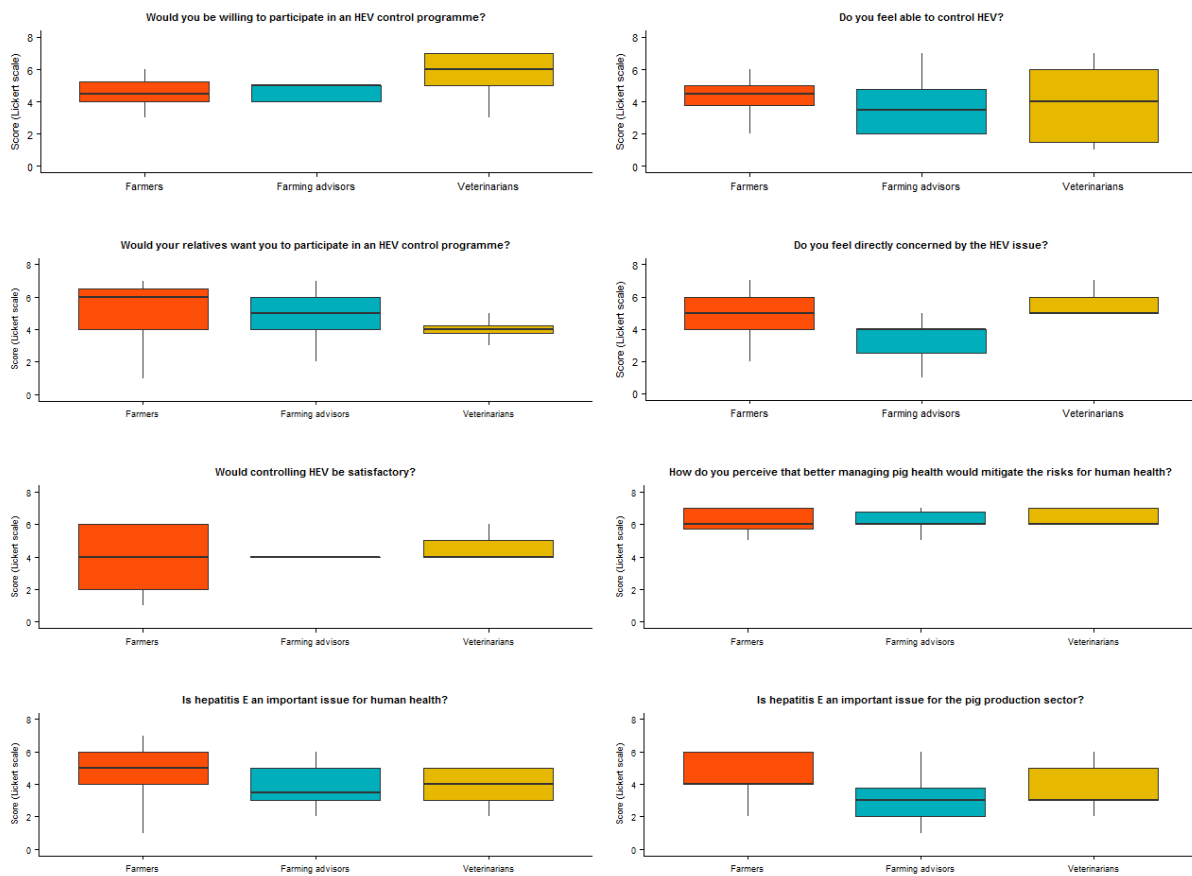
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337 **3.3.1. Descriptive results (Figure 3)**

338

339 Overall, the interviewees stated their intention to participate in an HEV control programme,
340 provided they would not act alone, as stated a veterinarian: “*Yes, as part of a collective control*
341 *plan*”. The willingness to participate seemed higher for veterinarians (average score: 5.8/7) than
342 for the other groups (farmers' score: 4.6/7; advisors' score: 4.5/7). High within-group
343 variability was evidenced regarding the actors' ability to participate in an HEV control plan
344 with average scores of 4.3, 3.8 and 3.9 for farmers, advisors and veterinarians, respectively.
345 The main reason for which the interviewees would not feel able to participate to an HEV control
346 programme was the lack of detailed and confirmed data and of concrete proofs of the efficacy
347 of the suggested control measures. When veterinarians and advisors were asked if, in their
348 opinion, farmers would be able to control HEV, their answers were highly heterogeneous. Some
349 of the interviewees believed that farmers would not be able to do this because they are unaware
350 of the existence of this disease and have other more important concerns. Others, on the other
351 hand, believed that farmers could be able to do so if good explanations are provided. Overall,
352 the question related to the influence of the relatives' opinions obtained high scores, with average
353 scores of 5, 4 and 4 for farmers, advisors and veterinarians, respectively. Farmers and
354 veterinarians said they feel directly concerned about this issue (average scores: 5 and 5.6,
355 respectively), more than advisors (average score: 3.6). With average scores of 6 in all
356 categories, the benefits of better managing pig health to reduce risks for human health appeared
357 highly interesting for all interviewees. Regarding the importance of the issue for human health
358 and for the pig production sector, answers were greatly variable and, on average, around the

359 middle score, essentially in relation with the quite low number of hepatitis E human cases.
 360 Farmers attributed higher scores to these two questions than the other interviewee categories.
 361



362
 363 **Figure 3. Distributions of the interviewees' answers to eight questions included in the**
 364 **framework of the theory of planned behaviour**
 365

366 If some veterinarians thought that it is important not to “*turn a blind eye*” but to “*remain*
 367 *attentive*” because “*it is a matter of consumer health, [one] cannot ignore it*”, others highlighted
 368 the risk of being too precautionous and of stigmatizing pig farms in an already touchy social,
 369 economic and political context: “*I mean, we’re in a context where we’re already pointing*
 370 *fingers at the animal sectors, so waving a small hepatitis E flag would be quite anxious without*
 371 *[hepatitis E] being really potentially serious for humans, we’ll say*”. This risk of a media crisis
 372 was addressed by the interviewees from two opposite angles: for some of them, the fear of a
 373 media crisis would be a positive incentive argument, which could push farmers to take an
 374 interest in the issue, while for others it would affect the entire sector negatively and lead to a
 375 crisis in consumer confidence. Among the barriers highlighted by the interviewees, the cost of
 376 implementing control measures (depending on the individual characteristics of each farm) was

377 the major one: “*The economic aspect remains the only obstacle that often prevents us from*
 378 *being positive and 100% committed to control plans*”, said one of the farmers surveyed.
 379 Financial incentives could then be considered, according to some of the surveyed persons.
 380 However, all questions related to the willingness to pay to a control programme were found
 381 hardly answerable by the interviewees and no outcomes could be drawn because of too much
 382 missing data. Unanimously, veterinarians considered themselves as the privileged interlocutors
 383 to provide advice and information on this topic during farm visits. They highlighted the annual
 384 sanitary check-up, meetings, documents and social network as good opportunities to talk about
 385 this issue. All farmers and advisors also appointed the veterinarian as their main contact person.

386

387 3.3.2. Statistical analysis

388

389 The univariate analysis showed a positive association between the willingness of veterinarians
 390 to participate in an HEV control programme and the influence of their relatives’ opinion
 391 (Spearman correlation coefficient = 0.75, p-value < 0.05) (Table 2). The same tendency was
 392 observed for farming advisors (CC = 0.60, p-value < 0.10). There was also a tendency of
 393 association between veterinarians’ intention to control HEV and the value they give to improve
 394 pig health in order to reduce the risks for human health (CC = 0.60, p-value < 0.10).

395

396 **Table 2. Correlation between the interviewees’ willingness to participate in an HEV**
 397 **control programme and seven explanatory variables fitting to the framework of the**
 398 **theory of planned behaviour**

399

CC: Spearman correlation coefficient

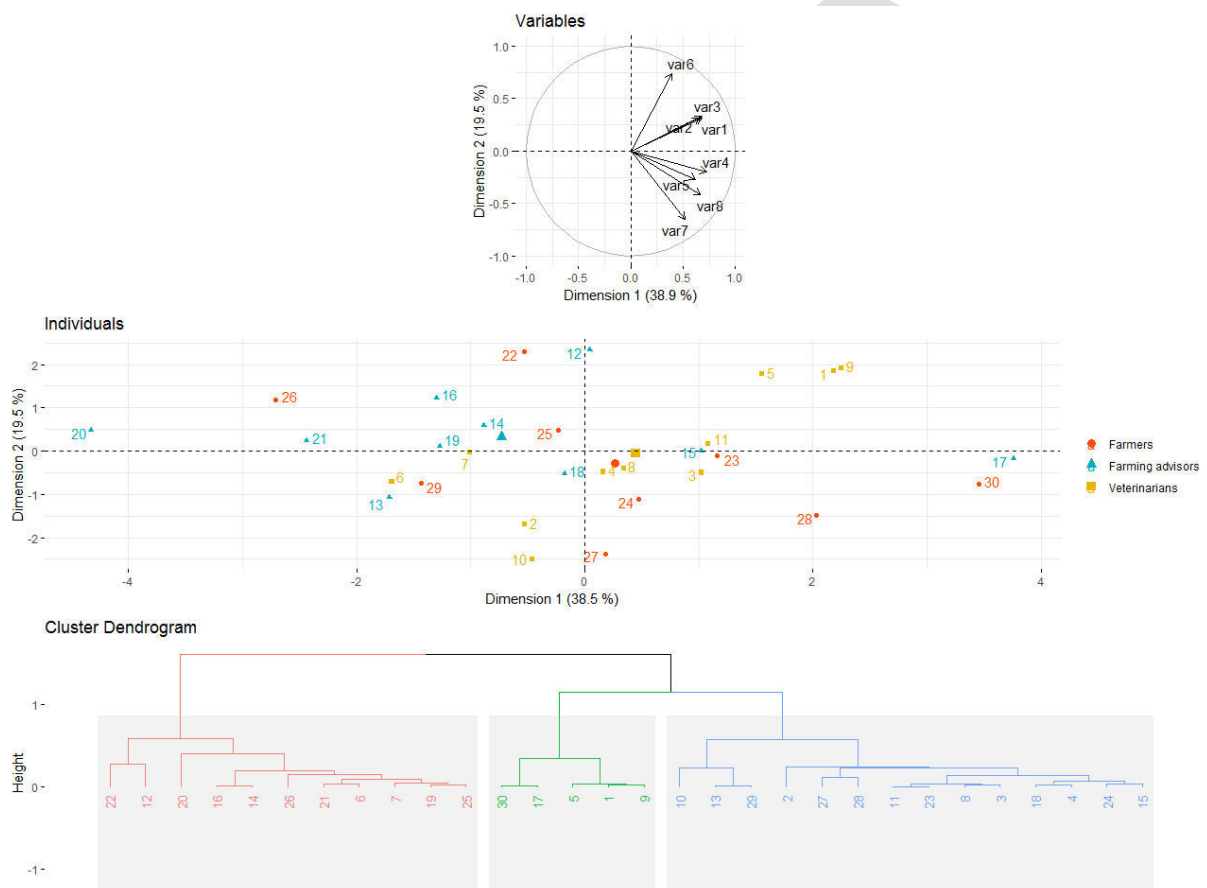
	Farmers		Farming advisors		Veterinarians	
	CC	p-value	CC	p-value	CC	p-value
Do you feel able to control HEV?	- 0.15	p > 0.10	0.33	p > 0.10	0.45	p > 0.10
Would your relatives want you to participate in an HEV control programme?	0.56	p > 0.10	0.60	p < 0.10	0.75	p < 0.05
Do you feel directly concerned by the HEV issue?	0.08	p > 0.10	0.44	p > 0.10	0.28	p > 0.10
Would controlling HEV be satisfactory?	- 0.25	p > 0.10	0.61	p > 0.10	0.28	p > 0.10
How do you perceive that better managing pig health would mitigate the risks for human health?	0.53	p > 0.10	0.67	p > 0.10	0.60	p < 0.10
Is hepatitis E an important issue for human health?	- 0.19	p > 0.10	0.24	p > 0.10	0.08	p > 0.10

Is hepatitis E an important issue for the pig production sector?

0.02 $p > 0.10$ 0.42 $p > 0.10$ 0.13 $p > 0.10$

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The multivariate analysis (PCA followed by HC) made it possible to evidence three clusters (Figure 4). The first axis was mainly represented by var4 (“Do you feel directly concerned by the HEV issue?”); the second axis was mainly represented by var6 (“How do you perceive that better managing pig health would mitigate the risks for human health?”) and var7 (“Is hepatitis E an important issue for human health?”). Var6 and var7 appeared orthogonal, thus independent.



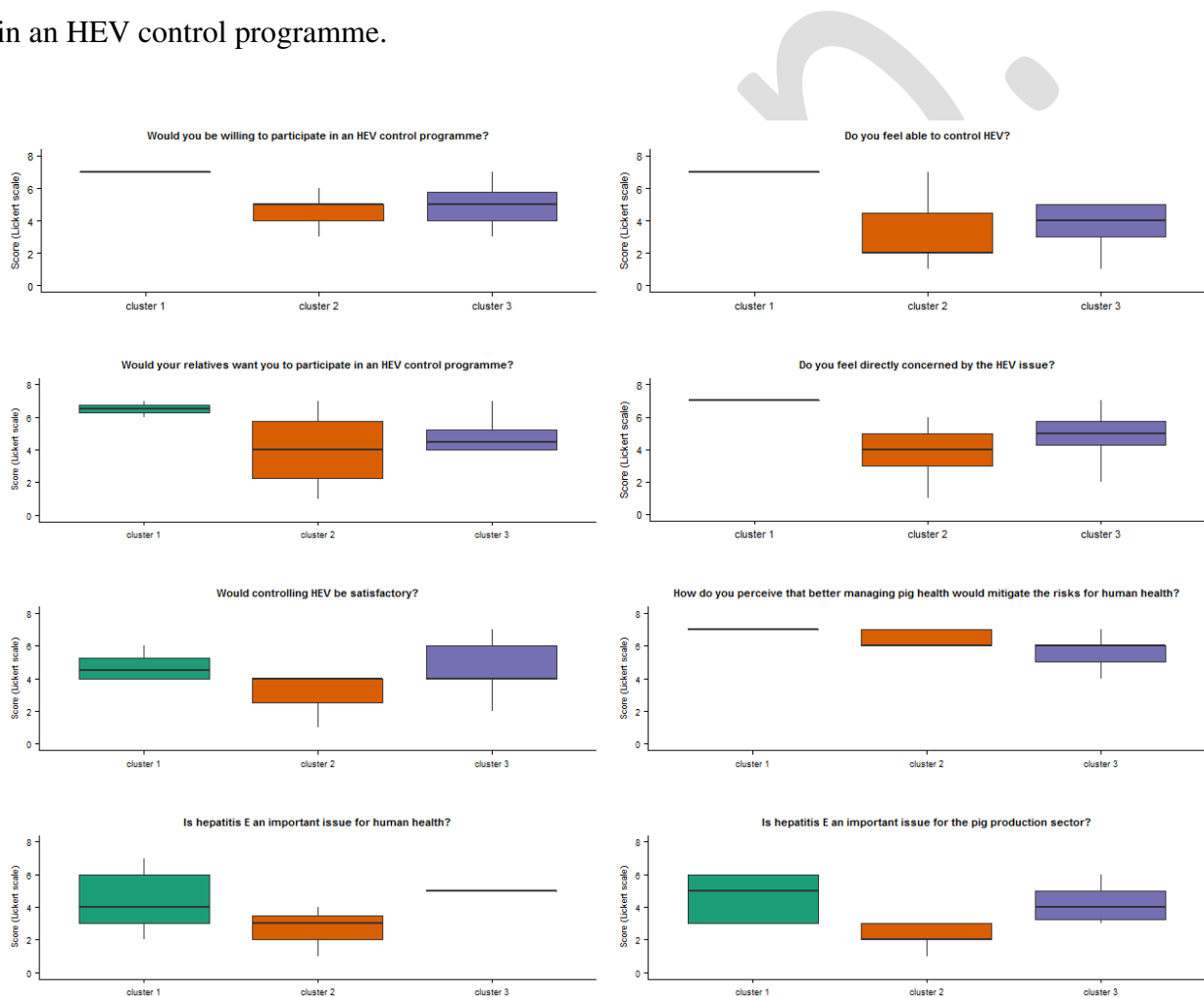
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Figure 4. Interviewees’ characteristics regarding behavioural determinants, as represented thanks to a principal component analysis followed by hierarchical clustering

Var1: Would you be willing to participate in an HEV control programme? Var2: Do you feel able to control HEV? Var3: Would your relatives want you to participate in an HEV control programme? Var4: Do you feel directly concerned by the HEV issue? Var5: Would controlling HEV be satisfactory? Var6: How do you perceive that better managing pig health would mitigate the risks for human health? Var7: Is hepatitis E an important issue for human health? Var8: Is hepatitis E an important issue for the pig production sector?

417 The smallest cluster (cluster 1) contains one farmer, one farming advisor and three
 418 veterinarians. With high scores to the eight questions, these interviewees were highly motivated
 419 by taking part in an HEV control programme and felt directly concerned by the issue (Figure
 420 5). The second one gathers three farmers, six advisors and two veterinarians. They had the
 421 lowest scores to all but one questions, especially to those regarding their ability to control HEV
 422 and the importance of the issue for human health and the pig production sector. However, they
 423 found particularly interesting the fact that better pig health management would help reduce risks
 424 for human health. The last one (cluster 3) hosts five farmers, three advisors and six veterinarians
 425 who had middle scores to most questions and a low score concerning their ability to participate
 426 in an HEV control programme.

427



428

429 **Figure 5. Distributions of interviewees' answers to eight questions included in the**
 430 **framework of the theory of planned behaviour depending on the cluster they belong to**

431

432

433

434 **4. Discussion and conclusion**

435

436 The primary interest of our study lies in the in-depth exploration of the potential barriers and
437 challenges that would arise from the implementation of an HEV control programme and in the
438 suggestion of levers favouring the stakeholders' involvement in HEV management strategies.
439 Assuming that pig farmers would be the major actors involved in the implementation of a future
440 on-farm HEV control plan, our study was designed as a three-level survey targeting similarly
441 farmers and their direct collaborators, i.e. farming advisors and veterinarians. This approach
442 made it possible to cross-reference the views of three categories of complementary actors,
443 working together on several technical and health issues of pig production. In this study, we
444 decided to focus on up-stream stakeholders only, but downstream surveys would be needed to
445 investigate the possibility of control plans at the slaughterhouse and/or processing plant levels.
446 The sample size was deliberately small to allow for a more detailed discussion of the topics
447 covered, hence increasing the validity of the investigation compared to short interviews which
448 would have been necessary to achieve a larger sample size (Crouch and McKenzie, 2006;
449 Alarcon et al., 2014). The surveyed sample cannot be considered as representative because of
450 the non-random selection procedure. Indeed, diverse interviewees' profiles were purposely
451 looked for, for instance to ensure that different farm types and the three major French producer
452 organisations were represented in the study. The interviewees were not nationally distributed
453 but mainly located in North-Western France, which is the biggest pig production area of the
454 country (Agreste, 2013; Salines et al., 2017b). It is therefore worth mentioning that the sample
455 composition is not adequate to extrapolate findings to the overall French pig farmer, advisor
456 and veterinarian population. Indeed, the respondents were voluntary to participate in the study,
457 thus suggesting that they are more involved in animal and public health issues. It is very likely
458 that a true random sample of interviewees would have yielded few or no people with intent to
459 be part of an HEV control programme and thereby not have been able to inform our study about
460 extrinsic and intrinsic barriers.

461

462 The interview template was designed in a way that the interviewees were first asked to give
463 their opinion on technical questions, which were considered easy, comfortable and non-
464 personal, before being led to broader considerations needing personal thinking. By doing so,
465 the interviews were conducted in a fluid manner and the questions were overall well understood.
466 Including information points during the interview appeared also relevant. Indeed, it made

467 possible for the interviewees to give their opinion in an informed way and to ask for
468 clarifications if needed. Moreover, it helped to raise their awareness on the issue and, by starting
469 with such small-scale awareness-raising operations, one could hope information and knowledge
470 to be disseminated through spill-over effects. The theory of planned behaviour was used as a
471 framework for the purpose of describing the decision-making process involved on the control
472 of HEV by farmers, advisors and veterinarians (Ajzen, 1991). This model presents several
473 limitations, notably the fact that it assumes that peoples' behaviour fits to a rational and
474 systematic decision-making process, which might not always be the case in real situations.
475 Nevertheless, this concept has already been used in several other studies dealing with risk
476 management in animal production sectors (Gunn et al., 2008; Ellis-Iversen et al., 2010; Alarcon
477 et al., 2014) and has enabled to evidence barriers to and/or drivers for disease control. In the
478 present case, our study was designed to identify and accurately understand behaviour's
479 determinants through stakeholders' own perceptions. Finally, combining qualitative and
480 quantitative analysis by alternating open- and closed-ended questions allowed for a more
481 comprehensive assessment of the stakeholders' opinions and behaviour.

482

483 The results from this study led to a number of noteworthy conclusions as regards barriers to and
484 drivers for the potential implementation of an HEV control programme by pig farming
485 stakeholders. One of the major outcomes of our survey is that most participants did not appear
486 reluctant to help tackle the HEV issue, with high scores concerning their willingness to
487 participate to an HEV control plan (86% of answers being above the mean score). This intent
488 to adopt HEV control measures was found affected by both extrinsic **(1)** and intrinsic **(2)** factors.

489

490 **(1)** First of all, like in the large-scale survey we have previously conducted (Salines et al., 2018),
491 the present study highlighted the lack of knowledge of and about HEV in all stakeholder
492 categories. As veterinarians have been identified as the main referent by the other actors, they
493 could act as a knowledge transfer channel. Other studies have shown that this lack of knowledge
494 was one of the reasons affecting people's decision-making process, e.g. explaining why farmers
495 did not implement biosecurity measures, some control programmes or adopt new technologies
496 on their farms (Gunn et al., 2008; Benjamin et al., 2010; Ellis-Iversen et al., 2010; Alarcon et
497 al., 2014; Ritter et al., 2015). However, if these studies alerted to the lack of awareness by
498 producers on current scientific research, the case of HEV appeared more complex to interpret.
499 Indeed, if all participants admitted their lack of knowledge about HEV, they also stressed the
500 numerous gaps in scientific knowledge that prevent them from considering disease control in

501 concrete terms. They would appreciate the effectiveness of the presented control strategies to
502 be confirmed with more solid data, for instance on-farm tested measures. Moreover, the absence
503 of any clinical signs or performance losses due to HEV infection of pigs was recognized as a
504 factor that would hinder the interviewees to implement on-farm risk mitigation strategies, as
505 evidenced in other studies (Alarcon et al., 2014). Regarding the technical aspects, they were
506 found closely related to the individual situation of farmers. For some of them, the required
507 changes in their farming practices would be marginal and would not necessitate much effort
508 from them. For the others, whose farm facilities appeared to be risky regarding HEV infection,
509 major investments would be needed and farmers seemed reluctant to make them, as it was
510 confirmed by veterinarians and advisors. Besides, if knowing farms' and livers' HEV status
511 sounded as a relevant option, the participants were not in favour of systematically testing all
512 traded live animals, this kind of highly restrictive measure being considered far-fetched and
513 impractical. Moreover, human resources were mentioned by the interviewees as a critical point
514 in farmers' business, meaning that these latter would have trouble with affording additional
515 labour (e.g. for an extra vaccination) or recruiting new employees. As shown in other
516 publications, the farmers' evaluation of a problem is generally performed in relation to other
517 hot topics or areas of focus which could overshadow other problems (Leach et al., 2010a;
518 Bruijnis et al., 2013; Horseman et al., 2014); this is currently the case e.g. with the external
519 threat linked to the African swine fever virus. Economics was also one of the major themes
520 identified consistently throughout the template, alternatively in a negative or positive manner.
521 Indeed, against the backdrop of global competition between markets and trading systems,
522 farmers also expressed concern that new standards or regulations would be imposed on them,
523 thereby distorting competition in comparison with foreign markets. For others, losing consumer
524 confidence in the product, e.g. due to a media scare, would have far-reaching consequences.
525 They also wish to overcome potential obstacles by turning challenges into opportunities: to their
526 mind, being involved in an HEV control programme would be a positive differentiating factor
527 on the market, like other labels, which would help them and the whole production sector to
528 move towards higher quality standards that could be financially valued. Financial incentives
529 could then be effective to stimulate producers' enrollment in such programmes. As wished by
530 the survey's participants, reducing external pressure would also be achievable through a
531 collective approach. It would mitigate the sense of isolation often felt by farmers, as described
532 in Alarcon et al. (2014), and provide them with collective support. Being part of an organized
533 and well-considered strategy would also help reduce potential mistrust and skepticism of
534 stakeholders, as well as the financial and technical burden. Most of the interviewees were in

535 favor of dedicating specific HEV-free farms for the production of liver-based products. This
536 kind of collective but targeted approach would make it possible to secure the sector without
537 impacting too many producers. The interviewees also would need organisational and
538 institutional support that would facilitate recommended changes and they mentioned other
539 organized systems existing for the control of zoonotic pathogens, such as *Salmonella* and
540 *Trichinella*. Interviewing actors from the downstream part of the production chain would be
541 highly relevant to discuss this risk mitigation strategy. Segmenting slaughter and process chains
542 to guarantee livers' traceability would probably be the major obstacles to this kind of specific
543 HEV-free production chain.

544

545 (2) The interviews also made it possible to highlight several intrinsic barriers to or, on the
546 contrary, motivators for HEV control. In the multivariate analysis, the most discriminant
547 variables were the ones related to the feeling of being directly concerned by the issue, to the
548 influence of better pig health management on the reduction of the risk for human health, and to
549 the importance of the issue for human health. This analysis made it possible to separate three
550 clusters of individuals. (i) One cluster gathers interviewees who did not feel able to participate
551 in HEV control, did not attach particular importance to their relatives' opinions and did not
552 consider HEV as an important issue, either for human health or for the pig production sector. It
553 highlights the fact that, despite the probably high number of HEV infections in industrialised
554 countries (Van Cauteren et al., 2017), the low number of actually reported cases leads to
555 underestimating the importance of the disease. However, they were highly interested in the fact
556 that better managing pig farms would help mitigate the risk of HEV for human health. This
557 cluster hosts mainly farming advisors and farmers. They can be considered as the most reluctant
558 group of people who would be probably the last to embrace the change. (ii) Another cluster
559 contains individuals who gave middle scores to almost all questions but who felt particularly
560 unable to participate in HEV control. This group gathers mainly veterinarians and farmers. One
561 could say that these people would not be either reluctant to or proactive in fighting HEV. They
562 would probably adopt a wait-and-see posture and would be willing to participate in HEV control
563 once the efficacy of the mentioned strategies would have been proven. (iii) Finally, the smallest
564 cluster contains individuals with high scores to all questions, with high motivation and self-
565 efficacy for an HEV control plan. In particular, helping tackle the HEV issue would give them
566 professional satisfaction and family recognition. This cluster gathers mostly veterinarians. It
567 could be considered as a group of leaders, who will take initiatives and stimulate change. This
568 clustering process allowed to identify where in the pathway to pathogen control a person – or a

569 group of persons – is. If information and awareness campaigns would be useful for all
570 stakeholders, one could say that involvement efforts should be focused on people being in the
571 pre-contemplation, contemplation or preparation stage of the transtheoretical model of change
572 (Prochaska and Di Clemente, 1982; Bamberg, 2013), corresponding to the two last clusters
573 described. Indeed, a control programme for such a non-regulated pathogen would need to be
574 incrementally set up, using the theories of increasing adoption rates (Vanloqueren and Baret,
575 2009). Leaders, for instance duos of highly engaged veterinarians and farmers, would help
576 unlock reluctance, disseminate innovations and better agricultural practices to the followers
577 (Rogers, 2003). They should be supported in their involvement, for instance if they get
578 committed to a niche market delivering HEV-free livers for liver-based products. Interfaces
579 between leaders and other producers should also be encouraged, in order for these local
580 innovations to be compatible with the dominating model (Geels and Schot, 2007; Bidaud,
581 2013).

582

583 In conclusion, collecting and analysing opinions from stakeholders before proposing HEV
584 control strategies was of major importance to guarantee the proper implementation of such a
585 plan. Our interview-based research has proven to be relevant for capturing the high variation of
586 opinions and perceptions amongst farmers, advisors and veterinarians but also for identifying
587 shared ideas and define three stakeholder clusters. From our results, potential hurdles (lack of
588 knowledge, scientific gaps, perceived inability to control HEV, low perception of the
589 importance of the issue) and preferred motivators (professional satisfaction, family recognition,
590 opportunity to achieve higher quality standards) have been highlighted. The importance of these
591 intrinsic and extrinsic circumstances highlights the need for socio-ecological behavioral
592 models, which acknowledge and incorporate the influences of external and internal factors on
593 someone's decision-making process. From a practical point of view, these outcomes are also
594 likely to help risk managers facilitate the implementation of an HEV control programme by
595 steering efforts to remove specific barriers and thereby creating favorable conditions for
596 zoonotic control on pig farms.

597 **Authors' contributions**

598 NR, MA and MS initiated and coordinated the project. CT and MS designed the questionnaires.
599 CT contacted the study participants, conducted the interviews, recorded and analysed the data.
600 CT and MS drafted the manuscript. All the co-authors revised the manuscript and approved the
601 final submitted version.

602

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607

608 **Competing interests**

609 The authors declare that they have no competing interests.

610

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Compte-rendu

Le virus de l'hépatite E dans la filière porcine. Compte rendu de la
réunion d'information et d'échanges du 25/06/2019

Le virus de l'hépatite E dans la filière porcine

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Objectifs de la réunion

Les objectifs de la réunion étaient d'une part de fournir un point d'information sur les données et travaux récents sur le VHE dans la filière porcine, d'autre part de susciter réflexions et échanges sur les stratégies pouvant être mises en place par les acteurs de la filière porcine.

Déroulé de la réunion

La réunion s'est tenue le 25 juin 2019, de 9h30 à 13h, dans les locaux de la Direction Générale de l'Alimentation à Paris. En amont de la réunion, les participants avaient reçu un rapport préliminaire synthétisant (i) les données récentes disponibles dans la littérature concernant le risque présenté par les produits porcins et l'épidémiologie du VHE dans les élevages de porcs ; (ii) les résultats du projet de recherche sus-cité, notamment en ce qui concerne l'influence des co-infections immunomodulatrices ainsi que la diffusion du virus dans la filière porcine. Le document s'accompagnait d'une synthèse des pistes d'action identifiées, formulées sous la forme de propositions à discuter avec les organisations publiques et privées gestionnaires du risque. La réunion s'est déroulée en deux phases : une première phase de présentation, suivie d'une discussion autour de trois thèmes : (i) quelle gestion possible du VHE dans l'amont de la filière porcine ? (ii) quelle gestion possible du VHE dans l'aval de la filière porcine ? (iii) quels futurs besoins de recherche ? La réunion a été co-animée par Morgane Salines, Nicolas Rose et Charlotte Teixeira-Costa, de l'unité de recherche en Epidémiologie, Santé et Bien-Être du laboratoire de l'Anses de Ploufragan/Plouzané/Niort.

Participants

La réunion a regroupé 38 participants. Les représentants de plusieurs organisations étaient présents : l'Anses, Santé Publique France (SPF), la Direction Générale de l'Alimentation (DGAI), la Direction Générale de la Santé (DGS), le Centre National de Référence (CNR) des virus hépatiques à transmission entérique, la Fédération française des Industriels Charcutiers Traiteurs (FICT), la Confédération Nationale des Charcutiers Traiteurs (CNCT), la

Confédération Française de la Boucherie, Charcuterie, Traiteurs (CFBCT), Coop de France, l'Interprofession Nationale Porcine (INAPORC), l'Association Nationale Sanitaire Porcine (ANSP), la Fédération Nationale Porcine (FNP), la Fédération du Commerce et de la Distribution (FCD), le Syndicat National de l'Industrie de la Nutrition Animale (SNIA), Cirhyo, Tradival.

Première partie : Présentation

Une version papier de la présentation a été remise aux participants en début de réunion. La présentation a porté sur (i) les caractéristiques de l'hépatite E chez l'homme, (ii) les voies de transmission zoonotique du VHE, et en particulier le risque posé par les produits à base de foie de porc et les autres produits à base de porc, (iii) les caractéristiques épidémiologiques du VHE dans les élevages de porcs (voie de transmission, prévalence, facteurs de risque, (iv) les pistes de maîtrise possibles du VHE en élevage déterminées, entre autres, par des approches de modélisation, (v) les premiers retours des acteurs de terrain (éleveurs, vétérinaires praticiens, conseillers d'élevage) quant à la mise en place pratique de ces mesures dans les élevages. Les diapositives ont été envoyées par courriel à l'issue de la réunion.

Seconde partie : Discussion des mesures de gestion possibles dans la filière porcine et des futurs besoins de recherche

Axe I : Possibilités de maîtrise du VHE dans l'amont de la filière

Les pistes d'action suivantes ont retenu toute l'attention des participants :

- ✓ Sensibiliser les éleveurs à la problématique et aux possibilités de réduction du risque de propagation et de persistance du VHE dans leur élevage.
- ✓ En ce sens, retenir le VHE (éventuellement en complément d'autres pathogènes zoonotiques) comme thème de sensibilisation lors de la prochaine campagne de visite sanitaire porcine.
- ✓ Accompagner les élevages (particulièrement ceux de grande taille ayant un mode de production intensif) vers des pratiques de biosécurité externe (sas sanitaire, quarantaine) et interne (compartimentation, gestion des flux) et de conduite (limitation des adoptions et des mélanges, gestion des effluents) plus sûres.
- ✓ Soutenir la mise en place de programmes d'éradication des pathogènes immunomodulateurs, notamment du virus du SDRP.

Axe II : Possibilités de maîtrise du VHE dans l'aval de la filière

Les éléments suivants ont été jugés prioritaires pour la gestion du VHE dans les produits :

- ✓ Envisager la qualification d'élevages indemnes de VHE qui pourraient approvisionner en foies le marché des produits à base de foie susceptibles d'être consommés crus.

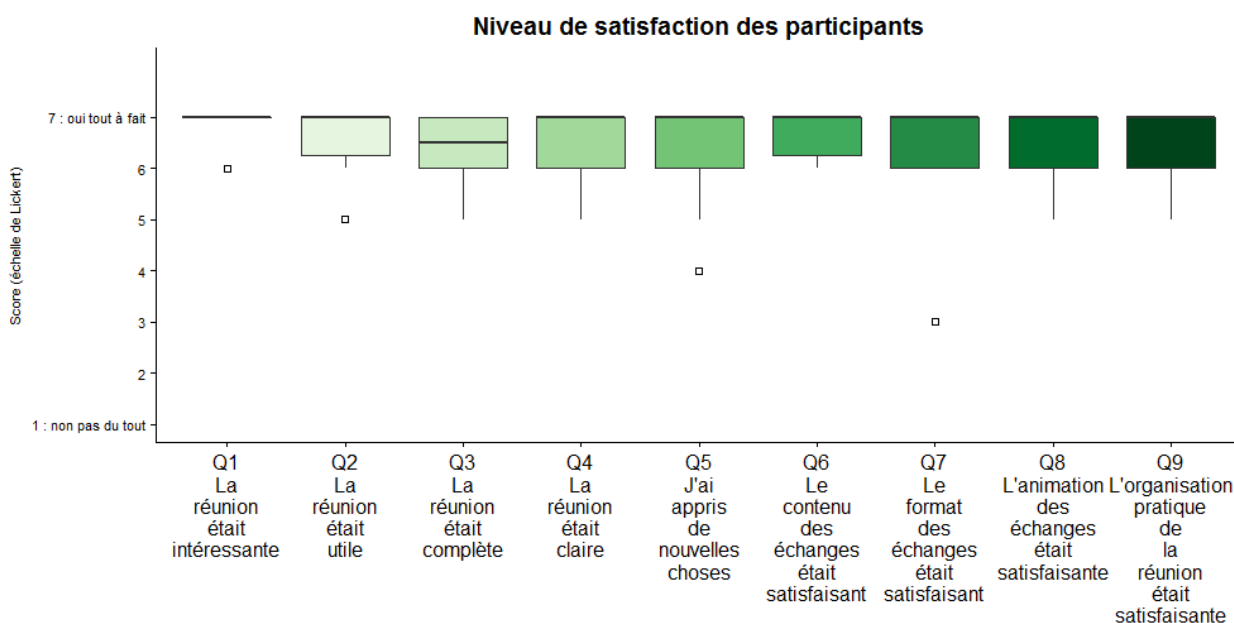
- ✓ Inclure le VHE dans les prochains plans de surveillance et plans de contrôle, en utilisant les facteurs de risque identifiés dans la littérature.
- ✓ En ce sens, inclure le VHE comme future thématique d'intérêt pour la plateforme de Surveillance de la Chaîne Alimentaire.
- ✓ Renforcer (i) le contrôle de l'étiquetage des produits à risque et de l'information des consommateurs et (ii) la sensibilisation des personnes à risque.

Axe III : Futurs besoins de recherche sur la thématique du VHE

Nombreux ont été les besoins de recherche dans la filière porcine identifiés par les participants :

- ✓ Réaliser une étude d'intervention en élevage pour tester l'efficacité en conditions réelles des mesures identifiées.
- ✓ Explorer la situation sanitaire des élevages dits alternatifs (non-conventionnels) et identifier les facteurs de risque associés à ces modes de production.
- ✓ Investiguer la situation sanitaire des élevages de sélection et de multiplication.
- ✓ Identifier les génotypes circulant actuellement dans la population porcine pour les comparer avec ceux circulant dans la population humaine.
- ✓ Poursuivre les travaux de recherche relatifs à la présence du VHE dans les muscles, le sang, et autres tissus de porcs ainsi que dans les produits à base de porc ne contenant pas de foie.
- ✓ Poursuivre les travaux concernant l'efficacité et la faisabilité de possibles traitements assainissants des produits finis, notamment la pascalisation, le séchage et la salaison.
- ✓ Concevoir un plan d'échantillonnage pour la recherche du VHE dans les foies destinés à la fabrication de produits crus.

Au bilan, la réunion a été jugée satisfaisante par l'ensemble des participants. Il est souhaitable que ce type d'initiative soit régulièrement renouvelé pour une information et une concertation efficaces de tous les acteurs de la filière porcine et de la santé humaine.





Ce qu'il faut retenir

En mobilisant différentes méthodes de sciences humaines et sociales, la dernière partie de ce projet de recherche a permis de mettre en évidence des freins pouvant compromettre l'effectivité de stratégies de maîtrise du HEV dans les élevages de porcs. Des leviers ont cependant pu être dégagés et permettront aux gestionnaires du risque de créer un contexte favorable à la mise en place d'un programme de lutte contre le HEV dans la filière porcine.



Take home message

By mobilizing different methods from social sciences, the last part of this research project highlighted obstacles that could jeopardize the effectiveness of HEV control strategies on pig farms. Nevertheless, levers have been identified that will allow risk managers to create a favourable context for the implementation of an HEV control programme in the pig production sector.



Chapitre V

Discussion générale

PARTIE 5.1. Analyse critique de la stratégie et des choix méthodologiques

I. Conception et organisation du projet

Ce projet de recherche a été conçu comme un **projet pluridisciplinaire**. En effet, ces travaux allient différentes disciplines, notamment l'**épidémiologie** et les **sciences humaines et sociales**. Au sein même de l'épidémiologie, plusieurs approches ont été employées : **suivi de cohortes, modélisation à plusieurs échelles** et dans différents langages de programmation, complétés par des **essais expérimentaux**. Cette démarche pluridisciplinaire est, d'une part, cohérente avec un cursus de formation préalable qui joignait sciences dures (études vétérinaires, master de santé publique, CEAV en santé publique) et sciences molles (master de management, master de sciences politiques). D'autre part, cette approche globale de la thématique du HEV est la garantie d'une **réponse plus adaptée et plus juste à la problématique**, car la biologie des populations animales ne peut être détachée des comportements humains.

La pluridisciplinarité ne va pas sans l'inclusion de **partenaires aux compétences variées** dans le montage et le déroulement du projet. Ainsi, en plus de l'encadrement rapproché de la thèse, les travaux ont été réalisés en collaboration avec des équipes de virologie et des équipes techniques de plusieurs sites de l'Anses, un vétérinaire informaticien suédois (SVA), ainsi qu'avec une psychologue de l'université de Rennes. Le comité de suivi de la thèse a également réuni des professionnels de différentes disciplines pour bénéficier de propositions hors du cœur de notre champ de compétences. Enfin, tout au long de ce **projet multipartenarial**, nous avons également veillé à travailler en lien avec les financeurs du projet, aussi destinataires et utilisateurs finaux de nos résultats (Direction générale de l'alimentation du Ministère en charge de l'Agriculture, Interprofession Nationale Porcine), et à leur fournir des **éléments d'aide à la décision pertinents**.

En effet, le projet de thèse vise autant à apporter des connaissances scientifiques fondamentales qu'à fournir des **recommandations pratiques, réalistes et réalisables** aux

organisations publiques et privées en charge de la gestion du risque HEV. Ainsi, dans l'ensemble des études réalisées, y compris les essais expérimentaux et les approches de modélisation, nous avons tenu à dégager des **résultats connectés au terrain**. Cet attachement à la réalité du terrain s'est aussi traduit par un **ancrage territorial**, puisque toutes les données utilisées ou produites correspondent à la situation française (données de mouvement, suivi de cohortes, niveaux de prévalence des pathogènes intercurrents, *etc.*). Par ailleurs, le projet s'est focalisé sur **l'amont de la filière porcine**, dans une optique **de la fourche à la fourchette** qui met la prévention de la contamination des produits au cœur de la gestion des risques, plutôt que la gestion des produits contaminés. Les deux démarches ne sont évidemment pas mutuellement exclusives mais l'approche par l'amont correspond davantage à la stratégie sanitaire adoptée en France. Elle permet aussi de **décloisonner la santé animale et la sécurité sanitaire des aliments**, par exemple en tenant compte, dans notre cas, de l'effet majeur des pathogènes intercurrents de santé animale sur la diffusion d'un pathogène de santé publique.

En termes d'organisation pratique, le projet a été conçu plus d'un an à l'avance ce qui a permis une **planification juste et cohérente des travaux**. Les trois volets présentés dans les chapitres précédents ont été conduits en parallèle et dans un ordre dépendant de la disponibilité des données. Deux comités de suivi ont permis de dresser des bilans intermédiaires et d'apporter de nouvelles orientations. Un attachement particulier a été apporté à la **valorisation régulière** des résultats afin de diffuser les nouvelles connaissances à chaque étape du projet. Si les publications scientifiques internationales sont certainement incontournables d'un point de vue académique, nous avons aussi veillé à transmettre les résultats aux principales parties prenantes au travers d'articles en français dans des journaux scientifiques, des revues professionnelles, des fiches d'information, *etc.* Les communications orales dans des congrès nationaux et internationaux et des journées professionnelles ont également fait partie de la valorisation de nos travaux, ainsi que quelques séances d'enseignement. Dans le souci constant de rendre compte de la progression du projet à une hiérarchie géographiquement distante, des rapports annuels d'avancement et des présentations régulières ont aussi été réalisés. Finalement, cette **stratégie de valorisation ciblée et différenciée** est, selon nous, garante de l'information du plus grand nombre. Cette communication n'incluait pas les étapes aval de la chaîne de production, comme les consommateurs, qui ne sont pas directement concernés par des mesures de maîtrise du risque dans les élevages. Il serait néanmoins judicieux que les services vétérinaires départementaux, les vétérinaires officiels en abattoir et les responsables privés des abattoirs bénéficient d'informations actuelles et ciblées sur la thématique du HEV.

II. Orientations et choix méthodologiques

Différents **niveaux de représentation** ont été adoptés dans les études précédemment présentées. **(1)** Par exemple, dans le modèle intra-troupeau, une **représentation individu-centrée** a été choisie pour représenter la diffusion du HEV dans un élevage de porcs naisseur-engraisseur. L'échelle individu-centrée permet de caractériser explicitement chaque individu du système par un ensemble de variables (âge, sexe, statut physiologique, localisation physique, *etc.*). Ainsi, notre modèle a permis d'incorporer le degré d'hétérogénéité de la population et les interactions entre individus, ainsi que de gérer les statuts infectieux de manière individuelle. Ce niveau de détail a toute son importance pour la représentation fine des co-infections par les virus immunomodulateurs à l'échelle individuelle. En revanche, dans le modèle inter-troupeaux, nous avons opté pour un moindre niveau de détails avec une **représentation à l'échelle de la case**. Ce choix découle de plusieurs raisons. D'une part, l'objectif de ce modèle est de représenter et comprendre la diffusion du HEV à l'échelle d'un territoire : pour ce faire, la simple représentation d'une dynamique intra-élevage cohérente avec les données de la littérature et les résultats du modèle intra-troupeau a été suffisante, et une représentation fine à l'échelle individuelle (comme dans le modèle intra-troupeau) n'a pas été nécessaire. D'autre part, l'échelle de la case permet de représenter la diffusion environnementale locale intra-case, telle que décrite par les études précédemment menées. Enfin, en termes d'efficacité computationnelle, une représentation plus fine était difficilement envisageable dans les délais contraints de notre projet. **(2)** Dans le dernier chapitre de la thèse mobilisant les sciences sociales, nous nous sommes également placés à deux échelles différentes. Dans la première étude, l'objectif était d'évaluer le niveau de connaissances du HEV des acteurs de la filière, et ce à l'échelle d'une **large population** d'éleveurs et de vétérinaires, considérés comme représentatifs de leur profession. A l'inverse, la seconde enquête visait à analyser en détail les freins et motivations qui, à l'échelle individuelle, seraient susceptibles d'influencer la mise en place d'un plan de lutte contre le HEV dans les élevages de porcs, d'où le choix méthodologique des **entretiens individuels**. Là encore, la stratégie du niveau de représentation a été dictée par les objectifs poursuivis.

L'une des principales caractéristiques des deux modèles développés dans ce projet est qu'ils sont **fondés sur données réelles**. **(1)** En effet, les paramètres épidémiologiques du modèle intra-troupeau dérivent majoritairement d'essais expérimentaux réalisés dans les

installations de la structure d'accueil. L'**approche expérimentale** permet l'estimation de paramètres épidémiologiques associés à un phénomène infectieux tout en contrôlant parfaitement les conditions environnementales (logement, conduite des animaux, statut sanitaire - notamment vis-à-vis des pathogènes intercurrents), ce qui limite ainsi la variabilité observée en conditions naturelles. Cet outil, dont l'utilisation a d'ores et déjà montré son efficacité dans divers travaux menés au sein du laboratoire, a permis d'estimer les paramètres de la dynamique d'infection du HEV chez des animaux exempts de pathogènes intercurrents, mais également de quantifier l'effet strictement spécifique de la co-infection par des virus immunomodulateurs. Cette forte spécificité des résultats obtenus en conditions expérimentales, combinée au faible effectif d'animaux inclus dans les expérimentations, peut néanmoins limiter l'extrapolation possible des résultats en conditions naturelles. Ceci a été contrebalancé par la réalisation d'un **suivi longitudinal individuel en conditions naturelles** sur les animaux de trois bandes dans trois élevages. Grâce à cette enquête de cohorte, les résultats obtenus en situation expérimentale ont été confirmés et d'autres effets individuels (sexe, parité de la truie) ont été mis en évidence. Cette étude a également montré l'effet additif des co-infections par le virus du SDRP et le PCV2, ce qui aurait pu être intéressant de tester en conditions expérimentales pour quantifier l'impact d'une triple infection, fréquemment rencontrée sur le terrain. Il convient aussi de noter que, pour les études observationnelles, les résultats observés reposent sur une notion probabiliste d'augmentation d'un risque, sans possibilité de mettre en évidence une relation causale comme c'est le cas dans le cadre d'expérimentations animales.

(2) De la même manière, le modèle inter-troupeaux repose sur des données réelles, puisque les paramètres épidémiologiques sont les mêmes que dans le modèle intra-troupeau, et les paramètres démographiques ont été adaptés à partir des données de mouvements enregistrées dans la base de données nationale BDporc. Ceci permet donc une **représentation explicite des échanges de porcs**, plutôt que d'inclure dans le modèle inter-troupeaux un réseau théorique. Notons néanmoins que, si la démographie inter-élevages repose bien sur des données réelles, elle a été simplifiée et approchée de manière probabiliste, qui ne permet pas de prendre en compte l'évolution des échanges dans le temps de manière fine. De plus, la démographie intra-élevage a elle aussi été standardisée (taille des élevages, type de conduite), ce qui ne permet pas d'explorer certains facteurs de variation des conditions réelles.

Une autre spécificité de ce projet est de proposer des **méthodes à la fois théoriques et appliquées** à la thématique d'intérêt pour en dégager des résultats. (1) Par exemple, le modèle intra-troupeau multi-pathogènes que nous avons développé peut être vu comme un **cadre**

conceptuel et méthodologique adaptable à la diffusion conjointe d'autres pathogènes. C'est une méthode particulièrement originale et innovante puisqu'elle permet de prendre en compte, de manière stochastique, quantitative et individualisée, les interactions entre deux pathogènes, ce qui, à notre connaissance, n'est actuellement pas décrit dans la littérature. Au-delà de son utilité théorique, cette méthode nous a permis de répondre de manière pertinente et ciblée à la problématique de ce volet de la thèse en dégagant des facteurs explicatifs de la dynamique d'infection par le HEV dans un élevage naisseur-engraisseur dont les animaux peuvent être infectés par des pathogènes intercurrents. (2) De même, nous avons présenté dans le chapitre II de ce manuscrit des méthodes génériques appliquées au HEV. Ainsi, les données de mouvements de porcs ont été modélisées sous la forme de deux types de réseaux : l'un prenant en compte uniquement la transmission de pathogènes par le transfert d'animaux infectés d'un élevage à un autre, l'autre intégrant en sus la possibilité d'une contamination vectorielle mécanique par le seul passage d'un camion de transport dans un élevage sans introduction d'animaux. Si le premier réseau est adapté à la problématique du HEV et a ainsi fondé la structure du modèle inter-troupeaux, le second pourra à l'avenir être utilisé pour l'étude d'autres pathogènes porcins, comme les virus de la diarrhée épidémique porcine ou de la peste porcine africaine. (3) De plus, le modèle inter-troupeaux que nous avons développé a représenté un défi méthodologique et informatique certain et cette approche complexe n'avait jusqu'à ce jour pas été utilisée dans une filière de production porcine. En ce sens, nous avons conçu le modèle de telle sorte qu'il puisse être facilement adapté dans des travaux futurs pour explorer la transmission entre fermes, ou l'introduction dans un territoire indemne, d'autres agents d'intérêt pour la filière porcine, comme le virus de la peste porcine africaine. Néanmoins, en l'état, notre modèle inter-troupeaux pêche par son manque de souplesse, notamment pour effectuer des manipulations du réseau, simulant par exemple des restrictions dans les échanges de mouvements d'animaux, qui seraient nécessairement imposées dans le cas d'une maladie règlementée comme la peste porcine africaine. (4) Enfin, le chapitre IV du présent manuscrit s'attache à la mise en place pratique des mesures de maîtrise dégagées, c'est-à-dire à l'évaluation de leur faisabilité et de leur acceptabilité par l'ensemble des acteurs concernés, **phase finale indispensable dans la conception d'un plan de lutte**. L'originalité de ce volet est d'allier **différentes méthodes d'enquête sociologique** : questionnaire quantitatif diffusé par voie électronique à une large population représentative, entretiens semi-directifs auprès d'un petit échantillon d'acteurs à une échelle locale, approche participative avec une réunion de concertation collective de représentants d'organisations publiques et privées évaluateurs et gestionnaires du risque. Faute de temps, les acteurs de l'abattage (notamment responsables

qualité des abattoirs et services vétérinaires d'inspection) n'ont pas été inclus dans l'échantillon des entretiens semi-directifs, mais les représentants de l'aval de la chaîne de production porcine étaient présents à la réunion de concertation et ont ainsi pu faire émerger de nouvelles orientations possibles sur la gestion de cette problématique à l'abattoir.

Au bilan, nous nous sommes efforcés, tout au long de ce projet de recherche, de proposer des **contributions méthodologiques innovantes et adaptables** et qui permettent, en même temps, de dégager des **éléments de réponse scientifiques et pragmatiques** à la problématique spécifique du HEV.

PARTIE 5.2. Discussion des principales conclusions et mise en perspective du projet de recherche

I. Contribution à la compréhension et à la maîtrise de l'infection par le virus de l'hépatite E dans les élevages de porcs

Ce projet de recherche a contribué à l'acquisition de nouvelles connaissances relatives à la dynamique de l'infection par le HEV dans la filière porcine. Tout d'abord, le volet intra-élevage de la thèse a permis de fournir des **éléments d'explication de la variabilité de la dynamique d'infection** qui prennent en compte à la fois la structure de l'exploitation, les pratiques d'élevage et la situation sanitaire. Le suivi longitudinal sur le terrain a permis d'objectiver la variabilité inter-bandes et inter-élevages, de mettre à jour de nouveaux facteurs individuels de variation qui n'avaient pas été rapportés dans la littérature jusqu'ici (sexe, parité de la truie) et de quantifier le rôle des virus immunomodulateurs sur l'infection par le HEV. De manière quasi-concomitante à la publication de nos travaux, l'équipe de Krog *et al.* (2019) a également réalisé un suivi longitudinal pour étudier la dynamique de l'infection par le HEV dans un élevage unique. Si cette enquête apporte quelques éléments additionnels concernant l'effet de l'immunité maternelle, cohérents avec la littérature déjà publiée, elle reste néanmoins assez descriptive et ne met pas en évidence d'autres facteurs de variation de la dynamique d'infection. En complément de notre étude longitudinale, nous avons conduit deux essais expérimentaux (l'un avant la thèse, l'autre pendant la thèse) qui ont **confirmé et quantifié l'impact des pathogènes intercurrents** (virus du SDRP, PCV2) sur la dynamique d'infection et sur le risque HEV pour la santé publique. Parallèlement à nos travaux, l'étude de Jackel *et al.* (2019) a également suggéré l'existence d'une corrélation entre l'infection par le HEV et celle par le PCV2. Grâce à notre approche expérimentale, nous avons aussi mis en évidence la présence du HEV dans le sang et les muscles de porcs co-infectés par le virus du SDRP, ce qui apporte de nouveaux éléments d'analyse sur la question d'un **éventuel risque HEV lié à la consommation de produits à base de porc ne contenant pas de foie**. Le modèle que nous

avons développé à la suite de ces études contribue à une vision plus globale de l'infection par le HEV dans un élevage naisseur-engraisseur, ce type d'élevage représentant plus d'un tiers des exploitations porcines françaises. C'est le premier modèle de ce type publié à ce jour, **alliant dynamique démographique et dynamique infectieuse, de surcroît multi-pathogènes**. A partir de ce modèle, nous avons notamment montré que le type de conduite en bandes et le mode de logement des truies en gestation avaient un impact majeur sur les caractéristiques de diffusion du HEV, avec une augmentation du risque HEV en lien avec l'intensification de la conduite et l'allotement des truies en grands groupes. La modification de ces deux facteurs n'a pas été testée en tant que mesures de maîtrise, car ce ne sont pas des pratiques à proprement parler mais bien des éléments structurels de l'exploitation : il est par exemple impensable qu'un éleveur modifie son type de conduite en bandes du seul fait d'un risque HEV plus élevé (même si des changements de type de conduite en bandes sont réalisés en pratique pour d'autres motifs, telles que l'organisation du travail ou des raisons sanitaires). Mais ces deux indicateurs peuvent néanmoins permettre de **caractériser les élevages selon leur niveau de risque** et d'exercer ainsi une vigilance accrue sur certains types d'exploitations ; cela pourrait alors participer de la conception d'une **stratégie de surveillance ciblée, fondée sur le risque**. Par la mise en lumière d'autres facteurs de variation de la dynamique de l'infection par le HEV, le modèle a également permis de dégager des **pistes de maîtrise du HEV** au sein d'un élevage, tant de nature zootechnique que sanitaire, corroborées par des données de terrain : réduction des adoptions, meilleure gestion des mélanges de porcs en croissance, éradication ou contrôle vaccinal des pathogènes intercurrents. Ces stratégies représentées et évaluées dans le modèle intra-troupeau correspondent à des **mesures réalistes** que les éleveurs pourraient mettre en place dans leur élevage. Certaines stratégies qui ne pourraient être développées pour des raisons économiques ou techniques n'ont délibérément pas été représentées, comme une vaccination anti-HEV. Les résultats que nous avons obtenus dans cette partie du projet constituent des pistes pertinentes de maîtrise du HEV.

Mais une gestion globale du HEV dans la filière porcine ne saurait être envisagée à l'échelle stricte des élevages pris isolément. En effet, nos travaux préliminaires ont permis de mettre en évidence l'impact des échanges d'animaux sur le risque de positivité HEV des élevages, et donc l'importance de prendre en compte ces mouvements pour comprendre la dynamique du HEV dans la filière. Ainsi, le modèle que nous avons développé par la suite à l'échelle territoriale a révélé des **différences de patterns de diffusion spatiale du HEV** selon le type d'élevage d'introduction. Bien que le haut de la pyramide de production porcine soit

souvent considéré, à raison, comme un maillon ayant un effet majeur sur la situation sanitaire globale de la filière, nous avons ici montré que la problématique du HEV était sensiblement plus complexe, et qu'au type d'exploitation devait être associé le statut sanitaire de l'élevage vis-à-vis des pathogènes immunomodulateurs pour une compréhension fine de la dynamique du HEV à l'échelle d'une population d'élevages. En faisant varier la proportion d'élevages de production infectés par des virus intercurrents, nous avons pu confirmer le rôle crucial de ces derniers, à l'échelle plus globale d'un territoire cette fois-ci, et ainsi souligner une nouvelle fois la nécessité de renforcer les programmes d'éradication ou de contrôle de ces co-infectants. Le modèle que nous avons développé, qui a le mérite d'être particulièrement novateur, reste néanmoins encore **expérimental**. En effet, si nous avons exploré la diffusion du HEV dans une communauté d'élevages, nous ne nous sommes pas placés dans une situation réaliste correspondant au contexte français actuel : nous avons simulé une introduction unique du HEV dans un cluster d'élevages indemnes du HEV, alors qu'à ce jour 65 % des élevages français sont séropositifs et qu'il est fort probable que les introductions d'animaux infectés par le HEV soient multiples. **Les résultats obtenus sont donc théoriques et ne peuvent être extrapolés tels quels en conditions réelles**. Ils donnent également des indications sur les principaux éléments conduisant à une diffusion ou non dans une population d'élevages, de manière à évaluer l'effet relatif de certaines conditions sur le potentiel de diffusion par rapport à d'autres, sans établir des prédictions strictes et absolues. Ils permettent également d'évaluer le risque que représenterait l'introduction du HEV dans une potentielle filière spécifique labellisée « HEV-free ».

Au bilan, cette approche multi-échelles de la diffusion et la persistance du HEV a permis de proposer un plan d'action pour réduire le risque de présence de produits porcins contaminés dans la chaîne de production. Ces pistes de maîtrise, formulées sous cinq axes complémentaires (élevage, filière, surveillance, communication, recherche), ont été soumises à l'avis des parties prenantes afin de garantir leur faisabilité de mise en place. A l'issue d'une réunion de concertation avec ces acteurs, **15 pistes d'action** ont finalement été retenues. L'enquête individuelle que nous avons conduite auprès d'éleveurs, de techniciens d'élevage et de vétérinaires a quant à elle permis de mettre en évidence un certain nombre de facteurs qui pourraient limiter la mise en place effective d'un plan de lutte et de dégager des leviers pour motiver les acteurs. Fournir de tels éléments aux gestionnaires du risque permettra de veiller à **créer un contexte favorable** à la mise en place d'un programme de lutte contre le HEV dans la filière porcine.

II. Pistes de maîtrise du risque lié au virus de l'hépatite E dans la filière porcine

Plusieurs mesures de maîtrise ont été proposées dans les chapitres précédents de la thèse, et notamment dans les rapports d'aide à la décision. A ce stade, **des préconisations aux éleveurs et à leurs accompagnants peuvent être prudemment formulées**. S'il est sûrement trop tôt pour fournir des conseils très spécifiques, il convient d'insister une nouvelle fois sur des **recommandations générales de biosécurité externe et interne** (présence et utilisation correcte d'un sas sanitaire, nettoyage et désinfection efficaces des installations, *etc.*). La **réduction des adoptions et des mélanges des porcs en croissance** doit également être favorisée, de même que la **maîtrise des pathogènes intercurrents**. A l'échelon des élevages, une **stratégie de surveillance fondée sur le risque** pourrait être développée, en ciblant les élevages à risque selon leur localisation géographique, l'intensité de leurs échanges commerciaux de porcs, leur taille, leur type de conduite en bande, le type d'installations pour le logement de leurs truies gestantes, leur type de production. La surveillance « aval » pourrait également être fondée sur le risque, en **incluant le HEV dans les prochains plans de surveillance et de contrôle (PSPC) annuels sur carcasses, abats et produits transformés** et en ciblant les produits à surveiller selon le niveau de risque de leur élevage d'origine et/ou le niveau de risque qu'ils présentent pour le consommateur (fonction des ingrédients, du mode de cuisson, *etc.*). Ces PSPC HEV permettraient de suivre l'évolution des niveaux de contamination des produits et de détecter la présence éventuelle du virus dans de nouveaux produits.

En plus de ces réflexions à l'échelle individuelle (des animaux, des produits, des élevages), nos résultats ont montré qu'il serait possible d'envisager la **structuration d'une filière spécifique** permettant aux élevages reconnus indemnes de HEV de fournir des foies sains pour la fabrication des produits à risque. Ceci permettrait de répondre aux exigences sanitaires spécifiques d'un marché restreint, de niche - celui des produits contenant du foie de porc et susceptibles d'être consommés crus ou insuffisamment cuits - tout en n'engageant des mesures de maîtrise que dans un nombre limité d'élevages. Ceci permettrait de garantir un haut niveau de sécurité sanitaire tout en appliquant des **principes pragmatiques et rationnels de parcimonie** et en veillant à **une utilisation judicieuse et raisonnée des ressources publiques et privées**. La sélection des élevages indemnes ou souhaitant devenir indemnes devrait alors se faire selon les facteurs de risque énoncés ci-dessus. Néanmoins, la mise en place d'une telle

démarche de segmentation nécessiterait au préalable *(i)* le **développement de tests** rapides et peu onéreux à l'abattoir ; *(ii)* de **connaître l'évolution dans le temps du statut des élevages** et de quantifier le risque pour un élevage de perdre son statut indemne ; *(iii)* d'acquérir davantage de données quant à la **contamination possible du sang et de la viande** de porcs infectés par le HEV ; *(iv)* de mettre en place une **traçabilité rigoureuse** à tous les échelons de la filière ; *(v)* de prévoir une **stratégie commerciale adaptée**, avec probablement une rémunération appropriée des éleveurs s'engageant dans cette qualification. Ceci participerait donc d'une **politique globale de filière**. Dans un contexte économique mondial très concurrentiel et face à des pays « gros » producteurs, miser sur cette **qualité sanitaire** serait en parfaite cohérence avec les stratégies actuelles de **démarquage commercial sur des signes de qualité**.

La mise en place d'un plan de lutte contre le HEV nécessite de **définir les responsabilités et les actions respectives du secteur public et du secteur privé**. A ce jour, **aucune réglementation spécifique** n'existe concernant le HEV dans la filière porcine. De manière générale, il appartient aux professionnels de **garantir la mise sur le marché de produits propres à la consommation humaine**, c'est-à-dire (entre autres) ne présentant pas de risque pour la santé humaine (Anonyme, 2002). Le règlement (CE) n° 2160/2003 rappelle aussi qu'il « importe que la responsabilité principale en matière de sécurité alimentaire incombe aux exploitants des secteurs de l'alimentation humaine et de l'alimentation animale » (Anonyme, 2003). Ils s'appuient pour cela sur leur **plan de maîtrise sanitaire** (PMS) qui comprend notamment les mesures d'application des **bonnes pratiques d'hygiène** (BPH), ainsi que les mesures de maîtrise définies dans le cadre d'une analyse HACCP (*Hazard Analysis – Critical Control Point*). Les professionnels peuvent également décider de renforcer le dispositif de maîtrise d'un pathogène par des initiatives allant plus loin que la réglementation en mettant en place des **chartes qualité d'application volontaire** et des **outils de surveillance renforcée**. En tant qu'agent zoonotique, le HEV peut aussi être considéré comme faisant partie de la **responsabilité sociétale des entreprises** (RSE)²⁷. Le guide de l'Association Nationale des Industries Agro-alimentaires (ANIA) indique par exemple dans la catégorie « protection de la santé et de la sécurité des consommateurs » que l'entreprise se doit d'anticiper les évolutions

²⁷ La responsabilité sociétale des entreprises (RSE) désigne la prise en compte par les entreprises, sur base volontaire, des enjeux sociaux et éthiques dans leurs activités.

réglementaires, d'être dans une démarche d'amélioration continue et d'adopter un mode proactif sur cette thématique²⁸.

A titre d'exemple, la problématique de la gestion du HEV peut être comparée à celle de ***Salmonella* dans la filière porcine**, pour laquelle un dispositif de maîtrise existe (Figure 28). Les objectifs et modalités du contrôle de *Salmonella* sont définis dans le règlement européen (CE) n°2160/2003 sur le contrôle des salmonelles et d'autres agents zoonotiques spécifiques présents dans la chaîne alimentaire (Anonyme, 2003). Ainsi, chaque Etat membre doit atteindre des objectifs de réduction de la contamination des élevages de porcs (entre autres) dans un délai fixé par le règlement. Pour atteindre ces objectifs communautaires, les États membres établissent des programmes de contrôle nationaux pour *Salmonella*. Par ailleurs, en plus des bonnes pratiques d'hygiène appliquées en élevage et chez les fabricants d'alimentation animale, l'aval de la filière met également en place des mesures de maîtrise du danger, conformément aux exigences réglementaires fixées par le Paquet Hygiène. Les exploitants doivent notamment définir un plan d'autocontrôles qui s'intègre dans une démarche préventive de la maîtrise de la sécurité et la salubrité de ses fabrications. Pour ce qui relève des analyses microbiologiques des aliments, les professionnels doivent *a minima* intégrer, dans leur plan d'autocontrôles, les microorganismes pour lesquels des critères sont définis dans le règlement (CE) n°2073/2005. Dans ce règlement, les salmonelles sont considérées comme un critère de sécurité de certaines denrées d'origine animale (viande hachée, préparation de viande, denrées alimentaires prêtes à être consommées, *etc.* : absence dans 25 grammes), ou comme un critère d'hygiène des procédés d'abattage des porcs (absence dans la partie de la carcasse examinée). En plus de ces obligations réglementaires, les professionnels peuvent aller plus loin en s'engageant dans des chartes d'application volontaire, telle que la charte « Saucisson sec » qui s'intéresse spécifiquement au risque Salmonelles.

²⁸ ANIA, mars 2015. Kit RSE – Grilles d'évaluation. Disponible à : https://www.ania.net/wp-content/uploads/2015/07/KIT-RSE-ANIA-ACTIA-3.-Grilles_dévaluation_v.mars_20151.pdf

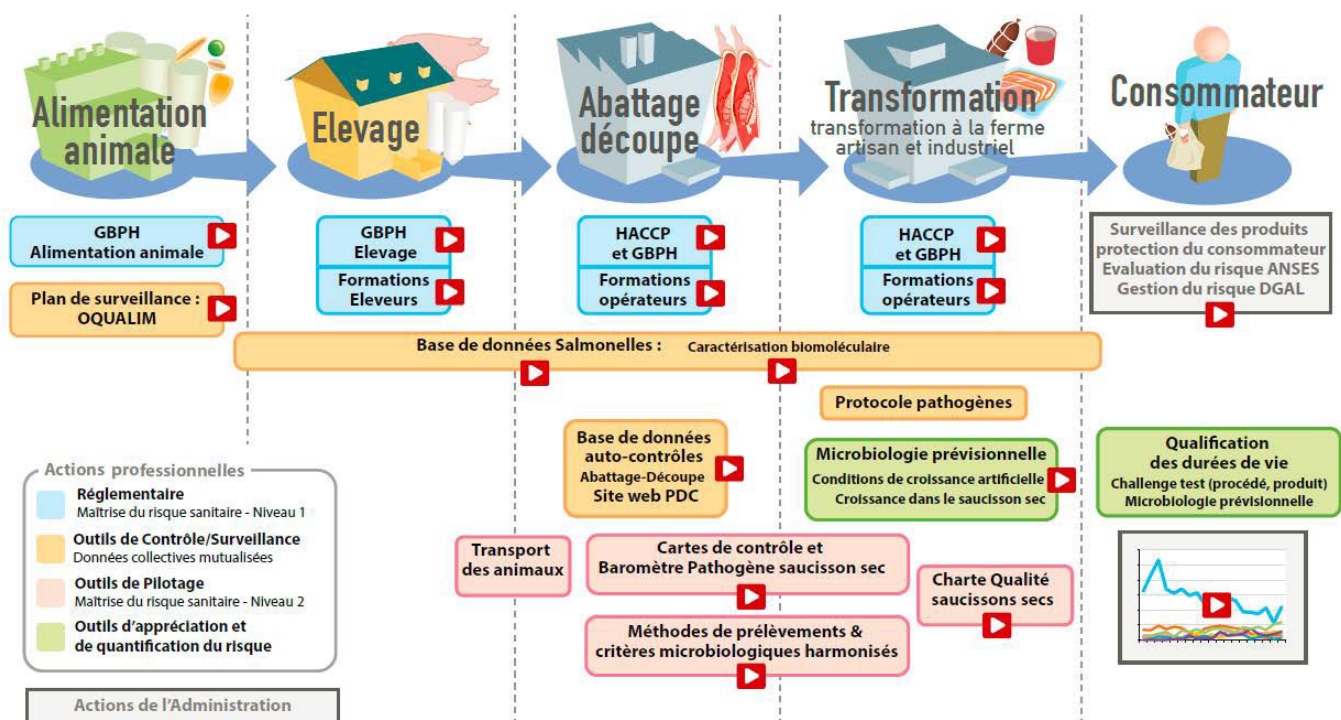


Figure 28 - Dispositif de maîtrise des Salmonelles dans la filière porcine française

Source : d'après IFIP, INAPORC²⁹

Un dispositif de maîtrise similaire pourrait être envisagé pour le cas du HEV. En ce qui concerne la surveillance et le contrôle encadrés par les services de l'Etat, il serait envisageable que le HEV entre dans les **prochains PSPC annuels** réalisés par le Ministère en charge de l'Agriculture au titre du règlement (CE) n°2060/2003, comme cela avait été le cas en 2011. Le HEV pourrait également être un sujet d'intérêt pour la récente **plateforme de Surveillance de la Chaîne Alimentaire** qui a pour objectif d'optimiser les dispositifs de surveillance mis en œuvre tout au long de la chaîne alimentaire en mettant en place des actions concertées entre les différents acteurs du secteur agro-alimentaire. Dans le cadre du règlement (CE) n°2073/2005, le HEV pourrait aussi être considéré comme **critère de sécurité** dans certains produits, par exemple le foie ou produits à base de foie. Dans le cadre d'une adaptation des méthodes d'inspection des viandes, il serait également intéressant de développer des **indicateurs épidémiologiques harmonisés** (*harmonised epidemiological indicators*³⁰) permettant de

²⁹ IFIP, INAPORC, 2018, Dispositif de maîtrise des Salmonelles dans la filière porcine française. Disponible à : https://www.ifip.asso.fr/sites/default/files/pdf-documentations/ref_salmonelle_2018_m.pdf

³⁰ "An epidemiological indicator is defined as the prevalence or incidence of the hazard at a certain stage of the food chain or an indirect measure of the hazards that correlates to human health risk caused by the hazard.", EFSA, 2011.

caractériser des élevages et/ou des abattoirs sur leur niveau de risque vis-à-vis du HEV, comme cela a été proposé par l'EFSA pour d'autres pathogènes comme *Salmonella* et *Trichinella*³¹. Par exemple, le danger *Trichinella* est géré à l'abattoir grâce à l'indication, sur la fiche d'Information sur la Chaîne Alimentaire (ICA), de l'une des trois mentions HRT (Hébergement Reconnu Trichine), HNRT (Hébergement Non Reconnu Trichine) ou PA (Plein Air). Selon la mention indiquée, les modalités de prélèvements sur les carcasses sont différentes³². Concernant le HEV, la catégorisation des élevages se fonderait notamment sur les facteurs de risque identifiés dans la littérature et dans notre projet de recherche, celle des abattoirs porterait sur leur capacité à détecter les foies contaminés et/ou à garantir la traçabilité de lots reconnus indemnes de HEV.

Ces engagements des services de l'Etat ne sauraient se substituer à une **implication forte du secteur privé**, de l'élevage à la distribution, en passant par l'abattage/découpe et la transformation, dans la mise en place de toutes les mesures nécessaires pour réduire le risque d'exposition humaine au HEV et ainsi favoriser le développement de **chartes qualité d'application volontaire pour les acteurs du marché « foie » de la filière porcine, qui doivent veiller à la qualité microbiologique de leurs matières premières.**

³¹ European Food Safety Authority, 2011. Scientific Report of EFSA. Technical specifications on harmonised epidemiological indicators for public health hazards to be covered by meat inspection of swine. EFSA Journal 2011; 9(10): 2371

³² Instruction technique DGAL/SDSSA/2018-551 du 23 juillet 2018 relative à la mise en œuvre par les services vétérinaires d'inspection des règles applicables aux contrôles officiels concernant la présence de *Trichinella* dans les viandes.

III. Perspectives de recherche sur la thématique du virus de l'hépatite E

Les résultats obtenus au cours de ce projet et les perspectives envisagées suscitent de nouvelles questions et hypothèses, nécessitant idéalement des études complémentaires pour y répondre. Tout d'abord, le modèle intra-troupeau développé pourrait faire l'objet d'**adaptations** et d'**extensions**. Par exemple, la diffusion du HEV dans des élevages avec d'**autres types de conduite en bandes** (actuellement implémentés dans le modèle mais non analysés) pourrait être étudiée. Le modèle pourrait également être modifié de manière à tester l'influence de **mesures de biosécurité** (efficacité des procédures de nettoyage-désinfection, présence et utilisation correcte d'un sas, modalités de quarantaine, *etc.*). Il serait également judicieux de pouvoir **modifier les pratiques d'élevage en cours de simulation**, de manière à simuler la mise en place de nouvelles pratiques dans un élevage infecté, contrairement à la situation actuelle où nous avons simulé l'introduction du HEV dans différentes configurations d'élevage, sans modification ultérieure de cette structure et des pratiques. Un **nouvel essai** pourrait également être conduit afin de quantifier l'**impact d'une triple infection HEV/SDRP/PCV2** sur la dynamique de l'infection par le HEV (comme observé dans le suivi longitudinal des trois élevages) et ainsi paramétrer cette modalité dans le modèle intra-troupeau. Le modèle peut également être paramétré de manière à représenter plus spécifiquement l'effet d'un pathogène donné, plutôt que celui d'un virus immunomodulateur générique. Enfin, dans une perspective plus lointaine, il serait envisageable de développer, à partir du modèle, un outil d'aide à la décision qui s'adapterait précisément à des typologies d'élevage pour évaluer leur risque et faire le lien avec les stratégies de surveillance et de qualification des élevages.

Le modèle intra-troupeau pourrait également **inclure des composantes économiques et sociales**. Ainsi, la **dynamique comportementale** des éleveurs pourrait être modélisée et incorporée à la dynamique infectieuse, en prenant en compte les **interactions réciproques** entre elles (Hidano *et al.*, 2018). Par exemple, des études ont montré que la diminution de la prévalence d'un danger réduisait la perception du risque par les éleveurs : c'est notamment le cas des éleveurs de chevaux qui vaccinent leurs animaux contre le virus Hendra et veillent alors moins aux bonnes pratiques d'élevage, du fait de leur confiance en la vaccination (Wiethoelter *et al.*, 2017). Les pratiques d'un éleveur n'apparaissent donc pas comme un facteur figé dans le temps et cette évolution pourrait être prise en compte dans les approches de modélisation. Une

analyse coût-bénéfice pourrait également être réalisée, en comparant le coût des mesures mises en place avec le bénéfice (sanitaire, économique) pour la santé humaine et la filière.

De plus, il serait nécessaire de conduire une **étude d'intervention en élevage** afin de tester en conditions réelles l'efficacité des mesures de maîtrise dégagées du modèle. La réalisation de ce type d'étude est particulièrement lourde, chronophage et coûteuse. En effet, cela nécessite en premier lieu de trouver des élevages HEV-positifs, ayant des pratiques ou configurations à risque, et volontaires pour modifier une ou plusieurs de leurs pratiques. Après un bilan initial de leur situation sanitaire vis-à-vis du HEV, il faudrait suivre l'évolution de la prévalence HEV de manière régulière et sur un pas de temps suffisamment long. C'est la raison pour laquelle il nous a semblé pertinent d'évaluer en premier lieu la faisabilité des stratégies de maîtrise identifiées à partir du modèle, afin de ne tester sur le terrain que les mesures réalistes. Dans l'objectif de développer un système d'aide à la décision pour une surveillance fondée sur le risque (*cf. supra*), il serait aussi intéressant de pouvoir **catégoriser les élevages sur leur niveau de risque**. Cette catégorisation pourrait servir à *(i)* mettre en place des mesures de maîtrise adaptées selon le niveau de risque des élevages ; *(ii)* approvisionner la filière « foie » par des élevages à faible niveau de risque ; *(iii)* suivre l'évolution dans le temps de la situation sanitaire des élevages de porcs. A ce stade, il est sûrement prématuré de proposer des critères de catégorisation des élevages mais quelques suggestions pourraient découler des facteurs de risque mis en évidence dans la littérature et notre projet. D'autres études de terrain seraient également nécessaires pour, par exemple, évaluer la situation sanitaire dans les **élevages de sélection et de multiplication** et analyser la dynamique de l'infection par le HEV dans les **élevages alternatifs** de porcs (agriculture biologique ou sous label de qualité impliquant un élevage des porcs sur litière et un accès à l'extérieur) qui, d'après la littérature, semblent plus à risque que les élevages conventionnels.

Quant au **modèle inter-troupeaux** que nous avons conçu, il devra nécessairement être **complexifié** afin *(i)* d'inclure différents types de conduite en bandes et différentes tailles d'élevage ; *(ii)* de tester des modifications de pratiques d'élevage et de biosécurité ; *(iii)* de permettre des adaptations du réseau simulant par exemple des restrictions de mouvements ou une réorganisation de la filière. La mise en place d'une démarche collective dans la filière pourrait également être intégrée dans un modèle, avec une uniformisation des mesures appliquées dans un sous-groupe d'élevages.

Concernant l'aval de la filière, il apparaît primordial d'acquérir davantage de **données relatives à la contamination des viandes de porc et produits à base de viande ne contenant pas de foie** afin d'évaluer et de gérer correctement le risque d'exposition humaine. Pour ce faire, une nouvelle enquête pourrait être réalisée dans des abattoirs, mais cette fois ci en ciblant l'échantillonnage sur le niveau de risque présenté par les éleveurs et en prélevant les mêmes muscles que ceux analysés dans l'essai de transmission HEV/SDRP que nous avons réalisé. Par ailleurs, l'**efficacité des traitements de séchage et de salaison** des produits à base de porc devrait être évaluée. Ceci pourrait être réalisé en conditions expérimentales : par exemple, il serait envisageable de répéter l'essai de transmission HEV/SDRP, de collecter des muscles contenant du HEV, d'utiliser ces pièces pour fabriquer de la charcuterie (saucisson, jambon sec), de leur appliquer le processus de transformation habituellement employé par les professionnels de la charcuterie-salaisonnerie et de tester leur statut vis-à-vis du HEV à l'issue de la période de séchage-salaison. Dans l'éventualité où du HEV serait détecté à la fin de l'expérimentation, il serait également nécessaire de **confirmer le caractère infectieux** des particules virales présentes (par un bio-essai ou des modèles *in vitro*). L'ensemble de ces résultats permettrait de réaliser une **analyse quantitative des risques** incluant des données de contamination des produits et des données de consommation de ces produits par les Français. A titre de comparaison, l'EFSA (*European Food Safety Authority*) a développé des modèles d'analyse quantitative des risques concernant *Salmonella* chez le porc et *Campylobacter* chez le poulet de chair, qui se sont révélés être des outils utiles aux gestionnaires du risque dans l'évaluation de la faisabilité et du ratio coût/bénéfice de la mise en place de mesures de maîtrise de ces deux pathogènes zoonotiques (Romero-Barrios *et al.*, 2013). Toujours au sujet de l'aval de la filière, une **enquête similaire** à celle conduite auprès des éleveurs, conseillers d'élevage et vétérinaires pourrait être réalisée **auprès des professionnels de l'abattage, de la transformation et de la distribution** pour évaluer la faisabilité de mesures de lutte contre le HEV dans ces maillons de la filière.

Conclusion générale

En combinant sciences du vivant, mathématiques et sciences sociales, nos travaux de recherche ont permis de répondre aux objectifs scientifiques et opérationnels initialement poursuivis à savoir *(i)* expliquer la propagation et la persistance du HEV au sein d'un élevage de porcs naisseur-engraisseur, *(ii)* explorer les modalités de diffusion du HEV à l'échelle de la filière porcine, *(iii)* fournir au gestionnaire du risque des éléments d'aide à la décision pour l'élaboration et la mise en place effective d'un plan de maîtrise du HEV dans la filière porcine. **(1)** Ainsi, les études présentées en début de Chapitre II, réalisées en conditions naturelles et expérimentales, ont permis, entre autres, de mettre en évidence et de quantifier le rôle central des co-infections immunomodulatrices dans la dynamique de l'infection par le HEV chez les porcs. Ces infections intercurrentes, notamment celle par le virus du SDRP, favorisent la chronicité de l'infection par le HEV, augmentant ainsi significativement le risque de présence du HEV dans le foie lors de l'abattage des porcs. De plus, en situation expérimentale, il a été montré que des porcs co-infectés par le virus du SDRP présentent une virémie persistante et de l'ARN du HEV dans plusieurs de leurs muscles, ce qui apporte de nouveaux éléments de réponse aux questions soulevées quant au risque de présence du HEV dans des produits à base de porc ne contenant pas de foie. Pour comprendre les modalités de propagation et de persistance du HEV dans un élevage de porcs, en intégrant la dimension liée à la population de porcs et celle relative aux caractéristiques épidémiologiques de l'infection par le HEV chez le porc, un modèle stochastique individu-centré a été développé en couplant un modèle de dynamique de population avec un modèle épidémiologique multi-pathogènes représentant la diffusion conjointe et les interactions du HEV et d'un virus immunomodulateur. Ce modèle a permis de mettre en évidence l'influence majeure de la structure de l'élevage (type de conduite en bandes, système de logement des truies gestantes) ainsi que de certaines pratiques d'élevage (modalités d'adoption, taille des cases en post-sevrage, modalités de mélange au post-sevrage) et sanitaires (vaccination des truies contre les pathogènes intercurrents). **(2)** Dans le chapitre III, après avoir mis en évidence des associations statistiques entre mouvements inter-élevages et prévalence HEV à l'échelle des élevages, d'une part, et à l'échelle des départements, d'autre part, un modèle dynamique inter-troupeaux, couplant la dynamique infectieuse du HEV circulant dans un élevage avec les échanges de porcs, a été développé. Il a permis de mettre en évidence l'influence du type d'élevage d'introduction, du type d'élevage exposé et du statut

sanitaire de la population vis-à-vis des pathogènes intercurrents sur la diffusion du HEV dans une communauté d'élevages. (3) A partir des éléments dégagés dans ces travaux, nous avons proposé dans le chapitre IV plusieurs axes d'amélioration de la maîtrise et la surveillance du HEV dans la filière porcine, en fondant les actions à mener sur les facteurs de risque (structurels, zootechniques et sanitaires) mis en évidence. Ces pistes d'action ont été formulées sous forme de propositions, soumises aux organisations publiques et privées gestionnaires du risque dans un rapport préliminaire d'aide à la décision. Une réunion de concertation a permis de montrer tout à la fois la volonté de ces acteurs de s'impliquer pour la sécurisation de la filière vis-à-vis du HEV et les probables difficultés qu'il y aura à mettre en place des mesures dans les élevages, notamment du fait du caractère asymptomatique du HEV et des inconnues existant encore. A l'échelle individuelle, les acteurs de l'amont de la filière ont également confirmé les contraintes techniques et économiques qu'imposeraient des mesures de gestion du HEV dans les élevages, tout en affirmant leur forte volonté de garantir la sécurité sanitaire des produits commercialisés. Les acteurs individuels et leurs représentants ont souligné l'importance que les mesures de lutte envisagées fassent partie d'une démarche collective de la filière, qui inclurait aussi des mesures de maîtrise significatives de la part des acteurs de l'aval de la filière, et - éventuellement - la structuration d'une filière spécifique dédiée à la fabrication des produits contenant du foie de porc. Finalement, toutes les parties prenantes identifiées peuvent et doivent contribuer à la réduction du nombre de cas d'hépatite E, y compris le monde de la recherche, puisque les perspectives envisagées dans notre dernier chapitre prouvent que nombre de questions restent encore en suspens, mais aussi le consommateur, premier acteur de sa santé, qui doit veiller au respect des recommandations sanitaires en matière d'alimentation.

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Annexe 1 - Liste des valorisations associées à la thèse

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Teixeira-Costa C., Andraud M., Rose N., **Salines M.** Controlling hepatitis E virus in the pig production sector: assessment of the technical and behavioural feasibility of on-farm risk mitigation strategies. In prep.

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Salines M., Dumarest M., Andraud M., Mahé S., Cineux M., Eveno E., Eono F., Dorenlor V., Grasland B., Bourry O., Pavio N., Rose N., 2018. Understanding hepatitis E virus (HEV) spread and persistence in pig farms using longitudinal field data. *HEV2018, Hepatitis E workshop, Hepatitis E: Paradigm of a food-borne zoonotic emerging disease in Europe*, Madrid, Spain, 4-5 June 2018.

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** Valorisations du stage de master 2 réalisé en 2014 avec la même équipe

Annexe 2 - Publication dans les *Journées Recherche Porcine 47*

Salines M., Andraud M., Barnaud E., Eono F., Renson P., Bourry O., Pavio N., Rose N., 2015. L'excrétion et la transmission du virus de l'hépatite E (VHE) sont augmentées lors d'une co-infection par le virus du Syndrome Dysgénésique et Respiratoire Porcin (SDRP). *Journées Recherche Porcine 47*.

L'excrétion et la transmission du virus de l'hépatite E (VHE) sont augmentées lors d'une co-infection par le virus du Syndrome Dysgénésique et Respiratoire Porcin (SDRP)

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L'excrétion et la transmission du virus de l'hépatite E (VHE) sont augmentées lors d'une co-infection par le virus du Syndrome Dysgénésique et Respiratoire Porcin (SDRP)

La probabilité d'abattre des porcs dont le foie contient du virus de l'hépatite E (VHE) est conditionnée par le moment où les animaux sont infectés avant l'abattage et par la durée d'excrétion du virus. Le virus du Syndrome Dysgénésique et Respiratoire Porcin (SDRP), virus immunosuppresseur très prévalent, pourrait influencer l'infection par le VHE. L'impact du virus du SDRP sur les caractéristiques de l'infection par le VHE a été étudié chez des porcs Exempts d'Organismes Pathogènes Spécifiques (EOPS) par l'intermédiaire d'une co-infection expérimentale par le VHE et le virus du SDRP, comparée à une infection par le VHE seul.

Le suivi des animaux a montré que l'excrétion virale et la réponse immunitaire humorale sont retardées chez les porcs co-infectés (d'un facteur 1,9 et 1,6 respectivement). L'excrétion du VHE est significativement plus importante et considérablement prolongée (48,6 jours contre 9,7 jours pour le VHE seul). L'allongement de la durée d'excrétion est significativement lié au défaut de réponse humorale chez les porcs co-infectés. Le taux de transmission directe du VHE est 4,7 fois plus élevé (0,70 par jour contre 0,15 pour le VHE seul). La sensibilité à l'infection par le VHE est augmentée d'un facteur 3,3 ($1,41 \cdot 10^5$ GE/g nécessaires pour infecter un porc co-infecté, $5,00 \cdot 10^5$ GE/g en cas d'infection par le VHE seul). Ces résultats montrent un impact important du virus du SDRP sur la dynamique d'infection du VHE et une potentielle chronicité chez des porcs co-infectés, augmentant considérablement le risque de présence du virus dans le foie des porcs abattus.

Hepatitis E virus (HEV) shedding and transmission are increased in the case of co-infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

The probability of slaughtering pigs whose liver contains hepatitis E virus (HEV) is related to the date of infection before slaughter and to the duration of viral shedding. Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is a highly prevalent immunosuppressive virus and it is suspected of influencing HEV infection dynamics. The impact of PRRSV on the features of HEV infection was studied through an experimental HEV/PRRSV co-infection of Specific-Pathogen-Free (SPF) pigs compared to an infection with HEV alone.

The follow-up of the animals showed that shedding and humoral immune response were delayed in co-infected pigs (by a factor of 1.9 and 1.6 respectively). HEV shedding was significantly increased in the case of co-infection and dramatically extended (48.6 days as against 9.7 days for HEV only). The increase in duration of the infectious period was significantly correlated with the impaired humoral response in co-infected pigs. HEV direct transmission rate was estimated to be 4.7 times higher in the case of co-infection than in HEV-only infected pigs (0.70 per day *versus* 0.15 for HEV only). HEV infection susceptibility was also increased by a factor of 3.3 ($1.41 \cdot 10^5$ GE/g being required to infect a co-infected pig, $5.00 \cdot 10^5$ GE/g for a pig infected with HEV alone). These results show that PRRSV has a major impact on HEV infection and transmission and that HEV/PRRSV co-infection could lead to chronic HEV infection. This chronicity would dramatically increase the risk of having pig livers containing HEV at slaughter time.

INTRODUCTION

Le virus de l'hépatite E est un virus à ARN simple brin de polarité positive, non enveloppé, agent étiologique d'une hépatite aiguë chez l'homme. Transmis principalement par voie oro-fécale, il est responsable de signes cliniques similaires à ceux de l'hépatite A mais en moyenne plus sévères (Emerson et Purcell, 2003). Des formes chroniques peuvent être observées, notamment chez des individus immunodéficients (Gerolami *et al.*, 2008; Kamar *et al.*, 2008). Dans les pays développés, l'hépatite E est reconnue aujourd'hui comme une zoonose alimentaire potentiellement grave, dont le nombre de cas apparents est en constante augmentation, et pour laquelle les porcs domestiques sont considérés comme le principal réservoir (Pavio *et al.*, 2008). La prévalence du VHE en élevage porcin est élevée ; différentes dynamiques d'infection sont observées et sont en lien direct avec la probabilité de contamination des foies des porcs abattus (Rose et Pavio, 2014). Ces variations inter-élevages de la dynamique d'infection du VHE ne sont pas entièrement expliquées à ce jour. L'influence de certaines maladies intercurrentes, et notamment du Syndrome Dysgénésique et Respiratoire Porcin (SDRP), due à un virus immunodépresseur fortement prévalent dans les régions de production porcine en France, est ainsi suspectée (de Deus *et al.*, 2007; Martelli *et al.*, 2010; Mao *et al.*, 2013). La présente étude vise à objectiver et quantifier l'impact d'une co-infection par le virus du SDRP sur l'infection par le VHE chez le porc (excrétion, transmission, réponse immunitaire humorale) grâce à une co-infection expérimentale VHE/SDRP de porcs Exempts d'Organismes Pathogènes Spécifiques (EOPS) comparée à une infection par le VHE seul (Andraud *et al.*, 2013).

1. MATERIEL ET METHODES

1.1. Collecte des données

1.1.1. Dispositif expérimental

L'expérimentation a été conduite au sein des animaleries protégées du laboratoire de l'Anses de Ploufragan, niveau 3 de biosécurité sous air filtré. Vingt porcs EOPS âgés de 5 semaines ont été utilisés ; ils sont exempts du VHE et du virus du SDRP et n'ont aucun anticorps maternel spécifique de ces virus. Dix-huit porcelets ont été répartis aléatoirement (stratification en fonction du sexe, de la portée et du poids) dans trois parcs contenant chacun 6 animaux : 3 porcs inoculés par le VHE et le virus du SDRP, et 3 animaux contacts. Deux porcelets ont été utilisés comme témoins non infectés (figure 1).

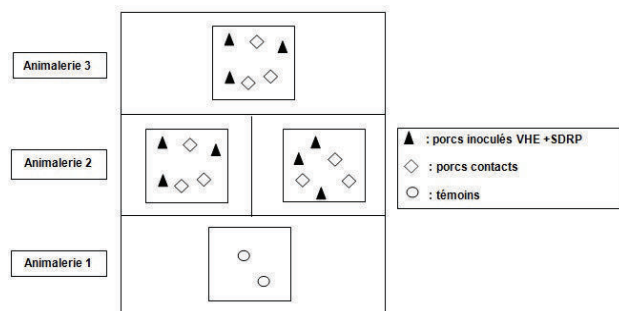


Figure 1 – Schéma du dispositif expérimental de co-infection.

1.1.2. Virus et inoculum

A J0, 3 porcs par parc ont été inoculés : (i) par voie orale (sondage œsophagien) avec un inoculum de VHE génotype 3

titrant à 10^8 génomes équivalents (GE) sous un volume de 10 mL, préparé selon le protocole décrit par Andraud *et al.* (2013) et (ii) par voie intranasale avec un inoculum d'une souche de virus du SDRP de génotype 1, sous-type 1 (PRRS/FR/29/24/1/2005, souche FINISTERE) titrant à 5.10^5 DCP₅₀ (dose cytopathogène 50) pour un volume de 2,5 mL par narine.

1.1.3. Prélèvements et observations

Les matières fécales des animaux ont été prélevées individuellement 3 fois par semaine dès J-3 et jusqu'à 49 jours post-infection (JPI). Une prise de sang a été réalisée avant inoculation puis une fois par semaine. Des observations cliniques ont également été collectées (relevé des symptômes en cas de manifestations cliniques, prise de température quotidienne, *etc.*). Les animaux ont été euthanasiés à la fin de l'essai, soit 49 JPI, par injection intraveineuse de thiopental sodique 1g/50kg (Nesdonal®, Merial, Lyon, France) suivie d'une saignée. A l'autopsie, des prélèvements de foie ont été effectués.

1.1.4. Analyses virologiques et sérologiques

La quantification des ARN du virus de l'hépatite E dans les matières fécales et dans le foie a été réalisée par une RT-PCR quantitative en temps réel (Barnaud *et al.*, 2012). Les résultats sont exprimés en nombre de copies de génome par gramme de fèces ou de foie (GE/g). La détection dans le sérum des anticorps dirigés contre le VHE a été réalisée avec le kit HEV ELISA 4.0v (MP Diagnostics, Illkirch, France) (Barnaud *et al.*, 2012). La détection des ARN du virus du SDRP dans le sérum a été réalisée à l'aide d'une RT-PCR en temps réel (Charpin *et al.*, 2012). Les résultats sont exprimés en Ct (cycle seuil).

1.2. Analyses statistiques et modèles

1.2.1. Dynamique d'infection du VHE

La période de latence, la période infectieuse et le délai nécessaire à la production d'anticorps anti-VHE ont été estimés par l'intermédiaire d'analyses de données de survie. Pour les animaux inoculés, la période de latence correspond au délai entre la date d'inoculation et la date du premier échantillon de matières fécales positif en RT-PCR VHE. La période de latence a été modélisée selon une distribution gamma, dont les paramètres de forme (α) et d'échelle (s) ont été estimés par la méthode du maximum de vraisemblance. L'intervalle de confiance des paramètres estimés a ensuite été calculé par une technique de bootstrap non paramétrique. Des analyses de données de survie ont été effectuées afin d'estimer la durée de la période infectieuse et d'évaluer l'impact de l'infection par le virus du SDRP sur le délai nécessaire à la production d'anticorps spécifiques vis-à-vis du VHE. Deux modèles paramétriques ont été testés pour ces analyses (distribution log-normale ou de type Weibull des temps de survie) et comparés en utilisant le Critère d'Information d'Akaike (AIC). L'influence du délai nécessaire à la production d'anticorps anti-VHE sur la durée de la période infectieuse a été étudiée par l'intermédiaire d'un modèle semi-paramétrique de Cox. La réponse immunitaire humorale a été qualifiée d'absente ou de tardive si le délai infection-séroconversion était supérieur ou égal à 25 JPI et de précoce s'il était strictement inférieur à 25 JPI (Satou et Nishiura, 2007).

1.2.2. Quantification de l'excrétion, de l'accumulation environnementale et de la transmission du VHE

La distribution de l'excrétion virale dans le temps pour les porcs co-infectés et non co-infectés a été représentée par une

série de « boxplot » (boîtes à moustache). Un modèle linéaire mixte prenant en compte des données répétées dans le temps a été construit afin d'objectiver la différence entre les charges virales selon le statut des animaux vis-à-vis du virus du SDRP. La charge virale accumulée dans l'environnement, correspondant à l'accumulation de particules virales excrétées par les porcs infectés, partiellement compensées par le taux de clairance, a également été estimée selon la méthode décrite par Andraud *et al.* (2013). Le taux de clairance, noté δ , prend en compte l'élimination des matières fécales au travers du caillebotis de la case et l'inactivation intrinsèque du VHE dans l'environnement.

Deux voies de transmission du VHE ont été investiguées : (i) la transmission due aux contacts directs entre les porcs infectés et les porcs sensibles et (ii) la transmission indirecte oro-fécale à partir d'un réservoir environnemental au sein d'une même case. Le modèle utilisé est similaire au modèle décrit par Andraud *et al.* (2013). Les paramètres de transmission directe et indirecte, respectivement β_w et $\beta_E^{(w)}$, la durée de la période de latence λ_j de chaque individu contact et le taux de clairance du virus δ ont été estimés par une méthode de Monte-Carlo par Chaînes de Markov, l'algorithme de Metropolis-Hastings, dans le cadre de l'inférence bayésienne.

Les résultats obtenus dans le cadre de cet essai sont comparés aux résultats issus d'un précédent essai d'infection par le VHE seul (Andraud *et al.*, 2013). Bien que les deux essais n'aient pas été conduits concomitamment, ils ont été réalisés dans des conditions expérimentales strictement identiques, ce qui autorise la comparaison des résultats (même lieu d'expérimentation, structure de contact identique, porcs EOPS génétiquement comparables, âges des animaux, souches de VHE inoculée, doses et protocoles identiques).

2. RESULTATS

2.1. Description des données d'infection

Les données d'infection sont présentées en figures 2 et 3. L'ensemble des individus inoculés et contacts sont virémiques pour le virus du SDRP avant la première excrétion du VHE détectée, excepté chez 2 porcs contacts. L'ensemble des individus exposés au VHE, contacts et inoculés, sont excréteurs du VHE jusqu'à la fin de l'essai (49 JPI). A l'autopsie, seuls 4 foies sur 18 sont négatifs en RT-PCR VHE. Seuls 4 individus inoculés sur 9 et 7 individus contacts sur 9 présentent une réponse immunitaire humorale anti-VHE (entre 35 et 49 JPI, et 42 et 49 JPI respectivement, données non montrées).

	JPI	2	4	7	9	11	14	16	18	21	25	26	32	35	39	42	44	46	49	Autopsie (foie)	
Témoin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Hémost	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Groupe 1																					
Inoculé*	0	0	0	1,08.10 ²	0*																
Inoculé*	0	0	0	0	0	2,21.10 ⁴	2,21.10 ³	1,24.10 ⁴	2,62.10 ⁴	1,08.10 ⁷	1,75.10 ⁷	5,96.10 ⁸	8,27.10 ⁸	7,51.10 ⁸	2,95.10 ⁷	4,43.10 ⁷	8,02.10 ⁸	8,76.10 ⁸	1,49.10 ⁷	1,46.10 ⁴	
Inoculé*	0	0	0	0	0	2,23.10 ⁴	1,72.10 ⁴	1,51.10 ⁴	0,80.10 ⁴	1,24.10 ⁷	3,56.10 ⁷	7,06.10 ⁷	1,52.10 ⁸	5,51.10 ⁷	0,76.10 ⁷	1,51.10 ⁷	1,09.10 ⁸	4,61.10 ⁷	5,85.10 ⁷	1,87.10 ⁴	
Inoculé*	0	0	0	0	0	0	0	1,50.10 ⁴	1,53.10 ⁴	5,85.10 ⁷	1,14.10 ⁷	1,30.10 ⁷	4,06.10 ⁷	3,99.10 ⁷	9,27.10 ⁷	4,28.10 ⁷	5,02.10 ⁷	7,20.10 ⁷	5,46.10 ⁷	1,02.10 ⁴	
Contact	0	0	0	0	0	0	0	0	0	0	0,57.10 ⁷	1,16.10 ⁷	1,49.10 ⁷	1,54.10 ⁷	8,75.10 ⁷	1,92.10 ⁷	1,42.10 ⁷	5,51.10 ⁷	0,74.10 ⁷	8,42.10 ⁴	
Contact	0	0	0	0	0	0	0	0	0	0	0	0	2,01.10 ⁷	5,58.10 ⁷	5,51.10 ⁷	5,24.10 ⁷	1,58.10 ⁷	4,51.10 ⁷	1,17.10 ⁷	5,49.10 ⁴	
Contact	0	0	0	0	0	0	0	0	0	0	1,78.10 ⁷	8,84.10 ⁷	1,48.10 ⁷	5,04.10 ⁷	5,48.10 ⁷	1,20.10 ⁷	4,20.10 ⁷	1,91.10 ⁷	1,20.10 ⁷	0	
Groupe 2																					
Inoculé	0	0	0	0	0	0	1,56.10 ²	1,09.10 ⁴	1,57.10 ⁴	2,81.10 ⁴	1,57.10 ⁷	2,50.10 ⁷	8,71.10 ⁷	6,98.10 ⁷	4,44.10 ⁸	5,92.10 ⁸	1,64.10 ⁷	9,02.10 ²	1,45.10 ²	1,56.10 ⁴	
Inoculé	0	0	0	0	0	0	2,19.10 ⁴	3,74.10 ⁴	0,85.10 ⁴	1,86.10 ⁴	1,51.10 ⁷	2,01.10 ⁷	1,55.10 ⁷	1,79.10 ⁷	2,57.10 ⁷	7,88.10 ⁷	1,37.10 ⁷	1,97.10 ⁷	0,80.10 ⁴	2,72.10 ⁴	
Inoculé	0	0	0	0	0	1,08.10 ⁴	6,17.10 ⁴	5,06.10 ⁴	8,85.10 ⁴	4,20.10 ⁷	8,06.10 ⁷	6,72.10 ⁷	1,25.10 ⁸	1,99.10 ⁸	5,08.10 ⁸	8,10.10 ⁸	1,82.10 ⁸	1,19.10 ⁷	4,86.10 ⁷	9,59.10 ⁴	
Contact	0	0	0	0	0	0	0	0	0	0	1,05.10 ⁸	0*	1,45.10 ⁸	4,12.10 ⁸	4,54.10 ⁸	abs	1,18.10 ⁷	2,51.10 ⁷	5,75.10 ⁷	1,12.10 ⁴	
Contact	0	0	0	0	0	0	0	0	0	0	0	0	1,82.10 ⁸	5,38.10 ⁸	8,40.10 ⁸	4,43.10 ⁸	8,37.10 ⁷	1,43.10 ⁷	5,24.10 ⁷	1,09.10 ⁴	
Contact	0	0	0	0	0	0	0	0	0	0	0	0	1,47.10 ⁸	7,26.10 ⁸	1,57.10 ⁷	5,95.10 ⁷	4,57.10 ⁷	2,46.10 ⁷	5,07.10 ⁷	5,44.10 ⁴	
Groupe 3																					
Inoculé	0	0	0	0	0	0	2,37.10 ²	1,12.10 ²	3,85.10 ⁴	0,79.10 ⁴	7,98.10 ⁴	1,09.10 ⁷	4,21.10 ⁷	1,04.10 ⁷	4,26.10 ⁷	1,51.10 ⁷	2,90.10 ⁷	1,00.10 ⁷	2,08.10 ⁷	1,28.10 ⁴	
Inoculé	0	0	0	0	0	0	0	0	0,06.10 ²	9,54.10 ²	4,08.10 ⁴	3,22.10 ⁴	1,55.10 ⁷	0,02.10 ⁷	1,76.10 ⁷	1,80.10 ⁷	1,88.10 ⁷	8,52.10 ⁷	1,85.10 ⁷	5,75.10 ⁷	2,04.10 ⁴
Inoculé	0	0	0	0	0	0	8,04.10 ⁴	4,85.10 ⁴	2,26.10 ⁴	1,14.10 ⁴	2,25.10 ⁷	1,78.10 ⁷	2,87.10 ⁷	8,56.10 ⁷	9,17.10 ⁷	1,04.10 ⁸	4,89.10 ⁷	4,09.10 ⁷	1,48.10 ⁷	5,61.10 ⁷	9,68.10 ⁴
Contact	0	0	0	0	0	0	0	0	0	0	0	0	8,80.10 ⁷	2,19.10 ⁷	2,19.10 ⁷	1,50.10 ⁷	3,09.10 ⁷	1,02.10 ⁷	2,19.10 ⁷	0	
Contact	0	0	0	0	0	0	0	0	0	0	0	0	8,56.10 ⁷	1,50.10 ⁷	8,47.10 ⁷	0*	1,45.10 ⁷	8,67.10 ⁷	1,01.10 ⁷	5,87.10 ⁴	
Contact	0	0	0	0	0	0	0	0	0	0	0	0	1,51.10 ⁷	8,42.10 ⁷	1,12.10 ⁷	0*	4,41.10 ⁷	7,78.10 ⁷	8,29.10 ⁷	0	

Figure 2 – Résultats de RT-PCR quantitative VHE sur les échantillons individuels de fèces (GE/g de fèces). Les zones grisées correspondent aux périodes d'excrétion du VHE. JPI : jours post-infection, * testé en duplicat, abs : absent, 0 : non détecté

	JPI	7	14	21	28	35	42	49
Groupe 1	Témoin	nt	nt	N/A	nt	N/A	N/A	nt
	Témoin	nt	nt	nt	N/A	N/A	N/A	N/A
	Inoculé	28,94	26,39	35,52	33,43	28,08	N/A	N/A
	Inoculé	30,74	29,27	33,43	39,25	N/A	N/A	N/A
	Inoculé	32,30	29,88	33,30	38,68	35,92	N/A	N/A
	Contact	31,76	29,16	29,64	28,96	37,73	N/A	N/A
	Contact	N/A	30,19	35,32	29,44	N/A	N/A	N/A
	Contact	N/A	N/A	N/A	N/A	N/A	31,39	22,26
Groupe 2	Inoculé	28,26	28,19	31,27	27,42	N/A	N/A	N/A
	Inoculé	28,36	30,33	33,20	26,4	29,4	N/A	N/A
	Inoculé	28,69	30,37	35,00	26,61	N/A	N/A	N/A
	Contact	35,11	28,68	31,97	24,27	31,2	26,03	N/A
	Contact	N/A	N/A	36,69	25,4	44,63	N/A	N/A
	Contact	36,95	N/A	33,13	24,37	29,28	N/A	N/A
	Inoculé	29,12	26,03	32,21	30,89	N/A	N/A	N/A
	Inoculé	30,49	26,82	32,39	27,37	N/A	36,54	N/A
Groupe 3	Inoculé	29,04	38,31	43,52	N/A	N/A	N/A	N/A
	Contact	N/A	32,15	29,99	25,74	N/A	28,43	N/A
	Contact	N/A	34,22	33,05	36,73	27,66	N/A	N/A
	Contact	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Figure 3 – Résultats de RT-PCR SDRP sur les sera individuels (Ct). Les zones grisées correspondent aux résultats positifs en RT-PCR dans le sérum. JPI : jours post-infection, nt : non traité, N/A : non amplifié.

2.2. Dynamique d'infection du VHE

La durée de la période de latence a été estimée par une loi gamma avec les paramètres de forme $a = 25,7$ [11,6 ; 180,4] et d'échelle $s = 0,5$ [0,08 ; 1,1] conduisant à une période de latence moyenne de 12,9 jours [12,8 ; 14,4] (tableau 1). Pour les individus contacts, la durée moyenne de la période de latence est de 13,4 jours [8,6 ; 17,1].

Avec une distribution log-normale présentant un AIC plus faible que celui obtenu avec la distribution Weibull, la durée de la période infectieuse a été estimée à 48,6 jours [27,9 ; 84,6] (tableau 1). La durée moyenne nécessaire à la production d'anticorps spécifiques du VHE, également modélisée selon une distribution log-normale, a été estimée à 43,14 jours [35,66 ; 52,19] lors de co-infection par le virus du SDRP et à 26,33 jours [23,49 ; 29,50] en l'absence de co-infection (tableau 1). La durée de la période infectieuse est significativement associée à la précocité de mise en place de la réponse immunitaire humorale. Une réponse immunitaire humorale tardive ou absente (> 25 JPI) est associée à une augmentation de la durée de la période infectieuse (la fin d'excrétion étant retardée, Hazard Ratio (ou rapport de risques) = 0,35, $P < 0,01$).

2.3. Quantification de l'excrétion, de l'accumulation environnementale et de la transmission du VHE

2.3.1. Excrétion du VHE et accumulation environnementale

La distribution de la charge génomique virale VHE excrétée au cours du temps en présence et en l'absence de co-infection par le virus du SDRP est représentée en figure 4. Chez les individus inoculés, l'infection par le virus du SDRP est significativement associée à une augmentation de la charge génomique virale excrétée ($P = 0,05$). L'interaction entre le temps et l'infection par le virus du SDRP est significative et positive, *i.e.* l'impact du virus du SDRP augmente au cours du temps ($P = 0,04$).

En revanche, l'effet de l'infection par le virus du SDRP n'est pas statistiquement significatif chez les individus contacts ($P > 0,05$, données non montrées).

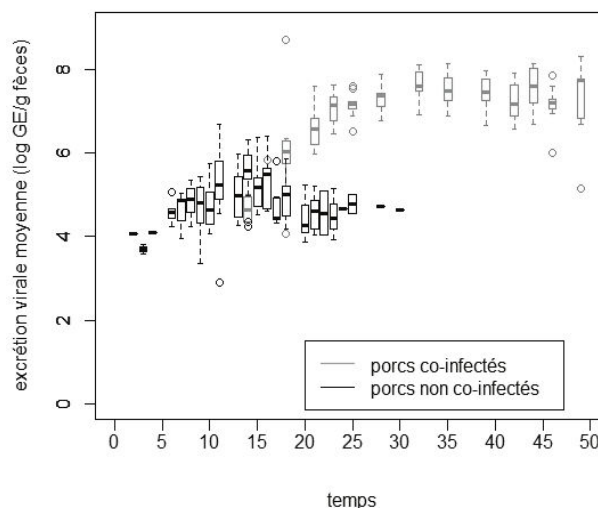


Figure 4 – Distribution des charges génomiques VHE excrétées au cours du temps chez les porcs inoculés par le VHE co-infectés ou non par le virus du SDRP.

La charge virale accumulée dans l'environnement modélisée pour les 3 cas d'expérimentation est représentée en figure 5. La charge environnementale est nulle jusqu'à 15 à 20 jours post-inoculation puis est croissante au cours du temps, atteignant à la fin de l'essai $1,0 \cdot 10^8$ à $1,5 \cdot 10^8$ GE/g de fèces, avec un taux de clairance du virus estimé à 30 % par jour [0,15 ; 0,44].

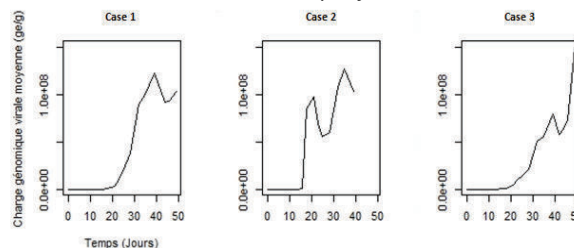


Figure 5 – Accumulation du VHE dans l'environnement lors de co-infection VHE/virus du SDRP. Paramètres de transmission du VHE

Transmission directe. Les résultats montrent que, dans ces conditions expérimentales, un porc infectieux est capable d'infecter 0,70 porc par jour par contact direct ($\beta_w = 0,70$ [$1,18 \cdot 10^{-3}$; 3,67]) (tableau 1).

Transmission indirecte. Le taux de transmission indirecte au sein d'une case peut être considéré comme le nombre moyen d'animaux qui peuvent être infectés par une seule particule virale présente dans l'environnement ($\beta_E^{(w)} = 6,59 \cdot 10^{-6}$ g/GE/jour [$1,43 \cdot 10^{-10}$; $1,27 \cdot 10^{-4}$]). Autrement dit, l'inverse de $\beta_E^{(w)}$ correspond au nombre moyen de particules virales par gramme de fèces dans l'environnement nécessaires pour infecter un animal par jour, soit $1,51 \cdot 10^5$ GE/g/jour [$7,86 \cdot 10^3$; $7,00 \cdot 10^9$] (tableau 1).

Tableau 1 – Récapitulatif des résultats obtenus et comparaison avec les données d'infection par le VHE seul.

	VHE + virus du SDRP	VHE seul (Andraud <i>et al.</i> (2013))
Période de latence (jours)	12,9 [12,8 ; 14,4]	6,9 [5,8 ; 7,9]
Période infectieuse (jours)	48,6 [27,9 ; 84,6]	9,7 [8,2 ; 11,2]
Transmission directe [β_w] (jour ⁻¹)	0,70 [$1,2 \cdot 10^{-3}$; 3,67]	0,15 [0,03 ; 0,31]
Transmission indirecte [$\beta_E^{(w)}$] (g/GE/j)	$6,6 \cdot 10^{-6}$ [$1,4 \cdot 10^{-10}$; $1,3 \cdot 10^{-4}$]	$2,0 \cdot 10^{-6}$ [$1,1 \cdot 10^{-7}$; $7,0 \cdot 10^{-6}$]
Délai de séroconversion (jours)	43,1 [35,7 ; 52,2]	26,3 [23,5 ; 29,5]

3. DISCUSSION

Plusieurs publications ont présenté des essais d'infection expérimentale de porcs par le virus de l'hépatite E (Balayan *et al.*, 1990; Kasorndorkbua *et al.*, 2003; Kasorndorkbua *et al.*, 2004; Bouwknecht *et al.*, 2008; Bouwknecht *et al.*, 2009; Casas *et al.*, 2009; Bouwknecht *et al.*, 2011). En particulier, Bouwknecht *et al.* (2008) ont inoculé des porcs par voie intra-veineuse avec le VHE et ont quantifié la transmission du VHE par contact direct avec un porc infecté par ces animaux primo-infectés. Plus récemment, le modèle d'Andraud *et al.* (2013) prend en compte une transmission par contact direct et une transmission indirecte d'origine environnementale à partir de porcs inoculés par le VHE par voie orale, se rapprochant ainsi des conditions naturelles. Cependant, les résultats de ces études diffèrent de la dynamique d'infection observée en élevage, notamment en termes de durées de période de latence et de période infectieuse, notablement plus longues que ce qui est observé au cours de ces infections expérimentales (de Deus *et al.*, 2008; Casas *et al.*, 2011; Backer *et al.*, 2012). Plusieurs études suggèrent une association possible entre l'infection par le VHE et l'infection par le virus du SDRP, virus immunosuppresseur très prévalent dans certaines régions d'élevage, sans pour autant identifier formellement une interaction entre les deux agents (de Deus *et al.*, 2007; Martelli *et al.*, 2010; Mao *et al.*, 2013).

Le suivi des animaux montre que l'excrétion du VHE est retardée en cas de co-infection par le virus du SDRP, avec une période de latence estimée à 12,9 jours dans l'essai de co-infection, contre 6,9 jours lors d'infection par le VHE seul, soit un allongement d'un facteur 1,9. Une activation de la réponse immunitaire innée par le virus du SDRP, retardant ainsi l'excrétion du VHE pourrait expliquer l'allongement de la durée de la période de latence observé. Dans l'essai de Bouwknecht *et al.* (2008), la durée de la période de latence observée n'est que de 3 jours, ce qui conforte le fait que la voie d'inoculation est susceptible de modifier les mécanismes physio-pathogéniques du virus, et notamment la rapidité avec laquelle le virus est en contact avec les cellules cibles. La durée de la période infectieuse est allongée en cas de co-infection par le virus du SDRP : 48,6 *versus* 9,7 jours respectivement, soit un allongement d'un facteur 5. Ces données se rapprochent des données de terrain disponibles (27 jours [20 ; 39] (Backer *et al.*, 2012)). Dans l'essai décrit par Bouwknecht *et al.* (2008), la période infectieuse dure entre 13 et 49 jours. Cependant, l'origine des animaux inclus dans cet essai et leur statut vis-à-vis du virus du SDRP ne sont pas précisés. Les résultats de l'essai réalisé montrent que l'excrétion virale des individus inoculés est quantitativement plus importante lors de co-infection par le virus du SDRP. L'effet de l'infection par le virus du SDRP sur la quantité de VHE excrété n'est pas significatif chez les individus contacts. Ceci peut s'expliquer par le nombre modeste d'animaux inclus dans l'essai, la plus forte variabilité individuelle observée chez les animaux contacts et le fait que deux animaux contacts se sont infectés tardivement par le virus du SDRP.

La charge virale accumulée dans l'environnement est plus importante lors de co-infection par le virus du SDRP que pour l'infection par le VHE seul (respectivement de l'ordre de 10^8 GE/g, et de l'ordre de 10^5 GE/g). L'allongement de la durée d'excrétion chez les individus co-infectés par le virus du SDRP, associé à une augmentation de la quantité de particules virales excrétées, favorise le maintien du virus dans l'environnement,

induisant une pression d'infection accrue et persistante sur les individus sensibles. Le taux de transmission directe est 4,7 fois plus élevé lors de co-infection (0,70 par jour contre 0,15 avec le VHE seul). Ainsi, lors de co-infection par le virus du SDRP, la voie de transmission directe joue un rôle plus important dans la transmission du VHE, ce qui peut être mis en lien avec l'excrétion individuelle quantitativement plus importante. Le taux de transmission environnementale au sein d'une case (*i.e.* le nombre d'animaux qui peuvent être infectés par une seule particule virale présente dans l'environnement) est estimé 3,3 fois plus élevé lors de co-infection : autrement dit, 3,3 fois moins de particules virales sont nécessaires pour infecter un animal par le VHE en présence du virus du SDRP. Les animaux inoculés et contacts (sauf 2) s'infectant par le virus du SDRP avant l'infection par le VHE, ces données suggèrent une plus grande sensibilité des porcs à l'infection par le VHE en cas de co-infection. Dans un modèle construit à partir d'un essai d'infection VHE par voie intra-veineuse, Bouwknecht *et al.* (2011) rapportent que la dose orale de VHE pour laquelle la probabilité d'infection est égale à 50 % serait de $1,4 \cdot 10^6$ GE/g, ce qui est 10 fois supérieur à la dose nécessaire estimée dans notre étude. Ces données confortent l'hypothèse d'une plus grande sensibilité vis-à-vis du VHE chez des porcs co-infectés.

Le délai nécessaire à la mise en place de la réponse immunitaire humorale anti-VHE est 1,6 fois plus long chez des porcs co-infectés que chez les porcs infectés par le VHE seul (43,1 et 26,3 jours respectivement). Ce défaut de réponse sérologique est significativement associé à l'allongement de la durée de la période infectieuse lors de co-infection par le virus du SDRP et pourrait donc expliquer la présence de particules virales dans le foie des porcs abattus. Les mécanismes à l'origine d'une potentielle baisse de l'immunité liée au SDRP et à l'origine d'une infection chronique par le VHE n'ont cependant pas été explorés dans ce travail. Chez l'homme, les mécanismes de la pathogénèse de l'hépatite E chronique sont encore mal connus mais les données décrites dans la littérature suggèrent qu'une réponse immunitaire déficiente (innée et/ou adaptative, cellulaire et/ou humorale) pourrait être à l'origine de la chronicité de l'infection par le VHE (Kamar *et al.*, 2011; Kenfak-Foguena *et al.*, 2011; Lhomme *et al.*, 2012; Suneetha *et al.*, 2012; Moal *et al.*, 2013). Le virus du SDRP est connu pour interférer fortement avec le système immunitaire. En effet, la sécrétion précoce et importante d'IL10 chez des porcs infectés par le virus du SDRP oriente préférentiellement vers une réponse de type Th2, moins efficace que la réponse Th1, et entrave ainsi la production d'interféron γ qui constitue une des principales voies de défense de l'organisme contre l'infection par le virus du SDRP (Diaz *et al.*, 2005; Mateu et Diaz, 2008). Ainsi, le retard de séroconversion anti-VHE observé chez les porcs co-infectés par le virus du SDRP, associé à un allongement de la période infectieuse et à une potentielle chronicité de l'infection par le VHE pourrait s'expliquer par une orientation spécifique de la réponse immunitaire gouvernée en partie par le virus du SDRP. D'autres études sont cependant nécessaires pour identifier ces mécanismes.

CONCLUSION

Les résultats obtenus dans cette étude montrent un impact important du virus du SDRP sur la dynamique d'infection du VHE et une potentielle chronicité chez des porcs co-infectés, augmentant le risque de présence du virus dans le foie des porcs abattus. Les mécanismes immunopathogéniques à l'origine d'une infection chronique par le VHE chez le porc

méritent d'être étudiés (exploration de la réponse immunitaire innée et adaptative anti-VHE, investigation des phénomènes biologiques se déroulant au cours de la période de latence, étude du rôle d'autres agents immunodépresseurs). Enfin, cette étude a montré une interaction forte entre un pathogène de santé animale (le virus du SDRP), aux conséquences considérables sur la compétitivité de la filière porcine, et un pathogène zoonotique (le VHE), aux conséquences sanitaires potentiellement graves chez l'homme. Ces données soulignent la nécessité d'une

appréhension globale de la santé animale et de la santé humaine et l'importance des programmes d'assainissement du virus du SDRP en élevage porcin, qui pourraient se révéler être un levier majeur pour la maîtrise du VHE dans la filière.

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Annexe 3 - Publication dans le *Bulletin Epidémiologique santé animale – alimentation* 78

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L'infection par le virus du syndrome dysgénésique et respiratoire porcin (SDRP) entraîne chez le Porc une infection chronique par le virus de l'hépatite E

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Résumé

Le virus de l'hépatite E (VHE) est responsable d'une hépatite aiguë chez l'Homme. Dans les pays industrialisés, l'augmentation de la fréquence de cas sporadiques, suite à la consommation de viande de porc insuffisamment cuite, pose la question du risque de transmission zoonotique à partir du réservoir porcin. L'impact de l'infection par le virus du syndrome dysgénésique et respiratoire porcin (SDRP), virus immunosuppresseur fortement prévalent en élevage porcin, sur celle du VHE chez le Porc a été étudié : i) à partir de données observationnelles de suivis de cohortes en élevages infectés par le VHE et ii) par l'intermédiaire d'un essai de co-infection expérimentale VHE/SDRP de porcs EOPS (exempts d'organismes pathogènes spécifiés). Les résultats obtenus dans ces deux études montrent un impact important du virus du SDRP sur la dynamique d'infection du VHE et une potentielle chronicité chez des porcs co-infectés, augmentant le risque de présence du virus dans le foie des porcs abattus. La co-infection ou infection préalable par le virus du SDRP retarde l'excrétion du VHE et augmente la durée d'excrétion, la charge virale excrétée et donc la transmission du virus au sein de la population. Une telle synergie pourrait expliquer la persistance enzootique du VHE en élevage et favoriser une excrétion tardive chez les porcs charcutiers jusqu'à l'abattage.

Mots-clés

Virus de l'hépatite E, zoonose, porc, virus du SDRP

Abstract

The infection by the Porcine Reproductive and Respiratory Syndrome (PRRS) virus promotes chronic Hepatitis E virus infection in pigs.

Hepatitis E Virus (HEV) is responsible for acute hepatitis in humans. In industrialized countries, the increase of sporadic cases frequently due to the consumption of raw pork meat raises the question of zoonotic transmission originating in the pig reservoir. The impact of PRRSV, an immunosuppressive virus that is highly prevalent in pig populations, on HEV infection has been studied based on: i) observational cohort data on HEV-infected farms, and ii) a PRRSV/HEV co-infection experiment using SPF (specific pathogen-free) pigs. The results obtained from both studies showed that PRRSV had a major impact on the dynamics of HEV infection and potential chronic infections in co-infected pigs, increasing the risk of HEV detection in the liver at the time of slaughter. Co-infection or previous infection by PRRSV postponed HEV shedding and increased its duration as well as the viral load shed, and consequently increased transmission of the virus within the population. This type of synergic association could explain the enzootic persistence of HEV in pig farms and increase the likelihood of late shedding in finishing pigs up through slaughter.

Keywords

Hepatitis E virus, Zoonosis, Pig, PRRS virus

Le virus de l'hépatite E (VHE) est un virus à ARN simple brin de polarité positive, non enveloppé, agent étiologique d'une hépatite aiguë chez l'Homme. Transmis principalement par voie oro-fécale, il est généralement responsable de signes cliniques similaires à ceux de l'hépatite A mais en moyenne plus sévères (Emerson and Purcell, 2003). Des formes chroniques peuvent également être observées, notamment chez des individus sous traitement immunosuppresseur (Gerolami et al., 2008; Izopet et al., 2009; Kamar et al., 2008). En Europe, l'hépatite E est aujourd'hui considérée comme une zoonose émergente (Adlhoc et al., 2016). Avec plus de 1800 cas humains autochtones annuels recensés en France par le centre national de référence (CNR) en 2013 et en 2014, une origine alimentaire est le plus souvent suspectée même si l'origine exacte de l'infection n'est identifiée avec certitude que dans un nombre limité de cas. Les produits à base de porc, et notamment ceux contenant du foie cru, consommés crus ou peu cuits, constituent les aliments les plus à risque (Pavio et al., 2014; Renou et al., 2014). Il est établi que la population porcine domestique constitue un des principaux réservoirs du VHE dans la plupart des pays producteurs de porcs. En France, le virus circule dans plus de 65 % des élevages et 4 % des porcs en moyenne sont porteurs du virus au niveau du foie au moment de l'abattage (Rose et al., 2011). L'existence d'une virémie, le plus souvent transitoire, peut cependant conduire à la détection du virus au niveau du muscle. En France, la prévalence de porcs virémiques entrant dans la chaîne alimentaire a été estimée extrêmement basse lors de l'enquête nationale de prévalence réalisée en 2009 (0,4 % des porcs abattus en moyenne contre 4 % de foies positifs (Rose et al., 2011)). Au Royaume-Uni, une étude récente fait cependant état d'une

prévalence de porcs virémiques beaucoup plus élevée (3 % (Grierson et al., 2015)).

En élevage, même si la prévalence de porcs excréteurs est en moyenne maximale entre 90 et 120 jours d'âge selon les études, différentes dynamiques d'infection inter-individuelles et inter-élevages sont observées et sont en lien direct avec la probabilité de contamination des foies des porcs abattus (Rose and Pavio, 2014). Ces variations inter-élevages de la dynamique d'infection du VHE ne sont pas entièrement expliquées à ce jour, de même que certaines excrétions très prolongées (jusqu'à 60 jours) décrites dans la littérature (Kanai et al., 2011) qui contrastent avec les observations expérimentales en conditions contrôlées (une 10^{aine} de jours (Andraud et al., 2013)). Certains co-facteurs infectieux pourraient ainsi favoriser l'infection VHE, voire entraîner une forme de chronicité à l'instar de ce qui est observé chez l'Homme.

Le virus du SDRP (syndrome dysgénésique et respiratoire porcin) est extrêmement répandu dans les régions où la population porcine est très dense (environ 60 % de prévalence en Bretagne par exemple). Ce virus a des caractéristiques immunosuppressives qui suggèrent une interaction possible avec le VHE. Il existe peu de données sur l'association potentielle de ces deux virus, essentiellement des découvertes *post-mortem*, avec mise en évidence de la présence des deux virus, sans pour autant apporter la preuve de leur implication directe dans le tableau clinique observé (de Deus et al., 2007; Mao et al., 2013). Une autre étude n'a cependant pas mis en évidence d'association particulière entre la présence de VHE et d'autres infections dont le virus du SDRP (Martelli et al., 2010).

Les travaux décrits dans le présent article associent deux études: i) un suivi de cohorte en élevages infectés par le VHE et le virus du SDRP, ayant pour objectif d'identifier d'éventuels liens entre la dynamique d'infection SDRP et la durée d'excrétion du VHE, ii) une étude expérimentale sur la co-infection des deux virus destinée à quantifier l'impact de l'infection par le virus du SDRP sur l'excrétion du VHE et sa transmission à des porcs contacts.

Matériel et méthodes

Étude de cohorte en élevages infectés

Protocole d'étude

L'étude longitudinale a été conduite dans trois élevages porcins de type naisseur-engraisseur identifiés comme étant infectés par le VHE d'après les résultats d'une enquête de prévalence antérieure (Rose *et al.*, 2011). Ces trois élevages étaient également infectés par le virus du SDRP avec une présence avérée d'une circulation active du virus chez les porcs en croissance. Au sein des élevages sélectionnés, trois bandes successives de porcelets ont été suivies. Chaque bande comportait un total de 800, 322 et 265 porcelets pour les élevages A, B et C respectivement. Pour chaque bande, un échantillon représentatif de 40 porcelets a été constitué à la naissance par tirage au sort parmi les différentes portées de la bande, soit 120 porcelets par élevage et un total de 360 porcs (3 élevages) ont ainsi été suivis individuellement de la naissance à l'abattage.

En élevage, des prélèvements individuels sanguins et rectaux de matières fécales ont été effectués à 1, 6, 10, 14, 18 et 22 semaines d'âge sur les animaux suivis. À l'abattoir, les porcs suivis individuellement en élevage ont été soumis à des prélèvements de sang au poste de saignée, puis de foie sur la chaîne. La détection des ARN du virus de l'hépatite E dans les matières fécales a été réalisée à l'aide d'une RT-PCR en temps réel (Barnaud *et al.*, 2012; Jothikumar *et al.*, 2006). Les anticorps anti-VHE ont été détectés à l'aide d'un test commercial validé pour les analyses vétérinaires (HEV ELISA 4.0v - MP Diagnostics, Illkirch, France). Les anticorps dirigés contre le virus du SDRP ont été détectés à l'aide du kit ELISA HerdChek* PRRS X3 IDEXX (Liebefeld-Bern, Suisse) selon les instructions du fabricant. Pour des raisons logistiques, les analyses sérologiques spécifiques du virus du SDRP n'ont été effectuées que sur la moitié des animaux (20 animaux par bande, soit 180 individus au total).

Analyse statistique

L'âge à la séroconversion et à l'excrétion du VHE ainsi que la durée de la période infectieuse ont été estimés par une analyse de survie. Un modèle de survie à risques proportionnels (modèle de Cox) a été utilisé afin d'évaluer l'impact de la chronologie des infections entre le VHE et le virus du SDRP sur l'âge à l'excrétion et à la séroconversion vis-à-vis du VHE. L'effet élevage a été incorporé dans le modèle en tant qu'effet aléatoire (modèle de fragilité de Cox). L'impact de la chronologie des infections sur la durée de la période infectieuse VHE a été exploré par une analyse de survie paramétrique. La chronologie des infections a été estimée de la manière suivante:

- la date de première excrétion du VHE étant connue, la date de l'infection par le VHE a été estimée en considérant que le délai infection – excrétion était de 25 jours, conformément au délai moyen décrit dans la littérature (Satou and Nishiura, 2007),
- la date de séroconversion vis-à-vis du virus du SDRP étant connue, la date de l'infection par ce dernier a été estimée en considérant que le délai infection – séroconversion était de sept jours, conformément au délai moyen décrit dans la littérature (Diaz *et al.*, 2005).

Les effets du niveau de l'immunité maternelle anti-VHE, de l'élevage d'origine des animaux et de l'interaction entre l'élevage et la chronologie des infections ont également été testés dans le modèle.

L'influence de la chronologie des infections entre le VHE et le virus du SDRP sur la contamination des foies au stade de l'abattage a été étudiée par un modèle de régression logistique, avec comme variable

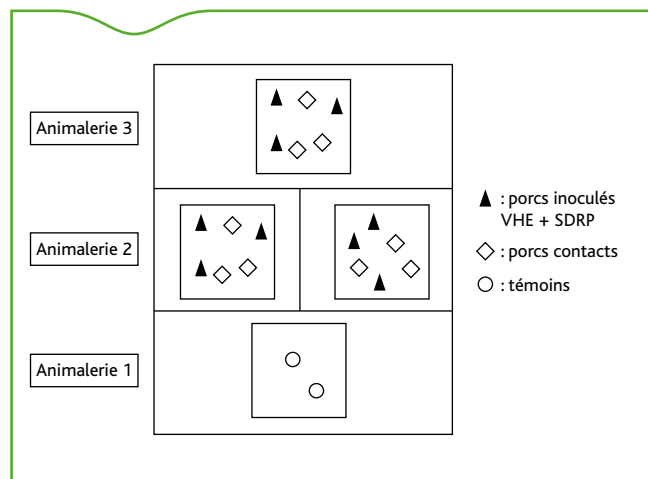


Figure 1. Schéma du dispositif expérimental de co-infection VHE/SDRP

à expliquer le statut VHE des foies à l'abattoir, et comme variable explicative la chronologie des infections virus SDRP/VHE. Les effets du niveau de l'immunité maternelle anti-VHE, de l'élevage d'origine des animaux et de l'interaction entre l'élevage et la chronologie des infections ont également été testés dans le modèle.

Étude expérimentale de co-infection VHE/ virus SDRP

Protocole expérimental

L'expérimentation a été conduite au sein des animaleries protégées du laboratoire de l'Anses de Ploufragan, niveau 3 de biosécurité sous air filtré. Vingt porcs EOPS âgés de cinq semaines ont été utilisés; ils étaient exempts du VHE et du virus du SDRP et n'avaient aucun anticorps maternel spécifique de ces virus. Dix-huit porcelets ont été répartis aléatoirement (stratification en fonction du sexe, de la portée et du poids) dans trois parcs contenant chacun six animaux: trois porcs inoculés par le VHE et le virus du SDRP, et trois animaux contacts. Deux porcelets ont été utilisés comme témoins non infectés (Figure 1).

À J0, trois porcs par parc ont été inoculés: i) par voie orale (sondage œsophagien) avec un inoculum de VHE génotype 3 titrant à 10^8 génomes équivalents (GE) sous un volume de 10 mL, préparé selon le protocole décrit par Andraud *et al.* (2013), et ii) par voie intranasale avec un inoculum d'une souche de virus du SDRP de génotype 1, sous-type 1 (PRRS/FR/29/24/1/2005, souche FINISTÈRE) titrant à $5 \cdot 10^5$ DCP₅₀ (dose cytopathogène 50) pour un volume de 2,5 mL par narine.

Les matières fécales des animaux ont été prélevées individuellement trois fois par semaine dès J-3 et jusqu'à 49 jours post-infection (JPI). Une prise de sang a été réalisée avant inoculation puis une fois par semaine. La quantification des ARN du virus de l'hépatite E dans les matières fécales a été réalisée par une RT-PCR quantitative en temps réel (Barnaud *et al.*, 2012). Les résultats sont exprimés en nombre de copies de génome par gramme de fèces (GE/g). La détection des anticorps dirigés contre le VHE a été réalisée avec le kit HEV ELISA 4.0v (MP Diagnostics, Illkirch, France) (Barnaud *et al.*, 2012). La détection des ARN du virus du SDRP dans le sérum a été réalisée à l'aide d'une RT-PCR en temps réel (Charpin *et al.*, 2012).

Analyses statistiques et modèles

Influence de l'infection SDRP sur les paramètres de l'infection par le VHE

Les durées d'excrétion du VHE avec ou sans infection SDRP associée ont été estimées et comparées par un modèle de survie paramétrique. L'influence du délai nécessaire à la production d'anticorps anti-VHE sur la durée de la période infectieuse a été étudiée par l'intermédiaire d'un modèle semi-paramétrique de Cox. La réponse immunitaire humorale a été qualifiée d'absente ou de tardive si le délai infection-séroconversion était supérieur ou égal à 25 JPI et de précoce s'il était strictement inférieur à 25 JPI (Satou and Nishiura, 2007).

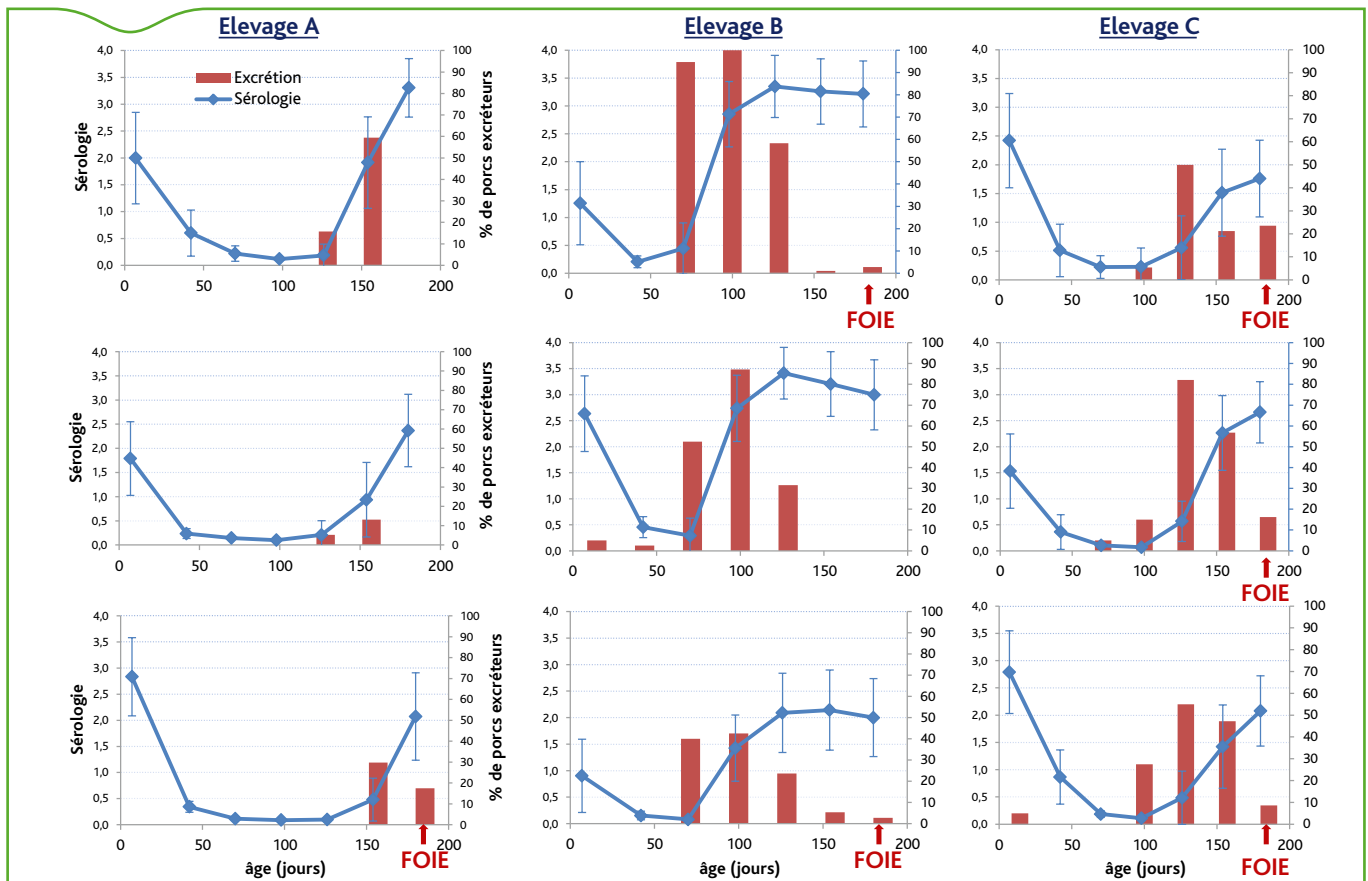


Figure 2. Dynamiques d'infection par le VHE observées dans chaque élevage suivi (3 bandes suivies par élevage)

Influence de l'infection SDRP sur l'excrétion et la transmission du VHE

Un modèle linéaire mixte prenant en compte des données répétées dans le temps a été utilisé afin d'évaluer la différence entre les charges virales VHE selon le statut des animaux vis-à-vis du virus du SDRP.

La transmission du VHE aux porcs contacts a été modélisée selon deux voies principales: i) la transmission due aux contacts directs entre les porcs infectés et les porcs sensibles, et ii) la transmission indirecte oro-fécale à partir d'un réservoir environnemental au sein d'une même case. Le modèle utilisé est similaire à celui utilisé pour une expérience de transmission du VHE sans co-infection (Andraud *et al.*, 2013). Les paramètres de transmission directe et indirecte, respectivement β_w et $\beta_e^{(w)}$, la durée de la période de latence de chaque individu contact et le taux de clairance du virus (qui représente l'élimination des matières fécales au travers du caillebotis de la case et la destruction du virus dans l'environnement) ont été estimés par une approche bayésienne.

Résultats

Étude de cohorte en élevages infectés

Description des dynamiques d'infection observées

Des profils d'infection très différents ont été mis en évidence selon les élevages. Dans l'élevage A, l'excrétion du VHE a été très tardive et de courte durée alors qu'elle a été relativement précoce et parfois très longue chez certains animaux dans les deux autres élevages (élevages B et C) (Figure 2). Dans ces deux élevages, des porcelets étaient excréteurs dès la phase de maternité. La prévalence maximale d'excrétion atteinte était aussi très variable selon les élevages et les bandes (jusqu'à 100 % dans la bande 1 de l'élevage B contre 10 % dans la bande 2 de l'élevage A). Les plus fortes proportions de foies positifs (jusqu'à 23 %) ont été atteintes dans l'élevage C associées à un pic d'excrétion atteint après 120 jours d'âge. L'analyse de ces données individuelles montre qu'il n'existe pas de relation stricte

entre la contamination des foies et l'âge à la première excrétion décelée, mais que la probabilité d'infection des foies augmente considérablement lorsque le délai infection-abattage est inférieur à 40 jours (OR=3,5 ; IC_{95%}[1,4-10,9]).

Effet de la chronologie de l'infection SDRP sur les caractéristiques de l'infection par le VHE

Les animaux préalablement infectés par le virus du SDRP ont une excrétion et une séroconversion vis-à-vis du VHE significativement plus tardives que les animaux qui ne se sont pas infectés par le virus du SDRP ou qui se sont infectés après leur infection par le VHE (Hazard Ratio (HR) = 0,49, p-value < 0,01 et HR = 0,46, p-value < 0,01 respectivement) (Figure 3). Les effets de l'immunité maternelle anti-VHE, de l'élevage et de l'interaction entre l'élevage et la chronologie des deux infections ne sont pas significatifs. La durée moyenne de la période infectieuse a été estimée à 27,6 jours [24,4 ; 31,2] par un modèle de survie paramétrique (distribution de type Weibull) pour l'ensemble des animaux suivis. Cependant, la durée de la période d'excrétion n'était pas significativement associée à une infection préalable par le virus du SDRP (p-value > 0,05).

Effet de la chronologie de l'infection SDRP sur la détection du VHE à l'abattoir à partir des foies

Une infection par le virus du SDRP préalable à l'infection VHE est significativement associée à une probabilité plus élevée que les foies soient positifs à l'abattoir (Odds Ratio (OR) = 2,67, p-value = 1,04*10⁻²). Les effets de l'immunité maternelle anti-VHE, de l'élevage et l'interaction entre l'élevage et la chronologie des deux infections ne sont pas significatifs.

Étude expérimentale de co-infection VHE/SDRPv

Description des données d'infection

L'ensemble des animaux inoculés et contacts sont virémiques pour le virus du SDRP avant la première excrétion du VHE détectée excepté pour deux porcs contacts (données non montrées). L'ensemble des individus exposés au VHE, contacts et inoculés, excrètent le VHE

Tableau 1. Résultats de la détection du VHE par RT-PCR quantitative sur les échantillons individuels de matières fécales (log[nombre de génomes équivalents/g])

	JPI	J2	J4	J7	J9	J11	J14	J16	J18	J21	J23	J25	J28	J32	J35	J39	J42	J44	J46	J49	FOIE	
Case 1	Témoin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Témoin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Case 2	Infecté	-	-	-	5,03	0*	4,35	5,35	6,09	6,42	7,14	7,13	6,78	6,92	6,88	7,47	7,65	6,90	6,94	7,17	6,16	
	Infecté	-	-	-	-	-	4,35	5,24	6,28	6,83	7,35	7,55	7,88	8,12	8,00	7,83	7,18	8,04	7,60	7,77	6,27	
	Infecté	-	-	-	-	-	-	-	5,18	6,18	6,77	7,06	7,11	7,61	7,60	7,97	7,63	6,70	7,86	7,81	6,01	
	Contact	-	-	-	-	-	-	-	-	-	-	5,43	6,06	7,25	7,19	7,57	7,28	7,15	7,73	7,69	5,94	
	Contact	-	-	-	-	-	-	-	-	-	-	-	5,46	6,57	6,55	5,80	5,18	5,69	6,07	5,54	-	
	Contact	-	-	-	-	-	-	-	-	-	-	-	2,25	5,95	6,16	6,48	5,73	6,08	6,63	6,28	6,08	-
Case 3	Infecté	-	-	-	-	-	-	5,19	6,04	6,20	6,45	7,22	7,43	7,94	7,80	6,65	6,59	7,21	6,00	5,16	3,19	
	Infecté	-	-	-	-	-	-	5,34	5,74	6,84	7,27	7,18	7,36	7,19	7,25	7,41	6,90	8,14	7,29	6,83	6,38	
	Infecté	-	-	-	-	-	5,01	5,84	8,70	7,59	7,62	7,60	7,83	8,10	8,14	7,77	7,91	8,12	7,05	6,69	5,56	
	Contact	-	-	-	-	-	-	-	-	-	-	6,22	0*	6,16	6,61	6,69	0*	7,07	7,40	7,74	6,05	
	Contact	-	-	-	-	-	-	-	-	-	-	-	-	6,26	6,73	6,92	6,65	7,92	7,16	6,72	6,23	
	Contact	-	-	-	-	-	-	-	-	-	-	-	-	6,17	6,86	7,29	7,48	7,64	8,40	8,49	6,54	
Case 4	Infecté	-	-	-	-	-	-	5,37	5,49	6,59	6,83	6,90	7,02	7,62	7,42	7,63	7,18	7,46	7,20	8,32	6,51	
	Infecté	-	-	-	-	-	-	-	5,78	5,98	6,61	6,51	7,19	7,48	7,25	7,26	7,27	7,93	7,27	7,76	4,31	
	Infecté	-	-	-	-	-	4,91	5,64	6,35	7,06	7,35	7,24	7,37	7,56	7,50	7,02	6,64	7,61	7,16	7,75	5,98	
	Contact	-	-	-	-	-	-	-	-	-	-	-	-	-	4,94	5,31	6,37	5,53	5,61	5,70	5,87	
	Contact	-	-	-	-	-	-	-	-	-	-	-	-	-	6,92	7,18	7,93	0*	7,16	7,94	8,01	5,77
	Contact	-	-	-	-	-	-	-	-	-	-	-	-	-	6,12	5,93	6,05	0*	4,64	5,89	5,92	-

JPI : jours post-infection
* : testés en duplicat

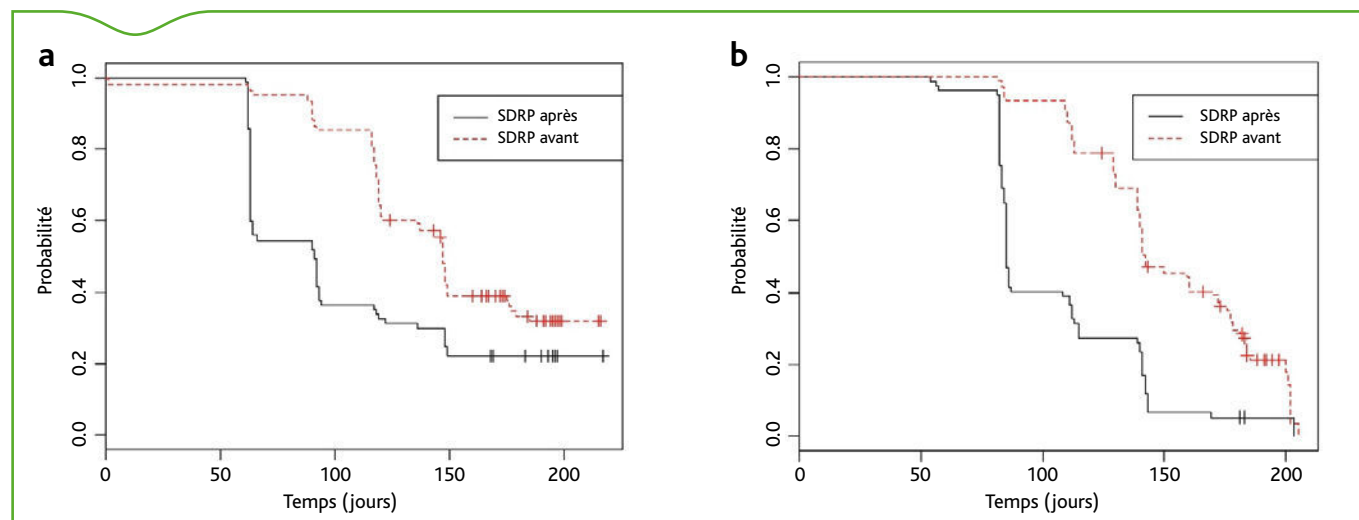


Figure 3. Effet de l'infection par le virus du SDRP sur l'âge à l'excrétion (a) et à la séroconversion (b) vis-à-vis du VHE (180 individus suivis individuellement) : courbes de survie

jusqu'à la fin de l'essai (49 JPI) (Tableau 1). À l'autopsie, quatorze foies sur dix-huit sont positifs en RT-PCR VHE. Seuls quatre individus inoculés sur neuf, et sept individus contacts sur neuf ont présenté une réponse immunitaire humorale anti-VHE (entre 35 et 49 JPI, et 42 et 49 JPI respectivement, données non montrées).

La durée de la période de latence a été estimée à 12,9 jours [12,8; 14,4] pour les porcs inoculés et 13,4 jours [8,6; 17,1] pour les porcs contacts. La durée de la période infectieuse a été estimée à 48,6 jours [27,9; 84,6] (Tableau 2). La durée moyenne nécessaire à la production d'anticorps spécifiques du VHE a été estimée à 43,1 jours [35,7; 52,2] lors de co-infection par le virus du SDRP et à 26,3 jours [23,5; 29,5] en l'absence de co-infection (Tableau 2). Une réponse immunitaire humorale tardive ou absente (>25 JPI) était associée à une augmentation de la durée d'excrétion et donc de la période infectieuse (la fin d'excrétion étant retardée, Hazard Ratio = 0,35, $P < 0,01$).

Quantification de l'excrétion et de la transmission du VHE > Excrétion du VHE

Chez les individus inoculés, l'infection par le virus du SDRP est significativement associée à une augmentation de la charge en VHE excrétée ($P = 0,05$, Figure 5), comparativement à l'infection par le VHE seul (Andraud *et al.*, 2013). L'interaction entre le temps et l'infection par le virus du SDRP est significative et positive, *i.e.* l'impact du virus du SDRP augmente au cours du temps ($p = 0,04$). En revanche, l'effet de l'infection par le virus du SDRP sur les charges VHE excrétées n'est pas statistiquement significatif chez les individus contacts ($p > 0,05$, données non montrées).

> Paramètres de transmission du VHE

Les résultats montrent que, dans ces conditions expérimentales, un porc excréteur de VHE est capable de transmettre l'infection à 0,70 porc par jour, par contact direct ($\beta_w = 0,70 [1,18 \times 10^{-3}; 3,67]$) (Tableau 2).

Tableau 2. Paramètres de transmission estimés au cours de l'essai expérimental de co-infection VHE/SDRP et comparaison avec les données d'infection VHE seul

	VHE + virus du SDRP	VHE seul*
Période de latence (jours)	13,4 [8,6 ; 17,1]	7,1 [3,2 ; 12,3]
Période infectieuse (jours)	48,6 [27,9 ; 84,6]	9,7 [8,2 ; 11,2]
Transmission directe [β_w] (jour ⁻¹)	0,70 [1,2*10 ⁻³ ; 3,67]	0,15 [0,03 ; 0,31]
Transmission indirecte [$\beta_E^{(w)}$] (g/GE/j)	6,6*10 ⁻⁶ [1,4*10 ⁻¹⁰ ; 1,3*10 ⁻⁴]	2,0*10 ⁻⁶ [1,1*10 ⁻⁷ ; 7,0*10 ⁻⁶]
Délai de séroconversion (jours)	43,1 [35,7 ; 52,2]	26,3 [23,5 ; 29,5]

*d'après Andraud et al. (2013)

Le taux de transmission indirecte au sein d'une case peut être considéré comme le nombre moyen d'animaux qui peuvent être infectés par une seule particule virale présente dans l'environnement ($\beta_E^{(w)} = 6,59*10^{-6}$ g/GE/jour [1,43*10⁻¹⁰ ; 1,27*10⁻⁴]) (Tableau 2). Autrement dit, l'inverse de $\beta_E^{(w)}$ correspond au nombre moyen de particules virales par gramme de fèces dans l'environnement, nécessaires pour infecter un animal par jour, soit 1,51*10⁵ GE/g/jour [7,86*10³ ; 7,00*10⁹].

Discussion

L'impact d'une co-infection par le virus du SDRP sur l'infection, l'excrétion et la transmission du virus de l'hépatite E, a été étudié, d'une part en comparant des données observationnelles obtenues en conditions réelles dans des élevages infectés par ces deux virus et d'autre part en situation expérimentale. Les deux approches complémentaires suggèrent que l'infection préalable par le virus du SDRP modifie le processus infectieux du VHE en retardant l'excrétion, mais en augmentant quantitativement la charge virale excrétée au niveau individuel, ainsi que la durée d'excrétion favorisant ainsi sa propagation et sa persistance dans la population.

Les données issues d'élevages suggèrent que la séquence des événements entre les deux infections joue un rôle important. Ainsi, une infection préalable par le virus du SDRP retarde l'âge à l'excrétion du VHE et est associée à une séroconversion retardée. Il est également montré à partir de ces mêmes données que la probabilité de détecter du VHE au niveau du foie des animaux abattus est aussi significativement augmentée chez ces porcs préalablement infectés par le virus du SDRP. Ce délai d'excrétion observé à partir des données de terrain est cohérent avec la latence augmentée mesurée dans l'essai expérimental (13,4 jours dans l'essai de co-infection, contre 7,1 jours lors d'infection par le VHE seul). Une activation de la réponse immunitaire innée par le virus du SDRP, retardant ainsi l'excrétion du VHE pourrait expliquer l'allongement de la durée de la période de latence observée.

La durée d'excrétion du VHE estimée à partir des données terrain n'était pas significativement différente selon la séquence d'infection avec le virus du SDRP (27 jours en moyenne) contrairement à l'augmentation considérable observée au cours de l'essai expérimental: 48,6 versus 9,7 jours pour l'infection VHE simple, soit un allongement d'un facteur 5. Ces données se rapprochent des données de terrain de la littérature (27 jours [20 ; 39]) (Backer *et al.*, 2012)). Dans l'essai décrit par Bouwknegt *et al.* (2008), la période infectieuse a été évaluée entre 13 et 49 jours, les animaux utilisés provenant d'un élevage conventionnel de haut niveau sanitaire *a priori* indemne de SDRP. Les résultats de l'essai réalisé montrent que l'excrétion virale des individus inoculés est quantitativement plus importante lors de co-infection par le virus du SDRP. Le taux de transmission directe est 4,7 fois plus élevé lors de co-infection (0,70 par jour contre 0,15 avec le VHE seul (Andraud *et al.*, 2013)). Ainsi, lors de co-infection par le virus du SDRP, la voie de transmission directe joue un rôle plus important dans la transmission du VHE, ce qui peut être mis en lien avec l'excrétion individuelle quantitativement plus importante. Le taux de transmission environnementale au sein d'une case (*i.e.* le nombre d'animaux infectés par particule virale présente dans l'environnement) est estimé 3,3 fois

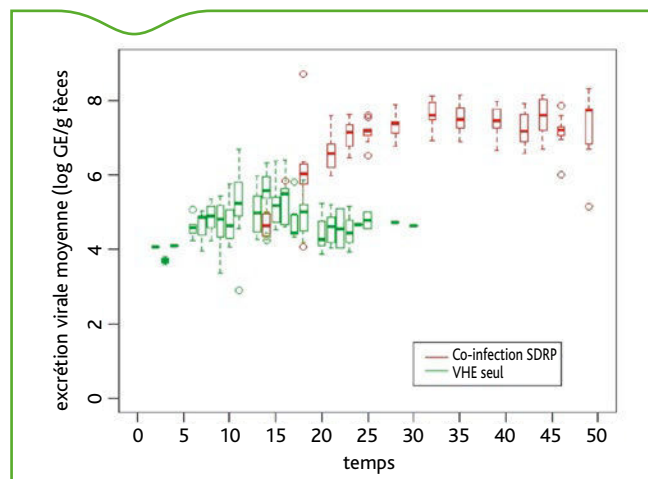


Figure 5. Distribution des charges génomiques en VHE excrétées au cours du temps, chez les porcs inoculés par le VHE co-infectés ou non par le virus du SDRP

plus élevé lors de co-infection qu'en l'absence. Autrement dit, 3,3 fois moins de particules virales sont suffisantes pour infecter un animal en présence du virus du SDRP. Dans un modèle construit à partir d'un essai d'infection VHE par voie intraveineuse, Bouwknegt *et al.* (2011) rapportent que la dose orale de VHE pour laquelle la probabilité d'infection est égale à 50 % serait de 1,4*10⁶ GE/g, ce qui est dix fois supérieur à la dose nécessaire estimée dans notre étude. Ces données confortent l'hypothèse d'une plus grande sensibilité vis-à-vis du VHE chez des porcs co-infectés par le virus du SDRP.

En conditions réelles, la séroconversion à l'égard du VHE est significativement retardée chez les animaux préalablement infectés par le virus du SDRP, ce qui est corroboré par une durée de 43,1 jours pour observer une séroconversion chez les animaux co-infectés en situation expérimentale versus 26,3 jours en moyenne chez des porcs infectés par VHE seul. Ce défaut de réponse sérologique est significativement associé à l'allongement de la durée de la période infectieuse lors de co-infection par le virus du SDRP et pourrait donc expliquer la présence de particules virales dans le foie des porcs abattus.

Conclusion

Les résultats obtenus dans ces deux études montrent un impact important du virus du SDRP sur la dynamique d'infection du VHE et une potentielle chronicité chez des porcs co-infectés, augmentant le risque de présence du virus dans le foie des porcs abattus. Ces résultats mettent en évidence une interaction forte entre un agent pathogène non zoonotique (le virus du SDRP), aux conséquences considérables sur la compétitivité de la filière porcine, et un agent pathogène zoonotique n'affectant pas les animaux (le VHE), aux conséquences sanitaires potentiellement graves chez l'Homme. Ces données soulignent encore la nécessité d'une appréhension globale de la santé animale et de la santé humaine et l'importance des programmes d'assainissement du virus du SDRP en élevage porcin, qui pourraient se révéler être un levier majeur pour la maîtrise du VHE dans la filière.

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Brève. Nouveau foyer de maladie de l'œdème chez le Sanglier, massif des Albères, Pyrénées-Orientales *Short item. A new outbreak of edema disease in wild boar, Albères Mountain, Pyrénées-Orientales, France*

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Mots-clés: Maladie de l'œdème, *Sus scrofa*, France/**Keywords:** Edema disease, *Sus scrofa*, France

De début septembre à fin novembre 2016, le réseau Sagir (réseau de l'Office national de la chasse et de la faune sauvage et de la Fédération nationale des chasseurs) a enregistré des signaux de mortalité anormale de sangliers dans le massif des Albères (Pyrénées-Orientales). La mortalité était d'amplitude anormale pour le département et très agrégée d'un point de vue spatio-temporel. Deux foyers de mortalité ont été identifiés, centrés sur Maureillas et Argelès. Les premières estimations de terrain chiffrent la mortalité/morbidité observée à environ 75 sangliers. Les juvéniles ont principalement été touchés (sangliers de 4-6 mois, en phase post-sevrage, en bon état corporel). Quelques adultes ont également été observés malades. Certains animaux présentaient une condition corporelle dégradée, associée à une strongylose respiratoire sévère. Des signes nerveux de type convulsions, tremblements et ataxie ont été observés. Les investigations épidémiologiques, cliniques, anatomo-pathologiques, bactériologiques ont permis de conclure avec un haut degré de certitude à l'émergence d'un nouveau foyer de maladie de l'œdème. La maladie de l'œdème se caractérise par une entéro-toxémie aigue souvent fatale, provoquée par quelques sérotypes d'*Escherichia coli*. Les *E. coli* pathogènes prolifèrent dans l'intestin grêle et produisent des shigatoxines (vérotoxines)

responsables de lésions artérielles. En résultent des œdèmes, des morts subites, et des signes neurologiques consécutifs à l'œdème cérébral (Imberechts *et al.* 1992). Il s'agit du deuxième foyer détecté chez des Suidés sauvages en France et dans le monde, dans des conditions naturelles. Le premier foyer avait été identifié en Ardèche, durant l'été 2013 (Decors *et al.* 2015). En Ardèche comme dans les Pyrénées-Orientales, le sérotype identifié est *E. Coli* O139k82.

Des investigations vont être mises en œuvre dans le cadre d'un travail universitaire, pour identifier les facteurs de risque associés à l'émergence de la maladie dans les populations de sangliers ardéchoises et pyrénéennes.

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Annexe 4 - Publication dans les *Journées Recherche Porcine 51*

Salines M., Rose N., Andraud M., 2019. Analyse des conditions de propagation et de persistance du virus de l'hépatite E en élevage porcin et identification de mesures de maîtrise : une approche par modélisation multi-pathogènes. *Journées Recherche Porcine 51*.

Analyse des conditions de propagation et de persistance du virus de l'hépatite E en élevage porcin et identification de mesures de maîtrise : une approche par modélisation multi-pathogènes

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Analyse des conditions de propagation et de persistance du virus de l'hépatite E en élevage porcin et identification de mesures de maîtrise : une approche par modélisation multi-pathogènes

Le virus de l'hépatite E (VHE) est un agent zoonotique dont les porcs représentent le principal réservoir dans les pays industrialisés. Une grande variabilité de dynamique d'infection du VHE en élevage porcin a été décrite et peut être due à l'influence d'autres pathogènes, en particulier d'autres virus affectant la réponse immunitaire des porcs. L'objectif de cette étude est d'utiliser une approche de modélisation multi-pathogènes pour comprendre les conditions de propagation et de persistance du VHE dans un élevage naisseur-engraisseur dans lequel les porcs peuvent être co-infectés par un pathogène intercurrent.

Le modèle stochastique individu-centré construit dans ce but couple un modèle de dynamique de population, permettant de prendre en compte le type de conduite d'élevage, avec un modèle épidémiologique multi-pathogènes décrivant la transmission du VHE en présence du pathogène intercurrent. Il combine ainsi un modèle de diffusion d'un agent pathogène immunomodulateur, dont le prototype est le virus du SDRP, et un modèle de diffusion du VHE incluant plusieurs statuts (avec immunité maternelle - sensible - exposé - infecté - retiré - sensible). Basés sur des données expérimentales et de terrain, les paramètres épidémiologiques du modèle VHE varient selon le statut de co-infection avec le pathogène immunomodulateur.

Les résultats des simulations montrent que la co-infection avec un pathogène immunomodulateur favorise la diffusion et la persistance du VHE et augmente la prévalence de foies contaminés à l'abattage. Le type de conduite en bande et certaines pratiques d'élevage sont également apparus comme ayant un impact majeur sur la diffusion du VHE. La maîtrise des pathogènes intercurrents et l'amélioration des pratiques d'élevage sont ainsi des leviers essentiels permettant de limiter le risque de santé publique lié au VHE.

Analysis of conditions for spread and persistence of the hepatitis E virus in pig herds and identification of control measures: a multi-pathogen modelling approach

The hepatitis E virus (HEV) is a zoonotic agent for which domestic pigs have been recognised as the main reservoir in industrialised countries. HEV infection dynamics vary greatly among pig farms and may be related to the influence of other pathogens, in particular viruses affecting pigs' immune response. The objective of this study was to use a multi-pathogen modelling approach to understand conditions of HEV spread and persistence on a farrow-to-finish pig farm in which pigs may be co-infected with an intercurrent pathogen.

To do so, a stochastic individual-based model was developed. It combines a population dynamics model, which makes it possible to take several batch-rearing systems into account, with a multi-pathogen model representing HEV transmission in the presence of the intercurrent pathogen. It therefore couples the dynamics of an immunomodulating virus (e.g. PRRSV) with a MSEIRS (Maternally immune - Susceptible - Exposed - Infected - Recovered - Susceptible) model of HEV spread. Based on experimental and field data, epidemiological parameters of the HEV model varied according to the pig's immunomodulating virus status.

Co-infection with an immunomodulating pathogen was found to favour HEV spread and persistence and to increase the prevalence of livers containing HEV at slaughter. Herd structure, driven by the batch-rearing system, and certain farming practices were also identified as pivotal factors impacting HEV spread dramatically. Controlling intercurrent pathogens and improving farming practices thus appear to be major mechanisms for mitigating HEV-related risks to public health.

INTRODUCTION

Le virus de l'hépatite E est un virus non-enveloppé à ARN simple brin provoquant chez l'homme une infection souvent asymptomatique, mais parfois une hépatite aiguë ou chronique en fonction, entre autres, du contexte immunitaire du patient (Emerson et Purcell, 2003; Kamar *et al.*, 2011). Les génotypes 3 et 4 sont partagés par l'homme et d'autres espèces animales et sont responsables de cas sporadiques autochtones dans les pays industrialisés (Dalton *et al.*, 2008; Purcell et Emerson, 2008). En particulier, le génotype 3 est largement répandu dans la population porcine et un certain nombre de cas autochtones ont pu être reliés à la consommation de produits porcins crus ou insuffisamment cuits (Colson *et al.*, 2010; Moal *et al.*, 2012; Motte *et al.*, 2012; Guillois *et al.*, 2016). En ce sens, l'hépatite E est reconnue comme une zoonose alimentaire dont les porcs domestiques sont le principal réservoir dans les pays développés (Pavio *et al.*, 2010). Le risque que des produits contaminés soient commercialisés dépend étroitement de la dynamique d'infection du VHE dans les élevages de porcs. Cependant, l'épidémiologie du VHE dans la filière porcine est encore mal comprise et l'hétérogénéité des données de prévalence disponibles dans la littérature indique des variations de la dynamique d'infection du VHE (Salines *et al.*, 2017). Certains facteurs de risque spécifiques augmentant la persistance et la propagation du VHE en élevage ont été identifiés. Ainsi, Certaines pratiques d'élevage (en termes d'hygiène, de biosécurité et de condition d'élevage), les mouvements d'animaux entre élevages, des facteurs de risque individuels, liés aux caractéristiques propres des porcelets ou héritées de leur mère (sexe des porcelets, parité de la truie, ou protection partielle par les anticorps d'origine maternelle) se sont révélés être des facteurs impactant la dynamique d'infection en élevage (Andraud *et al.*, 2014; Walachowski *et al.*, 2014; Salines *et al.*, 2018a; Salines *et al.*, 2018b). Il a aussi été montré que la co-infection avec le virus du Syndrome Dysgénésique et Respiratoire Porcin (SDRP) conduit à une infection chronique par le VHE, tant en conditions expérimentales que naturelles (Salines *et al.*, 2015; Salines *et al.*, 2018b). Plusieurs études se sont attachées à décrire et quantifier la transmission du VHE entre les porcs. Ainsi, Satou et Nishiura (2007) ont estimé le nombre de reproduction de base ($R_0 = 4,02-5,17$) à partir de données sérologiques d'élevages de porcs au Japon. Backer *et al.* (2012) ont obtenu un R_0 similaire à partir de données britanniques et ont développé une approche par modélisation pour évaluer certaines stratégies de maîtrise, dont une vaccination fictive. Bouwknegt *et al.* (2009) ont développé un modèle stochastique SIR simple pour quantifier la transmission du VHE entre les porcs et ont obtenu des valeurs de R_0 plus élevées ($R_0 = 4,4-19$). La même équipe a ensuite montré que la voie de transmission oro-fécale directe était probable mais non suffisante pour expliquer la transmission du virus (Bouwknegt *et al.*, 2011). L'hypothèse d'une transmission par voie environnementale a été confortée par Andraud *et al.* (2013), qui ont démontré l'impact de l'accumulation et la persistance du VHE dans les cases dues à l'excrétion fécale dans la diffusion du virus entre les porcs. Plus récemment, Crotta *et al.* (2018) ont développé un modèle simple d'évaluation quantitative du risque qui a prédit 13,8 % de porcs virémiques à l'abattoir et a montré que l'augmentation de la proportion de porcs immunisés à la naissance augmenterait la proportion de porcs virémiques au moment de l'abattage (19,8 %).

Ces premiers résultats ont apporté des éléments centraux dans la compréhension de la transmission du VHE. Néanmoins, ils ne sont pas aisément transposables sur le terrain aux conditions réelles d'élevage, qui associent une population animale dynamique divisée en groupes d'animaux ayant une structure de contact hétérogène à de nombreux facteurs de variation liés à la conduite et aux pratiques d'élevage. A ce jour, il n'existe pas de modèle prenant en compte la population dynamique d'un élevage et la circulation virale au sein de cet élevage, seule assurance d'explorer des hypothèses de déterminisme de persistance extrapolables à la situation réelle. Ainsi, l'objectif de cette étude est d'utiliser une approche par modélisation multi-pathogènes afin de comprendre les conditions de la diffusion et de la persistance du VHE dans un élevage naisseur-engraisseur dans lesquels les porcs sont susceptibles d'être co-infectés avec un pathogène intercurrent. Pour ce faire, un modèle stochastique individu-centré a été développé en couplant un modèle de dynamique de population avec un modèle épidémiologique multi-pathogènes représentant la diffusion conjointe du virus de l'hépatite E et d'un virus immunomodulateur (ci-après noté IMV, dont le prototype est le virus du SDRP). Ce modèle a aussi été utilisé pour évaluer l'influence du type de conduite en bandes ainsi que l'efficacité de stratégies de contrôle sur la dynamique et la persistance du VHE dans l'élevage.

1. MATERIEL ET METHODES

1.1. Description du modèle de dynamique de population

Un élevage de porcs de type naisseur-engraisseur est classiquement conduit en bandes : la population d'animaux est divisée en groupes d'individus de même stade physiologique, i.e. les bandes, ce qui induit des sous-populations relativement indépendantes sur le plan infectieux et immunitaire. Selon leur stade physiologique, les animaux évoluent dans cinq secteurs : les secteurs de quarantaine, gestation et maternité pour les truies ; les secteurs de maternité, post-sevrage et engraissement pour les porcs en croissance. Ces trois derniers secteurs sont divisés en salles, gérées selon une stratégie tout plein - tout vide, i.e. tous les animaux d'une bande quittent une salle simultanément et entrent dans une salle vide en même temps. Les mouvements d'animaux sont ainsi réalisés à temps fixes. Les deux sous-populations (animaux reproducteurs et porcs en croissance) n'interagissent physiquement qu'en maternité.

Le cycle reproducteur d'une truie dure 145 jours. Les cochettes restent en quarantaine pendant 42 jours. Après la quarantaine ou le sevrage, les cochettes et les truies sont déplacées en verraterie, où elles sont inséminées cinq jours plus tard (insémination artificielle - IA). Elles restent dans cette salle jusqu'au diagnostic de gestation (21 jours post-IA). Les truies gestantes passent alors en salle de gestation jusqu'à 107 jours de gestation. En cas d'échec de l'IA ou d'avortement, les truies sont transférées dans la bande suivante ou réformées. Sept jours avant la mise-bas, les truies entrent dans une salle de maternité. Le sevrage des porcelets a ensuite lieu 21 ou 28 jours après la mise-bas (selon le type de conduite). A la fin de la lactation, les truies retournent en verraterie pour un nouveau cycle ou sont réformées ; les porcelets passent dans une salle de post-sevrage jusqu'à 86 jours d'âge, où ils sont transférés en engraissement. Lorsque leur poids excède 115 kg ou leur âge 180 jours, ils sont vendus à l'abattoir.

1.2. Description du modèle épidémiologique multi-pathogènes

1.2.1. Modèle épidémiologique du VHE

Le modèle épidémiologique du VHE est un modèle MSEIRS – Avec anticorps d'origine maternelle (M), Sensible (S), Exposé (E), Infecté (I), Retiré (R), Sensible (S) – qui prend en compte une protection partielle conférée par les AOM et inclut également un compartiment environnemental. Plus précisément, les porcelets nés de truies immunisées (R) acquièrent des AOM anti-VHE via la prise de colostrum, ce qui leur confère une protection partielle contre l'infection. Un porcelet sensible (S) ou partiellement protégé (M) peut devenir infecté non excréteur (E) par voie féco-orale, soit par contact direct avec un porc infectieux soit par contact indirect avec du virus viable dans l'environnement contaminé de la case. Avec une probabilité plus faible, l'infection peut aussi survenir par contact indirect avec des porcs logés dans les cases adjacentes, par projection de fèces contaminées d'une case à l'autre. Après une période de latence, les animaux infectieux (I) excrètent du VHE dans l'environnement. La quantité excrétée dans les fèces évolue en fonction du temps selon une loi normale pondérée par un coefficient d'excrétion maximale Q_{max} . La charge virale globale accumulée dans la case augmente avec les fèces excrétées par les porcs infectieux et diminue avec le passage des fèces par le caillebotis, la mortalité naturelle du virus dans l'environnement et le retrait des fèces lors des opérations de nettoyage lorsque les cases sont vides. Les porcs acquièrent ensuite une immunité active (R), qu'ils perdent pour finalement redevenir sensibles (S).

1.2.2. Modèle épidémiologique de l'IMV

Le modèle épidémiologique de l'IMV est un modèle générique de type MSIRS. Un porcelet sensible (S) ou partiellement protégé (M) peut devenir infectieux (I) par voie oro-nasale, soit par contact direct avec un porc infectieux, soit par contact indirect via l'air circulant dans les cases et les salles. Après une période d'immunité active (R), il redevient ensuite sensible (S).

1.2.3. Couplage et paramétrage

Les deux modèles épidémiologiques fonctionnent simultanément dans la population. En fonction de leur statut vis-à-vis de l'IMV, les caractéristiques individuelles des porcs vis-à-vis du VHE varient (durée de la latence, taux de transmission, quantité de VHE excrétée). Les paramètres ont fait l'objet d'une estimation à partir d'études expérimentales préalablement conduites pour explorer la dynamique d'infection du VHE, notamment en relation avec l'influence de co-infections immunomodulatrices. Les paramètres des modèles sont détaillés dans les Tableaux 1 et 2.

Tableau 1 - Paramètres du modèle épidémiologique du VHE

Notation	Description du paramètre (unité)	Valeur / Distribution		Source
		VHE-seul	VHE/IMV	
D_{HE}^M v	Durée de l'immunité maternelle (jour)	$\Gamma(7,9 ; 5,8)$		Andraud <i>et al.</i> (2014)
p_{HEV}^{IMDA}	Probabilité d'infection sous immunité maternelle	0,08		Andraud <i>et al.</i> (2014)
D_{HEV}^E	Durée de la période de latence (jours)	$\Gamma(5,2 ; 1,3)$	$\Gamma(25,7 ; 0,5)$	(Andraud <i>et al.</i> , 2013;
β_{HEV}	Taux de transmission directe (porc/jour)	0,15	0,69	Salines <i>et al.</i> , 2015)
β_{E}^{WP}	Taux de transmission environnementale	6.10^{-6}		

	intra-case (g/génome équivalent (ge)/jour)		
θ_{E}^{BP}	Taux de transmission environnementale inter-cases (g/ge/jour)	7.10^{-8}	
$\omega(t)$	Quantité de VHE excrétée dans les fèces en fonction du temps (ge/g/jour)	$\mathcal{N}(5 ; 1)$ $Q_{max} = 10^6$	$\mathcal{N}(25 ; 5)$ $Q_{max} = 10^8$
f	Quantité moyenne de fèces excrétée par porc (g/jour)	Porcelets : 100 Porcs : 1000 Truies : 2000	Murai <i>et al.</i> (2018)
fQ	Quantité moyenne de fèces ingérée par un porc (g/jour)	25	Bouwknegt <i>et al.</i> (2011)
dr	Taux de décroissance du VHE dans l'environnement (/jour)	0,08	Johne <i>et al.</i> (2016)
sf	Taux d'élimination des fèces par le caillebotis (/jour)	0,70	Avis d'expert
cr	Taux d'élimination des fèces par le nettoyage	0,98	Avis d'expert
D_{HE}^R v	Durée de l'immunité active (jours)	$\Gamma(6,3 ; 29,4)$	Avis d'expert

Tableau 2 - Paramètres du modèle épidémiologique de l'IMV

Notation	Description du paramètre (unité)	Valeur / Distribution	Source
D_{IMV}^M	Durée de l'immunité maternelle (jour)	$\mathcal{N}(45 ; 8)$	Paramètres consensus représentant la diffusion d'un virus respiratoire immunomodulateur tel que le SDRP, PCV2,...
p_{IMV}^{IMDA}	Probabilité d'infection sous immunité maternelle	0,3	
β_{IMV}	Taux de transmission directe (porc/jour)	0,13	
D_{IMV}^R	Durée de l'immunité active (jours)	$\Gamma(6,3 ; 29,4)$	
C_{IMV}^{cap}	Coefficient de transmission entre deux cases adjacentes	0,1	
C_{IMV}^{intra}	Coefficient de transmission intra-salle	0,05	
$C_{IMV}^{intra,salle}$	Coefficient de transmission intra-salle en salle de maternité	0,1	
$C_{IMV}^{intra,troupeau}$	Coefficient de transmission intra-troupeau	0,01	

1.3. Initialisation et simulations

Au début de chaque simulation, le troupeau est uniquement composé de cochettes de 100 jours. La première bande est inséminée 21 jours après le début de la simulation. La dynamique de population est évaluée sur dix années en l'absence d'agent infectieux, afin d'obtenir une population démographiquement stabilisée. La onzième année, une cochette infectée par l'IMV est introduite en quarantaine pour initier le processus infectieux de l'IMV. De la même manière, la quinzième année, une cochette infectée par le VHE est introduite en quarantaine. Ni l'IMV ni le VHE ne sont ultérieurement réintroduits. Les simulations sont conduites sur 20 ans au total. Pour chaque scénario représenté, 200 simulations sont réalisées, nombre nécessaire pour stabiliser la variance des sorties du modèle.

1.4. Evaluation de l'efficacité de mesures de maîtrise

Plusieurs facteurs ont été testés : (i) type de conduite en bandes, (ii) pratiques d'élevage : type de logement des truies gestantes, gestion des adoptions, mélanges au sevrage, (iii) maîtrise de l'IMV par une vaccination anti-IMV des truies (qui

sont alors de statut épidémiologique R) à chaque cycle reproducteur deux ans post-introduction (Tableau 3). L'influence de ces pratiques sur l'âge à l'infection, la prévalence du VHE, et la prévalence à l'âge d'abattage a été testée par des tests non paramétriques basés sur les rangs de

Wilcoxon. L'impact de ces variables sur la probabilité de persistance du VHE cinq ans post-introduction a été étudié par des analyses de survie (modèle de Cox à risques proportionnels). Ces analyses ont été réalisées avec le logiciel R version 3.5.1. (Ihaka et Gentleman, 1996).

Tableau 3 – Description des scénarii de maîtrise testés dans le modèle

Scénario	Logement des truies			Pratiques d'adoption			Modalités de mélange au sevrage			Contrôle de l'IMV		
	Grands groupes (collectif)	Groupes moyens (1 case par bande)	Petits groupes (6 truies par case)	Pas d'adoption	Peu d'adoptions (15 %)	Beaucoup d'adoptions (> 15 %)	Petites cases (< 50)	Grandes cases (> 50)	Par portée	Au hasard	Pas de vaccination	Vaccination anti-IMV des truies
1												
2												
3												
4												
5												
6												
7												
8												

2. RESULTATS

2.1. Description des simulations après introduction du VHE dans un élevage IMV-positif

A la suite de l'introduction d'une cochette infectieuse en verrerie, une propagation rapide du VHE est observée au sein du troupeau reproducteur (Figure 1). L'entrée des truies infectieuses en maternité initie alors le processus infectieux chez les porcs en croissance *via* l'infection des porcelets sous la mère qui propagent ensuite l'infection en post-sevrage et en engraissement. En l'absence de réintroduction ultérieure, le virus persiste ensuite de manière enzootique dans 60 % des simulations dans un élevage conduit en 7 bandes au terme des cinq années post-introduction.

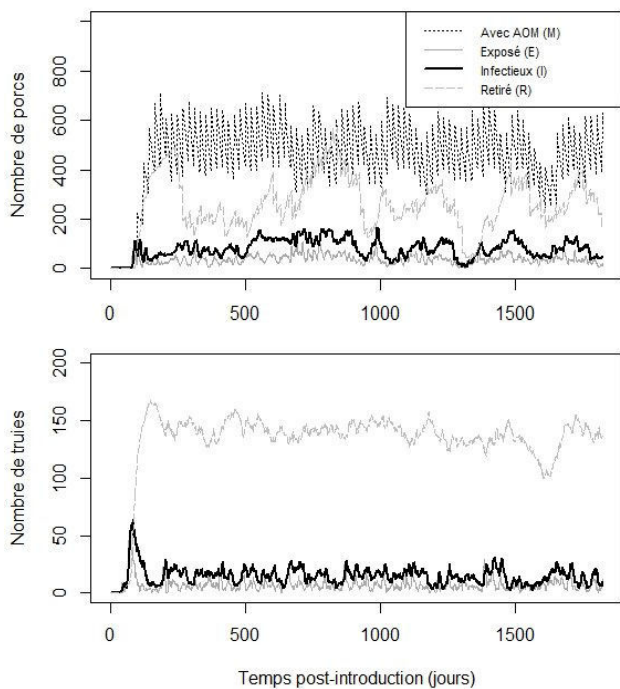


Figure 1 - Exemple d'une simulation conduisant à la propagation du VHE dans un élevage conduit en 7 bandes

2.2. Impact du système de conduite en bandes sur la dynamique infectieuse

L'infection par le VHE est significativement plus précoce dans un élevage conduit en 20 bandes (84 jours en moyenne) que lors de conduites en 4 ou 7 bandes (87 jours) ($p < 0,01$). Par ailleurs, la prévalence moyenne du VHE est plus élevée en conduite 20 bandes qu'en conduite 4 et 7 bandes, tant chez les truies que chez les porcs en croissance ($p < 0,01$). La prévalence moyenne du VHE à l'âge d'abattage est également plus élevée en conduite 20 bandes qu'en conduite en 4 et 7 bandes ($p < 0,01$) (3,9, 2,4 et 2,9 %, respectivement). Enfin, la probabilité de persistance du VHE cinq ans post-introduction dans un élevage conduit en 20 bandes est significativement plus élevée que dans un élevage conduit en 4 ou 7 bandes ($p < 0,01$) (97, 55 et 60 %, respectivement) (Figure 2). Seules les dynamiques en conduites 7 et 20 bandes divergeant significativement, les scénarii qui suivent n'ont été évalués que sur ces deux types de conduite.

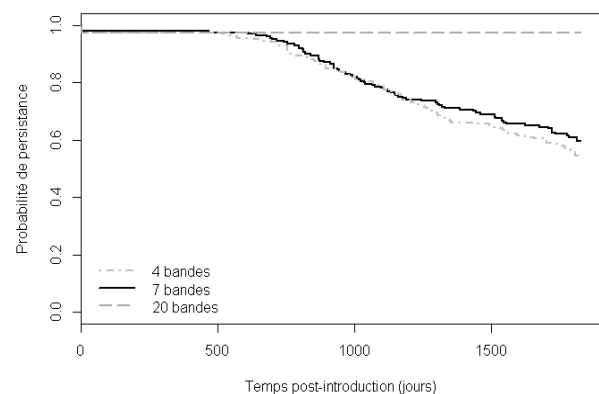


Figure 2 - Probabilité de persistance du VHE dans l'élevage pour trois types de conduites en bandes (n = 200 simulations)

2.3. Evaluation de mesures de maîtrise

Les résultats sont détaillés dans le Tableau 4.

2.3.1. Impact des pratiques d'élevage

Dans un élevage dans lequel les truies sont logées en grands groupes, les porcs s'infectent plus précocement, le virus circule plus largement – tant chez les porcs que chez les truies, la probabilité de persistance du VHE à cinq ans post-introduction et la prévalence de porcs positifs à l'abattage sont plus élevées ($p < 0,01$).

De plus, dans un élevage qui pratique largement les adoptions, l'infection est plus précoce de quelques jours, le virus circule davantage dans l'élevage, et la prévalence à l'abattage est plus élevée ($p < 0,01$). Enfin, dans un élevage dans lequel le nombre de portées mélangées par case est élevé, la circulation virale est plus intense, et l'âge à l'infection est plus tardif et la prévalence à l'abattage est plus élevée ($p < 0,01$). Dans un élevage conduit en 20 bandes, l'impact de ces pratiques est moindre.

Tableau 4 – Impact de pratiques d'élevage et de gestion sanitaire sur les caractéristiques de l'infection par le VHE dans un élevage conduit en 7 ou en 20 bandes ($n = 200$ simulations) (-) : référence ; ** $p < 0,01$; * $p < 0,1$

Scenario			Conduite en 7 bandes			Conduite en 20 bandes		
			Age moyen à l'infection par le VHE (jours)	Prévalence moyenne du VHE à l'âge d'abattage (%)	Probabilité de persistance du VHE 5 ans post-introduction	Age moyen à l'infection par le VHE (jours)	Prévalence moyenne du VHE à l'âge d'abattage (%)	Probabilité de persistance du VHE 5 ans post-introduction
1	Logement des truies	Grands groupes	87,0 (-)	2,9 (-)	59,5 (-)	84,1 (-)	3,9 (-)	97,5 (-)
6		Groupes moyens	89,7 (**)	1,7 (**)	28,5 (**)	86,7 (**)	2,8 (**)	95,0
7		Petits groupes	103,5 (**)	0,1 (**)	0 (**)	102,5 (**)	0,9 (**)	94,0 (*)
1	Pratiques d'adoption	Peu d'adoptions	87,0 (-)	2,9 (-)	59,5 (-)	84,1 (-)	3,9 (-)	97,5 (-)
2		Pas d'adoption	87,5 (**)	2,6 (**)	55,0	83,7 (**)	3,6 (**)	99
5		Beaucoup d'adoptions	84,3 (**)	3,9 (**)	61,0 (*)	79,4 (**)	5,0 (**)	93,3
1	Modalités de mélange au sevrage	Petite cases, par portées	87,0 (-)	2,8 (-)	59,5 (-)	84,1 (-)	3,9 (-)	97,5 (-)
3		Grandes cases, par portées	91,6 (**)	3,5 (**)	66,0	90,3 (**)	5,2 (**)	97,0
4		Grandes cases, au hasard	86,9 (**)	2,4 (**)	58,0	84,4 (**)	4,3 (**)	97,0
1	Contrôle de l'IMV	Non	87,0 (-)	2,9 (-)	59,5 (-)	84,1 (-)	3,9 (-)	97,5 (-)
8		Oui	93,5 (**)	1,7 (**)	34 (**)	93,0 (**)	3,7 (**)	98,7

3. DISCUSSION

Les modèles mathématiques, en tenant compte des caractéristiques épidémiologiques des pathogènes, s'avèrent être des outils pertinents pour une compréhension fine des dynamiques d'infection et des facteurs qui l'influencent. A ce jour, aucun modèle dynamique du VHE n'est disponible dans la littérature. Notre étude présente ainsi l'intérêt de développer le premier modèle de la dynamique d'infection du VHE prenant en compte la dynamique de population détaillée et la circulation virale au sein de cet élevage. La spécificité et l'originalité de ce modèle résident aussi dans l'approche multipathogènes utilisée : le modèle intègre, à l'échelle individuelle, les interactions épidémiologiques entre le VHE et un pathogène immunomodulateur générique, interactions dont l'impact majeur a été prouvé tant en conditions expérimentales que naturelles pour le virus du SDRP (Salines *et al.*, 2015; Salines *et al.*, 2018b).

Le modèle ainsi construit a permis de représenter la diffusion et la persistance du VHE conformément aux données de terrain. En effet, le scénario de référence (scénario 1, correspondant à la situation la plus fréquemment rencontrée dans les élevages français) montre que les porcs s'infectent en moyenne à 87 jours d'âge, ce qui est cohérent avec l'étude de terrain de Salines *et al.* (2018b) qui rapportent un âge moyen de l'infection des porcs à 91 jours d'âge. Les simulations permettent d'obtenir une prévalence moyenne de porcs infectieux à l'âge d'abattage de 2,9 %, ce qui est également cohérent avec la littérature : par exemple, l'étude nationale française conduite par Rose *et al.* (2011) rapporte une prévalence de foies positifs à l'abattoir de 4 % [2-6] et la méta-analyse réalisée par Salines *et al.* (2017) à partir de 31 publications internationales conclut à une prévalence de 6,1 % [1,2-15,4 %] de porcs infectieux au stade de l'abattage. Enfin, le premier scénario de notre modèle met en évidence une persistance du VHE dans environ 60 % des simulations à cinq ans post-introduction, sans réintroduction ultérieure du virus dans l'élevage. A notre connaissance, aucune donnée relative

2.3.2. Gestion sanitaire : impact de la vaccination contre le pathogène intercurrent

La vaccination des truies contre l'IMV conduit à une infection VHE plus tardive des porcs mais à une nette diminution de la prévalence du VHE à l'âge d'abattage et de la probabilité de persistance du VHE cinq ans post-introduction, particulièrement dans un élevage conduit en 7 bandes.

à la persistance du VHE en élevage n'est disponible dans la littérature, mais des cas d'éradication naturelle du virus ont été rapportés dans quelques élevages (Anses, communication personnelle).

Les élevages conduits en 20 bandes apparaissent particulièrement à risque vis-à-vis de la diffusion et la persistance du VHE. En effet, la persistance virale à cinq ans post-introduction est 1,6 fois plus probable, et la prévalence du VHE à l'abattage est en moyenne 1,3 fois plus importante dans un élevage conduit en 20 bandes que dans un élevage conduit en 7 bandes, toutes choses égales par ailleurs.

Le modèle a permis de tester l'efficacité de trois types de pratiques d'élevage. D'une part, le type de logement des truies apparaît jouer un rôle majeur dans la dynamique d'infection du VHE : loger les truies gestantes dans des groupes de plus faible effectif réduit drastiquement la prévalence du VHE à l'âge d'abattage et la persistance virale dans l'élevage cinq ans post-introduction, notamment dans un élevage conduit en 7 bandes. Ceci s'explique par une circulation virale plus limitée dans le cheptel reproducteur liée à la forte ségrégation, et donc ensuite dans la population de porcs en croissance. Ainsi, même si les porcs s'infectent en moyenne plus tardivement, la diffusion virale est plus restreinte. D'autre part, le modèle a permis de montrer que limiter les adoptions entraînait un moindre risque de diffusion et de persistance du VHE dans l'élevage, ce qui est cohérent avec l'étude de terrain de Walachowski *et al.* (2014). Là encore, la réduction drastique des adoptions permet de circonscrire la circulation virale à un plus faible nombre de portées. Enfin, les résultats mettent en évidence une moindre prévalence du VHE à l'âge d'abattage lorsque la taille des groupes en post-sevrage est faible. Ceci est cohérent avec les résultats de Walachowski *et al.* (2014) qui ont montré qu'un fort taux de mélange au sevrage augmentait largement la prévalence de foies positifs à l'abattoir. Notons que les différences concernant les âges à l'infection par le VHE selon les scénarii sont à interpréter avec précaution. En effet, des différences de quelques jours, même statistiquement significatives, ont des conséquences pratiques limitées, contrairement aux différences de prévalence à l'abattage.

Le modèle met en évidence l'efficacité de la vaccination des truies contre l'IMV sur le risque lié au VHE, avec une prévalence du VHE à l'abattage 1,7 fois moins élevée et une persistance à cinq ans 1,8 fois moins fréquente dans un élevage conduit en 7 bandes dans lequel les truies sont vaccinées par rapport au scénario de référence et sous l'hypothèse d'efficacité du vaccin représentée dans le modèle. Les mesures de gestion sanitaire contre les pathogènes immunomodulateurs en élevage porcin s'avèrent donc être un levier potentiel pour le contrôle du risque lié au VHE eu égard la disponibilité d'un vaccin permettant de contrôler efficacement le pathogène en question.

Ces mesures de maîtrise se révèlent moins efficaces dans un élevage conduit en 20 bandes que dans un élevage conduit en 7 bandes. La taille de population et les intervalles inter-bandes jouent sans doute un rôle dans les différences obtenues avec les deux conduites, la circulation virale étant plus difficilement contrôlable dans une population d'effectif élevé.

CONCLUSION

Cette étude a permis de développer le premier modèle dynamique représentant la circulation du VHE dans un élevage

de porcs naisseur-engraisseur, tout en intégrant les interactions avec des pathogènes immunomodulateurs affectant la dynamique d'infection du VHE. Cette approche originale multi-pathogènes a permis d'étudier à la fois le rôle du type de conduite en bandes, l'impact de certaines pratiques d'élevage et l'influence de la vaccination contre les pathogènes immunomodulateurs. En conclusion, il apparaît que le VHE est plus difficilement contrôlable dans un élevage en 20 bandes et que, dans un élevage conduit en 7 bandes, le type de logement des truies et la gestion sanitaire des pathogènes intercurrents sont des leviers d'action centraux pour la maîtrise du risque VHE pour la santé publique. D'autres types de mesure pourront être étudiés (amélioration des pratiques de biosécurité, modalités d'auto-renouvellement ou de mélanges en engraissement, éradication des pathogènes intercurrents). Il sera également particulièrement intéressant d'évaluer l'efficacité de combinaisons de mesures de maîtrise contre le VHE, afin de pouvoir élaborer un plan exhaustif et global de lutte contre le VHE dans la filière porcine.

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Annexe 5 - Publication dans le *Bulletin des Groupements Techniques Vétérinaires*

Salines M., Andraud M., Rose N., 2019. Le virus de l'hépatite E dans la filière porcine : point d'information et pistes de maîtrise. *Bulletin des Groupements Techniques Vétérinaires* 94, 105-110.

RÉSUMÉ Le virus de l'hépatite E (VHE) est responsable d'une hépatite chez l'homme, principalement transmise par l'alimentation dans les pays industrialisés et pour laquelle les porcs sont reconnus comme le principal réservoir. En France, plus de 2 200 cas ont été rapportés en 2016. Si les produits à base de foie de porc consommés crus ou insuffisamment cuits sont la source majeure de l'infection, la question d'une éventuelle contamination de la viande ou produits à base de viande de porc est soulevée. Le VHE circule dans 65,3 % des élevages français et 4 % des foies à l'abattoir sont infectés par le VHE. Chez le porc, l'infection est asymptomatique et le virus est transmis par voie féco-orale, l'accumulation virale dans l'environnement des cases jouant un rôle central dans la transmission et la persistance du virus en élevage. Plusieurs facteurs de risque relatifs à la structure des élevages et aux mesures d'hygiène et de biosécurité ont été mis en évidence. Il a aussi été montré que les co-infections avec des pathogènes immunomodulateurs (virus du SDRP, PCV2) modifient la dynamique d'infection du VHE et augmentent le risque de positivité des foies. Conjointement avec l'amélioration des pratiques d'élevage, il a été montré dans une étude de modélisation de la dynamique d'infection en élevage que la vaccination des truies contre ces pathogènes intercurrents serait un levier majeur pour réduire le risque pour la santé publique.

Le virus de l'hépatite E dans la filière porcine : point d'information et pistes de maîtrise

L'hépatite E est une problématique de santé publique majeure pour la filière porcine. Les connaissances scientifiques accumulées ces dernières années permettent de dégager des pistes d'action pour réduire le risque d'exposition humaine au VHE par la consommation de produits à base de porc.

Le virus de l'hépatite E (VHE) est un virus non-enveloppé à ARN simple brin provoquant chez l'homme une infection souvent asymptomatique, mais parfois une hépatite aiguë ou chronique selon, entre autres, le contexte immunitaire du patient. Les génotypes 3 et 4 sont partagés par l'homme et d'autres espèces animales et sont responsables de cas sporadiques autochtones dans les pays industrialisés. En particulier, le génotype 3 est largement répandu dans la population porcine et un certain nombre de cas autochtones ont pu être reliés à la consommation de produits porcins crus ou insuffisamment cuits (4, 7). En ce sens, l'hépatite E est reconnue comme une zoonose alimentaire dont les porcs domestiques sont le principal réservoir dans les pays

développés. Le risque que des produits contaminés soient commercialisés dépend étroitement de la dynamique d'infection du VHE dans les élevages de porcs. Cependant, l'épidémiologie du VHE dans la filière porcine est encore mal comprise et l'hétérogénéité des données de prévalence disponibles dans la littérature indique des variations de la dynamique d'infection du VHE (18). Cet article synthétise les données récentes disponibles sur le risque présenté par certains produits porcins et l'épidémiologie du VHE dans les élevages de porcs.

Transmission zoonotique du virus de l'hépatite E

Transmission du VHE par voie alimentaire à partir de viande et de produits à base de porc

En France, le nombre de cas rapportés au Centre National de Référence des hépatites à transmission entérique (CNR) en 2016 s'élève à 2 302, dont 2 292 cas autochtones, et 653 hospitalisations pour cas d'hépatite E ont été déclarées. En prenant en compte la grande proportion d'infections asympto-

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matiques (environ 70 %) et la sous-déclaration de la maladie, un modèle statistique a permis d'estimer le nombre réel de cas à plus de 68 000 par an (24). Plusieurs cas d'hépatite E ont été attribués avec certitude à la consommation de produits à base de porc contaminés par le VHE. Par exemple, Colson et al. (4) ont décrit des cas groupés d'hépatite E en France chez sept personnes qui avaient consommé des figatelles (ou figatelli, saucisses fraîches composées de viande et de foie de porc, spécialités corses). En 2013, des infections groupées par le VHE ont été rapportées à Belle-Île en Mer, parmi lesquelles trois cas cliniques. Les patients avaient consommé un porcelet rôti à la broche farci avec une farce crue contenant le foie du porcelet. Les mêmes souches de VHE que celles impliquées dans les cas humains ont aussi été détectées dans le lisier échantillonné dans l'élevage d'origine du porcelet et dans des eaux usées non traitées provenant du système d'épuration de la commune (7).

Plusieurs études visant à identifier des facteurs de risque expliquant l'infection ou la séroprévalence VHE ont été conduites dans différents pays. Par exemple, en France, une récente enquête nationale rapporte les facteurs de risque suivants associés à une sérologie VHE positive : la consommation de viande de porc ou de gibier (Risque Relatif (RR) = 1,53), de saucisses à base de foie de porc (RR = 1,30), d'abats (RR = 1,25) (11). Plusieurs études néerlandaises ont également pointé la consommation de viande comme un facteur de risque, par exemple Mooij et al. (12) ont décrit la consommation de saucisses traditionnelles néerlandaises (de type salami, cervelas, etc.) comme un facteur de risque (Odds Ratio (OR) = 1,5). En Allemagne, Faber et al. (6) ont montré, à partir de 270 cas et 1 159 contrôles, que l'infection par le VHE était associée, entre autres, à la consommation de foie de porc insuffisamment cuit (OR = 5,3), de viande de porc (OR = 3), de saucisses de Francfort (OR = 1,9) et de saucisses ou pâté de foie (OR = 2,1). Une étude particulièrement médiatisée en Angleterre a mis en évidence que l'infection par une souche particulière de VHE était liée à la consommation de saucisses et de jambons provenant de la chaîne de supermarchés britannique Tesco (RR = 1,85) (16).

En outre, de nombreuses données sont disponibles dans la littérature pour évaluer la contamination des produits à base de porc. Parmi eux, les foies de porcs se révèlent être particulièrement à risque, avec une prévalence de foies contaminés à l'abattoir ou en supermarché variant entre 1 et 21 % selon les études et les

pays. En France, par exemple, une enquête nationale a montré que 4 % des foies de porc à l'abattoir contenaient de l'ARN viral du VHE (14). Par conséquent, les produits à base de foie de porc (saucisse de foie, pâté de foie) sont également à risque vis-à-vis du VHE, en particulier s'ils sont consommés crus ou insuffisamment cuits. Par exemple, une étude menée en France à partir de 394 produits achetés en supermarchés a montré que 30 % des figatelles et fitones contenaient de l'ARN viral du VHE, ainsi que 3 % des foies séchés et salés, 25 % des quenelles de foie et 29 % des saucisses de foie sèches ou fraîches (13). Les charges génomiques virales retrouvées dans les foies, muscles, ou produits à base de foie ou de viande de porc sont comprises entre 10^1 et 10^7 copies d'ARN par gramme, selon les études et les produits et sont donc compatibles avec une infection humaine productive. Si le risque représenté par les produits à base de foie de porc est largement admis par la communauté scientifique et les autorités sanitaires, le risque lié à la consommation de viande de porc ou produits à base de porc ne contenant pas de foie est plus discuté. Expérimentalement, il a été montré que les muscles et le sang des porcs peuvent contenir du VHE, notamment lorsque les porcs sont co-infectés par le virus du Syndrome Dysgénésique et Respiratoire Porcin (SDRP) (19), et l'enquête de Di Bartolo et al. (5) rapporte 3 % de muscles linguaux de porcs positifs VHE à l'abattoir. En Allemagne, l'enquête de Szabo et al. (22) a montré que 21,7 % des salamis échantillonnés contenaient du VHE.

La détection du VHE dans un grand nombre de produits à base de foie de porc, et les questions émergeant ces dernières années sur la possible présence du VHE dans la viande et les produits à base de viande de porc, conduisent à insister sur la nécessité de consommer ces produits cuits à cœur. Rappelons qu'un traitement thermique à 71°C pendant 20 minutes a prouvé son efficacité pour inactiver le virus dans des matrices complexes (3).

Exposition professionnelle au VHE

La transmission du VHE par contact avec des porcs a été mise en évidence par de nombreuses études sérologiques conduites auprès de personnes ayant une exposition professionnelle à ces animaux (éleveurs de porcs, employés d'abattoir, bouchers, vétérinaires porcins), en comparaison à un groupe contrôle. En revanche, si plusieurs études décrivent une exposition plus élevée dans ces catégories professionnelles, la transmission du VHE par contact semble rarement conduire à

une hépatite clinique chez l'homme (pas d'excès de cas cliniques dans ces catégories socio-professionnelles).

Epidémiologie de l'infection par le virus de l'hépatite E chez le porc

Histoire naturelle de l'infection par le VHE chez le porc

Chez le porc, l'infection naturelle ou expérimentale par le virus de l'hépatite E est asymptomatique. Comme chez l'homme, la réplication virale a lieu principalement dans les hépatocytes. L'infection est accompagnée d'une hépatite légère à modérée, révélée par des examens histopathologiques, mais aucune lésion macroscopique du foie n'est observée. Un porc dit infectieux désigne un porc excréteur de particules virales dans ses matières fécales. La majorité des études réalisées en conditions d'élevage indique que les principaux excréteurs du VHE sont les porcs en croissance. L'excrétion fécale par les animaux reproducteurs a aussi été montrée. Les truies pourraient ainsi entretenir et maintenir la propagation du virus dans les élevages. Le virus est principalement excrété par voie fécale chez le porc, conduisant à une accumulation du VHE dans l'environnement des animaux en élevage infecté. Une méta-régression réalisée à partir de données issues de 31 publications internationales publiées entre 2002 et 2016 met en évidence une probabilité maximale d'excrétion aux alentours de 90 jours d'âge, conduisant à une prévalence de porcs infectieux à 185 jours d'âge (âge courant d'abattage) de 6,1 % [1,2-15,4] (18). Le virus se transmet principalement par voie oro-fécale. Expérimentalement, une charge minimale de 10⁶ génome équivalent (ge) par gramme de fèces semble nécessaire pour infecter des porcs per os et pour qu'ils soient capables d'excréter le virus et le transmettre à des congénères (1). Un modèle mathématique a montré que la transmission par contact direct était un facteur de persistance de l'infection en élevage porcin avec un taux de transmission directe de 0,15 [0,03-0,31] porc par jour (1). Néanmoins, le facteur majeur de propagation et de maintien de l'infection dans la population réside dans l'accumulation du virus dans l'environnement des animaux favorisant le processus de contamination par voie féco-orale via l'environnement. La transmission d'une case à l'autre est limitée et repose exclusivement sur le transfert de matières fécales d'une case adjacente à l'autre. Des mesures de biosé-

LE VIRUS DE L'HÉPATITE E

L'hépatite E est une maladie virale qui se manifeste par une inflammation du foie le plus souvent inapparente, mais pouvant parfois conduire à une hépatite aiguë (grande fatigue, signes digestifs, jaunisse, fièvre) avec une guérison progressive spontanée. Dans de plus rares cas, de graves complications peuvent survenir (chez les femmes enceintes par exemple) et aller jusqu'au décès pour des personnes immunodéprimées ou ayant déjà une maladie du foie. En 2016, plus de 2 000 cas humains ont été rapportés au Centre National de Référence.

Le porc est le principal réservoir du virus de l'hépatite E. Le virus est transmissible du porc à l'homme, notamment par consommation de produits à base de porc crus ou insuffisamment cuits (figatelles, saucisses de foie, pâtés de foie...). L'exposition au virus est plus élevée chez les professionnels en contact avec les porcs (éleveurs, vétérinaires...), sans toutefois que le nombre de cas observés ne soit plus important.

65 %

des élevages français sont infectés par le virus de l'hépatite E.

4 %

des foies des porcs abattus en France contiennent du virus de l'hépatite E.



L'infection par le virus de l'hépatite E est asymptomatique chez le porc : pas de signes cliniques, pas de mortalité, pas de baisse des performances. Il n'existe ni traitement ni vaccin pour les porcs.



Le virus de l'hépatite E se transmet par voie oro-fécale entre les porcs. La transmission est augmentée lors de co-infections (virus du SDRP).



ÉLEVEURS, POUR LIMITER LA PRÉSENCE DU VIRUS :

- ✓ Renforcez le protocole de nettoyage désinfection et la biosécurité interne
- ✓ Limitez les mélanges d'animaux
- ✓ Distribuez une eau de bonne qualité
- ✓ Maîtrisez les co-infections



L'Anses conduit actuellement un programme de recherche pour comprendre la dynamique du virus de l'hépatite E dans la filière porcine et dégager des mesures de maîtrise efficaces en élevage pour réduire l'exposition au virus.

curité internes drastiques seraient donc a priori très efficaces pour limiter la propagation à l'échelle de la population (1).

La présence d'anticorps maternels chez le porcelet n'empêche pas l'infection mais retarde le début de la virémie ainsi que la séroconversion chez celui-ci. La durée de présence des anticorps est également fonction du titre en anticorps chez la mère, ce dernier étant fortement influencé par l'âge de la truie. Les IgG perdurent jusqu'à 9 semaines d'âge chez les porcelets nés de truies fortement séropositives contre 1 à 3 semaines pour les porcelets issus de truies faiblement séropositives. L'impact des anticorps d'origine maternelle sur la transmission du VHE a été évalué en utilisant des données issues de suivis longitudinaux d'élevage (2) : la transmission du VHE chez les porcs ayant une immunité passive a été estimée 13 fois plus faible que chez des porcs sensibles, avec une grande variabilité selon les élevages [5-21].

Éléments d'épidémiologie descriptive et analytique

En France, une enquête nationale a été réalisée afin d'estimer la prévalence et la séroprévalence

Photo 1
Plaquette d'information sur le VHE à destination des éleveurs de porcs et des vétérinaires.

du VHE dans les élevages de porcs (14). Au total, 6565 sérums et 3715 foies ont été collectés dans 35 abattoirs répartis sur l'ensemble du territoire français. Les résultats de cette étude montrent que le VHE circule dans 65,3 % des élevages et que 31 % des animaux présentent des anticorps anti-VHE à l'âge d'abattage (i.e. vers 26 semaines d'âge). La séroprévalence intra-élevage du VHE varie de 5 à 90 % selon les élevages. A l'abattoir, les résultats suivants ont été obtenus : 24 % des élevages ont au moins un animal à foie positif au moment de l'abattage et au global, 4 % des foies sont infectés par le VHE. La grande variabilité inter-élevages suggère l'existence de facteurs de risque spécifiques aux élevages. Peu d'études ont exploré et quantifié les facteurs de risque associés à une prévalence ou

une séroprévalence élevée du VHE dans les élevages (**Tableau 1**) : la taille des élevages et les conditions de biosécurité apparaissent être des facteurs de risque majeurs.

Les sangliers sont reconnus comme un réservoir potentiel du VHE et des études expérimentales ont montré la possibilité d'une transmission du virus du sanglier au porc domestique (9, 23). Bien qu'aucune étude ne mette en relation la prévalence du VHE chez les porcs domestiques avec le contact avec des sangliers, ces derniers sont susceptibles de jouer un rôle dans l'épidémiologie du VHE dans les élevages de porcs en plein air.

Focus sur l'influence des co-infections immunomodulatrices

Tant en conditions naturelles qu'en conditions expérimentales, il a été montré que deux virus immunomodulateurs, le virus du SDRP (syndrome dysgénésique et respiratoire porcin) et le PCV2 (circovirus porcin de type 2) ont un impact sur la dynamique d'infection par le VHE. Ainsi, un suivi longitudinal dans trois élevages de porcs (trois bandes par élevage, au total 360 porcs suivis individuellement) a permis de montrer que la co-infection avec le virus du SDRP retarde l'âge à l'excrétion et à la séroconversion VHE, augmente la durée d'excrétion du VHE et le risque de positivité des foies à l'abattoir. L'effet du virus du SDRP est majoré lorsque les animaux sont également co-infectés par le PCV2, mais l'effet du PCV2 seul est plus difficile à mettre en œuvre à partir des données de terrain (20). Ces résultats de terrain ont été confirmés par des données expérimentales. En effet, le suivi d'animaux expérimentalement co-infectés par le virus du SDRP et le VHE montre que l'excrétion du VHE et la réponse immunitaire humorale anti-VHE sont retardées chez les porcs co-infectés, que l'excrétion du VHE est significativement plus importante et prolongée, que la transmission du virus entre les animaux est augmentée, et que par conséquent, le risque de positivité des foies à l'abattoir est augmenté (17). La co-infection expérimentale de porcs par le VHE et le PCV2 a des effets plus limités : elle n'augmente pas la durée de la période infectieuse ni la quantité de VHE excrétée dans les fèces, mais elle augmente la transmission directe du virus et retarde la mise en place de la séroconversion VHE.

Apports de la modélisation dynamique dans la compréhension et la maîtrise du VHE

Les travaux de recherche de l'Anses sur l'épidémiologie du VHE se sont poursuivis avec le

Tableau 1. Facteurs de risque associés à une séroprévalence VHE élevée dans les élevages de porcs

Facteurs liés...	Références
<p>À la taille de l'élevage</p> <p>Nombre de truies > 1000 (séroprévalence 54,2 % versus 18,9 %)</p> <p>Elevages de taille moyenne versus élevages de grande taille (OR = 4,95)</p> <p>Nombre de porcs > 600 (séroprévalence comprise entre 78 % et 100 %, versus 0 % à 29 %)</p> <p>Petite taille versus grande taille (séroprévalence 90 % versus 76 %)</p> <p>Taille des cases en post-sevrage > 26 porcs par case (OR = 2,4)</p>	(17, 18)
<p>À la biosécurité</p> <p>Durée du vide sanitaire en post-sevrage < 4 jours (OR = 1,7)</p> <p>Distance lisier-caillebotis en engraissement < 80 cm (OR = 1,9)</p> <p>Absence de sas sanitaire (OR = 3,6)</p> <p>Absence de période de quarantaine (OR = 2,7)</p> <p>Présence de carnivores domestiques (chiens, chats) (OR = 3,9)</p> <p>Présence de fèces d'oiseaux dans les bâtiments (OR = 2,9)</p>	(17-19)
<p>Aux mélanges</p> <p>Mélange d'animaux issus de salles différentes entre la maternité et le post-sevrage (OR = 1,8)</p>	(17)
<p>Au type de production</p> <p>Agriculture biologique versus conventionnel (séroprévalence 89 % versus 72 %)</p> <p>Plein air versus conventionnel (séroprévalence 76 % versus 72 %)</p> <p>Elevage extensif versus intensif (OR = 2,2)</p>	(19, 20)

développement d'un modèle mathématique permettant de représenter et de comprendre la diffusion et la persistance du VHE dans un élevage de porcs naisseur-engraisseur (21). Le modèle construit dans ce but couple un modèle de dynamique de population, permettant de prendre en compte différents types de conduite d'élevage, avec un modèle épidémiologique multi-pathogènes décrivant la transmission du VHE en présence d'un pathogène intercurrent (virus du SDRP, PCV2). Basés sur les données expérimentales et de terrain pré-citées, les paramètres épidémiologiques du modèle VHE varient selon le statut de co-infection avec le pathogène immunomodulateur. Le modèle développé permet de représenter une dynamique d'infection par le VHE similaire à celle décrite dans la littérature. En effet, à la suite de l'introduction d'une cochette infectieuse en verrerie, une propagation rapide du VHE est observée au sein du troupeau reproducteur (**Figure 1**). L'entrée des truies infectieuses en maternité initie alors le processus infectieux chez les porcs en croissance via l'infection des porcelets sous la mère qui propagent ensuite l'infection en post-sevrage et en engraissement. En l'absence de réintroduction ultérieure, le virus persiste ensuite de manière enzootique dans 60 % des simulations dans un élevage conduit en 7 bandes au terme des cinq années post-introduction.

De plus, le modèle indique que la persistance du VHE et la prévalence de foies positifs à l'abattoir sont plus importantes dans un élevage conduit en 20 bandes que dans un élevage conduit en 7 bandes (persistance cinq ans post-introduction dans 97 % des élevages versus 60 % ; prévalence à l'abattage de 3,9 versus 2,9 %). Certaines configurations ou pratiques d'élevage s'avèrent également à risque vis-à-vis du VHE. En effet, dans un élevage dans lequel les truies sont logées en grands groupes, le virus circule plus largement – tant chez les porcs que chez les truies, la probabilité de persistance du VHE à cinq ans post-introduction est plus élevée (0,6 contre une extinction complète lors de logement par cases de 6 truies), de même que la prévalence de porcs positifs à l'abattage (2,9 % contre 0,1 %). De plus, dans un élevage qui pratique largement les adoptions, le virus circule davantage induisant une prévalence à l'abattage plus élevée. Enfin, dans un élevage dans lequel le nombre de portées mélangées par case est élevé, la circulation virale est plus intense et la prévalence à l'abattage est plus élevée. Particulièrement dans un élevage conduit en 7 bandes, la vaccination des truies contre le pathogène immunomodulateur

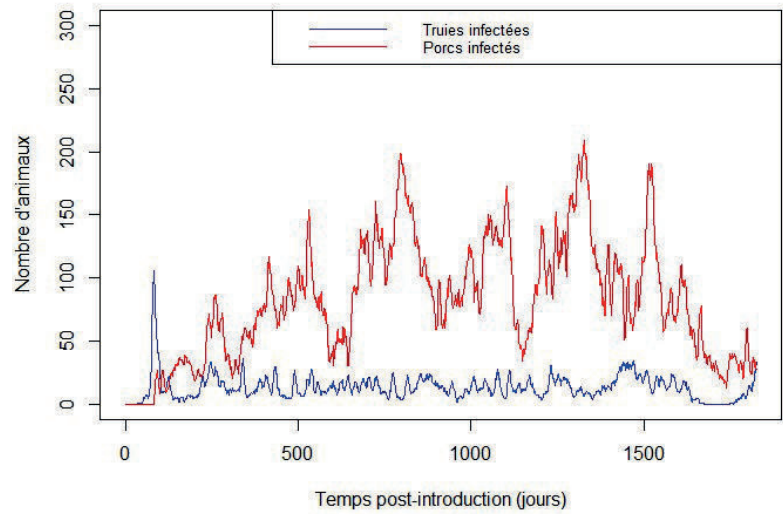


Figure 1
Simulation illustrant la propagation du VHE chez les porcs en croissance et les truies d'un élevage conduit en 7 bandes

conduit à une nette diminution de la prévalence du VHE à l'âge d'abattage (qui passe de 2,9 à 1,7 %) et de la probabilité de persistance du VHE cinq ans post-introduction (qui passe de 0,6 à 0,3). Lorsqu'elles sont combinées, ces mesures se révèlent d'autant plus efficaces pour la maîtrise du risque VHE. Néanmoins, il apparaît que le VHE est plus difficilement contrôlable dans un élevage en 20 bandes. La taille de population et les intervalles interbandes jouent sans doute un rôle dans les différences obtenues avec les deux conduites. L'efficacité de ces pistes de maîtrise du VHE devra être évaluée en conditions réelles, par exemple par le biais d'une étude d'intervention en élevage.

Conclusion

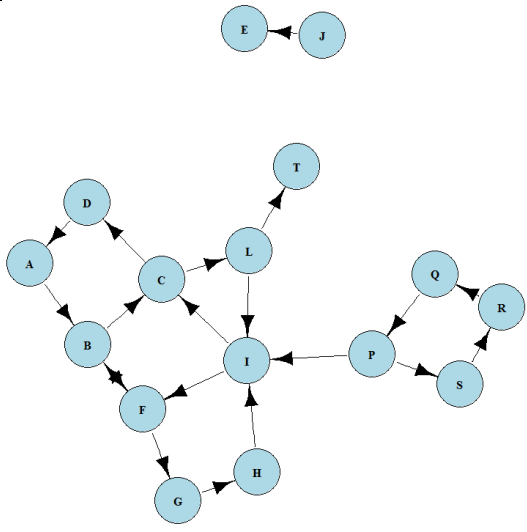
En associant des études en élevage, des essais expérimentaux et des approches de modélisation, il est apparu que les pratiques d'élevage, d'hygiène et de biosécurité, associées au rôle majeur des pathogènes intercurrents immunomodulateurs, jouaient un rôle central dans la persistance et la propagation du VHE dans les élevages porcins. La maîtrise des pathogènes intercurrents et l'amélioration des pratiques d'élevage permettraient ainsi de limiter le risque de santé publique lié au VHE. Une approche par modélisation multi-échelle permettra de comprendre les voies de diffusion préférentielles du VHE entre les élevages et ainsi la persistance du virus dans la filière de production porcine. Ces deux modèles permettront alors d'élaborer un plan de lutte contre le VHE tout au long de la filière, dont la faisabilité technique et économique sera évaluée conjointement avec les différents acteurs de la filière.

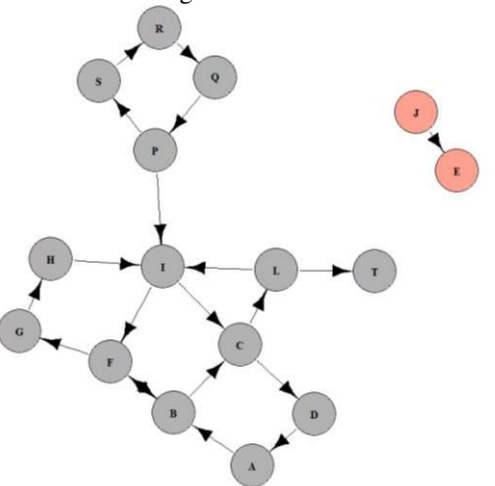
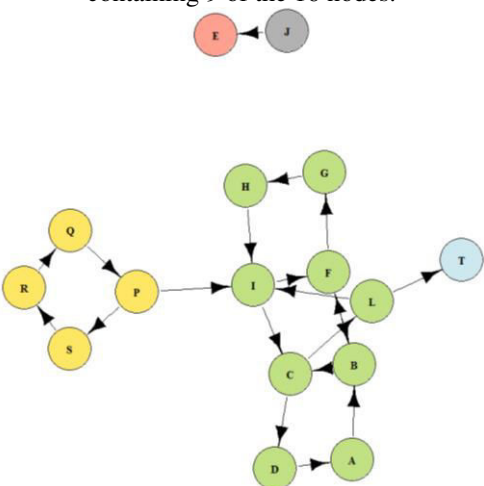
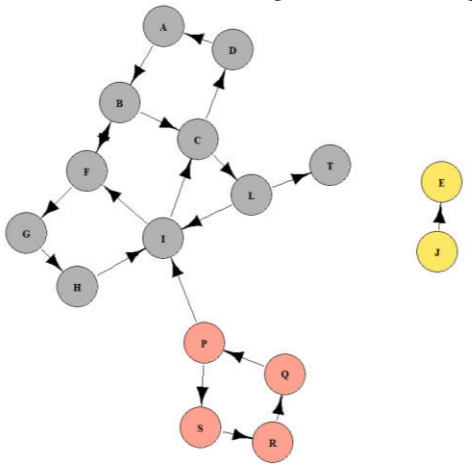
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Annexe 6 - Indicateurs classiquement étudiés pour l'analyse des réseaux sociaux

La littérature relative à l'analyse de réseaux étant principalement anglophone, et pour faciliter la compréhension de l'article publié en anglais dans *PLoS One*, ce tableau descriptif a volontairement été écrit en anglais.

Descriptive indicator	Definition	Example
<p>Graph</p>	<p>A graph is an ordered pair $G = (V, E)$ comprising a set V of vertices (or nodes) together with a set E of edges (or links). A graph can be directed or undirected. In a directed graph: $A \rightarrow B \neq B \rightarrow A$</p>	 <p>Vertices in the directed graph g: A, B, C, ... Number of vertices in the graph $g = 16$</p> <p>Edges in the graph g: $A \rightarrow B, B \rightarrow C, \dots$ Number of edges in the graph $g = 20$</p>
<p>Total degree</p>	<p>Number of adjacent edges to a vertex (going to and coming from a vertex).</p>	<p>Degree of the node B = 4. Degree of the node I = 5</p>
<p>In-degree</p>	<p>Number of edges going to a vertex.</p>	<p>In-degree of the node B = 2. In-degree of the node I = 3</p>
<p>Average degree of a graph</p>	<p>Mean of the degree of all vertices.</p>	<p>Average degree of the graph $g = 2.5$</p>
<p>Shortest path = geodesic</p>	<p>The shortest path length or geodesic distance is the smallest number of edges to connect a given pair of vertices.</p>	<p>The shortest path from A to I is: $A \rightarrow B \rightarrow C \rightarrow L \rightarrow I$</p>
<p>Average path length</p>	<p>The average path length is the length of all the shortest paths from or to the vertices in the network.</p>	<p>In the graph g, the average path length is 3.15</p>
<p>Diameter</p>	<p>The diameter of a graph is the length of the longest geodesic.</p>	<p>Diameter of the graph $g = 7$ (corresponding to the path from S to A)</p>
<p>Density</p>	<p>The density of a graph is the ratio of the number of edges and the number of possible edges.</p>	<p>Density of the graph $g = 0.0833$</p>
<p>Clustering coefficient = transitivity</p>	<p>The clustering coefficient measures the probability that the adjacent vertices of a vertex are connected. It is calculated for undirected graphs only.</p>	<p>Clustering coefficient of the node B = 0 (F, C and A are not connected to each other) Clustering coefficient of the node I = 0.1 (L and C are linked together)</p>
<p>Jaccard similarity ratio (JSC)</p>	<p>The JSC of two vertices is the number of common neighbours divided by the number of vertices that are neighbours of at least one of the two vertices considered.</p>	<p>JSC of the nodes B and I = $(F \text{ and } C) / (A, F, C, H, P, L) = 2/6 = 3$</p>
<p>Assortativity degree</p>	<p>The assortativity degree is the Pearson coefficient correlation between the degrees of linked nodes. It is positive if vertices having a similar degree tend to connect to each, and negative otherwise.</p>	<p>Assortativity degree of the graph $g = 0.25$</p>

Reciprocity ratio	The measure of reciprocity defines the proportion of mutual connections, in a directed graph. It is most commonly defined as the probability that the opposite counterpart of a directed edge is also included in the graph.	Reciprocity of the graph $g = 0.1$ (2 bidirectional edges out of 20 edges).
Betweenness	The vertex betweenness is defined as the number of geodesics (shortest paths) going through a vertex.	Betweenness of the node B = 20 Betweenness of the node I = 67
Closeness	Closeness centrality measures the number of steps required to access every other vertex from a given vertex.	Closeness of the node B = 0.00862 Closeness of the node I = 0.00854
Weakly connected components (WCCs) Strongly connected components (SCCs)	WCCs are sections of the graph where every vertex can be reached from every other vertex <u>whatever the edge direction</u> . SCCs are subgraphs in which every vertex can be reached from every other vertex via one or several <u>directed paths</u> .	<p>In the graph g, there are 2 WCCs, the largest containing 14 of the 16 nodes.</p>  <p>In the graph g, there are 5 SCCs, the largest containing 9 of the 16 nodes.</p> 
Community	A community is a subset of vertices in which there are significantly more edges than expected by chance, i.e. a group of highly connected vertices.	<p>In the graph g, there are 3 communities, the largest one containing 10 of the 16 nodes.</p> 

Annexe 7 - Publication dans les *Journées Recherche Porcine* 50

Salines M., Andraud M., Rose N., 2018. Importance de la prise en compte des mouvements de porcs pour l'organisation de la surveillance des pathogènes d'intérêt pour la filière porcine : application au virus de l'hépatite E. *Journées Recherche Porcine* 50.

Importance de la prise en compte des mouvements de porcs pour l'organisation de la surveillance des pathogènes d'intérêt pour la filière porcine : application au virus de l'hépatite E

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Importance de la prise en compte des mouvements de porcs pour l'organisation de la surveillance des pathogènes d'intérêt pour la filière porcine : application au virus de l'hépatite E

Les mouvements d'animaux entre élevages constituent une voie prépondérante de propagation des agents pathogènes dans la filière porcine. La présente étude a visé à évaluer l'impact des mouvements de porcs sur la prévalence d'un pathogène et le risque d'exposition des départements français en fonction de l'importance de leurs échanges. Le virus de l'hépatite E (VHE), agent zoonotique fortement prévalent dans les élevages de porcs, a constitué l'objet d'application de cette étude via (i) la mise en relation des caractéristiques de mouvements des élevages avec leur niveau de séroprévalence et (ii) l'évaluation du risque d'exposition des départements au VHE par l'intermédiaire des échanges interdépartementaux.

Le réseau de mouvements de porcs (données BDporc, 2013) et les résultats d'une enquête nationale de prévalence du VHE (données de 172 élevages dans 49 départements, 2009) ont été modélisés et analysés. Le lien entre les indicateurs de centralité des élevages et leur niveau de séroprévalence VHE a été exploré grâce à un modèle linéaire généralisé. La multiplicité des approvisionnements d'un élevage (degré entrant) ainsi que sa centralité de proximité par ses liens entrants apparaissent statistiquement associées à une séroprévalence VHE élevée dans l'élevage ($p < 0.05$). Le risque d'exposition au VHE d'un département a été évalué en combinant la distribution de la prévalence du VHE dans les départements sources avec le nombre de mouvements en provenance de ces départements. Le risque d'exposition des départements a ainsi été cartographié. Cette étude montre que la répartition d'un pathogène tel que le VHE est liée aux mouvements d'animaux, qui doivent être pris en considération pour l'élaboration de stratégies de surveillance ciblées et fondées sur le risque.

Pig movements should be taken into account to organise pathogen surveillance in the pig production sector: application to hepatitis E virus

Animal movements between farms are a major transmission route of pathogens in the pig production sector. The present study aimed to assess the impact of pig movements on the prevalence of a pathogen and the risk of French *départements* being exposed to pathogens because of pig trade. Hepatitis E virus (HEV) is a zoonotic agent highly prevalent in pig farms. We applied our methodology to HEV by (i) linking farms' movement features with their HEV seroprevalence level and (ii) assessing the risk of *départements* being exposed to HEV because of their external incoming movements.

Pig movement network (BDporc data, 2013) and results of a nation-wide HEV prevalence survey (data from 172 farms located in 49 departments, 2009) were modelled and analysed. The link between farms' centrality indices and their HEV seroprevalence level was explored with a generalised linear model. Farms' in-degree and ingoing closeness were found statistically associated with high HEV within-farm seroprevalence ($p < 0.05$). The risk of a *département* being exposed to HEV was calculated by combining the distribution of HEV farm-level prevalence in source *départements* with the number of movements coming from those source *départements*. By doing so, the risk of exposure of *départements* was mapped. This study shows that pathogen distribution is linked to animal movements and that they should be taken into account to develop targeted and risk-based surveillance strategies.

INTRODUCTION

Les maladies infectieuses des porcs ont des conséquences économiques pour la filière et peuvent affecter la santé publique. Les pathogènes peuvent être transmis d'élevage en élevage par l'intermédiaire des mouvements d'animaux et la diffusion des maladies est étroitement liée aux caractéristiques du réseau de mouvements d'animaux. Comprendre la topologie des échanges d'animaux permet d'expliquer la dynamique d'infection et la propagation des pathogènes, d'identifier des facteurs de risque liés aux caractéristiques du réseau et ainsi de développer des programmes de surveillance fondés sur le risque. C'est la raison pour laquelle les données de mouvements d'animaux sont de plus en plus étudiées par des méthodes d'analyse de réseau, dans lesquelles les élevages sont considérés comme des nœuds et les mouvements d'animaux entre élevages comme des liens (Wasserman, Faust, 1994; Bigras-Poulin *et al.*, 2006; Bigras-Poulin *et al.*, 2007; Natale *et al.*, 2009; Ribbens *et al.*, 2009; Nöremark *et al.*, 2011; Lindstrom *et al.*, 2012; Rautureau *et al.*, 2012; Buttner *et al.*, 2013; Dorjee *et al.*, 2013; Guinat *et al.*, 2016; Thakur *et al.*, 2016). Bien que la plupart des publications présentant des analyses de réseau soient motivées par l'étude des conséquences des échanges d'animaux sur l'épidémiologie des maladies, le rôle spécifique des transports d'animaux dans la transmission des pathogènes et/ou l'exposition à une maladie n'a été que rarement quantifié, particulièrement dans la filière porcine (Keeling, 2005; Lloyd-Smith *et al.*, 2005; Ortiz-Pelaez *et al.*, 2006; Green *et al.*, 2008; Martin *et al.*, 2011; Porphyre *et al.*, 2011; Frössling *et al.*, 2012; Nicolas *et al.*, 2013; Beaunee *et al.*, 2015; Lee *et al.*, 2017; Salines *et al.*, 2017b; Sintayehu *et al.*, 2017).

L'hépatite E est une zoonose alimentaire émergente pour laquelle les porcs sont reconnus comme le réservoir principal dans les pays industrialisés (Dalton *et al.*, 2008; Pavo *et al.*, 2010; EFSA *et al.*, 2017). En effet, plusieurs cas humains d'hépatite E ont été reliés à la consommation de produits contenant du foie de porc consommés crus ou insuffisamment cuits (Colson *et al.*, 2010; Moal *et al.*, 2012; Motte *et al.*, 2012). Le virus de l'hépatite E (VHE) est fortement prévalent dans les élevages de porcs et est susceptible de diffuser entre les élevages par l'introduction de porcs infectés, notamment du fait de l'organisation pyramidale de la production porcine (Salines *et al.*, 2017a).

La présente étude vise à développer une méthode quantitative combinant analyse de réseau et données épidémiologiques pour évaluer le rôle des mouvements d'animaux à deux échelles différentes : tout d'abord en mesurant l'impact des mouvements d'animaux sur la prévalence d'un pathogène en élevage, puis en évaluant le risque pour les départements français d'être exposés à un agent infectieux du fait de mouvements en provenance de départements infectés. Notre méthodologie a été appliquée au virus de l'hépatite E dans la filière porcine.

1. MATERIEL ET METHODES

1.1. Données

1.1.1. Données de mouvements

Base de données. Comme décrit par Salines *et al.* (2017a), les données de mouvements de porcs proviennent de la base de données nationale BDporc, gérée par les professionnels de la

filière porcine et reconnue par le ministère en charge de l'agriculture. Tous les mouvements de porcs entre élevages ou vers un abattoir, un site d'équarrissage ou un centre de rassemblement sont enregistrés dans BDporc. Les mouvements ne sont pas saisis à l'échelle individuelle mais pour un lot d'animaux : des groupes de porcs quittent des sites de production (mouvements de chargement, notés C) et sont envoyés dans d'autres sites (mouvements de déchargement, notés D). Un camion de transport peut charger et décharger des animaux dans plusieurs sites : une tournée correspond à une série de mouvements d'un camion, de la première opération de chargement au dernier mouvement de déchargement au terme duquel le camion est vide.

Modélisation du réseau de mouvements (Figure 1). Les données de mouvements enregistrées de janvier à décembre 2013 ont été représentées par un réseau dirigé à un mode, agrégé sur l'année. Les mouvements entre deux élevages donnés ayant eu lieu plusieurs fois dans l'année ont été agrégés en un lien unique entre ces deux élevages. Les mouvements intermédiaires composant une tournée ont été remplacés par des mouvements directs entre les élevages, en négligeant les mouvements de transit des camions sans déchargement d'animaux. Au sein d'une tournée, tous les sites dans lesquels des animaux ont été déchargés ont été reliés à tous les sites de chargement antérieurs dans la tournée.

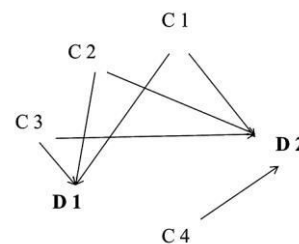


Figure 1 – Représentation du réseau de mouvements de porcs en France en 2013. Les nœuds C et D correspondent respectivement à des élevages dans lesquels des mouvements de chargement et de déchargement ont lieu. Les numéros correspondent à la chronologie de la tournée d'un camion.

1.1.2. Données de prévalence du VHE

Une enquête nationale de prévalence du VHE a été conduite en 2009. Comme décrit par Rose *et al.* (2011), 6 565 échantillons de sérum provenant de 186 élevages localisés dans 49 départements ont été collectés à l'abattoir. Les échantillons de sérum ont été testés avec le kit EIAgen HEV Ab Kit® Adaltis (Ingen, France).

1.2. Analyses statistiques

1.2.1. Centralité des élevages et séroprévalence VHE intra-élevage

Indicateurs de centralité des élevages. Parmi les 186 élevages inclus dans l'étude de prévalence, seuls 172 étaient enregistrés dans la base de données des mouvements. Plusieurs mesures de centralité ont été calculées à partir du réseau de mouvements de 2013 : le degré entrant, i.e. le nombre de mouvements entrant dans un élevage ; le degré sortant, i.e. le nombre de mouvements sortant d'un élevage ; les centralités de proximité entrante et sortante qui traduisent la proximité d'un élevage en termes de nombres de liens entrants ou sortants le séparant des autres élevages du réseau ; la centralité d'intermédiarité, i.e. la proportion de plus courts chemins sur lesquels se trouve l'élevage étudié ; les chaînes de contact entrante et sortante mensuelles, i.e. le nombre

d'établissements connectés à une exploitation en incluant les contacts directs (adjacents) et indirects (tous les chemins menant à cette exploitation) sur une période d'un mois et en tenant compte de la chronologie des contacts ; la loyauté des nœuds qui mesure la fraction des contacts d'un nœud qui sont préservés entre deux fenêtres de temps consécutives (ici d'un semestre à l'autre). Du fait de leur faible variabilité, les indicateurs de centralité de proximité ont été catégorisés selon leur valeur moyenne.

Séroprévalence VHE intra-élevage. Pour chacun des 172 élevages, la séroprévalence VHE a été définie comme le nombre de porcs séropositifs parmi le nombre total de porcs échantillonnés dans l'élevage. La sensibilité et la spécificité individuelles du test (Rose *et al.*, 2010) ont été utilisées pour corriger la séroprévalence apparente (Rogan, Gladen, 1978).

Modèle statistique. Une analyse univariée a été conduite pour évaluer le lien statistique entre chacune des variables explicatives (i.e. les indicateurs de centralité des élevages) et la variable à expliquer (i.e. la prévalence du VHE intra-élevage corrigée). Pour ce faire, une régression logistique (*generalised estimating equation*) a été réalisée, avec la variable « élevage » en effet répété (procédure GENMOD dans SAS 9.4) (SAS, 2014).

1.2.2. *Risque d'exposition des départements au VHE*

Mouvements de porcs à l'échelle départementale. Pour chaque département de France métropolitaine, le nombre de mouvements de porcs provenant de chacun des autres départements en 2013 a été calculé.

Prévalence du VHE à l'échelle des élevages. La prévalence du VHE a cette fois été définie au niveau départemental comme le nombre d'élevages ayant au moins un porc séropositif parmi le nombre total d'élevages échantillonnés dans le département (Figure 2). L'écart type de la prévalence a été calculé par un test binomial exact et a été pondéré avec un facteur de correction lié au taux de sondage (i.e. la proportion d'élevages échantillonnés parmi le nombre total d'élevages dans le département). Pour tenir compte de l'incertitude relative à l'estimation de la prévalence du VHE (Figure 2), la prévalence a été représentée par une distribution beta, avec la prévalence observée et son intervalle de confiance pondéré comme paramètres de la distribution.

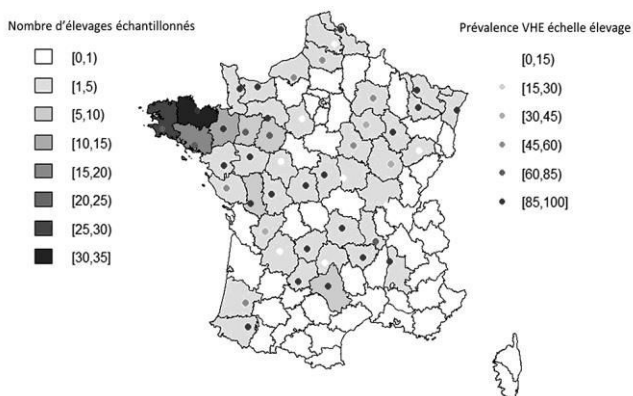


Figure 2 – Nombre d'élevages échantillonnés par département dans l'enquête de prévalence de 2009 et prévalence du VHE observée à l'échelle des élevages par département.

Evaluation du risque d'exposition au VHE. Le risque d'exposition au VHE d'un département a été calculé comme suit : tout d'abord, une valeur de prévalence du VHE a été tirée aléatoirement dans la distribution beta ; le nombre

correspondant d'élevages positifs dans le département a ensuite été dérivé de cette valeur de prévalence sélectionnée et les statuts individuels des élevages ont été aléatoirement attribués. Les élevages sources ont ensuite été aléatoirement sélectionnés selon le nombre de mouvements ayant quitté le département en 2013, ce qui a permis d'obtenir un nombre de mouvements sortants infectés. Enfin, le risque d'exposition d'un département au VHE a été défini comme le nombre de mouvements infectés qu'il a reçus depuis des départements sources, divisé par son nombre total de mouvements entrants. Ce calcul a été répété 10 000 fois, ce qui a conduit à une distribution du risque d'exposition au VHE pour chaque département. Ce modèle de risque d'exposition a été développé sous R (R, 2017).

2. RESULTATS

2.1. Centralité des élevages et séroprévalence VHE intra-élevage

Les valeurs moyennes des degrés entrants et sortants des élevages étaient respectivement de 2,46 (de 0 à 22) et 5,14 (de 0 à 134). Les centralités de proximité entrante et sortante étaient en moyenne de $2,17 \cdot 10^{-9}$ et $2,18 \cdot 10^{-9}$ respectivement, avec une faible variabilité. La centralité d'intermédiarité moyenne était de 27,06 (de 0 à 1 439). Les chaînes de contact mensuelles entrante et sortante étaient en moyenne égales à 0,98 (de 0 à 5) et à 1,15 (de 0 à 29) respectivement. La loyauté moyenne des nœuds était de 0,65 (de 0 à 1). Dans les 172 élevages, la séroprévalence corrigée variait de 0 à 100 % de porcs séropositifs (moyenne : 29 %, médiane : 17 %).

L'analyse univariée a montré qu'un fort degré entrant et une valeur de centralité de proximité entrante élevée étaient significativement et positivement associés à une séroprévalence VHE intra-élevage élevée ($p < 0,05$) (Tableau 1). Ces deux variables étant corrélées entre elles (coefficient de corrélation de Pearson égal à 0,6, $p < 0,01$), elles n'ont pas été incluses dans un modèle multivarié.

Tableau 1 – Lien entre les indicateurs de centralité des élevages et la séroprévalence VHE intra-élevage.

Indicateurs de centralité	Odds ratio (intervalle de confiance 95%)	p-value
Degré entrant	1,07 [1,01 – 1,13]	0,02
Degré sortant	0,99 [0,98 – 1,01]	> 0,1
Centralité de proximité entrante	1,91 [1,08 – 3,38]	0,02
Centralité de proximité sortante	0,54 [0,18 – 1,6]	> 0,1
Centralité d'intermédiarité	0,99 [0,99 – 1,01]	> 0,1
Chaîne de contact entrante	1,23 [0,96 – 1,58]	0,09
Chaîne de contact sortante	0,98 [0,92 – 1,04]	> 0,1
Loyauté des nœuds	0,72 [0,37 – 1,38]	> 0,1

2.2. Risque d'exposition des départements au VHE

Prévalence du VHE à l'échelle élevage dans les départements et incertitude liée. Les distributions de la prévalence apparente du VHE à l'échelle élevage ont été représentées (exemples en Figure 3). Du fait du nombre variable d'élevages échantillonnés selon le département (Figure 2), certains départements montrent une forte incertitude quant à leur niveau de prévalence (par exemple le département A de la Figure 3).

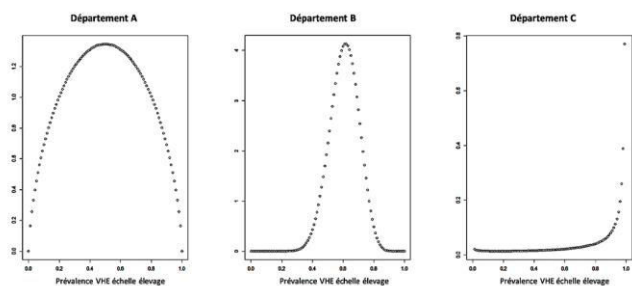


Figure 3 – Exemples de distributions de la prévalence du VHE à l'échelle élevage dans trois départements.

Estimation du risque d'exposition au VHE. Les distributions du risque pour les départements français d'être exposés au VHE ont été représentées (exemples en Figure 4).

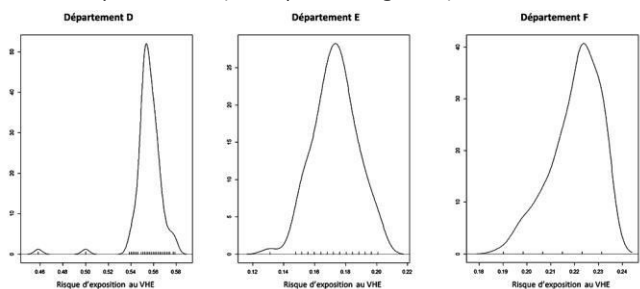


Figure 4 – Exemples de distribution du risque d'exposition au VHE de trois départements.

Le risque d'exposition médian de chaque département a été cartographié (Figure 5). Les répartitions géographiques de la prévalence du VHE et du risque d'exposition au VHE présentent des différences majeures (Figure 2 et Figure 5).

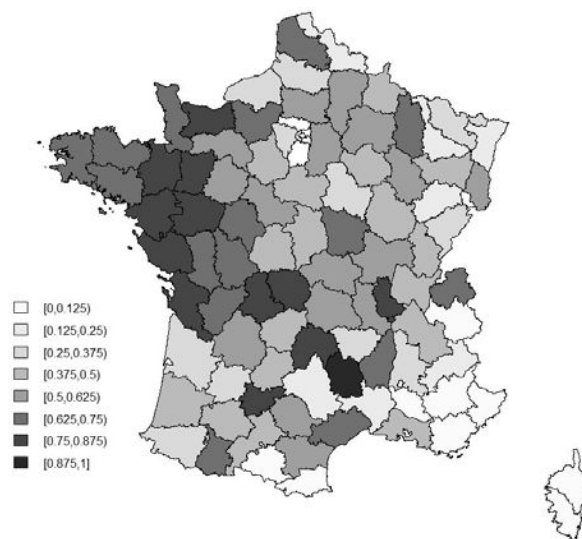


Figure 5 – Risque médian d'exposition des départements français au VHE par l'intermédiaire de leurs mouvements entrants de porcs.

3. DISCUSSION

L'analyse des caractéristiques des réseaux de mouvements d'animaux est indispensable à la compréhension de la dynamique d'infection des pathogènes, à l'exploration des facteurs de risque relatifs aux mouvements d'animaux et à la mise en place de stratégies de surveillance fondées sur le risque. Notre étude présente l'intérêt de développer une méthode couplant épidémiologie et analyse de réseau pour quantifier l'impact des mouvements de porcs à la fois sur la

prévalence des pathogènes dans les élevages et sur le risque pour des zones géographiques d'être exposées à des maladies du fait de leurs mouvements entrants. Le VHE a été choisi comme pathogène d'application. En effet, les mouvements de porcs sont susceptibles de jouer un rôle central dans l'épidémiologie du VHE (Nantel-Fortier *et al.*, 2016; Salines *et al.*, 2017a). De plus, aucun programme de surveillance du VHE n'est actuellement mis en place dans les pays industrialisés (Salines *et al.*, 2017a). Notre étude a ainsi visé à évaluer le rôle des mouvements de porcs en lien avec la prévalence du VHE intra-élevage et le risque d'exposition des départements français au VHE, afin d'orienter le développement de stratégies de surveillance du VHE fondées sur le risque lié aux mouvements d'animaux.

Les données de mouvements de porcs utilisées dans notre étude proviennent de la base de données BDporc dans laquelle tous les échanges de porcs sont enregistrés. Cette base de données est reconnue par le ministère en charge de l'agriculture, ce qui augmente la fiabilité des données. De plus, un nettoyage minutieux de la base de données a permis de traiter les données incorrectes ou incomplètes. Ainsi, la qualité des données en termes d'exhaustivité, de fiabilité et de précision assure la robustesse des résultats obtenus (Salines *et al.*, 2017b). La procédure de sélection aléatoire des porcs et des élevages testés dans l'enquête de prévalence, couplée à la correction de la séroprévalence apparente pour tenir compte des qualités diagnostiques du test, a permis d'estimer la prévalence du VHE intra-élevage (Rose *et al.*, 2011). Au niveau départemental, la faible précision de certaines valeurs de prévalence dans les départements où peu d'élevages avaient été échantillonnés a été prise en compte par un modèle impliquant la sélection aléatoire de valeurs de prévalence du VHE à l'échelle élevage dans des distributions beta, avec un intervalle de confiance pondéré par le taux de sondage. L'une des limites de l'étude réside dans la discordance temporelle des données, puisque les données de mouvements et de prévalence n'ont pas été simultanément collectées. Néanmoins, le réseau des mouvements de porcs en France est stable dans le temps (Salines *et al.*, 2017b), de sorte que les données de prévalence de 2009 peuvent être légitimement combinées avec les données de mouvements de 2013. L'agrégation des données de mouvements sur une base annuelle apparaît également justifiée du fait de l'absence de saisonnalité du réseau porcin français (Relun *et al.*, 2016; Salines *et al.*, 2017b) et permet d'obtenir des indicateurs représentant l'activité globale des élevages sur une année entière de production.

De récentes publications ont identifié plusieurs indicateurs de connectivité des élevages comme étant des facteurs de risque de la survenue et de la diffusion de pathogènes (Martin *et al.*, 2011; Frössling *et al.*, 2012; Lee *et al.*, 2017; Sintayehu *et al.*, 2017). Notre étude a mis en évidence que le degré entrant d'un élevage était positivement associé à un haut niveau de séroprévalence VHE intra-élevage. Ce résultat est en accord avec plusieurs études conduites dans différents secteurs de production animale (Martin *et al.*, 2011; Frössling *et al.*, 2012; Lee *et al.*, 2017; Sintayehu *et al.*, 2017). De plus, les échanges répétés d'animaux entre un même couple fournisseur-acheteur ont été condensés en un seul lien dans le modèle de réseau. L'association ainsi mise en évidence dans notre étude entre la séroprévalence VHE intra-élevage et le degré entrant ne traduit pas seulement que la séroprévalence VHE augmente avec le nombre de mouvements entrants, mais aussi que l'approvisionnement en animaux chez différents fournisseurs

explique en partie une séroprévalence VHE plus élevée. Nos résultats ont également montré que plus la centralité de proximité entrante de l'élevage était importante, plus son niveau de séroprévalence VHE était élevé. Une valeur élevée de la centralité de proximité entrante d'un élevage indique que l'élevage en question peut être atteint par ses partenaires commerciaux en seulement quelques mouvements. Ainsi, la centralité d'un élevage dans le réseau apparaît être un facteur de vulnérabilité au VHE. Ceci est cohérent avec les résultats de précédentes études (Lee *et al.*, 2017; Sintayehu *et al.*, 2017). Comme dans l'étude de Lee *et al.* (2017) au sujet du virus du SDRP, nos résultats mettent en évidence que le risque d'avoir une séroprévalence VHE intra-élevage plus élevée augmente plus avec la centralité de proximité entrante qu'avec le degré entrant, ce qui signifie que le niveau de connectivité global d'un élevage dans le réseau est un meilleur indicateur que le nombre d'élevages auxquels il est directement relié.

A l'inverse de ce qu'ont montré Lee *et al.* (2017) pour le virus du SDRP, aucune association significative entre la séroprévalence VHE intra-élevage et le degré sortant ni la centralité de proximité sortante n'a été mise en évidence. L'absence d'effet de ces indicateurs est cohérente : en effet, le VHE est principalement transmis par l'introduction de porcs infectés dans une population naïve ; l'introduction du virus dans un élevage du fait du seul transit d'un camion contaminé chargeant des porcs dans l'élevage, sans introduction d'animaux infectés, est ainsi peu probable. A l'inverse de Sintayehu *et al.* (2017) à propos de la tuberculose bovine, nos résultats ne montrent pas d'effet significatif de la centralité d'intermédiarité d'un élevage sur son niveau de séroprévalence VHE. Les élevages ayant une centralité d'intermédiarité élevée jouent un rôle central dans la diffusion de maladies dans un réseau car ils peuvent faire office de pont entre deux composantes séparées du réseau. Comme nous avons étudié l'impact des mesures de centralité sur la prévalence du VHE dans un élevage et non sur sa capacité à diffuser le virus vers d'autres élevages, l'absence d'effet de la centralité d'intermédiarité est cohérente. Les chaînes de contact entrante et sortante n'apparaissent pas associées à la séroprévalence VHE intra-élevage. Là encore, comme nous n'avons pas exploré la capacité d'un élevage à transmettre le VHE, aucun lien entre la chaîne d'infection sortante et la séroprévalence VHE n'était attendu. Une association entre la chaîne d'infection entrante et la séroprévalence VHE aurait pu être observée. Néanmoins, Frössling *et al.* (2012) ont montré que cette association dépendait du pathogène.

A notre connaissance, l'exposition d'une aire géographique à un pathogène du fait des mouvements d'animaux n'a jamais été quantifiée. Le choix du niveau départemental pour notre étude est lié au fait que les départements sont les zones administratives locales à l'échelle desquelles les programmes de surveillance sont généralement conçus et mis en œuvre. Du fait de la faible précision des données de prévalence à l'échelle des élevages dans certains départements, la distribution du risque d'exposition est large dans ces départements.

Néanmoins, les sorties de la procédure utilisée pour évaluer le risque d'exposition ont été stabilisées grâce à un nombre élevé de simulations. Une grande variabilité du risque d'exposition médian au VHE est observée selon les départements français, ce qui confirme l'intérêt de développer des stratégies de surveillance ciblées et différenciées selon le niveau de risque de la zone géographique. De plus, les différences entre les niveaux de prévalence et ceux du risque d'exposition des départements justifient de surveiller non seulement les zones fortement prévalentes mais aussi celles recevant des animaux en provenance de zones infectées.

Plusieurs équipes de recherche ont développé des scores de risque à l'échelle des élevages prenant en compte les mouvements d'animaux. Par exemple, Schärer *et al.* (2015) ont utilisé un score cumulatif incluant différents paramètres, dont la chaîne de contact entrante, le nombre d'animaux par mouvement entrant, le type de pâture et le nombre de semaines dans l'année durant lesquelles des échanges ont eu lieu. Une autre publication présente une méthode pour calculer un risque accru d'infection dû à l'introduction d'animaux (Frössling *et al.*, 2014). Ribeiro-Lima *et al.* (2015) ont aussi développé un score fondé sur les mouvements pour identifier les élevages ayant un risque plus élevé de tuberculose bovine. Une prochaine étape de notre étude pourrait être de construire un score à l'échelle des élevages qui inclurait à la fois des facteurs de risque liés aux mouvements d'animaux et d'autres facteurs de risque vis-à-vis du VHE qui ont déjà été identifiés (certaines pratiques d'élevage, le type d'élevage,...) (Walachowski *et al.*, 2014). Un tel score permettrait de cibler uniquement les élevages à risque et d'optimiser ainsi la surveillance.

CONCLUSION

Le couplage de méthodes d'analyse de réseau avec des données épidémiologiques a permis de montrer que la connectivité directe des élevages et leur centralité de proximité dans le réseau sont associées à la prévalence du VHE intra-élevage et que certaines aires géographiques sont plus à risque vis-à-vis du VHE du fait de leur approvisionnement depuis des zones infectées. Plus généralement, les méthodes proposées dans cette étude démontrent que des paramètres à l'échelle de l'élevage ou du département dérivés des caractéristiques du réseau de mouvements d'animaux peuvent orienter la sélection des élevages pour des programmes de surveillance fondés sur le risque, ou la mise en place de stratégies de surveillance différenciées selon les caractéristiques de mouvements des zones géographiques.

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**Annexe 8 - Rapport préliminaire d'aide à la décision
pour la maîtrise du risque lié à l'hépatite E dans la
filère porcine**

La maîtrise du risque lié au virus de l'hépatite E dans la filière porcine : éléments d'aide à la décision

Rapport préliminaire

Le projet

Les travaux de l'Anses sur la thématique du virus de l'hépatite E, initiés au début des années 2000, se sont poursuivis par une thèse d'université sur la période 2016-2019. Cette thèse en Formation Complémentaire Par la Recherche a eu comme objectif de comprendre la propagation et la persistance du virus de l'hépatite E dans les élevages de porcs et dans la filière porcine, afin de proposer des stratégies de réduction de l'exposition humaine au virus. Ce projet de recherche s'est articulé autour d'études en élevage, d'essais expérimentaux, et d'un modèle mathématique représentant la diffusion du VHE au sein d'un élevage de porcs naisseur-engraisseur.

Le rapport

Ce document est un rapport intermédiaire qui synthétise (i) les données récentes disponibles dans la littérature concernant le risque présenté par les produits porcins et l'épidémiologie du VHE dans les élevages de porcs ; (ii) les résultats du projet de recherche sus-cité, notamment en ce qui concerne l'influence des co-infections immunomodulatrices ainsi que la diffusion du virus dans la filière porcine. Il s'accompagne d'une synthèse des pistes d'action identifiées, formulées sous la forme de propositions à discuter avec les organisations publiques et privées gestionnaires du risque. Un rapport final sera adressé à ces mêmes organisations à l'automne 2019.

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Dix pistes d'action pour la maîtrise du risque VHE dans la filière porcine

AXE I - Lutte en élevage

Piste 1 : Accompagner les élevages (particulièrement ceux de grande taille ayant un mode de production intensif) vers des pratiques de biosécurité externe (sas sanitaire, quarantaine) et interne (limitation des adoptions et des mélanges, gestion des effluents) plus sûres.

Piste 2 : Soutenir la mise en place de programmes d'éradication des pathogènes immunomodulateurs, notamment du virus du SDRP.

Piste 3 : En collaboration avec les vétérinaires sanitaires, réaliser des dépistages VHE dans les élevages souhaitant s'engager dans un programme de maîtrise du VHE et assurer le suivi de leur situation sanitaire.

AXE II - Organisation de la filière

Piste 4 : Envisager la structuration d'une filière spécifique permettant aux élevages reconnus indemnes de VHE de fournir des foies sains pour la fabrication des produits à risque.

AXE III - Surveillance

Piste 5 : Inclure le VHE dans les prochains plans de surveillance et de contrôle annuels sur carcasses, abats et produits transformés.

AXE IV - Communication

Piste 6 : Réaliser une campagne d'information pour sensibiliser les acteurs de la filière porcine, notamment à l'échelon de l'élevage, à la problématique du VHE.

AXE V - Recherche

Piste 7 : Evaluer la faisabilité technique et l'acceptabilité sociale d'un plan de lutte par les différents acteurs de la filière.

Piste 8 : Réaliser une étude d'intervention en élevage permettant d'évaluer l'efficacité en conditions réelles des mesures de lutte proposées.

Piste 9 : Evaluer la prévalence de carcasses contaminées à l'abattage à partir d'un échantillonnage ciblé sur les facteurs de risque identifiés en élevage.

Piste 10 : Evaluer, à partir de viande de porcs infectés en conditions expérimentales, l'efficacité des processus de séchage et de salaison utilisés dans les IAA sur la diminution de la charge virale dans les produits de charcuterie et salaison.

Le virus de l'hépatite E (VHE) est un virus non-enveloppé à ARN simple brin provoquant chez l'homme une infection souvent asymptomatique, mais parfois une hépatite aiguë ou chronique selon, entre autres, le contexte immunitaire du patient [1, 2]. Les génotypes 3 et 4 sont partagés par l'homme et d'autres espèces animales et sont responsables de cas sporadiques autochtones dans les pays industrialisés [3, 4]. En particulier, le génotype 3 est largement répandu dans la population porcine et un certain nombre de cas autochtones ont pu être reliés à la consommation de produits porcins crus ou insuffisamment cuits [5-8]. En ce sens, l'hépatite E est reconnue comme une zoonose alimentaire dont les porcs domestiques sont le principal réservoir dans les pays développés [9]. Le risque que des produits contaminés soient commercialisés dépend étroitement de la dynamique d'infection du VHE dans les élevages de porcs. Cependant, l'épidémiologie du VHE dans la filière porcine est encore mal comprise et l'hétérogénéité des données de prévalence disponibles dans la littérature indique des variations de la dynamique d'infection du VHE [10].

1. Transmission zoonotique à partir du réservoir porcine

1.1. Transmission du VHE par voie alimentaire

En France, le nombre de cas rapportés au Centre National de Référence des hépatites à transmission entérique (CNR) en 2016 s'élève à 2 302, dont 2 292 cas autochtones. Plusieurs cas d'hépatite E ont été attribués avec certitude à la consommation de produits à base de porc contaminés par le VHE. Par exemple, Colson et al. ont décrit des cas groupés d'hépatite E en France chez sept personnes qui avaient consommé des figatelles (ou figatelli, saucisses fraîches composées de viande et de foie de porc, spécialités corses) [5]. En 2013, des infections groupées par le VHE ont été rapportées à Belle-Île en Mer, parmi lesquelles trois cas cliniques confirmés. Les patients avaient consommé un porcelet rôti à la broche farci avec une farce crue contenant le foie du porcelet. Les mêmes souches de VHE que celles impliquées dans les cas humains ont aussi été détectées dans le lisier échantillonné dans l'élevage d'origine du porcelet et dans des eaux usées non traitées provenant du système d'épuration de Belle-Île en Mer [8].

Plusieurs études visant à identifier des facteurs de risque expliquant l'infection ou la séroprévalence VHE ont été conduites dans différents pays. Par exemple, en France, une récente enquête nationale rapporte les facteurs de risque suivants associés à une sérologie VHE positive : la consommation de viande de porc ou de gibier (Risque Relatif (RR) = 1,5), de saucisses à base de foie de porc (RR = 1,3), d'abats (RR = 1,3) [11]. Trois études néerlandaises ont également pointé la consommation de viande comme un facteur de risque : Slot et al. ont montré que la séroprévalence VHE chez les donneurs de sang consommant de la viande était significativement plus élevée que chez les végétariens (20,5 % *versus* 12,4 %) [12] ; Mooij et al. ont quant à eux décrit la consommation de saucisses traditionnelles néerlandaises (de type salami, cervelas, *etc.*) comme un facteur de risque (Odds Ratio (OR) = 1,5) [13] ; de même, Tulen et al. ont montré que la consommation de saucisses traditionnelles crues à base de viande de porc (type cervelas) était associée à un risque plus élevé d'hépatite E aiguë (OR = 3) [14]. En Allemagne, une étude incluant 45 cas et 135 témoins a mis en évidence que la consommation

d'abats était associée à l'infection par le VHE (OR = 2,7) [15]. Plus récemment, toujours en Allemagne, Faber et al. ont montré, à partir de 270 cas et 1 159 contrôles, que l'infection par le VHE était associée, entre autres, à la consommation de foie de porc insuffisamment cuit (OR = 5,3), de viande de porc (OR = 3), de saucisses de Francfort (OR = 1,9) et de saucisses ou pâté de foie (OR = 2,1) [16]. En Angleterre et en Ecosse, Said et al. ont rapporté, à partir de 25 cas et 75 témoins, que l'infection par le VHE était associée à la consommation de tourte de porc (*pork pie*, spécialité britannique, OR = 6,3) et de saucisses et jambon provenant d'une grande chaîne de supermarchés britannique (OR = 10,1) [17]. Une étude particulièrement médiatisée des mêmes auteurs a mis en évidence que l'infection par une souche particulière de VHE était liée à la consommation de saucisses et de jambons provenant de la chaîne de supermarchés britannique Tesco (RR = 1,9) [18].

En outre, de nombreuses données sont disponibles dans la littérature pour évaluer la contamination des produits à base de porc. Parmi eux, les foies de porcs se révèlent être particulièrement à risque, avec une prévalence de foies contaminés à l'abattoir ou en supermarché variant entre 1 et 21 % selon les études et les pays. En France, par exemple, une enquête nationale a montré que 4 % des foies de porc à l'abattoir contenaient de l'ARN viral du VHE [19]. Par conséquent, les produits à base de foie de porc (saucisse de foie, pâté de foie) sont également à risque vis-à-vis du VHE, en particulier s'ils sont consommés crus ou insuffisamment cuits. Une étude menée en France à partir de 394 produits achetés en supermarchés a montré que 30 % des figatelles et fitones contenaient de l'ARN viral du VHE, ainsi que 3 % des foies séchés et salés, 25 % des quenelles de foie et 29 % des saucisses de foie sèches ou fraîches [20]. Les charges génomiques virales retrouvées dans les foies, muscles, ou produits à base de foie ou de viande de porc sont comprises entre 10 et 10⁷ copies d'ARN par gramme, selon les études et les produits [21] et sont donc compatibles avec une infection humaine productive.

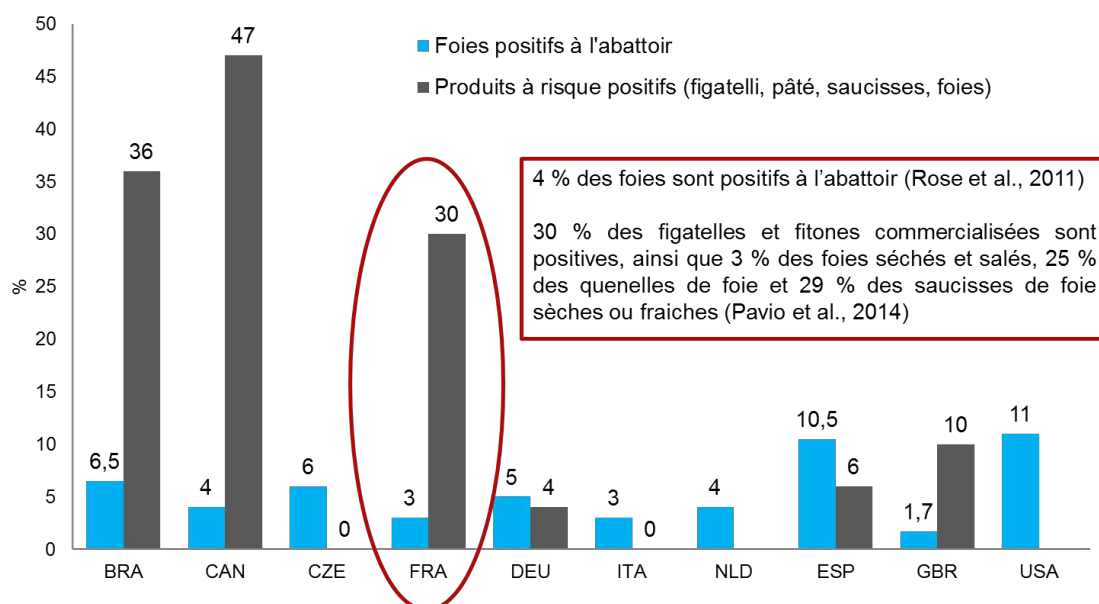


Figure 1 : Principales données de contamination des foies de porcs et produits à base de foies de porcs dans 10 pays industrialisés (d'après [10])

Si le risque représenté par les produits à base de foie de porc est largement admis par la communauté scientifique et les autorités sanitaires, le risque lié à la consommation de viande de porc ou produits à base de porc ne contenant pas de foie est plus discuté. Expérimentalement, il a été montré que les muscles et le sang des porcs peuvent contenir du VHE [22], notamment lorsque les porcs sont co-infectés par le virus du Syndrome Dysgénésique et Respiratoire Porcin (SDRP) [23], et l'enquête de Di Bartolo et al. rapporte 3 % de muscles linguaux de porcs positifs VHE à l'abattoir [24]. En Allemagne, l'enquête de Szabo et al. a montré que 21,7 % des salamis échantillonnés contenaient du VHE [25]. L'étude de Cossaboom et al. rapporte également que 25 % des andouillettes échantillonnées dans un supermarché de Virginie aux Etats-Unis sont positives VHE [26]. Enfin, une enquête conduite récemment au Pays-Bas décrit une contamination de produits sanguins de porcs, entrant dans la fabrication de produits à base de viande [27]. En France en revanche, l'étude conduite par Feurer et al. sur 1084 échantillons de muscles de porcs collectés à l'abattoir n'a pas mis en évidence la présence d'ARN du VHE dans ces muscles [28].

La détection du VHE dans un grand nombre de produits à base de foie de porc, et les questions émergeant ces dernières années sur la possible présence du VHE dans la viande et les produits à base de viande de porc, conduisent à insister sur la nécessité de consommer ces produits cuits à cœur. Rappelons qu'un traitement thermique à 71°C pendant 20 minutes a prouvé son efficacité pour inactiver le virus dans des matrices complexes [29]. L'efficacité des process de séchage et de salaison n'a pas été démontrée.

1.2.Exposition professionnelle au VHE

La possible transmission du VHE par contact avec des porcs a été suggérée par de nombreuses études sérologiques conduites auprès de personnes ayant une exposition professionnelle à ces animaux (éleveurs de porcs, employés d'abattoir, bouchers, vétérinaires porcins), en comparaison à un groupe contrôle. En revanche, si plusieurs études décrivent une exposition plus élevée dans ces catégories professionnelles, la transmission du VHE par contact semble rarement conduire à une hépatite clinique chez l'homme (pas d'excès de cas cliniques dans ces catégories socio-professionnelles).

2. Epidémiologie de l'infection par le virus de l'hépatite E chez le porc

2.1.Histoire naturelle de l'infection par le VHE chez le porc

Une revue exhaustive de la littérature sur l'épidémiologie du VHE chez le porc a été publiée en 2017 [10]. Chez le porc, l'infection naturelle ou expérimentale par le virus de l'hépatite E est asymptomatique [30-32]. Comme chez l'homme, la réplication virale a lieu principalement dans les hépatocytes. L'infection est accompagnée d'une hépatite légère à modérée, révélée par des examens histopathologiques, mais aucune lésion macroscopique du foie n'est observée [30, 33]. Un porc dit infectieux désigne un porc excréant des particules

virales dans ses matières fécales. La majorité des études réalisées en conditions d'élevage indique que les principaux excréteurs du VHE sont les porcs en croissance [34]. L'excrétion fécale par les animaux reproducteurs a aussi été montrée dans plusieurs études [33-35]. Les truies pourraient ainsi entretenir et maintenir la propagation du virus dans les élevages. Le virus est principalement excrété par voie fécale chez le porc, conduisant à une accumulation du VHE dans l'environnement des animaux en élevage infecté. Une méta-régression réalisée à partir de données issues de 31 publications internationales publiées entre 2002 et 2016 met en évidence une probabilité maximale d'excrétion aux alentours de 90 jours d'âge, conduisant à une prévalence de porcs infectieux à 185 jours d'âge (âge courant d'abattage) de 6,1 % [1,2-15,4] [10]. Le virus se transmet principalement par voie féco-orale. Expérimentalement, une charge minimale de 10^6 génome équivalent (ge) par gramme de fèces semble nécessaire pour infecter des porcs per os et pour qu'ils soient capables d'excréter le virus et le transmettre à des congénères [36]. Un modèle mathématique a montré que la transmission par contact direct était un facteur de persistance de l'infection en élevage porcin avec un taux de transmission directe de 0,15 [0,03-0,31] porc par jour. Néanmoins, le facteur majeur de propagation et de maintien de l'infection dans la population réside dans l'accumulation et la survie du virus dans l'environnement des animaux favorisant le processus de contamination par voie féco-orale via l'environnement. La transmission d'une case à l'autre est limitée et repose exclusivement sur le transfert de matières fécales d'une case adjacente à l'autre. Des mesures de biosécurité internes drastiques seraient donc a priori très efficaces pour limiter la propagation à l'échelle de la population et limiter ainsi l'exposition au virus à une fraction très faible de la population de l'élevage [36].

La présence d'anticorps maternels chez le porcelet n'empêche pas l'infection mais retarde le début de la virémie ainsi que la séroconversion chez celui-ci [37]. La durée de présence des anticorps est également fonction du titre en anticorps chez la mère, ce dernier étant fortement influencé par l'âge de la truie. Les IgG perdurent jusqu'à 9 semaines d'âge chez les porcelets nés de truies fortement séropositives contre 1 à 3 semaines pour les porcelets issus de truies faiblement séropositives [33]. L'impact des anticorps d'origine maternelle sur la transmission du VHE a été évalué en utilisant des données issues de suivis longitudinaux d'élevage [38] : la transmission du VHE chez les porcs ayant une immunité passive a été estimée 13 fois plus faible que chez des porcs sensibles, avec une grande variabilité selon les élevages.

2.2.Premiers éléments d'épidémiologie descriptive et analytique

En France, une enquête nationale a été réalisée afin d'estimer la prévalence et la séroprévalence du VHE dans les élevages de porcs [19]. Au total, 6565 sérums et 3715 foies ont été collectés dans 35 abattoirs répartis sur l'ensemble du territoire français. Les résultats de cette étude montrent que le VHE circule dans 65,3 % des élevages et que 31 % des animaux présentent des anticorps anti-VHE à l'âge d'abattage (*i.e.* vers 26 semaines d'âge). La séroprévalence intra-élevage du VHE varie de 5 à 90 % selon les élevages. A l'abattoir, les résultats suivants ont été obtenus : 24 % des élevages ont au moins un animal à foie positif au moment de l'abattage et au global, 4 % des foies sont infectés par le VHE. La grande variabilité inter-élevages suggère l'existence de facteurs de risque spécifiques aux élevages. Peu d'études

ont exploré et quantifié les facteurs de risque associés à une prévalence ou une séroprévalence élevée du VHE dans les élevages (Table I) : la taille des élevages et les conditions de biosécurité apparaissent être des facteurs de risque majeurs.

Table I : Facteurs de risque associés à une séroprévalence et/ou une prévalence VHE élevée dans les élevages de porcs

Facteurs liés...	
À la taille de l'élevage	<p>Nombre de truies > 1000 (séroprévalence 54,2 % versus 18,9 %) Elevages de taille moyenne versus élevages de grande taille (OR = 4,95) Nombre de porcs > 600 (séroprévalence comprise entre 78 % et 100 %, versus 0 % à 29 %) [39-43] Petite taille versus grande taille (séroprévalence 90 % versus 76 %) Taille des cases en post-sevrage > 26 porcs par case (OR = 2,4)</p>
À la biosécurité	<p>Durée du vide sanitaire en post-sevrage < 4 jours (OR = 1,7) Distance lisier-caillebotis en engraissement < 80 cm (OR = 1,9) Absence de sas sanitaire (OR = 3,6) [39, 40, 44] Absence de période de quarantaine (OR = 2,7) Présence de carnivores domestiques (chiens, chats) (OR = 3,9) Présence de fèces d'oiseaux dans les bâtiments (OR = 2,9)</p>
Aux mélanges	<p>Mélange d'animaux issus de salles différentes entre la maternité et le post-sevrage (OR = 1,8) [39]</p>
Au type de production	<p>Agriculture biologique versus conventionnel (séroprévalence 89 % versus 72 %) Plein air versus conventionnel (séroprévalence 76 % versus 72 %) [44, 45] Elevage extensif versus intensif (OR = 2,2)</p>

2.3. Focus sur l'influence des co-infections immunomodulatrices

Tant en conditions naturelles qu'en conditions expérimentales, il a été montré que deux virus immunomodulateurs, le virus du SDRP (syndrome dysgénésique et respiratoire porcin) et le PCV2 (circovirus porcin de type 2) ont un impact sur la dynamique d'infection par le VHE. Ainsi, un suivi longitudinal dans trois élevages de porcs (trois bandes par élevage, au total 360 porcs suivis individuellement) a permis de montrer que la co-infection avec le virus du SDRP, seule ou en association avec le PCV2, retarde l'âge à l'excrétion et à la séroconversion VHE, augmente la durée d'excrétion du VHE et le risque de positivité des foies à l'abattoir [46]. Ces résultats de terrain ont été confirmés par des données expérimentales. En effet, le suivi d'animaux expérimentalement co-infectés par le virus du SDRP et le VHE montre que l'excrétion du VHE et la réponse immunitaire humorale anti-VHE sont retardées chez les porcs co-infectés, que l'excrétion du VHE est significativement plus importante et prolongée, que la transmission du virus entre les animaux est augmentée, et que par conséquent, le risque de positivité des foies à l'abattoir est augmenté [47]. La co-infection expérimentale de porcs par

le VHE et le PCV2 a des effets plus limités : elle n'augmente pas la durée de la période infectieuse ni la quantité de VHE excrétée dans les fèces, mais elle augmente la transmission directe du virus et retarde la mise en place de la séroconversion VHE [48].

2.4. Apports de la modélisation dynamique dans la compréhension et la maîtrise du VHE

Les travaux de recherche de l'Anses sur l'épidémiologie du VHE se sont poursuivis avec le développement d'un modèle mathématique permettant de représenter et de comprendre la diffusion et la persistance du VHE dans un élevage de porcs naisseur-engraisseur [49]. Le modèle construit dans ce but couple un modèle de dynamique de population, permettant de prendre en compte différents types de conduite d'élevage, avec un modèle épidémiologique multi-pathogènes décrivant la transmission du VHE en présence d'un pathogène intercurrent (virus du SDRP, PCV2). Basés sur les données expérimentales et de terrain pré-citées, les paramètres épidémiologiques du modèle VHE varient selon le statut de co-infection avec le pathogène immunomodulateur. Le modèle développé permet de représenter une dynamique d'infection par le VHE similaire à celle décrite dans la littérature. En effet, à la suite de l'introduction d'une cochette infectieuse en verraterie, une propagation rapide du VHE est observée au sein du troupeau reproducteur (Figure 2). L'entrée des truies infectieuses en maternité initie alors le processus infectieux chez les porcs en croissance via l'infection des porcelets sous la mère qui propagent ensuite l'infection en post-sevrage et en engraissement. En l'absence de réintroduction ultérieure, le virus persiste ensuite de manière enzootique dans 60 % des simulations dans un élevage conduit en 7 bandes au terme des cinq années post-introduction.

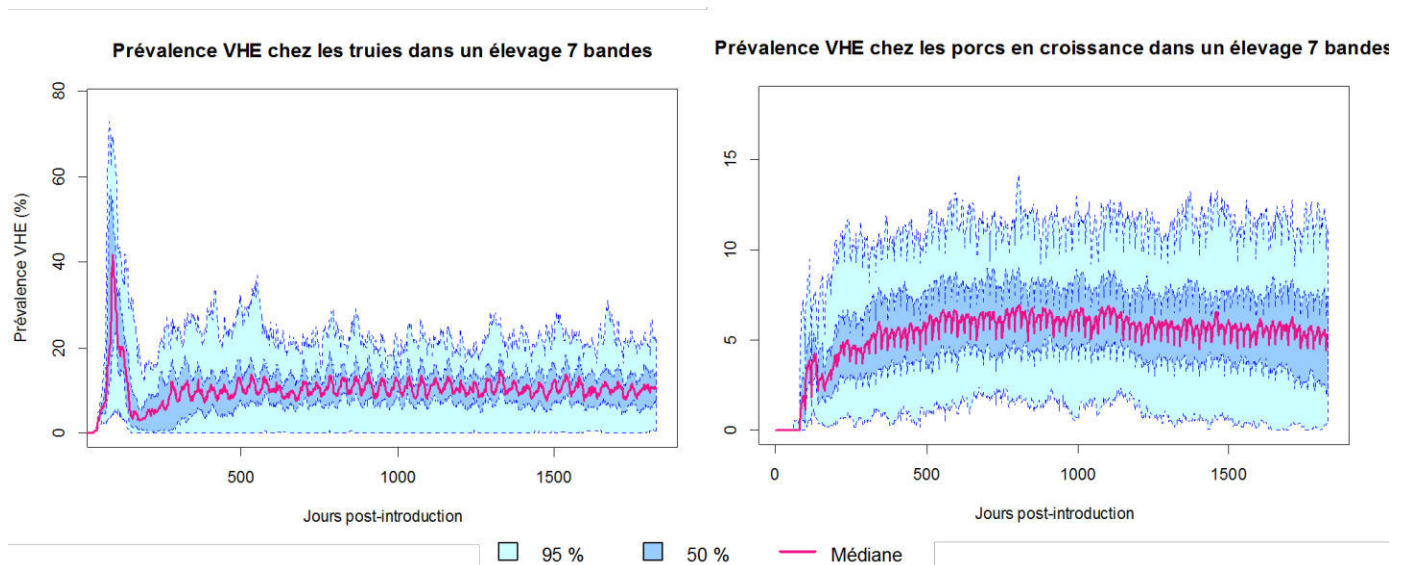


Figure 2 : Prévalence du VHE chez les truies (gauche) et les porcs en croissance (droite) dans un élevage conduit en 7 bandes après introduction d'une cochette infectée par le VHE

De plus, le modèle a permis de montrer que la persistance du VHE et la prévalence de foies positifs à l'abattoir sont plus importantes dans un élevage conduit en 20 bandes que dans un élevage conduit en 7 bandes (persistance cinq ans post-introduction dans 97 % des élevages

versus 60 % ; prévalence à l'abattage de 3,9 versus 2,9 %). Certaines configurations ou pratiques d'élevage s'avèrent également à risque vis-à-vis du VHE. En effet, dans un élevage dans lequel les truies sont logées en grands groupes, le virus circule plus largement – tant chez les porcs que chez les truies, la probabilité de persistance du VHE à cinq ans post-introduction est plus élevée (0,6 contre une extinction complète lors de logement par cases de 6 truies), de même que la prévalence de porcs positifs à l'abattage (2,9 % contre 0,1 %). De plus, dans un élevage qui pratique largement les adoptions, le virus circule davantage dans l'élevage et la prévalence à l'abattage est plus élevée. Enfin, dans un élevage dans lequel le nombre de portées mélangées par case est élevé, la circulation virale est plus intense et la prévalence à l'abattage est plus élevée. Particulièrement dans un élevage conduit en 7 bandes, la vaccination des truies contre le pathogène immunomodulateur conduit à une nette diminution de la prévalence du VHE à l'âge d'abattage (qui passe de 2,9 à 1,7 %) et de la probabilité de persistance du VHE cinq ans post-introduction (qui passe de 0,6 à 0,3). Lorsqu'elles sont combinées, ces mesures se révèlent d'autant plus efficaces pour la maîtrise du risque VHE. Néanmoins, il apparaît que le VHE est plus difficilement contrôlable dans un élevage en 20 bandes. La taille de population et les intervalles inter-bandes jouent sans doute un rôle dans les différences obtenues avec les deux conduites.

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**Annexe 9 - Plaquette d'information relative au virus de
l'hépatite E élaborée à destination des éleveurs et des
vétérinaires**

LE VIRUS DE L'HÉPATITE E

L'hépatite E est une maladie virale qui se manifeste par une inflammation du foie le plus souvent inapparente, mais pouvant parfois conduire à une hépatite aiguë (grande fatigue, signes digestifs, jaunisse, fièvre) avec une guérison progressive spontanée. Dans de plus rares cas, de graves complications peuvent survenir (chez les femmes enceintes par exemple) et aller jusqu'au décès pour des personnes immunodéprimées ou ayant déjà une maladie du foie. En 2016, plus de 2 000 cas humains ont été rapportés au Centre National de Référence.

Le porc est le principal réservoir du virus de l'hépatite E. Le virus est transmissible du porc à l'homme, notamment par consommation de produits à base de porc crus ou insuffisamment cuits (figatelles, saucisses de foie, pâtés de foie,...). L'exposition au virus est plus élevée chez les professionnels en contact avec les porcs (éleveurs, vétérinaires,...), sans toutefois que le nombre de cas observés ne soit plus important.

65 %

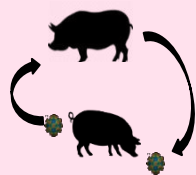
des élevages français sont infectés par le virus de l'hépatite E.

4 %

des foies des porcs abattus en France contiennent du virus de l'hépatite E.



L'infection par le virus de l'hépatite E est asymptomatique chez le porc : pas de signes cliniques, pas de mortalité, pas de baisse des performances. Il n'existe ni traitement ni vaccin pour les porcs.



Le virus de l'hépatite E se transmet par voie oro-fécale entre les porcs. La transmission est augmentée lors de co-infections (virus du SDRP).



ÉLEVEURS, POUR LIMITER LA PRÉSENCE DU VIRUS :

- ✓ Renforcez le protocole de nettoyage désinfection et la biosécurité interne
- ✓ Limitez les mélanges d'animaux
- ✓ Distribuez une eau de bonne qualité
- ✓ Maîtrisez les co-infections

Annexe 10 - Curriculum vitae



Morgane SALINES

Dr vétérinaire, MSc, ISPV

Résidente du Collège Européen de Santé Publique Vétérinaire



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Expérience professionnelle

2019 - ...

Direction Générale de l'Alimentation, Référente Nationale Abattoir

Appui technique opérationnel aux services (DGAI, DDecPP, SVI), missions de veille, de diffusion scientifique et de formation, exploitation des données d'inspection.

Membre du comité de management de l'action CoST (European Cooperation in Science and Technology) RIBMINS : *Risk based meat inspection and integrated meat safety assurance*.

2016 - 2019

Anses, laboratoire de Ploufragan, Unité Epidémiologie, Santé et Bien-Être SVA (Statens Veterinärmedicinska Anstalt), Uppsala, Suède



Doctorat : *Modélisation de la propagation du virus de l'hépatite E dans la filière porcine et évaluation de stratégies de réduction du risque d'exposition humaine*

Modélisation dynamique, statistiques en épidémiologie, expérimentation animale, études de terrain & audits, enquête auprès des acteurs de la filière, recherche de financement & réponse à des appels à projets, veille, valorisation et vulgarisation scientifique, encadrement d'un stagiaire, enseignement.

2016



Ambassade de France aux Etats-Unis, Washington D.C., USA (stage de 5 mois)

Mémoire : *Mettre en œuvre la diplomatie agricole collectivement. Etude du dispositif français de soutien aux exportations agroalimentaires aux Etats-Unis*

Veille réglementaire et sanitaire relative aux produits agro-alimentaires, appui aux exportateurs pour la mise en place du *Food Safety Modernization Act*, rédaction de notes administratives, opérations de communication.

2015

Anses, laboratoire de Ploufragan, Unité Epidémiologie, Santé et Bien-Être Oniris, campus vétérinaire de Nantes, UMR SECALIM (stage de 6 mois)

Thèse d'exercice vétérinaire : *Missions d'inspection sanitaire des produits en abattoir de volailles : indicateurs de réalisation et enquête psycho-sociale*

Statistiques en épidémiologie, enquête auprès des acteurs de la filière dans le cadre du projet pilote, valorisation et vulgarisation scientifique, formation.

2013



Royal Veterinary College, Veterinary Epidemiology and Public Health Group, Londres, Royaume-Uni (CDD de 3 mois)

Rapport de mission : *Ecopathologie de la trypanosomose animale en Afrique subsaharienne et développement d'un outil d'évaluation de stratégies de lutte*

Enquête de type Knowledge Attitudes Practices, statistiques en épidémiologie.

2012

DGAI, Bureau des établissements d'abattage et de découpe (stage de 2 mois)

Révision du GBPH pour l'abattage de volailles. Elaboration de grilles d'inspection pour le projet pilote de réorganisation de l'inspection en filière volailles.



Formation

2014-2016

Inspecteur élève de la Santé Publique Vétérinaire, ENSV, Lyon

Master 2 Politiques de l'alimentation et de gestion des risques sanitaires (PAGERS)

Master 2 Management de l'action collective en santé publique vétérinaire (MAC-SPV)

Certificat d'Etudes Approfondies Vétérinaires en Santé Publique Vétérinaire (CEAV-SPV)

2013-2014

Master 2 MAN-IMAL (en anglais) Man-Animal-Food Health: transdisciplinary management of global health and nutritional safety, Oniris, Nantes

2010-2015

Etudes vétérinaires, Oniris, Nantes

2008-2010

Deux années de licence de biologie, Université Paris VI, Paris

2008

Baccalauréat Série S, Mention TB, Lycée Louis le Grand, Paris

IT & Langues

Microsoft Office (Word, Power point, Excel, Access)
Programmation en R, SAS, C/C++, qGIS/arcGIS
Gestion de base de données

FRANCAIS

Langue maternelle

ANGLAIS

Courant : C1 Cambridge
English Language Assessment

ALLEMAND

Courant : C1 Goethe Institut

Autres diplômes

D.I.U. CESAM, modules "Methodologie statistique" et "Principes et méthodes quantitatives en épidémiologie", université Paris VI

D.U. "Modelling of infectious diseases", institut Pasteur, Paris VI

Engagements

Responsable de projet au sein du Réseau de Santé Publique Vétérinaire

Loisirs

Course à pied, escalade, équitation, voile
Photographie



Morgane SALINES

DVM, MSc, Official Veterinarian
Resident of the European College of Veterinary Public Health

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IT & Languages

Microsoft Office (Word, Power point, Excel, Access)
Programming in R, SAS, C/C++, qGIS/arcGIS
Database management

FRENCH
Native speaker

ENGLISH
Fluent: C1 Cambridge English Language Assessment

GERMAN
Fluent: C1 Goethe Institut

Other degrees

CESAM, "Statistical methods" and "Theories and quantitative methods in epidemiology", Paris VI university

"Modelling of infectious diseases", Pasteur Institute, Paris VI university

Volunteering

Project manager in the French network for Veterinary Public Health

Hobbies

Running, climbing, horse riding, sailing
Photography



Professional experience

2019 - ...

French Ministry of Agriculture, National expert for slaughterhouses

Technical and scientific support to veterinary services, scientific monitoring and communication, training, data analysis.

Management Committee member of CoST Action (European Cooperation in Science and Technology) RIBMINS : *Risk based meat inspection and integrated meat safety assurance*.

2016 - 2019



ANSES, Ploufragan laboratory, Epidemiology, Health and Welfare unit SVA (Statens Veterinärmedicinska Anstalt), Uppsala, Sweden

Ph.D: *Modelling the spread of hepatitis E virus in the pig production sector and evaluating strategies to mitigate the risk of human exposure*

Dynamic modelling, statistics in epidemiology, animal experiments, field studies & audits, socio-economic surveys with production stakeholders, search for funding & response to calls for projects, scientific watch, scientific communication & popularisation, intern supervision, teaching.

2016



Embassy of France in the United States, Washington D.C. (5-month internship)

Masters' thesis: *Collectively implementing agricultural diplomacy. Study of the French support system to agri-food exports to the United States of America*

Monitoring health and regulation changes, supporting exporters for compliance to Food Safety Modernization Act, drafting memoranda, making communication operations.

2015

ANSES, Ploufragan laboratory, Epidemiology, Health and Welfare unit Oniris, Nantes Veterinary College, Food Hygiene unit (6 month-internship)

Veterinary thesis: *Missions of meat inspection in poultry slaughterhouses: implementation indicators and psychosociological survey*

Statistics in epidemiology, sociological surveys with production stakeholders, scientific communication & popularisation, teaching.

2013



Royal Veterinary College, Veterinary Epidemiology and Public Health Group, London, United Kingdom (3-month consultancy)

Analytical report: *Ecopathology of African Animal Trypanosomiasis in Sub-Saharan Africa and development of an impact assessment tool*

Knowledge Attitudes Practices survey, statistics in epidemiology.

2012

French Ministry of Agriculture, General Directorate for Food (2-month internship)

Development of a Guide for Good Hygiene Practices and of inspection grids for veterinary inspection in poultry slaughterhouses.



Education

2014-2016

Training as Official Veterinarian, National College of Veterinary Services, Lyon

Master's degree "Food policy and health risk management"

Master's degree "Management of joint action in veterinary public health"

Advanced certificate in veterinary public health

2013-2014

MSc MAN-IMAL Man-Animal-Food Health: transdisciplinary management of global health and nutritional safety, Nantes Veterinary College, Oniris

2010-2015

Veterinary Studies, Nantes Veterinary College, Oniris

2008-2010

Bachelor's degree in Biology, Paris VI University, Paris

2008

Scientific Baccalauréat, Louis le Grand High School, Paris

Titre : Modélisation de la propagation du virus de l'hépatite E dans la filière porcine et évaluation de stratégies de réduction du risque d'exposition humaine

Mots clés : virus de l'hépatite E, modélisation, modèle multi-échelles, modèle multi-pathogènes, plan de maîtrise, santé publique, sciences humaines et sociales

Résumé : Le virus de l'hépatite E (HEV) est un agent zoonotique dont les porcs représentent le principal réservoir dans les pays industrialisés. Le présent projet de recherche a combiné études épidémiologiques, modélisation mathématique et sciences sociales pour proposer des leviers de réduction du risque d'exposition humaine au HEV par consommation de produits à base de porc. Deux essais expérimentaux et une étude en conditions naturelles ont mis en évidence le rôle majeur des co-infections immunomodulatrices dans la dynamique de l'infection par le HEV chez le porc, ces pathogènes intercurrents conduisant à une infection chronique par le HEV et à un risque augmenté de présence du virus dans le foie, le sang et les muscles des animaux abattus. Le développement d'un modèle intra-élevage, stochastique, individu-centré et multi-pathogènes, a permis de dégager des pistes de maîtrise à la fois zootechniques et sanitaires pour réduire la prévalence du virus en élevage. En complément, la conception d'un modèle inter-troupeaux a rendu possible l'analyse des facteurs de diffusion du virus dans un réseau d'élevages français. L'ensemble de ces mesures de gestion du HEV a été soumis à l'avis des organisations publiques et privées et des acteurs individuels de la filière porcine (éleveurs, conseillers, vétérinaires) par des approches de sciences humaines et sociales. Finalement, ce projet transversal et multi-disciplinaire a permis de définir des axes d'action tangibles et réalisables de gestion du HEV dans la filière porcine tout en apportant des contributions méthodologiques significatives en épidémiologie et en modélisation.

Title: Modelling the spread of hepatitis E virus in the pig production sector and evaluating strategies to mitigate the risk of human exposure

Keywords: hepatitis E virus, modelling, multi-scale model, multi-pathogen model, risk mitigation plan, public health, social sciences

Abstract: Hepatitis E virus (HEV) is a zoonotic pathogen whose main reservoir in industrialised countries is pigs. This research project combined epidemiological studies, mathematical modelling and social sciences to propose levers for reducing the risk of human exposure to HEV through the consumption of pork products. Two experimental trials and one study under natural conditions highlighted the major role of immunomodulating co-infections on the dynamics of HEV infection in pigs, as these intercurrent pathogens led to chronic HEV infection and an increased risk of the virus in the liver, blood and muscles of slaughtered animals. The development of a within-herd, stochastic, individual-based and multi-pathogen model has made it possible to identify both zootechnical and sanitary control measures to reduce the prevalence of the virus on farms. In addition, the design of a between-herd model has enabled to analyse the factors responsible for the spread of the virus in a network of French farms. All these HEV control measures have been submitted for the opinion of public and private organisations and individual players in the pig sector (farmers, farming advisors, veterinarians) through social science approaches. Finally, this transversal and multidisciplinary project made it possible to define tangible and achievable lines of action for the management of HEV in the pig sector while making significant methodological contributions in epidemiology and modelling.