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Lore Merdrignac

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**THÈSE DE DOCTORAT DE
SORBONNE UNIVERSITÉ**

Spécialité Epidémiologie

Ecole Doctorale Pierre Louis de Santé Publique à Paris :
Epidémiologie et Sciences de l'Information Biomédicale

Présentée par
Mme Lore MERDRIGNAC

Pour obtenir le grade de
DOCTEUR de SORBONNE UNIVERSITE

**Incidence de la coqueluche et efficacité post autorisation
de mise sur le marché de la vaccination contre la coqueluche,
chez le nouveau-né et le nourrisson âgé de moins d'un an,
en Europe**

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Articles

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Figure 1. Type of effects of vaccine interventions, adapted from Halloran et al.

List of abbreviations and acronyms

PV	Primary Vaccination
EU/EEA	European Union / European Economic Area
ECDC	European Centre for Disease Prevention and Control
PERTINENT	Pertussis in Infants European Network
VE	Vaccine Effectiveness
aP	acellular Pertussis vaccine
wP	whole-cell Pertussis vaccine
DAILYs	disability-adjusted life-years
TND	Test-Negative Design
PCR	Polymerase Chain reaction
DTaP	Diphtheria, Tetanus and acellular Pertussis vaccine
Tdap	Tetanus, diphtheria and acellular pertussis vaccine
RSV	Respiratory Syncytial Virus Infection
COVID-19	Coronavirus Disease of 2019
NPS	Nasopharyngeal Swab
NPA	Nasopharyngeal Aspirate
IgG	Immunoglobulin G
CDC	Centers for Disease Control and Prevention
ACIP	Advisory Committee on Immunization Practices
RCT	Randomised Clinical Trials
PEWS	Paediatric Early Warning Scores
ICU	Intensive Care Unit
WHO	World Health Organization
EUPert-LabNet / ERLNPert-Net	European Laboratory Network for Pertussis
EuPertStrain / EUPertGenomics	European network for pertussis strain characterisation
VC	Vaccination Coverage
EMA	European Medicines Agency

i. Résumé

Introduction

La coqueluche est une infection respiratoire causée par la bactérie *Bordetella pertussis*. C'est une maladie hautement contagieuse, particulièrement grave chez les nourrissons qui représentent une grande proportion des hospitalisations et décès [1]. A l'échelle mondiale, on estimait en 2014 que 160 700 décès étaient liés à la coqueluche chez les enfants âgés de moins de 5 ans, 53 % de ces décès survenant chez des nourrissons âgés de moins d'un an [2]. Malgré une couverture vaccinale élevée à l'échelle Européenne, supérieure à 90% pour les trois premières doses du nourrisson [3,4], la bactérie continue de circuler, à l'origine d'un important problème de santé publique. Le premier objectif de cette thèse est d'estimer en Europe, chez le nourrisson âgé de moins d'un an, l'incidence et la sévérité des hospitalisations pour coqueluche. Le second objectif est de mesurer l'efficacité de la primo-vaccination, en termes d'hospitalisations évitées pour coqueluche. Enfin, notre troisième objectif consiste à mesurer l'efficacité de la vaccination de la femme pendant la grossesse sur les hospitalisations pour coqueluche lors de ses premiers mois de vie et d'étudier sa potentielle interaction avec la primo-vaccination (PV) du nourrisson.

Méthodes

En 2015, le Centre Européen de prévention et contrôle des maladies (ECDC) lança le projet PERTINENT, « Pertussis in Infants European Network », un système de surveillance active de la coqueluche chez le nourrisson en milieu hospitalier, incluant sept sites d'étude dans six pays Européens. Ensemble, nous avons développé un protocole d'étude générique ainsi que des recommandations de bonnes pratiques détaillées à l'usage des laboratoires de microbiologie, qui ont été implémentés dans chacun des 41 hôpitaux participants. Nous avons inclus dans l'étude tout nourrisson se présentant à l'hôpital avec des signes évocateurs ou sur simple suspicion de coqueluche par le clinicien. Une aspiration et/ou écouvillonnage nasopharyngé ont été réalisés puis analysés par PCR et/ou culture.

Les mesures d'efficacité vaccinale (EV) reposaient sur un schéma d'étude cas-témoins de type « test-négatif » (TND). Les cas étaient les nourrissons positifs à *Bordetella pertussis*. Les témoins étaient ceux négatifs à toutes bactéries du genre *Bordetella*. Nous avons effectué une comparaison des rapports de cotes (OR, odds ratio) pour la vaccination entre les cas et les témoins. Nous avons utilisé une régression logistique pour estimer l'OR, incluant les sites d'étude comme effet fixe. Autant que la taille d'échantillon le permettait, nous avons ajusté sur la date de survenue des symptômes et l'âge du nourrisson et calculé l'EV ($1 - OR$) pour chacune des analyses.

Afin d'étudier la potentielle interaction de la vaccination pendant la grossesse avec la PV, nous avons effectué une analyse d'indicateurs basée sur quatre catégories : (a) les nourrissons dont la mère n'a pas été vaccinée pendant la grossesse et n'ayant pas reçu de PV (*catégorie de référence*) ; (b) les nourrissons ayant reçu au moins une dose de PV mais dont la mère n'a pas été vaccinée ; (c) les nourrissons dont la mère a été vaccinée pendant la grossesse mais n'ayant pas reçu de PV ; (d) les nourrissons dont la mère a été vaccinée pendant la grossesse et ayant reçu au moins une dose de PV. En prenant comme catégorie de référence les nourrissons dont la mère n'a pas été vaccinée pendant la grossesse et n'ayant pas reçu de PV (a), nous avons effectué une comparaison des rapports de cotes (OR) pour la vaccination entre chaque catégorie de vaccination (b), (c) et (d) et avons estimé l'EV correspondant à l'aide d'une régression logistique ajustée sur la date des symptômes et l'âge.

Résultats

De décembre 2015 à décembre 2019, nous avons recruté et testé pour la coqueluche 2346 nourrissons âgés de moins d'un an. Les résultats de laboratoires étaient disponibles pour 2313 (99%) d'entre eux. Nous avons exclu 36 nourrissons positifs à un autre genre de *Bordetella* : 3 infections à *Bordetella holmesii*, 20 infections à *Bordetella parapertussis*, 13 infections dont l'espèce du genre *Bordetella* est restée indéterminée. Au total, 557 nourrissons étaient positifs à *Bordetella pertussis* (24%) incluant une co-infection à *Bordetella parapertussis*. En 2016 et 2017, la plupart des cas ont été signalés au cours de l'été. Un cas de coqueluche sur quatre a été admis aux soins intensifs. La plus forte proportion de cas en soins intensifs concernait des nourrissons âgés de 0 à 3 mois. Cinq nourrissons sont décédés de coqueluche confirmée pendant la période d'étude, aucun n'était vacciné car trop jeunes pour la première dose de PV.

Parmi les 2346 nourrissons inclus dans l'étude PERTINENT, 1393 (59%) étaient dans le groupe d'âge éligible à la PV (2-11 mois). L'EV mesurée après au moins une dose était de 59% (Intervalle de Confiance à 95%, IC 95% : 36-73). L'EV après une dose seulement était de 56% (IC 95% : 28-73) chez les nourrissons âgés de 2-11 mois. Restreignant l'analyse chez les nourrissons éligibles uniquement à la première dose et donc âgés de 2-5 mois, l'EV après une dose était de 48% (IC 95% : 5-72). Après deux doses, l'EV mesurée était de 73% (IC 95% : 50-86) chez les nourrissons âgés de 2-11 mois. Restreignant l'analyse chez les nourrissons éligibles uniquement à la seconde dose et donc âgés de 3-10 mois, l'EV après deux doses était de 76% (IC 95% : 43-90).

Pour l'étude de l'efficacité de la vaccination de la femme pendant la grossesse sur les hospitalisations du nourrisson, quatre des six pays Européen participants au réseau PERTINENT recommandaient cette stratégie vaccinale pendant la période d'étude, représentant un échantillon de 829 nourrissons inclus. Parmi eux, 336 (41 %) étaient trop jeunes pour recevoir la première dose de PV (âgés de moins de 2

mois) et 493 (59 %) étaient éligibles à la PV (âgés de 2 à 11 mois). L'EV de la vaccination pendant la grossesse chez les nourrissons de moins de 2 mois, non éligibles à la PV, était comprise entre 75 % (IC 95 % : 35-91 %) et 88 % (IC 95 % : 57-96 %). Chez les nourrissons éligibles à la PV et âgés de 2-11 mois, l'EV de la vaccination pendant la grossesse combinée à au moins une dose de la PV, se situait entre 74 % (IC 95 % : 33-90) et 95 % (IC 95 % : 69-99). Dans ce même groupe d'âge, l'EV d'au moins une dose de PV (non-combinée à la vaccination pendant la grossesse) était comprise entre 68% [95%CI : 27-86] et 94% [95%CI : 59-99].

Discussion

Nos résultats suggèrent que les coqueluches les plus graves, et nécessitant un passage en soins intensifs, touchent principalement les nourrissons dans leurs premiers mois de vie, lorsqu'ils sont trop jeunes pour la PV. Une fois éligible à la PV, les résultats d'EV après au moins une dose, après une seule dose, ou encore après deux doses, sont satisfaisants et similaires à ceux déjà rapportés dans la littérature. Afin de palier au déficit de protection vaccinale lors des premiers mois de vie, nos résultats indiquent que la vaccination pendant la grossesse confère une bonne protection contre les coqueluches sévères. De plus, les EV après au moins une dose de PV semblent aussi bonnes que ce soit chez les nourrissons dont les mères ont été vaccinées pendant la grossesse ou non, suggérant ainsi l'absence d'une interaction de la vaccination maternelle sur l'efficacité des premières doses de PV. Cependant, ces résultats doivent être interprétés avec prudence en raison de la petite taille d'échantillon de chacune de nos analyses.

Conclusion

Le projet PERTINENT montre l'intérêt d'une surveillance active en Europe chez les nourrissons. Un tel réseau hospitalier Européen peut ainsi être le support de nombreuses études pour estimer le fardeau de la maladie ainsi que l'EV des diverses stratégies de vaccination actuellement en place dans chaque pays Européen. Il permet également un monitoring des cycles épidémiques coquelucheux et des espèces du genre *Bordetella* en circulation qui défient la forte couverture vaccinale et échappent pour une part au vaccin acellulaire actuellement utilisé. Le maintien mais surtout l'accroissement de ce réseau hospitalier sont essentiels pour obtenir une taille d'échantillon suffisante pour mesurer l'effet modificateur de la vaccination pendant la grossesse sur chacune des trois doses de la PV, sur l'EV de la stratégie du cocooning combinée ou non à la stratégie de la vaccination pendant la grossesse, sur l'EV de la primo-vaccination sur les autres espèces du genre *Bordetella* ou encore sur l'EV des potentiels nouveaux vaccins vivants atténués. Un tel réseau hospitalier pourrait préfigurer une plateforme Européenne pour la surveillance active de l'ensemble des maladies respiratoires préoccupantes.

ii. Summary

Title: Incidence of the disease and effectiveness of pertussis vaccination in infants aged less than one year in Europe.

Introduction

Pertussis is a respiratory infection caused by the bacterium *Bordetella pertussis*. It is a highly contagious disease that is particularly serious in infants, who account for a large proportion of hospitalisations and deaths due to pertussis [1]. Yeung et al. estimated 160,700 pertussis related deaths worldwide in children aged <5 years in 2014, 53% of these deaths occurring in infants aged <1 year [2]. Despite high vaccination coverage across Europe, with over 90% coverage for the first three doses in infants [3,4], the bacterium continues to circulate and remains a major public health concern. The first objective of this thesis is to estimate the incidence and severity of whooping cough in hospital in infants under one year of age in Europe. The second objective is to measure the effectiveness of primary vaccination against hospitalisation due to laboratory-confirmed pertussis. Finally, our third objective was to measure the effectiveness of vaccination during pregnancy against hospitalisation of infants in the first months of life, and to study its potential interaction with primary vaccination (PV) of infants.

Methods

In 2015, the European Centre for Disease Prevention and Control (ECDC) launched the PERTINENT project, "Pertussis in Infants European Network", a hospital-based active surveillance system for pertussis in infants, including seven study sites in six European countries. We developed a generic study protocol and laboratory guidelines. They were implemented in each of the 41 participating hospitals. We included in the study any infant presenting to hospital with pertussis-like symptoms or with suspicion of pertussis by the clinician. Nasopharyngeal aspirates and/or swabs were taken and analysed by PCR and/or culture.

For the vaccine effectiveness (VE) analyses, our protocol was based on a "test-negative" (TND) case-control study design. Cases were infants positive for *Bordetella pertussis*. Controls were infants who were negative for any *Bordetella* species. We then compared the odds of vaccination between cases and controls. We used logistic regression to model the odds ratio (OR), including study sites as a fixed effect. Where sample size permitted, we adjusted for symptom onset date and infant age and calculated VE (as $1 - OR$) for each analysis.

To investigate the potential interaction between vaccination during pregnancy and PV, we performed an indicator analysis based on four categories: (a) infants with no vaccination during pregnancy nor PV

(reference category); (b) infants with PV only (at least one dose); (c) infants with vaccination in pregnancy only; (d) infants with both vaccination in pregnancy and PV (at least one dose). Using infants with no vaccination in pregnancy nor PV (a) as reference category, we compared the odds of vaccination exposure in each category (b), (c) and (d) between cases and controls and estimated the corresponding EV using logistic regression adjusted for symptom date and age.

Results

From December 2015 to December 2019, we recruited and tested 2346 infants aged <1 year for pertussis. Of these, 2313 (99%) had laboratory results available. We excluded 36 infants positive for another *Bordetella* species: 3 *Bordetella holmesii* infections, 20 *Bordetella parapertussis* infections, 13 infections where the *Bordetella* species remained undetermined. A total of 557 infants were positive for *Bordetella pertussis* (24%) including one co-infection with *Bordetella parapertussis*. In 2016 and 2017, most cases were reported during the summer. One out of four pertussis cases was admitted to intensive care. The highest proportion of cases in intensive care involved infants aged 0-3 months. Five infants died during the study period, none of whom had been vaccinated because they were too young for the first dose of pertussis vaccine.

Of the 2346 infants included in the PERTINENT study, 1393 (59%) were in the age group eligible for PV and aged 2-11 months. Estimated VE after at least one dose was 59% (95% Confidence Interval, 95% CI: 36-73). VE after only one dose was 56% (95% CI: 28-73) in infants aged 2-11 months. Restricting the analysis to infants eligible for the first dose only and therefore aged 2-5 months, VE after one dose was 48% (95% CI: 5-72). After two doses, VE was 73% (95% CI: 50-86) in infants aged 2-11 months. Restricting the analysis to infants eligible only for the second dose and therefore aged 3-10 months, VE after two doses was 76% (95% CI: 43-90).

For the VE in pregnancy analysis, four of the six European countries participating in the PERTINENT network recommended this vaccination strategy during the study period, representing a sample of 829 infants included. Of these, 336 (41%) were too young to receive the first dose of PV (aged less than 2 months) and 493 (59%) were eligible for PV (aged between 2 and 11 months). VE in pregnancy in infants aged < 2 months, not eligible for PV, ranged from 75% (95% CI: 35-91%) to 88% (95% CI: 57-96%). Among PV-eligible infants aged 2-11 months, VE of vaccination during pregnancy combined with at least one dose of PV ranged from 74% (95% CI: 33-90%) to 95% (95% CI: 69-99%). In the same age group, VE of at least one dose of PV (not combined with vaccination during pregnancy) ranged from 68% [95%CI: 27-86] to 94% [95%CI: 59-99].

Discussion

Our results suggest that severe pertussis admitted to intensive care unit, mainly affects infants in their first months of life, when they are too young for PV. Once eligible for PV, VE results after at least one dose, after one dose, or even after two doses, are good and in line with the literature. In order to fill the immunisation gap of the first months of life, our results indicate that vaccination during pregnancy confers very good protection against severe pertussis, and does not seem to interact significantly with the first doses of PV. However, these results must be interpreted with caution due to the small sample size of each of our analyses.

Conclusion

The PERTINENT project emphasizes the importance of active surveillance of infants in Europe. A European hospital network of this kind can provide support for numerous studies addressing the burden of the disease and estimating VE of various vaccination strategies currently in place in each European country. It also allows to monitor pertussis epidemic cycles and circulating strains that defy high vaccination coverage and seem to escape the acellular vaccine currently in use. It is essential to maintain and, above all, expand this hospital network if we are to obtain a sufficient sample size to verify if vaccination during pregnancy modifies the VE of each of the three doses of pertussis vaccine, the VE of the cocooning strategy combined with the vaccination during pregnancy strategy, the VE of primary vaccination on other strains of *Bordetella*, and the VE of potential new live attenuated vaccines. Such an hospital network could represent a European platform for active surveillance of all respiratory diseases of concerns.

iii. Résumé substantiel

Introduction

La coqueluche est une infection respiratoire causée par la bactérie *Bordetella pertussis* et dont le niveau de transmission après exposition est des plus élevés, son ratio de reproduction de base (R_0) étant estimé entre 5 et 17 [1]. C'est une maladie d'évolution longue et responsable d'une toux quinteuse, répétée, prolongée (plusieurs semaines), épuisante et dont la reprise respiratoire est souvent difficile (« chant du coq »). Ces quintes peuvent provoquer des apnées, cyanoses, vomissements et autres complications. Elle est particulièrement grave, voire mortelle chez les nourrissons, chez qui la toux coquelucheuse caractéristique est parfois absente, conduisant ainsi à de nombreux sous-diagnostics [5]. Ce groupe d'âge représente une grande proportion des hospitalisations et décès dus à la coqueluche [1]. Malgré une couverture vaccinale élevée et supérieure à 90% à l'échelle Européenne pour les trois premières doses chez le nourrisson [3,4], la bactérie continue de circuler et une recrudescence est observée depuis plusieurs années aussi bien dans les pays développés qu'en voie de développement [6].

Vaccins contre la coqueluche

La primovaccination (PV) contre la coqueluche, administrée dès l'âge de deux mois selon les recommandations nationales, a pour objectif principal de réduire le risque de coqueluche grave chez le nourrisson, en raison de la forte létalité observée dans cette tranche d'âge. Deux types de vaccins contre la coqueluche sont disponibles : un vaccin à germes entiers et un vaccin acellulaire. Dans les années 90, la plupart des pays membres de l'Union Européenne (UE)/Espace Economique Européen (EEE) ont remplacé les vaccins à germes entiers par les vaccins acellulaires. Bien que ces derniers soient potentiellement moins efficaces dans la prévention et la transmission de l'infection, et associés à une plus faible durée de protection, ils entraînent un nombre moins important d'effets indésirables [7]. Ces vaccins sont administrés en association aux vaccins contre la diphtérie et le tétanos. La PV contenant les trois doses du vaccin DTCa (Diphtérie, Tétanos, Coqueluche acellulaire) ou DTCe (Diphtérie, Tétanos, Coqueluche à germes entiers) est habituellement réalisée entre l'âge de 2 et 12 mois. Un rappel est recommandé entre 11 et 24 mois, ainsi qu'une autre dose entre 3 et 6 ans. Les calendriers nationaux de vaccination de chaque pays de l'UE/EEE diffèrent cependant quant aux stratégies et délais d'administration de ces doses. Certains pays recommandent également des doses de rappel chez l'adolescent, la femme enceinte ou la femme venant d'accoucher, ainsi que chez les adultes en contact avec le nourrisson (stratégie dite du « cocooning »).

Épidémiologie de la coqueluche au sein de l'UE/EEE

Le nombre de cas de coqueluche a très fortement baissé dans chaque pays de l'UE/EEE depuis l'introduction des premiers vaccins à germes entiers dans les années 1950. Pour autant, la bactérie continue à circuler car le vaccin, tout comme la maladie, ne protège pas à vie. Au cours des 10 dernières années, la coqueluche est réapparue dans de nombreuses régions de l'UE/EEE, en dépit d'une couverture vaccinale forte et stable de la PV. Le nombre annuel de cas de coqueluche déclarés au Centre Européen de prévention et contrôle des maladies (ECDC) a fortement augmenté à partir de 2011, avec un pic de 42 500 cas atteint en 2012. Cette recrudescence de la coqueluche est observée chez l'adolescent et l'adulte, ainsi que chez l'enfant trop jeune pour être vacciné ou n'ayant pas encore terminé sa PV. Elle pourrait être expliquée par plusieurs facteurs : disponibilité de meilleures méthodes diagnostiques (introduction en 2012 d'une PCR en temps réel plus sensible que les précédents tests), sensibilisation accrue à la maladie ou véritable augmentation de l'incidence due au cycle épidémique naturel, au déclin de la protection induite par le vaccin ou à l'adaptabilité de la bactérie aux protections vaccinales actuelles [8,9].

Systèmes de surveillance au sein de l'UE/EEE

Les systèmes de surveillance de la coqueluche mis en place au sein des pays membres de l'UE/EEE sont hétérogènes : identification clinique et/ou microbiologique des cas, définitions de cas différentes d'un pays à un autre, qualité et complétude variables des données collectées, sensibilité des systèmes de surveillance [10]. En raison de ces limites, les données de surveillance collectées en routine ne permettent pas d'estimer l'incidence de la maladie de manière appropriée et représentative à l'échelle européenne, ni d'évaluer l'impact des différentes stratégies de vaccination.

En Novembre 2012, face à une épidémiologie changeante de la coqueluche en Europe, un groupe d'experts de l'ECDC concluait à la nécessité d'une étude exhaustive dans tous les pays membres [11]. Ainsi, le projet pilote PERTINENT (Pertussis in Infants European Network), financé par l'ECDC, était mis en place en Décembre 2015. En collaboration avec 11 instituts partenaires de ce réseau, nous avons rédigé un protocole d'étude générique pour estimer le fardeau de la maladie (en termes d'incidence et sévérité) et mesurer l'efficacité vaccinale (EV) contre la coqueluche du nourrisson hospitalisé, confirmée en laboratoire, suivant le schéma d'étude cas-témoins de type « test négatif » (TND) [12]. Ce protocole décrit les objectifs et les méthodes courantes devant être utilisées par les sites d'étude et inclut un plan d'analyse groupée. Les sites d'étude doivent préciser les spécificités qui leur sont propres dans les annexes relatives aux sites d'étude. Le protocole PERTINENT a été validé par les comités d'éthiques des hôpitaux participants. Nous avons ainsi mis en place une étude multicentrique

incluant 7 sites d'étude répartis dans 6 pays européens : France, Irlande, Italie, Norvège, République Tchèque, Espagne.

Objectifs

Ce projet doctoral repose principalement sur le réseau PERTINENT que nous avons coordonné tout au long du projet [13]. Le premier objectif de cette thèse était d'estimer en Europe, chez le nourrisson âgé de moins d'un an, l'incidence et la sévérité de la coqueluche confirmée en laboratoire à l'hôpital. Le second objectif était d'estimer l'efficacité de la PV contre l'hospitalisation avec une coqueluche confirmée en laboratoire. Enfin, notre dernier objectif consistait, si la taille de l'échantillon le permettait, d'estimer l'efficacité de la vaccination de la femme pendant la grossesse contre l'hospitalisation pour coqueluche du nourrisson. S'appuyant sur le réseau PERTINENT, nous avons également tâché de répondre à des questions supplémentaires, notamment celle de la potentielle interaction entre la vaccination pendant la grossesse et celle du nourrisson lors des premières dose de PV.

Méthodes

Lors de la mise en place de l'étude multicentrique PERTINENT, la coordination ciblait des sites d'études suffisamment importants pour permettre d'atteindre la taille d'échantillon requise et permettant d'assurer une représentation non biaisée à l'échelon européen. Les critères de choix des sites étaient ceux de la Commission Européenne relatifs aux projets multicentriques représentatifs. Les capacités financières du projet ont permis de sélectionner 7 sites d'études avec 41 hôpitaux répartis dans 6 pays européens. Chaque site d'étude devait respecter les exigences des comités d'éthique nationaux. Tous les participants ou tuteurs légaux devaient donner leur consentement éclairé. Les comités d'éthique nationaux précisaient si un consentement écrit ou oral est nécessaire.

Fin 2015 - début 2016, l'ensemble des sites d'études ont progressivement mis en place le protocole PERTINENT et la surveillance active a pu débuter dans les 41 hôpitaux participants. Au cours de l'année 2017, l'équipe italienne a progressivement perdu contact avec l'un des hôpitaux. En mai 2018, quatre des cinq hôpitaux norvégiens ont dû se retirer du projet PERTINENT en raison de l'expiration du cofinancement dont elle bénéficiait. En février 2019, le dernier hôpital du site d'étude norvégien a dû quitter l'étude en raison de l'absence de cas et d'une difficulté à poursuivre la surveillance. Ces problèmes rencontrés pendant la réalisation de l'étude multicentrique ont réduit le nombre d'hôpitaux participants de 41 à 35.

Incidence et sévérité de la maladie

La population de l'étude comprenait tous les nourrissons de moins d'un an, résidants dans la zone couverte par l'hôpital et susceptibles d'y être admis pour une coqueluche. Nous avons inclus tout nourrisson se présentant à l'hôpital avec des signes évocateurs ou sur simple suspicion de coqueluche par le clinicien. Pour chacun, une aspiration et/ou un écouvillonnage nasopharyngé ont été réalisés puis analysés par PCR et/ou culture. Un cas de coqueluche était défini comme un nourrisson de moins d'un an ayant consulté dans l'un des hôpitaux participants, testant positif à *Bordetella pertussis*.

Afin d'assurer une identification exacte des différentes espèces du genre *Bordetella*, les recommandations pour les laboratoires du projet PERTINENT incluent un algorithme de diagnostic pour la détection de l'ADN de *Bordetella*, comprenant une série de trois PCR : une PCR en temps réel triplex ciblant le gène IS481 (chez *Bordetella pertussis*, *holmesii* et certaines souches de *bronchiseptica*), pIS1001 (*Bordetella parapertussis*) et la RNase P comme contrôle interne humain ; suivi de deux tests singleplex de confirmation ciblant *ptxA-Pr* (*Bordetella pertussis*) et HIS1001 (*Bordetella holmesii*) [14].

Nous avons défini un cas de coqueluche sévère comme un nourrisson admis en soins intensifs et présentant une coqueluche confirmée en laboratoire.

À l'aide d'un questionnaire standardisé, l'ensemble des sites ont recueilli un set commun d'informations : données démographiques, épidémiologiques, cliniques, de laboratoire, statut vaccinal du nourrisson et des membres du foyer, facteurs de risque et de protection, ainsi que la source présumée de l'infection.

Ont été exclus de l'étude les nourrissons dont les parents n'ont pas souhaité participer, dont les données de laboratoires étaient manquantes, ainsi que les nourrissons testant positifs à une autre bactérie du genre *Bordetella*.

Efficacité de la primovaccination (PV)

Pour l'analyse de l'EV de la PV, nous avons restreint la population d'étude aux nourrissons éligibles à la PV, selon les spécificités nationales de chaque site d'étude. Notre protocole repose sur un schéma d'étude cas-témoins de type « test-négatif » (TND) [12] où les cas étaient les nourrissons dont la PCR ou la culture était positif à *Bordetella pertussis*, les témoins étaient ceux négatifs à toutes bactéries du genre *Bordetella*.

Nous avons distingué le niveau de vaccination contre la coqueluche des nourrissons comme suit :

- 4 doses du vaccin, s'il a reçu les 4 doses dont la dernière > 14 jours avant l'apparition des premiers symptômes ;

- 3 doses du vaccin, s'il a reçu les 3 doses > 14 jours avant l'apparition des premiers symptômes, ou si la 4ème dose a été reçue ≤ 14 jours avant ;
- 2 doses du vaccin, s'il a reçu 2 doses > 14 jours avant l'apparition des premiers symptômes, ou si la 3ème dose a été reçue ≤ 14 jours avant ;
- 1 dose du vaccin, s'il a reçu 1 dose > 14 jours avant l'apparition des premiers symptômes, ou si la 2ème dose a été reçue ≤ 14 jours avant.

Un nourrisson n'ayant reçu aucune dose ou une seule dose ≤ 14 jours avant l'apparition des premiers symptômes était considéré comme non vacciné.

Ont été exclus de l'analyse les nourrissons présentant une contre-indication à la vaccination, ne présentant pas les critères cliniques d'éligibilité, dont la date d'apparition des symptômes, la date de vaccination ou le statut vaccinal étaient manquants, ainsi que ceux dont l'échantillonnage nasopharyngé datait de plus de 4 semaines après l'apparition des symptômes.

Les caractéristiques du nourrisson susceptibles de modifier ou biaiser la mesure de l'EV ont été collectées via le questionnaire standardisé (type d'accouchement, âge gestationnel à la naissance, allaitement et durée de l'allaitement, prise en charge du nourrisson en crèche, mode de garde du nourrisson, nombre de frères et sœurs, statut vaccinal du père, de la mère et de la fratrie).

Les caractéristiques des cas et des témoins (âge, sexe, type d'échantillonnage et autres covariables) ont été comparées par le test du χ^2 , test exact de Fisher, t-test ou test de Wilcoxon-Mann-Whitney (selon la nature de la variable et de la taille de l'échantillon).

Afin d'estimer l'EV, nous avons ensuite comparé les cotes de la vaccination parmi les cas et témoins. Les tailles d'échantillon par site d'étude étant trop petites pour l'estimation de l'EV contrôlant l'ensemble des facteurs de confusion potentiels, une approche par analyse groupée a été utilisée. Ainsi, nous avons réalisé sur les données groupées une analyse multivariable par régression logistique pour modéliser le rapport de cotes (OR, odds ratio), incluant les sites d'étude comme effet fixe et ajustant sur les potentiels facteurs de confusion. Autant que la taille d'échantillon le permettait, nous avons ainsi pu ajuster sur la date de survenue des symptômes et l'âge du nourrisson. L'efficacité vaccinale a ensuite été calculée de la manière suivante : $EV = (1 - OR) * 100$. Un intervalle de confiance à 95 % a été calculé autour de l'estimation ponctuelle.

Efficacité de la vaccination pendant la grossesse chez le nourrisson <2 mois

Parmi les sept sites d'étude participant au réseau PERTINENT, cinq disposent de la recommandation de la vaccination pendant la grossesse à l'échelle nationale : la République Tchèque (depuis 2016), l'Irlande (depuis 2013), l'Italie (depuis 2017) et l'Espagne avec la Catalogne (depuis 2014) et Navarre (depuis 2015). L'objectif de cette vaccination étant de protéger les nourrissons dans leurs premières

semaines de vie avant l'administration de leur première dose de PV, nous nous sommes intéressés à l'EV de cette stratégie vaccinale chez les nourrissons <2 mois.

Pour cette sous-analyse, seuls ces cinq sites ont pu être inclus. Nous avons ensuite restreint la population d'étude à tous les nourrissons non éligibles à la PV (c'est-dire âgé <2 mois) dont la mère était éligible à la vaccination durant sa grossesse, résidaient dans la zone couverte par l'hôpital, et étaient susceptibles d'y être admis pour une coqueluche.

Ont été exclus les nourrissons dont le statut vaccinal de la mère était manquant, dont la vaccination de la mère a eu lieu ≤ 14 jours avant l'accouchement ou avant/après la grossesse, dont la mère présentait des contre-indications à la vaccination ou dont l'âge ne permettait pas une exposition suffisante (âgés de moins de 4 jours, i.e., la période d'incubation minimum pour la coqueluche).

Puis de la même manière que l'EV de la PV, nous avons calculé l'EV (1 - OR de la vaccination) sur les données groupées, et ajusté par site, date d'apparition des symptômes et groupes d'âge.

Vaccination pendant la grossesse combinée à la primovaccination du nourrisson

Parmi les cinq sites d'étude PERTINENT concernés par la vaccination pendant la grossesse, la population d'étude a été ici restreinte à tous les nourrissons éligibles à la PV (c'est-dire âgé <2 mois) dont la mère était éligible à la vaccination durant sa grossesse, résidant dans la zone couverte par l'hôpital, susceptibles d'y être admis pour une coqueluche.

Telle que pour l'EV pendant la grossesse, ont été exclus les nourrissons dont le statut vaccinal de la mère était manquant, dont la vaccination de la mère a eu lieu ≤ 14 jours avant l'accouchement ou avant/après la grossesse, dont la mère présentait des contre-indications à la vaccination. De plus, nous avons exclus les nourrissons dont le statut vaccinal de la PV était manquant ou présentant des contre-indications à la vaccination.

Puis, afin d'étudier la potentielle interaction entre la vaccination pendant la grossesse et la PV, nous avons effectué sur les données groupées une analyse par construction de variables indicatrices basées sur quatre catégories : (a) les nourrissons n'ayant aucune vaccination (i.e., dont la mère n'a pas été vaccinée pendant la grossesse et n'ayant pas reçu de PV) (*catégorie de référence*) ; (b) les nourrissons ayant uniquement reçu au moins une dose de PV (mais dont la mère n'a pas été vaccinée) ; (c) les nourrissons dont la mère a été vaccinée pendant la grossesse uniquement (mais n'ayant pas reçu de PV) ; (d) nourrissons ayant à la fois leur mère vaccinée pendant la grossesse et ayant reçu au moins une dose de PV. En prenant comme catégorie de référence les nourrissons dont la mère n'a pas été vaccinée pendant la grossesse et n'ayant pas reçu de PV (a), nous avons comparé les cotes de chaque catégorie d'exposition à la vaccination (b), (c) et (d) entre les cas et les témoins et avons estimé l'EV correspondant à l'aide d'une régression logistique ajustée sur la date des symptômes et l'âge.

Puissance de l'étude et taille d'échantillon

Pour toutes analyses d'EV, sous l'hypothèse d'une couverture vaccinale de 70% de la population d'intérêt, et d'une proportion de positivité à *Bordetella pertussis* de 20% chez les enfants présentant les critères cliniques et testés pour *Bordetella pertussis*, nous avons estimé que 186 cas et 745 témoins étaient nécessaires pour détecter une efficacité vaccinale de 75% avec une précision absolue de 10% pour chaque strate ou chaque analyse réalisée.

Résultats

Depuis le lancement du protocole PERTINENT dans le premier site d'étude en Décembre 2015, quatre années de données ont pu être collectées au sein d'une quarantaine d'hôpitaux européens. Le protocole générique a permis une homogénéisation de cette collecte, mais également des techniques de laboratoire, permettant ainsi une mise en commun des données des six pays participants.

Incidence et sévérité (données mises à jour en 2020 et donc différentes de l'article référencé)

De décembre 2015 à décembre 2019, 2346 nourrissons de moins de un an ont été recrutés et testés pour la coqueluche. Parmi eux, 2313 avaient des résultats de laboratoires disponibles (99%). Nous avons exclu 36 nourrissons positifs à une autre espèce du genre *Bordetella* : 3 infections à *Bordetella holmesii*, 20 infections à *Bordetella parapertussis*, 13 infections dont l'espèce de *Bordetella* est restée indéterminée. Un total de 557 nourrissons étaient positifs à *Bordetella pertussis* (24%) incluant une co-infection à *Bordetella parapertussis*.

Sur les 557 cas de coqueluche identifiés, 293 étaient des garçons (53%). L'âge médian à l'inclusion était de 2,6 mois (intervalle : 0-11,6 ; écart interquartile (EI) : 2,6). Le poids de naissance médian était de 3250g (intervalle : 601-4925; EI : 710) avec un âge gestationnel médian de 39 semaines (intervalle : 25-42 ; EI : 2).

Le groupe d'âge le plus impacté était les nourrissons de 0-2 mois (n=328) avec un pic durant le deuxième mois de vie (n=147) avant un déclin progressif par mois d'âge supplémentaire.

Trente-neuf nourrissons présentaient une coqueluche dite « atypique » (7%) avec 26 présentant une toux seulement, cinq une toux avec cyanose, deux une cyanose uniquement et six avaient une absence documentée de l'ensemble des symptômes typiques de la coqueluche. Enfin, sur les 39 coqueluches atypiques identifiées, six avaient au moins une donnée manquante parmi ces symptômes cliniques.

Sur la période d'étude, malgré un déclin notoire des cas à partir de 2017, la plupart ont été signalés au cours de l'été avec un pic en Aout 2016 (n=30), Juin 2017 (n=29), Aout 2018 (n=19) et Juin 2019 (n=10).

Les ratios de taux d'incidence entre 2018 et 2017 étaient de 1,43 en République tchèque ($p = 0,468$), 0,25 en Catalogne ($p = 0,002$), 0,71 en France ($p = 0,0335$), 0,14 en Irlande ($p = 0,002$), 0,63 en Italie ($p = 0,053$), 0,21 en Navarre ($p = 0,148$) et zéro en Norvège.

Cinq nourrissons sont décédés pendant la période d'étude. Ils étaient âgés de 2 à 10 semaines et aucun n'était vacciné car trop jeune pour la première dose de PV. Un cas de coqueluche sur quatre a été admis aux soins intensifs. Parmi les nourrissons âgés de 0-3 mois, 98 (33%) étaient admis en soins intensifs, contre 8 (8%) chez les nourrissons de 4-11 mois ($p < 0,001$).

Parmi les cas de coqueluche éligibles à la PV et âgés de 2-11 mois, huit des 36 nourrissons admis en soins intensifs (22%) et 82 des 192 nourrissons non admis en soins intensifs (43%) ont reçu au moins une dose de PV ($p = 0,025$). Aucun des nourrissons admis en soins intensifs et 40 des 192 nourrissons non admis en soins intensifs (21%) ont reçu deux à trois doses de PV ($p = 0,001$).

Efficacité de la primovaccination après au moins une dose

Parmi les 2346 nourrissons hospitalisés présentant des signes évocateurs de coqueluche et inclus dans l'étude PERTINENT, 1393 (59%) étaient dans le groupe d'âge éligible à la PV et âgés de 2-11 mois, incluant 259 cas et 746 témoins. Le nombre de cas ainsi que le ratio témoins/cas étaient hétérogènes par site, allant de 1 cas en Norvège à 102 cas en France, et d'un ratio de 248 témoins pour 1 cas en Norvège à 1,2 témoins pour 1 cas en République Tchèque.

L'âge médian était de 16 semaines pour les cas et de 19 semaines pour les témoins ($p < 0,001$). Le poids médian et l'âge gestationnel à la naissance étaient de 3235 g et 39 semaines pour les cas, 3113 g et 39 semaines pour les témoins.

Les cas étaient plus enclins que les témoins à présenter une apnée (49% des cas contre 22% des témoins, $p < 0,001$), une cyanose (48% des cas contre 17% des témoins, $p < 0,001$), une toux coquelucheuse ou paroxystique (53% des cas contre 13% des témoins, $p < 0,001$), ainsi que d'être cliniquement diagnostiqués par un médecin (80% des cas contre 29% des témoins, $p < 0,001$) ou de présenter un lien épidémiologique avec un cas confirmé (39% des cas contre 2% des témoins, $p < 0,001$). Aucune condition sous-jacente prévue par le protocole générique ne semble davantage présente chez les cas que les témoins.

Parmi les cas, 119 (46%) ont été vaccinés : 74 avec une dose, 37 avec deux doses, 8 avec trois doses. Parmi les témoins, 469 (63%) ont été vaccinés : 233 avec une dose, 206 avec deux doses, 30 avec trois doses. L'EV après au moins une dose de PV était de 59% (Intervalle de Confiance à 95%, IC 95% : 36-73).

Efficacité de la primovaccination après une seule dose

L'EV après une dose seulement était de 56% (IC 95% : 28-73) chez les nourrissons âgés de 2-11 mois. Cette analyse inclut les nourrissons ayant reçu leur première dose mais étant déjà éligibles à la deuxième ou même troisième dose et en retard sur leur calendrier vaccinal. En restreignant l'analyse aux nourrissons éligibles à la première dose uniquement et donc âgés de 2-5 mois, l'EV après une dose seulement était de 48% (IC 95% : 5-72).

Efficacité de la primovaccination après deux doses

Après deux doses, l'EV mesurée était de 73% (IC 95% : 50-86) chez les nourrissons âgés de 2-11 mois. De la même manière que pour l'EV après une dose, en restreignant l'analyse chez les nourrissons éligibles uniquement à la seconde dose et donc âgés de 3-10 mois, l'EV après deux doses était de 76% (IC 95% : 43-90).

Concernant l'efficacité de la primovaccination après trois doses, c'est-à-dire après un schéma complet, seuls 42 nourrissons étaient éligibles à la troisième dose. La taille de l'échantillon ne nous a pas permis de réaliser l'analyse correspondante.

Efficacité de la vaccination pendant la grossesse chez les nourrissons <2mois

Pour l'étude de l'efficacité de la vaccination de la femme pendant la grossesse contre l'hospitalisation du nourrisson, quatre des six pays Européen participants au réseau PERTINENT recommandaient cette stratégie vaccinale pendant la période d'étude, représentant un échantillon de 829 nourrissons hospitalisés avec des signes évocateurs de coqueluche et inclus dans l'étude. Parmi eux, 336 (41%) étaient trop jeunes pour recevoir la première dose de PV (âgés de moins de 2 mois) et 493 (59%) étaient éligibles à la PV (âgés de 2 à 11 mois).

Après exclusion des enfants dont le statut vaccinal de la mère était manquant, dont la mère a été vaccinée \leq 14 jours avant l'accouchement ou dont l'âge ne permet pas une exposition suffisante à la maladie, 75 cas de *B. pertussis* et 201 témoins étaient disponibles pour l'analyse de l'EV chez les nourrissons <2 mois.

Cas et témoins présentaient un âge à l'admission à l'hôpital similaire. L'âge gestationnel à la vaccination pendant la grossesse ainsi que l'âge gestationnel à la naissance étaient similaires entre cas et témoins, mais également le poids à la naissance et la proportion de nourrissons allaités. En revanche, les cas de coqueluche étaient plus enclins à être admis en soins intensifs que les témoins.

En termes de vaccination, les mères ayant reçu pendant la grossesse une dose du vaccin contre la coqueluche concernaient 9 nourrissons parmi les cas (12%) et 92 parmi les témoins (46%). L'EV du

vaccin reçu pendant la grossesse chez les nourrissons non-éligibles à la PV et âgés <2 mois était de 75% (IC 95% : 35-91%).

Afin de confirmer la robustesse de nos résultats, plusieurs analyses de sensibilité ont été réalisées. En effet, les espèces du genre *Bordetella* peuvent être isolées à partir d'écouvillons (NPS) ou d'aspirations (NPA) nasopharyngés. Cependant, un gain de 15 % dans le taux d'isolement peut être obtenu en utilisant des aspirations chez les nouveau-nés et les nourrissons [15]. De plus, deux sites d'études ont rencontré d'importantes difficultés à l'échelle nationale en terme d'adhésion au programme de vaccination pendant la grossesse [16–18] et aucun nourrisson inclus pendant l'étude n'avait de mère vaccinée. En conséquence, nous avons exclu l'ensemble des nourrissons ayant reçu un écouvillonnage nasopharyngé, afin de limiter l'inclusion de faux-négatifs. D'autre part, nous avons exclu les deux sites d'études n'ayant inclus aucun nourrisson exposé à la vaccination pendant la grossesse. L'EV de la vaccination pendant la grossesse chez les nourrissons de moins de 2 mois, non éligibles à la PV, était, selon l'analyse de sensibilité, alors comprise entre 75% (IC 95% : 35-91%) et 88% (IC 95% : 57-96%).

Efficacité de la vaccination pendant la grossesse combinée à la primovaccination

Au sein des cinq sites recommandant la vaccination pendant la grossesse, 493 nourrissons âgés de 2-11 mois et éligibles à la PV ont été hospitalisés avec des signes évocateurs de coqueluche. Après exclusion des nourrissons dont le statut vaccinal ou celui de leur mère était maquant, dont la mère a été vaccinée ≤14 jours avant l'accouchement, 123 cas de *Bordetella pertussis* et 253 témoins étaient disponibles pour l'analyse de l'EV du vaccin reçu pendant la grossesse, combiné à la PV du nourrisson éligible et âgé de 2 à 11 mois.

De la même manière que pour l'estimation de l'EV du vaccin pendant la grossesse chez les nourrissons <2 mois, nous avons réalisés différentes analyses de sensibilité excluant d'une part les nourrissons échantillonnés sur la base d'un écouvillon nasopharyngé, d'autre part les nourrissons issus des deux sites d'étude ne comptabilisant aucune mère vaccinée.

Chez les nourrissons éligibles à la PV et âgés de 2-11 mois, l'EV ajustée en fonction du site d'étude, de la date d'apparition des symptômes et du groupe d'âge se situait, selon l'analyse de sensibilité, entre 74 % (IC 95 % : 33-90) et 95 % (IC 95 % : 69-99) pour les nourrissons exposés à la fois à la vaccination pendant la grossesse et à au moins une dose de la PV. Dans ce même groupe d'âge, l'EV ajustée était comprise, selon l'analyse de sensibilité, entre 68% [95%CI : 27-86] et 94% [95%CI : 59-99] pour les nourrissons exposés à au moins une dose de PV (non-combinée à la vaccination pendant la grossesse).

Discussion

Nos résultats indiquent que, chez le nourrisson de <1 ans, environ une coqueluche confirmée à l'hôpital sur quatre nécessite un passage en soin intensif. De plus, ces coqueluches sévères touchent principalement les nourrissons dans leurs premiers mois de vie, lorsqu'ils sont trop jeunes pour la PV. Une fois éligible à la PV, notre analyse suggère que les cas vaccinés sont beaucoup moins enclins à développer une forme sévère. Lors de ces quatre années d'étude, nous avons comptabilisé cinq décès, soit environ 1% des cas, tous étant non-vaccinés.

Trente-neuf nourrissons présentaient une coqueluche atypique, en faveur de la nécessité de sensibiliser les cliniciens, mais également les parents, à la question de l'absence de toux coquelucheuse et de sous-diagnostic de la coqueluche dans ce groupe d'âge. Les systèmes de surveillance européens actuels pourraient ainsi potentiellement sous-estimer l'incidence de la coqueluche dans cette population. De plus, notre étude souligne que les cas de 0-3 mois étaient plus enclins à présenter une cyanose ou apnée, symptômes ne faisant pas partie des critères uniques suffisants dans la définition de cas de l'Organisation Mondiale de la Santé (OMS) ni du Centre de prévention et de contrôle des maladies américain (CDC).

En termes d'incidence, sur les quatre années de surveillance active 2016-2019, et malgré une première année d'implémentation progressive et une dernière année sujette à des problèmes financiers engendrant le retrait des hôpitaux norvégiens, nous avons observé une diminution du nombre de cas par habitants depuis 2017.

S'appuyant ensuite sur le schéma cas-témoin du TND, notre étude PERTINENT a permis l'estimation de différentes expositions vaccinales : la PV après au moins une dose, après une dose, après deux doses ; la vaccination pendant la grossesse chez les nourrissons non-vaccinés ; la combinaison de ces deux vaccinations chez les nourrissons éligibles.

D'après nos résultats, le vaccin réduit de moitié l'incidence de la coqueluche dès la première dose administrée. L'efficacité de la première dose est de 56% chez les nourrissons de 2 à 11 mois et celle de la deuxième dose de 73%, résultats similaires avec la littérature.

Afin de palier au déficit de protection vaccinale lors des premières semaines/mois de vie, nos résultats indiquent une efficacité de la vaccination pendant la grossesse estimée à entre 75% et 88%, même si la taille d'échantillon limite encore la précision de ces résultats.

Dans notre analyse d'indicateurs combinant la vaccination pendant la grossesse et la PV chez les nourrissons éligibles et âgés de 2 à 11 mois, nous avons également observé une bonne efficacité

vaccinale après au moins une dose de PV seule et après au moins une dose de PV chez les nourrissons dont la mère a été vaccinée pendant la grossesse.

Limites

Nos résultats doivent être interprétés avec prudence en raison de plusieurs limites.

En effet, pour chacune des analyses réalisées, la taille d'échantillon limitait fortement la précision de nos résultats et les stratifications possibles. En effet, l'immunogénicité des vaccins anticoquelucheux mesurée au cours des études cliniques avant mise sur le marché étant très élevée, le nombre potentiel de cas de coqueluche hospitalisée utilisable pour nos études spécifiques est très impacté. Seul un nombre d'hôpitaux et de laboratoires élevé permet d'assurer une taille d'échantillon suffisante pour une mesure précise de l'efficacité des différentes stratégies vaccinales.

De plus, les différents problèmes rencontrés par les sites pendant la réalisation de l'étude a réduit le nombre d'hôpitaux participants de 41 à 35. Le maintien d'un tel réseau dans un contexte financier difficile représente un véritable défi à l'échelle Européenne.

Lors du développement des recommandations pour les laboratoires ainsi qu'au cours des visites de sites, nous avons constaté une grande disparité dans les techniques de laboratoires utilisées : techniques d'échantillonnage, test laboratoire, type de PCR et capacité à identifier et distinguer chaque espèce de *Bordetella*. D'important efforts tout au long de l'étude ont permis une très nette amélioration et homogénéisation de ces méthodes. Un nombre non-négligeables d'infections à d'autre espèces du genre *Bordetella* a pu en effet être identifié.

L'ensemble des analyses présentées utilisent les données collectées en routine dans les hôpitaux et laboratoires de référence. Ces données comportent un nombre non négligeable de données manquantes. Cependant, la qualité des données recueillies et leur complétude se sont améliorées tout au long du projet, notamment au travers de formations continues du personnel en charge de la collecte, de différentes réunions techniques du réseau PERTINENT et de la poursuite d'un monitoring régulier.

Un des défis de cette étude est l'analyse groupée de données provenant de différents pays avec des politiques vaccinales et systèmes de soins différents, des couvertures vaccinales variables, etc. Pour diminuer l'hétérogénéité entre pays, les données étaient collectées en utilisant un protocole standard. En règle générale, l'hétérogénéité statistique entre les sites est calculée et, en fonction de l'hétérogénéité présente, les données groupées sont analysées en utilisant : un procédé dit « one stage » si l'hétérogénéité est faible ; un procédé dit « two stages » si l'EV varie selon les sites d'étude. Cette deuxième méthode correspond à une méta analyse. Cependant, dans notre étude PERTINENT, le nombre de cas par site était trop faible pour une telle analyse et ne permettaient pas le calcul de

l'EV par site contrôlant pour les facteurs de confusion nécessaires. Seule une analyse groupée dite « one stage » a pu être réalisée. Ces résultats doivent donc être interprétés avec prudence car l'analyse suppose non seulement que le véritable effet de l'exposition est le même pour tous les sites d'étude, mais aussi que l'association de toutes les covariables avec la survenue du critère de jugement est la même pour tous les sites. Si cette association est différente en fonction des sites, les interactions entre sites d'étude et covariables doivent être introduites.

Enfin, à notre connaissance, le schéma du « test-negative design » a été utilisé ici pour la première fois dans une étude hospitalière multi-centrique pour mesurer l'efficacité de la vaccination contre la coqueluche. La validation de cette méthode repose sur l'hypothèse que le risque de syndromes cliniques compatibles avec la coqueluche mais non confirmés au laboratoire est identique chez les enfants vaccinés et non vaccinés. En d'autres termes, ceci implique que la proportion de vaccinés parmi les enfants remplissant les critères d'inclusion mais testant négatifs est identique à la proportion d'enfant vaccinés (couverture vaccinale) dans la population d'enfants source des cas de coqueluche hospitalisés. Cette hypothèse devra être vérifiée à l'aide d'études de cohorte effectuées sur des bases de données électroniques disponibles au long cours.

Conclusion

Tenant compte des limites mentionnées ci-dessus, notre réseau hospitalier PERTINENT a permis de montrer que la coqueluche du nourrisson demeure une question de santé publique cruciale à l'échelle Européenne, notamment chez les nourrissons trop jeunes pour être éligibles à la PV.

La vaccination contre la coqueluche pendant la grossesse vise à pallier ce manque d'immunité des premières semaines de vie du nourrisson. Un nombre croissant de pays, notamment à l'échelle européenne, incluent progressivement cette nouvelle stratégie vaccinale (Belgique, Espagne, Irlande, Italie, Lettonie, Pays Bas, Portugal, République Tchèque, Slovaquie, Royaume Uni et la France depuis Avril 2022). Nos résultats sont en faveur de cette évolution de la politique vaccinale et suggèrent une EV élevée chez les nourrissons <2 mois.

A partir du troisième mois de vie, le nourrisson est éligible à la première dose de PV. Nos résultats indiquent une bonne EV dès la première dose. Cette EV semble renforcée après l'injection de la deuxième dose. De plus, que la mère ait été vaccinée ou non pendant la grossesse, l'EV après au moins une dose de PV est bonne. Cependant, la taille limitée de notre échantillon n'a pas permis une analyse stratifiée suffisamment précise pour déterminer si la vaccination pendant la grossesse modifie l'efficacité vaccinale après au moins une dose de PV. Elle n'a pas non plus permis de mesurer l'interaction entre les deux vaccinations. Par conséquent, même si nos résultats peuvent indiquer une efficacité vaccinale toute aussi bonne après au moins une dose de PV, quel que soit le statut vaccinal

de la mère, nous ne pouvons pas exclure une éventuelle atténuation immunologique de la vaccination maternelle sur la réponse immunitaire des nourrissons à leur primovaccination (i.e., « blunting effect »).

Le projet PERTINENT a montré l'importance de l'existence d'une surveillance active standardisée en Europe chez les nourrissons. Incluant six pays européens, il a permis de rassembler des données de qualité, comportant relativement peu de données manquantes, mais aussi de mettre en place une surveillance standardisée, utilisant des protocoles communs, des définitions de cas identiques et un algorithme commun de diagnostic de *Bordetella pertussis* à l'aide d'un nombre de PCR spécifique et similaire entre les sites d'étude.

Un tel réseau hospitalier Européen peut ainsi être le support de nombreuses études adressant le fardeau de la maladie ainsi que l'EV des diverses stratégies de vaccination actuellement en place dans chaque pays Européen. Il permet également un monitoring des cycles épidémiques coquelucheux et des espèces du genre *Bordetella* en circulation qui défient la forte couverture vaccinale et semblent échapper, du moins pour une part, au vaccin acellulaire actuellement utilisé. Le maintien mais surtout l'accroissement de ce réseau hospitalier sont essentiels pour obtenir une taille d'échantillon suffisante pour l'analyse de l'efficacité de la troisième dose de la PV, l'identification de la fenêtre optimale de vaccination de la mère (de 16 à 37 SA selon les pays), la durée de la protection du nourrisson conférée par la vaccination de la mère, l'effet modificateur la vaccination pendant la grossesse sur chacune des trois doses de la PV, l'EV de la stratégie du cocooning combinée ou non à la stratégie de la vaccination pendant la grossesse, l'EV de la PV sur les autres espèces du genre *Bordetella* ou encore de l'EV des potentiels nouveaux vaccins vivants atténués. Un tel réseau sentinelle hospitalier pourrait constituer une plateforme Européenne pour la surveillance active de l'ensemble des maladies respiratoires préoccupantes chez le nourrisson.

1. Introduction

Justification of the thesis

Pertussis, also known as whooping cough or even the 100-day cough, is a highly contagious acute respiratory infection caused by the bacterium *Bordetella pertussis*. It is an endemic disease with epidemic peaks every two to five years. The most severe symptoms occur in infants and young children whereas the disease is usually milder in adolescents and adults, who are a source of spread to infants and children. Deaths mostly occur in infants aged < 6 months, who are often too young to have completed the primary vaccination series (PV). The main objective of pertussis vaccination is to reduce the risk of severe pertussis in infants, due to the high morbidity in this age group [19]. Vaccines are not available before two months of age, and therefore infants must be protected indirectly. Despite high vaccination coverage, countries such as Australia, USA, and UK have experienced increase in pertussis activity over the last few decades. Clinical diagnosis is challenging, and under-diagnosis occurs, especially due to existence of atypical presentation, mostly in neonates and infants often misdiagnosed as bronchiolitis [20].

Even though the number of pertussis cases and deaths have fallen substantially since the introduction of the pertussis vaccines in the 1950's, *Bordetella pertussis* is still one of the leading causes of vaccine-preventable deaths and morbidity globally [2,21]. Worldwide, it has been estimated that there were 5.1 million pertussis cases and 85,900 estimated deaths in infants aged less than one year in 2014 [2]. After a dramatic decline of the reported incidence of pertussis over the last 50 years following the introduction of pertussis vaccines into national immunization programmes, reported pertussis incidence has increased markedly in almost all EU/EEA Member States, the USA and Canada, with a marked upsurge since 2012. This increase in reported incidence has occurred despite high and stable vaccination coverage (> 90% coverage of the first three doses in the European Region over the last two decades [4]) and has been observed in all age groups including adolescents and adults, and notably among infants <1 year of age.

While all European countries have at least four doses of pertussis-containing vaccines in their childhood vaccination schedules (PV with three doses plus one booster dose), they all have different immunisation strategies and schedules. The United Kingdom was the first country in Europe to start a maternal programme in 2012 to respond to an outbreak that led to an increase in infant deaths. Since then, an increasing number of EU/EEA countries introduced vaccination in pregnancy: Belgium, Czech Republic, France, Ireland, Italy, the Netherlands, Portugal, Slovenia, Spain. Other countries completed the pertussis primary series with the so-called "cocoon strategy", such as France in 2004. This vaccination strategy aims to protect infants too young to be vaccinated by providing them indirect protection through vaccination of household and close contacts, including post-partum women [22].

Surveillance systems in EU/EEA Member States are also heterogeneous: some systems rely only on identification of clinical cases, while others have strong laboratory components [10]. Even among the latter, differences in the use of laboratory tests and methods are responsible for wide variation in the number of tests performed and the number of cases detected. The European Centre for Disease Prevention and Control (ECDC) sponsored European networks such as EuPertStrain and EUPertLabNet that developed and promoted laboratory recommendations for *Bordetella pertussis* across EU/EEA countries. However, not all countries have the capacity to implement these recommendations, mainly due to financial constraints.

Different case definitions are in use in European countries and the completeness of the data collected is low for important variables such as vaccination status [23]. In addition, most of the case definitions in use do not take into account that whooping cough can be caused not only by *Bordetella pertussis* but also 3 other *Bordetella* species – *parapertussis*, *holmesii*, and *bronchiseptica*, which most often cause less severe disease in adults as well as in infants.

Due to all these limitations, the routine surveillance data collected does not allow for an assessment of the real burden of disease in the population and, most importantly, of the impact of different immunisation strategies on the disease epidemiology in Europe.

The PERTINENT Network

In November 2012, in response to the changing epidemiology of pertussis in Europe, a panel of experts from the ECDC concluded: “ECDC should implement a comprehensive study across all Member States to examine the epidemiology of pertussis among infants <1 year of age in order to obtain more complete epidemiological, clinical and laboratory data with which to characterise the burden of disease, diagnosis and control of whooping cough. To optimise comparison between countries, hospital admission data might be useful in this respect” [11]. Consequently, the pilot project PERTINENT, “Pertussis in Infants European Network”, funded by ECDC, was established in December 2015. Our group proposed a hospital-based pilot project with the aim of assessing the burden of whooping cough but also the effectiveness of pertussis vaccines in infants. It is the first EU/EEA collaboration allowing for large, independent and multi-country pertussis vaccine effectiveness (VE) studies.

In order to carry out this EU/EEA multicentre study, it was necessary to identify a sufficient number of study sites to achieve the required sample size and ensure unbiased representation at European level. The project's financial resources enabled to select seven study sites in six European countries (France, Spain, Ireland, Norway, the Czech Republic and Italy). The selection criteria corresponded to the European Commission's criteria for representative multicentre projects. At the beginning of the project, study sites were able to enrol 41 participating hospitals to implement this enhanced active

surveillance for pertussis in infants (Table 1). We developed a generic protocol [24], laboratory guidelines [14] and organised sites visits to ensure harmonisation of hospital practices and to allow pooling of sites' data. To maximise the sensitivity of the surveillance, we raised hospital physicians' awareness of pertussis clinical presentation and asked them to test all infants presenting at hospital with pertussis-like symptoms, even if some 'typical' symptoms were missing. 'Typical' pertussis presentation was defined either by a presence of apnoea; or by a cough associated with at least one of paroxysms, whoop or post-tussive vomiting. When physicians suspected pertussis even though some typical symptoms were missing, pertussis was considered as 'atypical'.

Regarding pertussis vaccination, all sites used the acellular pertussis (aP) vaccines for the primary series in infants. Estimated vaccination coverage for the first three doses exceeded >90% in all participating study sites [3]. However, both national vaccine recommendations and primary schedules varied across sites and over time (also during the study period) (Table 1). Sites recommended the first three doses of PV at either at 2, 4, 6 months; 2, 4, 11 months; 3, 5, 11 months or 3, 5, 12 months. Regarding vaccination in adults, four of the six European countries participating in PERTINENT recommended pertussis vaccination in pregnancy. While several studies report a good effectiveness of this passive immunisation strategy to fill the susceptibility gap during the first months of life [25,26], several immunological studies have raised concerns about a potential "blunting effect" from two months of age onwards, where maternal antibodies could interact with the infant's immune response to PV and thus reduce their immune response [27].

To estimate pertussis VE in our PERTINENT study, we used the test-negative design approach (TND). This approach has been widely used to measure influenza VE and has been showed to be very cost-effective for that matter [12]. TND studies consist in comparing the odds of vaccination between patients testing positive and patients testing negative for the given disease. In PERTINENT, we included all infants aged <1 year presenting with pertussis-like symptoms and tested them for *Bordetella pertussis* pathogen. Cases were infants testing positive for *Bordetella pertussis* by polymerase chain reaction (PCR) or culture (included in the disease burden analysis). Controls were those testing negative for all *Bordetella* species. Restricting the analyses to the study population of interest, we could use the TND setting in PERTINENT as a foundation for several VE analysis: investigating the effectiveness of PV with at least one dose; dose-specific VE for the first and second dose of primary schedule; effectiveness of vaccine in pregnancy in infants too young for vaccination and aged <2 months; effectiveness of vaccine in pregnancy together with at least one dose of PV in infants' vaccine-eligible and aged 2-11 months.

Objectives

This doctoral project is based on the ECDC-funded PERTINENT network, which we coordinated throughout the project on behalf of the Epiconcept group. The first objective of this thesis was to estimate the incidence and severity of whooping cough in hospital in infants under one year of age in Europe. The second objective was to measure the effectiveness of PV against hospitalisation due to laboratory-confirmed pertussis. Finally, if the sample size allowed, our third objective was to measure the effectiveness of vaccination during pregnancy against hospitalisation of infants in the first months of life, and to explore the potential interaction (i.e., “blunting effect”) between mother and infant vaccination.

Thesis overview

This thesis is divided into three chapters. **Chapter 1** presents the first article investigating the first objective of this thesis published in *Eurosurveillance*: estimating the incidence and severity of laboratory-confirmed *Bordetella pertussis* cases in hospital in infants aged less than one year in Europe. We have described the PERTINENT project and the implementation of the active hospital-based surveillance Network across the seven study sites located in the six participating EU/EEA countries. We will present the preliminary results of the pilot surveillance in terms of severity and disease incidence.

Chapter 2 includes the second article showing the results of the second objective of this thesis estimating the effectiveness of pertussis PV in infants. We present PERTINENT VE point estimates for three different vaccine exposures: (a) after at least one dose of aP vaccine; (b) after one dose; (c) after two doses of aP vaccines in infants eligible for each corresponding vaccine exposure.

Chapter 3 focusses on infants non eligible for PV yet and aged less than 2 months. We present PERTINENT VE point estimates of vaccination in pregnancy in infants aged <2 months. In this third article of the thesis published in *Vaccine*, we also investigate the effect of vaccination in pregnancy combined with at least one dose of PV in infants eligible for vaccination and aged 2-11 months.

Table.1 Characteristics of PERTINENT study sites, vaccination recommendations, introduction year and vaccine coverage at the start of the study, PERTINENT, 1st December 2015 - 31st December 2019

Study sites		Czech Republic	France	Ireland	Italy	Spain, Catalonia	Spain, Navarra	Norway
Vaccination recommendations								
Primary schedule	Year of introduction	2018 ^a	2013	1995	1995	2016 ^b	2016 ^b	1998
	First dose	3-month-old	2-month-old	2-month-old	3-month-old	2-month-old	2-month-old	3-month-old
	Second dose	5-month-old	4-month-old	4-month-old	5-month-old	4-month-old	4-month-old	5-month-old
	Third dose	11–13-month-old	11-month-old	6-month-old	11-month-old	11-month-old	11-month-old	12-month-old
	VC % in 2016	94.4%	95.8% at 24 months	90% at 24 months	96.8% at 24 months	94.0%	96.8%	96% at 24 months
Pregnancy	Year of introduction	2016	No	2013	2017	2014	2015	No
	VC %	<i>Not determined</i>	NA	49.9% in 2017-2018	<i>Not determined</i>	79% in 2017	89% in 2017	NA
Cocooning	Year of introduction	No	2004	2013	No	No	No	No
	VC %	NA	<50% in 2004-2015	<i>Not determined</i>	NA	NA	NA	NA
Booster doses	Schedule	5-6 yo 10-11 yo	6 yo 11-13 yo	4-5 yo 12-13 yo	6 yo 12-18 yo	6 yo	6 yo	7 yo 15 yo
Adults	Schedule	1 dose in life	1 dose every 10 years	No	1 dose every 10 years	No	No	1 dose every 10 years
Participating hospitals and catchment population estimation								
Number of hospitals participating in PERTINENT		6	21	2	2	1	4	5 (2016–2018/05) 1 (from 2018/05)
Method used to estimate hospital catchment population		National census	National census × estimation of PERTINENT hospital coverage	National census	Regional census	National census × estimation of PERTINENT hospital coverage	National census	Regional census prorata temporis

NA: not applicable; PERTINENT: Pertussis in Infants European Network; VC: Vaccine Coverage.

^a Before 2018: doses at 2, 3, 4 and 10 months.

^b Before 2016: doses at 2, 4 and 6 months.

2. State of the art

2.1. History of the disease

Bordetella pertussis is the causative agent of whooping cough. The first documented outbreak of pertussis was recorded by Guillaume De Baillou in Paris in the summer on 1578 and resulted in many deaths among infants and young children. Jules Bordet and Octave Gengou from the Pasteur Institute in Brussels were the first microbiologists to grow the bacterium in 1906, later categorised into a new genus called *Bordetella* in honour of Bordet. In the 1920s, *Bordetella pertussis* was found to be the causative agent of whooping cough, but three other organisms from the genus *Bordetella* were later found to be also able to cause pertussis-like symptoms in humans, usually less severe (*Bordetella parapertussis*, *Bordetella holmesii* and *Bordetella bronchiseptica*). Vaccination of children with whole-cell pertussis (wP) vaccines started in late 1950's in Europe and was followed by a substantial decrease of reported cases [6]. Unfortunately, several studies linked the use of wP vaccines prepared directly from *Bordetella pertussis* pathogen, with serious adverse reactions. As an example, a population-based studies in Sweden, England, and Wales reported very high rates for vaccine encephalopathy (with incidence of neurological reactions increasing from 1:6,000 children in the pre-vaccine area to 1:3,600 vaccinated children in 1959 to 1965) [28,29]. Therefore, vaccination coverage fell in many countries in the 1970's which led to a progressive resurgence of pertussis cases. Nevertheless, it is crucial to note that more recent studies concluded that the role of wP vaccines as a prime or concomitant factor in the aetiology of these serious acute neurological illnesses cannot be determined in any individual case, and encouraged the use of the vaccine [30]. In the 1980's, acellular pertussis (aP) vaccines were developed and extensively tested. Many countries progressively replaced wP vaccine by the safer aP vaccine in the 1990's. Introduction of aP vaccines also allowed the vaccination of older children, adolescents and adults which was not possible with the previous wP vaccines side effects [31]. Despite widespread routine administration of aP vaccines (combined with diphtheria and tetanus toxoids, DTaP vaccine), pertussis is still one of the leading causes of vaccine-preventable deaths and morbidity globally with more than 150,000 cases of pertussis reported in 2018 according to the World Health Organisation (WHO) [32]. Pertussis was also ranked as the ninth causes of disability-adjusted life-years (DALYs) in children younger than 10 years in 2019 [33].

2.2. Epidemiology

Despite the introduction of pertussis vaccination programmes and high vaccination coverage (VC) in Europe, pertussis remains an endemic disease in all countries with epidemic peaks every 2-5 years.

The last major peak incidence year in Europe occurred in 2012 with 42,500 reported cases, impacting 19 of 28 countries with nationwide epidemics of pertussis of different magnitude [34]. Since then, the overall notification rate remained higher than this pre-2012-epidemic levels according to ECDC, until a recent sharp decline from 2020 due to the COVID-19 pandemic and its masking practices [23,35]. In term of seasonality, despite the absence of strong pattern in the disease incidence trend, several studies suggested an increase in pertussis notification rates in the summer [13,36,37].

The disease is highly contagious and transmitted person to person by air droplets. Pertussis basic reproduction number (R_0), i.e., the average number of cases that would be expected from a single case of pertussis in a completely susceptible population, is estimated between 5 to 17 cases [1]. As a comparison, for measles, often cited as the most contagious infectious disease, R_0 is ranging from 12 to 18. In susceptible contacts, the *Bordetella pertussis* transmission rate is close to 90% of household contacts and 50% to 80% of schoolroom contacts of a case [38]. Additionally, silent transmission can occur from asymptomatic or mild infections, likely to represent an important reservoir for community transmission of infection [39].

Pertussis affects people of all ages, but is particularly severe in infants who experience the most complications and account for almost all pertussis hospitalisations and deaths. One large study of pertussis in infants and children in Germany in 1997 found that the overall rate of major complications was 6%, but up to 24% among infants aged less than 6 months [40]. More recently, among pertussis cases reported to the ECDC in 2018, infants were the most affected age group, with the highest rate of 44.4 per 100 000 population and 64% of the reported cases being hospitalised [23].

Pertussis typical clinical presentation consists of long-lasting episodes of coughing, marked by paroxysms of repeated coughs that end with an inspiratory “whoop” and often post-tussive vomiting. However, in neonatal infection, the cough with the characteristic whoop is much less common and this atypical clinical presentation similar to several other respiratory diseases may often lead to underdiagnosis in that age group [41]. Adolescents (≥ 11 years of age) and adults (≥ 18 years of age) can develop a mild, also often undiagnosed, form of the disease representing an important reservoir of transmission for infants. The other *Bordetella* infections cause usually less severe pertussis-like symptoms and are frequently misclassified as pertussis because routine diagnosis tests are not *Bordetella* species-specific [14].

2.3. Laboratory diagnosis

Bordetella pathogens can be detected by culture of the organism or DNA detection using PCR based on nasopharyngeal specimens obtained early in the illness and prior to antibiotic therapy. While culture remains the gold standard for laboratory diagnosis, its sensitivity is very low. Diagnosis by PCR

has the advantage that the organisms do not have to be viable to be detected. Therefore, *Bordetella pertussis* could still be detected by PCR at a more advanced stage of the disease or after antibiotic treatment. For routine diagnosis at hospital level, multiplex PCR assays are available and allow to test for a variety of organisms (e.g., *Bordetella pertussis*, RSV, influenza, etc.) from a single nasopharyngeal specimen. However, they usually include only one PCR target for *Bordetella pertussis* (i.e., IS481) which does not allow to distinguish between all *Bordetella* species. A series of three PCRs is recommended to ensure an accurate identification of the species, targeting successively IS481, pIS1001, hIS1001 and ptxA-Pr or IS1002 [1,14].

In terms of specimen collection, *Bordetella pertussis* can be isolated from a nasopharyngeal swab (NPS) or nasopharyngeal aspirate (NPA). However, it has previously been shown that a 15% gain in isolation rate can be obtained using NPA compared to NPS in neonates and infants [15].

2.4. Vaccination

Even though the wP vaccine is still the predominant vaccine used worldwide (64% of countries), all EU/EEA countries except Poland are now using the aP vaccine across all age groups [1,42].

The primary goal of all pertussis vaccination programs is to reduce the risk of severe pertussis in infants and young children, due to the high morbidity and mortality caused by the disease in this age group [19]. Despite a coverage > 90% for the first three doses of primary vaccination (PV) in infants over the last two decades [4], there are still significant challenges to controlling pertussis in Europe. There is a need to protect infants from the pertussis reservoir that represent other age groups.

2.4.1. Active immunisation

2.4.1.1. Primary schedule in infants

Across EU/EEA countries, PV starts as early as six weeks of age as part of a combined vaccine, most often the hexavalent vaccine: hexavalent combined diphtheria (D), tetanus toxoids (T), acellular pertussis (aP), inactivated poliovirus (IPV), Haemophilus influenzae type-b (Hib) conjugate and hepatitis B (HepB). However, the primary series administered by the age of 12 months vary a lot between countries but can be grouped as follows [42]:

- **“3p+0” schedule** including three primary doses at 2, 4 and 6 months of age in Ireland. There are no booster doses given in the second year of life but rather given at four to five years of age.
- **“2p+1” schedule** including two primary doses and a booster dose, with doses administered either at:

- 3, 5 and 11 or 12 months (booster) in Austria, Czech Republic, Denmark, Finland, Italy, Denmark, Iceland, Liechtenstein, Netherlands, Norway, Slovakia, Slovenia and Sweden;
 - or at 2, 4 and 11- 12 months (booster) in France, Germany, Romania, Spain.
- **“3p+1” schedule** including three primary doses given in the first year of life, starting as early as 2 months, with a booster in the second year of life (in Belgium, Bulgaria, Croatia, Cyprus, Estonia, Germany, Greece, Hungary, Latvia, Lithuania, Luxembourg, Malta, Portugal and in Poland but with wP vaccines).

According to the recent thorough review carried by Decker and Edwards (2021), at least one dose of aP vaccine provides important protection against death and hospitalisation related to pertussis [38]. But the best protection is achieved after three primary doses. The “2p+1” schedule at 3, 5 and 12 months would provide little less protection between the second and third doses but better protection afterwards [1]. Then, in older children, most countries in Western Europe recommend a booster at 4 to 7 years of age, whereas most in Eastern Europe do not.

2.4.1.2. Adolescent-adult vaccination

Contrary to popular belief of the pre-pertussis vaccine era, pertussis is not solely a childhood disease and its infection does not protect for life. Following aP vaccines rollout in the 1990’s, the age distribution of pertussis changed, affecting progressively older age groups. Thanks to their good safety and immunogenicity but also to the improved tolerability of aP vaccines compared with wP vaccines, pertussis vaccination was extended to adolescents and adults with the combined tetanus toxoid, diphtheria and acellular pertussis vaccine (Tdap, with lower antigen content for the latter two components). At EU/EEA level, national vaccination recommendations for adolescents and adults are also extremely heterogeneous (e.g., 1 dose in adolescents, then every 10 years between 18 to 60 years and finally every 5 years from 65 years onwards; 1 dose in adolescents and 1 dose in adulthood; 1 dose in adolescents only; 1 dose in adults only; no vaccination either in adolescent nor adults, etc. See also the PERTINENT countries recommendations in [Table 1](#)) [42].

Even though it is important to protect older age groups from the disease, pertussis vaccination in adolescents and adults also plays an important role with an indirect protection of infants and neonates (see definition of *indirect effect* in [Figure 1](#)). In 2007, Wendelboe et al. identified the most probable source of infection being the parents for 55% of infants followed by the siblings for 16% of infants [43]. For this reason, they estimated that vaccination of adolescents and adults in close contact with young infants may substantially reduce pertussis disease in infants. More recently, additional studies observed a shift of the source of transmission from the mother (21%) to the siblings (36%), older age groups remaining an important pertussis reservoir for infants [44].

2.4.2. Immunisation gap

Despite an effective and well-accepted PV with high coverages in infants in Europe (>90% for the three doses), pertussis remains an endemic disease with the highest notification rates reported to ECDC in 2018 being among infants aged less than one year (44.4 per 100 000 population). Out of them, 41% were hospitalised for severe pertussis and 58% were unvaccinated, having not received yet the first dose of PV [23]. This reflects the need to overcome the immunisation gap in infants too young for PV, harbouring the highest risk of severe pertussis and related death.

2.4.2.1. Active immunisation: Vaccination at birth?

In the early ages, vaccination at birth has been considered as a strategy worth pursuing. Even though several studies exist, this strategy has never been approved due to the lack of data on the safety of the vaccines in newborns [19]. Some studies as early as in 1960's would suggest a low protection, some others would provide conflicting results [45,46]. Several schedules were investigated such as a first injection at birth, a second at 4 weeks of age and then the conventional primary series, resulting in not less than four doses given in the first 4 months of life. Additionally, a possible blunting effect of these early doses on infants' PV was also mentioned [45]. Therefore, none of these rather aggressive schedules have ever led to the introduction of vaccination at birth to fill the immunisation gap before 2 months of age.

2.4.2.2. Indirect protection: Cocooning strategy

"Cocooning" is a strategy consisting in vaccinating all individuals in close contact with infants too young to be vaccinated with PV (mother, father, siblings, grandparents, caregiver, etc.). It is based on the potential *indirect effect* of the vaccination of all these individuals who are likely to be the source of infection for the infant by reducing the circulation of the pathogen among their close relatives [43,44]. It has been recommended in some developed countries such as France or Germany and was shown to be effective when well implemented with high coverage among siblings achieved in a timely manner. However, implementing an effective cocooning strategy with high coverage has proved challenging even in countries with extensive cocooning experience [47]. A modelling study in 2008 showed that a vaccination coverage of at least 65% of close contacts of new-borns was needed to control the disease in infants [48]. In the context of a low pertussis incidence, Skowronsky et al. showed in 2011 that the number needed to vaccinate was very high to prevent severe pertussis outcome in early infancy with at least 1 million vaccination needed to prevent 1 infant death, approximately 100,000 for ICU admission and 10,000 for hospitalisation [49]. This immunisation strategy is resource intensive, especially when disease incidence is low between epidemic peaks.

2.4.2.3. *Passive immunisation: Vaccination in pregnancy*

Whereas the cocooning strategy requires multiple doses for parents and close relatives, maternal vaccination requires a unique dose during pregnancy to protect the newborn. And the challenges in implementing the cocooning strategy generated increased interest in this maternal vaccination. In 2013, Terranella et al. compared these two vaccination strategies and estimated that the overall impact and cost-effectiveness of high vaccination coverage for the cocoon strategy was lower than maternal immunisation [50]. They found that vaccination in pregnancy would be more effective than the cocooning strategy with a reduction by 33% of annual infant pertussis incidence compared to 20%, by 38% of hospitalisations compared to 19%, and by 49% of deaths compared to 16% for cocooning [50].

History

The potential of pertussis vaccination in pregnancy was already discussed in the pre-vaccine era. Several studies from the 1940's and 1950's suggested that, when mothers had received wP vaccine during pregnancy, a higher level of pertussis antibodies in newborns was found, protecting infants from the disease in early life [51,52]. Observing a steady increase in pertussis deaths among infants aged less than 3 months in the US (49 deaths, 84 deaths and 175 deaths during the years 1980-1989, 1990-1999 and 2000-2009 respectively), the Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP) recommended pertussis vaccination in 2011 to all pregnant women during the third trimester of every pregnancy in the United States (US) [38]. Shortly after, in response to an increase of hospitalisations and deaths in unvaccinated infants aged less than 3 months, the United Kingdom (UK) also introduced vaccination in pregnancy in 2012, as the first EU/EEA countries. Since then, an increasing number of European countries are now introducing this immunisation strategy among their national recommendations: Belgium, Czech Republic, Germany, Ireland, Italy, Latvia, Portugal, Slovenia, Spain, Switzerland, the UK and more recently Netherlands in 2019 and France in 2022 [23,38,42].

Concept

This vaccination strategy relies on the transplacental transfer of antibodies from the mother to the foetus. Between 17 and 22 weeks of gestation, the level of maternal antibody for the foetus is approximately 10% of the maternal concentration. Around 28-32 weeks, this fraction rises to 50%, and then the majority of IgG is transferred to the foetus after 36 weeks [53]. At term, transplacental transfer of maternal antibodies is significantly higher than the maternal concentration with about 120 to 130% of the maternal antibody concentrations [54]. Even though there is no correlation between the level of pertussis antibodies and the level of protection against the disease, additional studies soon

suggested that a high level of vaccine-induced IgG in newborns is associated with protection against the disease in children [55]. However, such an association does not imply causation. Additionally, studies such as Taton et. al. suggested that pregnancy does not affect the quality of IgG and memory B cell responses to pertussis immunisation and that antibodies IgG are effectively transferred across the placenta [56].

Effectiveness

One year after the programme was introduced in the UK, Dabrera et al. observed a decrease in pertussis mortality [57]. In the following years, the effectiveness of the vaccine in pregnancy against pertussis in infants remained stable around >90% [58]. Since then, numerous studies suggested that Tdap in pregnancy was safe and provided a good passive protection to the infants. A systematic review carried out in 2020 suggested that vaccination in pregnancy would prevent about 70-90% of pertussis disease and up to 90.5% of pertussis hospitalisation in infants aged less than 3 months [26].

Timing of vaccination

Vaccination in pregnancy is recommended for each pregnancy. In 2013, Healy et al. showed that infants of mothers immunised preconception or in early pregnancy had insufficient pertussis-specific antibodies to protect against infection [59]. Even though the recommendations differ from one country to another (e.g., 27-36 weeks in the US; 24-32 weeks in Belgium; from 22 weeks in the Netherlands; 16-36 weeks in Ireland), there is a general consensus to vaccinate pregnant women during the second and beginning of the third trimester. The sooner vaccination is recommended in pregnancy, the higher the probability to protect preterm babies from the disease, the most vulnerable population [60]. The larger the time windows, the higher the probability that the pregnant women get a chance to get vaccinated when seeking healthcare.

Adverse events

Since its first implementation in 2011 in the US, maternal vaccination safety has been the subject of many studies. For most of them, receipt of pertussis vaccine during pregnancy was not associated with increased risk of adverse events neither for the mother nor the newborn. Two studies in 2017 reported a potential association with chorioamnionitis [61,62]. A systematic review performed in 2020 also detected an increased risk of chorioamnionitis but with no association with relevant sequelae [63]. A meta-analysis of RCT carried out in 2022 did not identify any difference of risk of serious adverse events in women and infants [64]. In 2020, Andersen et al. conducted a systematic review and meta-analysis of the effect of pertussis vaccine in pregnancy on the risk of chorioamnionitis, non-pertussis infectious diseases and other adverse pregnancy outcomes and confirmed an increased risk of chorioamnionitis [65]. These safety data are reassuring but ongoing safety assessment will continue.

The “blunting effect”

Despite encouraging results of vaccination in pregnancy in infants aged <2 months, immunological studies also suggest that maternal vaccination could possibly interact with the infant PV and reduce the corresponding immune response [27]. However, little evidence exists about the clinical implications of this potential “blunting effect” of vaccination in pregnancy.

Other passive immunisations

To protect infants too young for vaccination, other passive protections have also been investigated, such as the effect of breastfeeding when the mother has recently been vaccinated against pertussis [66,67]. Indeed, pertussis antibody levels in colostrum is higher in women vaccinated in pregnancy, suggesting that lactation could play a role in the infant’s protection against pertussis. Additionally, vaccinating the mother during pregnancy also provides with an indirect protection of the newborn since the mother used to be the first most likely source of infection in infants [44].

Nevertheless, because passively acquired maternal antibodies to pertussis are unlikely to persist in protective concentrations beyond 3 months of age, cocooning strategy could be an interesting strategy to be combined with vaccination in pregnancy [53]. Asymptomatic human carriage may also be a target for future vaccine strategies [21].

2.4.3. Measures of the effect of pertussis vaccine

To inform policy decisions regarding pertussis immunisation strategies, measuring the effects of pertussis vaccination is essential.

2.4.3.1. Type of effects in vaccinology

Halloran and Struchiner (1991) defined four population-level effects of particular intervention program [68]. First, they distinguished interventions in individuals and intervention programs in populations as the following. Individuals may receive interventions such as vaccination, personal protective measures or treatment directly. But if many individuals in the population receive this intervention, thus the population is considered as having received an intervention program. Population-level effects of this intervention can then be evaluated as direct, indirect, total but also overall effects.

[Figure 1](#) illustrates these different types of effects of vaccination programme. In this example, no vaccination has taken place in the “control population”, and a proportion of individuals has been vaccinated on the “intervention population”.

The *direct effect* of vaccination is the effect that is measured by comparing vaccinated and unvaccinated individuals belonging to the same population that was exposed to the same vaccination programme. We measure the percentage of reduction of disease incidence in vaccinated individuals

that may be due to vaccination [68]. In vaccinology, the primary focus is often on assessing this direct effect of the vaccine among within the population that receives the vaccination program.

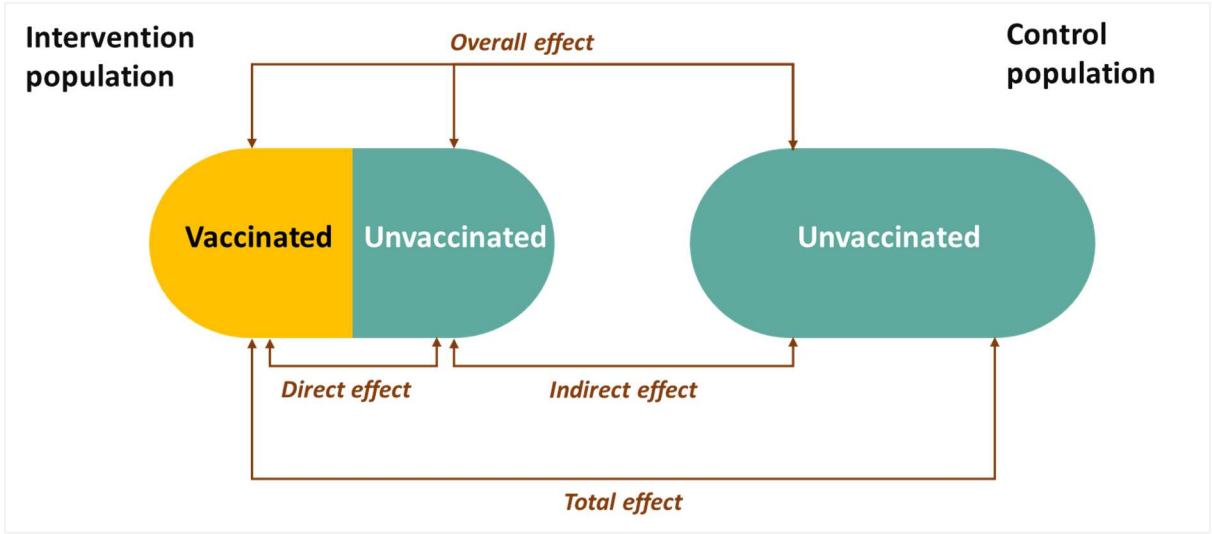
Nevertheless, a vaccination programme can potentially reduce the disease incidence across the entire population. The indirect, total and overall effects are then evaluated by comparing two populations: the population with a vaccinated programme (i.e., “intervention population”) and the population without a vaccination programme (i.e., “control population”). Note that the “control population” can basically be the “intervention population” but before the intervention.

The *indirect effect* is the effect that is measured by comparing the incidence in the unvaccinated individuals in the population with a vaccination programme, with the unvaccinated individuals in the population without the vaccination programme. We thus measure the risk reduction between the two unvaccinated groups which reflects the reduced transmission among the unvaccinated population, also called herd immunity.

The *total effect* is the combined effect of being vaccinated and being in a population with a vaccination programme. We estimate the risk reduction between vaccinated individuals in the population with a vaccination programme and unvaccinated individuals in the population without a vaccination programme. This is the sum of the direct and the indirect effects.

The *overall effect* is the comparison of the disease incidence in a population with a vaccination programme and in a population without a vaccination programme. It is a measure of the risk reduction in the entire population attributable to the vaccination programme. We usually estimate the overall effect by comparing the incidence of a disease in a given population before and after the introduction of a vaccination programme. In other words, in a population in which a new vaccination programme has been introduced, we can estimate the effect on the entire population, even though only a fraction is actually vaccinated.

Figure 1. Type of effects of vaccine interventions, adapted from Halloran et al. [68]



As above-mentioned, the indirect, total and overall effects are based on the comparison of two populations including one population where pertussis vaccination has not yet been introduced. For pertussis PV based on the aP vaccine, only a few studies have estimated population-level effects of pertussis immunisation programme in infants. From 1984 to 1996, an observational prospective cohort study of children aged less than 15 years in Senegal investigated the effect of the introduction of vaccination programs using both wP and aP vaccines. They observed an overall effect of the vaccination with a reduction in incidence in the 0-14 year olds of 46% [69,70]. Nowadays, in a context with a pertussis PV in infants included in all European national immunisation programs since at least the 1950's, studies to assess population-level VE such as the indirect effect would require expensive randomized trials that would mostly be non-ethical and non-feasible. Rane and Halloran (2021) recently estimated population-level effects for aP vaccine administered in a non-randomised way for the first time, using routinely collected surveillance data. The estimated indirect VE was 45% (95% CI, 1–70), the total VE 94% (91–96), and the overall VE 42% (19–60) [71].

In this context, the PERTINENT network focused on the analysis of the direct vaccine effect in infants in a given population in which an immunisation program based on the aP vaccine is currently in place.

2.4.3.2. Vaccine efficacy and effectiveness

Before the introduction of a new vaccine in the population, its immunogenicity, protective efficacy and quality must be demonstrated based on clinical trials. Vaccine efficacy is defined as the direct effect of a vaccine, i.e., direct protection induced by vaccination against the disease, measured in pre-licensure randomised trial conducted under optimal conditions.

Once the vaccine has been rolled-out in the population, post-licensure studies are required to estimate vaccine effectiveness and impact of vaccination programmes based on observational population-based studies. Vaccine effectiveness is defined as a measure of protection attributable to the vaccine administered under field conditions [72]. Both vaccine efficacy and effectiveness are measured with the same formula:

$$VE = \frac{R_{Unvacc.} - R_{Vacc.}}{R_{Unvacc.}} = 1 - \frac{R_{Vacc.}}{R_{Unvacc.}} = 1 - RR$$

Where: $R_{Unvacc.}$ is the risk among unvaccinated individuals, $R_{Vacc.}$ is the risk among vaccinated individuals, RR represents the risk ratio. When risk cannot be measured directly, we approach the RR by measuring the odds ratio OR .

2.4.3.3. Study designs

Post-licensure observational population-based studies allowing estimation of direct vaccine effectiveness, may lead to confounding or biases that are usually controlled in RCT by random

allocation of exposure. Several study design allow to account for these biases and can be used to measure the direct pertussis vaccine effectiveness, providing that the two groups compared belongs to the same population (see [Figure 1](#), “Intervention population”) and are eligible to the same vaccination programme (i.e., same chance of being exposed to the intervention) [73].

Cohort studies

In *cohort studies*, being the “gold standard” for all epidemiological studies, we follow groups of people over time and we compare the risk or rate (person-time) of developing the disease among vaccinated and unvaccinated of the same population. We estimate the direct vaccine effectiveness on the basis of a risk ratio (RR):

$$VE = 1 - RR.$$

In such a setting, we need information on vaccine status but also disease status throughout the study period and for the entire study population. This may require electronic databases including at least vaccination information, but also routine testing of individuals from the population. Randomised vaccine studies are cohort studies in which the vaccine has been randomly allocated.

Case-control studies

In *case-controls studies*, cases are ascertained and controls are selected from the source population of cases. Basically, a case-control study can be seen as a sample of a hypothetical cohort study. It aims at achieving the same goals as a cohort study but more efficiently, using sampling and thus usually at considerably less cost and time [74]. We estimate direct vaccine effectiveness from the comparison of the odds of vaccination among cases and the odds of vaccination among controls. We compute the ratio of these two odds (Odds Ratio, OR), thus approximating the RR and then deriving VE as:

$$VE = 1 - OR$$

We will obtain a good estimation of the VE if the OR properly approximates the RR. To ensure this assumption, the selected controls must have had the same vaccination experience than the source population of cases.

Depending on how controls are selected, there is a variety of alternative case control study designs.

PERTINENT study design

In the context of the PERTINENT hospital-based sentinel network, the study population is defined as all infants aged less than one year, likely to be hospitalised in one of the participating hospitals if developing pertussis-like symptoms.

An infant can be considered as vaccinated, for each aP vaccine dose, if she/he had received the corresponding dose > 14 days before symptom onset. Unvaccinated infants would then be those who had not received any dose.

Cases and controls are identified among hospitalised infants presenting with any pertussis-like symptoms, or any respiratory symptoms and an epidemiological link with a pertussis confirmed case or those not meeting the above clinical presentation but with clinical suspicion for pertussis by a physician.

Cases are defined as any infants hospitalised with criteria fulfilling the inclusion and with laboratory-confirmed *Bordetella pertussis* infection. Controls are those that are laboratory-negative for all *Bordetella* species. This design is the test-negative case-control design (TND) and is supposed to control for biases in case ascertainment, access to care and health seeking behaviour [75]. This design is relatively simple, requires less work for the teams in the field, can be implemented prospectively or retrospectively and can also be convenient for studying many exposures (e.g., infants PV, vaccination of the infants' mother during pregnancy). The TND is the predominant observational study design for influenza VE studies, and is increasingly used for COVID-19 VE, especially in hospital-based studies.

However, TND also relies on several hypotheses that need to be tested and validated. One of the core assumption is that the pertussis vaccine has no effect on other than pertussis diseases that result with similar respiratory presentation [76]. In a cohort study this would imply that the risk of being hospitalised for pertussis-like symptoms that are not laboratory confirmed as *Bordetella pertussis* disease is equal between those vaccinated and unvaccinated against pertussis. In a TND, the control group thus selected should reflect the vaccination coverage experience of the source population giving rise to cases. Additionally, TND assumes a high sensitivity of the diagnostic tests as false negatives would be misclassified as controls and bias the VE estimates.

To our knowledge, the PERTINENT study is the first study to estimate pertussis VE using TND in multi-country paediatric hospital settings. Therefore, validation of the hypothesis on which the TND design relies is needed for pertussis infection in infants at hospital level.

3. Thesis work

3.1. Incidence and severity of pertussis in infants

3.1.1. Summary

The first objective of the PERTINENT Network was to estimate the burden of laboratory-confirmed pertussis in hospitalised infants aged less than one year in terms of severity and incidence of the disease.

This chapter presents the first published article of the PERTINENT network describing pertussis severity and trends in incidence in hospitalised infants, during the first three pilot years of continuous data collection in the six participating EU/EEA countries.

From December 2015 to December 2018, we identified 469 laboratory-confirmed *B. pertussis* cases. The highest number of cases was in infants in their second month of life, prior to PV. Heterogeneous clinical practices by country and by hospital prevented us from ascertaining the severity of the disease using quantifiable criteria such as Paediatric Early Warning Scores (PEWS) or length of hospital stay [77]. One pertussis case out of four was admitted to the intensive care unit (ICU). During this three-year study period and in the seven study sites, we identified five deaths related to pertussis. All were unvaccinated yet. Out of the three infants with available information on maternal vaccination status, one had a mother who received pertussis vaccination 5 days before delivery (therefore, not yet considered as vaccinated), two had a mother vaccinated 5 to 10 years earlier.

Most of the cases were reported during summer, supporting the hypothesis of a seasonality of the disease already described in the literature [36,37]. The most probable source of pertussis infection reported by the parents were the siblings followed by the mother. These results are consistent with former studies describing a shift in the source of infant pertussis infection from the mother to the siblings [44] which might result from an increase of maternal vaccination across Europe (either before, during or after pregnancy with the cocooning strategy). This emphasises the need to protect infants from a pertussis reservoir in older age groups.

After a progressive implementation of the study in 2016, our findings suggest a decrease in pertussis incidence in 2018 compared with 2017, across all sites except for the Czech Republic site where incidence remained stable. The heterogeneity of surveillance systems [10] but also the low number of participating study sites in each country, did not allow us to extrapolate our results to national nor EU/EEA level.

This pilot study shows the importance of raising disease awareness especially in infants aged less than one year, when the disease is the most life-threatening and the most difficult to diagnose. Infants and neonates often have an atypical clinical presentation of the disease which may lead to under-diagnosis in that age group [41]. Our findings support the existence of atypical presentation and reinforce that pertussis cases aged less than 3 months are more likely to present with apnoea or cyanosis. These results support the inclusion of these criteria in the World Health Organization (WHO) and United States Centers for Disease Control and Prevention (US CDC) pertussis case definitions for younger age groups.

This paper also illustrates the need to improve laboratory diagnosis in pertussis routine surveillance in Europe in order to accurately identify and distinguish all *Bordetella* species. Indeed, five out of the seven sites were able to provide information on other *Bordetella* species relying on the PERTINENT diagnostic algorithm for DNA detection of *B. pertussis* [14]. The proportion of infants with a *Bordetella* infection other than *B. pertussis* among those testing positive for any *Bordetella* species was 11% (40/357) and included 16 *B. parapertussis*, 4 *B. holmesii* (usually rarely isolated in infants [78]), 19 undetermined *Bordetella* species and one co-infection with *B. parapertussis* and *B. pertussis*. Identifying all *Bordetella* species is crucial for pertussis surveillance but also VE studies as they may not have the same pathogenicity and may not be all directly targeted by pertussis-containing vaccines [41,79].

This article was published in *Eurosurveillance* as an original research article.

3.1.2. Article 1

Incidence and severity of pertussis hospitalisations in infants aged less than 1 year in 37 hospitals of six EU/EEA countries, results of PERTINENT sentinel pilot surveillance system, December 2015 to December 2018

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Introduction: PERTINENT is a pilot active surveillance system of infants hospitalised with pertussis in six European Union/European Economic Area countries (37 hospitals, seven sites). **Aim:** This observational study aimed to estimate annual pertussis incidence per site from 2016 to 2018 and respective trends between 2017 and 2018. Pertussis cases were described, including their severity. **Methods:** We developed a generic protocol and laboratory guidelines to harmonise practices across sites. Cases were hospitalised infants testing positive for *Bordetella pertussis* by PCR or culture. Sites collected demographic, clinical, laboratory data, vaccination status, and risk/protective factors. We estimated sites' annual incidences by dividing case numbers by the catchment populations. **Results:** From December 2015 to December 2018, we identified 469 cases (247 males; 53%). The median age, birthweight and gestational age were 2.5 months (range: 0–11.6; interquartile range (IQR): 2.5), 3,280 g (range: 700–4,925; IQR: 720) and 39 weeks (range: 25–42; IQR: 2),

respectively. Thirty cases (6%) had atypical presentation either with cough or cyanosis only or with absence of pertussis-like symptoms. Of 330 cases with information, 83 (25%) were admitted to intensive care units including five deceased infants too young to be vaccinated. Incidence rate ratios between 2018 and 2017 were 1.43 in Czech Republic ($p=0.468$), 0.25 in Catalonia ($p=0.002$), 0.71 in France ($p=0.034$), 0.14 in Ireland ($p=0.002$), 0.63 in Italy ($p=0.053$), 0.21 in Navarra ($p=0.148$) and zero in Norway. **Conclusions:** Incidence appeared to decrease between 2017 and 2018 in all but one site. Enhanced surveillance of hospitalised pertussis in Europe is essential to monitor pertussis epidemiology and disease burden.

Introduction

Most severe cases of pertussis (whooping cough) occur below 5 years of age. Worldwide, it causes substantial mortality in infants (85,900 estimated deaths in 2014) [1]. Sixty-three percent of cases aged less than 1 year

TABLE 1

 Characteristics of PERTINENT study sites, number of cases positive to *Bordetella* species and annual incidence by study site and year, Europe, 1 December 2015–31 December 2018 (n = 41 sites)

Study sites		Czech Republic	France	Ireland	Italy	Spain, Catalonia	Spain, Navarra	Norway
Vaccination recommendations: year introduction and doses recommended								
Primary schedule	Year of introduction	2018 ^a	2013	1995	1995	2016 ^b	2016 ^b	1998
	Ages for different doses	3, 5, 11–13 months	2, 4, 11 months	2, 4, 6 months	3, 5, 11 months	2, 4, 11 months	2, 4, 11 months	3, 5, 12 months
Pregnancy, year of introduction		2016	No	2013	2017	2014	2015	No
Cocooning, year of introduction		No	2004	2013	No	No	No	No
Participating hospitals and catchment population estimation								
Number of hospitals participating in PERTINENT		6	21	2	2	1	4	5 (2016–2018/05) 1 (from 2018/05)
Method used to estimate hospital catchment population		National census	National census × estimation of PERTINENT hospital coverage [13]	National census	Regional census	National census × estimation of PERTINENT hospital coverage	National census	Regional census prorata temporis
Number of screened infants and cases per <i>Bordetella</i> species								
Screened infants in PERTINENT		73	546	138	509	207	118	523
<i>Bordetella pertussis</i> cases ^c		25	199 ^d	30	145 ^e	50	13	7
<i>Bordetella parapertussis</i> cases		0	10 ^d	2	NA	1	4	NA
<i>Bordetella holmesii</i> cases		0	2	0	NA	1	1	NA
Other <i>Bordetella</i> species ^f		0	16	0	NA	3	0	NA
Total <i>Bordetella pertussis</i> cases and incidence, by year of study								
2016								
Number of cases 2016		8	45	6	61	19	7	4
Catchment population 2016		65,638	190,077	25,110	34,428	12,138	5,875	25,545
Incidence per 100,000 infants		12.2	29.1 ^g	57.2 ^g	211.9 ^g	156.5	119.1	17.1 ^g
January 2016–December 2016								
95% exact confidence interval		(5.3–24.0)	(21.2–38.9)	(21.0–124.4)	(162.1–272.1)	(94.3–244.3)	(47.9–245.3)	(4.7–43.8)
2017								
Number of cases 2017		7	91	21	47	25	5	3
Catchment population 2017		68,128	185,420	23,267	33,811	12,056	5,856	25,479
Incidence per 100,000 infants		10.3	49.1	90.3	139.0	207.4	85.4	11.8
January 2017–December 2017								
95% exact confidence interval		(4.1–21.2)	(39.5–60.3)	(55.9–137.9)	(102.2–184.8)	(134.2–306.0)	(27.7–199.1)	(2.4–34.4)
2018								
Number of cases 2018		10	63	3	28	6	1	0
Catchment population 2018		68,061	181,481	23,191	31,953	11,593	5,708	14,308 ^h
Incidence per 100,000 infants		14.7	34.7	12.9	87.6	51.8	17.5	0.0
January 2018–December 2018								
95% exact confidence interval ⁱ		(7.0–27.0)	(26.7–44.4)	(2.7–37.8)	(58.2–126.6)	(19.0–112.6)	(0.4–97.6)	(0–25.8)

NA: not available; PERTINENT: Pertussis in Infants European Network.

^a Before 2018: doses at 2, 3, 4 and 10 months.

^b Before 2016: doses at 2, 4 and 6 months.

^c Percentage of positivity among screened infants is expected to vary across sites due to the variation of hospitalisation likelihood of infants with pertussis-like symptoms.

^d Including one co-infection of *Bordetella pertussis* and *Bordetella parapertussis*.

^e Including nine cases recruited in 2015.

^f Laboratory could not confirm and differentiate the *Bordetella* species.

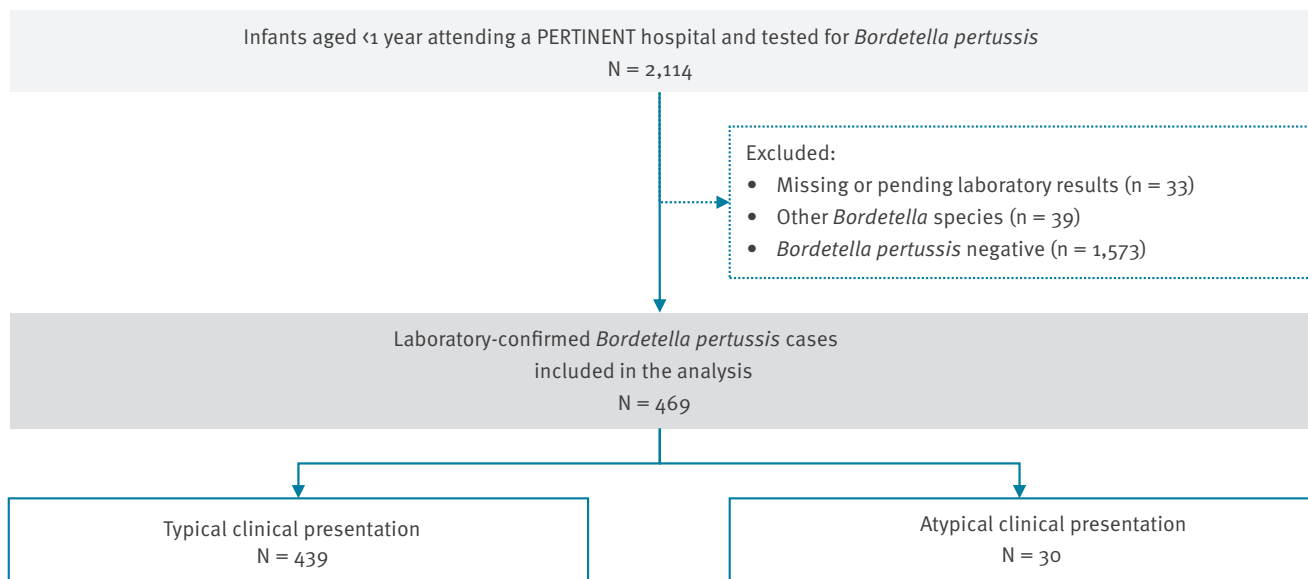
^g Incidence calculated *prorata temporis* based on available data in 2016 due to a progressive implementation of the surveillance.

^h Withdrawal of four hospitals.

ⁱ 'Exact confidence interval' for incidence as the exact binomial confidence interval (i.e. Clopper–Pearson interval) was computed.

FIGURE 1

Flowchart of hospitalised infants' (<1 year) inclusion in or exclusion from the PERTINENT study, Europe, 1 December 2015–31 December 2018 (n=2,144 hospitalised infants)



PERTINENT: Pertussis in Infants European Network.

reported to the European Centre for Disease Prevention and Control (ECDC) in 2017 required hospitalisation [2]. Severe complications include pneumonia, seizures, encephalopathy and death.

In neonatal infection, the cough with the characteristic whoop might be absent. The initial finding for *Bordetella pertussis* infection is frequently apnoea [3]. Since infants and neonates often have a clinical presentation that is atypical or similar to several other respiratory diseases, Vittuci et al. support a routine pertussis laboratory diagnosis in all infants aged less than 3 months with acute respiratory symptoms [4]. Adolescents (≥ 11 years of age) and adults (≥ 18 years of age) can develop a mild, often undiagnosed, form of the disease and represent a reservoir of transmission for infants.

Pertussis is one of the least controlled vaccine-preventable diseases in European Union/European Economic Area (EU/EEA) countries [5]. The number of pertussis cases reported to ECDC increased since 2011 despite a primary series of acellular pertussis vaccine coverage exceeding 90% in most countries [6]. The last peak incidence year occurred in 2012 with 42,500 reported cases, impacting 19 of 28 countries with different magnitude [5]. It affected adolescents, adults and particularly children too young to be vaccinated or to have completed the primary series. This increase may be explained by improved diagnostic methods, increased disease awareness, waning of acellular-vaccine-induced protection, or a lower vaccine effectiveness (VE) due to bacterial mutation [7].

At EU/EEA level, the routine pertussis surveillance systems are heterogeneous. Under-diagnosis and under-notification also differ across EU/EEA countries [8]. Even though a majority uses the EU case definition [9,10], differences in laboratory procedures, completeness of reporting and differences in disease awareness still remain [8]. Most case definitions do not distinguish between all *Bordetella* species that can have similar respiratory presentation and include indifferently *B. pertussis*, *B. parapertussis*, *B. holmesii* and *B. bronchiseptica*. *Bordetella* species may not have the same pathogenicity and may not be all directly targeted by pertussis-containing vaccine [3]. Therefore, existing surveillance systems make comparison of pertussis immunisation strategies and estimates of pertussis incidence at EU/EEA level difficult. Following the 2012 outbreak, an ECDC consultation resulted in a recommendation to conduct studies in the EU/EEA to measure the burden of pertussis including infant hospitalisations [11]. In recent years, some countries have enhanced surveillance of pertussis at hospital [12-14] or population level [15].

In 2015, ECDC initiated PERTINENT, 'Pertussis in Infants European Network', a hospital-based active pilot surveillance system in seven study sites using the same pertussis surveillance protocol. The surveillance system has two main objectives. The first is to identify the trends in incidence of laboratory-confirmed pertussis in hospitalised infants (≤ 1 year old) for the respective study sites. The second is to estimate VE using the test-negative design. In the current study, annual incidences per site are estimated from 2016 to 2018 as well as the change in incidence in 2018 compared

TABLE 2A

 Hospitalised *Bordetella pertussis* cases aged < 1 year by age group, sex, laboratory components, clinical presentation, most likely source of infection and severity criteria, PERTINENT study, Europe, 1 December 2015–31 December 2018 (n = 469 cases)

Characteristic		Cases (all < 1 year old) (n = 469)		Cases 0–3 months old (n = 354)		Cases 4–11 months old (n = 115)		p value
		Number	%	Number	%	Number	%	
		Demographic						
Sex (n = 469)	Female	222	47.3	165	46.6	57	49.6	0.593
	Male	247	52.7	189	53.4	58	50.4	
Laboratory components								
Nasopharyngeal specimen collection (n = 448)	Aspirate only	346	77.2	252	75.4	94	82.5	0.321
	Swab only	80	17.9	64	19.2	16	14.0	
	Both	22	4.9	18	5.4	4	3.5	
PCR (n = 465)	Positive	465	100.0	351	100.0	114	100.0	NA
	Negative	0	0.0	0	0.0	0	0.0	
Culture (n = 211) ^a	Positive	108	51.2	89	51.7	19	48.7	0.859
	Negative	103	48.8	83	48.3	20	51.3	
Clinical presentation								
Cough (n = 469)	Yes	456	97.2	342	96.6	114	99.1	0.202
	No	13	2.8	12	3.4	1	0.9	
Cough with paroxysms (n = 456)	Yes	393	86.2	298	87.1	95	83.3	0.347
	No	63	13.8	44	12.9	19	16.7	
Whooping cough (n = 269)	Yes	122	45.4	91	47.2	31	40.8	0.415
	No	147	54.6	102	52.8	45	59.2	
Post-tussive vomiting (n = 449)	Yes	219	48.8	164	48.7	55	49.1	1.000
	No	230	51.2	173	51.3	57	50.9	
Apnoea (n = 466)	Yes	235	50.4	193	55.0	42	36.5	0.001
	No	231	49.6	158	45.0	73	63.5	
Cyanosis (n = 467)	Yes	239	51.2	192	54.5	47	40.9	0.013
	No	228	48.8	160	45.5	68	59.1	
Epidemiological link (n = 457)	Yes	167	36.5	124	35.8	43	38.7	0.651
	No	290	63.5	222	64.2	68	61.3	
Diagnosis by a clinician (n = 469)	Yes	368	78.5	275	77.7	93	80.9	0.516
	No	101	21.5	79	22.3	22	19.1	
Reported source of infection								
Mother (n = 424)	Yes	106	25.0	88	27.5	18	17.3	0.038
	No	318	75.0	232	72.5	86	82.7	
Father (n = 419)	Yes	82	19.6	66	21.0	16	15.4	0.255
	No	337	80.4	249	79.0	88	84.6	
Sibling (n = 416)	Yes	128	30.8	102	32.8	26	24.8	0.143
	No	288	69.2	209	67.2	79	75.2	
Grandparents (n = 409)	Yes	41	10.0	33	10.8	8	7.7	0.451
	No	368	90.0	272	89.2	96	92.3	
Caregiver (n = 240)	Yes	0	0.0	0	0.0	0	0.0	NA
	No	240	100.0	175	100.0	65	100.0	

ECMO: Extracorporeal membrane oxygenation; ICU: intensive care unit; PERTINENT: Pertussis in Infants European Network.

^a Cultures were done for 255 cases, but the results were only available for 211 cases.

Number of cases presented for the different characteristics are those with information available.

TABLE 2B

Hospitalised *Bordetella pertussis* cases aged < 1 year by age group, sex, laboratory components, clinical presentation, most likely source of infection and severity criteria, PERTINENT study, Europe, 1 December 2015–31 December 2018 (n = 469 cases)

Characteristic		Cases (all <1 year old) (n = 469)		Cases 0–3 months old (n = 354)		Cases 4–11 months old (n = 115)		p value
		Number	%	Number	%	Number	%	
		Severity criteria						
Death (n = 466)	Yes	5	1.1	5	1.4	0	0.0	0.340
	No	461	98.9	346	98.6	115	100.0	
ICU (n = 330)	Yes	83	25.2	80	31.5	3	3.9	0.000
	No	247	74.8	174	68.5	73	96.1	
ECMO (n = 336)	Yes	7	2.1	7	2.7	0	0.0	0.360
	No	329	97.9	251	97.3	78	100.0	
Pneumonia (n = 327)	Yes	14	4.3	12	4.8	2	2.7	0.745
	No	313	95.7	240	95.2	73	97.3	
Encephalopathy (n = 327)	Yes	3	0.9	3	1.2	0	0.0	1.000
	No	324	99.1	249	98.8	75	100.0	
Seizure (n = 328)	Yes	10	3.0	8	3.2	2	2.7	1.000
	No	318	97.0	245	96.8	73	97.3	
Eating difficulties (n = 269)	Yes	62	23.0	50	25.4	12	16.7	0.144
	No	207	77.0	147	74.6	60	83.3	
Kidney failure (n = 267)	Yes	4	1.5	4	2.0	0	0.0	0.576
	No	263	98.5	192	98.0	71	100.0	
Dehydration (n = 300)	Yes	12	4.0	11	4.8	1	1.4	0.305
	No	288	96.0	216	95.2	72	98.6	

ECMO: Extracorporeal membrane oxygenation; ICU: intensive care unit; PERTINENT: Pertussis in Infants European Network. Number of cases presented for the different characteristics are those with information available.

to 2017. Pertussis cases are described by severity and other characteristics. VE results are not presented as VE will be assessed once the required sample size is reached.

Methods

Study design and setting

The PERTINENT coordination selected seven study sites (Czech Republic; France; Ireland; Italy; Norway; Catalonia, Spain and Navarra, Spain) willing to participate and able to comply with the generic PERTINENT sentinel surveillance protocol [16] and laboratory guidelines [17] and to estimate the participating hospitals' catchment population. We organised site visits and a laboratory workshop to ensure the harmonisation of site-specific protocols allowing pooling of sites' data.

From December 2015 to September 2016, study sites progressively implemented active surveillance in 41 hospitals. A large proportion of hospitals were situated in France (n = 21 hospitals) and other countries had one to six participating hospitals. Each site complied with the local ethical procedures. In May 2018, the number of participating hospitals was reduced to

37 after withdrawal of four Norwegian hospitals (Table 1). All sites use the acellular pertussis vaccine for the primary series in infants, but national vaccine recommendations and primary schedules vary across sites (Table 1 and supplementary Table S4).

Case identification and recruitment

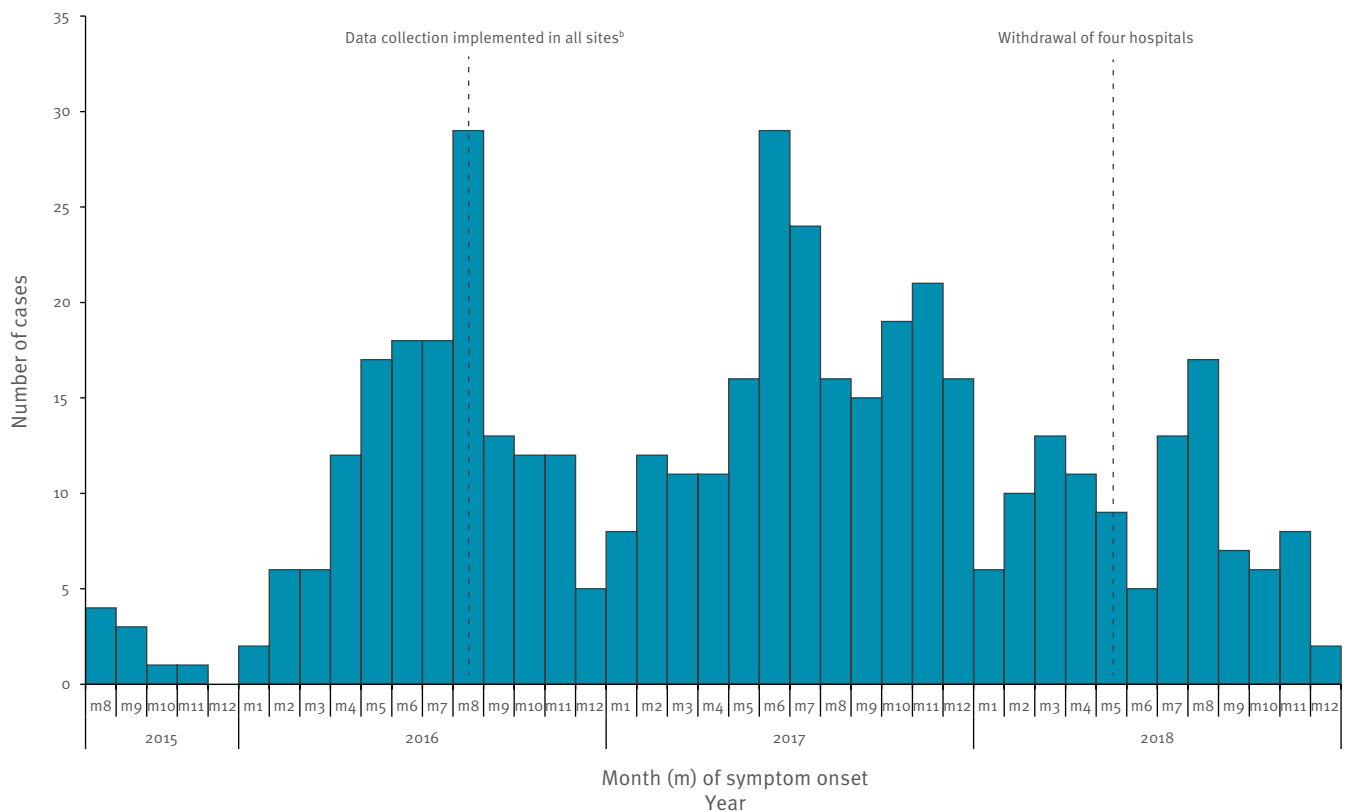
The study population consisted of all infants aged less than 1 year, likely to be hospitalised in one of the participating hospitals if developing pertussis-like symptoms.

To maximise the sensitivity of the surveillance, we raised hospital physicians' awareness of pertussis clinical presentation [3] and asked them to test all infants presenting at hospital with pertussis-like symptoms. 'Typical' pertussis presentation was defined either by a presence of apnoea; or by a cough associated with at least one of paroxysms, whoop or post-tussive vomiting. When physicians suspected pertussis even though some typical symptoms were missing, pertussis was considered as 'atypical'.

We identified all infants attending the hospital who were tested for pertussis and invited their parents to participate in the study. When required by the local

FIGURE 2

Hospitalised *Bordetella pertussis* cases aged < 1 year by month of symptom onset, PERTINENT study, Europe, 1 December 2015–31 December 2018 (n = 464 cases^a)



PERTINENT: Pertussis in Infants European Network.

^a Five patients with missing date of symptom onset are not included in the figure.

^b Data collection started in 2015, but was fully operational in all sites in August 2016 only.

ethical committee, parents or legal guardians were requested to provide an informed consent.

We excluded all patients with missing or pending laboratory results, testing positive to other *Bordetella* species than *B. pertussis* or whose legal guardian was unwilling to participate or unable to communicate and give consent. All laboratory-confirmed *B. pertussis* cases aged less than 1 year at the time of hospitalisation were included in the study (Figure 1).

Definitions

We defined a laboratory-confirmed *B. pertussis* case as an infant attending one of the participating hospitals (irrespective of the length of stay), aged less than 1 year and testing positive for *B. pertussis* by PCR (DNA detection of *B. pertussis* using PCR or real-time PCR in a nasopharyngeal aspirate or swab) or culture (isolation of *B. pertussis* from the prior-mentioned clinical specimen) regardless of the clinical criteria. We defined a severe case as a case admitted to the intensive care unit (ICU).

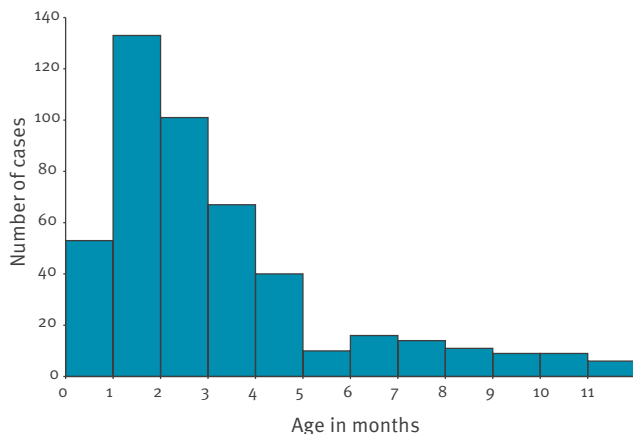
We defined a likely source of infection as a person with a cough who had contact with the case in the 7 to 20 days before the date of symptom onset of the case. For each pertussis vaccine dose, we defined an infant as vaccinated if she/he had received the dose of interest >14 days before symptom onset. Unvaccinated infants were those who had not received any dose or who had received the first dose ≤14 days before symptom onset.

Laboratory methods

To ensure an accurate identification of the *Bordetella* species, the PERTINENT laboratory guidelines recommend a diagnostic algorithm for DNA detection of *Bordetella* including a series of three PCRs: a triplex real-time PCR targeting IS481 gene (in *B. pertussis*, *holmesii* and some *bronchiseptica* strains), pIS1001 (*B. paraptussis*) and RNase P as the human internal control; followed by two confirmatory singleplex tests targeting *ptxA-Pr* (*B. pertussis*) and hIS1001 (*B. holmesii*) genes [17].

FIGURE 3

Hospitalised *Bordetella pertussis* cases aged < 1 year by age in months, PERTINENT study, Europe, 1 December 2015–31 December 2018 (n=469 cases)



PERTINENT: Pertussis in Infants European Network.

Data collection

Using a standard questionnaire, all sites collected a common set of information: demographic, epidemiological, clinical, laboratory data, vaccination status of the infant and household members, risk and protective factors and suspected source of infection. The list of potential sources of infection included close relatives and caregivers. Each study site translated the questionnaire available in English in its country's language. Data were collected through review of clinical case notes, extraction from patient registries or, if not available, interviews with parents or legal guardians.

Denominators

The denominator was the estimated population of infants aged less than 1 year likely to receive care at hospitals participating in the study. The methods to estimate the hospitals' catchment population varied across sites (Table 1). Throughout the study period, we adjusted the catchment population to the loss of the four Norwegian hospitals.

Analysis

We described cases by age, clinical presentations, risk and protective factors, severity. We used median for continuous variables (age in months, gestational age and weight at birth) and frequencies for categorical variables. We estimated the incidence by site and year. In four sites, the protocol was implemented during 2016 and we estimated 2016 incidence *prorata temporis*. We calculated incidence rate ratios (IRR) for 2018 compared with 2017.

We used Fisher's exact test to compare clinical signs and symptoms by age group and characteristics of cases admitted to ICU with those not admitted to ICU.

Ethical statement

The planning, conduct and reporting of the study was in line with the Declaration of Helsinki [18]. Ethical approval was not needed in Navarra as the PERTINENT study came under the umbrella of the mandatory surveillance system. Other study sites sought ethical approval from a review board according to country-specific regulations (Catalonia: PIC-31-16, Czech Republic: SZU/05992/2019, France: n°449199 v 1, Ireland: REC reference number 16.058 and Gen/499/16, Italy: Bambino Gesù Children's Hospital Ethical Committee: protocol n. 1064_OPBG_2016, Norway: REC register number 2015/956).

Results

Description of cases

Of the 2,114 infants tested for *B. pertussis*, 2,081 had laboratory results available (98%) (Figure 1). We excluded 39 cases caused by other *Bordetella* species (2%). A total of 469 infants were positive for *B. pertussis* (23%) including one co-infection with *B. pertussis* and *parapertussis*. The number of pertussis cases by site ranged from seven in Norway to 199 cases in France (Table 1).

Among the 469 laboratory-confirmed *B. pertussis* cases, information on the sample type was available for 448, with 368 (82%) having had a nasopharyngeal aspirate, 102 (23%) a nasopharyngeal swab and 22 (5%) both. Of all 469 confirmed cases, 465 had a PCR (99%) and 255 a culture done (54%). All PCRs and 108 of 211 culture results available (51%) were positive to *B. pertussis* (Table 2).

Of the 469 cases, 247 were males (53%). The median age was 2.5 months (range: 0–11.6; interquartile range (IQR): 2.5). The median weight at birth was 3,280g (range: 700–4,925; IQR: 720). The median gestational duration was 39 weeks (range: 25–42; IQR: 2).

The number of reported cases by month of symptom onset (Figure 2) was highest in August 2016 (n=29) and in June 2017 (n=29). Excluding the first months of progressive surveillance implementation, less cases were observed at the transition between years, such as December 2016 (n=5), January 2018 (n=6) and December 2018 (n=2), but not always, as for example June 2018 (n=5).

The highest number of cases was reported in the age group 0–2 months (n=287), with a peak in the second month of life (n=133), then the number decreased by age in months (Figure 3).

Of the 469 cases, 456 (97%) had a cough, among whom 86% had paroxysm. For those who had cough and additional information on post-tussive vomiting (n=449) and whoop (n=269), 49% had post-tussive vomiting and 45% whoop. For cases with available data on apnoea (n=466), 235 (50%) had apnoeic episodes

(Table 2), including six cases without cough. Pertussis was typical in 439 cases (94%) (Figure 1). Thirty cases had an atypical pertussis clinical presentation (6%), among whom 20 had a cough only, three had cough and cyanosis only, two had a cyanosis only and five had documented absence of all pertussis-like symptoms. Four of these atypical pertussis cases had at least one missing clinical information.

Among 354 cases aged 0–3 months, 250 (71%) had either apnoea or cyanosis compared with 62 (54%) among 115 cases aged 4–11 months ($p=0.001$) (Table 2).

Clinical presentation did not differ between vaccinated and not vaccinated infants (data not shown). Of all cases, 290 (62%) were eligible for vaccination. Of the 273 with information on vaccination status, 106 (39%) were vaccinated: 59 had received one dose, 39 two doses and eight three doses of pertussis vaccine. Of the 30 atypical cases, 18 were eligible for vaccination and, of the 17 with related data, about half ($n=9$) had received one or two doses.

Potential source of infection

Information on the potential source of infection was available for 441 cases (94%). Infants' sibling was mentioned for 128 cases (31%), the mother for 106 cases (25%), the father for 82 cases (20%) and the grandparents for 41 cases (10%) (Table 2).

Severity

Five (1%) of 466 cases with information died. They were aged from 2 weeks to 10 weeks and not vaccinated against pertussis. In two of the five cases, the mother was vaccinated 5 to 10 years earlier. For one case, the mother was vaccinated 5 days before delivery. Vaccination status of the mother was unknown for the remaining two cases.

Eighty-three cases (25%) were admitted to ICU, including seven cases treated with extracorporeal membrane oxygenation (ECMO) and the five infants who died (Table 2). Among infants aged 0–3 months, 80 (32%) were admitted to ICU compared with three (4%) among infants aged 4–11 months ($p<0.001$) (Table 2). The median age in months was 1.4 (range: 0–4.6; IQR: 1.3) in cases who required ICU admission and 2.8 (range: 0.1–11.6; IQR: 2.7) in those who did not ($p<0.001$). Of ICU cases, 66 (80%) had either apnoea or cyanosis compared with 160 (65%) in non-ICU cases ($p=0.014$). The proportion of cases with comorbidities was similar between the two groups. Among cases with available gestational week, 21 of the 82 ICU cases (26%) and 26 of 247 non-ICU cases (11%) were preterm infants born before gestational week 37 ($p=0.002$) (Table 3).

Among infants eligible for vaccination, seven of the 25 ICU cases (28%) and 65 of the 163 non-ICU cases (40%) were vaccinated with at least one dose of pertussis vaccine ($p=0.279$). None of the ICU cases and 35 of the

non-ICU cases (21%) had received two or more doses of pertussis vaccine ($p=0.005$).

Incidence by site

Incidence rate ratios between 2018 and 2017 were 1.43 in Czech Republic ($p=0.468$), 0.25 in Catalonia ($p=0.002$), 0.71 in France ($p=0.0335$), 0.14 in Ireland ($p=0.002$), 0.63 in Italy ($p=0.053$), 0.21 in Navarra ($p=0.148$) and zero in Norway (Table 1).

Other *Bordetella* species

Five of the seven sites provided information on other *Bordetella* species. We identified 40 respiratory infections caused by other *Bordetella* species, including 17 *B. parapertussis* (cases aged 6 to 51 weeks) and four *B. holmesii* (cases aged 7 to 14 weeks). Of the 17 *B. parapertussis* cases, two did not have typical pertussis-like symptoms but only cough and three of 12 *B. parapertussis* cases with information were admitted to ICU. Patients infected with *B. holmesii* had pertussis-like symptoms and did not require ICU admission. Among infants eligible for vaccination, eight of 13 *B. parapertussis* cases and two of three *B. holmesii* cases were vaccinated with at least one dose of pertussis vaccine.

Discussion

Over the three pilot years of the PERTINENT active hospital-based surveillance system, we identified 469 laboratory-confirmed *B. pertussis* cases. We observed a higher incidence in 2017 and a decrease in 2018 in all but one study site. In 2016 and 2017, most of the cases were reported during summer. One of four pertussis laboratory-confirmed hospitalised cases was admitted to ICU. The highest proportion of ICU cases was in infants aged 0–3 months. Five infants died, all were unvaccinated: either too young to be vaccinated or in the month of life targeted for the first dose of the primary schedule.

Despite standard protocols proposed, the PERTINENT pilot surveillance still has limits to consider when examining the findings. Different methods were used by study sites for estimating hospital catchment population. This may have biased the measured incidence and made annual comparison between sites difficult. However, those methods did not change over time and allowed to compute and compare incidence by sites. During the pilot phase, one country had to decrease the number of participating hospitals and we adjusted the incidence denominator accordingly.

Among the five sites that provided information on other *Bordetella* species, the proportion of patients with other *Bordetella* species among those testing positive for any *Bordetella* species was 11% (40/357) on average, ranging from 0 of 25 to 5 of 18. Two sites were not able to differentiate *B. pertussis* from the other *Bordetella* species that may induce a similar respiratory presentation upon infection. This likely decreased the specificity of the laboratory testing and increased reported incidences.

TABLE 3

Hospitalised *Bordetella pertussis* cases aged < 1 year by ICU admission, clinical presentation, comorbidities and risk/protective factors, PERTINENT study, Europe, 1 December 2015–31 December 2018 (n = 330 cases^a)

Characteristic	Admitted to the ICU		Not admitted to the ICU		p value	
	(n = 83)		(n = 247)			
	Number	%	Number	%		
Clinical presentation						
Cough (n = 330)	Yes	83	100.0	238	96.4	0.119
	No	0	0.0	9	3.6	
Paroxysms among those with cough (n = 321)	Yes	73	88.0	203	85.3	0.714
	No	10	12.0	35	14.7	
Whoop (n = 252)	Yes	17	43.6	101	47.4	0.728
	No	22	56.4	112	52.6	
Post-tussive vomiting (n = 320)	Yes	45	54.2	110	46.4	0.251
	No	38	45.8	127	53.6	
Apnoea (n = 328)	Yes	55	66.3	128	52.2	0.030
	No	28	33.7	117	47.8	
Cyanosis (n = 329)	Yes	59	71.1	106	43.1	0.000
	No	24	28.9	140	56.9	
Comorbidities						
Cardiovascular (n = 330)	Yes	4	4.8	6	2.4	0.278
	No	79	95.2	241	97.6	
Respiratory (n = 330)	Yes	2	2.4	4	1.6	0.644
	No	81	97.6	243	98.4	
Immunodeficiencies (n = 129)	Yes	0	0.0	0	0.0	NA
	No	57	100.0	72	100.0	
Risk/protective factors						
Breastfeeding (n = 327)	Yes	50	62.5	166	67.2	0.497
	No	30	37.5	81	32.8	
Premature 37 weeks (n = 329)	Yes	21	25.6	26	10.5	0.002
	No	61	74.4	221	89.5	

ICU: intensive care unit; PERTINENT: Pertussis in Infants European Network.

^a 330 cases of 469 had information on ICU or non-ICU admission.

Numbers of cases with available information are presented.

PERTINENT laboratories did not so far sequence *B. pertussis* isolates, which prevented detection of changes in the pathogen. Isolates were stored in optimal conditions to be analysed later on.

Severity of the disease remains difficult to interpret because of different severity ascertainment and clinical practices by country and by hospital. Hospitalised cases are usually severe pertussis cases, however, the probability of being hospitalised for pertussis-like symptoms is heterogeneous across sites due to different referral to hospital practices. In France, any infant aged less than 3 months with pertussis-like symptoms will be admitted regardless of the severity of the disease. In contrast, a large proportion of Norwegian infants will first attend the 'out-of-office emergency primary care' services (legevakt) and only severe cases will be transferred to hospitals. This likely affected the comparison of clinical signs and incidence rate of hospitalised severe cases between sites.

The likely source of infection was ascertained by family interviews, which may require caution in interpreting the results. Caregivers were not reported as probable sources for any of the reported pertussis cases. As parents self-reported the likely source of infection, they may have better remembered signs of coughing in the household members than among the infant's caregivers. Additional questions, laboratory confirmation in suspected sources of infection, different study designs with increased data completeness are needed to identify source of infection.

Despite using a standard protocol in all sites, data completeness still needs improvement. In severity variables, completeness ranged from 57% (267/469) to 72% (336/469), except for death ascertainment with a completeness >99% (Table 2).

Taking the above limitations into account, our results suggest a decrease in 2018 in pertussis incidence

compared with 2017, across all sites except for the Czech Republic site where incidence remained stable. The low number of study sites does not allow to extrapolate results to national nor EU/EEA level. However, incidences reported to the European Surveillance System (TESSy) by five of the six countries involved in PERTINENT also suggest a decrease. Incidences reported for hospitalised and non-hospitalised cases in 2018 ranged from 42.9 to 85.3 per 100,000 infants in all infants aged less than 1 year, as compared with a range of 12.9 to 87.6 per 100,000 infants in PERTINENT for hospitalised infants only (excluding Norway where zero cases were observed) suggesting a better sensitivity of the PERTINENT data.

We suggest that the summer peak observed in reported cases might possibly reflect the seasonality of the disease. It is unlikely that this was due to improved diagnosis in those months as the PERTINENT surveillance system was stable over time. An increase in pertussis cases during summer was previously reported. In the Netherlands where pertussis is a statutory notifiable disease, the annual peak incidence of notifications for all age groups (0–4; 5–12; 13–18 and 19–99 years) between 1996 and 2006 was in August [19]. More recently, using the notifiable infectious disease reporting system in China from January 2004 to May 2018, Wang suggested a seasonality in pertussis cases and a summer peak with a maximum in August [20].

Thirty cases did not have a typical pertussis clinical presentation including four with at least one clinical sign not documented and five cases with documented absence of all pertussis-like symptoms (reason for hospitalisation unknown). The EU case definition for pertussis was revised in June 2018 [21] to draw attention on atypical symptoms in adults, adolescents or vaccinated children. Our results may suggest the existence of atypical pertussis in infants [3] and highlight the need to raise clinicians' awareness about possible under-diagnosis of pertussis in that age group. The World Health Organization and United States Centers for Disease Control and Prevention pertussis case definitions do not include isolated apnoea or cyanosis in the clinical criteria for pertussis surveillance. In our study, pertussis cases aged less than 3 months were more likely to present with apnoea or cyanosis, which supports the inclusion of these clinical criteria in the pertussis case definition.

Our results suggest that cases admitted to ICU were younger and less vaccinated than non-ICU cases. In the PERTINENT hospital-based sentinel network, four pertussis deaths were reported in 2017 across the seven study sites. As pertussis cases identified in the study were followed up during hospitalisation, we expect no under-reporting of deaths. Among pertussis cases aged less than 1 year reported to TESSy, there were three pertussis deaths in 2017 across the 29 EU/EEA reporting countries [2]. This may suggest that detection of pertussis hospital death is more sensitive in

the PERTINENT system. As described in other systems, deaths may be under-ascertained in routine hospital based surveillance in EU/EEA countries [22,23].

The most likely source of infection reported by the parents was firstly the patient's siblings followed by the mother. Recent studies have also shown an increased risk of transmission to siblings of primary cases [24] and a shift in the source of infection from the mother to the siblings [25]. This may be a consequence of vaccinating the mother either before, during or after pregnancy (cocooning strategy), therefore preventing transmission to infants.

Bordetella species can be isolated from both nasopharyngeal swabs or aspirates but a 15% gain in the isolation rate can be obtained by using aspirates in neonates and infants [26]. In our study, we reached a good quality of specimen collection with a high proportion of nasopharyngeal aspirates (82%). We identified 40 infections caused by other *Bordetella* species, including four with *B. holmesii*, which is rarely isolated in infants [27]. Even though other *Bordetella* species are not directly targeted by pertussis-containing vaccine, the later may also induce some cross-immunity for specific *Bordetella* species [28].

Conclusions

This pilot project shows that enhanced pertussis surveillance in Europe is possible. The generic protocol presented some challenges and efforts by all partners were needed to improve data quality and laboratory procedures but we believe this allowed to pool sites' data to better describe hospitalised laboratory-confirmed pertussis cases, as these were recruited using the same criteria across six EU/EEA countries. However, a larger sustained project is needed with additional countries to ensure representativeness in Europe and a particular emphasis on harmonisation of laboratory methods. In the future, this surveillance network should allow monitoring emergence of atypical pertussis presentation, identifying upcoming pertussis epidemic cycles and comparing incidence over time in Europe according to immunisation strategies. It will also allow measuring the effectiveness of infants' and mothers' vaccination.

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Conflict of interest

None declared.

Authors' contributions

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This supplementary material is hosted by Eurosurveillance as supporting information alongside the article [Incidence and severity of pertussis hospitalisations in infants aged less than 1 year in 37 hospitals of six EU/EEA countries, results of PERTINENT sentinel pilot surveillance system, December 2015 to December 2018.], on behalf of the authors, who remain responsible for the accuracy and appropriateness of the content. The same standards for ethics, copyright, attributions and permissions as for the article apply. Supplements are not edited by Eurosurveillance and the journal is not responsible for the maintenance of any links or email addresses provided therein.

Table S4. Vaccination recommendations, introduction year and vaccine coverage by PERTINENT study sites, PERTINENT, 1st December 2015 - 31st December 2018

Study sites	Czech Republic	France	Ireland	Italy	Spain, Catalonia	Spain, Navarra	Norway
Primary schedule in infants <1 year	Year introduction	2018	2013	1995	1995	2016	1998
	Schedule	3, 5, 11-13 months	2, 4, 11 months	2, 4, 6 months	3, 5, 11 months	2, 4, 11 months	3, 5, 12 months
	VC % in 2016	94.4%	95.8% at 24 mo	90% at 24 mo	96.8% at 24 mo	94.0%	96% at 2 yo
Pregnancy	Year introduction	2016	no	2013	2017	2014	2015
	VC %	<i>Not determined</i>	NA	49.9% in 2017-2018	<i>Not determined</i>	79% in 2017	89% in 2017
Cocooning	Year introduction	no	2004	2013	no	no	no
	VC %	NA	<50% in 2004-2015	<i>Not determined</i>	NA	NA	NA
Booster doses	Schedule	5-6 yo 10-11 yo	6 yo 11-13 yo	4-5 yo 12-13 yo	6 yo 12-18 yo	6 yo	6 yo
Adults	Schedule	1 dose in life	1 dose every 10 years	no	1 dose every 10 years	no	no
							1 dose every 10 years

3.2. Effectiveness of pertussis primary vaccination in infants

3.2.1. Summary

This chapter presents the second article of this thesis studying the second objective of the PERTINENT study and the estimation of the effectiveness of pertussis primary vaccination using the test-negative design, December 2015-December 2019.

As described in the first chapter of this thesis, infants aged less than 3 months are the age group being the most affected by severe *Bordetella pertussis*. Due to the high morbidity and mortality in this population, the main objective of pertussis vaccination is to reduce the risk of severe pertussis in infants [19] but also to protect infants from the pertussis reservoir in older age groups. In Europe, pertussis vaccination roll-out using wP vaccine occurred in the 1950's and was concomitant with a marked reduction in disease incidence. However, despite a vaccination coverage exceeding 90% for the past two decades [80], pertussis notifications notably increased in 2012, as reported by the ECDC, and continues to circulate since then, posing disease control challenges [23]. Possible reasons include a lower efficacy and duration of protection conferred by aP vaccines (introduced in the 1990's) compared to the traditional wP vaccine series, or possibly improved laboratory methods, increased awareness or bacterial genetic changes [6,81,82].

This work highlights the importance of a representative European pertussis surveillance network in infants using common laboratory methods and diagnosis strategies [14], common clinical disease awareness ensured by site visits, monitoring of *Bordetella pertussis* isolate genomes, and common standardised protocol to estimate VE of primary vaccination in infants. Using data collected as part of the PERTINENT Network, we estimated the dose-specific VE of pertussis PV in infants in Europe based on a "test-negative-design" approach. Over the study period, all sites used the aP vaccine for PV in infants, but vaccination schedule varied across sites.

From December 2015 to December 2019, we tested all hospitalised infants aged <1 year presenting with pertussis-like symptoms. Cases were vaccine-eligible infants testing positive for *Bordetella pertussis* by PCR or culture. Controls were those testing negative to all *Bordetella* spp. For each vaccine dose, we defined an infant as vaccinated if the infant received the corresponding dose >14 days before symptoms onset. Unvaccinated were those who did not receive any dose.

Our findings suggest that having received at least one dose of aP vaccine reduces the risk of being hospitalised for pertussis by 59% (95%CI: 36-73) in infants aged 2-11 months. After only one dose, VE against hospitalisation for pertussis was between 48% and 56%. After two doses, VE was estimated between 73-76%. Due to the small sample sizes, we could not compute VE after three doses nor VE by

time since vaccination. Even though the sample size of each analysis did not allow for precise estimates, we observed an increasing VE from dose to dose with VE estimates consistent with existing literature. Sustaining and expanding the network is needed to consolidate our findings.

This paper has been submitted to *Vaccine* as an original research article.

3.2.2. Article

1 **Title:**

2 *“Effectiveness of one and two doses of acellular pertussis vaccines against laboratory-*
3 *confirmed pertussis requiring hospitalisation in infants: results of the PERTINENT sentinel*
4 *surveillance system in six EU/EEA countries, December 2015 – December 2019.”*

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35 * The members of the network are listed in the Acknowledgments

36 **Keywords**

37 Pertussis, whooping cough, pertussis vaccine, vaccine effectiveness, hospital surveillance.

38 **Abbreviations**

- 39 • PERTINENT: Pertussis in Infants European Network
- 40 • PV: primary vaccination
- 41 • wP vaccine: whole-cell pertussis vaccine
- 42 • aP vaccine: acellular pertussis vaccine
- 43 • VE: vaccine effectiveness

44 **Abstract**

45 **Background**

46 Monitoring effectiveness of pertussis vaccines is necessary to adapt vaccination strategies. PERTINENT,
47 Pertussis in Infants European Network, is an active sentinel surveillance system implemented in 35
48 hospitals across six EU/EEA countries. We aim to measure pertussis vaccines effectiveness (VE) by dose
49 against hospitalisation in infants aged <1 year.

50 **Methods**

51 From December 2015 to December 2019, participating hospitals recruited all infants with pertussis-
52 like symptoms. Cases were vaccine-eligible infants testing positive for *Bordetella pertussis* by PCR or
53 culture; controls were those testing negative to all *Bordetella* spp. For each vaccine dose, we defined
54 an infant as vaccinated if she/he received the corresponding dose >14 days before symptoms.
55 Unvaccinated were those who did not receive any dose. We calculated (one-stage model) pooled VE
56 as $100 \times (1 - \text{odds ratio of vaccination})$ adjusted by country, onset date (in 3-month categories) and age-
57 group (when sample allowed it).

58 **Results**

59 Of 1,393 infants eligible for vaccination, we included 259 cases and 746 controls. Median age was 16
60 weeks for cases and 19 weeks for controls ($p < 0.001$). Median birth weight and gestational age were
61 3,235 g and week 39 for cases, 3,113 g and week 39 for controls. Among cases, 119 (46%) were
62 vaccinated: 74 with one dose, 37 two doses, 8 three doses. Among controls, 469 (63%) were
63 vaccinated: 233 with one dose, 206 two doses, 30 three doses. Adjusted VE after at least one dose was
64 59% (95%CI: 36–73). Adjusted VE was 48% (95%CI: 5–71) for dose one (416 eligible infants) and 76%
65 (95%CI: 43–90) for dose two (258 eligible infants). Only 42 infants were eligible for the third dose.

66 **Conclusions**

67 Our results suggest moderate one-dose and two-dose VE in infants. Larger sample size would allow
68 more precise estimates for dose one, two and three.

69 **Article**

70 **1. Introduction**

71 Pertussis or whooping cough, caused by *Bordetella pertussis*, is a highly contagious vaccine-
72 preventable respiratory disease. It is characterised by a violent cough, and although it can present as
73 a mild disease in adults, the most severe complications usually occur in infants during the first weeks
74 and months of life, when the disease is the most life-threatening.

75 The main objective of the pertussis vaccination programmes today is to reduce the risk of severe
76 pertussis in infants, due to the high morbidity and mortality in this age group [1]. Yeung et al. estimated
77 160,700 pertussis related deaths worldwide in children aged <5 years in 2014, 53% of these deaths
78 occurring in infants aged <1 year [2]. In the pre-vaccine area, pertussis was a very common childhood
79 infectious disease worldwide, causing many deaths every year. Pertussis vaccines containing
80 inactivated whole *B. pertussis* bacterium cell were introduced in the 1950's in Europe and was followed
81 by a substantial decrease of reported cases. Unfortunately, several studies linked the use of whole-cell
82 pertussis (wP) vaccines with serious adverse reactions which led to substantial decrease in vaccination
83 coverage and pertussis resurgence in many countries in the 1970's [3]. In the 1980's, acellular pertussis
84 (aP) vaccines based on purified specific *B. pertussis* antigens were developed. Clinical trials in the
85 1990's suggested that they were safer and provided a similar efficacy as wP vaccines. Most European
86 countries progressively replaced wP with aP vaccines, recommending primary vaccination (PV) with
87 first dose as early as six weeks of age, and a total of three doses in the first year of life. Nevertheless,
88 the primary series varies a lot across countries and can be grouped with the "3p+0" schedule (three
89 primary doses at 2, 4 and 6 months), the "2p+1" schedule (two primary doses at 3 and 5 months or 2
90 and 4 months and a booster dose at 11 or 12 months) and the "3p+1" schedule (three primary doses
91 in the first year of life and a booster dose in the second year) [4].

92 Despite more than 90% coverage for the first three doses in most countries over the last two decades
93 [5], pertussis remains an endemic disease with epidemic peaks every 2–5 years. The last major peak

94 incidence in Europe occurred in 2012 and the European Centre for Disease Prevention and Control
95 (ECDC) reported a substantial increase in pertussis reports in many EU/EEA Member States [6], most
96 notable among infants and adolescents. Many hypotheses for the resurgence were postulated,
97 including improved diagnostic methods and disease awareness, or genetic changes in the organism
98 [7]. Additional studies suggested that aP vaccination might be less effective and lead to faster waning
99 of vaccination-induced immunity than the traditional wP vaccination [3,8]. Since then, the overall
100 notification rate remained high in many countries, the causal bacterial agent continues to circulate and
101 there are still significant challenges to controlling pertussis in Europe [6]. It remains one of the world's
102 leading causes of vaccine-preventable deaths with more than 150,000 cases of pertussis reported
103 globally in 2018 according to the World Health Organisation (WHO) [9]. According to the ECDC, infants
104 continued to be the group with the highest notification rate in all EU/EEA Member States in that same
105 year, except for Estonia and Norway [6]. From 2020, a dramatic decline in pertussis incidence was
106 observed at the EU/EEA level concomitant with the COVID-19 pandemic [10].

107 In response to the evolving epidemiology of the disease, from September 2015 to January 2020, the
108 ECDC created and funded PERTINENT, "Pertussis in Infants European Network", a multi-country
109 hospital-based active sentinel surveillance system to measure pertussis incidence and vaccine
110 effectiveness (VE) in infants aged <1 year [11].

111 In this article, we present the PERTINENT VE estimates against hospitalisation for laboratory-confirmed
112 pertussis after at least one dose of PV; after only one dose of PV and after two doses of PV in infants
113 eligible for PV and based on a prospective test-negative design (TND) [12].

114 **2. Methods**

115 **2.1. Study sites**

116 Seven study sites from six European countries participated in PERTINENT: Czech Republic, France,
117 Ireland, Italy, Norway, Catalonia and Navarra regions in Spain. All sites complied with the generic
118 PERTINENT sentinel surveillance and VE protocols [13] and laboratory guidelines [14]. We organised

119 site visits and a laboratory workshop to ensure harmonisation of practices and allow pooling of sites'
120 data.

121 From late 2015 to early 2016, all study sites gradually implemented the generic protocol and initiated
122 pertussis active surveillance in the 40 participating hospitals. The French site accounted for a large
123 proportion of hospitals (n = 21) located throughout the country. In 2018, four out of the five Norwegian
124 hospitals had to withdraw from the PERTINENT project. By February 2019, the remaining Norwegian
125 hospital had to leave the study due to surveillance challenges.

126 All sites used the aP vaccine for PV in infants, but vaccination schedule varied across sites (Table 1).

127 **2.2. Study population and eligibility criteria**

128 The study population consisted of all infants aged <1 year, likely to be hospitalised in one of the
129 participating hospitals if developing the following pertussis-like symptoms.

130 We raised participant hospital physicians' awareness of pertussis clinical presentation and asked them
131 to test all hospitalised infants aged <1 year presenting with apnoea or cough associated with at least
132 one symptom of paroxysmal cough, whoop or post-tussive vomiting. Infants with any respiratory
133 symptoms and an epidemiological link with a pertussis confirmed case or those not meeting the above
134 clinical presentation but with clinical suspicion for pertussis by a physician were also tested for
135 pertussis.

136 We asked parents or legal guardians of all infants tested for pertussis to participate in the study. When
137 required by site-specific research ethics committee, hospital teams requested an informed consent.

138 We restricted the analysis to infants eligible for vaccination according to sites' national immunisation
139 recommendations.

140 **2.3. Laboratory methods**

141 Since aP vaccines are prepared with *B. pertussis* major toxins and antigens, we asked the hospital
142 laboratories to ensure an accurate identification of the *Bordetella* species. The PERTINENT diagnostic

143 algorithm included a triplex quantitative PCR (qPCR): first targeting IS481 gene (in *Bordetella pertussis*,
144 *Bordetella holmesii*, and some *Bordetella bronchiseptica* strains), pIS1001 (*Bordetella parapertussis*-
145 specific) and RNase P as the human internal control and two confirmatory singleplex tests for
146 *Bordetella pertussis* (*ptxA-Pr*) and *Bordetella holmesii* (*hIS1001*) if IS481 was positive [14].

147 **2.4. Test-negative design and vaccination definition**

148 We conducted a multi-centre case control study using TND in the participating hospitals. We defined
149 a laboratory-confirmed *B. pertussis* case as an infant with suspicion of pertussis infection and testing
150 positive for *B. pertussis* by PCR (DNA detection of *B. pertussis* in a nasopharyngeal aspirate or swab) or
151 culture (isolation of *B. pertussis* from the prior-mentioned clinical specimen). Test-negative controls
152 were those testing negative to all *Bordetella* species by PCR or culture. Due the team's availability
153 constraints in the Catalan hospital, control recruitment was limited to the inclusion of three controls
154 per case using a systematic consecutive approach based on the date of specimen collection.

155 For each aP vaccine dose, we defined an infant as vaccinated if she/he had received the corresponding
156 dose > 14 days before symptom onset. Unvaccinated infants were those who had not received any
157 dose.

158 **2.5. Exclusion criteria**

159 We excluded all infants with missing information for laboratory results, date of onset, or vaccination
160 status. We also excluded infants with contra-indication for pertussis vaccination, those sampled >4
161 weeks after symptom onset, those testing positive to other *Bordetella* species than *B. pertussis*, those
162 with previous laboratory confirmed pertussis episode and those whose legal guardian did not give
163 consent to participate. Infants who did not meet the study eligibility criteria and testing negative to *B.*
164 *pertussis* were excluded from the analysis. We also excluded infants who received the first dose of PV
165 within 14 days before symptom onset.

166 **2.6. Analysis**

167 **2.6.1. VE after at least one dose of PV**

168 For the estimation of VE after at least one dose of aP vaccine, we restricted the analysis to infants
169 eligible to any of the three doses of PV and aged 2–11 months.

170 We described cases and controls by clinical presentations, severity, risk and protective factors (Table
171 2). We used Fisher’s exact test to compare those characteristics between cases and controls.

172 We compared the odds of vaccination with at least one dose between cases and controls. Based on
173 pooled site-specific data, we used a one-stage model with study site as a fixed effect. Using logistic
174 regression, we estimated the odds ratio (OR) and adjusted for date of symptom onset (in 3-month
175 categories) and age group (2, 3–11 months). We computed VE as 1 minus the adjusted OR, expressed
176 as a percentage and with 95% confidence intervals.

177 **2.6.2. One-dose VE**

178 To estimate VE after only one dose of pertussis PV, we excluded all infants who received more than
179 one dose. We estimated one-dose VE in infants aged 2–11 months adjusting for date of symptom onset
180 (in 3-month categories) and age group (2, 3, 4, 5–11 months).

181 This sub-population includes infants in the target age-group for the second, third or even fourth dose
182 (i.e., booster dose). To allow for more accurate one-dose VE estimate, we restricted the analysis to
183 infants in the target age group for the first dose only, according to sites’ national immunisation
184 schedule. We estimated one-dose VE in infants aged 2–5 months using logistic regression including
185 site as fixed effect and adjusted for date of symptom onset (in 3-month categories) and age group (2,
186 3, 4–5 months).

187 **2.6.3. Two-dose VE**

188 To estimate VE after two doses of pertussis PV, we excluded all infants who received one dose only,
189 those who received the second dose within 14 days before symptom onset and those who received

190 more than two doses. We estimated two-dose VE in infants aged 2–11 months adjusting for date of
191 symptom onset (in 3-month categories) and age group (2, 3–11 months).

192 This sub-population includes infants in the target age-group for the first but also the third or even the
193 fourth dose. To allow for more accurate two-dose VE estimate, we restricted the analysis to infants in
194 the target age group for the second dose only, according to sites' national immunisation schedule. We
195 estimated two-dose VE in infants aged 3–10 months using logistic regression including site as fixed
196 effect and adjusted for date of symptom onset (in 3-month categories). Sample size did not allow
197 adjustment for age group. When the number of cases per parameters in logistic regression was less
198 than ten, we carried out a sensitivity analysis using Firth's method of penalised regression to correct
199 for small sample bias [15].

200 **2.7. Data collection**

201 Using a standardised questionnaire, we collected a common set of information: demographic, clinical
202 and laboratory data, vaccination status of the infant, severity, risk and protective factors (Table 2).
203 Data were collected through review of clinical case-patient notes, vaccination cards, interviews with
204 parents or legal guardians, and extraction from patient registries.

205 **2.8. Ethical statement**

206 Each site complied with the local ethical procedures. The planning, conduct and reporting of the study
207 was in line with the Declaration of Helsinki [16]. Ethical approval was not needed in Navarra as the
208 PERTINENT study was considered part of the mandatory surveillance system. Other study sites sought
209 ethical approval from a review board according to country-specific regulations (Catalonia: PIC-31-16,
210 Czech Republic: SZU/05992/2019, France: CNIL authorisation for RENACOQ on 17 June 1996 and order
211 published in the Official Gazette (BO) no. 96/31, Ireland: Royal College of Physicians in Ireland REC
212 reference number 16.058 and Gen/499/16, Italy: Bambino Gesù Children's Hospital Ethical Committee:
213 protocol n. 1064_OPBG_2016, Norway: Regional Committees for Medical and Health Research Ethics,
214 South-East A (2015/956)).

215 3. Results

216 From 1 December 2015 to 31 December 2019, we screened and tested for *B. pertussis* 1,393 infants
217 eligible for vaccination and aged 2–11 months, attending a PERTINENT hospital with pertussis-like
218 symptoms.

219 3.1. VE after at least one dose of PV

220 After applying the exclusion criteria for the analysis of the VE after at least one dose of PV, we included
221 1,005 infants eligible for any dose, with 259 cases and 746 controls (Figure 1a). The ratio of the number
222 of controls per case ranged from 1.2 in the Czech study site, up to 248 controls per case in the
223 Norwegian site.

224 Over this four-year study period, the number of *B. pertussis* cases by month of symptom onset was
225 highest in August 2016 (n = 12), June 2017 (n = 16), July 2017 (n = 12) and August 2018 (n = 11). The
226 highest number of controls was over the periods February–March–April 2016 (n = 34, 38 and 38),
227 November–December 2016–January 2017 (n = 38, 62 and 38) and November 2017–December 2017 (n
228 = 30 and 29) (Figure 2a). Regardless of the year, August was the month of the year with the highest
229 mean number of *B. pertussis* cases with symptom onset (n = 8.5). February–March were the months of
230 the year with the highest mean number of *B. pertussis* negative controls with symptom onset (n = 22.3
231 and 22.8, respectively) (Figure 2b).

232 Although additional laboratory tests were not conducted systematically for every patient nor reported
233 by all study sites and all hospitals, test results for Respiratory Syncytial Virus (RSV) were available for
234 46 cases and 384 controls. Out of them, 10 cases (22%) and 202 controls (53%) were positive to RSV (p
235 < 0.001). Rhinovirus tests results were available for 50 cases and 273 controls and included 37 cases
236 (74%) and 123 controls (45%) positive ($p < 0.001$).

237 Out of the 253 cases and 734 controls with available type of specimen collection, 57 cases (23%) and
238 243 controls (33%) were diagnosed based on a nasopharyngeal swab collection only ($p = 0.002$) (Table
239 2).

240 One-hundred-forty-one cases (54%) and 386 controls (52%) were males ($p = 0.471$). The median age
241 at inclusion was 16 weeks for cases versus 19 weeks for controls ($p < 0.001$). The median birth weight
242 was 3,235 g (range: 700–4,780 g; interquartile range [IQR]: 780 g) for cases versus 3,113 g (range: 640–
243 5,006 g; IQR: 830 g) for controls ($p = 0.045$). The median gestational week at birth was 39 for both
244 cases (range: 25–42; IQR: 2) and controls (range: 24–43; IQR: 3) ($p = 0.215$). Out of the 257 cases and
245 497 controls with reported gestational week, 41 cases (16%) and 115 controls (23%) were born before
246 37 weeks ($p = 0.023$) (Table 2); 14 cases (5%) and 37 controls (7%) were born before 32 weeks ($p =$
247 0.360); 3 cases (1%) and 13 controls (3%) were born before 28 weeks ($p = 0.286$). Information being
248 available for 245 cases and 615 controls, 104 cases (42%) and 141 controls (23%) had a regular
249 babysitter at home ($p < 0.001$). Most of them were reported by the French study site (97% of cases
250 and 90% of controls). Excluding this site, only 3 cases (2%) and 14 controls (3%) were reported as having
251 a regular babysitter ($p = 0.074$). The proportion of cases and controls with risk and protective factors
252 such as delivery type, childcare and breastfeeding were similar.

253 Among cases, 119 (46%) were vaccinated: 74 with one dose, 37 two doses, 8 three doses. Among
254 controls, 469 (63%) were vaccinated: 233 with one dose, 206 two doses, 30 three doses. VE after at
255 least one dose, adjusted for study site, date of symptom onset (in 3-month categories) and age groups
256 (2, 3–11 months) was 59% (95%CI: 36–73) (Table 3a).

257 Out of the 182 cases with available information about ICU admission, 8 (27%) of the ICU cases and 72
258 (47%) of the non-ICU cases were vaccinated with at least one dose ($p = 0.044$).

259 **3.2. One-dose VE**

260 Out of the 1,005 infants eligible for any dose of PV, we excluded all infants vaccinated with more than
261 one dose. In this one-dose analysis, we included 210 *B. pertussis* cases and 476 controls aged 2–11
262 months (Figure 1b.1). Seventy cases (33%) and 199 controls (42%) were vaccinated with only one dose
263 of PV > 14 days before symptom onset ($p = 0.042$).

264 Over the study period, the Norwegian site did not report any cases either unvaccinated or vaccinated
265 with only one dose. Excluding this study site (135 controls including 53 vaccinated), one-dose VE
266 adjusted for site, date of symptom onset (in 3-month categories) and age group (2, 3, 4 months and
267 5–11 months) was 56% (95%CI: 28–73) (Table 3b.1).

268 According to participating countries immunisation recommendations, the above-mentioned one-dose
269 VE analysis includes infants already in the age group targeted for the second dose (n = 170), the third
270 dose (n = 14) and even the fourth dose of PV (n = 2). Restricting the analysis among infants eligible for
271 the first dose of PV only, we included 155 cases and 345 controls (Figure 1b.2). Excluding the
272 Norwegian site (84 controls including 9 vaccinated), 45 cases (29%) and 91 controls (35%) had received
273 only one dose (p = 0.236). Adjusted one-dose VE was estimated at 48% (95%CI: 5–72) in infants aged
274 2–5 months (Table 3b.2).

275 **3.3. Two-dose VE**

276 Out of the 1,005 infants eligible for any dose of PV, we excluded all infants who received either one or
277 three doses. In this two-dose analysis, we included 175 *B. pertussis* cases and 476 controls aged 2–11
278 months (Figure 1c.1). Thirty-five cases (20%) and 199 controls (42%) were vaccinated with two doses
279 of PV > 14 days before symptom onset (p < 0.001).

280 Two-dose VE adjusted for site, date of symptom onset (in 3-month categories) and age group (2, 3–11
281 months) was 73% (95%CI: 50–86) (Table 3c.1).

282 According to participating countries immunisation recommendations, the above-mentioned two-dose
283 VE analysis includes infants in the age group targeted for the first dose (n = 355) and not yet vaccinated
284 at all, in the age group targeted for the third dose (n = 32) and even the fourth dose of PV (n = 2).
285 Restricting the analysis among infants eligible for the second dose of PV only, we included 56 cases
286 and 206 controls (Figure 1c.2). Among them, 32 cases (57%) and 180 controls (87%) had received two
287 doses (p < 0.001).

288 Over the study period, the Irish site did not report any cases in the age group targeted for the second
289 dose only. Excluding this study site (4 vaccinated controls), adjusted two-dose VE was estimated at
290 76% (95%CI: 43–90) in infants aged 3–10 months using penalised logistic regression (Table 3c.2).

291 **4. Discussion**

292 Four years of active surveillance in 40 to 35 participating hospitals from 6 EU/EEA countries allowed us
293 to include 1,005 infants eligible for any dose of PV in the PERTINENT VE study. Our results suggest that
294 having received at least one dose of aP vaccine reduces the risk of being hospitalised for pertussis by
295 almost 60% in infants aged 2–11 months. Additionally, this comprehensive study served as a basis for
296 conducting several dose-specific effectiveness analyses. Our findings indicate that receiving only one
297 dose of aP vaccine halves the risk of being hospitalised for pertussis in infants eligible for the first dose
298 only and aged 2–5 months. After two doses, VE was estimated between 73–76%. Due to sample size
299 limitations, we did not compute VE after three doses, nor VE by time since vaccination nor by vaccine
300 brand. Even though the sample size of each analysis did not allow for precise estimates, we observed
301 an increasing VE from dose to dose with VE estimates aligned with existing literature. In 2014, based
302 on an Australian matched case-control study, Quinn et al. estimated that one-dose VE against
303 hospitalisation was 55% (95%CI: 43–65) in infants <4 months and two-dose VE against hospitalisation
304 was 83% (95%CI: 70–90) in infants <6 months of age [17]. Using a population-based retrospective case-
305 control study design in a more recent study in Switzerland, Mack et. al. estimated that one-dose VE
306 against hospitalisation was 42% (95%CI: 11–63) and two-dose VE against hospitalisation was 84%
307 (95%CI: 70–92) [18].

308 Our study is subject to several limitations. Despite the implementation of a standardised generic
309 protocol harmonising practices which enabled pooling of site data, there was a high heterogeneity
310 between PERTINENT study sites in terms on national vaccination recommendations (Table 1) but also
311 in terms of recruitment capacities. In the Catalan site, control recruitment was limited to three controls
312 per case, as described in the protocol. But some sites such as the Czech, French and Italian sites, hardly

313 managed to reach two controls per recruited case. In the Norwegian site, 248 controls were recruited
314 while only one case vaccinated with two doses met the eligibility criteria. Additionally, sample sizes
315 were too small to measure VE by study site, and we used a one-stage approach on pooled data instead,
316 with study site as fixed effect. In such analysis, we assume that the VE are the same in all sites, which
317 is unlikely in our settings due to differences of vaccine brand, vaccine schedule, age at first dose,
318 differences in circulating *Bordetella* strains or immunisation recommendations in adults. Therefore,
319 larger sample size is required to estimate site-specific VE, statistical heterogeneity between sites and
320 perform a “two-stage” model analysis including the confounding factors of interest.

321 Building upon the pilot study conducted by the PERTINENT Network which suggested a mild seasonality
322 of the disease during summer [11,19], in this VE study, we observed a distinct counter-cyclical
323 seasonality pattern between *B. pertussis* cases and controls, with peaks of disease incidence occurring
324 at opposite times compared to control incidence. During periods of low recruitment of *B. pertussis*
325 cases (i.e., winter period), there was a notable increase in the number of recruited controls.

326 Controls were more likely than cases to present with an RSV infection, aligning with the winter
327 seasonality observed during control recruitment. RSV is known to circulate predominantly during late
328 autumn, winter and early spring each year [20]. Date of symptom onset was an important potential
329 confounding factor that we have strived to include with the highest precision possible in the VE
330 estimation. In terms of co-infections, *B. pertussis* cases were more likely than controls to present with
331 a co-infection with rhinovirus. While studies have confirmed the occurrence of RSV and *B. pertussis* co-
332 infection in infants, limited information is available regarding rhinovirus co-infection [21,22]. This
333 potential bias in clinical presentation needs to be quantified.

334 Given the possibility of pertussis atypical presentation in infants [11], we asked hospital teams to test
335 for pertussis and include in the study any infants suspected for pertussis, even though some typical
336 symptoms were missing [23]. However, clinicians may be more likely or less likely to test suspected

337 pertussis cases according to vaccination status leading to selection bias. Including more unvaccinated
338 infants may lead to an increase of unvaccinated cases in the study and an overestimation of the VE.

339 As described in the methods, all infants presenting with pertussis-like symptoms received a
340 nasopharyngeal aspirate or swab that was then tested for pertussis by PCR or culture. However,
341 nasopharyngeal swabs can be less sensitive than aspirate to isolate *B. pertussis* in infants [24]. Inclusion
342 of false-negatives could lead to misclassification of unvaccinated cases as unvaccinated controls and
343 an underestimation of the VE. A larger sample size is needed to perform sensitivity analysis excluding
344 infants diagnosed only based on nasopharyngeal swabs.

345 TND is commonly used for assessment of influenza VE. Nevertheless, it is crucial to ascertain whether
346 this design constitutes an appropriate methodology for estimating VE against severe pertussis in
347 infants. To the best of our knowledge, we believe that this is the first prospective TND study at
348 European level and in hospital settings, implemented to estimate VE against severe pertussis in infants.
349 The main hypothesis of TND resides in the representation of the control group. Controls, consisting of
350 infants hospitalised for pertussis-like symptoms but diagnosed with alternative respiratory illnesses
351 (e.g., RSV), should mirror the pertussis vaccination experience of the source population. To confirm
352 that the likelihood of hospitalisation for non-pertussis respiratory infection is similar amongst both
353 vaccinated and unvaccinated infants, implementation of large ad-hoc cohort studies in Europe or
354 vaccination coverage studies in specific hospital catchment areas would be needed. Unfortunately,
355 such studies were not feasible at the PERTINENT network level.

356 PERTINENT dose-specific VE estimates tend to be lower than previous estimations based on different
357 study designs. One of the first studies estimating VE against hospitalisation due to pertussis in infants
358 was performed in 2002 in Germany, early after the introduction of aP vaccine in the country. Based on
359 a modified screening method, Juretzko and colleagues found a dose-dependent increase of VE against
360 hospitalised laboratory-confirmed pertussis with a one-dose VE of 68% and a two-dose VE of 92% in
361 infants [25]. However, comparing these estimates from the 2000's at the hospital level with those from

362 recent studies is challenging, mainly because of potential variations in hospitalisation behaviour over
363 time. Even though our VE estimates are aligned with the recent literature, confidence intervals are
364 large and we cannot conclude about a potential lower effectiveness that could explain the pertussis
365 resurgence observed over the past ten years in Europe, before the COVID-19 pandemic. However, it is
366 crucial to improve laboratory diagnostic methods across Europe to ensure the accurate differentiation
367 of *B. pertussis* from other *Bordetella* species. Although aP vaccine may confer cross-immunity against
368 other *Bordetella* thanks to some common virulence factors [26], it initially targets *B. pertussis* antigens.
369 Culturing the pathogen and sequencing its genome are also key to monitor genetic variations in
370 pertussis pathogens induced by vaccine selection pressure [7,27]. Considering other *Bordetella* species
371 in VE studies as well as describing the ongoing genetic shift in the *B. pertussis* organism, for instance
372 lacking pertactin (PRN), a common aP antigen [28], are factors that could contribute to a potential
373 lower VE and further map the circulation of the pathogen.

374 **5. Conclusion**

375 While existing literature includes several case-control studies investigating dose-specific acellular
376 pertussis VE against hospitalisation, our study stands out as the first independent and multi-country
377 pertussis VE study in infants within the EU/EEA region, using TND in hospital settings.

378 Despite the concerning resurgence of pertussis in recent decades, our findings indicate that aP vaccine
379 continues to offer a good effectiveness against hospitalisation for pertussis in infants aged 2–11
380 months. To further enhance protection for this vulnerable population, the consideration of pertussis
381 vaccination during pregnancy is essential to protect younger infants aged <2months who are not yet
382 eligible to receive the first dose of aP vaccine. This vaccination strategy was also observed as protective
383 in the context of the PERTINENT study [29]. Addressing this immunisation gap is of utmost importance
384 for this age group with the highest risk of severe complications and mortality.

385 The implementation and sustainability of a large hospital-based surveillance network in Europe for all
386 respiratory diseases including pertussis in infants, is crucial and can serve as a foundation for numerous

387 VE studies. Such studies are necessary to investigate the diverse immunisation strategies currently
388 implemented in EU/EEA (e.g., the so-called “2p+1” vs. “3p+1” primary course [6], vaccination in
389 pregnancy, cocooning strategy in adults, etc.). Expanding the PERTINENT Network to increase our
390 analysis sample sizes could allow for more robust and precise VE estimates, but also for estimating VE
391 in fully immunised infants, VE by vaccine product, and for addressing the concerning aP vaccine waning
392 immunity.

393 Additionally, the surveillance network has provided an opportunity to describe the circulating
394 *Bordetella* species, and could support monitoring their potential genetic evolution and the impact of
395 changes of vaccination strategies [30]. Even though most EU/EEA countries have sustained high routine
396 immunisation coverage during the COVID-19 pandemic, especially for DTP, a significant drop in
397 coverage worldwide was observed [31]; the impact of this drop needs to be closely monitored.

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493 **Conflict of interest**

494 No conflict of interest to declare except for Elmira Flem who has been employed since April 2019 by
495 Merck & Co., Inc., North Wales, PA, USA. The work for the current study was conducted by Dr. Flem
496 under the previous affiliation at the Norwegian Institute of Public Health.

497 **6. Illustrations**

498 **Table 1.** Characteristics of PERTINENT study sites, current vaccination recommendations during
 499 pregnancy and for the primary schedule, Europe, 1st December 2015–31st December 2019.

Study sites	Vaccination recommendations						Number of hospitals participating in PERTINENT	
	Year of introduction	Primary schedule in infants			Pregnancy			
		1 st dose	2 nd dose	3 rd dose	VC % in 2016 ^a			
				1 st dose	3 rd dose	Year of introduction		
Czech Republic	2018 ^b	3 ^b	5 ^b	11-13 ^b	98%	96%	2016	6
France	2013	2	4	11	99%	96%	2022	21
Ireland	1995	2	4	6	98%	95%	2013	2
Italy	1995	3	5	11	95%	94%	2017	1
Spain, Catalonia	2016 ^c	2 ^c	4 ^c	11 ^c	98%	97%	2014	1
Spain, Navarra	2016 ^c	2 ^c	4 ^c	11 ^c			2015	
Norway	1998	3	5	12	99%	96%	No	5 (2015-2017) 1 (2018-2019)

500 PERTINENT: Pertussis in Infants European Network ; VC : Vaccination coverage.

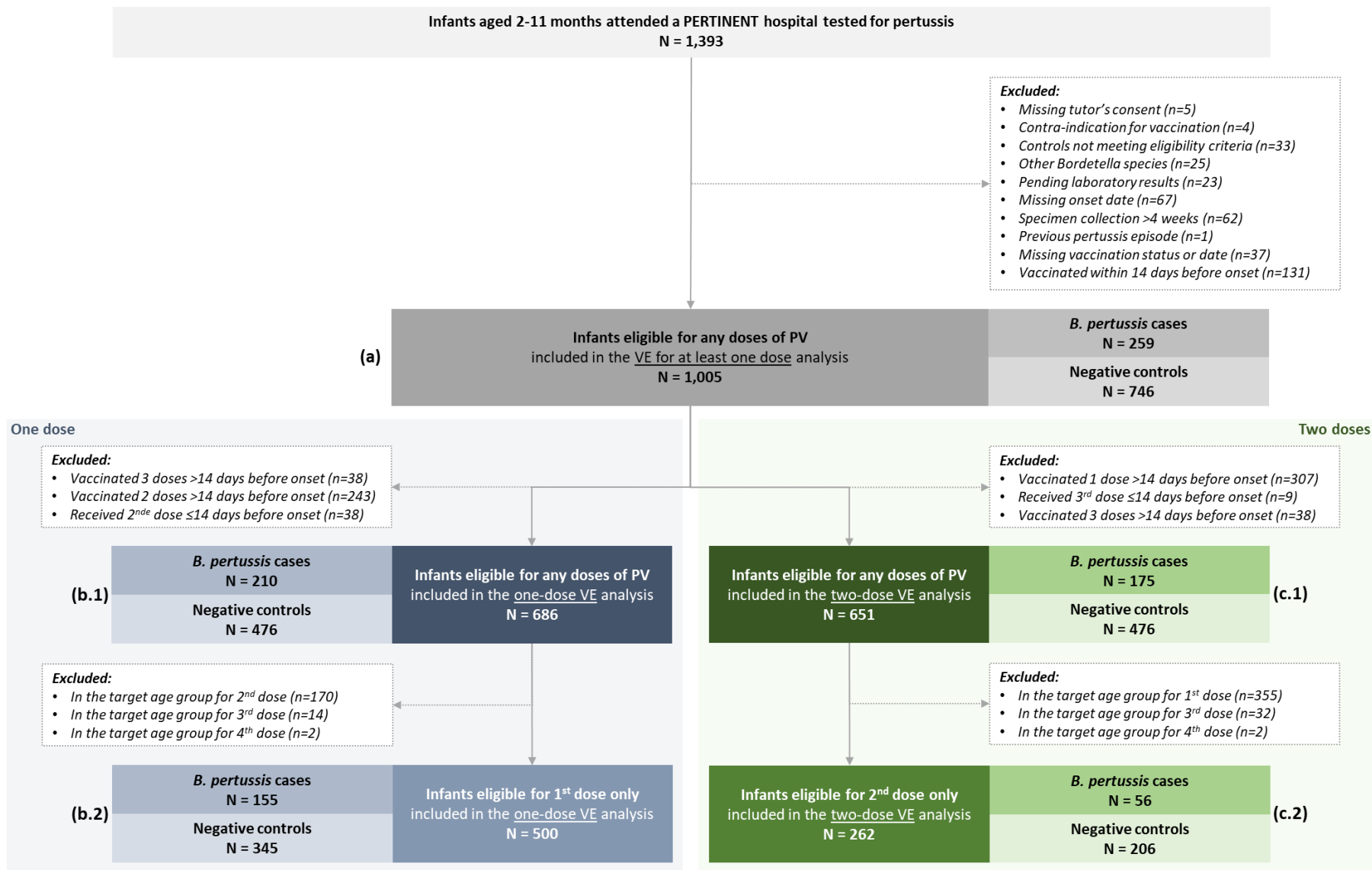
501 ^a Diphtheria tetanus toxoid and pertussis (DTP) vaccination coverage [Internet]. [cited 2023 Jun 7]. Available from:
 502 [https://immunizationdata.who.int/pages/coverage/dtp.html?CODE=CZE+FRA+IRL+ITA+ESP+NOR&ANTIGEN=DTPCV3+DTPCV1&YEAR=.](https://immunizationdata.who.int/pages/coverage/dtp.html?CODE=CZE+FRA+IRL+ITA+ESP+NOR&ANTIGEN=DTPCV3+DTPCV1&YEAR=)
 503

504 ^b Before 2018: doses at 2, 3, 4 and 10 months.

505 ^c Before 2016: doses at 2, 4 and 6 months.

506
507
508

509 **Figure 1.** Flowchart of hospitalised infants inclusion in or exclusion from the five analyses performed:
 510 (a) VE after at least one dose in infants 2-11 months; (b.1) One-dose VE in infants 2-11 months; (b.2)
 511 One-dose VE in infants 2-5 months; (c.1) Two-dose VE in infants 2-11 months; (c.2) Two-dose VE in
 512 infants 3-10 months of pertussis primary vaccination, PERTINENT study, Europe, 1st December 2015–
 513 31st December 2019.

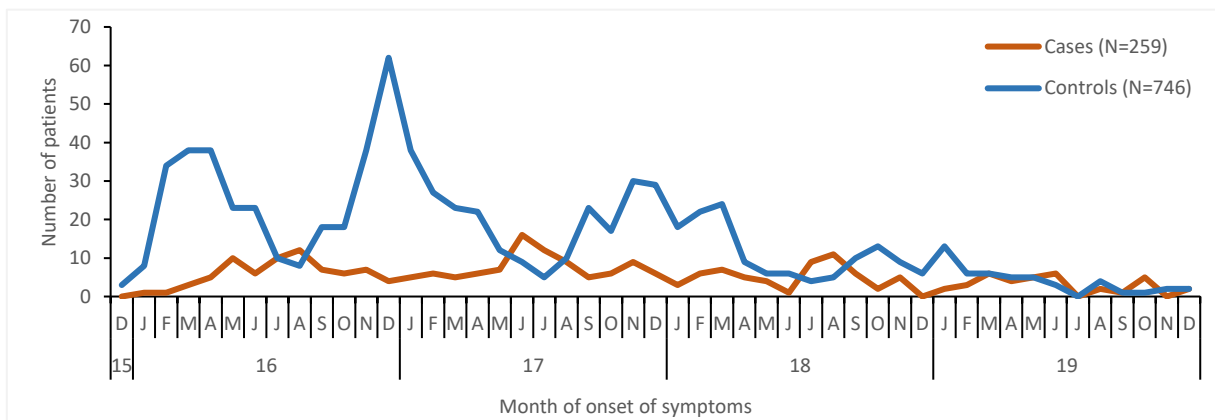


514

515 PERTINENT: Pertussis in Infants European Network; PV: primary vaccination; VE: vaccine effectiveness.

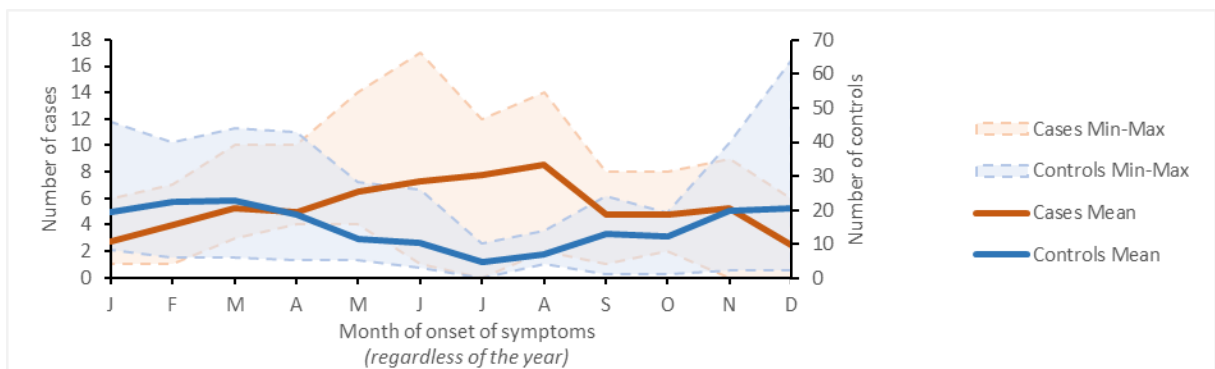
516 **Figure 2.** *Bordetella pertussis* cases (N = 259) and controls (N = 746) (a) by month and year of
 517 symptom onset, (b) by month of symptom onset (*regardless of the year*), hospitalised infants aged
 518 2-11 months, PERTINENT study, Europe, 1st December 2015–31st December 2019.

519 (a)



520

521 (b)



522

523 **Table 2.** Characteristics of *Bordetella pertussis* cases and controls by sex, laboratory components,
 524 clinical presentation, severity and risk/protective factors, hospitalised infants aged 2-11 months,
 525 PERTINENT study, Europe, 1st December 2015–31st December 2019.

Characteristics		Cases (n = 259)		Controls (n = 746)		p value
		N	%	N	%	
Demographic						
Sex	Female	118	45.6	360	48.3	0.471
	Male	141	54.4	386	51.7	
Laboratory						
Nasopharyngeal specimen collection	Aspirate or both aspirate and swab	196	77.5	491	66.9	0.002
	Swab only	57	22.5	243	33.1	
Clinical criteria						
Cough	Yes	256	98.8	720	96.8	0.115
	No	3	1.2	24	3.2	
Cough with paroxysms	Yes	227	87.6	487	68.3	<0.001
	No	32	12.4	226	31.7	
Whoop	Yes	84	52.8	80	13.1	<0.001
	No	75	47.2	531	86.9	
Post-tussive vomiting	Yes	120	47.2	384	53.0	0.126
	No	134	52.8	341	47.0	
Apnoea	Yes	126	48.6	163	22.2	<0.001
	No	133	51.4	571	77.8	
Cyanosis	Yes	123	47.7	127	17.2	<0.001
	No	135	52.3	610	82.8	
Epidemiological link	Yes	100	39.4	12	2.4	<0.001
	No	154	60.6	483	97.6	
Diagnosis by a clinician	Yes	205	79.5	216	29.3	<0.001
	No	53	20.5	522	70.7	
Severity						
Death	Yes	1	0.4	1	0.1	0.451
	No	257	99.6	738	99.9	
ICU	Yes	30	16.5	29	7.5	0.002
	No	152	83.5	357	92.5	
ECMO	Yes	3	1.6	0	0.0	0.011
	No	180	98.4	627	100.0	
Pneumonia	Yes	11	6.1	26	6.8	0.856
	No	170	93.9	356	93.2	
Encephalopathy	Yes	2	1.1	0	0.0	0.103
	No	179	98.9	382	100.0	
Seizure	Yes	6	3.3	1	0.3	0.005
	No	175	96.7	380	99.7	
Eating difficulties	Yes	34	21.8	277	46.5	<0.001
	No	122	78.2	319	53.5	
Kidney failure	Yes	3	1.9	0	0.0	0.029
	No	156	98.1	359	100.0	

Dehydration	Yes	8	4.7	55	9.6	0.04
	No	162	95.3	516	90.4	
Risk factors						
Premature <37 weeks	Yes	41	16.0	115	23.1	0.023
	No	216	84.0	382	76.9	
Delivery type	Vaginal	184	73.0	335	69.8	0.392
	C-section	68	27.0	145	30.2	
Episode in pregnancy	Yes	2	1.4	1	0.3	0.209
	No	145	98.6	350	99.7	
Infant going to day care	Yes	21	8.2	71	10.6	0.326
	No	234	91.8	600	89.4	
Infant with babysitter	Yes	104	42.4	141	22.9	<0.001
	No	141	57.6	474	77.1	
Infant staying regularly with grandparents	Yes	71	28.2	99	20.1	0.016
	No	181	71.8	394	79.9	
Protective factors						
Breastfeeding	Yes	168	65.6	465	64.5	0.761
	No	88	34.4	256	35.5	
Mother vaccination in pregnancy	Yes	84	35.4	215	47.8	0.002
	No	153	64.6	235	52.2	
Vaccinated at least 1 dose	Yes	119	45.9	469	62.9	<0.001
	No	140	54.1	277	37.1	
Number of doses	1 dose	74	28.6	233	31.2	<0.001
	2 doses	37	14.3	206	27.6	
	3 doses	8	3.1	30	4.0	

526

527 **Table 3.** Adjusted vaccine effectiveness of the five analyses performed: (a) VE after at least one dose
528 in infants aged 2-11 months; (b.1) One-dose VE in infants 2-11 months; (b.2) One-dose VE in infants
529 2-5 months; (c.1) Two-dose VE in infants 2-11 months; (c.2) Two-dose VE in infants 3-10 months of
530 pertussis primary vaccination, in hospitalised infants, PERTINENT study, Europe, 1 December 2015–
531 31 December 2019 (N = 1,005).

Adjustment variables	Df	N	Cases		Controls		Adjusted VE (95% CI)
			Vacc.	N	Vacc.	N	
VE after at least one dose							
(a)	<i>Infants eligible for any dose of PV (2–11 months; 7 sites; N = 1,005)</i>						
Site; Onset date (3-month); Age group (2, 3-11 months)	16	1,005	119	259	469	746	59 (36-73)
One-dose VE							
(b.1)	<i>Infants eligible for any dose of PV (2–11 months; 6 sites; N = 551)</i>						
Site; Onset date (3-month); Age group (2, 3, 4, 5-11 months)	16	551	70	210	146	341	56 (28-73)
(b.2)	<i>Infants eligible for 1st dose only (2–5 months; 6 sites; N = 416)</i>						
Site; Onset date (3-month); Age group (2, 3, 4-5 months)	15	416	45	155	91	261	48 (5-72)
Two-dose VE							
(c.1)	<i>Infants eligible for any dose of PV (2–11 months; 7 sites; N = 651)</i>						
Site; Onset date (3-month); Age group (2, 3-11 months)	15	651	35	175	199	476	73 (50-86)
(c.2)	<i>Infants eligible for 2nd dose only (3–10 months; 6 sites; N = 258)</i>						
Site; Onset date (3-month)	13	258	32	56	176	202	76 (43-90)

532 Df: degree of freedom; VE: vaccine effectiveness; CI: confidence interval; PV: primary vaccination.

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621

3.3. Effectiveness of pertussis vaccination in pregnancy

3.3.1. Summary

This chapter presents the third article of this thesis studying the effectiveness of vaccination in pregnancy. Using data collected as part of the PERTINENT Network, we measured first the effectiveness of vaccination in pregnancy to prevent hospitalisation in infants aged <2 months; second the effectiveness of both infant's PV and mother's vaccination in pregnancy to prevent hospitalisation in older infants, aged 2-11 months.

As described in the first chapter of this thesis, infants too young to be eligible for PV harbour the highest risk of severe illness and related deaths. Based on evidence of transplacental transfer of maternal antibodies, vaccination of pregnant women aims to protect their newborns during the first month of life, a period when they are not yet eligible for PV. In our study, we estimated that, in infants aged <2 months and not eligible yet for vaccination, pertussis vaccination in pregnancy reduces the risk of the infant of being hospitalised for pertussis by 75-88%. These results align with existing literature [25,26] and reflect a good effectiveness of this passive immunisation strategy to fill the susceptibility gap during the first months of life, when the disease is the most life threatening.

However, from two months of age onwards, several immunological studies have raised concerns about a potential "blunting effect" where maternal antibodies would interfere with the infant's immune response to PV and thus reduce their immune response [27]. Little evidence exists about the clinical implications of such an immunological interference. Therefore, we conducted an indicator analysis, in infants aged 2-11 months, based on four categories: (a) infants recording no vaccination in pregnancy nor PV (*reference category*); (b) infants recording PV only (at least one vaccine dose); (c) infants recording vaccination in pregnancy only; (d) infants recording both vaccination in pregnancy and PV (at least one vaccine dose). Using infants recording no vaccination in pregnancy nor PV (a) as reference category, we compared the odds of each category of vaccination exposure (b), (c) and (d) between cases and controls and estimated the corresponding VE. Our findings indicate that, regardless of the recommended schedule, when the infants are aged 2–11 months and eligible for vaccination, at least one dose of PV in infants whose mother had received vaccination in pregnancy would reduce the risk of hospitalisation for confirmed pertussis by 74–95%. Using the same reference group, at least one dose of PV in infants with unvaccinated mother would reduce the risk by 68–94%. However, our limited sample size did not allow a sufficiently precise estimate of the remaining effectiveness of the maternal vaccination in unvaccinated infants aged 2-11 months. Regarding a potential interaction between the two vaccinations and assuming an additive model [74], our results suggest that the observed VE point estimate of the combined maternal and infant vaccinations is different than the expected VE of these

combined exposures. Nevertheless, a larger sample size is needed for a more precise stratified analysis that would allow to fully investigate whether vaccination in pregnancy modifies VE after at least one dose of PV. Therefore, even if our results may indicate a similarly good VE of at least one dose of PV irrespective of the vaccination status of the mother, we cannot conclude about the absence of clinical significance of the immunological “blunting effect” of maternal vaccination in infants’ immune response to PV.

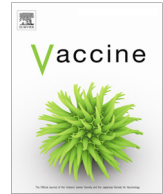
Our study also highlighted some challenges. The implementation and compliance with the maternal immunisation program were very heterogeneous across sites during the study period. It was well established in Spain, but programs for pregnant women were not fully implemented in the Czech Republic and Italy. Vaccine acceptance issues were documented in more recent studies [16–18].

We pointed out the need of a larger sample size to consolidate our results and allow additional stratification by effect modifiers and adjustments for potential confounding factors. The current sample size did not allow for more precise estimates or exploration of VE in pregnancy according to time of and since vaccination in pregnancy, VE for one dose of primary series only or VE by dose.

These findings underscore the public health importance of pertussis vaccination during pregnancy, but also highlights the need for further research and improved implementation of vaccination programs.

The detailed methods and results are presented in the next article below published in *Vaccine* as an original research article.

3.3.2. Article 3



Effectiveness of pertussis vaccination in pregnancy to prevent hospitalisation in infants aged <2 months and effectiveness of both primary vaccination and mother's vaccination in pregnancy in infants aged 2–11 months

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ABSTRACT

Background: PERTINENT is an active hospital-based surveillance system for pertussis in infants. In 2019, four of the six participating European countries recommended pertussis vaccination in pregnancy. Among infants aged <2 months, we measured the vaccine effectiveness (VE) in pregnancy; among infants aged 2–11 months, VE of vaccination in pregnancy and of primary vaccination (PV).

Methods: From December 2015 to 2019, we included all infants aged <1 year presenting with pertussis-like symptoms. Using a test-negative-design, cases were infants testing positive for *Bordetella pertussis* by PCR or culture. Controls were those testing negative for all *Bordetella* species. Vaccinated mothers were those who received vaccine in pregnancy. Vaccinated infants were those who received ≥ 1 dose of PV > 14 days before symptom onset. We excluded infants with unknown maternal or PV status or with mothers vaccinated ≤ 14 days before delivery. We calculated pooled VE as $100 * (1 - \text{odds ratio of vaccination})$ adjusted for study site, onset date in quarters and infants' age group.

Results: Of 829 infants presenting with pertussis-like symptoms, 336 (41%) were too young for PV. For the VE in pregnancy analysis, we included 75 cases and 201 controls. Vaccination in pregnancy was recorded for 9 cases (12%) and 92 controls (46%), adjusted VE was between 75% [95%CI: 35–91%] and 88% [95%CI: 57–96%]. Of 493 infants eligible for PV, we included 123 cases and 253 controls. Thirty-one cases and 98 controls recorded both PV with ≥ 1 dose and vaccination in pregnancy, adjusted VE was between 74% [95%CI: 33–90] and 95% [95%CI: 69–99]; 27 cases and 53 controls recorded PV only, adjusted VE was between 68% [95%CI: 27–86] and 94% [95%CI: 59–99].

Abbreviations: PV, primary vaccination; PERTINENT, Pertussis in Infants European Network.

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Conclusion: Our findings suggest that vaccination in pregnancy reduces pertussis incidence in infants too young for PV. In infants aged 2–11 months, PV only and both PV and vaccination in pregnancy provide significant protection against severe pertussis.

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1. Introduction

Pertussis (whooping cough) is a highly contagious acute respiratory infection caused by the bacterial pathogen *Bordetella pertussis*. In 2019, across the 30 European Union/European Economic Area (EU/EEA) Member States reporting pertussis data to the European Centre for Disease Prevention and Control (ECDC), infants aged <1 year were the most affected age group (46.8 per 100,000 population). Three deaths were reported that year in infants, all were too young to have received the first dose of primary vaccination (PV) [1].

Pertussis PV includes three doses in the first year of life and aims to reduce the risk of severe pertussis in infants.

After the introduction in the 1950s of pertussis vaccination with whole-cell (wP) vaccine in children in Europe, pertussis incidence and mortality markedly decreased [2]. Most European countries replaced wP with acellular-pertussis (aP) containing vaccine in the 1990s, which is less reactogenic. After a continued decline, reported cases have progressively increased again in recent years with the last peak incidence in 2012 with >42,000 reported cases in EU/EEA [3]. The World Health Organization (WHO) estimated that pertussis was still responsible for around 63,000 deaths in children aged <5 years worldwide in 2013, despite a global vaccination coverage estimated at 86% in 2014 [4]. Even with immunisation achievements, pertussis remains a major public health concern worldwide.

In September 2012, in response to an increase of hospitalisations and deaths in unvaccinated infants aged <3 months, the United Kingdom recommended for each pregnancy a single dose of aP-containing vaccine between 28 and 32 weeks of gestation. The programme was based on the evidence of transplacental transfer of maternal antibodies known to be maximal from the 34th week of gestation. One year after the programme was introduced, pertussis mortality decreased and VE in pregnancy remained stable around >90% in the following years [56]. Since 2012, an increasing number of EU/EEA countries introduced vaccination in pregnancy: Belgium, Czech Republic, Ireland, Italy, Portugal, Slovenia and Spain [1,7].

However, recent immunological studies suggest that vaccination in pregnancy could interfere with PV and reduce infants' immune response. But little evidence exists about the clinical implications of this potential “blunting effect” of vaccination in pregnancy with infants' PV [8].

From September 2015 to January 2020, ECDC created and funded PERTINENT, “Pertussis in Infants European Network”, a multi-country hospital-based active sentinel surveillance system to measure pertussis incidence and VE in infants aged <1 year [3]. For the first time in Europe, a prospective test-negative design (TND) [9] in hospital settings was used to estimate pertussis VE in a multi-country study.

In this study, we estimate VE in pregnancy in infants aged <2 months (i.e., too young to be eligible for PV) and investigate the effect of vaccination in pregnancy and PV in infants aged 2–11 months (i.e., eligible for PV).

2. Methods

2.1. Study sites

In 2019, four of the six European countries participating in PERTINENT recommended pertussis vaccination in pregnancy to pro-

tect infants too young for PV: Czech Republic, Ireland, Italy and Spain with two participating regions, Catalonia and Navarra (five study sites, 14 hospitals).

All sites complied with the generic PERTINENT sentinel surveillance and vaccine effectiveness protocol and laboratory guidelines [10] allowing to pool the data across sites.

All sites used the aP-containing vaccine for both PV and vaccination in pregnancy. Even though recommended schedules vary across countries, infants were eligible for the first dose of the primary series from their 61st day of life (2 months of age) in the four participating countries, including the two countries with a 3, 5, 11-month-old schedule.

The Czech Republic and Italy introduced vaccination in pregnancy during the course of the PERTINENT study and were included in both analyses only from that point onwards. Vaccine coverage estimates were not available for these two sites, and ranged from 50% to 90% in the other three sites (Table 1).

2.2. Study population and eligibility criteria

The study population consisted of all infants aged <1 year, likely to be hospitalised in one of the participating hospitals if developing pertussis-like symptoms.

All infants attending one of the participating hospitals and presenting with apnoea or cough associated with at least one of paroxysms, whoop or post-tussive vomiting were tested for pertussis. Infants with any respiratory symptoms and an epidemiological link with a pertussis confirmed case or those not meeting the above clinical presentation but diagnosed as pertussis by a physician were also tested for pertussis.

We included all infants who were tested for pertussis and invited their parents to participate in the study. When required by site-specific ethical committee, infants' legal guardians provided with an informed consent.

2.3. Laboratory methods

We recommended to the hospital laboratories to ensure an accurate identification of the *Bordetella* species using, as much as possible, a triplex quantitative PCR (qPCR): first targeting IS481 gene (in *B. pertussis*, *B. holmesii*, and some *Bordetella bronchiseptica* strains), pIS1001 (*B. parapertussis*-specific) and RNase P as the human internal control and two confirmatory singleplex tests for *B. pertussis* (*ptxA-Pr*) and *B. holmesii* (*hIS1001*) if IS481 was positive. Diagnostic algorithm was detailed in the PERTINENT laboratory guidelines [10].

2.4. Test-negative case control study

We conducted a multi-centre case control study using TND in the 14 participating hospitals.

We defined a laboratory-confirmed *Bordetella pertussis* case as an infant testing positive for *Bordetella pertussis* by PCR (DNA detection of *Bordetella pertussis* using PCR or real-time PCR in a nasopharyngeal aspirate or swab) or culture (isolation of *Bordetella pertussis* from the prior-mentioned clinical specimen) regardless of the clinical criteria. Test-negative controls were those testing negative to all *Bordetella* species by PCR or culture. In the Catalan hos-

Table 1

Characteristics of PERTINENT study sites, vaccination strategy during pregnancy, in adulthood, primary schedule in infants, Europe, 1 December 2015–31 December 2019.

Study sites	Vaccination strategy							Number of participating hospitals	
	Pregnancy			Cocooning	Adult	Primary schedule in infants (age in months)			
	Year of introduction	Gestational age (in weeks)	Estimated vaccination coverage	Year of introduction		1st dose	2nd dose		3rd dose
Czech Republic	2016	28–36	1.6% in 2021 ^a	No	At least once	3 ^b	5 ^b	11–13 ^b	6
Ireland	2013	16–36	49.9% in 2017/ 2018 [11]	2013	No	2	4	6	2
Italy	2017	≥ 27	NA	No	Every 10 years	3	5	11	1
Spain, Catalonia	2014	27–36	82.8% in 2019 [12]	No	No	2 ^c	4 ^c	11 ^c	1
Spain, Navarra	2015	27–36	91.1% in 2019 [12]	No	No	2 ^c	4 ^c	11 ^c	4

NA: not available.

^a Estimates from the final report of the project “Monitoring the vaccination of pregnant women against pertussis and influenza, 2020–2021” financed from NIPH Prague internal institutional funds. In this pilot prospective observational hospital-based study in the maternity hospital in Prague, 4617 women (84%) were included in the analysis out of the 5475 women who gave birth in 2021.

^b Before 2018: doses at 2, 3, 4 and 10 months.

^c Before 2016: doses at 2, 4 and 6 months.

pital, due to heavy workload, we selected systematically the next three controls per case matched for date of specimen collection.

2.5. Exposures

We defined infants as vaccinated with PV if they had received at least one dose of pertussis vaccine >14 days before symptoms onset. Unvaccinated infants were those who had not received any dose or who had received the first dose ≤14 days before symptom onset.

We defined an infant as having a mother vaccinated during her pregnancy if she had received a pertussis vaccine dose >14 days before delivery. We defined an infant as having a mother not vaccinated if she did not receive any dose during adulthood.

2.6. Exclusion criteria

We excluded all infants with missing information for laboratory results, date of onset, or vaccination status. We also excluded infants sampled >4 weeks after symptoms onset, those testing positive to other *Bordetella* species than *Bordetella pertussis*, those with previous laboratory confirmed pertussis episode and those whose legal guardian did not give consent.

For both analyses, we excluded infants with unknown maternal vaccination status, those whose mothers were vaccinated ≤14 days before delivery or before/after pregnancy or had contra-indication for pertussis vaccination.

2.6.1. Effectiveness of vaccination in pregnancy in infants too young for vaccination (<2 months)

To estimate VE in pregnancy, we restricted the analysis to infants too young to be vaccinated and aged <61 days of life. Additionally, we excluded infants too young to develop the disease and aged <4 days of life (4 days being commonly known as the minimum incubation period for pertussis [2]).

2.6.2. Effectiveness of vaccination in pregnancy and PV in infants (2–11 months)

To explore the effect of both vaccinations, we restricted the analysis to infants eligible for PV and aged 2–11 months. We excluded all infants with unknown PV status or with contra-indication for pertussis vaccination.

2.7. Analysis

For both analyses, we described cases and controls by clinical presentations, severity, risk and protective factors. We used Fisher's exact test to compare those characteristics between cases and controls.

2.7.1. Effectiveness of vaccination in pregnancy in infants too young for vaccination (<2 months)

We compared the odds of vaccination of the infants' mother between cases and controls. We used a logistic regression to model the odds ratio (OR), including study site as fixed effect. We adjusted for time of onset in quarter and age group (4–30 days; 31–60 days). We computed VE as 1 minus the OR, expressed as a percentage.

2.7.2. Effectiveness of vaccination in pregnancy and PV in infants (2–11 months)

We conducted an indicator analysis based on four categories: (1) infants recording no vaccination in pregnancy nor PV (reference category); (2) infants recording PV only (at least one dose); (3) infants recording vaccination in pregnancy only; (4) infants recording both vaccination in pregnancy and PV (at least one dose).

Using infants recording no vaccination in pregnancy nor PV (1) as reference category, we compared the odds of each category of vaccination exposure (2), (3) and (4) between cases and controls and estimated the corresponding OR using logistic regression. We refer to this analysis as the indicator analysis. We included study site as fixed effect in the model and adjusted for time of onset in quarter and age group (2 months; 3–11 months). We computed VE as 1 minus the OR, expressed as a percentage.

2.7.3. Sensitivity analyses

Bordetella species can be isolated from both nasopharyngeal swabs (NPS) or aspirates (NPA). However, a 15% gain in the isolation rate can be obtained by using aspirates in neonates and infants [13].

Additionally, the Czech Republic and Italy encountered difficulties of adherence to the maternal immunisation programme in the first years of its implementation. National vaccine coverage in both sites were assumed to be very low and the mothers of the children enrolled in the analysis were not vaccinated.

Therefore, we conducted sensitivity analyses: (I) excluding the two sites with no mother vaccinated in pregnancy included in

the study, (II) excluding all infants sampled with NPS, (III) excluding both the two sites and the infants with NPS.

If the number of events per parameter was lower than 10, we conducted an additional sensitivity analysis using Firth’s method of penalised logistic regression to assess small sample bias [14].

2.8. Data collection

Using a standardised questionnaire we collected demographic, epidemiological, clinical, and laboratory data, vaccination status of the infant and the mother, risk and protective factors. Hospital teams collected data through the review of clinical case-patient notes, vaccination cards, interviews with parents or legal guardians, and extraction from patient registries.

2.9. Ethical statement

Each site complied with the local ethical procedures. The planning, conduct and reporting of the study was in line with the Declaration of Helsinki [15]. Ethical approval was not needed in Navarra as the PERTINENT study was considered part of the mandatory surveillance system. Other study sites sought ethical approval from a review board according to country-specific regulations (Catalonia: PIC-31-16, Czech Republic: SZU/05992/2019, Ireland: Royal College of Physicians in Ireland REC reference number 16.058 and Gen/499/16, Italy: Bambino Gesù Children’s Hospital Ethical Committee: protocol n. 1064_OPBG_2016).

3. Results

From December 2015 to December 2019, 829 infants aged less than one year were tested for *Bordetella pertussis*. Among them, 336 (40.5%) were too young to receive the first dose of PV (aged < 2 months) and 493 (59.5%) were eligible for PV (aged 2–11 months). No death was reported during the study period.

3.1. Effectiveness of vaccination in pregnancy in infants too young for vaccination (<2 months)

After applying the exclusion criteria for VE in pregnancy analysis, we included 276 infants aged <2 months with 75 *Bordetella pertussis* laboratory confirmed cases (27%) and 201 test-negative controls (73%). Among excluded infants, 31 had a missing maternal vaccination status or date of vaccination (Fig. 1).

Twenty-six cases (35%) and 53 controls (26%) were aged 4–30 days (p = 0.181). The median-birthweight was 3320 g for cases (range: 1740–4925; interquartile range (IQR): 800) and 3260 g for controls (range: 1000–5150; IQR: 649) (p = 0.412). The median gestational week at birth was 39 for both cases (range: 29–42; IQR: 2) and controls (range: 28–42; IQR: 2) (p = 0.671).

Information on the type of specimen collection was available for the 75 cases and 199 controls with 18 cases (24%) and 71 controls (36%) only diagnosed based on NPS collection (p = 0.043) (Table 2).

Out of the 75 cases, 20 cases (27%) were both PCR and culture-confirmed, 17 cases (23%) were PCR-confirmed but culture-negative, 37 cases (49%) were PCR-confirmed (no culture result) and one case (1%) was culture-confirmed (no PCR performed). Out of the 201 controls, 6 (3%) were confirmed by culture only.

The proportion of cases and controls by risk and protective factors such as prematurity, delivery type, child care, breastfeeding was similar. Three cases (4%) and no controls had their mother experiencing pertussis in pregnancy. The mothers did not receive pertussis vaccine during their pregnancy.

The median gestational age at vaccination was 30.4 weeks for cases (range: 23–36; IQR: 4) and 30.1 for controls (range: 20–37; IQR: 3.5) (p = 0.741).

Out of the 276 infants too young to be vaccinated, nine cases (12%) and 92 controls (46%) had their mother vaccinated in pregnancy. VE in pregnancy adjusted for study site and time of onset (in quarter) was 76% (95% CI: 38–91) and 75% (95% CI: 35–91) when also adjusted for age group (Table 3).

In the sensitivity analysis excluding infants sampled only with NPS (N = 185), VE adjusted for site and time of onset (in quarter)

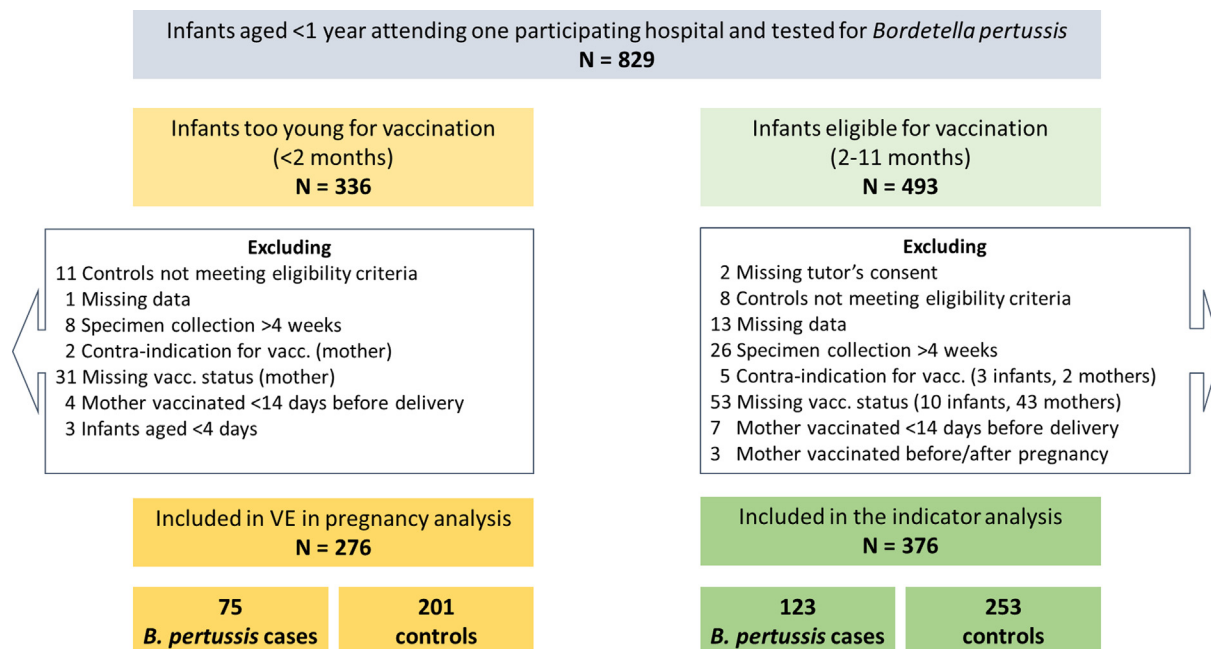


Fig. 1. Flowchart of hospitalised infants aged <1 year inclusion in or exclusion from the analysis of the effectiveness of vaccination in pregnancy, and the analysis of the effectiveness of vaccination in pregnancy combined with primary vaccinations after at least one dose, PERTINENT study, Europe, 1 December 2015–31 December 2019 (N = 829).

Table 2

Characteristics of *Bordetella pertussis* cases and controls by analysis (left: effectiveness of vaccination in pregnancy analysis in infants aged <2 months; right: vaccination in pregnancy and primary vaccination analysis in infants aged 2–11 months) and by sex, laboratory components, clinical presentation, severity and risk/protective factors, hospitalised infants aged <1 year, PERTINENT study, Europe, 1 December 2015–31 December 2019.

Characteristics		Cases <2mo (n = 75)		Controls <2mo (n = 201)		p value	Cases 2–11mo (n = 123)		Controls 2–11mo (n = 253)		p value
		N	%	N	%		N	%	N	%	
<i>Demographic</i>											
Sex	Female	39	52.0	91	45.3	0.345	57	46.3	126	49.8	0.583
	Male	36	48.0	110	54.7		66	53.7	127	50.2	
<i>Laboratory</i>											
Nasopharyngeal specimen collection	Aspirate or both aspirate and swab	57	76.0	128	64.3	0.043	92	75.4	159	62.9	0.019
	Swab only	18	24.0	71	35.7		30	24.6	94	37.2	
<i>Clinical criteria</i>											
Cough	Yes	72	96.0	186	92.5	0.415	121	98.4	249	98.4	1.000
	No	3	4.0	15	7.5		2	1.6	4	1.6	
Cough with paroxysms	Yes	64	85.3	120	59.7	<0.001	112	91.1	179	70.8	<0.001
	No	11	14.7	81	40.3		11	8.9	74	29.2	
Whoop	Yes	35	47.9	36	18.6	<0.001	66	53.7	47	18.9	<0.001
	No	38	52.1	158	81.4		57	46.3	202	81.1	
Post-tussive vomiting	Yes	39	52.0	77	38.5	0.055	56	45.5	124	49.0	0.582
	No	36	48.0	123	61.5		67	54.5	129	51.0	
Apnoea	Yes	50	67.6	92	46.0	0.002	61	49.6	62	25.0	<0.001
	No	24	32.4	108	54.0		62	50.4	186	75.0	
Cyanosis	Yes	47	63.5	62	30.8	<0.001	53	43.1	49	19.4	<0.001
	No	27	36.5	139	69.2		70	56.9	203	80.6	
Epidemiological link	Yes	43	58.9	3	1.5	<0.001	64	54.7	10	4.0	<0.001
	No	30	41.1	192	98.5		53	45.3	241	96.0	
Diagnosis by a clinician	Yes	71	94.7	74	36.8	<0.001	113	93.4	137	54.6	<0.001
	No	4	5.3	127	63.2		8	6.6	114	45.4	
<i>Severity</i>											
Death	Yes	0	0.0	0	0.0	NA	0	0.0	0	0.0	NA
	No	75	100.0	199	100.0		123	100.0	249	100.0	
ICU	Yes	26	34.7	24	12.0	<0.001	14	11.4	13	5.2	0.035
	No	49	65.3	176	88.0		109	88.6	238	94.8	
ECMO	Yes	0	0.0	0	0.0	NA	0	0.0	0	0.0	NA
	No	75	100.0	200	100.0		123	100.0	249	100.0	
Pneumonia	Yes	4	5.3	5	2.5	0.262	3	2.5	14	5.6	0.289
	No	71	94.7	195	97.5		119	97.5	237	94.4	
Encephalopathy	Yes	0	0.0	1	0.5	1.000	0	0.0	0	0.0	NA
	No	75	100.0	199	99.5		122	100.0	251	100.0	
Seizure	Yes	1	1.3	2	1.0	1.000	1	0.8	0	0.0	0.327
	No	74	98.7	198	99.0		121	99.2	251	100.0	
Eating difficulties	Yes	22	29.3	66	33.2	0.566	32	26.2	83	33.2	0.190
	No	53	70.7	133	66.8		90	73.8	167	66.8	
Kidney failure	Yes	0	0.0	2	1.0	1.000	0	0.0	0	0.0	NA
	No	75	100.0	198	99.0		123	100.0	250	100.0	
Dehydration	Yes	6	10.2	5	3.2	0.075	12	10.8	15	6.8	0.208
	No	53	89.8	151	96.8		99	89.2	207	93.2	
<i>Risk factors</i>											
Premature <37 weeks	Yes	7	9.5	23	11.4	0.828	14	11.4	43	17.0	0.170
	No	67	90.5	178	88.6		109	88.6	210	83.0	
Delivery type	Vaginal	53	70.7	145	73.6	0.649	90	73.8	182	74.3	1.000
	C-section	22	29.3	52	26.4		32	26.2	63	25.7	
Episode in pregnancy	Yes	3	4.1	0	0.0	0.020	2	1.8	1	0.4	0.233
	No	71	95.9	197	100.0		112	98.2	248	99.6	
Infant going to day care	Yes	5	6.7	6	3.0	0.178	6	4.9	26	10.3	0.114
	No	70	93.3	194	97.0		117	95.1	226	89.7	
Infant with babysitter	Yes	1	1.4	11	7.3	0.109	5	4.5	11	5.7	0.793
	No	69	98.6	139	92.7		105	95.5	182	94.3	
Infant staying regularly with grandparents	Yes	24	32.0	40	20.1	0.054	38	31.9	64	25.5	0.214
	No	51	68.0	159	79.9		81	68.1	187	74.5	
<i>Protective factors</i>											
Breastfeeding	Yes	55	73.3	157	78.5	0.421	84	68.3	174	69.3	0.905
	No	20	26.7	43	21.5		39	31.7	77	30.7	
Mother vaccination in pregnancy	Yes	9	12.0	92	45.8	<0.001	40	32.5	136	53.8	<0.001
	No	66	88.0	109	54.2		83	67.5	117	46.2	
Vaccinated at least 1 dose	Yes	0	0	0	0	NA	58	47.2	151	59.7	0.027
	No	0	0	0	0		65	52.8	102	40.3	
Number of doses	1 dose	0	0	0	0	NA	30	24.4	73	28.9	0.151
	2 doses	0	0	0	0		22	17.9	62	24.5	
	3 doses	0	0	0	0		6	4.9	16	6.3	

Table 3

Adjusted vaccine effectiveness of pertussis vaccination in pregnancy in hospitalised infants too young to be vaccinated (aged < 2 months), PERTINENT study, Europe, 1 December 2015–31 December 2019 (n = 276).

Adjustment variables	Df	N	Cases		Controls		VE (95% CI)
			Vacc.	N	Vacc.	N	
All infants, 5 sites (N = 276)							
Site; Onset quarter	9	276	9	75	92	201	76 (38–91)
Site; Onset quarter;	10	276	9	75	92	201	75 (35–91)
Age group (4–30d; 31–60d)							
All infants, 3 sites* (N = 165)							
Site; Onset quarter	7	165	9	30	92	135	76 (39–91)
Site; Onset quarter;	8	165	9	30	92	135	75 (35–90)
Age group (4–30d; 31–60d)							
Infants sampled with NPA, 5 sites (N = 185)							
Site; Onset quarter	9	185	6	57	51	128	88 (59–96)
Site; Onset quarter;	10	185	6	57	51	128	87 (55–96)
Age group (4–30d; 31–60d)							
Infants sampled with NPA, 3 sites ^a (N = 88)							
Site; Onset quarter	7	88	6	20	51	68	88 (57–96)
Site; Onset quarter;	8	88	6	20	51	68	87 (53–96)
Age group (4–30d; 31–60d)							

CI: confidence interval; Df: degree of freedom; NPA: nasopharyngeal aspirate; VE: vaccine effectiveness.

^a Excluding 2 sites due to the absence of vaccinated women.

and VE also adjusted for age group were, respectively 88% (95% CI: 59–96) and 87% (95% CI: 55–96).

The results were similar when excluding the two sites with no infant with mother vaccinated in pregnancy, or when using penalised logistic regression.

3.2. Effectiveness of vaccination in pregnancy and PV in infants (2–11 months)

After applying the exclusion criteria for the effectiveness of both vaccinations analysis, we included 376 infants eligible for PV (aged 2–11 months) with 123 *Bordetella pertussis* laboratory confirmed cases (33%) and 253 test-negative controls (67%). Among excluded infants, 43 had a missing maternal vaccination status or vaccination date and 10 had a missing PV status or vaccination date (Fig. 1).

Thirty-six cases (29%) and 97 controls (38%) were in their third month of life (p = 0.053). The median-birthweight was 3250 g for cases (range: 1160–4780; IQR: 750) and 3200 g for controls (range: 640–4500; IQR: 770) (p = 0.186). The median gestational week at birth was 39 for both cases (range: 28–42; IQR: 2) and controls (range: 24–43; IQR: 2) (p = 0.220).

Information on the type of specimen collection was available for 122 cases and the 253 controls with 30 cases (25%) and 94 controls (37%) only diagnosed based on NPS (p = 0.019) (Table 2). Out of the 123 cases, 32 cases (26%) were both PCR and culture-confirmed, 22 cases (18%) were PCR-confirmed but culture-negative, 65 cases (53%) were PCR-confirmed (no culture result) and four cases (3%) were culture-confirmed (no PCR performed). Two controls (<1%) were confirmed by culture only.

The median gestational age at vaccination was 30.1 weeks for cases (range: 19–36; IQR: 4) and 30.6 for controls (range: 14–36; IQR: 3) (p = 1.000).

Out of the 376 infants eligible for PV, 40 cases (33%) and 136 controls (54%) had their mother vaccinated in pregnancy (p < 0.001), 58 cases (47%) and 151 controls (60%) were vaccinated with at least one dose of PV (p = 0.027) (Table 2). Thirty-one cases (25%) and 98 controls (39%) had received both PV and vaccination in pregnancy, 27 cases (22%) and 53 controls (21%) had received PV only, 9 cases (7%) and 38 controls (15%) had received vaccination in pregnancy only (Table 4).

In the main analysis (N = 376), using unvaccinated infants and mothers as the reference group, VE adjusted for site, time of onset and age group was 74% (95% CI: 33–90) for infants with both PV and vaccination in pregnancy; 68% (95% CI: 27–86) for those with PV only; 36% (95% CI: -85–78) for those with vaccination in pregnancy only (Table 4).

In the sensitivity analysis excluding the two sites with no infants with vaccination in pregnancy (N = 257), VE adjusted for site, time of onset and age group was 90% (95% CI: 64–97) for infants with both PV and vaccination in pregnancy; 92% (95% CI: 69–98) for those with PV only; 63% (95% CI: -29–89) for those with vaccination in pregnancy only. When excluding infants sampled with NPS only (N = 251), VE adjusted for site, time of onset and age group was 88% (95% CI: 62–96) for infants with both PV and vaccination in pregnancy; 81% (95% CI: 46–93) for those with PV only; 44% (95% CI: -109–85) for those with vaccination in pregnancy only. Applying both exclusions (N = 164) provided with similar results (Table 4).

4. Discussion

After four years of PERTINENT data collection in 14 participating hospitals from four EU/EEA countries, we included 276 infants aged <2 months in the VE in pregnancy analysis and 373 infants aged 2–11 months in the indicator analysis of both vaccination in pregnancy and PV. Our results suggest that vaccination in pregnancy reduces the risk of being hospitalised for pertussis by 75–88% in infants aged <2 months too young to be vaccinated with PV. In the indicator analysis, regardless of the recommended schedule, when the infants are aged 2–11 months and eligible for vaccination, at least one dose of PV in infants whose mother had received vaccination in pregnancy would reduce the risk of hospitalisation for confirmed pertussis by 74–95%. Using the same reference group, at least one dose of PV in infants with unvaccinated mother would reduce the risk by 68–94%. Even though those results are based on small sample sizes, they suggest a good VE in pregnancy, consistent with existing literature [16] and also a similarly good VE after at least one dose of PV only and receiving both PV and mother vaccination.

However, these findings need to be interpreted with caution due to some existing limitations. Despite four years of active per-

Table 4

Adjusted effectiveness of three combinations of vaccine exposures in hospitalised infants eligible for vaccination (2–11 months): (1) mother vaccinated in pregnancy and infant vaccinated with at least one dose of PV; (2) infant vaccinated with PV only (at least one dose); (3) mother vaccinated in pregnancy only. PERTINENT study, Europe, 1 December 2015–31 December 2019 (n = 376).

Vaccination status		N	Cases	Controls	VE (95% CI) ^a
Infant	Mother				
All infants, 5 sites (N = 376)					
Unvaccinated	Unvaccinated	120	56	64	Ref.
Vaccinated	Vaccinated	129	31	98	74 (33–90)
Vaccinated	Unvaccinated	80	27	53	68 (27–86)
Unvaccinated	Vaccinated	47	9	38	36 (-85–78)
All infants, 3 sites ^b (N = 257)					
Unvaccinated	Unvaccinated	29	13	16	Ref.
Vaccinated	Vaccinated	129	31	98	90 (64–97)
Vaccinated	Unvaccinated	52	11	41	92 (69–98)
Unvaccinated	Vaccinated	47	9	38	63 (-29–89)
Infants sampled with NPA, 5 sites (N = 251)					
Unvaccinated	Unvaccinated	84	43	41	Ref.
Vaccinated	Vaccinated	90	24	66	88 (62–96)
Vaccinated	Unvaccinated	52	19	33	81 (46–93)
Unvaccinated	Vaccinated	25	6	19	44 (-109–85)
Infants sampled with NPA, 3 sites ^b (N = 164)					
Unvaccinated	Unvaccinated	21	11	10	Ref.
Vaccinated	Vaccinated	90	24	66	95 (69–99)
Vaccinated	Unvaccinated	28	7	21	94 (59–99)
Unvaccinated	Vaccinated	25	6	19	61 (-89–92)

CI: confidence interval; NPA: nasopharyngeal aspirate; PV: primary vaccination; VE: vaccine effectiveness.

^a Adjusted for site, onset quarters and age group (2; 3–11 months).

^b Excluding 2 sites due to the absence of vaccinated women.

tussis surveillance, the achieved sample sizes for maternal vaccination studies did not allow for more precise estimates. Increasing data collection in this multicentre study is needed to consolidate our results and to allow additional adjustments for potential confounding factors or stratification by effect modifiers (e.g., breastfeeding, repeated vaccination in pregnancy). Due to this substantial limitation in our study, we could not compute VE in pregnancy by site and estimate sites' heterogeneity. The current sample size also prevented us to explore VE in pregnancy according to time of and since vaccination in pregnancy, VE for one dose of primary series only, VE by dose or VE by time since vaccination. As described by Barug et al. [17], pertussis antibody responses in infants may differ depending on the infant vaccination schedule. Their study suggested a higher immunological effect when PV is starting at 2 months compared with starting at 3 months of age. Interaction between vaccination in pregnancy and PV may also differ according to the number of doses received, the time of and since vaccination of the mother and other additional factors [18].

Implementation and compliance to the maternal immunisation programme was very heterogeneous across sites during the study period. It was very well established in Spain with Catalonia and Navarra regions. Conversely, immunisation programmes for pregnant women were not being fully implemented in Czech Republic and Italy. Vaccine acceptance aspects were documented in Italy [19].

Hospital teams had to test for pertussis and include in the study any infants suspected for pertussis, even though some typical symptoms were missing [20]. However, clinicians may be more likely or less likely to test suspected pertussis cases according to vaccination status leading to selection bias. We believe this bias may had a very limited impact at least on the VE in pregnancy analysis as we assume that clinicians may not have direct access to the mother vaccination status at the infant's admission.

Vaccination status data were obtained by reviewing clinical case notes, vaccination cards, interviews with parents or legal guardians, and extraction from patient registries. The current small sample size did not allow to compare VE estimates by source of

information for the vaccine status. In the VE in pregnancy analysis, 31 infants were excluded due to missing values for mother vaccination status or vaccination date. Out of them, 21 were excluded due to missing vaccination date (2 cases and 19 controls), assuming that the mother was vaccinated. In the indicator analysis, 43 infants were excluded due to missing values for mother vaccination status or vaccination date. Out of them, 35 were excluded due to missing vaccination date (4 cases and 31 controls), assuming that the mother was vaccinated. For both analyses, this suggests that mother's vaccinations may be better documented among cases than among controls, which could lead to underestimation of VE in pregnancy.

A large proportion of the exclusions in the study are due to lack of information on vaccination status and vaccination date from the mother. Even though efforts done for an enhanced data collection at hospital level were successful, more efforts are needed to retrieve information outside of the hospital setting.

In our study population, the clinical case definition was associated with confirmed pertussis. Even if this lends support to the definition used, discussing pertussis clinical presentation was however not part of our study objectives.

To validate our findings, we would need to further study and confirm that TND in hospital settings is a proper study design for pertussis VE estimation in infants. This is the first time that a prospective TND is used in Europe in hospital settings for estimating pertussis vaccine effectiveness in infants. The rationale for TND is that the control group (infants hospitalised for pertussis-like symptoms but with other respiratory disease than pertussis) are representative of the vaccine coverage in the source population of pertussis cases. The risk of hospitalisation for non-pertussis respiratory infections should then be equal between vaccinated and unvaccinated infants. There is a need to validate this assumption using large cohorts in Europe. Unfortunately, we could not compare the proportion of our controls that were vaccinated to the vaccine coverage in the catchment area of the participating hospitals. Aiming to validate TND for pertussis, a recent Canadian study compared their results with a frequency-matched design (FMD) for

pertussis VE studies estimating waning immunity. In both designs, VE estimates were high and consistent with clinical trials at early stage after vaccination and in early years of life [21].

In both our analyses, we included all infants tested for pertussis and classified them as cases and controls according to PCR or culture results. Although PCR has a high sensitivity, culture sensitivity is only about 60% with the highest among unvaccinated infants [22]. Including false-negative, especially among vaccinated infants, could lead to overestimate VE in both analyses. However, only six controls (3%) aged <2 months and two controls (<1%) aged 2–11 months were confirmed by culture only, which lead us to assume a very minor impact on our results.

Even though our VE estimates for both analyses are consistent with existing literature [23], they tend to be in the lower range. In our study, controls were more likely than cases to have been diagnosed based on the laboratory results of a NPS only (Table 2). Since NPS can be less sensitive than NPA in infants to isolate *Bordetella pertussis* by PCR or culture [13], we cannot prevent inclusion of false-negative among controls. Misclassification of unvaccinated cases as controls would lead to underestimating the corresponding VE. Despite the very low sample size, when excluding infants sampled with NPS, we observed higher VE estimates, closer to existing literature. Overall, our results are in the range of VE observed in other studies reporting VE in pregnancy between 70% and 90% in infants aged <2 months [8,24] and additional protection from vaccination in pregnancy during the first year of life [16]. In our indicator analysis, we also observed a good VE after at least one dose of PV only and after at least one dose of PV in infants whose mother was vaccinated. However, our limited sample size did not allow a robust stratified analysis to investigate whether vaccination in pregnancy modifies VE after at least one dose of PV. It did not allow either to measure the interaction between the two vaccinations. Therefore, even if our results may indicate a similarly good VE of at least one dose of PV irrespective of the vaccination status of the mother, we cannot conclude about the absence of clinical significance of the immunological blunting effect of maternal vaccination in infants' immune response to PV.

5. Conclusion

The PERTINENT network is the only EU/EEA collaboration that allows for large, independent and multi-country pertussis vaccine effectiveness studies.

Despite PV starting at 2 months of age, infants too young to be eligible for vaccination still harbour the highest risk of illness and related deaths.

Our findings suggest that vaccination in pregnancy is an effective strategy to fill the immunisation gap of the first two months of life, when infants are not eligible for vaccination and the disease is the most life-threatening. From 2 months of age onwards, despite existing immunological studies suggesting a possible lower immunological response after PV in infants whose mother had received vaccination in pregnancy [8], our results suggest a good effectiveness of at least one dose PV in infants aged 2–11 months irrespective of the vaccination status of the mother.

In making decisions about vaccination strategies, countries take into account various factors, including cost-effectiveness evaluations. As health economic analyses are sensitive to local circumstances and are not easily generalisable, national health-economic studies may need to be conducted as part of such comprehensive evaluations.

In the up-coming post-acute COVID-19 pandemic times where an increase of vaccine-preventable respiratory infections such as bronchiolitis and pertussis is to be expected [25], consideration should be given to increase disease awareness, to improve pertus-

sis surveillance and laboratory diagnosis [3] but, above all, to enhance maternal vaccination in pregnancy, as well as ensuring that these recommendations are effectively implemented in accordance with national guidelines.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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As mentioned in the above article, because the number of events per parameter was lower than 10 in the presented VE analyses, we conducted an additional sensitivity analysis using Firth's method of penalised logistic regression to assess small sample bias. The results from this sensitivity analysis using penalised logistic regression are presented in the supplementary table below.

Table S5. Adjusted vaccine effectiveness of pertussis vaccination in pregnancy in hospitalised infants too young to be vaccinated (aged <2months) using Firth's method of penalised logistic regression to assess small sample bias, PERTINENT study, Europe, 1 December 2015–31 December 2019 (n = 276)

Adjustment variables	Df	N	Cases		Controls		VE (95% CI)
			Vacc.	N	Vacc.	N	
All infants, 5 sites (N = 276)							
Site; Onset quarter	9	276	9	75	92	201	74 (35-90)
Site; Onset quarter; Age group (4-30d; 31-60d)	10	276	9	75	92	201	73 (32-89)
All infants, 3 sites* (N = 165)							
Site; Onset quarter	7	165	9	30	92	135	74 (35-89)
Site; Onset quarter; Age group (4-30d; 31-60d)	8	165	9	30	92	135	73 (32-89)
Infants sampled with NPA, 5 sites (N = 185)							
Site; Onset quarter	9	185	6	57	51	128	86 (55-95)
Site; Onset quarter; Age group (4-30d; 31-60d)	10	185	6	57	51	128	84 (50-95)
Infants sampled with NPA, 3 sites ^a (N = 88)							
Site; Onset quarter	7	88	6	20	51	68	84 (51-95)
Site; Onset quarter; Age group (4-30d; 31-60d)	8	88	6	20	51	68	83 (45-95)

CI: confidence interval; Df: degree of freedom; NPA: nasopharyngeal aspirate; VE: vaccine effectiveness.

^a Excluding 2 sites due to the absence of vaccinated women.

4. Synthesis

This section details and interprets the main findings of each objective presented in previous sections. Major strengths and limitations of the work done and studies used for this thesis are discussed. We then conclude this synthesis with the implications of the findings for public health intervention and further research that is needed.

4.1. Summary of evidences

To the best of my knowledge, the PERTINENT network is the only EU/EEA multicenter collaboration that allows for large, independent and multi-country pertussis VE studies which is the greatest strength of this work.

4.1.1. *Setting up a sentinel surveillance system for severe pertussis in infants*

To set up this EU/EEA multicentre study, we identified the largest number of study sites that the project's financial resources would enable, and ensuring the most unbiased representation at European level. We selected seven study sites in six European countries: France, Spain, Ireland, Norway, the Czech Republic and Italy. We developed a generic protocol [24], laboratory guidelines [14] and organised sites visits to ensure harmonisation of hospital practices and to allow pooling of sites' data. To maximise the sensitivity of the surveillance, we trained the team and raised hospital physicians' awareness of pertussis clinical presentation. We developed statistical scripts (i.e., STATA dofiles) to analyse the pooled data. All the developed material throughout the project was shared with the partners, and when possible, made publicly available on the PERTINENT website:

<https://sites.google.com/a/epiconcept.fr/pertinent/pertinent-network>.

In the PERTINENT project, we built a strong multidisciplinary network with paediatricians, epidemiologists, nurses, clinicians from the hospital but also microbiologists and collaborated with the ERLNPert-Net, formerly known as EUPert-LabNet (European Laboratory Network for Pertussis). Indeed, the laboratory component proved to be crucial in pertussis surveillance at the early stage of the project. Hospitals, national and regional public health institutes as well as experts from the ECDC, US CDC and WHO, participated to our annual PERTINENT meetings, contributing insightful discussions that shaped the project.

With the enhanced pertussis surveillance, we aimed to provide better quality data than the routine national surveillance of the participating countries, but also to standardise clinical diagnosis and laboratory procedures as much as possible across the participating countries [10]. Ultimately, setting up this European sentinel network was an excellent opportunity to create an EU/EEA platform with

significant potential for addressing key public health questions related to pertussis and pertussis vaccine strategies, provided that the sample size would be sufficient.

4.1.2. Incidence and severity of the disease

Pertussis is an endemic disease with epidemic peaks occurring every two to five years. Bearing in mind that the last pertussis outbreak in Europe was in 2012, the PERTINENT project was in a good position when launching enhanced surveillance in 2015 in the 41 participating hospitals. According to ECDC annual reports on the disease and considering countries that reported consistently between 2014 and 2018, the number of reported cases increased between 2014 and 2016 and decreased thereafter [23].

Over four years of data collection from 2016 to 2019, we tested 2,346 infants aged <1 year for *Bordetella pertussis*. We excluded 36 respiratory infections caused by other *Bordetella* species including 20 *Bordetella parapertussis*, 3 *Bordetella holmesii*. A total of 557 infants were positive for *Bordetella pertussis*, including one co-infection with *Bordetella pertussis* and *parapertussis*, which suggest that out of all *Bordetella* species identified over the study period, 6% of infections are not caused by *Bordetella pertussis* but another strain, most of them being *Bordetella parapertussis*.

In 2016 and 2017, most of the cases were reported during summer which supports findings from previous studies in Europe suggesting a mild seasonality of the disease [23,37]. Incidence rate ratios between 2018 and 2017 were 1.43 in Czech Republic ($p = 0.468$), 0.25 in Catalonia ($p = 0.002$), 0.71 in France ($p = 0.0335$), 0.14 in Ireland ($p = 0.002$), 0.63 in Italy ($p = 0.053$), 0.21 in Navarra ($p = 0.148$) and zero in Norway, suggesting a decrease in pertussis incidence across all sites except one.

Thirty-nine cases had an atypical pertussis clinical presentation (7%), among whom 26 had a cough only, five had cough and cyanosis only, two had a cyanosis only and six had documented absence of all pertussis-like symptoms. Six of these atypical pertussis cases had at least one missing clinical information. Our findings highlight the need to raise clinician's awareness about pertussis underdiagnosis in infant.

Over the study period, five infants died. They were aged from 2 weeks to 10 weeks and not vaccinated against pertussis. One of four pertussis laboratory-confirmed hospitalised cases was admitted to ICU. The highest proportion of ICU cases was in infants aged 0–3 months.

The PERTINENT project ended in January 2020, after four years of data collection, owing to the cessation of funding. At this time, the COVID-19 pandemic emerged and disrupted healthcare but also surveillance systems in Europe. Implemented in 2020, mitigation measures such as mandatory masking-wearing, physical distancing recommendations but also remote schooling were used to reduce the spread of the disease. Concurrently, a global decrease of epidemic diseases in Europe,

including pertussis infections, was observed. Initially, this decline was likely attributable to surveillance shortfalls, but shortly later appeared to be related to new behaviours on personal protection measures [83–86]. Since then, pertussis incidence has remained low across Europe, altering the pathogen's typical circulation patterns. At a lower level, we recently observed an increase of *Bordetella parapertussis* cases in France with several local outbreaks in children aged less than 6 years in July 2022 [84]. In this post COVID-19 pandemic era, all *Bordetella* infections should be closely monitored to detect any resurgence in the community now that social restrictions have been relaxed across Europe.

4.1.3. Primary vaccination in infants

The PERTINENT project not only monitored pertussis infection in infants aged <1 year, but also allowed a prospective TND study aiming to estimate pertussis VE in infants eligible for PV and aged 2-11 months.

At least one dose of pertussis PV

In our network of paediatric hospitals, we estimated VE after at least one dose of aP PV against *Bordetella pertussis* infection in infants aged 2-11 months. VE estimate adjusted for study site, time of symptoms onset (in quarters) and age groups (2, 3-11 months) was 59% (95%CI: 36-73) suggesting that having received at least one dose of aP vaccine already offer a moderate protection to infants aged 2-11 months.

One-dose VE

Despite low sample sizes, we also aimed to estimate dose-specific VE of the infants PV. In infants aged 2-11 months and eligible for any doses of pertussis PV, only one-dose VE adjusted by site, time of symptoms onset (in quarters) and age group (2, 3, 4 months and 5-11 months) was 56% (95%CI: 28-73).

According to national immunisation recommendations of each study site, this analysis includes infants already in the age group targeted for the second, the third and the fourth dose of PV and who are late in their vaccination schedule. These older infants might not have the same immune response to a unique dose of PV and should be excluded for more robust estimations. Restricting the analysis among infants eligible for the first dose of PV only and aged 2-5 months, adjusted one-dose VE was estimated at 48% (95%CI: 5-72). Note that the precision of the estimate was impacted by the reduction of sample size.

Two-dose VE

In infants aged 2-11 months and eligible for any doses of pertussis PV, two-dose VE adjusted by site, time of symptoms onset (in quarters) and age group (2, 3-11 months) was 73% (95%CI: 50-86).

As for the one-dose analysis, this two-dose analysis includes infants in the age group targeted for the first dose and not yet vaccinated at all, but also infants in the age group targeted for the third and fourth dose of PV. Restricting the analysis among infants eligible for the second dose of PV only and aged 3-10 months, adjusted two-dose VE was estimated at 76% (95%CI: 43-90). Due to the drastic reduction of sample size, we performed an additional sensitivity analysis using penalised logistic regression which provided similar results.

4.1.4. Vaccination in pregnancy

Based on the four-year data collected within the PERTINENT study, our settings allowed to also investigate the vaccination in pregnancy recommended in four of the six European participating countries: Czech Republic, Ireland, Italy, Spain with the Catalonia and Navarra regions (five study sites, 14 hospitals). We restricted our study population to the infants from these countries, whose mother was eligible for vaccination in pregnancy, and likely to be hospitalised if developing pertussis-like symptoms.

In infants too young for vaccination (<2 months)

Vaccination in pregnancy aims to protect infants too young for vaccination with PV, based on the evidence of transplacental transfer of maternal antibodies from the mother to the foetus.

We estimated VE in pregnancy against laboratory-confirmed *Bordetella pertussis* infection in infants aged 4 to 60 days. VE estimate adjusted for study site and time of onset (in quarter) was 76% (95% CI: 38–91) and 75% (95% CI: 35–91) when also adjusted for age group (4-30 day-old; 31-60 day-old).

Bordetella species can be isolated from both NPS or NPA. However, a 15% gain in the isolation rate can be obtained by using NPS in neonates and infants [87]. Therefore, in an additional sensitivity analysis, we excluded infants sampled only with NPS. VE adjusted for site and time of onset (in quarter) and VE also adjusted for age group were, respectively 88% (95% CI: 59–96) and 87% (95% CI: 55–96). Results were similar when using a penalised logistic regression.

These findings suggest that vaccination in pregnancy is an effective strategy to fill the immunisation gap of the first two months of life, when the disease is the most life-threatening.

In the current post-COVID-19 era, we observe a global decrease of epidemic respiratory diseases in Europe, including pertussis infections, likely related to new behaviours on personal protection and social measures, but we also observe a significant coverage drop in 2021 for several routine childhood

vaccines [83–86,88]. This decrease in vaccine-induced but also disease-induced protection may lead to a new epidemic cycle for pertussis in the near future in Europe. Very recently, The Statens Serum Institut (SSI) in Denmark observed an increase in pertussis incidence in the summer 2023, with a particularly high incidence among infants aged less than one year. The institute assessed this increase as a new epidemic peak in the country (last epidemic known to be in 2019-2020, before the COVID pandemics). This recent whooping cough epidemic in Denmark led to a temporary offer of pertussis vaccination for pregnant women from 1st August 2023, like they already did for the 2019 pandemic and as the UK did for the first time during the 2012 pertussis pandemic [57,89].

In infants eligible for PV (2-11 months)

Study settings of the PERTINENT project also allowed us to investigate the clinical implications of the potential “blunting effect” of vaccination in pregnancy with infants’ PV [27].

Due to sample size limitations, we conducted an indicator analysis based on four categories: (a) infants recording no vaccination in their mother’s pregnancy nor own PV (*reference category*); (b) infants recording PV only (at least one dose); (c) infants recording vaccination in pregnancy only; (d) infants recording both vaccination in pregnancy and PV (at least one dose).

We estimated VE in each stratum using infants recording no vaccination in pregnancy nor PV (a) as reference category. VE adjusted for site, time of onset and age group (2; 3–11 months) was 74% (95% CI: 33–90) for infants with both PV and vaccination in pregnancy; 68% (95% CI: 27–86) for those with PV only; 36% (95% CI: -85–78) for those with vaccination in pregnancy only. When excluding infants sampled with NPS only (N = 251), VE adjusted for site, time of onset and age group was 88% (95% CI: 62–96) for infants with both PV and vaccination in pregnancy; 81% (95% CI: 46–93) for those with PV only; 44% (95% CI: -109–85) for those with vaccination in pregnancy only. These findings suggest a good effectiveness of at least one dose PV in infants aged 2–11 months, ranging from 68% to 88%, irrespective of the vaccination status of the mother. Unfortunately, current sample size did not allow us to restrict the analysis on one dose of PV only (instead of at least one dose). Additionally, results of this stratified analysis were not robust enough to conclude on a potential interaction of maternal vaccination with the infants’ PV.

4.2. Current limitations

Each article included in this thesis details its main limitations related to each objective of the thesis and the corresponding analysis carried out. Throughout the four years of the PERTINENT surveillance programme, we have identified overarching limitations that we aim to discuss further.

4.2.1. Heterogeneity inherent to a multi-country and multi-centre study

Despite development of a standardised generic protocol, laboratory guidelines, and organisation of site visits at the implementation phase of the study to ensure a homogenisation of protocol implementation, there was a strong heterogeneity between study sites. In terms of capacity, some sites were able to enrol a large number of hospitals spread out all over the country, ensuring representativeness of the population (e.g., France, Czech Republic, Navarra region, Norway). Some other could only include one or two hospitals, even though the largest paediatric hospitals of the region (e.g., the Catalonia region, Ireland and Italy). The French site accounted for about 50% of the participating hospitals. When computing overall VE pooling all sites' data at the PERTINENT level, we adjusted by study site to account for this heterogeneity.

When computing disease incidence, methods to estimate the catchment population (i.e., denominators) and their precision varied across sites (e.g., National/Region census vs. more complex estimations). These differences prevented us from estimating pertussis incidence at EU/EEA level but also from comparing pertussis incidences between sites. Nevertheless, we could monitor incidence trends by sites over the study period.

Due to funding issues, duration of the project was uncertain and only allowed short term plans for the PERTINENT project. After almost three years, five hospitals had to withdraw from the project due to the lack of long-term funding. We also had to cope with the turnover of key staff member in several study sites throughout the duration of the project. Due to heavy workload in hospital teams and the absence of funding for study-specific staff member, we had to adapt the protocol for a couple of sites. Cases and controls inclusion: We selected three controls per case, systematically matched for date of specimen collection in the Catalan site. On the opposite, the Norwegian site could include up to 248 controls for one case enrolled. We noticed that the probability of being hospitalised for pertussis-like symptoms is heterogeneous across countries due to different health care practices.

Laboratory diagnostic methods were also very heterogenous and we asked hospital teams to systematically perform a series of three PCRs on a nasopharyngeal aspirate to ensure a good quality of specimen collection and good sensitivity and specificity of laboratory diagnostic results [14]. We emphasised the importance of an adequate laboratory-based definition of *Bordetella pertussis* to the hospital teams. Misclassifying a *Bordetella parapertussis* or *Bordetella holmesii* case as a *Bordetella pertussis* would lead to a classification bias in all PERTINENT analyses. Pertussis incidence would then be overestimated. And because infections with *Bordetella parapertussis* or *Bordetella holmesii* tend to be less severe than *Bordetella pertussis*, misclassification would potentially also bias disease burden towards a lower severity [41,78]. Regarding VE analysis, the aP vaccine is based on *Bordetella pertussis*

pathogen, including up to five antigens of its genome. Even though other *Bordetella* species are not directly targeted by pertussis-containing vaccine, the later may also induce some cross-immunity [79]. Despite this unexpected cross-immunity, misclassifying other *Bordetella* species as *Bordetella pertussis* cases might lead to an underestimation of VE estimates.

Additionally, even though all sites were using the aP vaccine for infants' PV and maternal vaccination, vaccination recommendations and schedules varied between countries for both immunisation strategies (see [Table 1](#)). Recommendations also evolved during our four-year study period with the move from a primary schedule at 2, 3, 4, 10 months to 3, 5, 11-13 months in 2018 for Czech Republic; or the implementation of the maternal vaccination in 2017 in Italy.

4.2.2. Sample size limitations

We carried out four years of active surveillance in 41 participating hospitals spread out over six EU/EEA countries and an enhanced disease awareness among the hospital teams. However, the achieved sample sizes for all VE analysis that we carried out (i.e., dose-specific VE after infants' primary schedule, VE of vaccine in pregnancy against pertussis in infants) did not allow for a high precision in VE estimates nor further stratifications.

And despite the inherent wide heterogeneity across study sites above described, the small sample size available for our analysis did not allow to estimate VE by site. We used a "one-stage" approach on pooled data, with study site as fixed effect instead. To consolidate our findings in PERTINENT VE analysis, it is crucial to strengthen the sentinel network and to allow larger sample sizes and estimation of the statistical heterogeneity between sites.

To control for small sample biases, if the number of events per parameter in each analysis was lower than 10, we conducted a sensitivity analysis using Firth's method of penalised logistic regression [90]. Current sample size did not allow us to estimate PV dose-specific VE by age group nor by brand. The latter is nevertheless an important topic in the field. Pertussis-containing vaccines do not include the same antigens across all brands. Few data is available on that matter, but this could theoretically lead to a different VE by brand [1].

Analysis of pertussis VE by time since vaccination is also of the utmost importance for both PV and vaccination in pregnancy, as there has been doubts expressed recently on the duration of the protection conferred by the aP vaccine [6]. More robust findings on the duration of aP vaccine-induced immunity would help public health policy to target the best schedule for PV, but also the best timing for the first dose of PV in the infant after vaccination during the mother's pregnancy.

Additional stratifications were needed in our analysis in order to assess the presence of unmeasured confounding or modifier factors such as the interaction between vaccination in pregnancy and the first dose of PV in infants. Therefore, at this stage of the PERTINENT project, we cannot exclude the existence of unmeasured confounding biasing our results.

One of the main challenges related to the pooling of multi-centre data is to take heterogeneity into account. The “one-stage” approach assumes that the VE and the confounding are the same in all sites which is unlikely in our settings due to differences of vaccine brand, PV schedule, maternal vaccination window during pregnancy (e.g., from week 16th or week 27th of gestation), age at first dose, differences in circulating Bordetella strains or immunisation recommendations in adults. Larger sample size is needed to estimate site-specific VE, statistical heterogeneity between sites and perform a “two-stage” model analysis including the confounding factors of interest for each study site.

4.2.3. Information biases

Data quality was high in our study with less than 5% of missing exposure variable, less than 3% of missing outcome variables and less than 5% of missing information for confounding variables.

However, we found a large proportion of missing information for variables related to the severity of the disease. There was no common definition nor common indicators of pertussis severity. We intended to build a Paediatric Early Warning Scoring system (PEWS) specific to the PERTINENT project that could include: white cell counts, respiratory rate, respiratory effort, saturation, oxygen therapy, etc [77,91]. But not all participating hospitals had the capacity to measure all these severity indicators and the proportion of missing data could vary between cases and controls. Taking this limitation into account, we described the severity of cases with the data available but we did not measure VE by pertussis severity.

4.2.4. Test-negative design approach

Based on our review of the literature, the PERTINENT study appears to be the first multi-country study using a prospective TND approach in Europe in hospital settings for estimating pertussis VE. To validate our findings, we need to confirm that this is a proper study design to estimate pertussis VE in infants.

Since 2005, there is an increasing number of VE studies based on TND to estimate the effectiveness of the influenza vaccine, RSV vaccine, rotavirus vaccine, and more recently the COVID-19 vaccine. Several publications show attempts to validate the design for influenza [12,76]. But the validity of the design has not been explored for pertussis vaccine. As in all case control studies, in TND studies we assume that controls are representative of the source population in terms of vaccine coverage. Therefore, we need to compare the vaccine coverage in the control group of our analysis, with the vaccine coverage

of infants of the same age in the source population of cases. To validate this assumption, we would need to have access, if available, to the vaccine coverage by specific age group in each hospital catchment area participating in our network.

As illustrated during the COVID pandemic, vaccination against various respiratory diseases (influenza, COVID, RSV, pertussis) are likely to be correlated. Infants vaccinated against COVID-19, influenza or RSV are very likely to be vaccinated against pertussis. Several publications have suggested that each additional vaccine may be a confounding factor for a specific study [92]. Authors have proposed that for example in a VE study for Influenza, COVID cases should be excluded from the control group. If so, it is crucial to verify that the remaining control group still fulfils criteria for being a representative control group. Besides, such a selection constraint might represent a dramatic issue for the feasibility of TND study in the near future with many children vaccinated with other respiratory vaccines in the control group. In addition, but this goes beyond the scope of this thesis, another correlated vaccination may not fulfil the condition to be a confounder for the measured VE of interest.

In the post-COVID-19 era, we also saw the success of self-sampling and self-testing in the fight against the pandemic. If testing at home becomes the new norm for other diseases such as pertussis, (e.g., self-testing kits for HIV infection) beyond COVID-19 infections, there is a high probability that this new diagnostic method will affect our VE estimations based on the TND approach. In hospital-based TND studies such as the PERTINENT study, we can expect to more likely have the most severe cases with pertussis-like symptoms presenting at the hospital. Indeed, infants with mild symptoms might more likely be tested at home by their parents who may subsequently consult their general practitioner instead. Impact on VE studies against severe pertussis might therefore be limited.

4.3. Public health implications and further research needed

4.3.1. Public health implications

PERTINENT sentinel surveillance highlighted the existence of pertussis atypical clinical presentation in infants, especially in infants aged <3 months. These findings are in favour of including atypical symptoms such as apnoea or cyanosis in the pertussis case definition of infants, but also to increase disease awareness among clinicians in that age group for which the disease is particularly life-threatening. We believe that, as suggested by Vittuci et. al, the systematic use of a multiplex RT-PCR for any infants aged <3 months and presenting with acute respiratory symptoms, would be a major improvement in pertussis surveillance to limit its under-diagnosis [20]. These recommendations are particularly important in these post-COVID-19-pandemic times, when we could expect a resurgence of

the disease due to relaxing mitigation measures, but also an emergence of unexpected outbreaks of other *Bordetella* species [93].

4.3.2. Policy implications

Our findings suggest that the effectiveness of pertussis vaccination, aiming at reducing the risk of severe pertussis in infants, is moderate for both the first and second dose but also for at least one dose of PV. During the four years of PERTINENT surveillance, we identified five deaths in unvaccinated infants. About one hospitalised pertussis case out of four required ICU admission and cases admitted to ICU were younger (aged <3 months) and less vaccinated than non-ICU cases. Despite an overall vaccination coverage (VC) >90% in Europe, pertussis remains an important public health concern for infants. This work underscores the necessity to maintain a high coverage of the pertussis PV at the EU/EEA level. France and Italy recently moved pertussis primary vaccination among infants' mandatory vaccinations. Even though it allows to maintain a good VC, such policy should be considered with caution, bearing in mind the increasing vaccine hesitancy in the population. This is particularly valid these days, in the context of the COVID-19 pandemic that disrupted vaccination follow-up due to the lock-down, but also vaccination acceptance and trust all over Europe. Several countries recently reported an overall decline in routine childhood vaccine coverages[80]. In a recent paper, Ghaznavi et. al. estimated the global changes in routine childhood vaccination coverage in 2020-2021 from WHO/UNICEF Estimates of National Immunization Coverage. Though the past several decades have been characterised by widespread gains in vaccine coverage, they found widespread evidence in paediatric populations of a lower vaccination coverage globally, specially in 2021 compared to 2020 [88,94]. Such decline in VC accentuates the necessity of high VC to prevent large and uncontrolled pertussis epidemic peak incidence in the next years.

Another implication of our observation of more severe pertussis infections in younger and unvaccinated infants (aged <3 months), is the importance of maternal vaccination policy. This immunisation strategy is of utmost importance to protect infants too young for PV. Our results support the shift of an increasing number of European countries toward the implementation of vaccination in pregnancy. Indeed, it is encouraging to see this increasing number of European countries introducing this immunisation strategy among their national recommendations (Belgium, Czech Republic, Ireland, Italy, Latvia, Portugal, Slovenia and Spain and more recently Netherlands in 2019 and France in 2022) [23,42]. However, we noticed that adherence to maternal vaccination was very heterogenous across PERTINENT study sites and could be challenging in some countries such Italy and Czech Republic [16–18,95]. Improving knowledge of pregnant women is key for a good compliance of maternal immunisation. Recent literature has reported the essential role of healthcare workers in that matter. Training, improved information about vaccine efficacy and safety, and disease awareness among

midwives, obstetricians, gynaecologists or family physicians is strongly needed to increase overall vaccine uptake, especially in these times of high vaccine hesitancy [96,97]. And again, this is particularly important in this post-COVID-19-pandemic era with low pertussis incidence and pertussis VC [98].

4.3.3. Research needed

As previously mentioned, little evidence exists about the clinical implications of the potential “blunting effect” of vaccination in pregnancy on infants’ PV, as described in numerous immunological studies [27]. And despite our encouraging results, the limited sample size of our analysis in infants aged 2-11 months did not allow us to conclude on the absence of interaction between the two vaccinations. Further research with larger European studies is essential to clarify if but also how much maternal vaccination interacts with the infant’s first dose of PV. These findings could guide policy decision regarding the optimal timing for administering the first dose of PV to infants whose mothers received the vaccine during pregnancy. Indeed, given that vaccination in pregnancy is the most effective protective strategy for infants too young for vaccination, we find relevant to study the option of postponing the first dose of PV to the best time point of waning maternal antibodies.

A large cohort of pregnant women could be a good platform for studies on vaccination in pregnancy including pertussis but also influenza or the recently approved RSV vaccine.

Additionally, while we support the increasing implementation of vaccination in pregnancy in European countries, a question remains about the relevance of having both cocooning and maternal vaccinations in place, such as in Ireland and France. Cost-effectiveness with regards to vaccine adherence are needed in these countries to identify the best strategy and possibly simplify current recommendations. Last but not least, now that the PERTINENT surveillance has ended and that we can already detect the first *Bordetella* species local post-COVID-19-pandemic outbreaks in Europe [93], it is of critical importance to re-launch a large European surveillance for *Bordetella* infections in Europe, monitoring potential strain replacement or genome shift that would escape the current aP vaccine. Such information would be crucial in the race of development of new pertussis vaccine [38].

5. Conclusion

The results from our PERTINENT hospital network are very encouraging and suggest that pertussis in infant remains a health protection issue at the European level, especially among infants too young to be eligible for PV.

Vaccination against pertussis during pregnancy aims to address this lack of immunity in the infant's first weeks of life. An increasing number of countries, particularly in Europe, are progressively adopting this new vaccination strategy (Belgium, Spain, Ireland, Italy, Latvia, Netherlands, Portugal, Czech Republic, Slovenia, United Kingdom, and France since April 2022). Our results support this shift in vaccination policy and suggest a high VE in infants under 2 months while not being able to assess the epidemiological impact on VE of the hypothesis of a blunting effect.

In most of the vaccination schedules recommended in Europe, the infant is eligible for the first dose of PV from the third month of life. Our results indicate a moderate VE after one dose. The VE after the first dose of vaccine seems to be boosted following the injection of the second dose. Furthermore, whether or not the mother was vaccinated during pregnancy, the VE after at least one dose of PV is good. However, our limited sample size of included infants did not allow for a sufficiently precise stratified analysis to determine whether vaccination during pregnancy modifies the effectiveness of at least one dose of pertussis PV. It also did not allow the measurement of the interaction between the two vaccinations. Consequently, even if our results may indicate equally good vaccine effectiveness after at least one dose of PV, regardless of the mother's vaccination status, we cannot conclude on the absence of a clinical impact of the immunological blunting effect of maternal vaccination on the infants' immune response to their PV.

The PERTINENT project has highlighted the importance of active surveillance in Europe among infants. Including six European countries, it has enabled the collection of good-quality data, with few missing data, but also the establishment of standardised surveillance systems across seven study sites, using common protocols, identical case definitions and common laboratory guidelines for *Bordetella pertussis* identification based either on culture or on a diagnostic algorithm including a specific set of PCR targets.

Such a European hospital network could support numerous studies addressing the disease burden and the VE of various vaccination strategies currently in place in each European country. It also allows monitoring of pertussis epidemic cycles and circulating *Bordetella* species that challenge the high vaccination coverage in Europe and evade the currently used acellular vaccine. The sustainability, but especially the expansion, of such an hospital network is essential for obtaining a sufficient sample size for the analysis of the effectiveness of the third dose of PV, the identification of the optimal vaccination

window for the mother (from 16 to 37 weeks gestation depending on the country), the duration of infant protection conferred by maternal vaccination, the modifying effect of vaccination during pregnancy on each of the three doses of PV, the VE of cocooning strategy combined or not with the strategy of vaccination during pregnancy, the VE of PV against other *Bordetella* species, or the VE of potential new live attenuated vaccines. Such a sentinel hospital network could constitute a large European platform for the active surveillance of all concerning respiratory diseases in infants.

6. Perspectives

Paediatric hospital surveillance network in Europe for respiratory infections

The PERTINENT project emphasised the importance of pertussis surveillance in infants at European level to monitor disease incidence in this vulnerable population; to monitor the effectiveness of acellular pertussis vaccines in infants but also during pregnancy; and to measure impacts of changes of vaccination strategies. Such a network, including a strong laboratory component, would allow collection of *Bordetella* isolates over Europe, and follow their genetic evolution in the population over time. This is particularly crucial for pertussis surveillance due to the changes of vaccination strategies in several European countries, and the recent questions about a potential waning immunity related to *Bordetella* strains that would evade the acellular vaccine composed of up to five purified *Bordetella pertussis* antigens. There is an urgent need to build a strong paediatric hospital network in Europe for an enhanced active surveillance of all concerning respiratory diseases in infants that can be tested with a single multiplex PCR such as *Bordetella pertussis*, SARS-CoV-2 but also respiratory syncytial virus infection (RSV). In September 2022, the European Medical Agency (EMA) has recommended granting a marketing authorisation in the EU for the first monoclonal antibody therapy (Beyfortus, nirsevimab) to prevent RSV infection in infants. The European Commission then approved it in November 2022. More recently, the first vaccine to protect infants aged <6 months against RSV through passive immunisation following vaccination of the mother during pregnancy was authorised for use in the EU by the EMA in July 2023 but its effectiveness in field delivery conditions has not been evaluated yet. It is getting crucial to set up a large European hospital surveillance platform for respiratory infections in infants that would allow to conduct numerous analysis and answer burning research questions to support public health decision making.

New pertussis vaccines

Addressing the limitations of current pertussis vaccines is crucial for effective pertussis control. The current acellular vaccines, while safe and effective, do not similarly stimulate the immune response observed with wP vaccines or natural infections. This disparity not only affects the duration of immunity but also raises concerns about the potential for pathogen carriage and subsequent transmission. To address these issues, recent research has intensified to produce vaccines exploring three primary strategies: (i) modifying the *Bordetella pertussis* bacterium to produce a new whole-cell vaccine that is both safe and has reduced side effects; (ii) introducing additional *Bordetella pertussis* antigens to acellular vaccines; (iii) adding an adjuvant that drives immune responses toward the pattern seen with wP vaccines [38,99].

New diagnostic method

Pertussis is often underreported and particularly misdiagnosed among infants and neonates presenting with an atypical clinical presentation that can be very similar to several other respiratory diseases. The introduction of PCR diagnosis for pertussis in 2012 could explain part of the 2012 peak incidence of pertussis. First because this new diagnostic method has a higher sensitivity than the culture of the pathogen. Second because PCR can be used as a routine pertussis laboratory diagnosis testing a unique nasopharyngeal sample for several respiratory diseases within a single multiplex PCR test, and therefore identifying a significant additional number of unexpected pertussis cases. Facing a important underestimation of pertussis cases in infants, Vittuci et al. supported in 2016 a routinely use of RT-PCR for pertussis in all infants aged less than 3 months with any respiratory symptoms [20]. Over the past few years, new diagnosis methods for pertussis are being investigated, including the use of machine learning algorithm based on clinical signs and symptoms in the first year of age, such as cough sound evaluation [100,101]. For now, these new diagnostic methods are still under evaluation and there are few studies investigating that field. Even though the results are quite heterogenous, some models used seem to nicely support clinical decision and show promise. Creating accurate tools to help timely decision on clinical diagnosis of pertussis, combined with a sensitive laboratory diagnostic algorithm, could bring a huge change in pertussis surveillance in the future by preventing underestimation of the disease, especially in infants.

What to expect next?

Finally, it is important to remember that the COVID-19 pandemic disrupted surveillance systems, vaccination programs, population behaviours and social interaction, and led to a decrease in the pathogen circulation but also of vaccination coverage in several countries [83,84,88]. We need to be prepared to a strong resurgence of pertussis in the near future and a robust surveillance is needed over Europe to detect at early stages the upcoming epidemic crisis of pertussis.

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