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**Transmission and management of brucellosis
in a heterogeneous wild population
of Alpine ibex (*Capra ibex*)**

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J'espère que tu sais que je traverserais montagnes et océans pour toi,
Et que j'aimerais qu'on puisse partager tous nos moments de bonheur
pendant encore de nombreuses années
Je t'aime.

*To see the world,
Things dangerous to come to,
To see behind walls,
Draw closer,
To find each other,
And to feel.
That is the purpose of life.*

The secret life of Walter Mitty

SUMMARY

Transmission and management of brucellosis in a heterogeneous wild population of Alpine ibex (*Capra ibex*)

The management of infectious diseases in wildlife reservoirs is particularly challenging and faces several limitations. The development of appropriate management strategies requires a detailed understanding of the factors affecting the transmission and persistence of the infectious agent in the population. Among these factors, heterogeneity of transmission is a common characteristic in natural host-pathogen systems. Indeed, wild animals express a broad range of behaviours, are organised in a variety of social and spatial structures, occupy many areas with very different characteristics and belong to a large diversity of species. Such heterogeneities, from between-individuals to between-species, may result in different contributions to the overall number of new cases of infections. Thus, understanding transmission heterogeneity could provide valuable insights on how to effectively manage these systems, by targeting the individuals or areas that are responsible for most transmissions.

The aim of this thesis was to provide insights on the monitoring and management of infectious diseases in heterogeneous wild populations, using *Brucella melitensis* infection in a French population of wild Alpine ibex (*Capra ibex*) as a case study. The biology of brucellosis and the ecology of Alpine ibex makes this case study a good candidate for transmission heterogeneity at several levels.

Using bacterial examinations, we first established that only 58% of seropositive individuals were at risk to excrete *Brucella*, and that this risk decreased with increasing age. Then, we took advantage of detailed information available on ibex population dynamics, behaviour, and habitat use, and on epidemiological surveys, to build an individual-based model in order to quantify heterogeneity at the individual and spatial levels. The transmission is extremely heterogeneous between individuals, with females generating around 90% of the new cases of brucellosis infection, and between spatial units, three of the five socio-spatial units (the core area) accounting for more than 80% of brucellosis transmission. Using statistical models to estimate the temporal dynamics of the seroprevalence and of the force of infection in the population, we found evidence that the massive captures with test-and-remove operations that were conducted in 2015 managed to reduce brucellosis transmission in the population. Based on these results, we evaluated several predictive disease management strategies in the individual-based model. Our results confirmed that the primary strategy should be to remove as many infected individuals as possible through test-and-remove, and that strategies targeting females and/or the core area are more effective than untargeted management.

Although there is no silver bullet for the management of brucellosis in the population of study, targeted strategies offer a wide range of promising refinements to classical sanitary measures. We therefore encourage to look for heterogeneity in other infection-wildlife systems and to evaluate potential targeted strategies for improving management schemes in terms of efficiency and acceptability.

Keywords: epidemiology, infectious diseases, wildlife reservoirs, modelling, heterogeneity, force of infection, targeted management, *Capra ibex*

RESUME

Transmission et gestion sanitaire de la brucellose dans une population sauvage hétérogène de bouquetins des Alpes (*Capra ibex*)

La gestion des maladies infectieuses dans la faune sauvage se heurte à de nombreuses limites, et le développement de stratégies efficaces représente un défi de taille. Pour atteindre cet objectif, une compréhension fine des facteurs influençant la transmission et la persistance de l'infection est nécessaire. Parmi ces facteurs, l'hétérogénéité de transmission est une caractéristique importante des populations sauvages. En effet, la diversité des comportements, des structures sociales et spatiales, ou encore des espèces peut conduire à des contributions très variables au nombre de nouvelles infections. Par conséquent, quantifier l'hétérogénéité de transmission pourrait permettre d'améliorer l'efficacité des mesures de gestion sanitaire dans la faune sauvage, en ciblant les individus ou les unités de population qui sont responsables de la majorité des événements de transmission.

L'objectif de cette thèse était d'améliorer les connaissances sur la gestion des maladies infectieuses dans des populations sauvages hétérogènes, en utilisant la brucellose à *Brucella melitensis* dans une population de bouquetin des Alpes (*Capra ibex*) comme modèle d'étude. En effet, la biologie de la brucellose et l'écologie de l'espèce hôte se prêtent bien à l'existence et donc à l'étude d'une hétérogénéité de transmission à différentes échelles.

A l'aide de cultures bactériennes, nous avons tout d'abord montré que seulement 58 % des individus séropositifs sont à risque d'excréter la brucellose, et que ce risque diminue avec l'âge. Ensuite, mettant à profit l'existence d'informations détaillées sur la dynamique de population et le comportement du bouquetin, et de données épidémiologiques dans la population d'étude, nous avons développé un modèle individu-centré afin de quantifier l'hétérogénéité individuelle et spatiale de la transmission. Nous avons démontré que la transmission de la brucellose était hétérogène entre individus, les femelles provoquant environ 90% des nouvelles infections, et entre unités spatiales, plus de 80% des cas de transmission ayant lieu dans les trois sous-unités socio-spatiales qui forment la zone cœur du massif. Nous avons également estimé l'évolution temporelle de la séroprévalence et de la force d'infection, en utilisant différents modèles statistiques. Les résultats suggèrent que l'importante opération de capture menée en 2015, avec test systématique et élimination des individus séropositifs, a permis de diminuer la transmission de la brucellose dans la population. Sur la base de l'ensemble de ces résultats, nous avons évalué une série de stratégies de gestion sanitaire qui pourraient être utilisées à l'avenir dans la population. Les résultats, issus du modèle individu-centré, confirment que la stratégie prioritaire devrait être d'éliminer le plus d'individus infectés possible par test-et-élimination, et que cibler les femelles et/ou la zone cœur permet d'améliorer l'efficacité des mesures.

Bien qu'il n'y ait pas de solution évidente pour la gestion de la brucellose dans notre cas d'étude, les stratégies de gestion ciblées sont très prometteuses et permettent de raffiner les mesures sanitaires classiquement utilisées. Il est donc primordial de bien comprendre l'hétérogénéité de transmission dans les populations sauvages infectées, et de rechercher des stratégies ciblées qui peuvent permettre d'améliorer la gestion en termes d'efficacité et d'acceptabilité.

Mots-clés : épidémiologie, maladies infectieuses, réservoirs sauvages, modélisation, hétérogénéité, force d'infection, gestion ciblée, *Capra ibex*

RESUME DE LA THESE EN FRANCAIS

La gestion des maladies infectieuses dans la faune sauvage se heurte à de nombreuses limites, et le développement de stratégies efficaces représente un défi de taille. Pour atteindre cet objectif, une compréhension fine des facteurs influençant la transmission et la persistance de l'infection est nécessaire. Parmi ces facteurs, l'hétérogénéité de transmission est une caractéristique importante des populations sauvages. En effet, la diversité des comportements, des structures sociales et spatiales, ou encore des espèces peut conduire à des contributions très variables au nombre de nouvelles infections. Par conséquent, quantifier l'hétérogénéité de transmission pourrait permettre d'améliorer l'efficacité des mesures de gestion sanitaire dans la faune sauvage, en ciblant les individus ou les unités de population qui sont responsables de la majorité des événements de transmission.

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D'une part, l'excrétion dans les sécrétions génitales des femelles à l'occasion d'avortements induits par la bactérie ou au moment des naissances est la principale voie de transmission de la brucellose. Par conséquent, les femelles sont responsables de la majorité des cas de transmission. Cette excrétion est également plus importante chez les femelles qui viennent d'accéder à la maturité sexuelle que chez les femelles plus âgées. D'autre part, le bouquetin présente un comportement de ségrégation sexuelle, où les contacts entre mâles et femelles décroissent progressivement après la période du rut. Cela peut avoir des répercussions importantes sur les opportunités de transmission d'agents infectieux entre mâles et femelles. Enfin, dans notre système d'étude, la séroprévalence varie fortement entre sous-unités spatiales, ce qui pourrait être le signe d'une hétérogénéité de transmission à l'échelle spatiale.

Lors de la découverte du foyer en 2012, la dynamique de transmission de la brucellose chez le bouquetin était pour l'essentiel inconnue. En effet, avant cela, les autres populations de bouquetins infectées par *B. melitensis* avaient vu l'infection s'éteindre spontanément après quelques années. La première étude de la thèse avait donc pour objectifs de déterminer

les voies de transmission et d'évaluer l'existence d'une hétérogénéité individuelle, qui sont deux caractéristiques majeures à connaître pour proposer des stratégies de gestion efficaces.

Pour atteindre ces objectifs, évaluer le statut infectieux des individus en se basant uniquement sur la détection des anticorps n'est pas suffisant. Pour identifier les modes et voies de transmission ainsi que les classes d'animaux les plus susceptibles de constituer des sources de bactéries, 88 bouquetins séropositifs ont été autopsiés entre 2012 et 2017 et des cultures bactériennes ont été réalisées sur des échantillons d'organes.

Les résultats obtenus étaient en faveur de l'existence de quatre voies de transmission, comme cela est décrit chez les ruminants domestiques : la voie horizontale directe et indirecte par l'excrétion de *B. melitensis* dans les sécrétions génitales, la voie congénitale par transmission *in utero*, la voie pseudo-verticale par consommation de lait et enfin la voie vénérienne.

La bactérie a été détectée chez 51 (58%) des animaux autopsiés, qui ont donc été considérés comme infectés et pouvant excréter la bactérie. Chez les bovins infectés par *B. abortus*, la bactérie est détectée chez 45 à 50% des individus séropositifs. Nos résultats sont donc en faveur d'un fort potentiel excréteur chez les bouquetins infectés par *B. melitensis*, ce qui pourrait avoir contribué au maintien de l'infection à long terme dans cette population. La détection de la bactérie et l'intensité de l'excrétion bactérienne étaient liées à celle de la réponse sérologique (titres en anticorps mesuré par le test de fixation du complément), suggérant que cette réponse pourrait permettre d'identifier les femelles présentant un risque plus élevé d'excrétion. Enfin, un résultat important de cette étude était que la bactérie était le plus souvent détectée chez les animaux séropositifs les plus jeunes de notre échantillon (2 ans) et décroissait avec l'âge dans les deux sexes, ce qui suggère que les animaux jeunes sont des sources importantes d'infection, ce qui pourrait avoir d'importantes répercussions sur la gestion.

Nos résultats ainsi que ceux d'une étude précédente sur le comportement spatial et les patrons de contact des bouquetins dans la population d'étude sont en faveur de deux rôles épidémiologiques distincts pour les mâles et les femelles. Les mâles, qui se déplacent dans l'ensemble du massif en particulier au moment du rut, pourraient participer à la diffusion spatiale de l'infection par la voie vénérienne. D'autre part, les femelles, qui sont plus sédentaires et restent dans leur unité spatiale la plupart du temps, pourrait maintenir

l'infection au sein de chaque unité spatiale en excréant la bactérie au moment des avortements ou des naissances.

Mettant à profit l'existence d'informations détaillées sur la dynamique de population et le comportement du bouquetin, et de données épidémiologiques dans la population d'étude, nous avons développé un modèle individu-centré afin de déterminer l'importance relative des différentes voies de transmission et de quantifier l'hétérogénéité individuelle et spatiale de la transmission.

Les résultats du modèle révèlent que la voie de transmission majoritaire est la voie horizontale par l'excrétion de la bactérie dans les sécrétions urogénitales (responsable d'environ 70 % des nouveaux cas d'infection), en accord avec ce qui est connu de la brucellose chez les autres ruminants domestiques et sauvages. Les voies pseudo-verticales et congénitales jouent aussi un rôle non négligeable, et, au total, les femelles provoquent environ 90 % des nouvelles infections dans chaque unité. Cela confirme notre prédiction que les femelles jouent un rôle de maintien de l'infection au sein de chaque unité spatiale.

En ce qui concerne la transmission inter-unités, la transmission vénérienne était prédominante, mais contrairement à nos attentes, à la fois les mâles et les femelles étaient impliqués. Ce résultat est contre-intuitif par rapport au comportement sédentaire des femelles, mais s'explique en fait par le fait que des mâles sensibles se déplacent et acquièrent l'infection de la part de femelles d'autres unités spatiales. Par conséquent, à la fois les mâles infectés et les mâles sensibles jouent un rôle de diffusion spatiale de l'infection.

Enfin, nous avons montré que la transmission de la brucellose était hétérogène entre unités spatiales, plus de 80 % des cas de transmission intra-unité ayant lieu dans les trois sous-unités socio-spatiales qui forment la zone cœur du massif. De plus, les deux unités de la zone cœur avec les séroprévalences les plus élevées jouaient le rôle de sources de transmission inter-unité pour l'unité avec des séroprévalences modérées (zone cœur) et pour les deux unités avec des séroprévalences faibles (zone périphérique).

Ces résultats pourraient permettre d'améliorer la gestion sanitaire, par exemple en ciblant les unités spatiales responsables de la majorité des cas de transmission ou en ciblant plus particulièrement les bouquetins femelles. Nous avons testé ces améliorations dans la dernière étude de la thèse, en se basant sur les prédictions du modèle individu-centré.

Avant cela, nous avons également estimé l'évolution temporelle de la séroprévalence et de la force d'infection, en utilisant différents modèles statistiques. L'objectif était d'évaluer l'efficacité des mesures de gestion qui ont été mises en place dans le foyer depuis sa découverte en 2012. Ces opérations ont consisté en : 1) un abattage massif à la fin de 2013, au cours duquel environ un tiers des animaux ont été abattus ; 2) une importante opération de 71 captures en 2014, avec élimination de 31 individus testés séropositifs (test-et-élimination) ; 3) une opération de test-et-élimination au printemps 2015, avec 106 captures et 38 individus séropositifs euthanasiés, suivie à l'automne 2015 de 19 captures supplémentaires (aucun séropositif) et de l'abattage de 70 individus non marqués ; et 4) des opérations concernant de plus petits effectifs en 2016, 2017 et 2018, avec moins de 50 captures chaque année, et l'abattage de 5 individus non marqués en 2017 et 2018.

L'analyse des données disponibles suggèrent que les mesures de gestion ont permis de diminuer à la fois la séroprévalence, la prévalence de l'infection et la transmission de la brucellose dans la population. Plus précisément, une diminution de l'ensemble de ces paramètres a été démontrée entre 2015 et 2016, qui résulte probablement de l'effet cumulé des opérations menées en 2014 et en 2015.

L'efficacité des opérations menées entre 2016 et 2018 reste à déterminer. En effet, la séroprévalence et le risque de transmission aux animaux sensibles entre 2016 et 2018 ne semblent pas varier de façon significative. En revanche, la proportion de femelles séropositives excrétrices dans la zone cœur pourrait avoir diminué entre 2017 et 2018, ce qui pourrait indiquer que les mesures ont bien contribué à diminuer la prévalence de l'infection. Cependant, il faut rester prudent vis-à-vis de ce résultat étant donné qu'il est basé sur un faible nombre de bouquetins femelles séropositives en 2018 (n=2).

Enfin, dans la dernière étude de la thèse, nous avons évalué une série de stratégies de gestion sanitaire qui pourraient être utilisées à l'avenir dans la population. Nous nous sommes focalisés sur la stratégie de test-et-élimination, qui semble être la plus efficace pour réduire la transmission de la brucellose d'après les études précédentes, et qui permet de produire des données de suivi de la situation épidémiologique. De plus, nous avons évalué des stratégies faisables en pratiques sur le terrain, afin que les résultats des simulations puissent être adaptés à la réalité. Par exemple, le nombre de captures dans les simulations a

été maintenu à 15-30 animaux par an, qui correspond aux nombres d'animaux capturés ces dernières années dans la population (estimée à environ 300 individus).

Nous avons aussi évalué l'intérêt de combiner la vaccination ou l'abattage d'animaux non marqués au test-et-élimination. Le nombre d'animaux non marqués abattus par an était compris entre 5 et 20 animaux, encore une fois afin de rester réaliste par rapport à ce qui a été fait dans la population récemment.

Sur la base des résultats des études précédentes concernant l'hétérogénéité de transmission dans la population, nous avons prédit que 1) cibler les mesures de gestion à la zone cœur du massif devrait être plus efficace que de les appliquer à l'ensemble de la population ; 2) cibler spécifiquement les femelles devrait être plus efficace que de cibler à la fois les mâles et les femelles pour un même nombre d'individus ; et 3) combiner les deux approches (cibler les femelles dans la zone cœur) devrait être encore plus efficace.

D'après les résultats du modèle, une extinction spontanée de la brucellose sans mesures de gestion ne devrait pas être attendue dans la population du Bargy dans un futur proche. De plus, la probabilité d'extinction est fortement reliée à la démographie, étant donné que la transmission de l'infection est liée à la reproduction. Actuellement, il n'a pas été détecté d'augmentation du succès de reproduction des femelles dans la population, mais ce paramètre démographique doit continuer à être suivi dans les années à venir. Une augmentation de la transmission de la brucellose serait attendue si ce paramètre démographique venait à augmenter.

Les résultats issus du modèle individu-centré confirment que la stratégie prioritaire devrait être d'éliminer le plus d'individus infectés possible par test-et-élimination. Combiner cette mesure avec d'autres mesures comme la vaccination ou l'abattage d'animaux non marqués pourrait permettre d'améliorer encore l'efficacité de cette mesure. Cependant, l'usage de la vaccination présente de nombreuses limites et pourrait complexifier la surveillance de l'évolution du foyer, ce qui fait qu'il serait plus recommandé de combiner le test-et-élimination avec l'abattage d'individus non marqués.

Même à des niveaux de capture ou d'abattage faibles comme entre 2016 et 2018, les opérations de test-et-élimination plus ou moins combinées à l'abattage d'individus non marqués semble avoir un effet sur la séroprévalence dans la population. Cependant, le bénéfice associé à l'abattage de 5 individus non marqués comme en 2017 et 2018 n'apparaît

pas très marqué. Il semblerait plus intéressant de combiner le test-et-élimination avec l'abattage d'une vingtaine d'individus non marqués.

Enfin, nos prédictions concernant les mesures de gestion ciblées se sont vérifiées. Cibler spécifiquement les femelles ou la zone cœur permet d'améliorer l'efficacité des mesures, et combiner les deux approches est encore plus intéressant.

Bien qu'il n'y ait pas de solution évidente pour la gestion de la brucellose dans notre cas d'étude, les stratégies de gestion ciblées sont très prometteuses et permettent de raffiner les mesures sanitaires classiquement utilisées. Il est donc primordial de bien comprendre l'hétérogénéité de transmission dans les populations sauvages infectées, et de chercher des stratégies ciblées qui peuvent permettre d'améliorer la gestion en termes d'efficacité et d'acceptabilité.

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LIST OF ABBREVIATIONS

- ABC: Approximate Bayesian Computation
AIC: Akaike Information Criterion
ASF: African Swine Fever
bTB: Bovine Tuberculosis
BSL-3: BioSafety Level 3
CFT: Complement Fixation Test
CFU: Colony Forming Unit
CI: Confidence Interval or Credible Interval
CMR: Capture-Mark-Recapture
CWD: Chronic Wasting Disease
DD: Density-Dependent
DFTD: Devil Facial Tumour Disease
EID: Emerging Infectious Disease
ELISA: Enzyme Linked ImmunoSorbent Assay
 ↳ iELISA: indirect ELISA
 ↳ cELISA: blocking or competitive ELISA
EU: European Union
FAO: Food and Agriculture Organisation
FD: Frequency-Dependent
FeLV: Feline Leukemia Virus
GPS: Global Positioning System
GYA: Greater Yellowstone Area
HIV: Human Immunodeficiency Virus
LFIA: Lateral Flow Immuno-chromatographic Assay
OAT: One-At-a-Time
OIE: World Organisation for Animal Health
OR: Odds Ratio
PCR: Polymerase Chain Reaction
 ↳ qPCR: quantitative PCR
PI: Persistently Infected
RBCT: Randomised Badger Culling Trial
RBT: Rose Bengal Test
SARS: Syndrom Acute Respiratory Syndrom
TR: Test-and-Remove
TRC: Test-and-Remove and Culling
TVR: Test and Vaccinate or Remove

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PREAMBLE

The general aim of this thesis is to study the management of brucellosis in Alpine ibex (*Capra ibex*). Brucellosis is a major bacterial and zoonotic infectious disease that causes economic and public health issues worldwide. In the European Union, eradication programs have been implemented for more than fifty years, and France was declared officially free of brucellosis in domestic ruminants in 2005. However, in 2012-2013, a high seroprevalence of brucellosis was found in the Alpine ibex population of the Bargy massif (French Alps). The causative agent of brucellosis in this case, *Brucella melitensis*, probably spilled over from domestic animals to ibex before disease eradication in the domestic compartment, and probably spilled back from ibex to cattle recently, and then from cattle to humans in 2012.

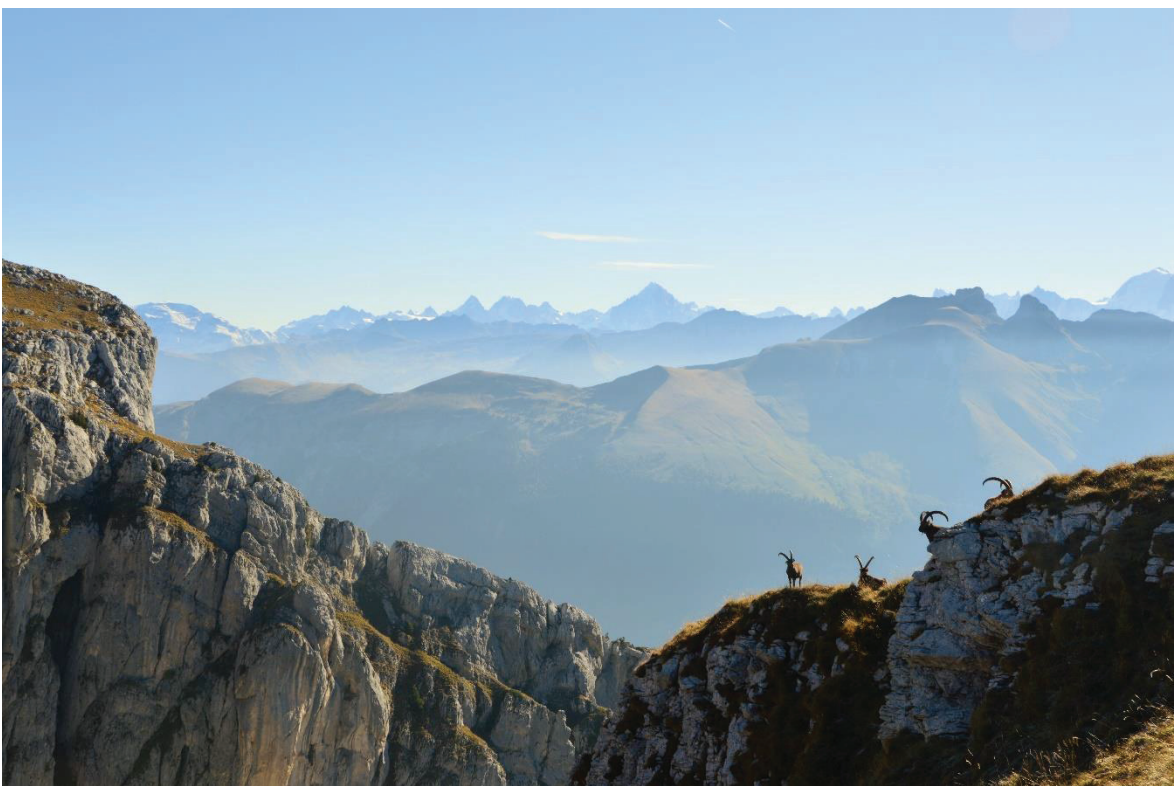
This system is worth of interest because it is the first example of a wild population that self-sustained brucellosis infection in European ungulates. This re-emergence represents a threat to the eradication program of brucellosis in domestic ruminants and to public health. Moreover, brucellosis in Alpine ibex raises a conservation issue since this species is protected. Indeed, it was restored in the Alps during the last decades, after being close to extinction at the beginning of the 20th century. In domestic herds in the context of brucellosis eradication, the preservation of the officially free status involves culling the whole herd when infected animals are detected. Such a management is hardly practicable in a wildlife population, and also conflicting the protection status of the Alpine ibex. Other disease management options would therefore be desirable to mitigate the risk to domestic ruminants and humans, while being relevant for the species conservation.

Since the infection was discovered in late 2012, the infected population of Alpine ibex was the target of disease management. In particular, the population was the subject of a massive culling operation at the end of 2013, during which about one third of the animals were removed. However, the available data did not support any differences in brucellosis seroprevalence between the year before or the two years after this culling operation. From 2015 onwards, the efficacy of management remains to be evaluated.

Further studies are therefore required to provide disease management strategies that are more effective and acceptable as regards the protected status of Alpine ibex. Determining (i) how brucellosis is transmitted in this population and (ii) what were the effects of the disease management methods implemented so far are two prerequisites that could come up with new strategies for the management of brucellosis in ibex.

Chapter 1

GENERAL INTRODUCTION



Personal picture

Managing infectious diseases in wildlife population is a challenge compared to what can be done in domestic populations. Compared to the latter, humans have limited access to wild animals, and weak control over their reproduction, movements and population structure. Therefore, methods for managing infectious diseases in wildlife such as brucellosis cannot be simply derived from knowledge on domestic animals. Their objectives and basic principles are similar, but they need to account for the specificities of wildlife populations. Variability is one striking example of such specificity that we will be particularly interested in over the course of this thesis. Compared to domestic animals, wild animals express a broad range of behaviours, they are organised in a variety of social and spatial structures, they occupy many areas with very different characteristics and they belong to a much wider diversity of species. Such heterogeneities, from between-individuals to between-species, are common places in natural systems and may result in as many different transmission dynamics of infectious agents. Thus, understanding transmission heterogeneity in wild host-pathogen systems could provide valuable insights on how to effectively manage these systems.

In the general introduction of this thesis, I first define infectious diseases and give some historical examples of anthropogenic attempts to eradicate these diseases in humans and domestic animals, before explaining the reasons for studying infections such as brucellosis in wildlife. I then describe *B. melitensis* infection in domestic species, and highlight the paucity of available knowledge regarding *Brucella* transmission and management in Alpine ibex. Given this lack of knowledge, I review in the subsequent parts how unsolved questions about brucellosis in ibex could benefit from answers to similar issues in other wild host-pathogen systems, and their limitations when applied to the system of interest. The third part presents general considerations on the study of the health status of wild animals and populations, and examples of available methods, their benefits, challenges and limitations. How the variability between individuals, populations or areas can shape the transmission and impact disease management strategies is explained in part 4. Then, I describe available options for disease management in wildlife, giving examples in various host-pathogen systems and highlighting their strengths and limits (part 5). Finally, I present how this thesis is structured and why brucellosis in Alpine ibex is a relevant biological model for studying infectious diseases management in heterogeneous populations.

1.1. INFECTIOUS DISEASES IN WILDLIFE

1.1.1. WHAT ARE INFECTIOUS DISEASES?

An infection is the entrance and development of a biological agent in a host organism (Vymnycky and White, 2010). Biological infectious agents can be transmitted from the infected host to a susceptible one, either through direct contacts, through the environment or through an arthropod vector (Barreto *et al.*, 2006). Infectious agents are highly diverse, as they include viruses, bacteria, fungi, protozoans, parasitic worms or even proteins in the case of prions (Alberts *et al.*, 2002).

Infectious diseases are infections where the presence of infectious agents or their products impairs the normal functioning of the host. The ability of an infectious agent to cause a disease is not always fixed and can evolve (Barreto *et al.*, 2006). It also depends on the context and on the dynamics of the interactions between the infectious agent and the host's response (Casadevall and Pirofski, 2014). An infectious agent can be harboured by a host without causing any harm while being pathogenic in other situations, for instance in an unusual location in the body (e.g., mastitis caused by *Escherichia coli* in cows – Hogan and Smith, 2003), depending on host condition (e.g., pulmonary disease by *Aspergillus fumigatus* in people with leukaemia – Brown *et al.*, 2012), in the presence of a virulent strain (e.g., high-virulence strains of pestivirus in Pyrenean chamois (*Rupicapra pyrenaica pyrenaica* – Colom-Cadena *et al.*, 2019) or in particular species (e.g., Ebola virus asymptomatic infection in bats but symptomatic in humans and non-human primates – Leroy *et al.*, 2005).

Infections and infectious diseases are widespread in living organisms (Alberts *et al.*, 2002; Vymnycky and White, 2010). Human infectious diseases have had a strong impact on the history and evolution of human societies, notably by causing severe declines in population demography through major epidemics such as the 'Black Death' in the 14th century, that killed around 50 million people in Europe and Asia, or the Spanish influenza in 1918, that killed approximately 50-100 million people around the world (Morens *et al.*, 2008). Infectious diseases of domestic animals also have had profound impacts, especially since the 18th century with the emergence of severe cattle epizootics such as rinderpest, anthrax and foot-and-mouth disease (Thrusfield, 2007; Morens *et al.*, 2008).



FIGURE 1-1 | Edward Jenner performing his first vaccination against smallpox in 1796 (left; painting: Ernest Board, commons.wikimedia.org [Accessed June 30, 2019], public domain) and cows dead from rinderpest in South Africa in 1896, a hundred years later (right; photo: Anon., commons.wikimedia.org [Accessed June 30, 2019], public domain). These two infectious diseases are the only ones for which global eradication was achieved.

These threats to human and animal health elicited disease management strategies, and large eradication campaigns were carried out in the late 19th century and the 20th century. At this time, huge efforts were directed towards the control and elimination of major infectious diseases of humans and animals. The discovery and the efficacy of vaccination largely benefited these efforts. The first vaccine was developed in 1796 by Edward Jenner to protect people against smallpox (Figure 1-1), and the global eradication was reached in 1980 (Strassburg, 1982). In animals, eradication campaigns started in the 1880s and involved mass testing of animals and combination of techniques such as vaccination, slaughter and movement restrictions (Thrusfield, 2007). It notably led to the global eradication of rinderpest (Figure 1-1 – Roeder *et al.*, 2013), and to the control of other diseases such as bovine tuberculosis – bTB – in many countries (Bénet *et al.*, 2006).

However, only small pox and rinderpest have been eradicated globally, and the management of infectious diseases still represents a challenge nowadays. For example, developing efficient vaccines for the ‘big three’ (the deadliest remaining infectious diseases in humans: malaria, Human Immunodeficiency Virus – HIV – and tuberculosis) has proven difficult and is not yet achieved despite the technological advances (Bourzac, 2014). Re-emergence of infectious diseases can also happen after successful reduction of the disease by management programs. For example, maintaining the vaccination coverage needed to protect populations against measles has proven difficult in the recent years because of anti-

vaccination movements, and as a result an increase in the number of measles cases has been observed (Paules *et al.*, 2019).

Moreover, opportunities for the emergence of new infectious diseases or the re-emergence of others, although they occurred throughout history, have been increasing in recent times especially with the growth of human population, exchanges and travel practices (Morens *et al.*, 2008). Contacts at the interface between humans, domestic animals and wildlife also underwent major changes on recent history, promoting the transmission of infectious agents between species and the occurrence of (re)emerging infectious diseases in those various hosts (Daszak *et al.*, 2000; Gortázar *et al.*, 2014). For example, the first recorded human outbreak of Ebola virus in the Democratic Republic of Congo in 1976 was attributed to increased bushmeat hunting, allowing for interspecies contacts between humans and non-human primates (Wolfé *et al.*, 2005). The emergence of Nipah virus in pigs and from pigs to humans in 1998 in Malaysia was also promoted by changing practices at the interface between natural environment and humans. Following deforestation, fruit bats, the natural reservoir of the virus, encroached into cultivated fruit orchards associated with piggeries, allowing for new interspecies transmission (Chua, 2003).

Although the existence of wildlife-associated risks have long been recognized, for example for rabies or plague, this increased frequency of emerging infectious diseases originating from wildlife (Jones *et al.*, 2008) illustrated the urgent need to better understand of their epidemiology, and to elaborate specific disease management strategies in wild populations and at the wildlife-domestic species-human interface (Rhyan and Spraker, 2010).

1.1.2.FIVE GOOD REASONS FOR STUDYING INFECTIOUS DISEASES IN WILDLIFE

Unsurprisingly, most of (60%) emerging infectious diseases (EID) in humans are zoonoses, i.e., come from animals, and among them 72% originate from wildlife (Jones *et al.*, 2008). This makes sense as wild animals form a varied reservoir for many infectious agents that are circulating and constantly evolving. Adaptation of new strains, or increased contacts between humans and animals are opportunities for cross-species transmission (Daszak *et al.*, 2000; Jones *et al.*, 2008), even though all potential zoonotic pathogens do not

achieve to infect humans (Lloyd-Smith, 2017) or to be transmitted between humans (Gortázar *et al.*, 2014). The impact of these emerging zoonoses on human health is a first reason for studying the epidemiology of wildlife diseases, identify the determinants of these EID events and predict where and when they will most probably happen (Jones *et al.*, 2008).

A second reason is the role of wildlife reservoirs for diseases shared with domestic farmed animals (Figure 1-2), representing a potential threat to livestock production, economy and animal welfare (Rhyan and Spraker, 2010). These diseases may also be of zoonotic importance through the consumption of animal products, such as bTB or brucellosis. This role of reservoir may become apparent only when disease management programs entail massive decrease of incidence and prevalence in domestic animals, up to a level when the eradication efforts are hampered by the wildlife reservoir as observed for example for bTB in several areas in the world (Corner, 2006). In some cases, these diseases originated from domestic animals and were transmitted to wildlife (“spill-over”), before being transmitted back to domestic animals by wildlife (“spill-back”).

Infectious diseases can also represent a threat to biodiversity, by causing severe declines of population or extinctions (Daszak *et al.*, 2000), representing another incentive for the study of wildlife diseases. This is particularly true for already endangered species, where small fragmented populations are more susceptible to stochastic events such as disease outbreak and population extinction. For example, the devil facial tumour disease (DFTD) represents an additional threat to the endangered Tasmanian devil (*Sarcophilus harrisii* – McCallum and Jones, 2006 – Figure 1-2). Diseases in endangered species can be the result of spill-over from domestic animals, such as rabies or canine distemper virus in Ethiopian wolves (*Canis simensis* – Haydon *et al.*, 2006), or from humans, such as measles in mountain gorilla (*Gorilla beringei* – Ferber, 2000). The introduction of new pathogens following voluntarily (i.e., for conservation purposes) or involuntarily (i.e., invasive species) introduction of new animals in an area can also represent a threat to endangered species (Daszak *et al.*, 2000). The management of these risks in endangered species requires specific tools, in particular to limit at-risk contacts (Breed *et al.*, 2009) and to avoid population extinction (Beeton and McCallum, 2011).



FIGURE 1-2 | An example of interface between wildlife and domestic animals, with close proximity between two goats (*Capra hircus* on the left) and an ibex (*Capra ibex* on the right) where interspecies transmission of shared diseases can occur (left; photo: B. Muffat-Joly, ONCFS, with kind permission of the author) and a case of facial tumour disease, which represents a threat to the already endangered Tasmanian devil (*Sarcophilus harrisii*) (right; photo: Menna Jones; McCallum and Jones, 2006, doi.org/10.1371/journal.pbio.0040342.g001 [Accessed June 30, 2019], licence CC-BY-2.5).

The fourth reason to study infectious diseases of wildlife is to better understand the behaviour of infectious diseases in natural populations, including those of humans (Jolles and Ezenwa, 2015). Experimental studies involving domestic animals under strictly controlled conditions have brought tremendous advances on disease mechanisms; however, in real life, humans and animals experience infectious diseases in varied contexts. Understanding questions such as the conditions of invasion and spread of an infectious agent in a population, the effects of social and spatial behaviour, population dynamics, and physiology on infection dynamics and vice versa, or the impact of the environment and its changes (e.g., climate change) on infectious diseases, requires the study of natural host-pathogen systems that include environmental, individual and populational variability (Jolles and Ezenwa, 2015).

Finally, studying infectious diseases in wild populations can also help understand the mechanisms that govern the evolution of infectious agents, including changes in virulence, the emergence of new strains or the adaptation to new hosts (Galvani, 2003; Alizon and Méthot, 2018). With a better understanding of these mechanisms, accurate predictions of the evolution of the pathogens will enable to improve disease management strategies, including by anticipating and therefore avoiding the evolutionary response of pathogens to these very same interventions (Galvani, 2003; Alizon and Méthot, 2018).

Studying brucellosis due to *Brucella melitensis* infection in Alpine ibex brings together at least two of the conditions described above. First, it is an example of a maintenance population at the interface between wild and domestic animals, which represents a threat to the eradication program of brucellosis in domestic ruminants, and is also of zoonotic importance. Second, as it is the first reported case of brucellosis maintenance in wild ungulates in Europe, its study could bring valuable insights into the transmission and the maintenance of the infection in natural populations.

In the following section, I describe the pathogenesis and the transmission of ruminant brucellosis, highlighting common features and dissimilarities between species, including wildlife, and explaining why mechanisms of transmission in ibex cannot be completely extrapolated from knowledge of *B. melitensis* or *B. abortus* in domestic ruminants. I also explain basic concepts on brucellosis management in domestic ruminants. They might not be directly applicable to wildlife populations, but they could represent valuable options based on their efficacy in domestic ruminants.

1.2. BRUCELLOSIS IN RUMINANTS

1.2.1. WHAT IS BRUCELLOSIS?

Brucellosis is one of the most common zoonosis in the world, with more than half a million new cases every year (Godfroid *et al.*, 2013) and a wide geographic distribution (European Commission, 2001). Among the ten species of the genus *Brucella*, which all have their preferred host but can infect many mammals (Godfroid *et al.*, 2011), humans are especially susceptible to three species (European Commission, 2001): *Brucella melitensis*, the main causative agent of brucellosis in humans (Godfroid *et al.*, 2013), *B. abortus* and *B. suis*.

Brucellosis in humans is highly variable, from asymptomatic to acute or chronic forms with flu-like symptoms (lassitude, headache, muscular and articular pain, fluctuating temperature), more or less associated to complications such as orchitis, spondylitis and peripheral arthritis, or even death as a result of thrombopenia or endocarditis (FAO and WHO, 1986). *B. melitensis* and *B. suis* are often associated with severe forms (European Commission, 2001).

Humans are considered as dead-end hosts of brucellosis (European Commission, 2001) and get the infection from animal maintenance populations (Godfroid *et al.*, 2013). The main sources of contamination for humans are direct or indirect contacts with infected animals for exposed professions such as farmers or veterinarians, and more generally consumption of raw dairy products from cattle, sheep and goats (European Commission, 2001).

Bovine brucellosis is mainly due to *B. abortus*, and brucellosis in small ruminants is mainly caused by *B. melitensis*, although both species of *Brucella* can be found in cattle, sheep and goats (World Organisation for Animal Health – OIE, 2016). Brucellosis in domestic ruminants causes significant economic losses by causing abortions and stillbirths, and by impeding international trade of animals and animal products (Diaz-Aparicio, 2013).

Because of this economic and zoonotic importance, eradication programs of brucellosis in domestic ruminants have been implemented in the European Union for more than fifty years (Godfroid, 2017). Thanks to these programs, no case of brucellosis (caused by *B. abortus* or *B. melitensis*) have been reported in domestic ruminants since 2003 in France, which was declared officially free of ruminant brucellosis in 2005 (Perrin *et al.*, 2016a,b).

B. abortus has been isolated from many wildlife species (Godfroid, 2002; Godfroid *et al.*, 2013), and there are some examples of maintenance reservoir populations: elk (*Cervus canadensis*) and bison (*Bison bison*) in the Wood Buffalo National Park (Canada – Tessaro, 1986) and in the Greater Yellowstone Area (Yellowstone National Park and surrounding areas, USA – Meagher and Meyer, 1994), as well as African buffalo (*Syncerus caffer*) in the Kruger National Park (South Africa – Herr and Marshall, 1981) and in Zimbabwe (Madsen and Anderson, 1995). Conversely, *B. melitensis* is rarely reported in wildlife (Godfroid, 2002; Godfroid *et al.*, 2013). Localized cases of spill-over that spontaneously faded out have been reported in chamois (*Rupicapra rupicapra*) and Alpine ibex (Garin-Bastuji *et al.*, 1990; Ferroglio *et al.*, 1998; Gauthier *et al.*, 1998; Hars and Garin-Bastuji, 2013). As a consequence, these species used to be considered as unable to maintain the infection and act as reservoirs (Godfroid *et al.*, 2013), until the discovery of *B. melitensis* in the ibex population of the Bargy massif.

1.2.2. PATHOGENESIS OF RUMINANT BRUCELLOSIS

Brucellae are facultative intracellular bacteria, and have the ability to replicate intracellularly, including in phagocytic cells, using different survival strategies (Carvalho Neta *et al.*, 2010). The main routes of infection for *B. melitensis* and *B. abortus* are the oral, nasal, and conjunctival mucosae (European Commission, 2001; Poester *et al.*, 2013). Other potential routes of entry are the mucosae of the female and male genital tracts (European Commission, 2001). From the entry point, infection spreads to local lymph nodes, then a bacteraemia allows the infection to spread to the spleen, other lymph nodes, genital tract and mammary glands (Carvalho Neta *et al.*, 2010; Poester *et al.*, 2013). After the initial infection, the presence of brucellae is restricted to genital and mammary lymph nodes (Fensterbank, 1987). On favourable occasions such as oestrus, pregnancy or immunosuppression, they can be reactivated, leading to new bacteraemia and reinvasion of the target organs and bacterial shedding (European Commission, 2001; Schlafer and Miller, 2007).

Elimination of the infection followed by resistance to reinfection is possible, after varying durations of infection depending notably on the species (European Commission, 2001). For example, goats frequently have infections persisting lifelong, while sheep have more self-limited infections with spontaneous recovery in a few months. In cattle, infection lasts for an intermediate duration of several years (European Commission, 2001; Schlafer and Miller, 2007; Olsen and Palmer, 2014). There is evidence that bison and elk have the ability to clear the bacteria after a few years (Thorne *et al.*, 1978; Rhyan *et al.*, 2009; Treanor *et al.*, 2011). The infection duration and the ability to clear the bacteria in Alpine ibex is currently unknown, and could be anywhere between shortly-infected sheep and persistently-infected goats.

The main lesions associated with *Brucella* infection are placentitis, metritis and mastitis in females, orchitis and epididymitis in males, joints affections such as arthritis, hygromas and tenosynovitis, and abscesses in various organs (Schlafer and Miller, 2007; Godfroid *et al.*, 2013). Similar lesions have been found in elk and bison (Thorne *et al.*, 1978; Roffè *et al.*, 1999; Rhyan *et al.*, 2009) and in Alpine ibex (Ferroglio *et al.*, 1998; Freycon *et al.*, 2017).

Lesions in the uterus can lead to infertility (Godfroid *et al.*, 2013). Seropositive females had a smaller reproductive success than seronegative ones in bison (Fuller *et al.*, 2007), but

not in African buffalo (Gorsich *et al.*, 2015). In ibex, seropositive females appear to be less sexually active and less often pregnant than seronegative ones (C. Toigo, unpubl. data).

Contrasting results on the effect of brucellosis on mortality were found, with no effect on bison (Joly and Messier, 2005; Fuller *et al.*, 2007) but strong impact on moose (*Alces alces* – Forbes *et al.*, 1996) and African buffalo (Gorsich *et al.*, 2015). In ibex, there is currently no evidence for brucellosis-related mortality (no important mortality detected, no seropositive individuals detected in the few individuals found dead).

Brucella infection induces both humoral and cellular immune responses, but the protective effect of the humoral response is low (Carvalho Neta *et al.*, 2010) because the bacteria can hide from antibodies inside the host's cells. The humoral immune response is nonetheless used for serological diagnosis (Carvalho Neta *et al.*, 2010). Serological response begins two to four weeks after infection (European Commission, 2001), and generally lasts for several years (Godfroid *et al.*, 2010). Similarly, long-lasting humoral responses have been demonstrated in bison infected by *B. abortus*, with rare events of seroconversion from positive to negative (Rhyan *et al.*, 2009).

1.2.3. TRANSMISSION OF *B. MELITENSIS* (AND *B. ABORTUS*)

Brucellae have a marked tropism for the pregnant uterus, male genital organs, mammary gland and associated lymph nodes (Poester *et al.*, 2013), leading to four transmission routes: direct or indirect horizontal transmission mainly by ingestion of contaminated genital secretions, venereal transmission, congenital transmission and pseudo-vertical transmission (Diaz-Aparicio, 2013).

Brucella shedding from the female genital tract is the main source of transmission in domestic ruminants (European Commission, 2001). During the next gestation after primo-infection, about 80% of females abort and shed huge amounts of bacteria (Godfroid *et al.*, 2004). Even in the absence of abortion, there is heavy shedding in the placenta, foetal fluids and vaginal exudates (Diaz-Aparicio, 2013). Shedding generally lasts three weeks after abortion or full-term parturition in small ruminants, even though the bacteria can be isolated for longer periods especially in goats (European Commission, 2001; Poester *et al.*, 2013). Most often, females abort only once (FAO and WHO, 1986), usually during the last third of

gestation (Diaz-Aparicio, 2013). Abortion generally does not occur when the female gets infection late during its gestation (European Commission, 2001). After the first gestation, reinvasion of the genital tract occurs with shedding in genital secretions (European Commission, 2001).

In bison and elk, bacterial shedding following abortion or live birth is also suspected to be the main source of bacteria (Thorne *et al.*, 1978; Rhyan *et al.*, 2009; Rhyan, 2013). Species-specific differences are quantitative rather than qualitative, with bison being reported as more susceptible to abortion caused by *B. abortus* than cattle, with higher abortion rates (Olsen and Johnson, 2011), while elk appears as the least sensitive to abortion (Thorne *et al.*, 1978). The rate of abortion caused by *B. melitensis* in ibex is currently unknown.

The persistence of *Brucella* in the environment is variable depending on the conditions. When favourable conditions are gathered, i.e., high humidity, low temperature, absence of direct sunlight and presence of organic materials such as aborted fetuses, Brucellae can remain viable for several months (European Commission, 2001). Environmental survival is generally lower in less favourable situations, e.g., less than five days in pastures exposed to sunlight (European Commission, 2001). Indirect transmission through the environment is therefore possible, but horizontal transmission to susceptible animals is rather the result of direct or indirect contacts with infected animals shedding the bacteria in close proximity (Olsen *et al.*, 2010).

Invasion or reinvasion of the uterus by the bacteria can lead to congenital transmission of the infection from the mother to its newborn (Renoux, 1962; Plommet *et al.*, 1973; Lapraik *et al.*, 1975). About 5% of kids born from infectious mothers develop the infection, and they remain latently infected without serological response up to their first gestation for females or to the age of sexual maturity for males (Plommet *et al.*, 1973; FAO and WHO, 1986; Godfroid *et al.*, 2013). Because they remain undetected until then, these animals represent a threat to eradication programs and have been associated with re-emergence and persistence of the infection in domestic herds (Plommet *et al.*, 1973; Wilesmith, 1978).

Shedding in the colostrum or milk is possible at every lactation, whether or not the female previously aborted (Philippon *et al.*, 1971). Milk consumption by kids born from infectious mothers can also lead to pseudo-vertical transmission of the infection (FAO and WHO, 1986; European Commission, 2001), although the frequency of transmission remains low

(Grilló *et al.*, 1997). In bison and elk, there have been reports of kids acquiring infection from their mother, although the transmission route (*in utero* or through milk consumption) was not clarified. Similarly to domestic ruminants, these transmission routes appear to be infrequent (Thorne *et al.*, 1978; Rhyan *et al.*, 2009).

In domestic males, shedding of *Brucella* in the semen is possible (European Commission, 2001). Venereal transmission was demonstrated experimentally (Thomsen, 1943; Islam *et al.*, 2013) and transmission of the infection through artificial insemination was also reported (Bendixen and Blom, 1947; Manthei *et al.*, 1950). However, several studies in cattle showed that naturally-infected bulls shedding *Brucella abortus* in the semen failed to infect susceptible females under natural mating conditions (King, 1940; Thomsen, 1943). This is the reason why this transmission route is not believed to be a major mode of transmission in domestic ruminants (King, 1940; Thomsen, 1943; FAO and WHO, 1986). In bison, although venereal transmission has not been demonstrated, further studies are required to determine if this is a possible transmission route. Indeed, shedding in the semen is possible (Frey *et al.*, 2013), and seroconversion was demonstrated after intravaginal experimental infection (Uhrig *et al.*, 2013).

Transmission routes in ibex are likely to be the same as in domestic ruminants, as for other wildlife species (European Commission, 2001; Diaz-Aparicio, 2013; Godfroid *et al.*, 2013). However, remaining grey areas such as the existence of venereal transmission, that was suggested to play an important role for brucellosis in ibex (Hars *et al.*, 2013), requires to further investigate whether there is evidence for all four transmission routes in Alpine ibex.

1.2.4. BRUCELLOSIS MANAGEMENT STRATEGIES IN SMALL RUMINANTS

1.2.4.1. The B. melitensis Rev. 1 vaccine

The live *B. melitensis* Rev. 1 strain is considered the only vaccine available for brucellosis management in small ruminants (Blasco, 1997). Indeed, the efficacy of killed vaccines is not high enough (Elberg and Faunce, 1957) and only live vaccines are considered suitable for brucellosis management (Blasco, 1997). Moreover, alternative live vaccines such as the *B. suis* strain 2, the *B. abortus* B19, the *B. abortus* RB51 or the rough mutants of

B. melitensis are less efficient than Rev. 1 (Morgan *et al.*, 1966; Verger *et al.*, 1995; El-Idrissi *et al.*, 2001; Barrio *et al.*, 2009) and are therefore not suitable (Blasco, 1997).

The Rev. 1 vaccine has an excellent efficacy against *B. melitensis* infection, although some vaccinated animals got infected in experimental conditions (Elberg and Faunce, 1957; Elberg, 1959). Vaccinated animals that get infected have at least half as much risk to abort, and shed less bacteria (Fensterbank *et al.*, 1985, 1987; Verger *et al.*, 1995). However, vaccination has no effect in previously infected animals (Blasco, 2010). The duration of immunity conferred by vaccination is long, at least 4.5 years, and could be considered lifelong in the context of small ruminants breeding (Alton, 1966, 1968). Conjunctival and subcutaneous vaccination have similar efficacies (Fensterbank *et al.*, 1985, 1987).

The vaccine has two major drawbacks: (i) it induces long-lasting serological responses in vaccinated animals, which cannot be distinguished from naturally-infected animals, and (ii) vaccine-induced abortions are possible when vaccinating pregnant animals (Blasco, 2010). A possible solution for avoiding these difficulties is to vaccinate young immature animals. Indeed, young animals develop only transient serological responses in response to vaccination, less than one year after sub-cutaneous vaccination (Alton, 1967), and less than four months after conjunctival vaccination (Fensterbank *et al.*, 1985, 1987; Muñoz *et al.*, 2008).

If vaccination of mature females must be done, the vaccination scheme should be adapted to limit the risk of vaccine-induced abortions, both for the safety of animals and to avoid the transmission of the vaccine strain and the induction of serological responses in unvaccinated animals (Zundel *et al.*, 1992). Reduced doses should never be used, as they neither confer an adequate level of immunity nor avoid vaccine-induced abortions (Blasco, 2010). However, the use of conjunctival vaccination should be preferred to the use of subcutaneous vaccination, because it reduces the risk of vaccine-induced abortions, although not completely (Zundel *et al.*, 1992). Moreover, vaccinating around the middle of gestation should be avoided (Zundel *et al.*, 1992), whereas the pre-mating period, the very beginning of gestation, its end and the lambing-lactation period minimise the risk of vaccine-induced abortions (Elberg and Meyer, 1958; Jiménez De Bagués *et al.*, 1989; Blasco, 1997).

A short-duration shedding of Rev. 1 in milk in lactating ewes and goats has been rarely observed (Jones and Marly, 1975; Zundel *et al.*, 1992; Blasco, 1997).

1.2.4.2. *Possible strategies for brucellosis management*

The choice of a strategy to achieve eradication mainly depends on the prevalence in the small ruminant population, i.e., the proportion of infected herds (Figure 1-3 – European Commission, 2001).

When the prevalence is high (more than 5-10% of infected herds), the mass vaccination of all animals is the only relevant strategy (European Commission, 2001; Blasco, 2010). In these situations, the serological interferences caused by Rev. 1 are irrelevant (Blasco, 2010). Therefore, the aim is to rapidly establish protective immunity and to reduce the level of abortions and *Brucella* shedding by vaccinating all animals (European Commission, 2001). Unfortunately, there is no entirely safe strategies for the use of Rev. 1, but the risk of vaccine-induced abortions can be minimised by performing vaccination at appropriate periods (1.2.4.1a – Blasco, 2010). Ideally, the mass vaccination campaign should be accompanied by appropriate individual identification of vaccinated animals. To decrease the vaccination rate, it has been proposed to use preliminary testing of the animals and removal of seropositive animals before applying mass vaccination (Garrido, 1992).

To be effective, vaccine immunity has to be maintained over time. Two strategies have been proposed to achieve this goal after the first vaccination campaign (Blasco, 2010): either vaccination of the young in the following years, still combined with suitable individual identification; or repeated mass vaccination in the whole population every two years, avoiding previously vaccinated animals if those are marked, as boost vaccination does not improve the level of efficacy (Elberg, 1959; Fensterbank *et al.*, 1985). Whatever the strategy adopted, mass vaccination should be implemented for at least one entire generation (6-12 years in the context of small ruminants breeding – Blasco, 2010).

After the implementation of the mass-vaccination programme, the prevalence of the infection should be effectively reduced (Blasco, 2010). In this case, a combined strategy is recommended to pursue towards eradication, using both vaccination and identification of young animals (3-4 months old), and test and remove of seropositive adult animals (European Commission, 2001; Blasco, 2010). During the transition from mass-vaccination to combined vaccination and test-and-remove, it is recommended to avoid the serological testing of the population for at least two years to avoid the unnecessary culling of seropositive vaccinated animals (Blasco, 2010). After these two years, testing of all adult

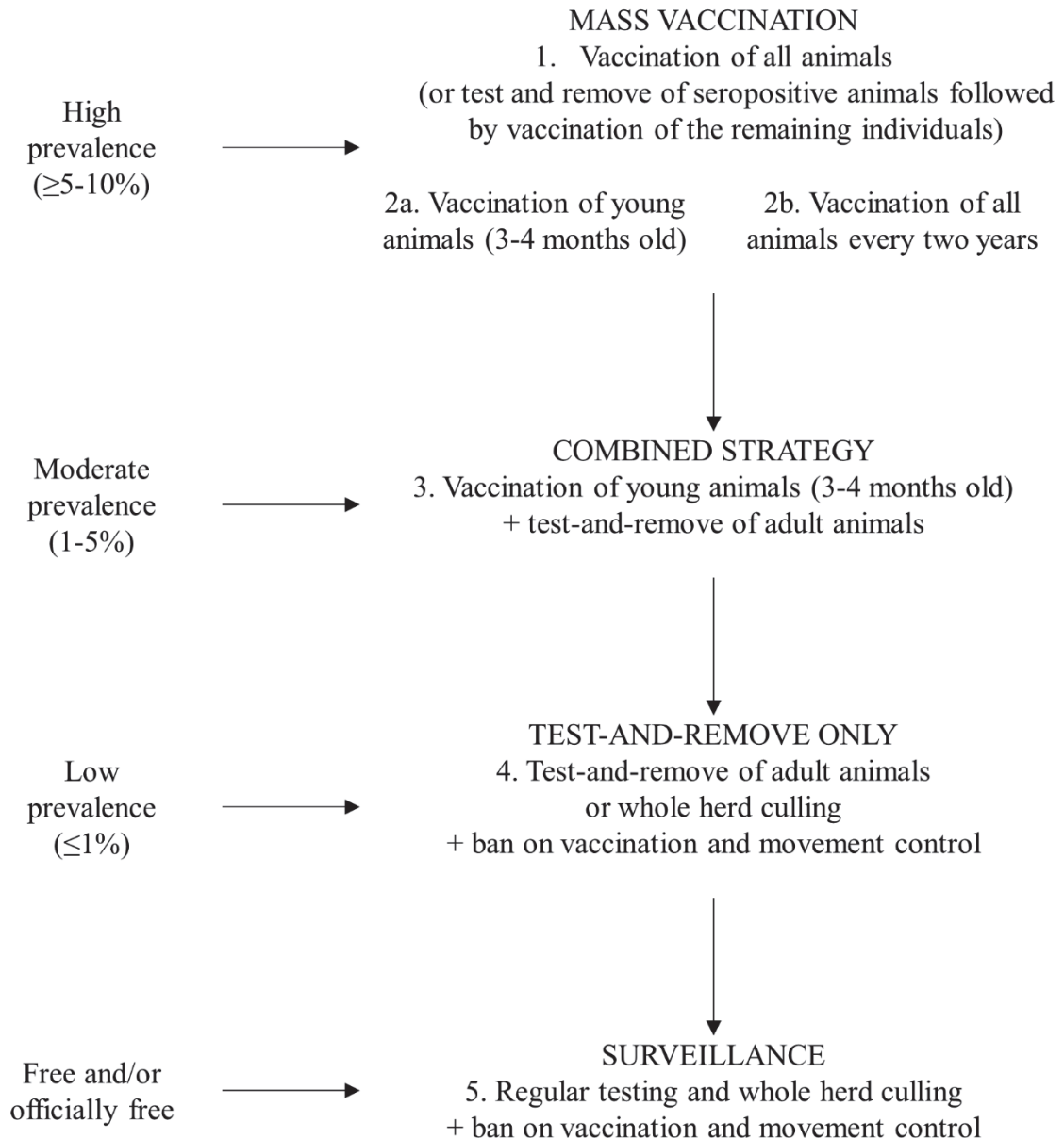


FIGURE 1-3 | Decision and succession of brucellosis infection management strategies in small domestic ruminants. Adapted from European Commission (2001). See also Blasco (2010).

animals over 12-16 months of age should be systematically performed every year. Because the vaccine-induced serological response is short in young animals, distinction between infected and healthy vaccinated animals is possible. This strategy should also be maintained for at least one generation to reach and maintain a prevalence close or equal to zero (Blasco, 2010).

When the situation is favourable and stable (prevalence $\leq 1\%$), banning vaccination and applying test-and-remove only is the next necessary step to obtain the officially-free status (Blasco, 2010). The decision to stop vaccination should always be made with caution and should not be premature (European Commission, 2001; Blasco, 2010). At this stage, effective control of animal movements to avoid the importation of the disease is critical (European Commission, 2001). Systematic testing of animals is required, and the detection of seropositive animals should be interpreted at the herd instead of the individual level: the whole culling of the herd is more practical and effective than removal of the infected animals only (Blasco, 2010). This strategy also avoids the possible resurgence due to latent vertically-infected animals (1.2.3 – Plommet *et al.*, 1973).

Finally, when the infection is fully eradicated and/or the officially-free status has been granted, movement controls and regular testing of animals should be maintained (Blasco, 2010).

To adapt management of brucellosis to Alpine ibex, a precise understanding of the transmission pathways, as well as efficient tools for the diagnosis of infected animals (to decide which individuals should be removed or vaccinated) and for the estimation of the prevalence in the population (to choose when to manage, which strategy to apply and then evaluate its effect on the infection dynamics) would be required. However, the study of infectious diseases in wild populations face difficulties inherent to the wild nature of the populations in consideration (Mörner *et al.*, 2002), and require the development of specific methods (Rhyan and Spraker, 2010; Ryser-Degiorgis, 2013). In the following section, I review some of the available methods for studying the health status of individuals and populations, developed for other host-pathogen systems. I discuss the insights those methods can bring and their limits.

1.3. STUDYING WILDLIFE DISEASES

1.3.1. SAMPLING COLLECTION AND DIFFICULTIES

Studying infectious diseases of wildlife is either for research, when the goal is to understand and gain knowledge, or for surveillance, when information on diseases is collected for management purposes (Ryser-Degiorgis, 2013). The objectives, concepts and methods of the investigations of wildlife infectious diseases are similar to those of the investigations of domestic animals diseases, but they necessitate to take into account the specific characteristics of wildlife populations (Ryser-Degiorgis, 2013).

Two types of sampling collection methods can be distinguished (Ryser-Degiorgis, 2013): the opportunistic collection of sick or dead animals and the identification of the underlying disease, called scanning or passive surveillance when there is a view to management, and the proactive sampling of live or dead animals (including apparently healthy ones) to detect a selected infectious agent in a population, called targeted (previously called active) surveillance.

Both sampling methods may concern found-dead animals, including road accidents (Courcier *et al.*, 2018), game meats inspection and sample collection of hunter-harvested animals (Mörner *et al.*, 2002). Live animals can also be sampled through captures, either for this specific purpose or for translocation, population monitoring or disease management purposes. One major hurdle in common between these various techniques is the non-random selection of the sampled individuals. Even for proactive sampling during hunting or captures for targeted surveillance, efforts to get representative samples are limited by availability and accessibility of the animals (Ryser-Degiorgis, 2013).

Other obstacles faced by wildlife disease surveillance include the difficulty to access the animals or carcasses in some natural habitats; diagnostic limitations due to quality and conservation of samples, as well as suitability of diagnostic tests for wildlife species; lack of knowledge about infectious diseases and the biology of many species; lack of previous data on the population of interest, both epidemiological and demographical; seasonal variations of population dynamics and infection levels; and possible logistical, political, financial and legal restrictions (Mörner *et al.*, 2002; Ryser-Degiorgis, 2013; Walton *et al.*, 2016).

Once the samples are collected, assessing the health status of individuals is the preliminary step to the study of wildlife diseases. In general, laboratory tests adapted from techniques used for domestic species are available. They can determine if individuals were exposed or not to the infectious agent of interest, if exposed individuals are still infected or if they eliminated the infectious agent, and whether infected animals are also infectious, i.e., able to transmit it to other individuals. Based on available data on the health status of individuals, the second step is to estimate the health status at the population level. Depending on the situation and the data at hand, methods have been developed to estimate the number of new infections in a population over a period of time, called incidence, or the proportion of infected individuals in the population, called prevalence. These two steps (assessing the health status of individuals and estimating incidence and prevalence) are described in the next sections.

1.3.2. ASSESSING THE HEALTH STATUS OF INDIVIDUALS

1.3.2.1. Exposure

Previous exposure of the individual to the infectious agent of interest is often revealed by the detection of specific antibodies against it (Gilbert *et al.*, 2013). Although some serological tests detect antigens (e.g., some Enzyme Linked ImmunoSorbent Assays – ELISA), the term “serology” usually refers to the testing of blood serum to detect the presence of antibodies against a specific antigen.

Basic serological tests are simple to use in practice: they are easily available, and can be used on blood serum, but also on fluids obtained from other organs such as lung extract (Jakubek *et al.*, 2012) or meat juice (Richomme *et al.*, 2010). Moreover, antibodies can often be detected more easily and for longer periods than the infectious agent itself. For these reasons, assessing the health status of wild animals is often performed using serology.

The use of antibody tests is often the basis for management decisions, such as the removal of exposed individuals or the vaccination of susceptible ones. Results from these tests on a sample of individuals can also be used to estimate population-level measures such as prevalence (especially if the antibodies are short-lived) or incidence (using repeated captures of animals or age-stratified seroprevalence data – see 1.3.3).

The interpretation of the tests should be made with caution, as they have limitations. All tests are imperfect, and may produce false negative (e.g., due to lack of detection of relevant antibodies) or false positive results (e.g., due to cross-reactivity, the detection of antibodies that were produced by other infectious agent with similar antigens). Antibody assay may also detect maternally-derived antibodies instead of the individual's own response (Gilbert *et al.*, 2013). It is possible to combine several tests to gain more information on the probability that the individual was indeed exposed to the infectious agent and developed antibodies (Buzdugan *et al.*, 2017).

1.3.2.2. Infection

Infection can be revealed directly using isolation techniques, antigen-detection tests or molecular diagnostic tools such as Polymerase Chain Reaction – PCR (Gilbert *et al.*, 2013). It is often the only diagnosis of certainty to confirm the presence of the infectious agent of interest. For example, the isolation and identification of *Brucella* is the only diagnosis of certainty for brucellosis infection (Alton *et al.*, 1988; OIE, 2016).

Compared to serology, these techniques have the additional interest to distinguish between infected and recovered animals when the antibodies are long-lived. Detecting the infectious agent may allow managers to avoid the unnecessary removal of seropositive animals that are no longer infected during management operations, an improvement that was suggested for more efficient and conservation-adapted test-and-remove management of brucellosis in bison (Treanor *et al.*, 2011), and could also apply to brucellosis in ibex. Conversely, these tests can detect infected individuals that would have been disregarded as negative on the basis of serologic tests alone. For example, PCR detected infection by Aujeszky's disease virus in wild boars (*Sus scrofa*) that were otherwise seronegative because infection was recent (Ruiz-Fons *et al.*, 2007).

At the population level, the distinction between infected and recovered animals can provide more accurate estimates of the prevalence of infection than antibody prevalence, especially when the antibodies are long-lived (see 1.3.3 – Gilbert *et al.*, 2013).

The detection of the infectious agent in specific organs also permits to infer contamination routes and shedding patterns. For example, the contamination route of *Mycobacterium bovis* is reflected by infected lymph nodes: mesenteric for the oral route versus pulmonary for the nasal route (Biet *et al.*, 2005). Conversely, high numbers of bacteria in the lungs are likely

to indicate shedding of *M. bovis* by the nasal route, as observed in red deer (*Cervus elaphus* – Johnson *et al.*, 2008). In the case of brucellosis in ibex, detection of infection and characterization of the within-host distribution of the bacteria allowed us to infer possible transmission routes and shedding patterns (Chapter 3.1).

Finally, the isolation or molecular detection of the agent can be associated to molecular typing, to infer epidemiological patterns. For example, in ibex, molecular characterization is a strong argument to think that the source of infection in ibex was cattle herds that were present in the area (Mick *et al.*, 2014). Molecular typing may even contribute to decipher transmission chains at fine scale using phylodynamics (Kamath *et al.*, 2016; Luzzago *et al.*, 2016).

Isolation techniques, antigen-detection tests or molecular diagnostic tools also have several limitations, in the case of low amount of infectious agent in sampled organs, latent infection or short infectious period. They also require to sample dead animals for instance for organ culture (Gilbert *et al.*, 2013). These factors make the detection of infectious agents in wildlife impractical in many cases, explaining why serology is more often used in wildlife for assessing the health status of individuals.

1.3.2.3. *Infectiousness*

Finally, in addition to the distinction between infected and recovered, it can be useful to distinguish between shedding and non-shedding individuals, in order to identify the individuals responsible for transmission and provide insights on the transmission mechanisms, with possible implications in surveillance and management. This is performed by the same detection techniques of infectious agent as before, but on individuals' nasal, oral, rectal and genital secretions.

An interesting example is Aujeszky's disease in wild boar, where shedding analysis explained the transmission mechanisms. Shedding in genital secretions was found in both males and females, suggesting venereal transmission as the main route of transmission, while oro-nasal shedding exists only in females and explains how the infection is transmitted within groups of females (González-Barrío *et al.*, 2015).

Once again, these techniques face limitations in case of low amounts of infectious agents in samples, intermittent shedding or short shedding periods. This is especially true for

Brucella, which is shed intermittently in infectious animals (e.g., at the time of abortion or birth, see 1.2.2) and therefore absence of positive results on secretions should not necessarily be associated with the absence and inability to shed the bacteria.

1.3.3. ESTIMATING PREVALENCE AND INCIDENCE IN THE POPULATION

1.3.3.1. Prevalence

Studies aiming at sampling individuals only once at a given time or period provide information on prevalence and/or seroprevalence, i.e., the proportion of infected or seropositive individuals in the population (Gilbert *et al.*, 2013). If antibodies do not last after the period of infection, seroprevalence may serve as a good proxy for prevalence of infection. A single study can evaluate if the infectious agent is present and possibly the level of infection in the population. Repeated samplings may enable to bring insights into temporal variations of prevalence and seroprevalence.

For pestivirus infection in a population of Pyrenean chamois, repeated follow-up with both antibodies and virus detection revealed seasonal and temporal variations, with highest viro-prevalences and lowest seroprevalences when there are high proportions of young susceptible animals, i.e., in autumn and during years of high recruitment (Pioz *et al.*, 2007). A few years later, the same survey detected of a strong decline in virus prevalence, followed by a decline in antibody prevalence, suggestive of a local extinction of the virus (Gilot-Fromont *et al.*, 2018b).

Many biases related to non-random sampling in the population can also occur during sampling, as well as possible interactions with population dynamics or even human interventions for disease management. Apparent prevalence data, the proportion of animals from the collected sample that are positive to the diagnostic method used, may thus constitute a biased estimator of infection prevalence, and statistical analyses taking into account the various biases that can intervene in the population of interest are required.

1.3.3.2. Incidence

Incidence is the number of new infections in a population over a period of time, and the incidence risk is the proportion of susceptible individuals that become infected in a given time. They can be derived from longitudinal survey, i.e., repeated sampling of the same individuals to detect new infections or seroconversions in originally negative animals, or from age-stratified seroprevalence data coming from individuals sampled only once.

Longitudinal studies can bring insights into the changes of the disease dynamics between seasons and over the years. For example, Buzdugan *et al.* (2017) developed a method for *M. bovis* in badgers (*Meles meles*) to estimate the probability of infection in each sampled individual given its test results during previous and current capture. Using these results, they showed seasonal and temporal variations of the incidence risk. However, repeated samplings of the same individuals can be difficult for wild animals, while sample size needs to be large enough to detect new cases, especially if incidence is low. This constitutes the main limit to the use of this method (Gilbert *et al.*, 2013). In the context of disease management, repeated samplings of individuals with positive or doubtful test results is also not necessarily feasible, if infected animals are supposed to be eliminated from the population.

If enough seroprevalence data is obtained from individuals sampled only once, they can also provide information on the force of infection (FOI), i.e., the rate at which susceptible individuals become infected per unit time, from which can be derived the incidence risk (Vynnycky and White, 2010). The methods used to estimate the FOI are based on the monitoring of seronegative individuals born into the population to determine the rate at which they become seropositive. Therefore, seroprevalence data of cross-sectional samples can be stratified by age to represent different cohorts of animals with various exposure times since they were born (i.e., age), and used to determine how fast individuals become seropositive, under the assumption that the force of infection was constant on average.

These methods have been used for a long time in the study of infectious diseases in humans (Muench, 1934; Hens *et al.*, 2010) and more recently for wildlife diseases (Heisey *et al.*, 2006). The latter study demonstrated a strong age-dependency of the FOI for bovine tuberculosis in bison, with most of the new cases occurring in young individuals (Heisey *et al.*, 2006). One limitation of these methods is that they estimate an average FOI over time, and cannot provide insights on its temporal variations. Further work has to be done to overcome this limitation. One recent development was proposed by Pepin and colleagues,

which inferred FOI using both age-seroprevalence data and experimental data on antibody kinetics, to add information on the time since infection (Pepin *et al.*, 2017). However, this method requires precise information on the antibody kinetics of the species of interest, which is not always available in wildlife.

1.3.3.3. Monitoring incidence and prevalence for disease management

Information on the health status of individuals, the transmission routes and shedding patterns, the prevalence and incidence, and when possible their temporal variations are of paramount importance for the design and the choice of appropriate disease management strategies. Monitoring also has to be carried out in parallel to management methods, to assess their efficacy and test if the objectives have been reached so as to stop or adapt the undertaken measures.

Under practical disease management conditions however, understanding disease dynamics can be complicated. For example, vaccination may interact with serological tests and make prevalence estimations difficult to obtain. To circumvent this limitation in the case of oral vaccination of wild boar against classical swine fever, the evolution of seroprevalence was measured in young animals born in the population after vaccination campaigns were completed. A strong decrease in seroprevalence revealed that vaccination successfully altered the circulation of the virus (Saubusse *et al.*, 2016).

In practice, however, the monitoring of disease dynamics is often not sufficient to detect the moment when the epidemiological situation has improved enough to stop, lighten or change management options. Decisions concerning changes in management have to be taken in a context of high level of uncertainty about the actual epidemiological situation. Relevant monitoring protocols and indicators for outbreak monitoring are still lacking though badly needed (e.g., Haydon *et al.*, 2002).

To effectively manage an infectious disease in a wild population, it is also necessary to understand precisely the transmission dynamics of the infection. As stated in the introduction of this chapter, variability, from between-individuals to between-species, is more pronounced in wild animal populations than in domestic ones. This may result in different

transmission dynamics of infectious agents depending on the individuals or the local situations, and therefore understanding how heterogeneity shapes these dynamics could provide valuable insights on how to effectively manage wild host-pathogen systems. In the following section, I explain the different levels of heterogeneity and some of the mechanisms generating heterogeneity. Then, I describe possible impacts of heterogeneity on disease transmission and management.

1.4. HETEROGENEITY OF TRANSMISSION

1.4.1. DIMENSIONS OF HETEROGENEITY

Transmission heterogeneity is defined as variability in the contribution of individuals, populations/species, or areas, to the overall number of new cases of infections (Paull *et al.*, 2012; VanderWaal and Ezenwa, 2016).

Considering populations as homogeneous is a common simplifying hypothesis. However, in general, the contribution of individuals to epidemiological dynamics is heterogeneous. For example, in several sexually transmitted and vector-borne infections, only 20% of cases contributed to at least 80% of transmission (Woolhouse *et al.*, 1997). For directly transmitted diseases, individuals infecting many others were used to be considered as exceptions, maybe because they remain undetectable in many cases using standard incidence and prevalence data (Kemper, 1980). However, since transmission heterogeneity was demonstrated for the Severe Acute Respiratory Syndrome (SARS) epidemic in 2003 (Galvani and May, 2005), transmission heterogeneity, with 20% of infected individuals contributing to the majority of transmission (40 to 90%), was evidenced for several directly transmitted infectious diseases of humans and animals (Lloyd-Smith *et al.*, 2005b; Matthews *et al.*, 2006).

Extreme cases of heterogeneity are referred to as “superspreading events”. Lloyd-Smith *et al.* (2005b) defined a superspreader as an individual that caused more infections than would occur in 99% of cases in a population in which transmission between individuals would be entirely homogeneous. Evidence for such superspreading events has been found in reported data from several infectious diseases (Lloyd-Smith *et al.*, 2005b).

Heterogeneity may also occur between demographic classes, with specific age- and sex-classes contributing to the majority of transmission. For example, males have been shown to

be much more important than females for transmission of some diseases (e.g., tick-borne encephalitis virus and nematodes in yellow-necked mouse – *Apodemus flavicollis* – Ferrari *et al.*, 2003; Skorpung and Jensen, 2004). Variability in transmission can also happen between different age-classes (e.g., higher transmission among adult wild boars than among juveniles for classical swine fever – Bolzoni *et al.*, 2007) or in individuals displaying certain types of behaviours (e.g., in meerkats – *Suricata suricatta*, subordinate individuals are more at risk of contracting bovine tuberculosis than dominant ones – Drewe, 2010).

Heterogeneities between different areas or populations can also be found, where some areas display particularly high transmission rates or prevalence (transmission foci or “hotspots”) or transmit infection towards other areas more than the others do (“source areas” – Paull *et al.*, 2012).

Finally, for multi-host infectious agents, each species can play different roles for transmission. Species can act as maintenance hosts (able to both maintain the infection without transmission from other species and to transmit it to other species), as spillover hosts (in which maintenance is not possible without incoming transmission from other species, but can still transmit to other species), or as dead-end hosts, which can neither maintain nor transmit the infection (Morris *et al.*, 1994; Caley and Hone, 2005; Rhyan and Spraker, 2010).

1.4.2. MECHANISMS GIVING RISE TO HETEROGENEITY

1.4.2.1. Individual level

Heterogeneity in the number of new infections caused by an infected individual depends on three components: the variability in the number of contacts during which transmission may occur (“supercontacting”), which can be subdivided in the variability of the rate of appropriate contacts for transmission and in the variability in the duration of the infectious period during which transmission can occur; and finally the variation in infectiousness (“supershedding”), i.e., the probability that contact leads to transmission (VanderWaal and Ezenwa, 2016).

Physiological and behavioural mechanisms can promote heterogeneity of these three components: behavioural mechanisms will for example lead to variation in the contact rate, while physiological mechanisms could make infectiousness or duration of the infectious period vary (VanderWaal and Ezenwa, 2016).

Immunity is one of the major physiological mechanisms that influences the amount and the duration of shedding (Pathak *et al.*, 2010). Several factors can influence the immune response to infectious agents, such as body condition (Gilot-Fromont *et al.*, 2012), genetic resistance (Borriello *et al.*, 2006; Luikart *et al.*, 2008) or co-infections (Ezenwa and Jolles, 2015). An extreme case of superspreading is the development of persistently infected (PI) individuals by pestivirus infections, for example in Pyrenean chamois: individuals infected vertically by the virus early during gestation do not develop an immune response against it, and therefore shed high amounts of the virus and remain infectious during their entire lifetime (Pioz *et al.*, 2007; Beaunée *et al.*, 2015).

Personality and behaviour may influence the rate of contacts in which individuals engage, either between individuals or with environmental sources of infection. For example, the risk of contracting bTB is highest in meerkats that are often the target of aggressions and groom other individuals (Drewe, 2010). In deer mice (*Peromyscus maniculatus*), bold individuals are responsible for a majority of transmission of the Sin Nombre virus (Dizney and Dearing, 2013).

Finally, interactions between behaviour and physiological factors can also lead to transmission heterogeneity. For instance, social behaviour can induce variability in individuals exposure and response to stress, which in turn can impact the immune response and the level of infection (VanderWaal and Ezenwa, 2016). In mandrills (*Mandrillus sphinx*), dominant males are more stressed than subordinate males when the dominance hierarchy is unstable, inducing immunosuppressive effects and higher levels of infection by gastrointestinal parasites; the opposite is true when the dominance hierarchy is stable (Setchell *et al.*, 2010). Another example is sickness behaviour, where the variability in physiological response to the infection, such as fever or lethargy, can induce changes in behaviour and therefore reductions in contact rates in infected animals (Lopes *et al.*, 2016; White *et al.*, 2018a).

1.4.2.2. *Demographic-classes level*

Variability between classes of individuals can be related to the above-mentioned behavioural or physiological mechanisms, coupled to the patterns of contacts between classes. For example, males can be more heavily infected than females due to physiological differences, therefore leading to more transmission. For example, rodents infected by the

same doses under experimental conditions have demonstrated higher loads of nematodes or protozoans in males than females (Zuk and McKean, 1996). Even in the absence of these male-biased levels of infection, for which there is no general rule (Poulin, 1996; Schalk and Forbes, 1997), sex differences in behaviour such as space use and larger home ranges can lead to male-biased transmissions. For example, in a population of yellow-necked mice infected by a nematode with similar prevalence and shedding between males and females, treating males decreased prevalence in both sexes whereas treating females decreased prevalence only in females. This result was related to different contact patterns between males and females (Ferrari *et al.*, 2003). Heterogeneity can also arise from sex-related differences in transmission routes, such as for Aujeszky's disease in wild boar, which is transmitted by the venereal route in both males and females but by the oronasal route exclusively in females (Ruiz-Fons *et al.*, 2007; González-Barrio *et al.*, 2015).

Transmission can also strongly depend on age: for example, shedding of brucellosis in bison is strongly age-dependent, with high risk of transmission in young animals at the age of first parturition (Treanor *et al.*, 2011). In wild boar, contact rates among adults strongly increases during mating season, which is not the case for juveniles (Bolzoni *et al.*, 2007).

Finally, differences in behaviour can also explain transmission heterogeneity between social classes such as dominant and subordinates, as shown for bTB in meerkats: because dominants are less likely to groom others and to be the target of aggressions (see 1.4.2.1), they are less at risk of contracting bTB (Drewe, 2010).

1.4.2.3. Spatial level

Hotspots of infection are characterized by environmental conditions that increase local survival of the infectious agent (Paull *et al.*, 2012). For example, the environmental survival of *Vibrio cholerae*, the agent of cholera, is enhanced by water hyacinths (*Eichornia crassipes*) and can lead to disease hotspots in areas where water hyacinths are abundant (Spira *et al.*, 1981). Another factor that can promote heterogeneity between populations or areas is the size or density of competent hosts: for instance, badgers act as maintenance hosts of bTB in the United Kingdom and Ireland, whereas they are spillover hosts in France and Spain for example, where badger populations experience much lower densities relatively to their British and Irish conspecifics (Gortázar *et al.*, 2012).

Transmission between areas is dependent on prevalence and movements of animals. Areas with high prevalence and high movement rates can act as sources of infection to other areas, while areas with low prevalence or low movement rates are less likely to transmit infection. For instance, the analysis of movement patterns of mule deer and differences in chronic wasting disease prevalence between different areas in Colorado demonstrated the existence of several spatial units acting as sources of infection through migration of animals towards areas with lower prevalence (Conner and Miller, 2004). Movements of migration or dispersal can be linked to several factors, such as resources, environmental factors or human management, that can vary between areas or populations and lead to these variations in spatial transmission.

1.4.2.4. Species level

Species can differ in their competence, which can be defined as the efficiency with which a host acquires, produces and transmits an infectious agent (Paull *et al.*, 2012). Variability in competence results from pathogen adaptation to a specific host and its ability to overcome specific cellular, tissue and immune barriers (Kuiken *et al.*, 2006). Behavioural differences can also be a source of heterogeneity between species. For example, roe deer (*Capreolus capreolus*), elk and white-tailed deer (*Odocoileus virginianus*) are all competent hosts for bTB, but the gregarious elk and white-tailed deer have been shown to maintain the infection in North America (Fitzgerald and Kaneene, 2013), while its solitary behaviour makes roe deer an occasional spill-over host (Lambert *et al.*, 2017).

1.4.3. WHY DOES HETEROGENEITY MATTER?

One major and classical assumption in epidemiology is that all infected individuals and groups are similar and that the entire population mixes homogeneously (Anderson and May, 1991). However, we saw in the previous section that, especially in wildlife, all infected individuals are not comparable and populations exhibit socio-spatial structure most often than not. These heterogeneities could provide a pathway towards better management, by allocating management resources on the effective sources of transmission instead of the whole population or all infected animals without distinction.

When only a small proportion of infected individuals contributes to the majority of transmission, invasion by infectious agents are less likely than in homogeneous populations because there is a greater chance that the early cases will not transmit the disease (Lloyd-Smith *et al.*, 2005b). However, when invasion succeeds, outbreaks are more likely to display high epidemic growth rates, because once superspreaders are infected they quickly spread the infection in the population (Lloyd-Smith *et al.*, 2005b).

If it is possible to identify and target the individuals most responsible for transmission, disease management should be more efficient (Lloyd-Smith *et al.*, 2005b; Matthews *et al.*, 2006), especially when disease management effort is low or intermediate (Lloyd-Smith *et al.*, 2005b). If individuals cannot be identified easily in the field, identifying and targeting classes of individuals are another valuable option, if transmission is associated to recognizable traits (see 1.4.2.2 – VanderWaal and Ezenwa, 2016). For example, there is evidence that gender-based harvesting in white-tailed deer, where males transmit bTB more than females, could achieve disease eradication where non-targeted strategies would fail (Fenichel and Horan, 2007). Similarly, targeting specific areas based on how infection is transmitted between areas could efficiently reduce transmission at the population level. In Ethiopian wolves (*Canis simensis*), vaccination of uninfected packs adjacent to nearby transmission hotspots and source areas effectively reduced the extent of rabies outbreaks (Haydon *et al.*, 2006).

In addition to a precise understanding of the transmission mechanisms and dynamics, the choice of disease management strategies in wildlife should be based on previous experiences in other systems (Delahay *et al.*, 2009). Although all host-pathogen systems are not necessarily comparable, wildlife populations share certain specificities that can limit the efficacy of some disease management methods compared to domestic animals. As an example, population dynamics are not under anthropogenic controls and can change in response to management leading to opposite changes to those expected in infection dynamics. The next section gives examples of disease management strategies used in wildlife and how they performed in various host-pathogen systems, highlighting effective options and also their challenges.

1.5. THE MANAGEMENT OF WILDLIFE DISEASES

1.5.1. WHY MANAGING WILDLIFE DISEASES?

Disease management strategies are generally implemented in wildlife when the disease represents a threat to public health, to veterinary health and/or to the conservation of a wild population (Cross *et al.*, 2007). When at least one of these three justifications is met, disease management strategies may have three possible goals depending on the situation at hand (Wobeser, 2002):

- Prevention, to avoid the introduction of an infectious agent into a susceptible population;
- Control or mitigation, to reduce the incidence, the prevalence and/or the spatial spread of an infection already present in a population, without aiming to complete elimination;
- Eradication, to remove the infectious agent from the population.

For many diseases in wildlife, no management strategies have been implemented, either because the possible strategies were not feasible or not expected as effective (Wobeser, 2002, 2007). Indeed, disease management strategies in wildlife face several difficulties and limits that do not exist in domestic species (Artois *et al.*, 2001), leaving few remaining options of known efficacy. Once the decision to implement disease management has been made and its goal has been determined, the choice of the method should be based on the results of previous approaches (Delahay *et al.*, 2009), some of which are discussed in the following section.

1.5.2. LIMITED OPTIONS IN WILDLIFE AND MANY CHALLENGES

Disease management measures can be classified either by distinguishing measures targeting the environment (Ward *et al.*, 2009), the infectious agent (Blancou *et al.*, 2009), or the hosts (Carter *et al.*, 2009); or by distinguishing offensive measures aiming at limiting infected individuals, and defensive measures, aiming at protecting susceptible individuals and preventing transmission (Table 1-1).

TABLE 1-1 | Examples of disease management measures available for wildlife, classified depending on which of the infectious agent, hosts or environment they are targeting, and on whether they are targeting infected individuals (offensive measures) or susceptible individuals or transmission itself (defensive measures).

	Infectious agent	Hosts	Environment
Offensive	Treatment	Test-and-remove	
Defensive	Vaccination	Culling	Fencing Hunting-offal disposal

1.5.2.1. Targeting the environment

Changes in the environment can help mitigating disease transmission. These changes can aim at modifying the distribution of hosts or limiting their movements, in order to decrease contact rates and the spread of infection. Other measures can aim at reducing the environmental load of the infectious agent, when the infection can be transmitted through the environment (e.g., soil, plants, water). Demonstrations of the efficacy of measures directed at the environment are rare, and these measures can have non-desired impacts on the ecological community (Ward *et al.*, 2009).

Fences is an example of a method that targets the environment. It is used in order to limit host movements and decrease contacts between populations, but their efficacy is controversial while their maintenance is difficult and expensive (Gortázar *et al.*, 2015; Mysterud and Rolandsen, 2019). This method is more likely to be effective to contain the infection in small areas and when the disease spread slower than the construction of the fence (e.g., Šatrán, 2017). Hunting-offal disposal, i.e., removal of harvested animals, viscera and other remains, is another example, that aims at limiting indirect transmission (Gortázar *et al.*, 2015). This method is used for example for bovine tuberculosis in wild boar and red deer (Zanella *et al.*, 2012).

1.5.2.2. Targeting the infectious agent

Treatment is generally administered in captive animals, e.g., treating against bTB in Arabian oryx (*Oryx leucoryx*) contributed to the production of bTB-free animals for their reintroduction in the wild (Greth *et al.*, 1994). In free-ranging wildlife, treatment against *Echinococcus multilocularis* using baits in foxes is a promising example (König *et al.*, 2008).

Several studies demonstrated a decrease in parasite prevalence after regular baiting campaigns, from four up to 35 times lower (Hegglin and Deplazes, 2013). However, the treatment is unlikely to result in the eradication of *E. multilocularis*, and only campaigns lasting for several decades can be cost-effective (Hegglin and Deplazes, 2013). Such a long-time investment is difficult to organise, and whether the epidemiological situation will be stable over long period is unclear. This example raises the question of the long-term monitoring and decision scheme for wildlife disease management.

Apart from these notable exceptions, medical treatment is rarely used in wildlife because of its difficult administration, high costs and associated concerns about biosafety for other species or development of resistance (Blancou *et al.*, 2009; Gortázar *et al.*, 2015). Coinfections can also be a limitation to the effect of medical treatment: for example, transmission of bTB by infected African buffalo unexpectedly increased after anthelmintic treatment, due to the increased survival of treated animals (Ezenwa and Jolles, 2015).

Vaccination was used successfully for rabies in wild foxes in Europe, using oral baits and achieving eradication in many countries of western and central Europe (Cross *et al.*, 2007; Müller *et al.*, 2015). This example and the effectiveness of oral mass vaccination for the control of classical swine fever in wild boar (Rossi *et al.*, 2015) have made oral vaccination a promising tool against wildlife diseases, which also receives better support from the public (Cross *et al.*, 2007). However, several difficulties can limit the efficacy of oral vaccination or its development: biosafety for other species, potential reversion to virulence, stability in the environment, difficulty to distinguish between vaccinated and naturally-infected individuals, sufficient distribution and attractiveness of the baits (Cross *et al.*, 2007; Blancou *et al.*, 2009; Artois *et al.*, 2011). Parenteral administration is a possible alternative, but necessitates rare conditions where the whole or almost whole population can be captured and vaccinated, so as to reach the critical level of vaccination needed to protect the population (herd immunity – Keeling *et al.*, 2013). It was used for instance in a population of endangered Florida pumas (*Puma concolor coryi*) in response to a Feline Leukemia Virus (FeLV) outbreak (Cunningham *et al.*, 2008). Preventive parenteral vaccination was also used recently in the Hawaiian monk seal (*Neomonachus schauinslandi*) in an attempt to protect them against a potential introduction of phocine distemper virus (Malakoff, 2016).

Unexpected outcomes can also arise from vaccination. For example, vaccination can increase host survival and thus the birth of larger number of susceptible individuals, which in turn may enhance the risk of transmission (Smith and Cheeseman, 2002; Blancou *et al.*,

2009). When vaccination does not confer a complete protection against the infection, increased host survival may increase infectious duration and therefore enhance disease spread (Lange *et al.*, 2012).

1.5.2.3. *Targeting the hosts*

The non-selective elimination of hosts is often the first measure proposed to manage a wildlife disease outbreak, as this method parallels the “stamping-out” used in domestic species, and generally appears as more feasible than others. The rationale behind non-selective culling is to reduce host density below a certain threshold under which stochastic fadeout of the disease is highly probable (Lloyd-Smith *et al.*, 2005a). However, evidence for such thresholds in wildlife populations is rare, for various reasons such as population dynamics, socio-spatial structure, behaviour or the existence of alternative host species (Lloyd-Smith *et al.*, 2005a). Consequently, removal rates necessary to achieve eradication of the disease may be too high to be achieved in practice, as demonstrated for example for Devil Facial Tumour Disease in Tasmanian devil (Beeton and McCallum, 2011). Moreover, such high levels of culling are ethically questionable and ecologically risky (Gortázar *et al.*, 2015). An exception to these limitations are introduced or invasive species. For example brushtail possums (*Trichosurus vulpecula*) are under extensive culling in New Zealand for bTB management (Livingstone *et al.*, 2015). Culling may also be performed when there is no alternative, at least based on the current scientific knowledge, such as the recent eradication of a wild reindeer population in Norway for Chronic Wasting Disease (CWD) management (Mysterud and Rolandsen, 2018).

In addition to these limitations, removal can lead to counterintuitive effects, when the disease increases transiently or persistently as a result of culling. This perturbation effect can be the result of interactions between culling and transmission dynamics, by increasing susceptibility of hosts because of stress, or by increasing the number of contacts by disrupting social structure and movements (Prentice *et al.*, 2014). Perturbations of the social structure leading to increased disease spread was for example observed as a result of non-selective culling for fox rabies (Aubert, 1992) or for bTB in badgers (McDonald *et al.*, 2008). These perturbations are more likely to occur when culling rates are too low or when culling is not applied to enough groups (Prentice *et al.*, 2014, 2019). Counterintuitive effects can also result from interactions between culling and population dynamics: when population size

is reduced, density-dependent parameters such as recruitment may increase, thereby increasing the number of susceptible individuals and therefore transmission (Choisy and Rohani, 2006).

Because of these ethical, practical, and efficacy limits, alternative methods to non-selective culling have to be developed (Artois *et al.*, 2011). One possible option is to proceed to selective culling, i.e., removing only infected individuals instead of any individuals regardless of their infection status (Carter *et al.*, 2009). This can be performed if infected individuals can be identified, for example due to disease symptoms or by capturing animals and using diagnostic tests. This option, called test-and-remove, is limited by the difficulty to capture large numbers of individuals and the existence of a diagnostic test that can be used in the field (Carter *et al.*, 2009). However, successful test-and-remove operations have been conducted for example for brucellosis (*Brucella abortus*) in wildlife from the Elk Island National Park, Canada (Tessaro, 1986) or for bTB in African buffalo in Hluhluwe–iMfolozi Park, South Africa (le Roex *et al.*, 2015).

In conclusion, numerous tools are available to combat or prevent disease in wildlife, however all have important limitations. It is then necessary to determine in which individuals, when and where, each tool should be used to obtain a relevant management strategy.

1.5.3. THE DEVELOPMENT OF WILDLIFE DISEASES MANAGEMENT STRATEGIES

The identification of all species or populations involved is required to choose the appropriate targets for disease management. Not targeting all populations or species involved can result in the failure of the strategy, especially when the aim is the eradication of the infectious agent. For example, several species of non-targeted species of wild carnivores, such as skunks (*Mephitis* spp.), were able to maintain rabies transmission in the United States, limiting the efficacy of oral vaccination compared to its success in Europe where foxes were the only maintenance hosts (Cross *et al.*, 2007).

Once the target(s) of management are identified, the choice of the method or combination of methods is based on several criteria. The first criterion is effectiveness, feasibility and

absence of undesired effect (or favourable balance between both): various methods, selected on the basis of past experiences or theoretical predictions, should be evaluated for their relative ability to reach the objective for the specific study system (Wobeser, 2007; Delahay *et al.*, 2009). The response of the system to management interventions can be evaluated through experimentation in the field (Delahay *et al.*, 2009). An outstanding example of such experimentation is the Randomised Badger Culling Trial (RBCT) carried in England for the management of bovine tuberculosis. In this large field experiment, 30 study areas of 100 km² were grouped in triplets with similar ecological and disease characteristics. Each study area in the triplet was then randomly assigned one out of three management interventions: proactive culling of badgers in the whole area, localized badger culling around cattle outbreaks or control area with no culling. This experiment allowed the researchers to compare the relative efficacy of these three methods on bTB prevalence in badgers and in cattle (Donnelly *et al.*, 2006; McDonald *et al.*, 2008). However, such experiments may be impractical, too expensive or unable to bring answers fast enough given the severity of the problem (Delahay *et al.*, 2009). For example, the RBCT costed more than £50 million and took 10 years to complete (McDonald *et al.*, 2008). Although scientific analyses brought significant results, study itself was not sufficient to close the strong debate over bTB management in the UK. In the case of ibex brucellosis, a single population of ibex is infected, making experimental comparison of different interventions in similar conditions impossible.

In such circumstances, mathematical modelling can be a useful tool. Models can enhance the knowledge on the mechanisms and dynamics of the system, and also provide the opportunity to simulate several management interventions and compare their likely outcome. Therefore, the results of mathematical models represent useful contribution to the decision-making process for developing management strategies (Smith *et al.*, 2009 – see Chapter 2 for more details on mathematical modelling).

The second criterion is the cost-benefit ratio of the strategy. The most effective interventions are also likely the costliest because they require the most efforts (Delahay *et al.*, 2009). If the financial requirements cannot be met, the interventions will most likely fail and it may be more useful to choose a less effective, but cheaper, intervention in order to increase cost-benefit ratio. The cost assessment is a sensitive step, as it must consider all costs, including long term ecological costs or benefits that may result from management (Wobeser, 2007). For example, management strategies should include the aim to maintain and protect wild populations and biodiversity (Delahay *et al.*, 2009; Rhyan and Spraker,

2010), a condition difficult to assess and to measure. Another possible adverse ecological effect of management interventions is the development of resistance to treatment that would be detrimental in the long term.

The last criterion is the acceptability of the intervention. This includes both acceptance by the general public and by the local stakeholders and parties involved. Culling for example is generally not supported by the public and is considered as less acceptable than other methods (Wobeser, 2007). However, it also depends on the perception of the wildlife species and of the disease in the local area where interventions are required. For example, culling may be considered as more acceptable for species considered as pests (Wobeser, 2007). Even if a strategy is effective, and with a good cost-benefit ratio, it may not be implemented by politicians if it is not acceptable by the public (Wobeser, 2007). Transparency and evidence-based political decisions are key elements for public acceptance. For example, the eradication of a whole herd of reindeer in Norway for management of CWD generated less negative reactions than expected, probably because the decision was based on experts opinion and the culling process was made fully transparent (Myrsterud and Rolandsen, 2018). Acceptance by the local stakeholders is also critical as they are in charge of implementing the disease management strategies, which demands huge efforts often for long periods (Cowie *et al.*, 2015). Contrary to the general population, local stakeholders possess information and knowledge that make them local experts, who are not always ready to accept national or international management policies. Informing stakeholders about the results of investigations and monitoring, and considering their point of view in the decision process along with experts for the choice of the method and how to implement them are likely to identify effective measures that they will readily accept (Dorn and Mertig, 2005; Cowie *et al.*, 2015; Myrsterud and Rolandsen, 2018). For example, banning of supplemental feeding was not supported by local stakeholders for the management of bTB in Spain, contrary to hunting-offal disposal and separate access of livestock and wildlife to waterholes (Cowie *et al.*, 2015).

As a conclusion, although many attempts have been performed to control wildlife diseases, all available methods have strong limitations, due to logistical and financial reasons, low efficacy due to interactions with population demography and social structure, possible adverse effects on the surrounding ecosystem and ethical and acceptability problems. There have been a few successes of wildlife disease management in the past, but there is room for improvement. Targeting more precisely the entities (individuals, populations, areas) responsible for most of transmission could improve available strategies. Indeed, they would be probably more effective because they focus on the real sources of infection. Thus, for similar efforts, they would have a better cost-benefit ratio than random selection (but could increase the efforts required as they are more selective). Finally, they could be more easily accepted as they concern a smaller fraction of the population.

1.6. AIM AND STRUCTURE OF THE THESIS

The aim of this thesis is to provide insights on the surveillance and management of infectious diseases in heterogeneous wild populations, using *Brucella melitensis* infection in a French population of wild Alpine ibex (*Capra ibex*) as a biological model. This host-pathogen system is a relevant model to study disease management in natural settings in the context of transmission heterogeneity. First, wild ungulates are good model systems because of their close phylogenetic relationships with domestic ungulates and because they share the same diseases, enabling for example to use already available diagnostic tests after validation (Jolles and Ezenwa, 2015). Second, both brucellosis and Alpine ibex are good candidates for the existence of heterogeneity of transmission. On the one hand, brucellosis is typically associated with abortions and genital shedding around gestation in females (FAO and WHO, 1986; Carvalho Neta *et al.*, 2010; Godfroid *et al.*, 2013), and also displays patterns of age-related bacterial shedding (Treanor *et al.*, 2011). On the other hand, ibex displays a behaviour of sexual segregation, where contacts between males and females decrease progressively after the mating period (Villaret and Bon, 1995; Bon *et al.*, 2001), which can have consequences on sex-related pathogen transmission (Ferrari *et al.*, 2010). Finally, in our study system, spatial differences in seroprevalence levels have been demonstrated (Marchand *et al.*, 2017), which could reveal heterogeneity of transmission at the spatial level.

Our study system is however associated with some difficulties. Indeed, this population was almost not monitored before the onset of infection. Thus, the population dynamics was

virtually unknown before the discovery of the infection, and opportunities to collect epidemiological data came directly from reactive disease management strategies, instead of being coupled with existing monitoring. Moreover, the implementation of disease management strategies from the very beginning of the study period make it more difficult to distinguish between the natural history of the disease and the anthropic impact.

Nonetheless, the fact that Alpine ibex is a protected species raises many questions about the acceptability of disease management in this species, and is an incentive to come up with improved disease management strategies. The possible existence of disease transmission heterogeneity both at the individual-classes level and at the spatial level make it a great opportunity to come up with new solutions that combine the beneficial effects of targeting at the different levels.

Following the general introduction above, the second chapter will introduce general considerations about the Materials and Methods I used for my PhD work. I first present the ecology and behaviour of the biological model, the Alpine ibex, before describing more precisely the population of study and data collection. As most of the PhD work was performed using mathematical modelling, I explain the basic concepts of modelling, and what can be expected from it.

In chapter 3, I present two studies in which we focused on characterizing transmission heterogeneity of brucellosis in Alpine ibex. First, we used available data on bacterial cultures to gain insights on *Brucella* shedding patterns and transmission routes in ibex. Serology is often used to evaluate the health status of individuals in wildlife (1.3.2), but is not sufficient to distinguish and therefore target the individuals responsible for transmission. Here, we used bacteriology to distinguish actively from non-actively infected individuals among seropositives, and to determine the factors associated with active infection. From the within-host distribution of the bacteria, we also inferred the transmission routes of brucellosis in ibex. Then, we used these results, as well as existing knowledge, in a mathematical model to reproduce the observed situation in the field and quantify transmission heterogeneity at the individual, demographic classes and spatial levels.

Chapter 4 had the objective to evaluate the efficacy of the disease management strategies implemented between 2012 and 2018, based on available surveillance data. The first study focused on seroprevalence, trying to overcome biased and limited sampling to provide reliable estimates of the temporal evolution of seroprevalence. The second study focused on

the evolution of the force of infection and incidence risk, providing a framework to estimate varying FOI instead of an average FOI over time (1.3.3).

Based on the results from the previous chapters, chapter 5 aimed at evaluating the efficacy of several disease management strategies using predictions from the model developed in chapter 3. Several strategies were evaluated, notably targeting specific classes of individuals and/or specific areas.

Finally, the last chapter summarises and discusses the findings reported in previous chapters, and proposes some perspectives of this work.

Chapter 2

MATERIAL AND METHODS – GENERAL CONSIDERATIONS



Personal picture

2.1. STUDY POPULATION AND MONITORING

2.1.1. THE BRUCELLOSIS OUTBREAK

In January 2012, two human cases due to *Brucella melitensis* biovar 3 were detected in the French Alps (le Grand Bornand, Bargy massif, Haute-Savoie), one of which presented symptoms of brucellosis (Mailles *et al.*, 2012). Epidemiological investigations related these cases to the consumption of raw milk cheese produced in a local farm, which was confirmed as infected by *B. melitensis* biovar 3 in April 2012 by bacterial isolation in a seropositive cow that aborted in late January (Mailles *et al.*, 2012). The whole herd (21 dairy cows) was culled following French regulations. All animals were seronegative except the cow that aborted, but the bacteria was isolated from a second animal and PCR-positive results were obtained for four others (Mailles *et al.*, 2012).

The last previous outbreak in domestic ruminants in the area (a few kilometres away) was reported in 1999, suggesting persistence or re-emergence of brucellosis in livestock. No other infected herd was detected, neither among the 40 herds having direct links to the outbreak, nor among the 205 herds that grazed during the summer of 2012 in the nearby massif (Mailles *et al.*, 2012; Garin-Bastuji *et al.*, 2014). Therefore, wildlife was suspected of being the source of infection for this re-emergence. No red deer (n=30) and roe deer (n=44) were found infected, and only one chamois out of 55 was seropositive and infected. However, an unprecedented high seroprevalence (38% in 2013, n=77 – Hars *et al.*, 2013; Garin-Bastuji *et al.*, 2014) was found in the local population of Alpine ibex in the Bargy massif (46°N, 6.5°E; elevation: 600-2 348 m; area: ca. 7 000 ha). *Brucella* genotyping confirmed that the same strains infected the dairy herd and the ibex, and that it was closely related to the strain observed during the 1999 cattle outbreak (Mick *et al.*, 2014). It was thus hypothesized that *B. melitensis* spilled over from domestic animals to ibex before disease eradication in the domestic compartment, and probably spilled back from ibex to cattle recently (ANSES, 2015). This long-term persistence and high prevalence had never been observed in any Alpine ibex population before.

2.1.2. THE ALPINE IBEX POPULATION

The Alpine ibex (*Capra ibex*) is a ruminant belonging to the Bovidae family and Caprinae subfamily (order Artiodactyla). The Alpine ibex presents a high sexual dimorphism in body and horn sizes. The adult male Alpine ibex is 150 cm long, measures 93 cm (at the shoulder) and weighs 67-117 kg, against 132 cm, 81 cm and 35-50 kg for adult females (Parrini *et al.*, 2009). Maximum length of horns can reach 100 cm in males and 35 cm in females (Parrini *et al.*, 2009). The horns grow throughout life, and age can be determined by counting horn growth annuli (Michallet *et al.*, 1988).

In the 19th century, the Alpine ibex nearly faced extinction, mainly due to hunting overexploitation and poaching. A single population remained in the Gran Paradiso (north-western Italian Alps). To protect this population against poaching, a royal hunting reserve was created in 1854 and then a National Park in 1922. From this population, multiple reintroductions in the 20th century allowed managers to re-establish Alpine ibex populations across the Alps (Couturier, 1962; Gauthier and Villaret, 1990; Parrini *et al.*, 2009). The number of Alpine ibex is now above 20 000 individuals in the Alps range, and the species is not considered as endangered anymore (Parrini *et al.*, 2009). In France, it remains a protected species and cannot be hunted.

The population of concern for brucellosis occupies the Bargy massif, an alpine massif located 46°N, 6.5°E, and well delimited by valleys (Figure 2-1). The adjacent areas (Aravis and Sous-Dine massifs – Figure 2-1) were also studied for brucellosis at the beginning of the study period. As a single seropositive result was obtained out of 60 Alpine ibex in the Aravis population and none in the Sous-Dine population (n=30 ibex), it was considered that the Bargy area is the sole area of concern (ANSES, 2015).

The population of Alpine ibex of the Bargy massif was reintroduced during two release events in 1974 and 1976, comprising a total of 14 released individuals (Gauthier and Villaret, 1990). Between 1982 and 1999, several population censuses were conducted to evaluate the growth of the population (ANSES, 2015). However, the population was not the object of any monitoring between 1999 and 2012 (ANSES, 2015).

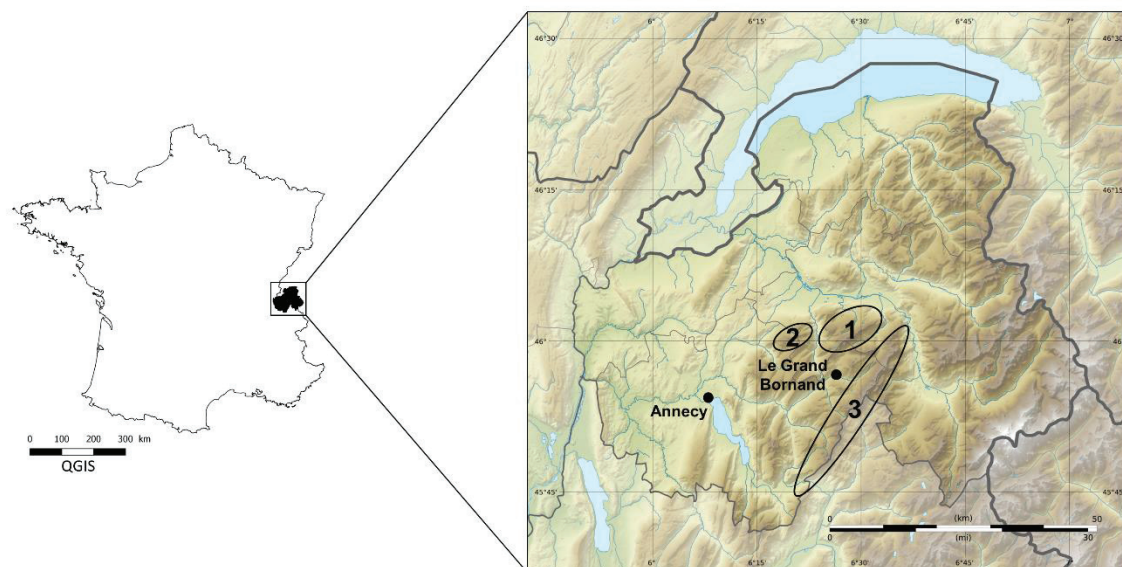


FIGURE 2-1 | Location of the Alpine ibex populations of the Bargy Massif (1) and adjacent areas (2: Sous-Dine Massif, 3: Aravis Massif) in the Haute-Savoie department (French administrative division – black area) and in France.

Right map: adapted from Flappiefh, commons.wikimedia.org [Accessed October 01, 2019], licence CC-BY-SA-4.0.

Since 2012, when ibex were discovered to be infected by Brucellosis, the population has been closely monitored by Capture-Mark-Recapture (Marchand *et al.*, 2017), allowing annual estimates of the Alpine ibex pre-breeding population size. Population size was estimated at 567 individuals in 2013 (95% Confidence Interval: [487-660]) in 2013, 310 [275-352] in 2014, 277 [220-351] in 2015, 272 [241-312] in 2016 and 291 [262-327] in 2017 (Marchand *et al.*, 2017; C. Toïgo, unpubl. data).

The monitoring by Capture-Mark-Recapture is detailed in the following section, as well as the disease management operations conducted in parallel. I also present the diagnostic tests that were performed on the animals to determined their health status.

2.1.3. DATA COLLECTION

2.1.3.1. *Capture-mark-recapture and disease management*

Between October 2012 and June 2018, nine Capture-Mark-Recapture (CMR) operations were conducted, six during spring (one per year) and three during autumn (in 2012, 2015 and 2016). A total of 387 captures and recaptures were performed (2012: 24, 2013: 57, 2014: 71, 2015: 125, 2016: 35, 2017: 27 and 2018: 48), concerning 319 different individuals. Captured animals were blood-sampled by trained technical staff and serological tests were performed on serum samples (see 2.1.3.2 for serological methods). For each individual, sex, age (by counting horn growth annuli, Michallet *et al.*, 1988), horn length and spatial location of capture were systematically recorded. Carcasses of seropositive animals were collected for necropsy whenever possible. Among marked and released ibex, 51 females and 39 males were equipped with Global Positioning System (GPS) collars, collecting hourly locations and providing information on space use and movements of animals (Marchand *et al.*, 2017).

Test-and-Remove (TR) operations were implemented on the basis of the results of the serological tests as part of management measures decided by the French Authorities. Seronegative individuals were marked and released while seropositive ones were euthanized. In 2014, a rapid serological test was validated for its use in the field, which facilitated TR operations since 2015. In addition to CMR monitoring and TR operations, culling operations were also implemented as part of the disease management strategy conducted by the French Authorities. In spring 2013, 5 individuals showing clinical signs (e.g., lameness or presence of visible gross lesions of the joints or the testes) were culled. In autumn 2013 and spring 2014, 233 and 18 individuals estimated to be older than 5 years were culled, based on the observation that seroprevalence was significantly higher for this age class (Hars *et al.*, 2013). Non-selective culling of individuals that were never captured was also performed in autumn 2015 (n=70), autumn 2017 (n=5) and spring 2018 (n=5). Non-captured individuals were considered as having a higher risk of being seropositive than previously captured individuals (which were all seronegative when marked and released). The same serological tests were performed on culled and captured animals when blood samples of good quality were available. Because of limitations in terms of time and costs, not all animals culled since the discovery of the outbreak were necropsied, but carcasses were collected and analysed whenever possible.

The diagnostic tests for brucellosis infection realised on blood samples and necropsied carcasses of captured and culled animals are detailed in the following section.

2.1.3.2. *Diagnosis of B. melitensis infection*

a. Serological tests

Although the only diagnosis of certainty is bacterial isolation and identification, serological tests are suitable for use for surveillance and disease management (FAO and WHO, 1986). The Rose Bengal Test (RBT), the Complement Fixation Test (CFT) and the Enzyme Linked ImmunoSorbent Assay (ELISA) methods are the most widely used tests (European Commission, 2001; Godfroid *et al.*, 2010) and are recognised by the OIE for serological diagnosis of brucellosis in domestic ruminants (OIE, 2016). All these tests can usually detect antibodies two to four weeks after infection (European Commission, 2001).

In wildlife, classical tests such as the RBT and the CFT are recommended (OIE, 2016). However, both tests require the use of high quality serum samples that are not necessarily easy to obtain in wildlife. It is therefore recommended to also use indirect ELISA (iELISA) and competitive ELISA (cELISA), that can be used with poor quality and haemolysed sera (OIE, 2016).

All serological tests have limitations (OIE, 2016), and the serial or parallel use of two or more tests is recommended (Godfroid *et al.*, 2004), especially for test-and-remove programmes (European Commission, 2001). In domestic ruminants, the RBT is the standard screening test for brucellosis and the CFT is widely recognized as the confirmatory test for the serological detection of infected animals (European Commission, 2001; Godfroid *et al.*, 2004, 2010). The RBT is very sensitive, while the CFT has a lower sensitivity but a very good specificity. The iELISA have greater or equal sensitivity than RBT or CFT in domestic ruminants, but lower specificity, and cELISA is more specific than iELISA, but less sensitive than iELISA or RBT (Table 2-1 – Godfroid *et al.*, 2010; OIE, 2016).

In Alpine ibex, all four tests were performed in parallel on serum samples. Individuals were considered as seropositive when at least two of the tests were positive. In 2012-2014, individuals were shot after the results of the serological tests were obtained in the laboratory. Since 2015, ibex were euthanized when they were positive to a rapid Lateral Flow

TABLE 2-1 | Diagnostic sensitivity and specificity of serological tests for the diagnosis of *B. abortus* and *B. melitensis* in domestic ruminants.

Test	Sensitivity (%)	Specificity (%)
RBT	92.1-100	100
CFT	81.9-97.6	99.4-100
iELISA	91.7-100	97-99.8
cELISA	75-100	97.9-99.9

Adapted from the systematic review of Ducrotoy et al. (2018) for bovine brucellosis, with additional values for brucellosis of sheep and goats. References: Ducrotoy et al., 2018 and references therein; Blasco et al., 1994a,b; Ferreira et al., 2003; Nielsen et al., 2004.

Immune-chromatographic Assay (LFIA) available for direct use in the field, before confirmation by the other tests in the laboratory. The LFIA had been validated on ibex samples by the EU/OIE/FAO and National reference laboratory, showing very good correlation with the other four tests (ANSES, 2014; Corde *et al.*, 2014).

Positive results to these serological tests (RBT, CFT, ELISAs) suggest past exposure to *Brucella*; however, they do not necessarily mean that the animal is actively infected at the time of sampling, i.e., is neither latently infected nor recovered (Godfroid *et al.*, 2010). The humoral immune response remains detectable over long periods of time (Godfroid *et al.*, 2010), even when the animals cleared the infection, and therefore seropositive results do not necessarily represent the true health status of individuals. However, high levels of antibody titres measured by the standard tests have been associated to active infection in pregnant domestic ruminants (Durán-Ferrer *et al.*, 2004), and *Brucella* isolation in cows was directly correlated with CFT titre (Huber and Nicoletti, 1986). Therefore, measuring antibody titres may be a way to distinguish between actively infected, possibly contagious individuals, and inactive infections.

Moreover, seropositivity does not indicate which species was involved, and false positive results due to cross-reactions with *Yersinia enterocolitica* O9 have been reported (European Commission, 2001). Therefore, if brucellosis is suspected because of positive serological results, isolation and identification of the bacteria should be performed (Godfroid *et al.*, 2010). Similarly, serological tests cannot differentiate vaccinated from naturally infected animals, but molecular typing of isolated *Brucella* vaccine from wild strains (Godfroid *et al.*, 2010).

b. Bacteriological tests

The isolation and identification of *Brucella* is the only diagnosis of certainty for brucellosis infection (Alton *et al.*, 1988; OIE, 2016). Once colonies showing morphological and microbiological characteristics of *Brucella* have been isolated from biological samples, definitive identification of the strains (species and biovar) using biotyping methods is required (OIE, 2016). These biotyping techniques follow standardized procedures (Alton *et al.*, 1988; OIE, 2016) and are performed by reference laboratories (Godfroid *et al.*, 2010).

Appropriate samples are: vaginal secretions, semen, colostrum/milk, aborted fetuses (stomach contents, spleen and lung), placentas, fluids collected from arthritis and hygromas, genital organs, mammary gland, spleen and various lymph nodes, in particular the supramammary lymph nodes (OIE, 2016).

Bacterial culture is a specific method but its sensitivity varies according to the bacterial loads, nature and number of samples (European Commission, 2001). Molecular methods such as PCR have been developed, but up to now, the sensitivity of these approaches remain lower than bacterial cultures (Godfroid *et al.*, 2010, 2013; OIE, 2016). However, the ability of PCR techniques to detect the DNA in samples with dead or very few live bacteria could improve diagnostic sensitivity if used in combination with bacterial culture (European Commission, 2001; Godfroid *et al.*, 2010). Molecular methods are also useful for identification and typing of *Brucella* strains (Godfroid *et al.*, 2010; OIE, 2016).

In ibex, adequate tissues were sampled during necropsy of seropositive carcasses. They were analysed by culture, performed by a local laboratory officially authorized to provide brucellosis diagnosis (biosafety level 3 facility): the veterinary laboratory of the Savoie department (LDAV 73). Biotyping of the isolated *Brucella* strains was performed at the French National Reference Laboratory for animal brucellosis (ANSES). All methods were performed according to OIE standards, as described previously (Alton *et al.*, 1988; Mick *et al.*, 2014; OIE, 2016).

2.1.4. AN HETEROGENEOUS SYSTEM

In the general introduction of the thesis, we discussed how heterogeneity influences the dynamics of infections from the individual to populations or communities, and the need to take into account such heterogeneities for understanding transmission and designing disease

management strategies (Chapter 1, 1.4). Both brucellosis and Alpine ibex are good candidates for the existence of such heterogeneities. First, brucellosis in domestic and wild ruminants is typically associated with abortions and genital shedding around gestation in females, and displays patterns of age-related bacterial shedding (Chapter 1, 1.2). This will be further investigated in the case of brucellosis in ibex as part of this thesis (Chapter 3). Second, ibex is a highly socially structured species, with differences in terms of mating behaviour, social-behaviour and space use between sex- and age-classes, that could lead to varying contact rates and transmission heterogeneity. In this section, I therefore describe these ecological characteristics of ibex and how they can interact with brucellosis transmission.

2.1.4.1. *Behavioural heterogeneity*

a. Mating behaviour

The mating period lasts from the beginning of December to mid-January (Couturier, 1962; Gauthier *et al.*, 1991; Willisch and Neuhaus, 2009). During this period, adult males and females aggregate. Most females are sexually receptive only once during the mating period, and for a brief period of time (around two days – Couturier, 1962; Willisch and Neuhaus, 2009).

When a female comes in oestrus, males approach the female depending on their rank status. In general, one dominant male adopts the tending tactic, which consists in consistently following the female and keeping exclusive access by defending her against other males (Willisch and Neuhaus, 2009). Subordinate males adopt another tactic, called coursing, which consists in waiting at a distance from the female and her tending male, and trying to bypass the tending male to copulate with the female when she moves or runs away from the tending male (Willisch and Neuhaus, 2009).

The adoption of one tactic over the other is strongly related to age: older males (≥ 8 years) are more often dominant and adopting the tending tactic, whereas younger males (2-7 years) are subordinates and adopt the coursing tactic (Willisch *et al.*, 2012). This age-related heterogeneity could be relevant for brucellosis transmission through the venereal route, as tending males are more successful in engaging in copulation with females (Willisch and Neuhaus, 2009; Willisch *et al.*, 2012).

After the female's oestrus, males leave the female to associate with another receptive female, where the courtship process can start over. Thus, contrary to females that are sexually receptive only once in most cases, males can copulate throughout the mating period. Males could therefore be qualitatively and quantitatively more important for the venereal route than females.

b. Social behaviour

Although the Alpine ibex is a gregarious species, social and spatial segregation occurs between sexes gradually after the mating period. Soon after the rut, old males over 6 years old and females segregate and use separate areas, whereas young males under 5 years of age segregate more gradually and stay in contact with females during spring (Villaret and Bon, 1995; Bon *et al.*, 2001).

In summer, all males equally segregate from females, except from yearlings (one-year-old) that can still be observed with females (Villaret and Bon, 1995; Bon *et al.*, 2001). Moreover, ibex females isolate in steep cliffs to give birth, and tend to remain alone with their newborn for about two weeks after parturition (Couturier, 1962; Villaret *et al.*, 1997; Bon *et al.*, 2001). Then, females and kids gather to form nurseries (Gauthier *et al.*, 1991). Males and females without kids are not in contact with the individuals in these nurseries, as they use separate areas (Grignolio *et al.*, 2007a,b). Whether females also isolate in steep cliffs when they abort is currently unknown, but could have strong implications for brucellosis transmission.

As a conclusion, males have varying probabilities of contact with females depending on their age and on the season. Transmission of brucellosis during pregnancy or around birth could therefore be higher between females than between males and females, and could decrease as male age increases.

2.1.4.2. *Spatial heterogeneity*

The last ecological characteristic of Alpine ibex that is expected to impact transmission is space use. Using GPS data on marked individuals collected since 2013 (see 2.1.3.1), a structuration in five socio-spatial units (Figure 2-2) was demonstrated in the population

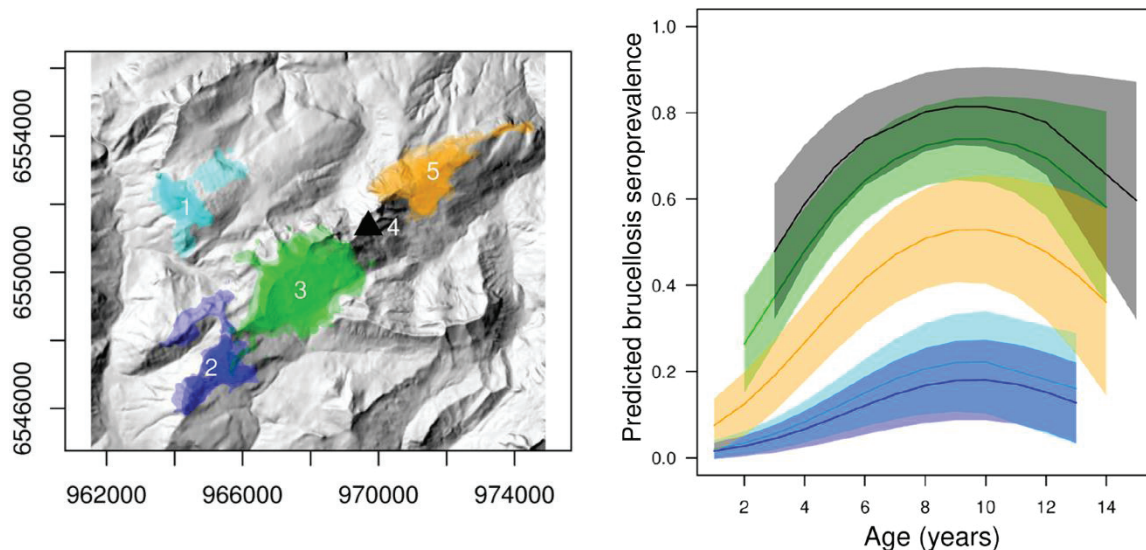


FIGURE 2-2 | The five socio-spatial units identified in the Alpine ibex (*Capra ibex*) population of the Bargy massif using GPS data (left) and model predictions describing the relationship between age, socio-spatial unit and brucellosis seroprevalence in females in 2012-2013.

The colours correspond to the different units identified using overlap between annual home ranges as a measure of distance between individuals: unit 1 is called “Leschaux-Andey” (light blue), unit 2 “Charmieux-Buclon” (dark blue), unit 3 “Jallouvre-Peyre” (green), unit 4 “Grand Bargy” (black) and unit 5 “Petit Bargy” (orange). Reproduced with kind permission of the authors from Figure 2 and Figure 4 in Marchand *et al.* (2017) (doi.org/10.1038/s41598-017-15803-w).

of the Bargy Massif by Marchand *et al.* (2017), with marked differences in space use between males and females. Indeed, females usually stay in their spatial unit, whereas males are more prone to move in the whole massif especially during the mating period (Marchand *et al.*, 2017). Marked differences in seroprevalence were recorded in individuals belonging to these five socio-spatial-units, ranging from <15% up to ~70%. Despite the very different movement pattern observed between males and females, these spatial variations of seroprevalence were observed in both sexes.

Such strong differences of seroprevalences between units could reveal heterogeneity of transmission at the spatial level. Host densities or movement patterns for example could create hotspots of transmission, and some spatial units could represent sources of infection to others.

2.1.5. HOW TO DEAL WITH HETEROGENEITY?

In order to understand brucellosis transmission dynamics in ibex and to evaluate possible management strategies, these potential sources of heterogeneity had to be taken into account. Statistical analyses of available data may provide insights on brucellosis transmission and the effects of past management strategies, but not on the predictions of the likely outcomes of various management strategies in similar conditions. To achieve this goal, mathematical models are required as they provide the opportunity to simulate several management interventions and compare their effectiveness (Smith *et al.*, 2009). Moreover, mathematical models may also increase the knowledge on the mechanisms and dynamics of the system. For instance, mechanisms generating heterogeneity of transmission at several levels can be integrated in such models, so as to explore their implications on epidemiological processes (VanderWaal and Ezenwa, 2016).

First results of modelling brucellosis in the study population indicated moderate effects of test-and-remove and vaccination strategies (6% of extinction probability with high capture rate, around 150 animals repeated each year during 5 years – ANSES, 2015; Thébault *et al.*, 2015). However, only few data were available at the time of this early work on mathematical model, and it did not take into account the spatial structure of the population and of the seroprevalence that was demonstrated later on by Marchand *et al.* (2016, 2017). Therefore, as new data and knowledge on the study system were becoming available, the development of a new mathematical model was required.

In the two following sections of this chapter, I will present mathematical models of infectious diseases, their principle and how they are developed, before explaining the successive steps that are classically followed in the modelling process, and the outcomes that can be expected from each step for understanding the past, current and future states of the system.

2.2. INFECTIOUS DISEASES MODELLING

2.2.1. DEFINITION OF A MODEL

A model is a simplified representation of a complex real-life system (Vynnycky and White, 2010). A mathematical model uses formal mathematical equations to precisely

describe the fundamental mechanisms that are supposed to drive the observed dynamics of the system (Keeling and Rohani, 2008). Starting from a set of initial conditions, these mathematical equations allow users to predict the state of the system at each time step, and to derive quantities such as the prevalence of infection or the population size, called model results or outputs, that can be compared to their real-life counterparts. Mathematical models can be solved by analytic methods, or by numerical simulations using computer software.

As simplified representations, there are no perfect models. Models reflect the actual knowledge of the system, and summarizes the mechanisms of interest to answer the research question (Ezanno *et al.*, 2012). Increasing the complexity of models by including additional biological details should improve the accuracy of the model, *i.e.* its ability to reproduce the observed dynamics. However, complexity also diminishes our understanding of how the different model components influence the dynamics, and is limited by available knowledge and data (Keeling and Rohani, 2008). Models should therefore aim at capturing the essential mechanisms necessary to improve our understanding of the system of interest, which does not requires perfect accuracy (Restif *et al.*, 2012).

2.2.2.SETTING UP MATHEMATICAL MODELS IN EPIDEMIOLOGY

2.2.2.1. Simple models

According to the natural history of the infection and of the host considered, to the research question and to the level of accuracy needed to answer it or to match the data, several choices of model structures are possible (Vynnycky and White, 2010). The basic structure of a model is based on the succession of health status of an individual, which is used to infer infection dynamics at the population level (Anderson and May, 1979; Ezanno *et al.*, 2012).

The simplest possible infection dynamics is when a susceptible individual becomes infectious for life once infected: it is the Susceptible-Infectious (*SI*) structure. Depending on the course of infection, other health status can be added such as the pre-infectious, *i.e.* when an individual has been infected but is not yet infectious, conventionally noted *E* for exposed. Another classical health status is *R* for recovered/immune, *i.e.*, when an individual stops being infectious but is not susceptible to infection anymore. Thus, various structures can be formulated, such as the simple *SIR* model used in the early work of Kermack and

McKendrick (1927) to describe an infection with no pre-infectious period and a lifelong immunity; or more complicated structures such as *SEIRS* model to represent the existence of a pre-infectious period and the possibility to return to the susceptible state (for instance because of changes in the circulating strains or because of immunity loss).

Two different types of models can be distinguished: compartmental models and individual-based models. For the former, each health status is considered as a compartment, where all individuals in the compartment are supposed to be identical. The model deals with the numbers of individuals in each compartment and the flows of individuals between compartments. For individual-based model, each individual is explicitly represented and the model deals with the transitions between health states of each single individual. In this case, the population level dynamics are obtained by summing the individuals in the same states at a given time. Individual-based models are used when individual specificities (such as age, sex, spatial location ...) that can significantly impact the infection dynamics are numerous and need to be taken into account. They also prove particularly useful when stochastic processes are important such as in small populations and for invasion or extinction processes (see 2.2.2.5).

The transition from the current state to the next depends on parameters, that are probabilities (risks) or rates depending on the choice of mathematical formulation. A simple example is the transition from *I* to *R*, whose associated parameter is the recovery rate, which can be simply defined as the inverse of the average duration of infectiousness (Vynnycky and White, 2010). If probabilities are needed, there is a mathematical relationship between risks and rates: $\text{risk} = 1 - e^{-\text{rate}}$ (Vynnycky and White, 2010).

How individuals get infection (i.e., how they leave the susceptible state) is the core of mathematical modelling of infectious diseases. It is defined by the force of infection (FOI – see also Chapter 1 section 1.3.2), i.e., the rate at which susceptible individuals becoming infected per unit time (Vynnycky and White, 2010). The FOI can be viewed as the product of three processes, c , ν , and p , where (i) c is the contact rate, i.e., the number of individuals that each (susceptible) individual contacts per unit time, where contacts are of an appropriate type for transmission to be possible if one of the hosts is infectious, (ii) ν is the probability of successful transmission, the probability that an appropriate contact between an infectious and a susceptible host does in fact lead to transmission, and (iii) p is the probability that a contact is indeed with an infectious host (Begon *et al.*, 2002).

Thus:

$$FOI = cvp$$

The probability of successful transmission v is usually assumed to be constant for a specific combination of host, pathogen and type of contact (Begon *et al.*, 2002). More realistic assumptions can be made, such as v depending on the pathogen load in the infectious individual and the dose acquired by the susceptible one (McCallum *et al.*, 2017).

The proportion of infectious individuals in the population, I/N , is usually used as the probability that a contact is with an infectious host (Begon *et al.*, 2002).

For the contact rate c , two main assumptions are usually used: frequency-dependence (FD) and density-dependence (DD). A FD contact rate is when a susceptible individual contacts the same number of individuals per unit time irrespective of the density of the population. This is how contact rate for sexually transmitted infections are represented, as contacts depend on the mating system of the host and usually do not depend on density (McCallum *et al.*, 2001). In this case, the FOI becomes: $\beta_{FD} \times \frac{I}{N}$, with β_{FD} the number of individuals effectively contacted by each individual per unit time (in t^{-1}), where an effective contact is a contact of appropriate type that successfully lead to transmission (Vynnycky and White, 2010). A DD contact rate is when the number of individuals contacted by each susceptible individual per unit time depends on the size of the population or its density, *i.e.* c is proportional to N . In this case, the FOI becomes: $\beta_{DD} \times I$, with $\beta_{DD} = \frac{cv}{N}$ the *per capita* rate at which two specific individuals come into effective contact per unit time (in $individual^{-1}.t^{-1}$) which does not depend on N (Vynnycky and White, 2010).

The choice between FD and DD contact rates depends not only on the infectious disease considered, but on the combination of its transmission route and the ecology and behaviour of the host species. These two representations may not represent the reality or may depend on the scale considered, and intermediate forms of contact rates between these two extremes have been proposed (McCallum *et al.*, 2001).

Simple models also assume (Anderson and May, 1979; Ezanno *et al.*, 2012): that all susceptible and infectious individuals have equal susceptibility and infectiousness; that all individuals are homogeneously mixed and therefore anyone can contact each other; that infection dynamics are short compared to population dynamics, which allows to neglect demographic processes such as births and deaths. Deterministic models also assume that

chance does not intervene in transmission, i.e., for two exactly identical populations at a given point in time, the exact same number of susceptible individuals would get the infection. As we will see in the following sections, these assumptions may not hold in every situations and may be refined according to the system studied and the needs of the research question.

2.2.2.2. *Individual heterogeneity*

As we saw in the introduction, all infectious individuals do not necessarily play the same role regarding transmission (see also Chapter 1 section 1.4.1). Variability of individual's susceptibility can also exist, for example due to individual characteristics (such as genetic resistance) or due to varying levels of existing immunity (due to previous exposure to the pathogen or cross-immunity generated by other infectious agents – Ezanno *et al.*, 2012). It is possible to model such heterogeneities, for instance by simply considering different categories of *S* and *I* individuals to adequately represent the existing variability (Keeling and Rohani, 2008; Ezanno *et al.*, 2012).

2.2.2.3. *Structured contacts*

Beside individual heterogeneity, heterogeneity of contacts can have profound implications on pathogen transmission. In most cases, individuals do not mix homogeneously in the whole population but are clustered in groups within which contacts occur more frequently than with the rest of the population (Keeling and Rohani, 2008). These groups can result from behavioural (for example linked to sexual behaviour), social (for example family groups) or spatial (for example subpopulations separated by landscape barriers) structuration.

In each case, within-group transmission by direct or indirect contacts occur more frequently than between-group transmission, that can be driven for example by individual movements (Jesse *et al.*, 2008). The structuration and the way groups are connected to each other can affect infectious disease invasion, spread and persistence in a population (Jesse *et al.*, 2008; Jesse and Heesterbeek, 2011), and are therefore important refinements of infectious diseases models.

TABLE 2-2 | Comparison of spatial model techniques reviewed in Keeling and Rohani (2008) and White *et al.* (2018b).

Model	Concept	Pros	Cons
Metapopulation	Subdivides the entire population into distinct subpopulations: - Within-unit independent dynamics - Between-unit transmission through aerial or vector-dispersal, permanent or punctual movements	Adapted to populations that are naturally subdivide into spatial units, with random interactions within groups. Simple models.	Ill-suited for heterogeneous contacts inside each subpopulation.
<u>Examples:</u> Fromont <i>et al.</i> , 1998 Fulford <i>et al.</i> , 2002 Swinton <i>et al.</i> , 1998			
Lattice	Uses a grid to partition the population in subpopulations (coupled-d-lattice) or in lone individuals (cellular automata). Uses square or hexagonal grid sites, with between-grid sites transmission only with the neighbouring sites.	Special forms of metapopulations. Adapted to populations approximately uniformly distributed, without clear subdivisions in spatial units, and to territorial species when one grid cell represents a vital area. Conceptual models that inform on non-random mixing.	Real populations seldom organised in grids. Ill-suited for long-distance interactions. Individuals within a grid have stronger contacts independently of their spatial location, whereas they may be closer to individuals from neighbouring sites.
<u>Examples:</u> Keeling and Gilligan, 2000 Highfield <i>et al.</i> , 2009 Rees <i>et al.</i> , 2013			
Continuous-space	Defines densities of individuals at all locations, without discretization. Individuals move at random through the entire space.	Avoid the discretization of space imposed by grids. Adapted to populations with dense distributions across the entire space. Theoretical models of spatial spread of infection.	Movements of individuals seldom truly random. Ill-suited for populations with low densities or with patchy distributions. Simple deterministic models only.
<u>Example:</u> Murray <i>et al.</i> , 1986			
Network	Describes each individual as a node in the network, and contacts between individuals as branches. Usually contacts are binary, either present or absent.	Adapted when individuals contact a small proportion of the population and contacts are highly heterogeneous. Simple representations of complex systems.	Require collection of high-resolution contact data that match the infection patterns (Craft, 2015). Ill-suited for homogeneous or random contacts.
<u>Examples:</u> Craft <i>et al.</i> , 2011 Drewe <i>et al.</i> , 2011 Hamede <i>et al.</i> , 2012			

Social and behavioural structures are often age and/or sex related, and a possible option for modelling is to subdivide each health status between different age and sex classes (e.g., Beaunée *et al.*, 2015). As regards spatial structuration, a variety of modelling techniques have been developed depending on the characteristics of the system (Table 2-2 – Keeling and Rohani, 2008; White *et al.*, 2018b).

2.2.2.4. *Population dynamics*

Neglecting population dynamics processes such as births and deaths is not possible when infection dynamics and population dynamics have similar durations, or when studying long-term dynamics of the system (Ezanno *et al.*, 2012). Population dynamics can have consequences on infection dynamics, by increasing (births) or decreasing (mortality) the number of susceptible individuals in the population. The other way around is also true, when infectious diseases impact fecundity or survival of the hosts.

Moreover, in wild populations, births and deaths can also be density-dependent, *i.e.*, depend on the population size (Bonenfant *et al.*, 2009). In this case, changes in population size (such as disease management strategies) can impact population dynamics and therefore infection dynamics (Choisy and Rohani, 2006 – see Chapter 1 section 1.5.2).

Another aspect of population dynamics that can affect transmission is seasonality, for example seasonal host social behaviour and contact rates or seasonal peak of births (Altizer *et al.*, 2006). These seasonal dynamics can be included in mathematical models and reproduce observed seasonal changes in incidence and prevalence (Beaunée *et al.*, 2015).

2.2.2.5. *Demographic stochasticity*

Demographic stochasticity can be defined as the variation in dynamics owing to the random nature of individual processes such as birth, death or pathogen transmission (Lloyd-Smith *et al.*, 2005a). These variations are small in large populations, and consequently models that do not include chance, called deterministic, are a good representation of the system. For small populations however, or when processes arise from a small fraction of individuals, which is favoured by individual heterogeneities or population structure, stochastic models represent the system better. Demographic stochasticity can drive population or pathogen extinction, which needs to be accounted for when trying to predict

epidemiological outcomes of pathogen invasion or evaluating the efficacy of disease management (Lambert *et al.*, 2018a).

2.3. WHAT CAN WE EXPECT FROM MATHEMATICAL MODELLING?

For this section, see also Appendix 1 (in French). Benefits that stem from mathematical models can be categorized as understanding and predictions (Keeling and Rohani, 2008). Three main steps of the modelling process are classically distinguished, each with their own benefits (Ezanno *et al.*, 2012):

- The first step is the construction of a formal and simplified representation of the system, which allows modellers to review available knowledge, to better understand the underlying mechanisms, to provide qualitative conclusions on the system dynamics and to present/discuss the modelling choices;
- The second step identifies the most important mechanisms in the system, by evaluating the parameters most influential on model results and estimating uncertain parameter values;
- While the two first steps permit a better understanding of the system, the last one is about predictions of its future evolution, after the model was validated using observations of past dynamics. This prediction step includes the evaluation of the relative efficacy of disease management strategies.

2.3.1. DESCRIBING AND REPRESENTING A COMPLEX SYSTEM

Building a mathematical model requires an in-depth review of the system under study, which enables to make a thorough assessment of available knowledge on the pathogen, the host population and interactions between the two. This review will enable to hypothesised which mechanisms are driving the observed dynamics of the system, and to provide a conceptual description of these mechanisms (Restif *et al.*, 2012). Relevant mechanisms include example (see also 2.2.2): the succession of health status at the individual level and

the transitions between them, such as transmission of infection or duration of infectious period; the transmission routes of the pathogen and their relative importance; the contacts between hosts that are appropriate for transmission given these transmission routes; the population dynamics mechanisms such as births and deaths.

Once the conceptual mechanistic model of the system has been described, it needs to be represented in a mathematical form, and then translated in a numerical form for simulation models. The fine description of the conceptual ecological and epidemiological model, and its translation into mathematical equations require a collaborative work between biologists (pathologists, epidemiologists, ecologists ...) and mathematical modellers (Restif *et al.*, 2012). On the one hand, biologists provide modellers with a list of mechanisms underlying the dynamics of observable variables, and check the appropriateness of the mathematical models' assumptions. On the other hand, modellers identify the parameters needed to run the model, and can help design experimental and/or field studies needed to inform these parameters (Restif *et al.*, 2012). When such studies are not an option, either because they are impractical or not feasible in a relevant time-scale, parameter values can also originate from scientific literature or experts' opinion.

At this stage of the modelling process, the dynamic patterns generated by the model should be compared with the qualitative features of observed data. If the model fails to reproduce the observed dynamics or to predict consistent levels of observed variables such as population size or prevalence of infection, it helps identify mechanisms that were missed or discarded when conceiving the conceptual model and need to be added to the model structure (Restif *et al.*, 2012).

2.3.2. VALIDATING AND RANKING THE MOST RELEVANT MECHANISMS

The second step of the modelling process is analytical. Among model parameters, some are documented in the scientific literature, while others need to be estimated, either by direct measurement using experimental or field studies, or by indirect inference by fitting the model to the observed data. The aim of this step is to identify the parameters that need to be estimated the most, so as to focus research on the estimation of these key parameters. This

step is essential to study the relative importance of the mechanisms involved in the system, and to be able to provide sound predictions in the last step of the modelling process.

The sensitivity analysis of a model aims at identifying the parameters that influence the most the variability of the model outputs, which should be estimated in priority, and conversely the parameters that influence the less the variability, which can be fixed without affecting model outputs (Cariboni *et al.*, 2007). Sensitivity analysis consists in varying the input parameters of the model in their range values (depending on mathematical properties and available biological knowledge) and evaluating how much model outputs are impacted by these variations. Various sensitivity analysis methods exist (Saltelli *et al.*, 2000). The simplest methods (called OAT for “One-At-a-Time”), measure the impact of a single parameter at a time, all the others being fixed (Cariboni *et al.*, 2007). Other methods estimate the global impact of a parameter when the others are also varying, with additional benefit compared to OAT being the quantification of interactions between parameters (Saltelli *et al.*, 2000). Most methods evaluate the sensitivity of each output separately; but recent developments enabled to evaluate the overall impact on several outputs at the same time (Lamboni *et al.*, 2009; Bidot *et al.*, 2017), giving insights on the broad effect of each parameter on the whole system.

The identification of key parameters using sensitivity analysis allows modellers to guide future experimental or field research that should focus on reducing the uncertainty around these parameters and therefore the variability of the model outputs; or to target these key parameters for inference by fitting the model to observed data (Cariboni *et al.*, 2007; Ezanno *et al.*, 2012).

Model fitting consists in simulating the model for varying parameter values, and selecting the parameter values that produced the simulated outputs that are closest to observed data. The results are then used to determine the most probable value of these key parameters. Such approaches have been used for many infectious diseases models (e.g., Bekara *et al.*, 2014; Brooks-Pollock *et al.*, 2014; Kosmala *et al.*, 2016). Among model fitting techniques, Approximate Bayesian Computation (ABC – Beaumont *et al.*, 2002; Beaumont, 2010; Csilléry *et al.*, 2010) is particularly relevant for complex infectious diseases models without tractable likelihood function (i.e., without the ability to directly calculate the probability of obtaining the observed data for each possible model parameterization – Hartig *et al.*, 2011). Indeed, ABC permits to approximate the likelihood by running many simulations of the model and comparing simulated outputs to the observed data (Toni *et al.*, 2009).

Matching model results with model data can provide better estimations of parameter values, reducing the uncertainty on model results and providing quantitative information on parameters that were previously unknown or very uncertain. Integrating this additional knowledge in the model can help comparing the relative importance of various mechanisms in the system. Moreover, alternative hypotheses around a specific mechanism can be tested using versions of the model that include these different hypotheses, all else being equal, and assessing which model provides the best fit to the available data (Ezanno *et al.*, 2012; Restif *et al.*, 2012). Both results (quantification and selection of the mechanisms that are the most supported by the available data) are important for the last step of the modelling process, that aims at making predictions of future states of the system based on observed dynamics to date.

2.3.3. EVALUATING DISEASE MANAGEMENT STRATEGIES

Among possible use of the model for prediction, the evaluation of disease management strategies, one of the major goal of this PhD thesis, is particularly useful as it allows to compare the efficacy of several scenarios, which is most often impractical in the field (Smith *et al.*, 2009). As management options are limited in wildlife (see Chapter 1 section 1.5.2), such an option is particularly relevant and can provide insights to decision makers, in addition to other aspects such as feasibility, cost-benefit analysis or acceptability by stakeholders and the general public.

The evaluation of the efficacy of various scenarios goes through the comparison of model outputs such as the evolution of the prevalence of infection or the probability that the pathogen will fade-out. For wild populations, the demographic impact of these management strategies is also relevant to account for in the efficacy evaluation, as the objective is not only to manage the infectious disease but also the infected population (Lambert *et al.*, 2018a – see also Chapter 1 section 1.5).

To conclude this section, it is necessary to underline the mutual inputs between mathematical models and the collect and analysis of biological data (Ezanno *et al.*, 2012; Restif *et al.*, 2012). The available data and existing biological knowledge allow modellers to represent the system and the underlying mechanisms, to estimate the parameters and to

validate the model. On the other hand, mathematical models can test biological hypotheses, compare different scenarios and guide future research by identifying gaps of knowledge that should be investigated in priority. For example, in the case of brucellosis in ibex, the first results of modelling were an incentive to perform an experimental study to determine if vaccinating ibex with the *B. melitensis* Rev. 1 vaccine commonly used in domestic ruminants was safe (Ponsart *et al.*, 2017; Ponsart *et al.*, in prep.). On the contrary, the new knowledge on the spatial structure of seroprevalence in the population raises new questions on disease management that can be answered by mathematical modelling.

Chapter 3

CHARACTERISING BRUCELLOSIS TRANSMISSION HETEROGENEITY



Personal picture

Foreword

Determining the routes of transmission and the categories of individuals that present the most infectiousness is of utmost importance for efficient disease management (Lloyd-Smith *et al.*, 2005b). To reach these goals, assessing the health status of individuals based on serological assays is not sufficient. Instead, it is necessary to assess within-individual pathogen distribution to infer a shedding pattern and individual heterogeneity in infectiousness (González-Barrío *et al.*, 2015).

Because the Alpine ibex population of the Bargy Massif was the very first case of self-sustained infection with *B. melitensis* in this species, knowledge of *Brucella* infection and pathogenesis was scant in ibex when the outbreak was discovered. Our first study in this chapter (3.1 – see also Appendix 2 in French) aimed to (i) identify possible transmission routes based on *Brucella* distribution in organs, (ii) determine the frequency of bacterial carriage in seropositive individuals and (iii) analyse the potential variations of excretion with sex and age.

Concerning transmission routes, we predicted that they would be the same as in domestic ruminants, namely direct or indirect horizontal transmission through *Brucella* shedding in genital secretions, venereal transmission, congenital transmission and pseudo-vertical transmission (Díaz-Aparicio, 2013). Therefore, we performed bacterial culture on the organs/samples that may be involved in these transmission routes (genital swabs, testes, genital tract, urine and/or bladder, udder, cotyledons, amniotic fluid and foetuses), as well as associated lymph nodes (e.g., supramammary, internal iliac and inguinal).

Because brucellosis was self-sustained in the ibex population, we made the prediction that a high proportion of seropositive individuals would be actively infected and at risk to shed the bacteria. We also hypothesised that high antibody titres measured with Complement Fixation Test would be correlated with active infection as in domestic ruminants (Huber and Nicoletti, 1986; Durán-Ferrer *et al.*, 2004), and that the probability of being actively infected would either be constant with age, indicating lifelong persistence of the infection as in goats, or decrease with age, indicating possible recovery from infection as in bison for example (European Commission, 2001; Treanor *et al.*, 2011). We tested these hypotheses using generalised linear mixed models.

3.1. High shedding potential and significant individual heterogeneity in naturally-infected Alpine ibex (*Capra ibex*) with *Brucella melitensis*

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ABSTRACT

Wildlife reservoirs of infectious diseases raise major management issues. In Europe, brucellosis has been eradicated in domestic ruminants from most countries and wild ruminants have not been considered important reservoirs so far. However, a high prevalence of *Brucella melitensis* infection has been recently identified in a French population of Alpine ibex (*Capra ibex*), after the emergence of brucellosis was confirmed in a dairy cattle farm and two human cases. This situation raised the need to identify the factors driving the persistence of *Brucella* infection at high prevalence levels in this ibex population. In the present paper, we studied the shedding pattern of *B. melitensis* in ibex from Bargy Massif, French Alps. Bacteriological examinations (1 to 15 tissues/samples per individual) were performed on 88 seropositive, supposedly infected and euthanized individuals. Among them, 51 (58%) showed at least one positive culture, including 45 ibex with at least one *Brucella* isolation from a urogenital sample or a lymph node in the pelvic area (active infection in organs in the pelvic area). Among these 45 ibex, 26 (30% of the total number of necropsied animals) showed at least one positive culture for a urogenital organ and were considered as being at risk of shedding the bacteria at the time of capture. We observed significant heterogeneity between sex-and-age classes: seropositive females were most at risk to excrete *Brucella* before the age of 5 years, possibly corresponding to abortion during the first pregnancy following infection such as reported in the domestic ruminants. The high shedding potential observed in young females may have contributed to the self-sustained maintenance of infection in this population, whereas males are supposed to play a role of transmission between spatial units through venereal transmission during mating. This heterogeneity in the shedding potential of seropositive individuals should be considered in the future to better evaluate management scenarios in this system as well as in others.

Keywords: *Brucella melitensis*; Alpine ibex (*Capra ibex*); wildlife disease; serology; bacteriology; pathogenesis; transmission; epidemiology

3.1.1. INTRODUCTION

The transmission of infectious diseases results from a complex interplay between the pathogen, the host and the environment, which generates highly variable dynamics at all scales from individuals to populations or communities (Tompkins *et al.*, 2011). In directly transmitted infectious diseases, heterogeneity of infectiousness has been neglected until some evidence of superspreading, *i.e.*, extreme heterogeneity, where the 20% most infectious individuals may be responsible for more than 80% of cases, has been found for example for the Severe Acute Respiratory Syndrome in humans (SARS, Galvani and May, 2005) or for *Escherichia coli* O157 in cattle (Matthews *et al.*, 2006). Generally speaking, heterogeneity increases the growth rate of outbreaks and the probability of stochastic extinction of the pathogen, and lowers the efficacy of control measures (Lloyd-Smith *et al.*, 2005b). Such heterogeneity may result from various factors, such as host age (Treanor *et al.*, 2011), sex (Silk *et al.*, 2018), immunity (Pathak *et al.*, 2010), behavior (Drewe, 2010), or genetic background (Borriello *et al.*, 2006).

Identifying the categories of individuals that are most responsible for disease transmission is a key towards targeting these individuals for efficient disease control (Matthews *et al.*, 2006). Assessing the health status of individuals, which is often based on serological assays in wildlife, is not sufficient to attain this goal. Instead, it is necessary to assess within-individual pathogen distribution to infer a shedding pattern and individual heterogeneity in infectiousness (González-Barrío *et al.*, 2015).

Here we deal with brucellosis, a major zoonosis that causes economic and public health issues worldwide. Infections with *Brucella abortus* or *Brucella melitensis* in ruminants mainly lead to late-term abortions and infertility, with substantial shedding of bacteria in the environment through genital fluids. Until recently, European wild ungulates were seen as dead-end hosts and had been thus considered as negligible hosts in Europe (Godfroid *et al.*, 2013). In particular, previous cases of *B. melitensis* in European wild mountain ruminants were localized events that spontaneously faded out (Garin-Bastuji *et al.*, 1990; Ferroglio *et al.*, 1998; Hars and Garin-Bastuji, 2013). However, an unprecedented high seroprevalence (38% in 2013) was reported in the Alpine ibex (*Capra ibex*) population of the Bargy area (French Alps) (Hars *et al.*, 2013; Garin-Bastuji *et al.*, 2014; Mick *et al.*, 2014). This unique situation constitutes the very first case of self-sustained infection with *B. melitensis* in wildlife in Europe. The public health and economic concerns, as well as the conservation

issues for this recently restored and protected species, raise many questions in terms of disease management in this population. Determining the drivers of pathogen persistence in this population, in order to further evaluate management strategies, is of the utmost importance. This unique situation has been investigated in-depth, through epidemiological surveys in local populations of ungulates, observation of contacts at the interface with domestic herds and bacterial typing (Mick *et al.*, 2014; Freycon, 2015; Marchand *et al.*, 2017). However, our knowledge of *Brucella* infection and pathogenesis is scant in this species, and cannot be completely extrapolated from knowledge of *B. melitensis* in domestic ruminants. Indeed, in studies on the wild reservoir of *B. abortus* in wildlife in the Greater Yellowstone Area (USA), some pathogenesis characteristics of *B. abortus* were similar between bison (*Bison bison*) and cattle, but specificities such as increased bison susceptibility were also demonstrated (Olsen and Johnson, 2011). Such specificities could also exist in wild Caprinae infected with *Brucella*, but similarities and differences are not currently known.

In the present study, we have addressed the distribution of *Brucella* in organs from seropositive, supposedly infected, ibex, and the consequences in terms of transmission pathways and potential heterogeneity in infectiousness at the individual level in naturally-infected Alpine ibex. Through the bacteriological examination of field or necropsy samples from seropositive ibex, we aimed to: (i) analyse the distribution of *Brucella* in different organs and estimate the frequency of bacterial carriage in seropositive individuals, (ii) identify the different transmission routes, and (iii) study the potential variation of excretion with sex and age. We provide here the first description of *B. melitensis* carriage and shedding in Alpine ibex and discuss the potential consequences of individual heterogeneity in terms of transmission, persistence and disease management in this population.

3.1.2. MATERIAL AND METHODS

3.1.2.1. Study area

Since no case of brucellosis had been reported in domestic ruminants since 2003, France had been declared officially free of ruminant brucellosis (caused by *B. abortus* or *B. melitensis*) by 2005 (Perrin *et al.*, 2016a,b). Nevertheless, in 2012, an outbreak due to

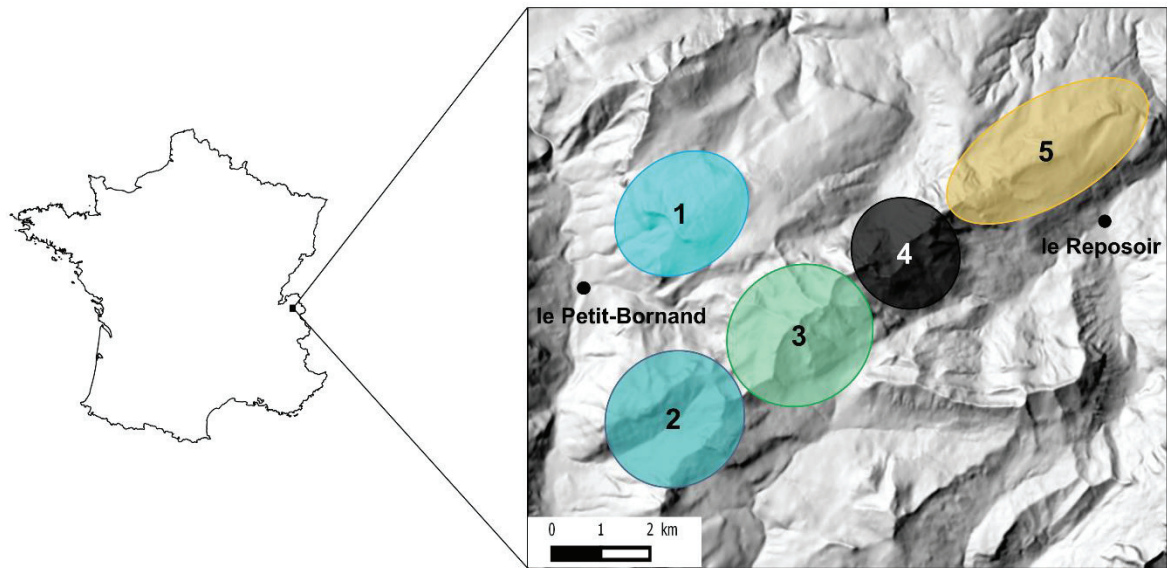


FIGURE 3.1-1 | Location of the study area in France and approximate localization of the five socio-spatial units identified in Marchand *et al.* (2017).

These five socio-spatial units correspond to the best number of spatially-segregated groups as determined by hierarchical classification methods on distances between individuals measured as overlap between annual home ranges of GPS-collared female Alpine ibex. See Marchand *et al.* (2017) for details.

B. melitensis biovar 3 occurred in a dairy herd in the northern French Alps (Bargy Massif, 46°N, 6.5°E; elevation: 600-2348 m; area: *ca.* 7000 ha) (Garin-Bastuji *et al.*, 2014). This re-emergence led to in-depth epidemiological surveys to find the origin of the outbreak. No potential source was identified in other domestic herds (not one infected domestic ruminant out of more than 12,000 tested), suggesting a possible implication of wildlife in this re-emergence. Indeed, an unprecedented high seroprevalence was reported in the Alpine ibex population living in the Bargy area, a remote mountain area. *Brucella* genotyping confirmed that the strain infecting the dairy herd and the ibex was the same, and closely related to the one observed in domestic livestock in 1999 during the last outbreak reported in domestic cattle in the area (Mick *et al.*, 2014). It was thus hypothesized that a probable spillover occurred from domestic animals to ibex in the 90's and a spillback from ibex to domestic cattle in 2011 (ANSES, 2015).

Alpine ibex were reintroduced in the Bargy Massif during two release events in 1974 and 1976 comprising a total of 14 released individuals (Gauthier and Villaret, 1990). Since the discovery of the outbreak in 2012, the Alpine ibex population of the Bargy Massif has been continuously monitored by Capture-Mark-Recapture (CMR). Because there were too few

physical recaptures to estimate the population size, data on visual recaptures (capture-mark-resight) were used instead. The population size without newborns was estimated at 567 (95% CI: [487-660]) in 2013, at 310 [275-352] in 2014 and at 277 [220-351] in 2015 (Marchand *et al.*, 2017). Using GPS data on marked individuals, Marchand *et al.* (2017) demonstrated that females are structured in 5 distinct socio-spatial units (Figure 3.1-1), whereas males were prone to move between units especially during the mating period. Noticeable differences in seroprevalence were recorded between these units: 2 units (1 and 2) showed seroprevalences <15%, whereas seroprevalences of the other three spatial units (3, 4 and 5) reached 54, 70 and 35% respectively (Marchand *et al.*, 2017).

3.1.2.2. *Sampling*

A total of 339 animals were captured or recaptured between 2012 and June 2017 (2012-2013: 81, 2014: 71, 2015: 125, 2016: 35 and 2017: 27). Most captures occurred between April and June, corresponding to the last third of pregnancy, on mature animals (≥ 2 years old). During captures, test-and-cull was implemented on the basis of serological tests (see below for serological methods) as part of management measures decided by the French Authorities (Hars *et al.*, 2013). Seronegative individuals were marked and released while seropositive ones were euthanized, and part of them were collected and necropsied in a biosafety level 3 (BSL-3) facility.

In addition to CMR monitoring and test-and-cull measures, the French Authorities implemented selective culling of animals with observed clinical signs (e.g., presence of visible gross lesions of the joints or the testes, lameness) or individuals older than five years, based on the observation that seroprevalence was significantly higher for this age class (Hars *et al.*, 2013). These selective culling operations occurred in spring 2013 (n=5), autumn 2013 (n=233) and spring 2014 (n=18). Non-selective culling on unmarked individuals, considered as having a higher risk of being seropositive than marked individuals (which were all seronegative when released), was also performed in autumn 2015 (n=70). The serological status of these animals was tested using the same assays as for captured animals, when a blood sample of good quality was available.

Carcasses of seropositive animals were collected for necropsy whenever possible, and sex, spatial location and age (estimated by counting horn growth rings, Michallet *et al.*, 1988), were recorded.

Ibex captures were performed by agents and researchers from the French Hunting and Wildlife Agency in accordance with legal and ethical regulations (French environmental code, 2005, 2006; Préfecture de Paris, 2009; Préfecture de la Haute-Savoie, 2013, 2015a,b; French Minister of Ecology, Sustainable Development and Energy, 2014).

Animals were captured by dart-gun xylazine-ketamine anesthesia (Rompun®, Bayer, Leverkusen, Germany and Imalgène®, Merial, France; 100 mg/individual) (Hars *et al.*, 2013; Marchand *et al.*, 2016, 2017). Seropositive ibex were shot (2012-2013) or euthanized by veterinarians using an embutramide intravenous injection (T61®, Intervet, Angers, France, 2014-2017).

3.1.2.3. *Serological analyses*

All captured animals were blood-sampled by trained technical staff. Four tests were performed in parallel on serum samples:

- The Rose Bengal Test (RBT) and the Complement Fixation Test (CFT), according to requirements of the European Union (EU) for diagnosis of brucellosis in small ruminants and following standards of the World Organisation for Animal Health (OIE). For CFT, a cut-off titer of 20 IU/mL was applied according to EU and OIE requirements (EU, 2008; OIE, 2016).
- The indirect Enzyme-Linked ImmunoSorbent Assay iELISA (Brucellosis Ovine/Caprine Ab Test, IDEXX, Montpellier, France) and the blocking cELISA (INgezim Brucella Compac 2.0, Ingenasa, Madrid, Spain).

In 2012-2014, individuals considered as seropositive on the basis of at least two of the four above-mentioned tests were shot after laboratory testing. In 2014, a rapid Lateral Flow Immune-chromatographic Assay (LFIA) (Rapid G.S. Brucella Ab test, Bionote, Gyeonggi-do, Rep. of Korea) had been validated on ibex samples by the EU/OIE/FAO and National reference laboratory (ANSES, 2014; Corde *et al.*, 2014). Results showed a very good correlation in laboratory conditions between the LFIA and the other four tests on serum samples from 2012-2013. Moreover, a trial in field conditions confirmed the reliability of the LFIA. Therefore, since 2015, ibex were euthanized when positive to LFIA, and their serostatus was further confirmed in the laboratory using the same four tests as in 2012-2014 and considered as seropositive when at least two of the laboratory tests were positive.

3.1.2.4. *Bacterial culture (for Brucella)*

For each necropsied animal, bacterial cultures were performed on 1 to 15 organs, as the list of organs selected for systematic bacteriological examination was only standardized in 2016. Statistical analyses were shaped to take into account this heterogeneity in the bacteriological examination between individual ibex (section Statistical analyses).

This list comprised organs that may have shedding potential (e.g., testes, genital tract, supramammary, internal iliac and inguinal lymph nodes, urine and/or bladder, udder), organs with lesions compatible with brucellosis (e.g., gross lesions of the joints or the testes, abscesses) and fetuses. Fetuses were analyzed independently from their mother on heart, kidneys, spleen, abomasum, liver and testes. Other organs (such as spleen, cotyledons and amniotic fluid) had been sampled on some ibex necropsied by 2013-2015, to better characterize *Brucella* distribution. Finally, genital swabs (either vaginal or preputial) were performed in the field (before necropsy) and were used for bacterial cultures in some individuals in 2013-2015.

Tissues were sampled during necropsy, and either directly analyzed by culture, or frozen for delayed culture. Cultures were performed by a local laboratory officially authorized to provide brucellosis diagnoses (BSL-3 facilities): the veterinary laboratory of the Savoie department (LDAV 73). Isolated *Brucella* strains were further bio-typed by the National Reference Laboratory for animal brucellosis (ANSES). All methods were performed according to OIE standards as described previously (Alton *et al.*, 1988; Mick *et al.*, 2014; OIE, 2016). Positive cultures were semi-quantitatively enumerated (1: less than 10 colony-forming units (CFU), 2: 10-50 CFU, 3: 50-100 CFU, 4: > 100 CFU). The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

3.1.2.5. *Definition of organ categories and ibex infection status*

We separated the analyzed organs into three categories in relation to their shedding potential:

- Urogenital organs (testes, genital tract, genital swab in the field, urine or bladder) can directly excrete the bacteria in the environment;

- Lymph nodes from the pelvic area (supramammary, internal iliac and inguinal lymph nodes), that drain the udder and the reproductive organs and can reflect a latent infection with potential further urogenital or milk excretion following recirculation of the bacteria;
- Other ‘entry’ or ‘closed’ organs, such as retropharyngeal lymph nodes, joints, visceral abscesses, which reflect the presence of the bacteria but with a much lower risk of excretion.

Since the risk of bacterial shedding varied according to the category of organ infected, we considered four infection statuses in ibex according to the previous organ categories and positive results from culture (Table 3.1-1). Individuals having at least one positive culture in any organ were considered as actively infected. Among them, animals being actively infected in organs in the pelvic area (at least one positive culture for organs in the pelvic area, i.e., urogenital samples or lymph nodes from the pelvic area) were considered as having a risk of potential shedding during present or future reproductive cycles. Finally, among them, individuals actively infected in urogenital organs (having at least one positive culture for the urogenital samples) were considered as having a risk of potential shedding at the time of capture. Individuals with negative bacterial cultures were called ‘bacteriologically unconfirmed’ but could have been misdiagnosed, since bacteriological examination is not a method with 100% diagnostic sensitivity.

TABLE 3.1-1 | Definition of infection classes for *Brucella melitensis* in Alpine ibex, depending on bacteriological results on tissue samples of the three categories of organ (+: at least one positive culture, -: all cultures negative).

Individual status		Urogenital samples	Lymph nodes in the pelvic area	Other organs
Bacteriologically confirmed (active infection in any organ)	Active infection in urogenital organs	+	+/-	+/-
	Active infection in organs in the pelvic area	-	+	+/-
		-	-	+
Bacteriologically unconfirmed		-	-	-

3.1.2.6. *Statistical analyses*

Statistical analyses were performed using the R software version R 3.4.1 R Core Development Team, 2017. We first intended to analyse the probability of an individual being actively infected in any organ, in organs in the pelvic area, or in urogenital organs (i.e., fetuses were not included in these analyses). However, the number of bacterial cultures varied among individuals, thus the probability of finding at least one positive culture in an ibex depended on the number of cultures assayed. To take into account this unequal number of samples between individuals, we used grouped binary data for each individual, where ‘successes’ and ‘failures’ were the numbers of positive and negative bacteriological results, respectively. The dependent variable was thus the probability of observing a positive bacteriological result on a panel of examined organs, using the number of organs tested per individual as weights. Therefore, we analyzed these data using a generalized linear model with a binomial distribution (Zuur *et al.*, 2009), and in order to account for random/cluster effects in our dataset, we used a mixed model with `glmer` from the *lme4* R package, with the spatial unit and the year fitted as random effects (Bates *et al.*, 2015). The binomial generalized linear mixed model expresses the log of the odds of the probability of a bacterial culture being positive as a linear function of the explanatory variables. For each explanatory variable, the odds ratio (OR) was therefore expressed as the exponential of the associated model coefficient (Dohoo *et al.*, 2009).

Three models were adjusted to analyse the probability of a bacterial culture being positive in (i) any organ, (ii) organs in the pelvic area, or (iii) urogenital organs. According to its capture or culling location, each individual was assigned to one of the five socio-spatial units structuring the population previously identified by Marchand *et al.* (2017). To control for possible variation in the epidemiological background among these socio-spatial units, we considered them as a random effect (ZONE) and we excluded from analyses one individual for which the capture location was missing. Because of small sample size, we also merged individuals belonging to units 1 and 2, since both have similar low seroprevalences. We tested for the influence of sex (SEX) and age (continuous variable in years, AGE) as fixed effects. We tested the influence of the CFT titer as a fixed effect (TITER) as a proxy for active infection, because high levels of circulating antibodies have been linked to active *Brucella* infection in the domestic species (Durán-Ferrer *et al.*, 2004). As longitudinal studies in Alpine ibex infected by *Brucella* are not easily conceivable, we could not test this hypothesis, but we assumed that this was also the case in this species. We excluded one

individual with a negative CFT (<20 UI/mL), and six animals on which CFT was not performed. We also tested for the period of sampling as a fixed effect (before/after the mass culling performed in autumn 2013: PERIOD). Indeed sampling biases were likely in 2012-2013 (captures focusing on animals with the most obvious clinical signs or gross lesions - Freycon et al., 2017). We also used the year of capture as a random effect (YEAR) to control for possible sampling biases and varying epidemiological situations among years (within periods), that could lead to varying shedding patterns. Finally, we tested whether freezing of samples/tissues would decrease the sensitivity of subsequent bacterial cultures by decreasing the amount of *Brucella*. To this end, we also considered sample treatment as a fixed effect (fresh or frozen: TREATMENT). We also tested for the effect of first-order interactions between sex, age and CFT because the course of *Brucella* infection may differ between males and females (Zuk and McKean, 1996).

The semi-quantitative assessment of bacterial load also allowed us to analyse the variations in *Brucella* carriage among organs, to test whether *Brucella* preferentially infected specific organs and whether this preference varied with age or sex. This expected heterogeneity would have direct implications on the shedding potential, as we assume that the higher the bacteria load in organs, the higher the shedding probability in associated regions (genital, inguinal, mammary) or fluids (urogenital secretions, semen, fetal fluids). After examining the variable distribution, we used a GLMM with a negative binomial distribution (`glmmadmb` function from the *glmmADMB* R package, Fournier *et al.*, 2012; Skaug *et al.*, 2013), where the response variable was the semi-quantitative assessment of the number of CFU per plate for each tissue sample. Since several samples originated from the same individual, we used individual identity (ID) as a random effect. We included the same fixed additive effects as before and added the organ category to evaluate potential differences in *Brucella* distribution in the organism (ORGAN). We also tested the first order interactions between sex, age, CFT titer and organ category in order to assess the variation over time of *Brucella* distribution in each sex.

For all analyses, after adjusting the complete model, we compared all possible sub-models using the dredge function (*MuMin* R package, Kamil, 2016). Model selection was performed using the Akaike Information Criterion corrected for small sample size (AIC_c), keeping models for which the difference between their AIC_c value and the lowest AIC_c value (ΔAIC_c) was less than 2 (Burnham and Anderson, 2002). For each candidate model, we examined the Akaike weights (W), which are relative model likelihoods normalized over the likelihoods

of all possible sub-models. A weight can be interpreted as the probability for a candidate of being the best model given the data and the set of possible sub-models (Wagenmakers and Farrell, 2004). We chose the final model based on the number of parameters, following the principle of parsimony: we chose the model with the fewest parameters from the set of ‘best models’ and when two models had the same number of parameters, we chose the model with the highest Akaike weight.

On the final model, we verified that random effects were normally distributed, and checked adequacy of the model with residual plots. The amounts of variability explained by the fixed and random effects of generalized linear mixed models were respectively estimated using marginal and conditional R^2 of Nakagawa and Schielzeth (2013).

3.1.3. RESULTS

3.1.3.1. Collected data

Bacterial cultures were performed on 88 seropositive individuals slaughtered between October 2012 and June 2017: 56 females aged from 2 to 14 (pregnant: 11, non-pregnant: 42, unknown: 3) and 32 males aged from 2 to 15. The sampling was heterogeneous among spatial units (Units 1-2: 6, Unit 3: 41, Unit 4: 23, Unit 5: 17, unknown: 1) and over time (2012: 11, 2013: 20, 2014: 30, 2015: 16, 2016: 5, 2017: 6). Among the 88 individuals, 81 were necropsied at the LDAV73, whereas only genital swab and blood sampling was performed on the seven remaining animals. Most individuals were captured or culled in spring during the last third of gestation (43 females, 28 males), and a few were culled or captured in autumn before the mating period (13 females, 4 males).

Among the 88 seropositive ibex, one was negative with RBT and CFT (<20 UI/mL) but was positive on LFIA, iELISA and cELISA; one was negative in RBT but positive on LFIA, CFT, iELISA and cELISA; one was negative in iELISA but positive on LFIA, RBT, CFT and cELISA; and six individuals culled in 2015 were only tested by LFIA because of small blood samples; all six LFIA tests were positive. The 79 remaining ibex were positive to all tests performed and did not show inconsistency between tests. Overall, CFT titer decreased with age in both males and females (data not shown).

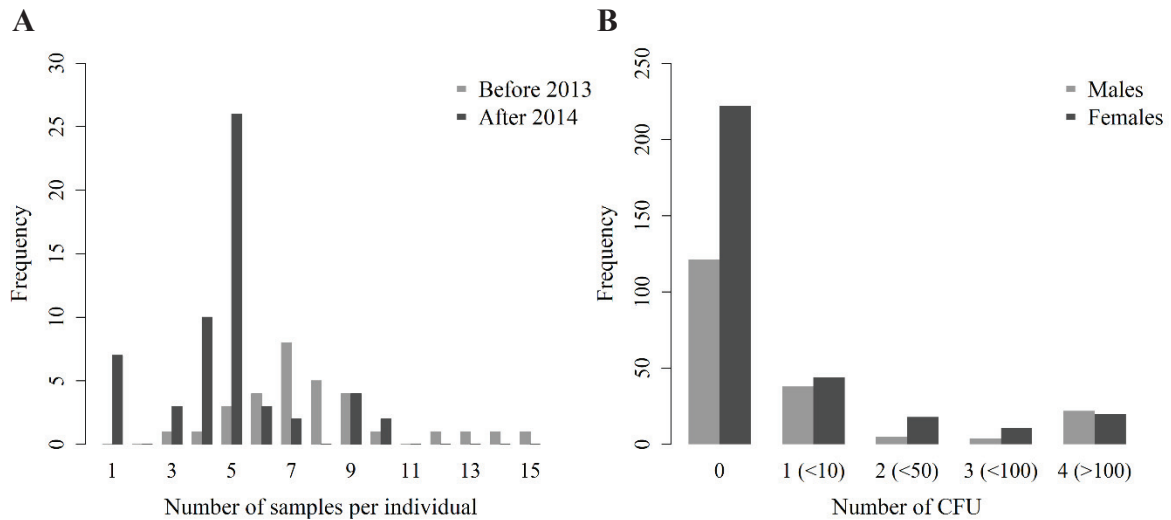


FIGURE 3.1-2 | Distribution of the number of samples per individual, before and after the culling implemented in 2013 (**A**) and number of CFU (Colony Forming Units) per plate for males and females (**B**).

TABLE 3.1-2 | Number of bacterial cultures performed in Alpine ibex between October 2012 and June 2017 in search for *Brucella melitensis*, and number of positive cultures for each tissue sample of three categories of organs.

Organ category	Tissue sample	Females	Males
Urogenital samples	Genital swab (in-the-field)	6/36 (17%)	5/23 (22%)
	Genital tract	9/41 (22%)	5/11 (45%)
	Urine or bladder	3/16 (19%)	5/15 (33%)
	Testes	-	12/32 (37%)
Lymph nodes in the pelvic area	Supramammary lymph nodes	17/48 (35%)	-
	Inguinal lymph nodes	1/1	6/19 (32%)
	Internal iliac lymph nodes	18/50 (36%)	12/29 (41%)
Other organs	Retropharyngeal lymph nodes	14/47 (30%)	12/28 (43%)
	Joints	11/19 (58%)	12/21 (57%)
	Abscesses	6/6	3/4
	Other organs	14/57 (25%)	2/13 (15%)
Total		99/321 (31%)	74/195 (38%)

One to 15 samples were cultured per individual, leading to 516 bacteriological results from 88 animals. Percentages of positive cultures are indicated in brackets except when the sample size was <10.

A total of 516 bacteriological results were obtained from these 88 animals. The number of analyzed organs per individual ranged from 1 to 15 (mean: 5.9, 95% CI: [5.3-6.4] – Figure 3.1-2A). In 2012-2013, the number of analyzed samples per individual was higher (7.8 [6.8-8.8]) than during the following years (4.8 [4.2-5.4]). Out of these 516 bacterial cultures, 173 tissues/samples, were positive (1 to 9 positive tissues/samples per individual, 2.0 on average [1.4-2.5]). The number of analyzed organs and the number of positive results are shown in detail in Table 3.1-2. Positive abscesses and positive samples from other organs came from the spleen (n=6), udder (5), other lymph nodes (5), lungs (4), nuchal ligament (2), C3/C4 cervical vertebrae (1), cotyledons (1) and mesentery (1).

The semi-quantitative evaluation of the number of CFU was available for all but 11 positive culture results. These 11 positive cultures were therefore excluded from the model analyzing the number of CFU in each organ. Among the 505 remaining tissues/samples, the mean semi-quantitative evaluation of the number of CFU was 0.6 in females (95% CI: [0.5-0.7]) and 0.8 in males ([0.6-1.0] – Figure 3.1-2B). In positive tissues/samples, the mean semi-quantitative number of CFU was 2.1 ([1.9-2.3]), meaning that there were between 10 and 50 CFU on average.

When gross lesions of the joints were present, associated bacterial culture of the joint fluid was positive in 17 cases, negative in 15 cases. On the other hand, six bacterial cultures were positive and two negative for joints without gross lesions. Bacterial cultures of joints with gross lesions were not significantly more often positive than those of joints without gross lesions (Fisher's exact test, p=0.428). Similarly, when gross lesions of the testes were present, bacterial culture was positive in six cases and negative in three. In the absence of gross lesions, six bacterial cultures were positive and 15 were negative. To conclude, cultures of testes in the presence of gross lesions were not significantly more often positive than in the absence of gross lesions (Fisher's exact test, p=0.102).

Bacteriological results were also obtained for three fetuses, two males and one female, for which four to six organs were analyzed: heart (n=2), kidneys (n=3), liver (n=3), spleen (n=2), abomasum (n=3) and testes (n=2). One of these fetuses was positive for all analyzed organs (heart, kidneys, liver, spleen and abomasum). Its mother was also positive for 8 out of 9 of the analyzed organs, including cotyledons (Table 3.1-3). The other two fetuses were negative for all analyzed organs, while their mothers were positive for 3 out of 10 and 1 out of 9 analyzed organs respectively. Cotyledons were negative for both, as well as for four other females analyzed in previous years.

TABLE 3.1-3 | Results of bacteriological detection of *Brucella melitensis* on three fetuses of Alpine ibex and their mothers in 2017.

	Organ category	Tissue sample	Foetus#1	Foetus#2	Foetus#3
Fetuses		Heart	4	-	0
		Kidneys	4	0	0
		Liver	1	0	0
		Spleen	4	-	0
		Abomasum	4	0	0
		Testes	-	0	0
	Total (positive/analyzed)			5/5	0/4
Mothers	Urogenital samples	Genital swab (in-the-field)	-	-	-
		Genital tract	-	-	-
		Urine or bladder	4	0	0
	Lymph nodes in the pelvic area	Supramammary lymph nodes	2	2	0
		Inguinal lymph nodes	-	-	-
		Internal iliac lymph nodes	4	1	0
	Other organs	Retropharyngeal lymph nodes	4	0	0
		Joints	0	0	0
		Abscesses	-	2 (udder)	-
		Spleen	4	0	0
		Lungs	4	0	0
		Cotyledons	4	0	0
		Superficial cervical (prescapular) lymph nodes	4	-	3
		Other	-	0	-
	Total (positive/analyzed)			8/9	3/10

0, negative bacterial culture; 1-4, positive bacterial culture (1: <10 CFU; 2: 10-50 CFU, 3: 50-100 CFU, 4: >100 CFU).

3.1.3.2. Bacterial distribution and shedding potential

Among the 88 seropositive animals (fetuses excluded), 37 (42%) were negative for all analyzed samples and thus infection was not bacteriologically confirmed. The remaining 51 (58%), which were positive for at least one sample, were considered as having active

infection. Among them, 45 were positive for at least one lymph node from the pelvic area (i.e., active infection in the pelvic area), including 26 positives for at least one urogenital sample (i.e., active infection in urogenital organs, Table 3.1-4). In seropositive females, the proportion of active infection in organs in the pelvic area was 45% (25/56), and 21% in urogenital organs (12/56). Among the 32 seropositive males, 20 (62%) were actively infected in organs in the pelvic area, and 14 (44%) were actively infected in urogenital organs (Table 3.1-4). When at least two organs were analyzed, the majority of bacteriologically confirmed ibex (active infection in any organ) had more than one positive sample (37/49 i.e. 76%, Table 3.1-4).

3.1.3.3. Probability of positive culture in a panel of organs

The model best fitting the probability of a bacterial culture being positive in any organ included the variables AGE, SEX, TITER and the interaction SEX:TITER. These variables were present in all models with $\Delta AICc < 2$ (Table 3.1-5).

TABLE 3.1-4 | Distribution of infected Alpine ibex according to classes established from the category of organs positive for *Brucella melitensis* and to the number of positive samples per individual.

Organ category	Positive samples	Bacteriologically unconfirmed		Active infection in any organ		Active infection in organs in the pelvic area		Active infection in urogenital organs	
		(n = 37)		(n = 51)		(n = 45)		(n = 26)	
		♀	♂	♀	♂	♀	♂	♀	♂
Urogenital samples	0	27	10	17	8	13	6	0	0
	1	-	-	7	9	7	9	7	9
	2+	-	-	5	5	5	5	5	5
LN in the pelvic area	0	27	10	6	9	2	7	2	7
	1	-	-	10	8	10	8	4	4
	2+	-	-	13	5	13	5	6	3
Other organs	0	27	10	7	7	7	7	4	3
	1	-	-	13	7	10	5	2	5
	2+	-	-	9	8	8	8	6	6

See Table 3.1-1 and text for definition of categories (bacteriologically unconfirmed, active infection in any organ/in organs in the pelvic area/in urogenital organs). Actively infected individuals in any organ include actively infected individuals in organs in the pelvic area and in urogenital organs.

TABLE 3.1-5 | Model selection table to analyse the probability of positive bacterial culture for any organ, for organs in the pelvic area, or for urogenital samples (random variables: ZONE and YEAR), and to analyse the number of CFU in each organ for females and for males (random variable: ID). The model selected is in bold.

Model	DF	LL	AICc	Δ	W
PROBABILITY OF POSITIVE BACTERIAL CULTURE FOR ANY ORGAN					
AGE+SEX×log(TITER)	7	-154.92	325.4	0.00	0.196
AGE+SEX×log(TITER)+AGE:log(TITER)	8	-154.05	326.1	0.73	0.136
AGE+SEX×log(TITER)+AGE:log(TITER)+TREATMENT	9	-153.01	326.6	1.20	0.107
AGE+SEX×log(TITER)+TREATMENT	8	-154.37	326.8	1.38	0.098
AGE+SEX×log(TITER)+PERIOD	8	-154.61	327.2	1.84	0.078
PROBABILITY OF POSITIVE BACTERIAL CULTURE FOR ORGANS IN THE PELVIC AREA					
AGE+SEX×log(TITER)	7	-112.66	240.9	0.00	0.271
AGE+SEX×log(TITER)+PERIOD	8	-112.27	242.6	1.71	0.115
AGE+SEX×log(TITER)+AGE:log(TITER)	8	-112.37	242.8	1.90	0.105
PROBABILITY OF POSITIVE BACTERIAL CULTURE FOR UROGENITAL ORGANS					
AGE+SEX×log(TITER)+TREATMENT	8	-69.01	156.1	0.00	0.124
AGE+SEX×log(TITER)+TREATMENT+PERIOD	9	-67.81	156.2	0.14	0.115
AGE+SEX×log(TITER)+TREATMENT+AGE:SEX	9	-68.43	157.4	1.37	0.062
AGE+SEX×log(TITER)+TREATMENT+PERIOD+AGE:SEX	10	-67.17	157.5	1.46	0.060
AGE+SEX×log(TITER)+AGE:SEX	8	-70.00	158.0	1.98	0.046
NUMBER OF CFU IN EACH ORGAN					
SEX×ORGAN+log(TITER)+SEX:log(TITER)+AGE	11	-477.79	978.2	0.00	0.071
SEX×ORGAN+log(TITER)×AGE+SEX:log(TITER)	12	-476.93	978.5	0.38	0.058
SEX×ORGAN+log(TITER)+SEX:log(TITER)	10	-479.10	978.7	0.53	0.054
SEX×ORGAN+log(TITER)+SEX:log(TITER)+AGE+AGE:SEX	12	-477.42	979.5	1.37	0.036
SEX×ORGAN+log(TITER)+SEX:log(TITER)+AGE+PERIOD	12	-477.52	979.7	1.56	0.032
SEX×ORGAN+log(TITER)×AGE+SEX:log(TITER) +PERIOD	13	-476.51	979.8	1.67	0.031
SEX×ORGAN+log(TITER)+SEX:log(TITER)+AGE+TREATMENT	12	-477.65	980.0	1.82	0.028
SEX×ORGAN+log(TITER)×AGE+SEX:log(TITER)+TREATMENT	13	-476.63	980.1	1.91	0.027

For each candidate model, the table gives the fixed variables (see text for definition), the number of Degrees of Freedom (DF), the Log-Likelihood (LL), the Akaike Information Criterion corrected for small sample size (AICc), $\Delta AICc < 2$ (Δ), and the Akaike Weight (W, see text for definition).

The probability of a bacterial culture being positive decreased significantly with increasing age (odds ratio $OR_{\text{per year}} = 0.85$, 95% CI: [0.77-0.93] – Table 3.1-6 and Figure 3.1-3A), increased with increasing TITER for females ($OR_{\text{per 1 unit in log(TITER)}} = 2.71$ [1.84-3.97]) but not for males ($OR_{\text{per 1 unit in log(TITER)}} = 0.83$ [0.45-1.52] – Table 3.1-6 and Figure 3.1-3B), and tended to be higher for males than for females ($OR_{\text{males vs females}} = 1.55$ [0.97-2.47]). It is important to note that, infection being generally active in young individuals, the CFT titer decreased with age ($OR_{\text{per year}} = 0.85$ [0.79-0.91], $p < 0.001$). Consequently, a confounding effect was possible between the effects of AGE and TITER, however both variables still appeared in the selected model.

The AGE:TITER interaction ($OR = 1.07$ [0.97-1.17]), the TREATMENT variable ($OR_{\text{fresh vs frozen}} = 1.37$ [0.76-2.47]) and the PERIOD variable ($OR_{2012-2013 \text{ vs } 2014-2017} = 1.79$ [0.43-7.51]) were also present in some of the selected models based on the AICc, but they did not significantly improve the model-data fit and were thus not retained in the most parsimonious model (Table 3.1-5).

Regarding the probabilities of positive culture in pelvic area organs and of positive culture in urogenital organs, both models retained the variables AGE, SEX, TITER, and the interaction SEX:TITER, and the results were qualitatively and quantitatively the same as before (Tables 3.1-5 and Table 3.1-6, Figure 3.1-3C-D-E-F). Unlike the other two, the best model for the probability of a bacterial culture being positive in urogenital organs also included the variable TREATMENT, which was present in all models with $\Delta AICc < 2$ but one. Fresh samples had higher probabilities of being culture-positive than frozen ones ($OR = 3.24$ [1.11-9.43]). The interaction AGE:SEX ($OR = 1.31$ [0.94-1.81]) was also present in some models with $\Delta AICc < 2$ (Table 3.1-5) but was not retained in the final model.

3.1.3.4. *Brucella carriage in organs*

Regarding bacteria carriage on the organ level, the final model included the SEX, ORGAN, TITER variables, and the SEX:ORGAN and SEX:TITER interactions. These variables were present in all models with $\Delta AICc < 2$ (Table 3.1-5). The average bacterial load was significantly higher in males than in females ($OR = 3.69$ [1.51-9.03]). In females, the urogenital organs had lower bacterial loads compared to lymph nodes from the pelvic area ($OR = 2.33$ [1.32-4.11] compared to urogenital organs) or to other organs

TABLE 3.1-6 | Parameters of selected models to explain the probability of positive bacterial culture for any organ, for organs in the pelvic area, or for urogenital samples, and to explain the number of CFU in each organ.

Response variable	Explanatory variable and modality	OR and 95% confidence interval	P-value of Wald test
Probability of positive bacterial culture for any organ	AGE	0.85 [0.77-0.93]	<0.001
	SEX (males)	1.55 [0.97-2.47]	0.066
	log(TITER)	2.71 [1.84-3.97]	<0.001
	SEX:log(TITER)	0.31 [0.19-0.49]	<0.001
Probability of positive bacterial culture for organs in the pelvic area	AGE	0.84 [0.74-0.95]	0.004
	SEX (males)	1.49 [0.82-2.70]	0.189
	log(CFT)	2.82 [1.70-4.67]	<0.001
	SEX:log(TITER)	0.27 [0.14-0.51]	<0.001
Probability of positive bacterial culture for urogenital samples	AGE	0.80 [0.66-0.97]	0.025
	SEX (males)	1.62 [0.65-4.05]	0.304
	log(TITER)	2.87 [1.30-6.30]	0.009
	SEX:log(TITER)	0.21 [0.08-0.57]	0.002
	TREATMENT (fresh)	3.24 [1.11-9.43]	0.031
Number of CFU in each organ	SEX (males)	3.69 [1.51-9.03]	0.004
	ORGAN (NLpelvic)	2.33 [1.32-4.11]	0.004
	ORGAN (others)	2.65 [1.53-4.59]	<0.001
	log(TITER)	2.37 [1.43-3.94]	<0.001
	SEX(males):ORGAN(NLpelvic)	0.45 [0.20-1.00]	0.050
	SEX(males):ORGAN(others)	0.33 [0.16-0.69]	0.003
	SEX:log(TITER)	0.39 [0.19-0.80]	0.010

The reference levels are females (for the variable SEX), frozen (for the variable TREATMENT) and urogenital (for the variable ORGAN).

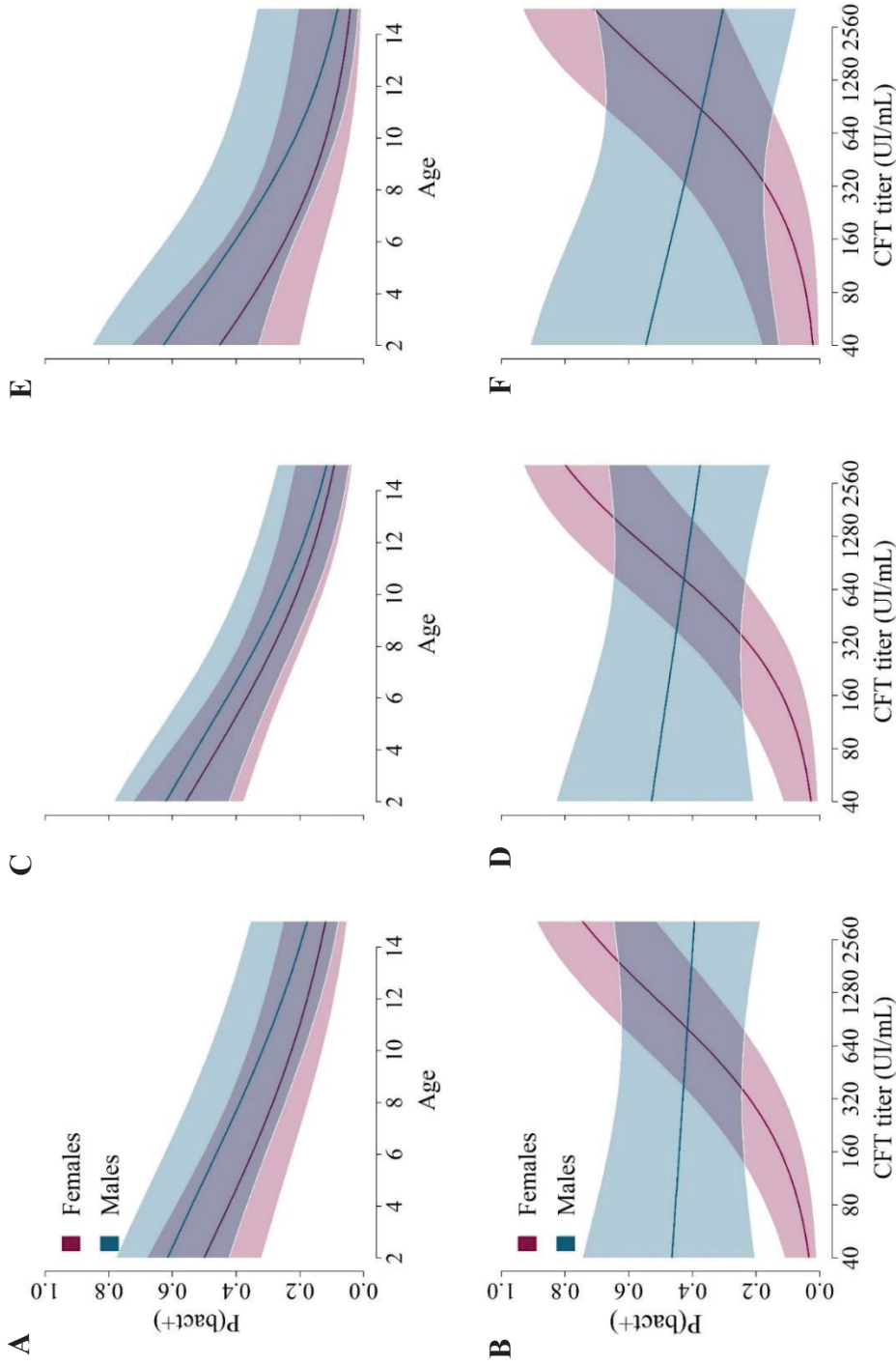


FIGURE 3.1-3 | Predictions from the best models describing the relationship between age/sex (A,C,E, on the top) or complement fixation titers/sex (B,D,F, on the bottom) and the predicted probability for ibex of belonging to a specific class:
 - bacteriologically confirmed (A, B, left: *Brucella* found in any organ)
 - active infection in organs in the pelvic area (C,D, middle: *Brucella* found in urogenital samples and/or lymph nodes in the pelvic area),
 - or active infection in urogenital organs (E,F, right: *Brucella* found in urogenital samples).

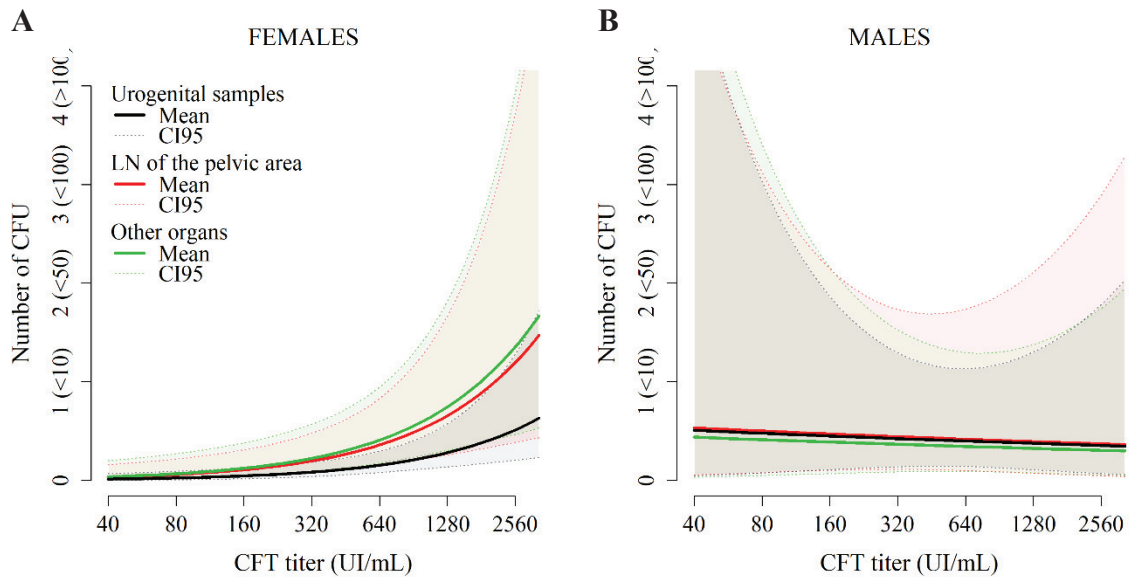


FIGURE 3.1-4 | Predictions from the model describing the number of CFU per plate for each organ in females **(A)** and in males **(B)** as a function of CFT titers and organ category.

(OR = 2.64 [1.53-4.59] compared to urogenital organs, Table 3.1-6 and Figure 3.1-4A). In males, bacterial loads were not significantly different between organ categories (OR = 1.05 [0.39-2.80] for lymph nodes from the pelvic area and OR = 0.86 [0.38-2.23] for other organs - Figure 3.1-4B). The bacterial load increased with increasing TITER for females (OR = 2.37 [1.43-3.94]) but not for males (OR = 0.92 [0.38-2.23]).

The AGE (OR_{per year} = 0.90 [0.79-1.02]), TREATMENT (OR_{fresh vs. frozen} = 1.23 [0.58-2.63]), PERIOD (OR_{2012-2013 vs. 2014-2017} = 1.38 [0.59-3.25]) variables and the AGE:SEX (OR = 0.89 [0.67-1.17]) and AGE:TITER (OR = 1.09 [0.96-1.24]) interactions were also present in models with low AICc values, but not in the most parsimonious model.

3.1.4.DISCUSSION

This study analyzes the largest dataset of bacteriological investigations on Alpine ibex infected by *Brucella* ever collected to date. Using semi-quantitative measurements of bacterial load in a large variety of organs allowed us to infer the pattern of bacteria shedding in infected animals as well as its variation among individuals, and to explore the level of active infection in seropositive individuals. In summary, the probability of bacteria presence

decreased with ibex age, in both males and females. For a given age, females with highest CFT titers exhibited the highest bacteria presence and load, a trend not observed in males. The average bacterial load was significantly lower in females than in males. Furthermore, in females, pathogen loads were lower in urogenital organs than in other organs, whereas no differences were observed between organ categories in males. We also reported the presence of *Brucella* at high bacterial load in fetuses in one out of three and in cotyledons of one out of seven necropsied females in the last third of pregnancy, confirming that pregnancy represents a particular at-risk situation for both horizontal and vertical transmission, as it does in domestic ruminant species.

Overall, our results revealed (i) a high probability of detecting *Brucella* in seropositive ibex (58% on average), (ii) a broad bacterial dissemination and high *Brucella* carriage both at individual and tissue/sample levels (2.0 infected tissues/samples per individual and around 10-50 CFU per infected tissues/samples on average) and (iii) a strong variation in the bacteriological carriage and shedding capacity according to ibex sex, age and serological status. In the following sections, we will discuss each of these results, each time highlighting their limitations and inferring on the epidemiology of the infection in Alpine ibex.

3.1.4.1. *Prevalence of bacterial detection*

We selected supposedly infected ibex based on serological test results. The diagnostic sensitivities of serological tests are good in domestic animals, i.e., up to 97% in cattle (Godfroid *et al.*, 2010, and references therein) and up to 100% in sheep and goats (European Commission, 2001, and references therein), but are still not well-defined in ibex. Even though the serological reaction of ibex seems strong and long-lasting, a lack of sensitivity cannot be excluded, which might represent a limit to our results by missing some infected animals, especially young sexually immature or old immunosenescent individuals. One possible option would have been to necropsy and perform bacterial cultures on seronegative individuals also, which was unfortunately not performed given many legal restrictions (mainly seropositive animals were euthanized), as well as practical and costs aspects (transportation of large carcasses from remote mountains areas, huge necropsy and bacteriological effort allowing only the analysis of some carcasses, therefore the choice was made to target seropositive individuals for investigating bacterial carriage). It should be noted, however, that six bacterial cultures were performed on four entirely seronegative

individuals from the same area, and that 17 bacterial cultures were performed on three found-dead animals on which serological tests could not be performed. All 23 bacterial cultures were negative (data not shown).

To detect active infection, we chose bacterial culture, the gold standard for brucellosis diagnosis (Alton *et al.*, 1988; OIE, 2016). We targeted organs that were described as the preferred tissues for bacteria isolation, i.e. retropharyngeal, supramammary, iliac and inguinal lymph nodes, as well as genital organs and vaginal secretions (Marín *et al.*, 1996; European Commission, 2001; Godfroid *et al.*, 2013). Because of obvious limitations in terms of time and costs, we were not able to necropsy all seropositive animals culled since the discovery of the outbreak, or perform bacterial cultures in all relevant organs from all individuals. In particular, the list of organs selected for systematic bacteriological examination was only standardized in 2016, which represents another limit of our study. In future studies, variability between samples would be reduced, if the standard protocol implemented since 2016 is still followed.

Brucella were not detected in 42% of seropositive individuals; these animals probably cleared the bacteria or had a low *Brucella* carriage in organs undetectable by the bacterial culture at the time of capture. Other techniques such as the quantitative polymerase chain reaction (qPCR) assay have been developed (Yu and Nielsen, 2010), but, up to now, there is no report of higher diagnostic sensitivity of a particular PCR method compared with bacterial culture (Godfroid *et al.*, 2013). In particular, PCR has been demonstrated as inaccurate to detect all culture-positive animals in bison (Treanor *et al.*, 2011). However, qPCR could have allowed us to improve diagnosis sensitivity, in particular if used in parallel with the bacterial culture, by detecting the DNA of the bacteria, even in samples with dead or very few live *Brucella*.

Even if the proportion of 58% of ibex with at least one positive bacterial result may be underestimated in our sample, this result remains high given the significance of the presence of live *Brucella* (including in terms of zoonotic risk). Proportion of animals with active infection in naturally-infected seropositive cattle was under 50% in several studies (e.g., 46% of 355 cattle in Harrington and Brown 1976, 49.2% of 2,570 cattle in Huber and Nicoletti 1986 or 47.6% of 21 cattle in O'Leary *et al.* 2006), as it was in wild bison (46% of 26 bison - Roffè *et al.*, 1999). In small domestic ruminants, proportions were more contrasted, with a proportion of 62.2% of actively infected animals among 45 naturally-

infected seropositive sheep (Ilhan *et al.*, 2008), whereas a lower proportion has been found in naturally-infected goats (1 out of 12 seropositive goats - Ribeiro *et al.*, 1990).

Our data supports a high shedding potential in ibex infected by *B. melitensis*, which may be a major factor of a self-sustained enzootic transmission. This could be explained by a high susceptibility of ibex to brucellosis. Specific differences in susceptibility to brucellosis have already been demonstrated between bison and cattle, with bison being more susceptible to the disease and getting infections in fetuses, the uterus or mammary glands more often (Olsen and Johnson, 2011). Moreover, the genetic diversity of reintroduced Alpine ibex populations is typically low, which can affect susceptibility to infection (Biebach and Keller, 2010), and this could explain, at least in part, the high susceptibility of the Alpine ibex population of the Bargy Massif to *B. melitensis*. In the future, experimental studies aiming at determining the relative susceptibility of ibex as compared to domestic species and other wild species such as Alpine chamois (*Rupicapra rupicapra*) would be interesting.

3.1.4.2. *Multiple transmission routes*

Several transmission routes have been demonstrated for brucellosis in domestic animals: horizontal direct or indirect transmission through genital secretions, aborted fetuses or products of live births (placenta, fetal fluids and vaginal exudate) from infected mothers, vertical transmission *in utero* or during birth, pseudo-vertical transmission through the consumption of colostrum or milk, and venereal transmission (Diaz-Aparicio, 2013).

Here, our data supported possible horizontal transmission through urogenital secretions, as we found positive bacterial cultures in genital swabs, genital tracts and urine/bladders of seropositive Alpine ibex of both sexes.

We did not test the semen or epididymis of males, but positive bacterial cultures of testes observed in 12/32 (37%) males with more than 100 CFU per plate for five individuals support the ability of males to transmit the infection through venereal transmission. In seropositive naturally-infected bulls, Hill (1983) found positive bacterial cultures of testes in 2/17 (12%) of animals. Shedding in the semen is not believed to be a major mode of transmission in domestic animals (King, 1940; FAO and WHO, 1986) but spread of the infection through artificial insemination has been reported (Bendixen and Blom, 1947; Manthei *et al.*, 1950). In the American bison (*Bison bison*), low amounts of bacteria in the semen outside the mating period did not support this transmission route, but it cannot be

excluded that the amount of bacteria could increase during the mating period (Frey *et al.*, 2013). Here, we observed high loads of *Brucella* in testicular tissues outside the mating period, so it is highly probable that it will also be the case during the rut, possibly allowing sexual transmission.

We also provided the very first indication of possible vertical transmission of *B. melitensis* in Alpine ibex, with one positive foetus out of the three fetuses analyzed. Besides, our results support a possible transmission from females to newborns through the consumption of colostrum or milk. *B. melitensis* was isolated from the udder of five females, and from 35% of supramammary lymph nodes (68% in actively infected females), whose infection is related to the presence of brucellae in milk in livestock (Philippon *et al.*, 1971). Our results are similar to those found in naturally-infected and actively infected cattle, in which Corner *et al.* (1987) found 70% of positive supramammary lymph nodes. As most captures occurred during the last third of pregnancy, i.e. before the lactating period, we were not able to sample milk in the field or during necropsy. Any opportunity to sample milk in culled or captured females in the future should be seized to evaluate milk shedding.

3.1.4.3. *Shedding potential among seropositive animals*

Thirty percent of the seropositive Alpine ibex we studied carried the bacteria in their urogenital organs or secretions and thus represented a potential source of bacteria for other ibex and susceptible species during the reproductive cycle at the time of capture. The proportion of active infections in urogenital organs was probably underestimated in the present study, due to the few fetuses and placentas analyzed. Moreover, our models reveal that frozen urogenital samples had a significantly lower probability of being culture-positive than fresh ones. The same trend was present but not significant in other organs. Thus, freezing carcasses before necropsy allows for a practical and efficient analysis process, but at the price of a slightly lower sensitivity of bacterial culture. qPCR could have allowed to improve the detection of the bacteria in urogenital organs, but it would not necessarily give us any further information on bacterial shedding as it could also detect DNA of dead bacteria, i.e., without any consequences on transmission.

Moreover, more than half were positive either in genital tract or in lymph nodes from the pelvic area, i.e., could be at-risk of potential shedding during future reproductive cycles. Indeed, the bacteria can persist in regional lymph nodes (Fensterbank, 1987) and be

reactivated on favorable occasions (i.e., estrus, pregnancy, immunosuppression) with shedding in genital secretions and milk (European Commission, 2001).

The strong variability of bacteriological results among seropositive individuals illustrates the heterogeneity of the host-pathogen relationship, and underlines the limits of serological investigations to infer the transmission dynamics of pathogens (González-Barrio *et al.*, 2015).

An important result of our study is that the probability of positive bacterial culture and bacterial carriage was at the highest level for the youngest animals in our sample (i.e., 2 years old) and then decreased with age, as described in wild bison (Treanor *et al.*, 2011).

The biology of infection in ibex under two years of age remains unknown, as our sample did not include individuals under two because these were seldom captured and always seronegative (only five individuals under 2 captured between 2012 and 2017, and all were negative to LFIA, RBT and CFT). However, one cannot exclude that young ibex individuals may play an unrecognized role in brucellosis dynamics since they might get infected early in life, either through transmission from infected females to viable foetus or through the colostrum or the infected environment after birth, and become shedders once sexually mature. In the future, depending on the management measures implemented to control this outbreak, analyzing young ibex might allow us to confirm that sexually immature animals may experience latent (undetected) infection, showing no or mild serological response until their sexual maturity or first gestation, as demonstrated in cattle with *B. abortus* (Plommet *et al.*, 1973; Lapraik *et al.*, 1975) or small ruminants with *B. melitensis* (Renoux, 1962; Grilló *et al.*, 1997).

In females, the probability of positive bacterial culture and bacterial carriage was highest for young animals and high CFT titers. Because CFT titer is also higher in young individuals, confounding effects could be expected between age and CFT titer; however, both variables were actually selected in the final model. In domestic ruminants, it is well-known that infection with *B. melitensis* in primiparous females often leads to abortion and subsequent *Brucella* shedding during the last third of the pregnancy (Carvalho Neta *et al.*, 2010; Godfroid *et al.*, 2013). Besides, high shedding was associated with high CFT titers: around abortion or births, CFT titers peaked and reached high values in ewes that shed brucellae, whereas they were notably low in ewes that did not shed the bacteria (Durán-Ferrer *et al.*, 2004). Sexual maturity in the Alpine ibex is reached at the earliest at the age of 1.5 years in

females (Couturier, 1962), with first access to reproduction ranging between 1.5 and 3.5 years depending on individuals and population density (Gauthier *et al.*, 1991). Therefore, the highest probability of bacterial shedding and the highest bacterial load in female ibex could correspond to the first pregnancy event. Most animals were captured between April and June, i.e., during the last third of pregnancy, thus in a period of potential abortion and shedding in female ibex (Gauthier *et al.*, 1991). Our results thus suggest the probability of bacterial shedding should be at the highest in primiparous females in relation to abortion, and should decrease during subsequent pregnancies, as observed in wild bison (Treanor *et al.*, 2011) or in domestic ruminants (Tittarelli *et al.*, 2005). Such a scenario might explain the decreasing probability of positive bacterial culture as age increases. Unfortunately, too few pregnant females were integrated in our sample to properly test a relationship between pregnancy status and *Brucella* shedding. Additionally, shedding in old females could have been underestimated, because too few captures occurred during the birth period, when shedding occurs in vaginal discharges and birth products. In contrast, evaluation of shedding in young females was probably more accurate, as captures occurred during the abortion period.

Interestingly, the probability of bacterial shedding and the bacterial load did not vary with CFT titers in males, i.e., males with low CFT titers could carry high amounts of *Brucella*. However, here most captures occurred in April-June. The behavior of *Brucella* shedding in sexually active males during the mating period (end of November to January) remains unknown. Active shedding with high titers of antibodies might happen then, in a similar way as females around abortion or birth. Season of sampling (April-June vs October-November) could also have influenced the bacterial load; unfortunately, too few animals were sampled in autumn to allow us to explore the effect of season on our results.

Males generally had higher probability of positive culture and higher bacterial load than females (Figure 3.1-3). However, it is unlikely that males play a more important role than females, as this result needs to be put in perspective with the possible transmission routes. Indeed, males, even with high bacterial loads, could not be responsible for disease transmission outside the mating period, as their only possible route of transmission is venereal transmission, each contact infecting a single female. In contrast, females can contaminate many susceptible individuals through indirect transmission following abortion or births.

Finally, we failed to detect any change in this shedding pattern before and after a mass culling had been performed in this ibex population, thus the selective captures performed before 2013 did not result in any obvious change in the bacterial shedding pattern.

3.1.4.4. *Two different epidemiological roles for males and females?*

Our results on shedding and previous results on the spatial behavior and contact patterns of Alpine ibex in the study population (Marchand *et al.*, 2017) support two different epidemiological roles for males and females. We argue that on the one hand, females play a role of maintenance of the infection inside each spatial unit of the Bargy Massif, by shedding bacteria especially through abortion during the first gestation following infection and then through parturition following subsequent gestations. On the other hand, males of all ages tend to visit all subunits during the mating period (Marchand *et al.*, 2017) and thus could play a role of transmission between spatial units through venereal transmission. This kind of pattern was hypothesized for instance for Aujeszky's disease in wild boar, with venereal male-to-female transmission during mating and oro-nasal female-to-female transmission only outside mating (Ruiz-Fons *et al.*, 2007; González-Barrio *et al.*, 2015). This difference could be even more marked in our case, as the efficacy of female-to-female transmission through indirect transmission following abortion or birth is likely to be high, consequently playing a crucial role in maintaining the infection inside spatial units.

3.1.4.5. *Management options*

As highlighted throughout the discussion, the biology of infection remains unknown as regards young or seronegative ibex for instance. Besides, we used here a cross-sectional study, as it is not easily conceivable to use experimental infection for the study of brucellosis in Alpine ibex (for instance, it would require building BSL-3 facilities respecting the welfare of a wild caprine, which in addition is a protected species in France). Therefore, further studies are needed to better understand the infection dynamics in the whole population. However, our results provide a new insight in the infection epidemiology, which is critical for future development of control strategies. Indeed, heterogeneity due to age and sex can impact the efficacy of management methods such as culling based on the serological status

of individuals or groups, for example by skewing population age structure in favor of highly susceptible juveniles (Bolzoni *et al.*, 2007). In the present case, removing old animals might have contributed to limited efficacy of the mass culling performed in 2013 (focused on animals aged more than 5 years old) since older animals were not necessarily the ones shedding the most bacteria. In the Yellowstone area, test-and-cull strategies were indeed most successful at reducing seroprevalences in bison when targeting young seropositive females (Ebinger *et al.*, 2011). Modelling approaches, including the different kinds of data (on *Brucella* epidemiology and ibex demography) that have been collected in the field from 2012 onwards, will now be used to infer disease management principles that may be relevant in this and other cases.

Interlude

The results obtained in the first part of this chapter (3.1) are compatible with the existence of four possible transmission routes, as described in domestic ruminants: horizontal transmission through urogenital excretion, congenital transmission *in utero*, pseudo-vertical transmission through milk consumption, and venereal transmission. The relative importance of these various transmission routes, unknown in Alpine ibex, constituted the first aim of the second part of this chapter (3.2).

Among 88 seropositive ibex, 58% were positive for at least one sample, and were considered as having active infection. In *B. abortus* naturally-infected cattle, proportions of active infections in seropositive animals have been reported between 45 and 50%. Our data thus supported a high shedding potential in ibex infected by *B. melitensis*, which may be a major factor of a self-sustained enzootic transmission. The probability of positive bacterial culture and bacterial carriage was highest for high CFT titres according with our prediction, but only for females. Therefore, the level of CFT titres could be used to infer the infection status of seropositive females (see 4.1). Finally, an important result of our study is that the probability of positive bacteriological culture was at the highest level for the youngest animals in our sample (i.e., 2 years old) and then decreased with age in both genders, which could have major repercussions on disease management.

Our results on shedding and previous results on the spatial behavior and contact patterns of Alpine ibex in the study population (Marchand *et al.*, 2017) support two different epidemiological roles for males and females. On the one hand, we predicted that males, which are more prone to move across the whole study area than females (especially during the mating period), should mostly spread the infection between socio-spatial units through venereal transmission. On the other hand, females, which are more sedentary and usually stay within their spatial unit, should maintain the infection inside each socio-spatial unit through bacterial shedding following abortion or birth.

Understanding and quantifying these heterogeneities is a necessary preliminary step towards designing and evaluating disease management strategies for Alpine ibex. To achieve this goal as well as quantify the importance of the different transmission routes, we developed in the second part of this chapter (3.2) an individual-based and metapopulation model of *Brucella* transmission in the study population.

3.2. An individual-based model to assess the spatial and individual heterogeneity of *Brucella melitensis* transmission in Alpine ibex

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ABSTRACT

Heterogeneity of infectious disease transmission can be generated by individual differences in the frequency of contacts with susceptible individuals, in the ability to transmit the infectious agent or in the duration of infection, and by spatial variation in the distribution, density or movements of hosts. Identifying spatial and individual heterogeneity can help improving management strategies to eradicate or mitigate infectious diseases, by targeting the individuals or areas that are responsible for most transmissions. Individual-based models allow quantifying the respective role of these sources of heterogeneity by integrating potential mechanisms that generate heterogeneity and then by tracking transmissions caused by each infected individuals. In this study, we provide an individual-based model of the population of Alpine ibex (*Capra ibex*) of the Bargy massif (France) facing endemic brucellosis by taking advantage of detailed information available on ibex population dynamics, behaviour, and habitat use, and on epidemiological surveys. This host-pathogen system is expected to be subject of both individual and spatial heterogeneity. We first estimated the transmission probabilities, hitherto unknown, of the two main transmission routes of the infection (i.e., exposure to infectious births/abortions and venereal transmission). Then, we quantified heterogeneity at both individual and spatial levels. We found that both transmission routes are not negligible to explain the data, and that there is a high amount of heterogeneity of the host-pathogen system at the individual level, with females generating around 90% of the new cases of brucellosis infection. Males transmit infection at a lesser extent but still play a non-negligible role because they move between subpopulations and thereby create opportunities for spreading the infection spatially by venereal transmission. Two particular socio-spatial units are hotspots of transmission, and act as sources of transmission for the other units. These results may have important implications for disease management strategies.

Keywords: wildlife disease, epidemiology, disease ecology, metapopulation, superspreading, transmission routes

3.2.1. INTRODUCTION

Heterogeneity in the transmission of infectious agents occurs when a small proportion of an animal population is responsible for the majority of transmission, whereas the rest of the population only generates few secondary cases. The '20-80' rule emerged from empirical data for several sexually-transmitted and vector-borne diseases, where 20% of cases contributed to at least 80% of transmission (Woolhouse *et al.*, 1997). When comparing several directly-transmitted diseases, Lloyd-smith *et al.* (2005b) found support for this pattern, but nonetheless found evidence for high variation in the amount of heterogeneity, with 20% of cases contributing to 40% up to around 90% of transmission depending on the disease considered. Such substantial levels of heterogeneities are common in infectious diseases of both humans and animals (Galvani and May, 2005; Matthews *et al.*, 2006; Brooks-Pollock *et al.*, 2014).

Individuals responsible for the majority of transmission are referred to as “superspreaders” (Lloyd-Smith *et al.*, 2005b). Their high success in transmission of infectious agents is related either to a high contact rate with susceptible individuals, to a long duration of infection, or to an especially high infectiousness, i.e., ability to transmit the infectious agent (VanderWaal and Ezenwa, 2016). These three components of transmission can be influenced by various traits such as age, sex, behaviour, immunity or genetics (Cross *et al.*, 2009; VanderWaal and Ezenwa, 2016).

Heterogeneous transmission can also occur when areas with a particularly high pathogen prevalence or transmission rate are responsible for the transmission to other areas (Paull *et al.*, 2012). This spatial heterogeneity can arise from various environmental factors that promote the transmission or persistence of the disease, but also from the distribution, density and movement patterns of hosts (Conner and Miller, 2004). Spatial heterogeneity can also be related to individual heterogeneity, as superspreaders are likely to enhance transmission around them more than in other areas (Paull *et al.*, 2012). Identifying spatial and individual heterogeneity can help improving management strategies to eradicate or mitigate infectious diseases, by targeting the individuals or areas that are responsible for most transmissions (Woolhouse *et al.*, 1997; Lloyd-Smith *et al.*, 2005b).

In the present study, we built an individual-based model of *Brucella melitensis* transmission in the wild population of Alpine ibex (*Capra ibex*) of the Bargy massif in the French Alps, the first reported case of persistence of brucellosis in European wild ungulates.

France is officially free of Brucellosis in domestic ruminants, a major zoonosis due to *B. abortus* and *B. melitensis* that causes economic and public health issues worldwide, the last cases having been reported in 2003 (Perrin *et al.*, 2016a,b). However, in 2012-2013, a high seroprevalence was found in the Alpine ibex population of the Bargy massif (Hars *et al.*, 2013). *B. melitensis* probably spilled over from domestic animals to ibex before disease eradication in the domestic compartment, and probably spilled back from ibex to cattle (ANSES, 2015), and then from cattle to humans in 2012 (Mailles *et al.*, 2012).

The persistence of brucellosis in a wildlife population raises serious public health and economic concerns. Effective disease management options would therefore be desirable to mitigate the risk to livestock and humans. Moreover, brucellosis in Alpine ibex raises a conservation issue because ibex have been restored in the Alps during the last decades, after being close to extinction at the beginning of the 20th century, and is now protected. The sanitary management of brucellosis usually operated in domestic herds, which involves culling both infected and exposed individuals, is hardly applicable to a wildlife population, especially in the case of a species with a protection status. Targeting individuals or areas that are responsible for most transmissions allows sparing other individuals and could be a relevant option for a more effective and more ethically-acceptable management strategy to eradicate brucellosis.

This host-pathogen system is expected to be subject of both individual and spatial heterogeneity. First, five socio-spatial units were identified in the population, associated with marked differences in seroprevalence, ranging from <15% up to ~70% depending on the unit considered (Marchand *et al.*, 2017). Second, individual heterogeneity could arise from sex and age differences, as typically observed in vertebrate populations (e.g., Plard *et al.*, 2015). Indeed, four transmission routes have been demonstrated in domestic ruminants for brucellosis: horizontal transmission after abortion or parturition when *Brucella* is shed in genital fluids, venereal transmission, congenital transmission and pseudo-vertical transmission (Diaz-Aparicio, 2013). Bacteriological data on necropsied seropositive ibex suggest that the same four routes may also exist in ibex (Lambert *et al.*, 2018b). All routes relate to reproduction, and thus involve specific age and sex classes. In this highly socially-structured species, contact rates vary between sex- and age-classes because of differences in terms of social-behaviour (Bon *et al.*, 2001), space use (Marchand *et al.*, 2017) but also mating behaviour (Willisch and Neuhaus, 2009). Moreover, the proportion of active infection is higher in young individuals than in old ones (Lambert *et al.*, 2018b).

We had two main objectives when building our model: (i) to determine the relative importance of the various transmission routes, which is currently unknown in this species, and (ii) to quantify transmission heterogeneity among individuals, classes of individuals and socio-spatial units in the ibex Bargy population. Based on general knowledge on brucellosis transmission in livestock and wildlife (FAO and WHO, 1986; European Commission, 2001; Godfroid *et al.*, 2013) and on preliminary studies on the Bargy case study (Marchand *et al.*, 2017; Lambert *et al.*, 2018b), we formulated two predictions:

- First, due to their distinct patterns of space use, males and females should play different epidemiological roles. Males, which are more prone to move across the whole study area than females (especially during the mating period), should mostly spread the infection between socio-spatial units through venereal transmission, whereas females, which are more sedentary and usually stay within their spatial unit, should maintain the infection inside each socio-spatial unit through bacterial shedding following abortion or birth.
- Second, units with the highest seroprevalences, which also occupy central positions in the Massif, were the first areas colonised by ibex and correspond to the largest socio-spatial units. These socio-spatial units should act as sources of infection, whereas the more peripheral units with both lower seroprevalence and lower abundance, should be sinks.

Understanding and quantifying these heterogeneities is a necessary preliminary step towards designing and evaluating disease management strategies for Alpine ibex.

3.2.2. MATERIAL AND METHODS

3.2.2.1. Study site and population monitoring

Our model was built on the kinetics of the Alpine ibex population of the Bargy massif in the French Alps (46°N, 6.5°E; elevation: 600-2348 m; area: *ca.* 7000 ha). The brucellosis outbreak was detected in 2012, and since, the ibex population has been closely monitored by Capture-Mark-Recapture, allowing estimating the pre-breeding Alpine ibex population size to 567 individuals (95% Confidence Interval: [487-660]) in 2013, 310 [275-352] in 2014, 277 [220-351] in 2015, 272 [241-312] in 2016 and 291 [262-327] in 2017 (Marchand *et al.*, 2017; C. Toigo, unpubl. data).

During captures, Test-and-Remove (TR) operations were implemented on the basis of serological tests performed according to requirements of the European Union (EU) for diagnosis of brucellosis in small ruminants and following standards of the World Organisation for Animal Health (OIE) – see Marchand *et al.* (2017) and Lambert *et al.* (2018b) for details. In addition, a rapid serological test was used in the field to screen the sanitary status of captured animals during their handling, and take the decision of removal or release (ANSES, 2014; Corde *et al.*, 2014). Between 2012 and 2018, 319 individuals were captured at least once. Among them, 115 were seropositive and removed, while 204 were seronegative and were marked and released. Fifty marked individuals were recaptured, sometimes several times, and among them, four were found positive and then removed. For each individual, sex, age (by counting horn growth annuli, Michallet *et al.*, 1988) and spatial location of capture were systematically recorded.

Culling operations were also conducted as part of the disease management strategy conducted by the French Authorities. In autumn 2013 and spring 2014, respectively, 233 and 18 individuals older than 5 years were culled, as apparent seroprevalence was the highest for this age class (Hars *et al.*, 2013). In autumn 2015, autumn 2017 and spring 2018, 70, 5 and 5 unmarked individuals were culled respectively, because they were considered as having a higher risk of being seropositive than marked individuals (which were all seronegative when released).

Finally, among marked and released ibex, 51 females and 39 males were equipped with GPS collars. We recorded hourly locations that provided information on ibex space use and movements. In particular, the locations of 27 females, which were followed for at least six months, revealed a spatial structuration including five socio-spatial units – Figure 3.2-1A, see Marchand *et al.* (2017) for further details.

3.2.2.2. *Individual-based model*

We describe the individual-based model we built following the updated ODD protocol (“Overview”, “Design concepts”, “Details”) of Grimm *et al.* (2006, 2010). The third part, “Details”, is described concisely in the main text below, while the full description of the initialization of the model and of the complete system of mathematical equations are provided in Appendix 3.2A.

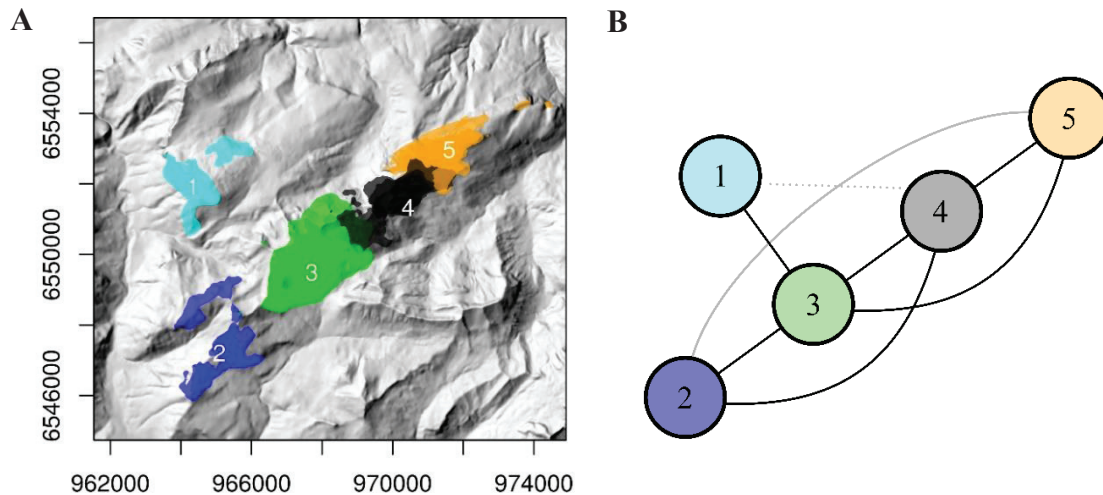


FIGURE 3.2-1 | The five socio-spatial units of the Bargy massif and representation of the associated conceptual metapopulation model.

A (left): identification of the five socio-spatial units. Unit 1 is called “Leschaux-Andey”, unit 2 “Charmieux-Buclon”, unit 3 “Jallouvre-Peyre”, unit 4 “Grand Bargy” and unit 5 “Petit Bargy”. This is an updated version of Figure 2 in (Marchand et al., 2017), using new data of GPS-collared Alpine ibex (*Capra ibex*), corroborating the socio-spatial structure of the population. See Marchand et al. (2017) for details on the method. B (right): configuration of the associated conceptual metapopulation model. Circles represent the five socio-spatial units. Females always stay in their unit, whereas male can make temporary visits to other units. Solid black lines represent between-unit movements of males that are possible during both mating and abortion period. The solid grey line represents movements of males that exist only during mating period. The dashed grey line represents movements of males that exist only during abortion period. Seroprevalence varied between units (Marchand et al., 2017): <15% for 1 and 2, 54% for 3, 70% for 4 and 35% for 5. See Appendix 3.2A for more details.

a. Purpose

We developed an individual-based, spatially implicit SEIR model to represent the ibex brucellosis dynamics in the Bargy massif. The purpose of the model was to quantify transmission of brucellosis among ibex, and to determine whether heterogeneity of transmission arises from differences among individuals in terms of social-behaviour, space use, mating behaviour, transmission routes or active infection. We used an individual-based model for this purpose because it offers the possibility to take into account numerous individual specificities that can impact the infection dynamics (Keeling and Rohani, 2008), and has the ability to track the number of secondary cases caused by each infected individuals (VanderWaal and Ezenwa, 2016).

b. Entities, state variables, and scales

To track information on each individual at each time step, the model included several state variables: individual ibex identity, sex, age in weeks, socio-spatial unit, health status (susceptible: *S*; exposed: *E*; actively infected: *I*; or non-actively infected, i.e., non-shedder: *R*), year of infection, year of recovery and reproductive status (sexually receptive, pregnant, abortion, birth, parity).

Males adopt different mating tactics according to their dominance status. Subordinate males adopt mainly the coursing tactic, whereas dominant males adopt the tending tactic (Willisch and Neuhaus, 2009; Willisch *et al.*, 2012). Tending males monopolise access to the female, whereas coursing males are more opportunistic and try to access the female temporarily when it starts to run (Willisch and Neuhaus, 2009). The adoption of one of the two tactics is strongly age-dependent. Younger males (2-7 years) mainly adopt the coursing tactic while older males (≥ 8 years) mainly engage in tending (Willisch and Neuhaus, 2009; Willisch *et al.*, 2012). Accordingly, in the model, we made the simple assumption that all males aged 2-7 years were coursing and all males aged 8-14 years were considered as tending.

The population is structured into five socio-spatial units, with contrasting seroprevalence. The units 1 and 2 had seroprevalence less than 15%, vs. 54% for the unit 3, 70% for the unit 4 and 35% for the unit 5 (Figure 3.2-1A – Marchand *et al.* 2017). Within each socio-spatial unit, ibex have the tendency to form groups, but they experience very loose bonds (Villaret and Bon, 1998) and groups are not stable and split and merge rapidly (fission-fusion dynamics; C. Calenge, pers. comm.). To account for this structure in the model, we used a spatial metapopulation model, often applied to populations naturally subdivided into spatial units, with homogeneous contacts within subpopulations (Keeling and Rohani, 2008). At birth, each individual was attributed to socio-spatial unit of its mother (permanent socio-spatial unit). All individuals living in the same socio-spatial unit constituted a subpopulation, each subpopulation being characterised by its name and its own relative carrying capacity, defined as a proportion of the carrying capacity of the whole population. The five subpopulations constituted the overall metapopulation (Figure 3.2-1B).

Females usually stay in their spatial unit, whereas males move between units (Marchand *et al.*, 2017). We assumed that females were faithful to their unit and did not engage in any movements between units. Males, however, were found to move between units during the

periods when they can transmit or acquire infection (Figure 3.2-1B). Therefore, the socio-spatial units males were temporarily visiting at each time step were also included as an individual state variable in the model. We defined between-unit transmission as a transmission that occurred during one of these temporary visit between a male and a female that do not share the same permanent socio-spatial unit (regardless of the direction of transmission, i.e., from male to female or vice versa).

Finally, for a susceptible individual that acquired infection, the transmission route by which it was infected, and whether transmission was between or within the unit was recorded. Similarly, the number of individuals a given actively infected individual transmitted infection to (secondary cases), written as Z (Lloyd-Smith *et al.*, 2005b), was included for between- and within-unit and for each transmission route. Z represents an individual equivalent of the population parameter R_n , the net or effective reproductive number, which is defined as the average number of secondary infections caused by an infected individual over the course of its infectious period in a given population (Vynnycky and White, 2010). However, due to the relatively short period considered in our model (i.e., 6 years, compared to a maximum lifespan >20 years in ibex), we could not compute the total number of secondary cases caused by each infected individual during all its infectious life. We thus computed the number of secondary cases caused by each case every year, keeping track of the transmission route and whether it corresponded to within- or between-unit transmission. When it was not possible to attribute a secondary case to a single primary case, the secondary case was shared uniformly between all possible primary cases involved.

The model was developed to represent the evolution of the population dynamics between December 1, 2012 and November 30, 2018 with a discrete weekly time step.

c. Process overview and scheduling

Simulated years began on December 1st, and were split into two periods: the mating period, which lasts from early December to mid-January in ibex (Couturier, 1962; Gauthier *et al.*, 1991; Willisch and Neuhaus, 2009) and was therefore fixed at 7 weeks in the model; and the non-mating period for the remaining 45 weeks (Figure 3.2-2). During the mating period, the events occurring successively at each discrete time step were mortality, spatial movements, reproduction, venereal transmission, incubation and recovery (Appendix 3.2A Figure 3.2A.1).

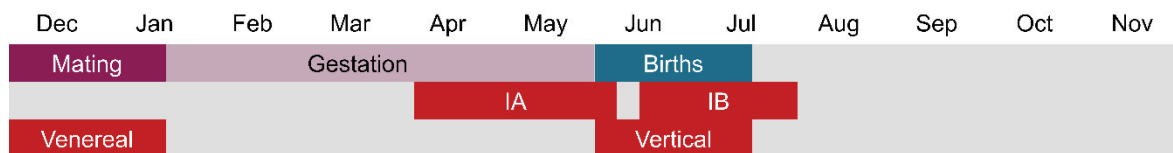


FIGURE 3.2-2 | Seasonal reproduction of the Alpine ibex and periods of potential *Brucella* shedding.

Mating season lasts from early December to mid-January (purple rectangle), leading to births from the end of May to mid-July (blue rectangle). *Brucella* shedding after an infectious abortion (IA) or birth (IB) lasts three weeks. Therefore, horizontal transmission by IA is possible from early April to the end of May. After parturition, a female is not in contact with other individuals for two weeks, therefore the beginning of horizontal transmission by IB is delayed for two weeks compared to the start of the birth period. Venereal transmission is possible during the entire mating period, and vertical transmission (congenital and pseudo-vertical) happens during the entire birth period.

During the non-mating period, the events occurring successively at each discrete time step were surveillance and management strategies, mortality, spatial movements, abortions or births, congenital/pseudo-vertical transmission, horizontal transmission, incubation and recovery (Appendix 3.2A Figure 3.2A-1). Such an order is artificial but is required to apply probabilities of dying or getting infected to the correct numbers of individuals. For each event, individuals were processed simultaneously.

There is a strong seasonal pattern of reproduction in ibex, and by extension of transmission. Gestation in ibex lasts 167 days (Stüwe and Grodinsky, 1987), which leads births to take place from the end of May to mid-July, i.e., 24 weeks after the mating period (Figure 3.2-2). Abortions caused by brucellosis occur during the last third of gestation (Diaz-Aparicio, 2013), i.e., 16 weeks after the beginning of gestation for an ibex. In domestic ruminants, shedding of *Brucella* through placenta, foetal fluids and vaginal discharges when a female aborts or gives birth may last *ca.* three weeks (European Commission, 2001), and we assumed a similar duration for ibex in the model. Therefore, horizontal transmission of *Brucella* through abortions is possible from early April to the end of May. However, after parturition, an ibex female tends to isolate itself in cliffs and rocky areas with its newborn for two weeks (Couturier, 1962; Villaret *et al.*, 1997; Bon *et al.*, 2001). Thus, other individuals were assumed to be in contact with a female only the third week after its parturition, reducing the period of exposure to this transmission route (Figure 3.2-2).

d. Design concepts

The model considers ibex demography, transmission of infection within- and between-unit, and surveillance and management strategies. Population dynamics emerge from individual reproduction and mortality, imposed by parameters dependent on age, sex and density. Density-dependence of survival and reproduction is a common process in populations of large herbivores (Bonenfant *et al.*, 2009). Density-dependent responses of population parameters were shaped to reproduce a logistic population growth, where the population size stabilises around the carrying capacity in the absence of management interventions. Indeed, previous studies of population dynamics of ibex have shown that the logistic model may be appropriate to describe the density regulation in ibex populations (Sæther *et al.*, 2002, 2007). Density-dependent processes play a crucial role in disease ecology because it can interact with transmission, for example by increasing the number of births and therefore the pool of susceptible individuals after population size reduction (Choisy and Rohani, 2006). Population dynamics is highly seasonal in ibex. The mating period is short, and *ca.* 70% of mating associations between males and females occur during the first 2 weeks (Willisch and Neuhaus, 2009), which produces a seasonal peak of births (i.e., susceptible individuals) in late May and early June. Social structure is also subject to seasonal variation, because of progressive sexual segregation (Villaret and Bon, 1995; Bon *et al.*, 2001) with consequences on relevant contact patterns for transmission. These seasonal patterns were important to take into account because of their possible impacts on disease dynamics (Altizer *et al.*, 2006).

Infection dynamics emerges from contacts between susceptible and actively infected individuals and from probabilities of successful transmission given those contacts for each transmission routes. For venereal transmission, contacts emerge from male mating tactic and from female sexual receptivity, described by empirical rules and probabilities. Thus, venereal transmission depended only on the mating system of the host and not on the population size, and can thus be labelled as frequency-dependent (McCallum *et al.*, 2001). For horizontal transmission of *Brucella* through infectious abortions or births, contacts were considered as homogeneous inside a socio-spatial unit, i.e., all individuals could contact each other. Thus, this horizontal transmission increases with the size of the population (i.e., positive density-dependence, McCallum *et al.*, 2001). For transmission between subpopulations, we used a mechanistic model that explicitly represented movements of individuals, representing opportunities of contacts and transmission between individuals

from different subpopulations (Jesse *et al.*, 2008). Heterogeneity of transmission can emerge from age- and sex-specific variation in these contact and transmission patterns.

For the model analysis, the individual statuses were used to derive population-level variables such as population size, prevalence or annual incidence (i.e., number of new cases) in the population. For the model fitting, data on the number of individuals captured and the number of seropositive individuals found among them was used, to match how data were collected in the field.

Transitions between states were modelled as stochastic flows assuming demographic stochasticity. Each transition was the outcome of a Bernoulli process.

e. Initialisation

See Appendix 3.2A.

f. Input data

The model does not use input data or an external model to represent time series of driving environmental variables.

g. Submodels

Surveillance and management strategies: Test-and-remove operations implemented between 2012 and 2018 on the Alpine ibex population of the Bargy massif were integrated in the model. The model reproduced the number of ibex captured and recaptured in each age- and sex-classes and each socio-spatial unit, sampling individuals at random inside each category. Seropositive individuals were removed, whereas seronegative ones were marked and released. In the model, *I* and *R* individuals were considered seropositive, but some were mistakenly detected as seronegative, as the sensitivity of serological tests was assumed to be 95% (ANSES, 2015). On the contrary, *S* and *E* individuals were considered seronegative and specificity of the serological tests was assumed to be 100% (ANSES, 2015). Although values of sensitivity and specificity of the serological tests that were used in ibex are unknown in this species, such high values were demonstrated when applied to the diagnosis of brucellosis in domestic ruminants (e.g., Ferreira *et al.*, 2003; Nielsen *et al.*, 2004;

Ducrotoy *et al.*, 2018). Moreover, the serological status in the data of the ibex Bargy population was based on several serological tests performed in parallel, which improves the accuracy of the diagnosis (Godfroid *et al.*, 2004).

In addition to captures, management strategies implemented by a decision of the French Authorities, were also integrated in the model. In autumn 2013 and spring 2014, the model reproduced the number of culled individuals over five years old of each sex class and each socio-spatial unit, sampling individuals for removal at random. During the culling operation in autumn 2013, errors were made on the estimation of age, which were more frequent for females (C. Calenge, pers. comm.). The model also reproduced these errors on age estimation. Finally, in autumn 2015, autumn 2017 and spring 2018, the model reproduced the number of unmarked individuals of each sex class in each socio-spatial unit that were culled, sampling individuals for removal at random.

The massive culling operation of autumn 2013 did not induce any increase in female reproductive success, despite the drop in population size (C. Toïgo, pers. comm.). This could be explained by a delay on density-dependent responses, that need several years before they can occur (ANSES, 2015). Therefore, in the model, density-dependent responses of population parameters between 2014 and 2018 were obtained from the population size in 2013, to reproduce this phenomenon.

Mortality: We used a complete age-dependent model, with a mortality estimate at each age and for each sex (Toïgo *et al.*, 2007 – Table 3.2-1 and Appendix 3.2A). Males lived up to 16 years and females up to 19 years (Toïgo *et al.*, 2007). The density-dependent response of winter juvenile survival was determined using a function that related survival to the carrying capacity and the abundance of the subpopulation (ANSES, 2015 – see Appendix 3.2A, Equation 3.2A-1 and Figure 3.2A-2A). The carrying capacity was estimated using Approximate Bayesian Computation (see 3.2.2.3).

Neonatal mortality was included in the probability to give birth to a live newborn, and therefore the mortality of juveniles was assumed to happen only in winter. In our model, we assumed no brucellosis-related mortality, as there is currently no evidence for it (no important mortality detected, no seropositive individuals detected in the few individuals found dead).

Spatial movements: Movements of males between units during the mating period and the abortion period, when they can transmit or acquire infection, were derived from data on space use collected by GPS collars (Appendix 3.2A). The probability and direction of movements were estimated separately for the two periods, because males are especially prone to visit other units during the mating period (Figure 3.2-1B and Appendix 3.2A Figure 3.2A-3). We considered all movements to be temporary, as the model randomly sampled a new temporary socio-spatial unit at each time step based on the movement probabilities.

Reproduction: In the model, reproductive success of females was the outcome of two processes: sexual receptivity during mating, and the probability to give birth to a living newborn. In ibex, most mature females are sexually receptive only once during the mating period (Couturier, 1962; Willisch and Neuhaus, 2009). We considered that a mature female could become sexually receptive during mating only once every year with probabilities depending on age (starting at 1.5 years old – Couturier, 1962; Gauthier *et al.*, 1991). The density-dependent response of female receptivity was determined using a function that related receptivity to the carrying capacity and the abundance of the subpopulation (ANSES, 2015 – Appendix 3.2A Equation 3.2A-2 and 3 and Figure 3.2A-2B).

Males, on the other hand, are sexually mature at 2.5 years of age and active every year throughout the mating period (Couturier, 1962; Willisch *et al.*, 2012). Sexual receptivity in females is short, less than two days (Couturier, 1962; Willisch and Neuhaus, 2009), so we assumed that mature males could be associated with up to 3 females per week (Table 3.2-1). During mating, a receptive female is associated with one tending male and a median of three coursing males (Willisch and Neuhaus, 2009). In the model, we associated a receptive female with up to 4 males: 1 tending male and 3 coursing males, randomly sampling males among those that were not already associated to another female and depending on age. We made the simple assumption that all males aged 2-7 years were coursing and all males aged 8-14 years were considered as tending. Among coursing males, those between 2 and 5 years old were around two times less frequent than males of 6 and 7 years of age (4/67 *versus* 3/23 – Table 3 in Willisch *et al.*, 2012).

In ibex, only one male succeeds in engaging sexual intercourse with the female (Willisch and Neuhaus, 2009; Tettamanti, 2015). Based on paternity data, males that we considered as tending (≥ 8 years) are around 6 times more successful in engaging sexual intercourse with

the female than coursing males (14/32 *versus* 7/90 – Table 3 in Willisch *et al.*, 2012). We used these probabilities in the model to sample the successful male among those that were associated to the receptive female. As a result of these two processes (association to a receptive female and selection of the successful male), offspring were sired by a small fraction of males, mainly dominant males engaging in the tending tactic (Willisch *et al.*, 2012). Every female who engaged in sexual intercourse with a male became pregnant. In our model, we assumed that *Brucella* infection decreased sexual receptivity during mating, thus leading to a lower probability of gestation (Table 3.2-2– C. Toïgo, pers. comm.).

Venereal transmission: High amounts of bacteria were found in testes of seropositive males (Freycon, 2015; Lambert *et al.*, 2018b). It was therefore hypothesised that venereal transmission could play a role in brucellosis transmission in the Alpine ibex population of the Bargy massif (Hars *et al.*, 2013; Lambert *et al.*, 2018b). The model thus assumed the existence of this transmission route for ibex, however the corresponding transmission probability was unknown and thus had to be estimated using Approximate Bayesian Computation (see 3.2.2.3).

During sexual intercourse with a female, a tending male often manages to copulate several times due to its almost exclusive access, contrary to a coursing male (2.22 copulations on average for tending males vs. 1 copulation for coursing males - Willisch and Neuhaus, 2009). Therefore, in the model, the probability of venereal transmission during a sexual intercourse between an infectious male and a susceptible female or vice versa was supposed to be higher for tending than for coursing males (Table 3.2-2). Venereal transmission of *Brucella abortus* experimentally-infected rats appeared more efficient from males to females (Islam *et al.*, 2013), and male-to-female transmission is more effective than female-to-male in several other sexually-transmitted diseases (European study group on heterosexual transmission of HIV, 1992; Brunham *et al.*, 1993; Nicolosi *et al.*, 1994; Augustine, 1998). Therefore, the efficacy of male-to-female transmission was supposed to be 1.67 times higher than female-to-male transmission (Table 3.2-2 – Augustine, 1998).

Abortions or births: In domestic ruminants, 80% of infectious females abort and most of them do so only once, during the first pregnancy post-infection (FAO and WHO, 1986; Godfroid *et al.*, 2004). If abortion does not occur, invasion of the uterus leads to *Brucella*

shedding in genital fluids at the time of parturition (European Commission, 2001). In the model, we assumed that pregnant females could abort only during their first pregnancy following infection with a probability of 80% (Table 3.2-2). When abortion does not occur, females give birth to a live newborn. In both cases, females shed *Brucella* in genital fluids for *ca.* three weeks.

For the other pregnant females, and for actively infected females that already went through their first pregnancy following infection, the probability to give birth to a live newborn that survives until winter depends on their parity (Table 3.2-1). We also assumed that active *Brucella* infection decreases the probability to give birth to a live newborn (Table 3.2-2 – C. Toïgo, pers. comm.). Actively infected females that give birth to a live newborn shed *Brucella* in genital fluids for three weeks. The sex-ratio at birth is expected to be 1.

Congenital/pseudo-vertical transmission: Congenital transmission from infected mothers to their offspring *in utero* is demonstrated in domestic ruminants (Renoux, 1962; Plommet *et al.*, 1973; Lapraik *et al.*, 1975) and happens in *ca.* 5% of kids born from infectious mothers (FAO and WHO, 1986; Godfroid *et al.*, 2013). Pseudo-vertical transmission from infectious mothers to kids can also happen through colostrum or milk consumption in all gestation events (Philippon *et al.*, 1971; European Commission, 2001), but this transmission only concerns a small proportion of kids (Grilló *et al.*, 1997). Both transmission routes are suspected in Alpine ibex (Lambert *et al.*, 2018b). Therefore, we included both transmissions in the model, with fixed low probabilities of 5% (ANSES, 2015 – Table 3.2-2). During the two weeks following parturition, we considered that an actively infected female that shed *Brucella* in genital fluids could only transmit infection to its newborn, which we considered as a special case of pseudo-vertical transmission because ibex females isolate themselves with their newborns during the parturition time.

Horizontal transmission: In domestic ruminants, excretion of the *Brucella* in female genital discharges through infectious abortions or births is considered as the main route of transmission to other individuals (European Commission, 2001). For this transmission route, contacts were not modelled explicitly. An individual was exposed to all abortions or births caused by brucellosis within the same socio-spatial unit. We assumed that at a given time step, the probability of an individual coming into effective contact (i.e., coming into a contact

that leads to infection) with one infectious abortion or with one infectious birth was the same (Table 3.2-2). This probability had to be estimated using Approximate Bayesian Computation (see 3.2.2.3).

In ibex, males over 5 years of age segregate from females both socially and spatially soon after the rut, whereas segregation is more gradual for males 5 years of age and under, which are still associated to females during spring (Villaret and Bon, 1995; Bon *et al.*, 2001). Thus, we assumed in the model that males under 5 were less exposed than females to *Brucella* shed following infectious abortions, and males over 6 even less (Table 3.2-2). After being isolated for two weeks following parturition, females and kids gather to form nurseries (Gauthier *et al.*, 1991), and have different space use than males older than 1 year old and females without kids (Grignolio *et al.*, 2007a,b). Thus, we considered that mothers began to gather in nurseries three weeks after parturition. Horizontal transmission through infectious births was assumed to occur only in nurseries, from infected mothers to non-infected mothers and susceptible newborns.

Incubation and recovery: Transmission of *Brucella* to a susceptible ibex S led to an incubation (state E) of three weeks (Table 3.2-2), which is the duration of the incubation period after which seroconversion generally occurs in domestic ruminants (European Commission, 2001). For kids born after congenital transmission, the incubation ends only at abortion or parturition after their first pregnancy for females or at the age of sexual maturity for males (Plommet *et al.*, 1973). After incubation, we considered that infected individuals first entered the I class, characterised by an active infection and therefore the ability to shed the bacteria.

Bacteriological analyses revealed that the probability of active infection decreases with increasing age (Lambert *et al.*, 2018b). Therefore, we assumed that I individuals could transit to the R class, i.e. infected but without active infection and unable to shed the bacteria, based on the probability of recovery. We derived the probability of recovery from these bacteriological data (see Table 3.2-2 and Appendix 3.2A for details).

The complete system of mathematical equations is provided in Appendix 3.2A. All simulations and further analyses were performed using R 3.4.1 (R Core Development Team, 2017).

TABLE 3.2-1 | Definition and value of demographic parameters included in the model of population dynamics of ibex facing a brucellosis outbreak in the Bargy massif.

	Description (dimension)	Value	References
k	Threshold for density-dependent effect	131	†
K	Carrying capacity of the metapopulation		‡
μ_0^{min}	Probability of winter mortality of newborns, minimum (over the duration of winter)	0	†
μ_0^{max}	Probability of winter mortality of newborns, maximum (over the duration of winter)	0.60	†
$\mu_{X\varphi}$	Probability of mortality of X-years old females (annual)	App. A	*
$\mu_{X\sigma}$	Probability of mortality of X-years old males (annual)	App. A	*
τ_X^{max}	Sexual receptivity probability of 1-years old females, maximum	App. A	†
τ_X^{min}	Sexual receptivity probability of X-years old females, minimum	App. A	†
p'_{ij}	Probability for a male whose permanent unit is i to visit the unit j during one time step (per week)		
p_1	Proportion of associations during the first 2 weeks of the mating period	0.70	Willisch and Neuhaus, 2009
p_2	Proportion of associations during the rest of the mating period	0.30	Willisch and Neuhaus, 2009
n_T	Number of tending males per female (over the duration of mating)	1	Willisch and Neuhaus, 2009
n_C	Number of coursing males per female (over the duration of mating)	3	Willisch and Neuhaus, 2009
p_{C1}	Proportion of coursing males 2-5 years old associated to females	1/3	Willisch <i>et al.</i> , 2012
p_{C2}	Proportion of coursing males 6-7 years old associated to females	2/3	Willisch <i>et al.</i> , 2012
τ_T	Copulation probability of tending males (8-14 years old)	6/7	Willisch <i>et al.</i> , 2012
τ_C	Copulation probability of coursing males (2-7 years old)	1/7	Willisch <i>et al.</i> , 2012
n^{max}	Maximal number of partners for males (per week)	3	Willisch and Neuhaus, 2009
η_1	Probability of giving birth for primiparous females	0.70	†
η_2	Probability of giving birth for multiparous females	0.90	†
δ	Sex ratio	0.50	†
d_{rut}	Duration of mating period (weeks)	7	Willisch and Neuhaus, 2009
d_{gest}	Duration of gestation (weeks)	24	Gauthier <i>et al.</i> , 1991
d_{isol}	Duration of postpartum isolation of the mother-offspring couple (weeks)	2	Gauthier <i>et al.</i> , 1991

App. A: see Appendix A

* Calibrated using field data

† Experts knowledge

‡ Approximate Bayesian Computation

TABLE 3.2-2 | Definition and value of epidemiological parameters included in the model of population dynamics of ibex facing a brucellosis outbreak in the Bargy massif.

	Description (dimension)	Value	References
d_{inc}	Duration of <i>Brucella</i> incubation (weeks)	3	European Commission, 2001
d_{shed}	Duration of shedding of <i>Brucella</i> in genital fluids when a female aborts or gives birth (weeks)	3	European Commission, 2001
d_{abo}	Delay between mating and abortion due to <i>Brucella</i> (weeks)	16	Diaz-Aparicio, 2013
Se	Sensitivity of serologic tests	0.95	ANSES, 2015
ρ	Probability of abortion during the first pregnancy following infection	0.80	Godfroid <i>et al.</i> , 2004
γ	Probability of recovery (annual)	0.16	*
β_{IA}°	Per capita probability of one female coming into effective contact with one infectious abortion due to <i>Brucella</i> (per week)		‡
σ	Reduction in the number of contacts with infectious abortion for males ≤ 5 years old	0.27	*
$\beta_{IA}^{\sigma \leq 5}$	Per capita probability of one male ≤ 5 years old coming into effective contact with one infectious abortion (per week)	$\sigma \times \beta_{IA}^{\circ}$	
ϕ	Reduction in the number of contacts with infectious abortion for males > 5 years old	0.06	*
$\beta_{IA}^{\sigma > 5}$	Per capita probability of one male > 5 years old coming into effective contact with one infectious abortion (per week)	$\phi \times \beta_{IA}^{\circ}$	
β_{IB}	Per capita probability of one female or one newborn coming into effective contact with one infectious birth (per week)		‡
$v_{ven}^{T\circ}$	Probability of successful venereal transmission from tending males to females given contact		‡
κ	Relative efficiency of coursing male-to-female transmission	0.45	Willisch and Neuhaus, 2009
$v_{ven}^{C\circ}$	Probability of successful venereal transmission from coursing males to females given contact	$\kappa \times v_{ven}^{T\circ}$	
ω	Relative efficiency of female-to-male transmission	0.60	Augustine, 1998
$v_{ven}^{\circ T}$	Venereal transmission coefficient from females to tending males	$\omega \times v_{ven}^{T\circ}$	
$v_{ven}^{\circ C}$	Venereal transmission coefficient from females to coursing males	$\omega \times v_{ven}^{C\circ}$	
β_{cong}	Congenital transmission coefficient by <i>in utero</i> infection	0.05	Godfroid <i>et al.</i> , 2013
β_{pseu}	Pseudo-vertical transmission coefficient by milk ingestion	0.05	†
ζ_{τ}	Impact of brucellosis on sexual receptivity probability of infectious females	0.80	†
ζ_{η}	Impact of brucellosis on probability of giving birth of infectious females in subsequent pregnancies	0.90	†

* Calibrated using field data

† Experts knowledge

‡ Approximate Bayesian Computation

3.2.2.3. *Simulation experiments*

The model was parameterised using Approximate Bayesian Computation (ABC) rejection algorithm (Beaumont *et al.*, 2002), based on the abc R package (Csilléry *et al.*, 2012). This approach has the advantage to approximate the likelihood by running the model a large number of times and comparing simulated outputs to the observed data (Toni *et al.*, 2009). The algorithm is fully described in Appendix B. We first determined the parameters to be estimated based on (i) identifying the most uncertain parameters, from literature survey and field data, and (ii) performing a sensitivity analysis to determine which parameters have the strongest influence on model outputs. These steps led us to estimate K , the carrying capacity; $\beta_{IA}^{\circ} = \beta_{IB}$, the per-capita probability of one female coming into effective contact with one infectious abortion (IA) or birth (IB) due to *Brucella*; and $v_{ven}^{T^{\circ}}$, the probability of successful venereal transmission from tending males to females given contact, which were both the most influential and uncertain parameters (Appendix 3.2B). For the ABC parameterisation, we fitted the model to two types of available data: the population size over time, and the results of test-and-remove operations in each sex and age classes and in each socio-spatial unit (number of captured and recaptured animals during test-and-remove operations, and seroprevalence in captured and recaptured animals – Appendix 3.2B). For model fitting, we chose to distinguish two age classes, individuals 5 years of age and under and individuals over 5 years of age, because it was the criterion used in the field during management interventions, especially culling operations, and because in males, these age classes corresponded to different levels of exposure to *Brucella* shed following infectious abortions.

We used 10^6 simulations of the model, using uniform prior distributions, and we kept the 10^3 sets of parameter values that produced the closest simulated outputs to the observed data to approximate the posterior distributions. To determine if the ABC approach was able to estimate accurately the parameters, we used cross-validation (Csilléry *et al.*, 2012). This procedure uses a subset of simulated outputs as “pseudo-data”, performing ABC on the remaining outputs. As the parameter values chosen to generate the pseudo-data are known, it is possible to check if ABC is accurately estimating them (see Appendix 3.2B for details).

3.2.2.4. *Model analysis*

The model allowed us to describe the main life events of each individual (i.e., birth, death, reproductive events and movements between units) as well as its epidemiological status at each step. These crude predictions allowed us to reconstruct population size over time from 2012 to 2018, also with the demographic, spatial and epidemiological distribution of the population.

For each individual, information about the year it got infection and whether it was due to within- or between-unit transmission and by which route was recorded. We derived from this information the annual incidence (i.e., number of new cases) in the population, and the proportion of these new cases in each age and sex class and in each socio-spatial unit, for each transmission route.

We also recorded each year the number of secondary cases caused by each actively infected individual, Z . Thus, the number and proportion of these actively infected individuals that caused secondary cases, called primary cases, were calculated. Moreover, we computed the number of secondary cases that originated from each primary case age and sex class as well as each socio-spatial unit, and their respective proportions.

3.2.3. RESULTS

3.2.3.1. *Parameter estimation and model predictions*

The estimated parameter values were (median and 95% credible interval): $K = 564 [535 - 591]$ for the carrying capacity; $\beta_{IA}^{\varnothing} = \beta_{IB} = 0.026 [0.001 - 0.128]$ for the per-capita probability of one female to come into effective contact with one infectious abortion or birth per week; and $\nu_{ven}^{T\varnothing} = 0.168 [0.005 - 0.682]$ for the probability of successful venereal transmission from tending males to females given contact. The posterior distributions of the parameter values are provided in Appendix 3.2B. We evaluated the correlations between the accepted parameter values (Appendix 3.2B), and we found a correlation of 0.018 between K and $\nu_{ven}^{T\varnothing}$, of about -0.14 between $\beta_{IA}^{\varnothing} = \beta_{IB}$ and K and of -0.25 between $\nu_{ven}^{T\varnothing}$ and $\beta_{IA}^{\varnothing} = \beta_{IB}$.

The cross validation procedure revealed that K was the most accurately estimated parameter, followed by $\beta_{IA}^{\varnothing} = \beta_{IB}$ and then by $\nu_{ven}^{T\varnothing}$ (see).

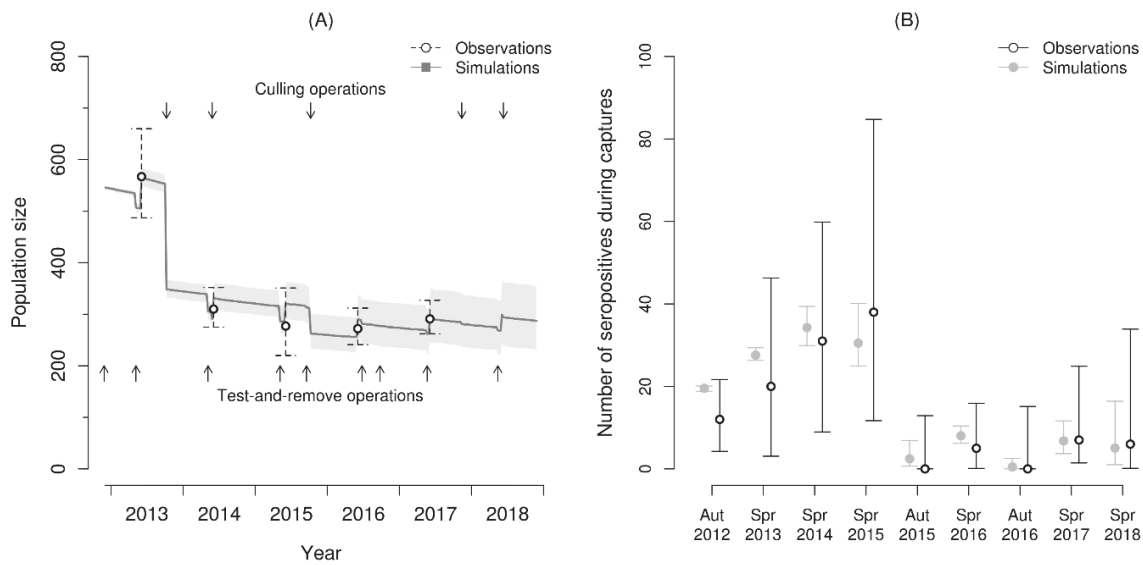


FIGURE 3.2-3 | Fit of the model to observed data.

A: Fit of the model to the estimated pre-breeding population size, also with capture, test-and-remove operations (bottom arrows) and culling operations (top arrows). B: Fit of the model to the expected number of seropositive animals during captures and recaptures. The open black circles and black lines are the observed data and the 95% confidence interval, respectively. Similarly, the grey line (left) or the grey points (right) represent the median of the simulated outputs over the 1000 iterations, while the grey area (left) or the grey lines (right) represent the 95% credible interval. See Appendix 3.2B for more details.

Simulations with 1000 iterations, each iteration using a set of parameter values from the 1000 best simulations from the ABC, produced predictions in accordance with observations both qualitatively and quantitatively (see Figure 3.2-3 and Appendix 3.2B). The population size decreased drastically after massive culling and to a much lesser extent due to birth and death processes (Figure 3.2-3A). Similarly, the number of actively infected individuals dropped after the massive culling of 2013, especially in the socio-spatial unit 3, which was the largest subpopulation and concentrated most of the culling operations (Figure 3.2-4A). It then decreased more gradually in 2014 and 2015, before stabilising at a roughly constant level during the last three years. The number of new cases per year decreased down to a minimum of 5.6 individuals on average in 2016, followed by a slight increase since 2016 (Figure 3.2-4B).

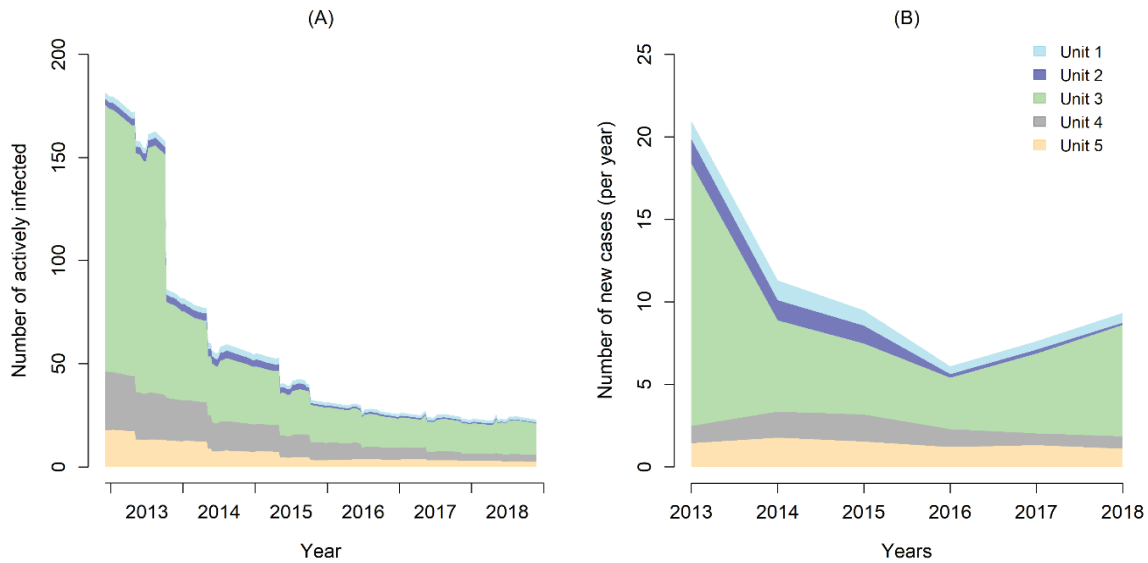


FIGURE 3.2-4 | Model predictions for the spatio-temporal dynamics of brucellosis infection in the wild population of Alpine ibex (*Capra ibex*) of the Bargy massif. A: evolution of the average number of actively infected (I) individuals in the model at each time step (weeks). B: evolution of the average number of new cases per annum in each socio-spatial unit.

3.2.3.2. Importance of transmission routes

New cases were in majority (95%) caused by within-unit transmission, while between-unit transmission caused less than 5% (see Figure 3.2-5A). Among the few cases of between-unit transmission, more than 75% on average were due to venereal transmission, while the remaining cases were due to horizontal transmission through abortions (i.e., susceptible males that visited other units and were infected by females there, before returning to their original unit – Figure 3.2-5B and Appendix 3.2C Figure 3.2C-1).

Among the cases due to within-unit transmission, horizontal transmission through infectious abortions and births were predominant (more than 58% on average of within-unit new cases), followed by vertical transmission (congenital and pseudo-vertical transmission) and then by venereal transmission (Figure 3.2-5C and Appendix 3.2C Figure 3.2C-1). We did not detect any variation in these proportions during the study period (see Appendix 3.2C Figure 3.2C-1).

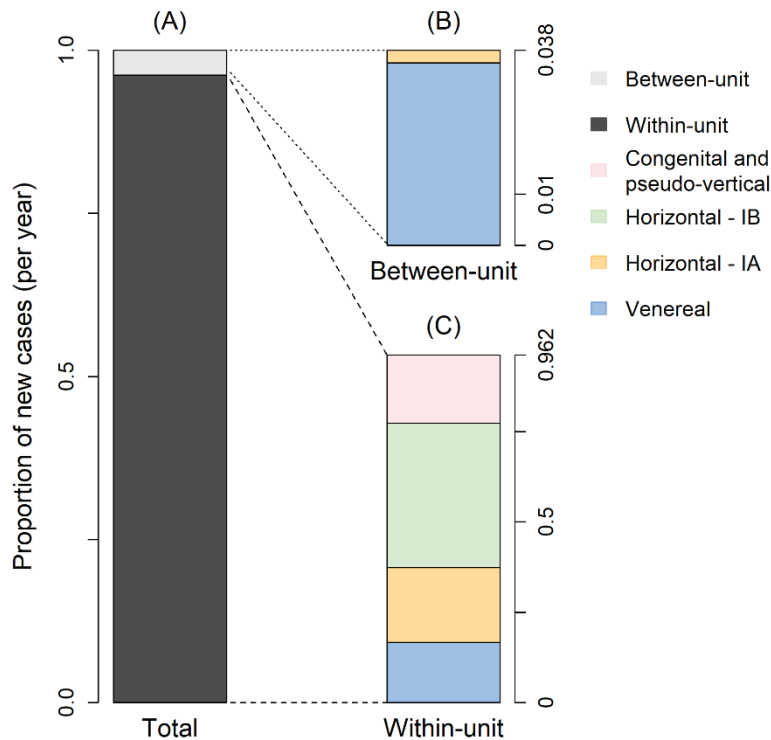


FIGURE 3.2-5 | Proportion of new cases caused by each transmission route.

A: Average proportion of new cases caused by between-unit (light grey) or within-unit (dark grey) transmission. B: Average proportion of new cases caused by each transmission route for between-unit transmission. C: Average proportion of new cases caused by each transmission route for within-unit transmission. Vertical transmission (congenital and pseudo-vertical) is in light pink, horizontal transmission through infectious births (IB) is in green, horizontal transmission through infectious abortions due to Brucella (IA) is in orange, and venereal transmission is in blue.

3.2.3.3. *Quantification of heterogeneity*

a. Individual-level heterogeneity

We first considered heterogeneity of transmission at the individual level. For each infectious individual, we recorded the number of secondary cases generated each year. Figure 3.2-6A shows the cumulative contribution of infectious individuals to the total transmission during one year. Each year, only *ca.* 20% of infectious individuals (i.e., so called primary cases) were responsible for all secondary cases. Among these primary cases, the majority generated one or two secondary cases (Figure 3.2-6B), but a few primary cases were responsible of some extremely high numbers of secondary cases that sometimes occurred (the maximal annual number of secondary cases was 57, with a median maximum of 2 [0-12]).

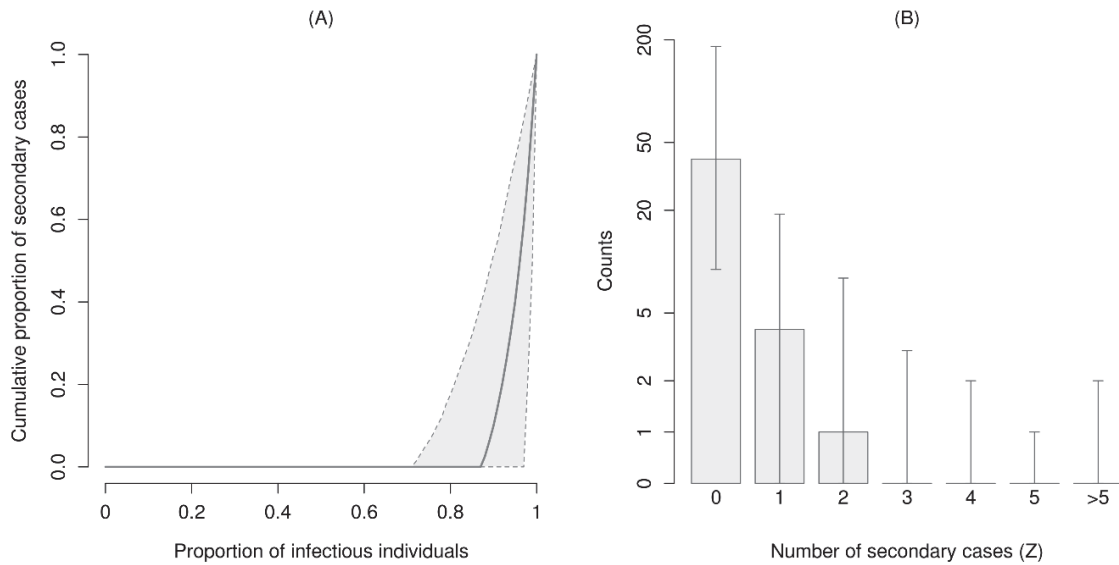


FIGURE 3.2-6 | Contribution of infectious individuals to the yearly number of secondary cases.

A: Cumulative contribution to transmission vs. proportion of infectious individuals, sorted by increasing contribution of secondary cases. The solid grey line represents the median of the output over the 1000 simulations and the six years of study, while the light grey area represents the 95% credible interval. B: absolute frequency of Z, the number of secondary cases caused by each infectious individual. Bars represent the median of the output over the 1000 simulations and the six years of study, while the grey lines represent the 95% credible interval.

b. Heterogeneity between demographic classes

Figure 3.2-7A shows the distribution of secondary cases by age and sex classes and by age and sex classes they originated from. In accordance with the previous result on heterogeneity, we show that the distribution of primary and secondary cases differs in term of age and sex classes, with primary cases (20% of infectious individuals each year) having specific demographic characteristics. In particular, females generated 89% on average of the new cases resulting from within-unit transmission (Figure 3.2-7A and Appendix 3.2C Figure 3.2C-3). Females over 5 years of age generated on average 56% of these new cases, especially through horizontal transmission following births, congenital/pseudo-vertical transmission and venereal transmission (Figure 3.2-7A and Appendix 3.2C Figures 3.2C-3-4-6-7), while younger mature females (2-5 years old) generated on average 33% of them but mainly through abortions (Figure 3.2-7A and Appendix 3.2C Figure 3.2C-5).

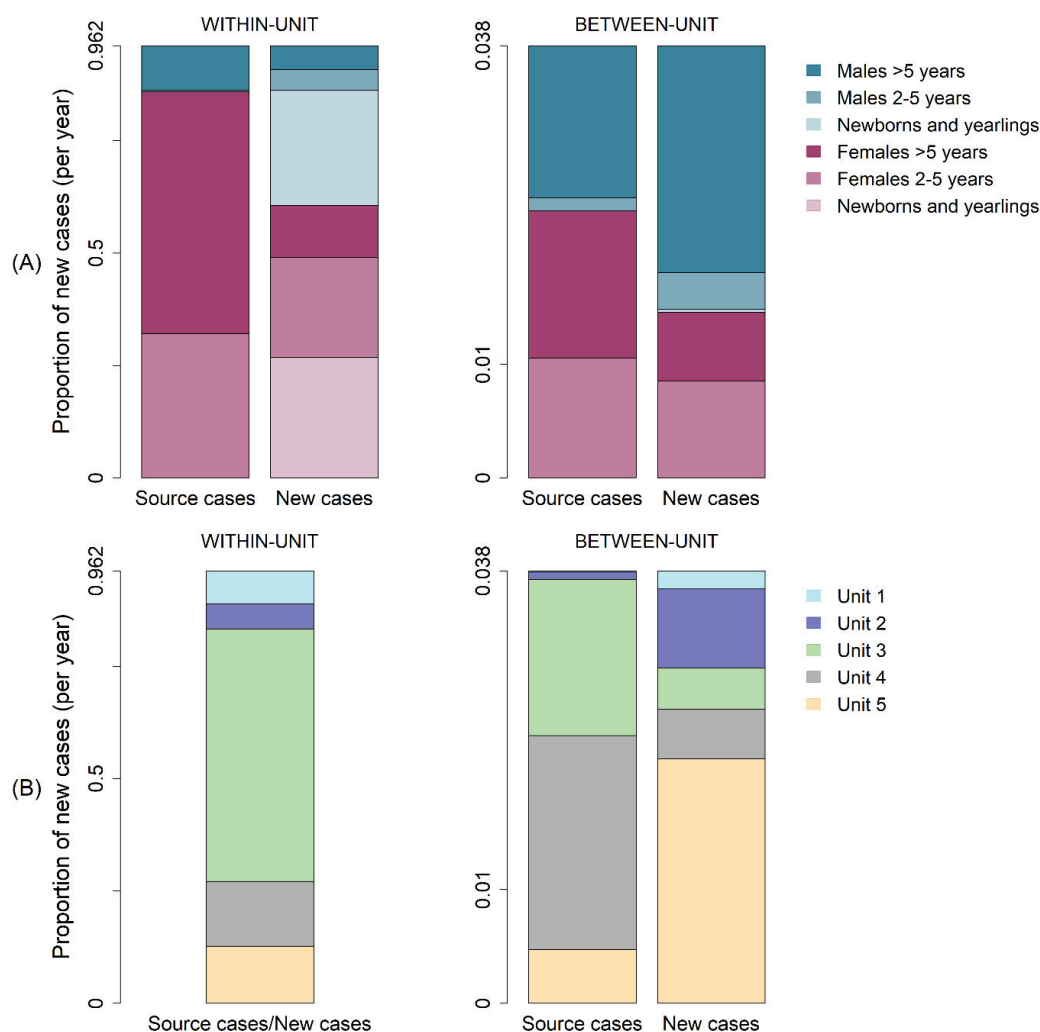


FIGURE 3.2-7 | Distribution of secondary cases of brucellosis transmission according to their age- and sex-classes and their socio-spatial unit, and according to the age- and sex-classes and socio-spatial unit they originated from.

A: average proportion of secondary cases that originated from primary cases of each age- and sex-class (“Source cases”), and average proportion of secondary cases that occurred in each age- and-sex class (“New cases”). For the sake of example, consider a situation where one actively infected female caused two secondary cases in females and one in males, and one actively infected male caused two secondary cases in females. The, a proportion of 60% (3/5) of secondary cases originated from females and 40% (2/5) from males (“Source cases”), while the proportion of secondary cases would be 80% (4/5) in females and 20% (1/5) in males (“New cases”). We separated within-unit (left, 96.2% of new cases) and between-unit (right, 3.8% of new cases) transmission. B: average proportion of secondary cases that originated from primary cases of the five socio-spatial units of the population (“Source cases”) and average proportion of secondary cases that occurred in each socio-spatial unit (“New cases”). For within-unit transmission (left), the socio-spatial unit of the secondary case and the socio-spatial unit it originated from were necessarily the same as transmission occurred between two individuals of the same unit; on the contrary, between-unit transmission (right) always occurred between two individuals from different units.

Secondary cases resulting from within-unit transmission occurred mostly in newborns and yearlings of both sexes (average proportion of around 27% for each sex) through horizontal transmission following births and through congenital and pseudo-vertical transmission (Figure 3.2-7A and Appendix 3.2C Figures 3.2C-6-7). Females 2-5 years old were also often infected (around 23% of new cases) mostly through indirect transmission following abortions (Figure 3.2-7A and Appendix 3.2C Figure 3.2C-5), while older age and sex classes were less concerned and mostly contaminated through venereal transmission (Figure 3.2-7A and Appendix 3.2C Figure 3.2C-4).

As regards between-unit transmission, which as a reminder occurs mainly through venereal transmission, females contaminated males slightly more often than the other way around (*ca.* 60 versus 40% – Figure 3.2-7A and Appendix 3.2C Figure 3.2C-4). Contrary to what was observed within-unit, the older age classes were the most represented in both primary and secondary cases in both sexes for the between-unit transmission (Figure 3.2-7A and Appendix 3.2C Figure 3.2C-4).

c. Socio-spatial units

Figure 3.2-7B shows the spatial distribution of where the secondary cases occurred and where the primary cases came from. Within-unit transmission mostly occurred in the socio-spatial unit 3 (Figure 3.2-7B and Figure 3.2-8 and Appendix 3.2C Figure 3.2C-8), where 58.5% of within-unit new cases arose, followed by decreasing order by the socio-spatial units 4 (14.9%), 5 (13.2%), 1 (7.6%) and 2 (5.8%). Between-unit transmissions mostly originated from both central units 3 (49.4% of between-unit new cases) and 4 (36.1%), which were mostly responsible for new cases in the units 2 (18.3%) and 5 (56.6% – Figure 3.2-7B and Appendix 3.2C Figure 3.2C-8). Units 3 and 4 generated more new cases of between-unit transmission than they received, while it was the opposite for units 1, 2 and 5 (Figure 3.2-8).

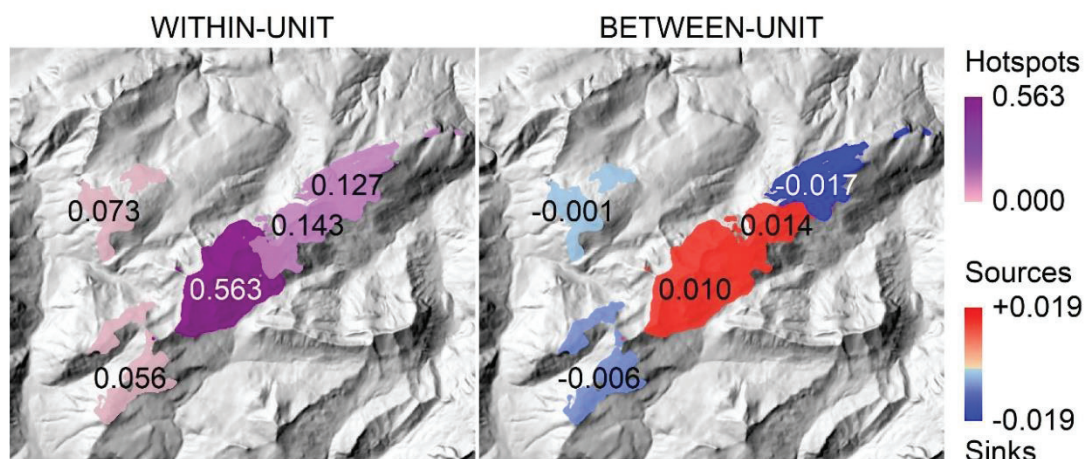


FIGURE 3.2-8 | Spatial transmission heterogeneity of brucellosis in the five socio-spatial units of the Alpine ibex population of the Bargy massif.

To the left, socio-spatial units are ranked according to the average proportion of secondary cases that occurred in each socio-spatial unit by within-unit transmission (96.2% of new cases), to identify hotspots of transmission. Proportions are the same than in Figure 3.2-7B. To the right, each unit is ranked according to the difference between the proportion of new cases that originated from it and the proportion of new cases that occurred in it because of the other units (between-unit transmission, 3.8% of new cases). This corresponds to making the difference between the “source cases” column and the “new cases” column in Figure 3.2-7B. If the difference is positive, the unit was considered as a source of transmission, because more cases were generated than received. In the case of a negative difference, the unit was considered as a sink of transmission, because more cases were received than generated. The differences were calculated as: $0 - 0.001 = -0.001$ for Unit 1, $0.001 - 0.007 = -0.006$ for Unit 2, $0.014 - 0.004 = 0.010$ for Unit 3, $0.018 - 0.004 = 0.014$ for Unit 4 and $0.005 - 0.022 = -0.017$ for Unit 5.

3.2.4. DISCUSSION

We built an individual-based model of *Brucella melitensis* transmission in the wild population of Alpine ibex (*Capra ibex*) of the Bargy massif in the French Alps, the first reported case of persistence of brucellosis in European wild ungulates. Our results revealed (i) an extremely high heterogeneity with only 20% of individuals which are responsible for new cases each year, (ii) a predominant role of females which are the most frequent source of brucellosis infection and (iii) source-sink metapopulation dynamics of infection between the five socio-spatial units in the population. In the following sections, we will discuss each of the results provided by our model on heterogeneity at the different levels and compare them to existing knowledge on this system and others, before discussing the potential implications of our work for disease management.

3.2.4.1. *A highly heterogeneous system*

Our model allowed us to track the annual number of secondary cases produced by each individual. We revealed an extremely high heterogeneity. Each year, only 20% of individuals are responsible for most new cases (Figure 3.2-6). However, this result is not strictly comparable to the proportion of transmission due to the most infectious 20% of cases reported by Woolhouse *et al.* (1997) and Lloyd-Smith *et al.* (2005b), since we considered in the present study the number of secondary cases caused by an infectious individual each year instead of summing them over the course of the individual infectious period. Therefore, the contribution of an individual to the proportion of transmission could vary between years, and we could reasonably expect that on longer periods more than 20% of infectious individuals would contribute to the infection transmission. It is also important to stress out that this high individual heterogeneity could be enhanced by disease management measures (Lloyd-Smith *et al.*, 2005b) implemented in the population during our study period. Indeed, removing an infectious individual a given year reduced its number of secondary cases that year, while infectious individuals that were not the subject to measures or were wrongly diagnosed as seronegative could continue to infect other individuals.

Even if individual heterogeneity may not be as marked as suggested by Figure 3.2-6, our work suggests that disease management targeting the most infectious individuals could be more efficient than that based on population-wide strategies. This is especially expected for low or intermediate control efforts (Lloyd-Smith *et al.*, 2005b), and therefore would be particularly relevant in the study case. However, although we were able to identify the individuals most responsible for infection using our model, doing so in the field would prove quite challenging. This is the reason why we focused on identifying the classes of individuals most responsible for infection rather than the individuals themselves (VanderWaal and Ezenwa, 2016), using traits associated with transmission that are identifiable in the field, such as sex, age or socio-spatial unit in our case.

3.2.4.2. *Brucellosis dynamics in ibex: asymmetric roles for males and females?*

Several years ago, a review of host-pathogen systems in mammals suggested that males play a critical role in diseases transmission compared to females, and proposed that disease

dynamics was “all caused by males” (Skorping and Jensen, 2004). To explain this pattern, the authors proposed either a biased prevalence or shedding intensity towards males (Poulin, 1996; Schalk and Forbes, 1997), or differences in behaviour (such as movements of males over large range) leading to biased exposure even in the absence of prevalence or shedding differences (Ferrari *et al.*, 2003; Skorping and Jensen, 2004). In contrast with this general rule, we found that females are the most frequent source of brucellosis infection for ibex, as for brucellosis in other species of domestic and wild ruminants (European Commission, 2001; Rhyan, 2013). In our study system, there were no or few differences between males and females in terms of seroprevalence (Marchand *et al.*, 2017) or probability of active infection (Lambert *et al.*, 2018b). However, we expected sex differences in disease transmission because mating behaviour (Willisch and Neuhaus, 2009), social behaviour (Bon *et al.*, 2001) and space use (Marchand *et al.*, 2017) all markedly differ between sexes.

The different transmission routes potentially involved in males and females (Lambert *et al.*, 2018b) could also be a potential driver of sex differences in transmission (Zuk and McKean, 1996). Indeed, males can only be involved in venereal transmission, which is frequency-dependent, whereas females also have the ability to transmit the infection horizontally by shedding *Brucella* in genital fluids. Females could therefore infect more individuals, because horizontal transmission route follows a positive density-dependence. The posterior distributions of horizontal and venereal transmission parameters were both narrower than their prior distributions, which suggests that available data are informative for both transmission routes, although uncertainty remains high especially for venereal transmission.

According to the socio-spatial behaviour of ibex and the transmission routes of brucellosis, we would have expected female-to-female transmission through bacterial shedding following abortion or birth to be predominant within units, and male-to-female transmission through venereal transmission to play a critical role between units. Instead, we found that bacterial shedding following abortion or birth was indeed the predominant transmission route within units (Figure 3.2-5), but was not restricted to female-to-female transmission. In particular, female-to-male transmission occurred frequently after birth, when susceptible newborn males were in contact with shedding females (Appendix 3.2C Figures 3.2C-6-7). Vertical transmission also played a significant role, and overall, as within-unit transmission was largely predominant, our results demonstrated a critical role of females in the studied system.

As regards between-unit transmission, venereal transmission was the predominant transmission route, but contrary to our expectation, again, transmission occurred slightly more often from female to male than from male to female (Figure 3.2-7A and Appendix 3.2C Figure 3.2C-4). This means that males got infected from females of other units almost as much as they transmitted infection to females. Therefore, females represent also an important source of transmission between units, which was counterintuitive given their sedentary behaviour. However, it is important to stress out that males are the only individuals that move between units in the ibex population, and are thus essential for the spatial spread of brucellosis. In bison, venereal transmission was assumed to play a negligible role compared to *Brucella* shedding in females following abortion or birth (Treanor *et al.*, 2010; Ebinger *et al.*, 2011). In our study, although the uncertainty around the venereal transmission parameter remains high, most transmission between units were accounted for by the venereal route. Our result suggest that this transmission route play a critical role for the spatial spread of brucellosis in ibex, and thus has to be taken into account.

The critical role of within-unit transmission, and especially through shedding following infectious birth, could explain the maintenance of the differences in seroprevalence among socio-spatial units, and the fact that males present the same spatial structuration in seroprevalence as females, despite their movements between units (Marchand *et al.*, 2017). Indeed, if there was a majority of between-unit transmissions, we would expect a homogenisation of seroprevalences in the different units.

3.2.4.3. *The role of old individuals*

For males, the age class that is both a source and a victim of transmission (through venereal transmission) clearly appears to be the individuals over 5 years old, even when compared to the proportion of males of this age class in the whole population (Figure 3.2-7A and Appendix 3.2C Figures 3.2C-2-3). This result is in accordance with the hypothesis made by Hars *et al.* (2013) based on serological data. This occurs because older males are more frequently in contact with females (“tending” mating tactic), and have thus a higher probability of effective contact than younger males. This pattern still held after the massive culling operation in Autumn 2013, despite the fact that males over 5 years old were specifically targeted. Although the number of older males in the population decreased (Appendix 3.2C Figures 3.2C-2-3), we did not detect a strong increase of the proportion of

younger males (≤ 5 years old) involved in venereal transmission (Appendix 3.2C Figure 3.2C-4), contrarily to what was suggested in Hars *et al.* (2015).

In contrast, females 5 years old and under were responsible for most new cases through infectious abortions (Appendix 3.2C Figure 3.2C-5), whereas older females were accountable for the majority of new cases through infectious births as well as congenital/pseudo-vertical transmission (Appendix 3.2C Figures 3.2C-6-7). Thus, despite the fact that young seropositive females have a higher probability of being actively infected (Lambert *et al.*, 2018b), young females play a major role through abortion during their first gestation post-infection, but transmission through shedding after birth in the following gestations (i.e., in older females) also plays a determinant role. This could be explained, although less categories of individuals are exposed to this route and for shorter duration than for abortions, by the fact that most of exposed individuals are newborns, fully susceptible to the infection. Therefore, ecological data in combination with epidemiological data on seroprevalence structure and shedding pattern suggest that brucellosis transmission is not limited to young age classes in ibex, in contrast to what is suggested by shedding patterns alone (Lambert *et al.*, 2018b). This result is similar to the pattern of transmission in domestic ruminants facing endemic brucellosis (European Commission, 2001; ANSES, 2015).

3.2.4.4. *Source, sink and metapopulation dynamics of brucellosis*

As regards our second hypothesis, we expected that units with the highest seroprevalence and the largest sub-population, that also occupy a central positions in the Massif (Marchand *et al.*, 2017), would act as sources of infection to the other units. We used explicit movements of animals, quantified through the use of GPS data, to model transmission between units, rather than a phenomenological model that links units between them without specifying any mechanism (Jesse *et al.*, 2008). Therefore, transmission between units was the result of both movement pathways of animals and characteristics of each unit such as abundance and seroprevalence.

Our prediction was supported by the outcome of the model (Figure 3.2-7B and Figure 3.2-8), with both units 3 and 4 (with high seroprevalence) being the main sources of between-unit new cases, while the units 2 (with low seroprevalence) and 5 (with intermediate level of seroprevalence) were the main units where new cases occurred from between-unit transmission. During the mating period, movements of males from unit 3 mostly occurred

towards unit 2 (Appendix 3.2A Figure 3.2A-3). Therefore, transmission between these units was mostly accounted for by movement of infectious males from unit 3 and contamination of susceptible females of unit 2. As for unit 4, it acted as a source through two main mechanisms: through movements of infectious males from unit 4 and contamination of susceptible females of unit 5; but also from movements of susceptible males from unit 5, which got infected by infectious females from unit 4.

We therefore confirmed the role of transmission source of the two central socio-spatial units in our population, which mainly infected the two peripheral socio-spatial units. The last socio-spatial unit, more isolated than the others (see Figure 3.2-1B and Appendix 3.2A), also received the infection from the two sources, but relatively less so. To attribute to the peripheral units the role of sink, in the context of metapopulation dynamics, would require confirming that infection would eventually fade-out from these units without movements and transmission from the sources (Pulliam, 1988), which was not explored by our model.

3.2.4.5. *Potential consequences for disease management*

Our study, although not aiming to analyse explicitly the relative relevance of different management scenarios, suggest that management interventions implemented so far have decreased brucellosis transmission, even if *Brucella* was still persistent in all 1000 simulations at the end of the study period. However, we could not disentangle the effects of each intervention or combination of interventions as we did not compare our results to other management scenarios. Our results also offer first insights on how to improve disease management. For example, previous studies suggested that disease management targeted towards a subset of units in a metapopulation, based on their position in the configuration of the metapopulation and their contribution to transmission, could be more effective (Hess, 1996; Fulford *et al.*, 2002; Haydon *et al.*, 2006). In our case, the three units acting as hotspots of transmission could be a possible way of improving the cost/benefit ratio of disease management.

Targeting specific age- or sex-classes could be an alternative (Ferrari *et al.*, 2003; Fenichel and Horan, 2007). In the ibex case, management strategies should probably focus on females, as they are responsible for the vast majority of new cases in the system. Predicting which female age class to target appears difficult because younger females are more often actively infected and abort, while older females play an important role as well

through shedding at birth and vertical transmission. In a model of brucellosis infection in bison from the Yellowstone National Park, test-and-remove was more effective when targeting pre-reproductive seropositive females rather than targeting all females regardless of age (Ebinger *et al.*, 2011). This selective strategy allowed to remove seropositive females before they could even shed bacteria following abortion or birth (Ebinger *et al.*, 2011). In ibex, individuals under two years of age are seldom captured, which would make this strategy difficult to apply.

Males play a less important role quantitatively, but qualitatively they connect socio-spatial units together and create opportunities for between-unit transmission. Therefore, if the aim of the management strategy is to reduce or prevent transmission between socio-spatial units, then males, especially older ones, should be targeted.

Future studies about the efficacy of disease strategies in our population are therefore required, and could be improved by the new insights we provided in this work. The model we proposed could be expanded for predictions on the future dynamics of the population, with and without disease management, so as to evaluate theoretically the relative efficacy of various strategies that could then be implemented in the field.

3.2.4.6. *Conclusion*

By integrating information issued from population demography, spatial use, management strategies and epidemiological surveys to build a realistic model, we provide an accurate picture of the population dynamics of ibex in the Bargy massif, which faced a brucellosis outbreak. This models allows unravelling the mechanisms involved in this dynamics. Considering the specific features of this population, particularly the management measures implemented since 2012, extrapolating our conclusions to other cases (if these arise), should be done with caution. In particular, the outcome of infection would have likely been different if management options had been different. Nonetheless, our results suggest that brucellosis in ibex is epidemiologically similar to *B. melitensis* and *B. abortus* infection in other species of domestic and wild ruminants, with mature females playing a predominant role of transmission through *Brucella* shedding in genital fluids (European Commission, 2001; Diaz-Aparicio, 2013; Rhyan, 2013). Our findings demonstrate that our general framework using individual-based models is suitable for the study of transmission heterogeneity in wild populations, and could be applied to other host-pathogen systems.

APPENDIX 3.2A: MODEL DETAILS

We developed an individual-based, spatially implicit SEIR model to represent the Alpine ibex (*Capra ibex*) brucellosis dynamics in the Bargy massif between December 1, 2012 and November 30, 2018 with a discrete weekly time step.

1. Initialisation

The initial population size for the first time step of the model (December 1, 2012) was calculated based on the population size estimate of 2013 and the assumption that the population is close to saturation (ANSES, 2015). Thus, the model started with 566 adult individuals and 144 newborns. Their distribution among socio-spatial units was determined based on the spatial distribution of individuals recorded during test-and-remove or removal operations. Between 2012 and June 2018, 508 different recorded individuals were old enough to be already present in the population in 2012. We considered that this sample was large enough to be representative of the initial population, and therefore we extrapolated the repartition of these 508 different individuals among socio-spatial units to the total population in our model. These proportions were: 7.5% for Unit 1 (Leschaux-Andey), 8.1% for Unit 2 (Charmieux-Buclon), 61.3% for Unit 3 (Jallouvre-Peyre), 10.1% for Unit 4 (Grand Bargy) and 13.0% for Unit 5 (Petit Bargy).

Age and sex composition was based on field data (Table 3.2A-1 – C. Toïgo, pers. comm.), and was assumed to be the same for each socio-spatial unit. The proportion of females in the population was 0.538, with different proportions for each unit: 0.605 for Unit 1, 0.683 for Unit 2, 0.508 for Unit 3, 0.627 for Unit 4 and 0.485 for Unit 5.

The proportions of seropositive individuals ($I + R$) among males and females of each age and socio-spatial unit (Table 3.2A-2) were drawn from the predictions of the statistical model of Marchand *et al.* (2017) for the period 2012-2013. The proportions of active infection (I individuals) among seropositive males and females of each age (Table 3.2A-3) were drawn from the predictions of the statistical model of Lambert *et al.* (2018b).

2. Input

The model does not use input data or an external model to represent time series of driving environmental variables.

TABLE 3.2A-1 | Initial proportions for each age among females and males.

Sex	X (age in years)																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
♀	0.189	0.108	0.108	0.087	0.087	0.086	0.086	0.086	0.022	0.022	0.022	0.015	0.015	0.015	0.014	0.012	0.012	0.012	0.002
♂	0.218	0.081	0.081	0.081	0.081	0.065	0.065	0.065	0.065	0.049	0.049	0.033	0.033	0.011	0.011	0.007	0.005	0	0

TABLE 3.2A-2 | Initial proportions of seropositive individuals among females and males of each age and socio-spatial unit.

Sex	Unit	X (age in years)																		
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
♀	1	0	0.020	0.034	0.056	0.083	0.116	0.151	0.183	0.207	0.220	0.222	0.201	0.181	0.161	0.141	0.121	0	0	0
	2	0	0.016	0.028	0.044	0.067	0.093	0.121	0.147	0.168	0.179	0.181	0.171	0.153	0.128	0.108	0.088	0	0	0
	3	0	0.130	0.264	0.373	0.480	0.572	0.643	0.693	0.724	0.739	0.739	0.725	0.695	0.637	0.581	0.499	0	0	0
	4	0	0.272	0.392	0.479	0.589	0.675	0.737	0.769	0.802	0.814	0.813	0.802	0.777	0.716	0.657	0.596	0	0	0
	5	0	0.075	0.125	0.191	0.267	0.345	0.415	0.471	0.509	0.528	0.529	0.512	0.477	0.426	0.361	0.307	0	0	0
♂	1	0	0.020	0.034	0.052	0.073	0.096	0.118	0.133	0.141	0.140	0.130	0.111	0.091	0.071	0.052	0.019	0	0	0
	2	0	0.016	0.026	0.040	0.058	0.076	0.094	0.108	0.115	0.116	0.108	0.095	0.078	0.060	0.036	0.008	0	0	0
	3	0	0.129	0.260	0.354	0.444	0.519	0.575	0.609	0.623	0.617	0.592	0.548	0.486	0.406	0.327	0.212	0	0	0
	4	0	0.239	0.362	0.459	0.553	0.627	0.678	0.700	0.720	0.715	0.692	0.651	0.589	0.499	0.408	0.318	0	0	0
	5	0	0.077	0.122	0.178	0.240	0.298	0.347	0.380	0.395	0.391	0.369	0.331	0.281	0.225	0.168	0.086	0	0	0

TABLE 3.2A-3 | Initial proportions of active infection among seropositive females and males of each age.

Sex	X (age in years)																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
♀	0.578	0.540	0.501	0.463	0.425	0.387	0.351	0.317	0.285	0.254	0.226	0.200	0.177	0.155	0.136	0.119	0.104	0.090	0.078
♂	0.686	0.651	0.616	0.578	0.540	0.502	0.463	0.425	0.388	0.352	0.318	0.285	0.255	0.227	0.201	0.177	0.156	0	0

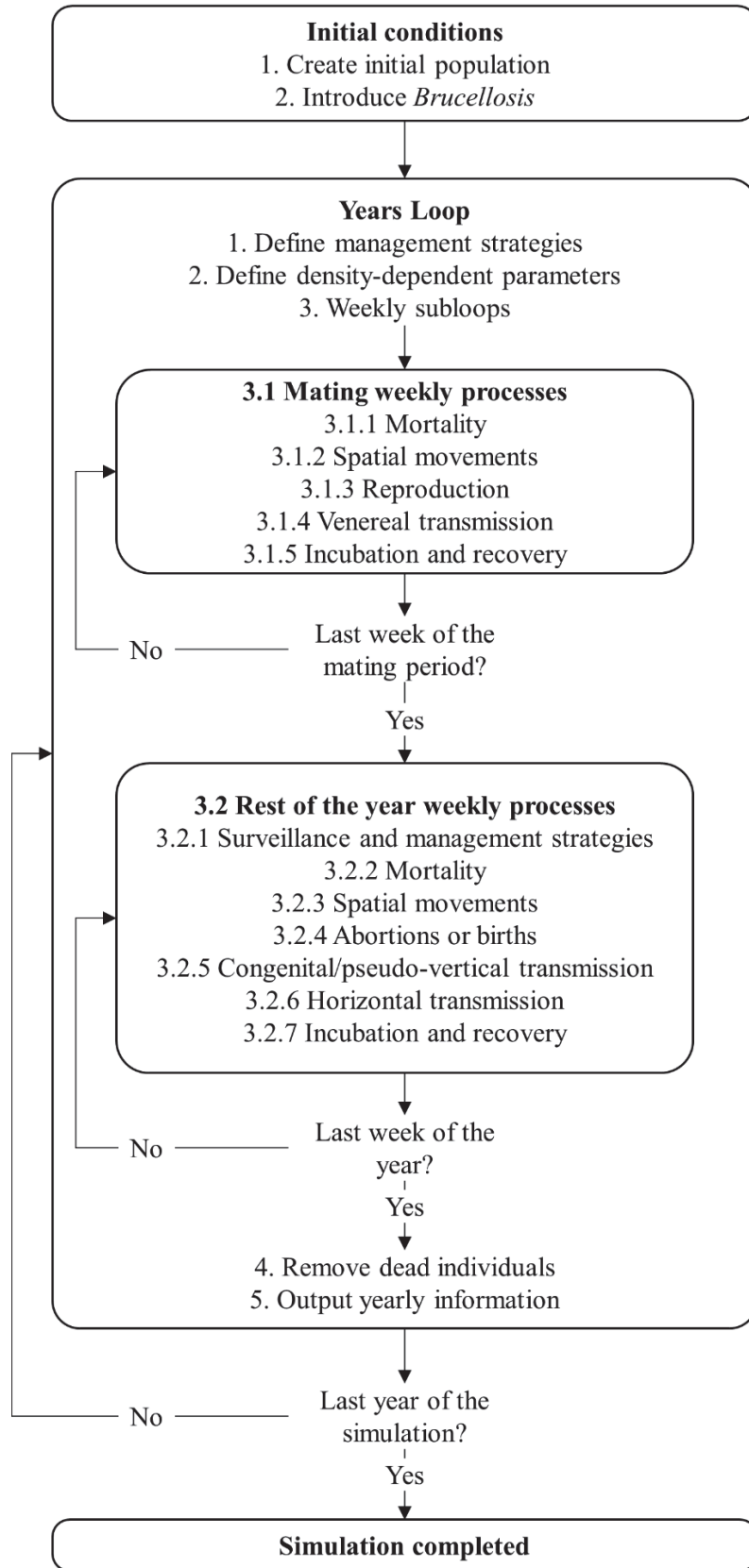


FIGURE 3.2A-1 | Flow diagram of annual and weekly individual-based model processes for a single simulation of *Brucella melitensis* infection in Alpine ibex (*Capra ibex*).

3. Submodels

Simulated years began on December 1st, and were split into two periods: the mating period, which lasts from early December to mid-January in ibex (Couturier, 1962; Gauthier *et al.*, 1991; Willisch and Neuhaus, 2009), and the non-mating period, for the remaining 45 weeks. During the mating period ($1 \leq t \leq d_{rut}$), the events occurring successively at each discrete time step were mortality, spatial movements, reproduction, venereal transmission, incubation and recovery (Figure 3.2A-1).

During the non-mating period ($d_{rut} + 1 \leq t \leq 52$), the events occurring successively at each discrete time step were management strategies, mortality, spatial movements, abortions or births, congenital/pseudo-vertical transmission, horizontal transmission, incubation and recovery (Figure 3.2A-1). Such an order is artificial but is required to apply probabilities of dying or getting infected to the correct numbers of individuals. For each event, individuals were processed simultaneously.

Each year, the probability of winter juvenile mortality μ_0 , as well as the probability for a female to become sexually receptive τ , had density-dependent responses at the socio-spatial unit level that were shaped to reproduce a logistic population growth (Sæther *et al.*, 2002, 2007). For these parameters, we used sigmoid functions (1-3) using explicit variables ($k(u)$: the threshold for density-dependent effect in unit u , $K(u)$: the carrying capacity of unit u , $N(u)$: the annual pre-breeding population size in unit u). See Table 3.2-1 in the main text and Table 3.2A-4 for the parameter values, and Figure 3.2A-2 for the graphical shape of the functions of equations 1-3 (ANSES, 2015). Each unit was characterized by its own relative carrying capacity, defined as a proportion of the carrying capacity of the whole population. Based on the assumption that the population was closed to its carrying capacity in 2012, we used the same proportions that were used for the repartition of individuals among spatial units for the initial population (see Initialisation).

TABLE 3.2A-4 | Values of sexual receptivity probability of females depending on age.

	X (age in years)					
	0	1	2-3	4-11	12-16	17-19
τ_X^{max}	-	0.50	0.80	1	0.80	-
τ_X^{min}	-	0	0	0.50	0.30	-

$$(1) \mu_0(u) = \begin{cases} \mu_0^{min}, & \text{when } N(u) < k(u) \\ 1 - \frac{1}{e^{-a} + 1}, & \text{when } k(u) < N(u) < K(u) \\ \mu_0^{max}, & \text{when } N(u) > K(u) \end{cases}$$

Where $a = \left(\frac{N(u) - k(u)}{K(u) - k(u)}\right) \text{logit}(1 - \mu_0^{max}) + \left(\frac{K(u) - N(u)}{K(u) - k(u)}\right) \text{logit}(1 - \mu_0^{min})$

$$(2) \tau_X(u) = \begin{cases} \tau_X^{max}, & \text{when } N(u) < k(u) \\ \frac{1}{e^{-a} + 1}, & \text{when } k(u) < N(u) < K(u) \\ \tau_X^{min}, & \text{when } N(u) > K(u) \end{cases}$$

Where $a = \left(\frac{N(u) - k(u)}{K(u) - k(u)}\right) \text{logit}(\tau_X^{min}) + \left(\frac{K(u) - N(u)}{K(u) - k(u)}\right) \text{logit}(\tau_X^{max})$

and X corresponds to age 1 – 11.

$$(3) \tau_{12-16}(u) = \begin{cases} \frac{1}{e^{-a} + 1}, & \text{when } N(u) < K(u) \\ \tau_{12-16}^{min}, & \text{when } N(u) > K(u) \end{cases}$$

Where $a = \left(\frac{N(u) - k(u)}{K(u) - k(u)}\right) \text{logit}(\tau_{12-16}^{min}) + \left(\frac{K(u) - N(u)}{K(u) - k(u)}\right) \text{logit}(\tau_{12-16}^{max})$

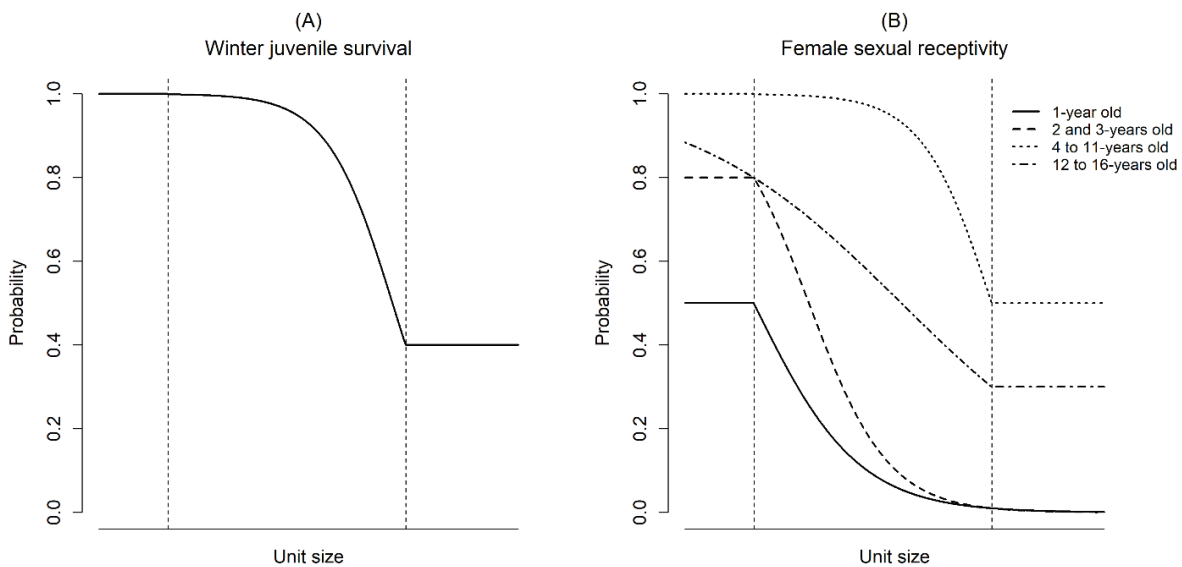


FIGURE 3.2A-2 | Shape of winter juvenile survival (A) and female sexual receptivity (B) function of population size in the unit considered.

Vertical dashed lines represent the values of $k(u)$: the threshold for density-dependent effect in unit u and $K(u)$: the carrying capacity of unit u .

The equations describing weekly processes during mating period are given below (4-22). See Table 3.2-1 and Table 3.2-2 in the main text for the parameter values.

Surveillance and management strategies

- (4) When test-and-remove was applied, each individual i in socio-spatial unit u was captured (or recaptured) following a Bernoulli process with probability:

$$p_i(t, u) = \frac{n_{X\varphi}(t, u)}{N_{X\varphi}(t, u)}$$

where $n_{X\varphi}(t, u)$ is the number of unmarked (marked) males or females φ of the age class X to be captured (recaptured) in socio-spatial unit u at time t , and $N_{X\varphi}(t, u)$ is the total number of unmarked (marked) individuals of the corresponding sex and age class in the socio-spatial unit u at time t .

Then, individuals detected as seropositive were removed, whereas individuals detected as seronegative were marked (if not yet the case) and released. S and E individuals were considered as seronegative, whereas I and R individuals were seropositive. Among seropositive individuals, $1 - Se$ were mistakenly detected as seronegative due to the lack of sensitivity of the serological tests (ANSES, 2015).

- (5) When removal of individuals over 5 years of age was applied, each individual i in socio-spatial unit u was removed following a Bernoulli process with probability:

$$p_i(t, u) = \frac{n_{X\varphi}(t, u)}{N_{X\varphi}(t, u)}$$

where $n_{X\varphi}(t, u)$ is the number of unmarked males or females φ over 5 years of age to be removed in socio-spatial unit u at time t , and $N_{X\varphi}(t, u)$ is the total number of unmarked individuals over 5 years of age of the corresponding sex in socio-spatial unit u at time t .

During the culling operation in autumn 2013, errors were made on the estimation of age, which were more frequent for females (C. Calenge, pers. comm.). The model also reproduced these errors on age estimation. The proportion of young males (under 5 years old) removed in autumn 2013 was 24.5%, and the proportion of young females (under 5 years old) was 63.5% (C. Calenge, pers. comm.).

- (6) When removal of unmarked individuals was applied, each individual i in socio-spatial unit u was removed following a Bernoulli process with probability:

$$p_i(t, u) = \frac{n_{X\varphi}(t, u)}{N_{X\varphi}(t, u)}$$

where $n_{X\varphi}(t, u)$ is the number of unmarked males or females φ to be removed in socio-spatial unit u at time t , and $N_{X\varphi}(t, u)$ is the total number of unmarked individuals of the corresponding sex in socio-spatial unit u at time t .

In each case, if the number $n_{X\varphi}(t, u)$ of individuals to be captured/recaptured/removed in socio-spatial unit u at time t was greater than the number $N_{X\varphi}(t, u)$ available, management action was applied to all available individuals.

Mortality

Annual probabilities of natural mortality depended on age and sex, and were derived from data of other ibex populations (Table 3.2A-5 – Toïgo *et al.*, 2007; Toïgo, pers. comm.).

- (7) For each X -year old individual i , mortality was the outcome of a Bernoulli process with the probability of dying at time t given by:

$$p_i(t) = 1 - (1 - \mu_{X\varphi})^{\frac{1}{52}}$$

with $\mu_{X\varphi}$ the annual probability of mortality (over 52 weeks) of X -year old male or female φ .

- (8) For juveniles of socio-spatial unit u , mortality was the outcome of a Bernoulli process with the probability of dying at time t given by:

$$\begin{cases} p_i(t, u) = 1 - (1 - \mu_0(u))^{\frac{1}{13}}, & \text{if } 1 \leq t \leq 13 \\ p_i(t, u) = 0, & \text{if } 14 \leq t \end{cases}$$

with μ_0 the probability of winter juvenile mortality (over 13 weeks).

TABLE 3.2A-5 | Values of annual probability of mortality depending on age and sex classes.

		X (age in years)							
		0	1-8	9-12	13-15	16	17	18	19
♀	See Fig S1.3.2A		0.01	0.14	0.15	0.15	0.15	0.9	1
♂			0.02	0.15	0.49	0.9	1	-	-

Spatial movements

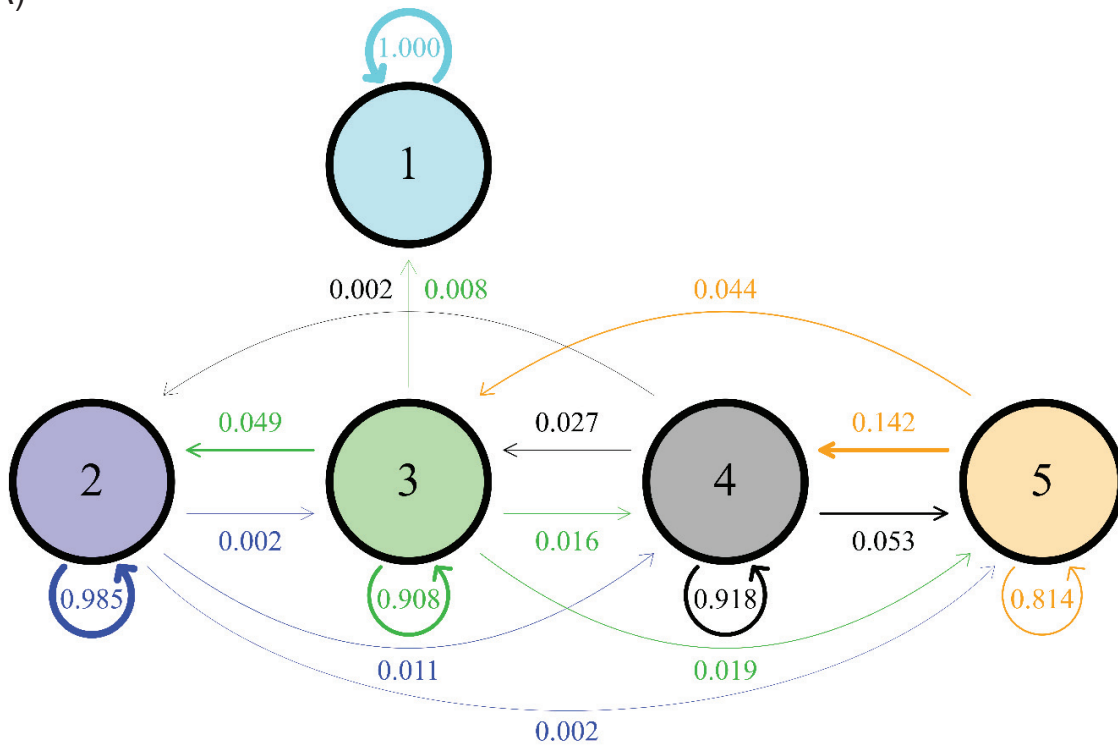
Using GPS data from 51 females and 39 males, we estimated the probability that an ibex belonging to one socio-spatial unit moved to another socio-spatial unit. The probability that an ibex from socio-spatial unit i was found in socio-spatial unit j , and therefore could be engaged in potentially infectious contacts was calculated as (Conner and Miller, 2004):

$$p_{ij} = \frac{\sum_{k=1}^{n_i} R_{ijk}}{n_i}$$

R_{ijk} was set to 1 if the k^{th} location of an ibex from unit i was in unit j and 0 otherwise, and n_i was the total number of locations for all ibex from unit i . For females, this probability was always $p_{ii} = 1$, and $p_{ij} = 0$ for $j \neq i$, thus females stayed in their socio-spatial unit lifelong. Thus, we assumed that females were faithful to their unit and did not engage in any movements between units.

For males, the probability and direction of movements were estimated separately for the two periods where males can be exposed to transmission, *i.e.* during mating period in winter, and in spring when infectious abortions due to *Brucella* occur, because males are especially prone to visit other units during the mating period (Marchand *et al.*, 2017). Then, we converted these probabilities p_{ij} in the corresponding probabilities of movements at each time step p'_{ij} for the two periods (Figure 3.2A-3). Thus, in the model, at each time step, males either stayed in their socio-spatial unit of origin, or moved to another socio-spatial unit with probabilities depending on socio-spatial unit of origin.

(A)



(B)

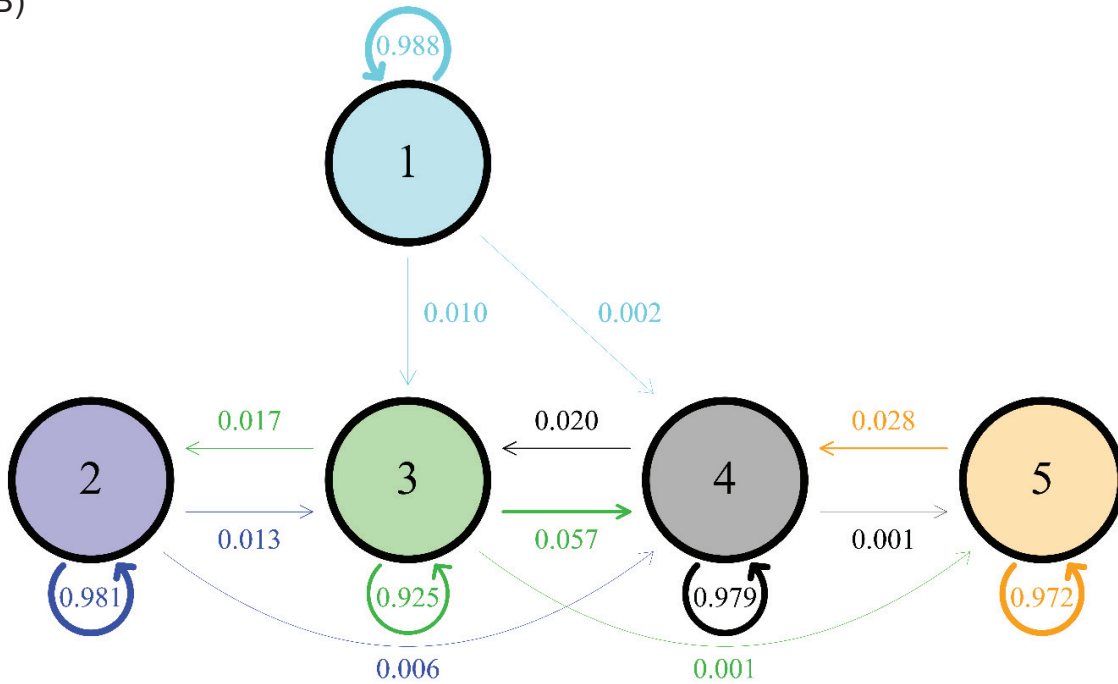


FIGURE 3.2A-3 | Weekly probabilities of movements between socio-spatial units for males during mating period (A) and abortion period (B)

Arrows represent movements and are drawn with the colour of the socio-spatial unit of origin (sky blue: Leschaux-Andey, navy blue: Charmieux-Buclon, green: Jallouvre-Peyre, black: Grand Bargy, orange: Petit Bargy). Arrows are proportional to the probability of movements, which are also explicitly specified for each movement.

(6) During mating period, the movement matrix of males was represented as:

$$M = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 0.985 & 0.002 & 0.011 & 0.002 \\ 0.008 & 0.049 & 0.908 & 0.016 & 0.019 \\ 0 & 0.002 & 0.027 & 0.918 & 0.053 \\ 0 & 0 & 0.044 & 0.142 & 0.814 \end{pmatrix}$$

(7) During abortion period, the movement matrix of males was represented as:

$$M = \begin{pmatrix} 0.988 & 0 & 0.010 & 0.002 & 0 \\ 0 & 0.981 & 0.013 & 0.006 & 0 \\ 0 & 0.017 & 0.925 & 0.057 & 0.001 \\ 0 & 0 & 0.020 & 0.979 & 0.001 \\ 0 & 0 & 0 & 0.028 & 0.972 \end{pmatrix}$$

where $m_{i,j}^\sigma$, the element in i^{th} row and j^{th} column of the movement matrix, is the weekly probability p'_{ij} for a male whose unit of origin is i to move to the unit j at the time step t . Thus, for a male of the socio-spatial unit of origin u ($u \in [1; 5]$), the outcome of the multinomial process to determine the socio-spatial unit at time t depended on the vector of probability $p_i(t, u) = m_{u,*}^\sigma$, where $m_{u,*}^\sigma$ represents all the elements of the u^{th} row of the movement matrix.

Reproduction

For each female, becoming sexually receptive during mating was the outcome of a Bernoulli process.

(8) For each S , E , and R female i in socio-spatial unit u , becoming sexually receptive during mating was the outcome of a Bernoulli process with the probability:

$$p_i(u) = \tau_X(u)$$

with τ_X the probability of being sexually receptive for a X -year old female.

(9) For each I female i in socio-spatial unit u , becoming sexually receptive during mating was the outcome of a Bernoulli process with the probability:

$$p_i(u) = \zeta_\tau \times \tau_X(u)$$

with τ_X the probability of being sexually receptive for a X -year old female and ζ_τ the impact of brucellosis on sexual receptivity probability.

- (10) Then, the week of receptivity was the outcome of a trial with the following probability distribution:

$$\begin{cases} p(\text{week} = t) = \frac{p_1}{2}, & \text{if } 1 \leq t \leq 2 \\ p(\text{week} = t) = \frac{p_2}{d_{rut} - 2}, & \text{if } 3 \leq t \leq d_{rut} \end{cases}$$

with p_1 the proportion of associations during the first 2 weeks of the mating period, p_2 the proportion of associations during the rest of the mating period, and d_{rut} the duration of the mating period.

For each receptive female, n_T tending males and n_C coursing males were sampled for mating association (11-12):

- (11) Tending males were sampled among all available tending males present in the same socio-spatial unit u as the female at time t , following a uniform distribution: for each individual i , the probability to be associated with this female was:

$$p_i(t, u) = \frac{1}{N_{T(t,u)}}$$

where $N_{T(t,u)}$ is the number of available tending males in unit u at time t .

- (12) Coursing males were sampled following a bimodal distribution according to age. For each individual i in socio-spatial unit u , the probability to be associated with this female was:

$$\begin{cases} p_i(t, u) = \frac{p_{C1}}{p_{C1} \times N_{C1(t,u)} + p_{C2} \times N_{C2(t,u)}}, & \text{for coursing males } \leq 5 \text{ years old} \\ p_i(t, u) = \frac{p_{C2}}{p_{C1} \times N_{C1(t,u)} + p_{C2} \times N_{C2(t,u)}}, & \text{for coursing males } 6 - 7 \text{ years old} \end{cases}$$

where $N_{C1(t,u)}$ and $N_{C2(t,u)}$ are the number of available coursing males under 5 years old and 6-7 years old in unit u at time t , respectively.

- (13) Only one male among those associated to a receptive female engaged in copulation. The probability to engage in copulation was:

$$\begin{cases} p_i(t) = \frac{\tau_T}{n_T}, & \text{for tending males} \\ p_i(t) = \frac{\tau_C}{n_C}, & \text{for coursing males} \end{cases}$$

where τ_T and τ_C are copulation probabilities of tending males (8-14 years old) and coursing males (2-7 years old) respectively, and n_T and n_C the number of tending and coursing males respectively associated to the female considered.

Venereal transmission

We considered that all actively infected males and females I shed the bacteria during mating, and could therefore be responsible for venereal transmission, until they became R (non-actively infected), based on the probability of recovery γ .

(14) For male-to-female transmission, a S female i engaged in sexual intercourse with a I male became E following a Bernoulli process with probability:

$$\begin{cases} p_i(t) = v_{ven}^{T\varphi}, & \text{for a female with a tending male} \\ p_i(t) = v_{ven}^{C\varphi} = \kappa \times v_{ven}^{T\varphi}, & \text{for a female with a coursing male} \end{cases}$$

with $v_{ven}^{T\varphi}$ the probability of successful venereal transmission from tending males to females given contact, $v_{ven}^{C\varphi}$ the probability of successful venereal transmission from coursing males to females given contact, and κ the relative efficiency of coursing male-to-female transmission.

(15) For female-to-male transmission, a S male i engaged in sexual intercourse with a I female became E following a Bernoulli process with probability:

$$\begin{cases} p_i(t) = v_{ven}^{\varphi T} = \omega \times v_{ven}^{T\varphi}, & \text{for a tending male} \\ p_i(t) = v_{ven}^{\varphi C} = \omega \times v_{ven}^{C\varphi}, & \text{for a coursing male} \end{cases}$$

with $v_{ven}^{\varphi T}$ the probability of successful venereal transmission from females to tending males given contact, $v_{ven}^{\varphi C}$ the probability of successful venereal transmission from females to coursing males, and ω the relative efficiency of female-to-male transmission.

Abortions or births

For actively infected females I , the shedding status varied according to various factors. Abortion events occurred only once, during the first pregnancy post-infection. The possibility of an abortion to occur depended on females' parity and transmission route. We considered that pregnant females infected by horizontal transmission caused by infectious abortions or births of other females during the same gestation did not abort, as infection was acquired during late gestation (European Commission, 2001). In the case of females infected

by horizontal transmission caused by infectious abortions or births before pregnancy, abortion could occur only on primiparous females. For all other transmission routes (venereal, congenital and pseudo-vertical transmission), abortion could occur during the first pregnancy post-infection (Figure 3.2A-4). In all cases where abortion could occur, abortion probability was $\rho = 0.80$ (FAO and WHO, 1986; Godfroid *et al.*, 2004). When abortion did not occur, females gave birth to a live newborn and shed the bacteria at birth (Figure 3.2A-4). All actively infected females that already went through their first pregnancy following infection shed the bacteria at birth, until infection became non-active and females transitioned to the R class, based on the probability of recovery γ .

- (16) For pregnant I females infected for the first time during mating period, for the first pregnancy of I females vertically infected, and for I primiparous females infected before pregnancy (horizontally or pseudovertically), the occurrence of infectious abortion (IA) due to *Brucella* was the outcome of a Bernoulli process with probability:

$$p_i(t) = \rho$$

with ρ the probability of abortion during the first pregnancy following infection.

If abortion did not occur for those females, their probability of giving birth was 1 and associated births were considered as infectious births (IB).

- (17) In subsequent gestations of I females, as well as for I primiparous females infected horizontally during gestation, and for the first gestation following horizontal infection in I multiparous females, giving birth was the outcome of a Bernoulli process with probability:

$$\begin{cases} p_i(t) = \zeta_\eta \times \eta_1, & \text{for primiparous females} \\ p_i(t) = \zeta_\eta \times \eta_2, & \text{for multiparous females} \end{cases}$$

with η_1 the probability of giving birth for primiparous females, η_2 the probability of giving birth for multiparous females, and ζ_η the impact of brucellosis on probability of giving birth of infectious females.

Associated births were considered as infectious births.

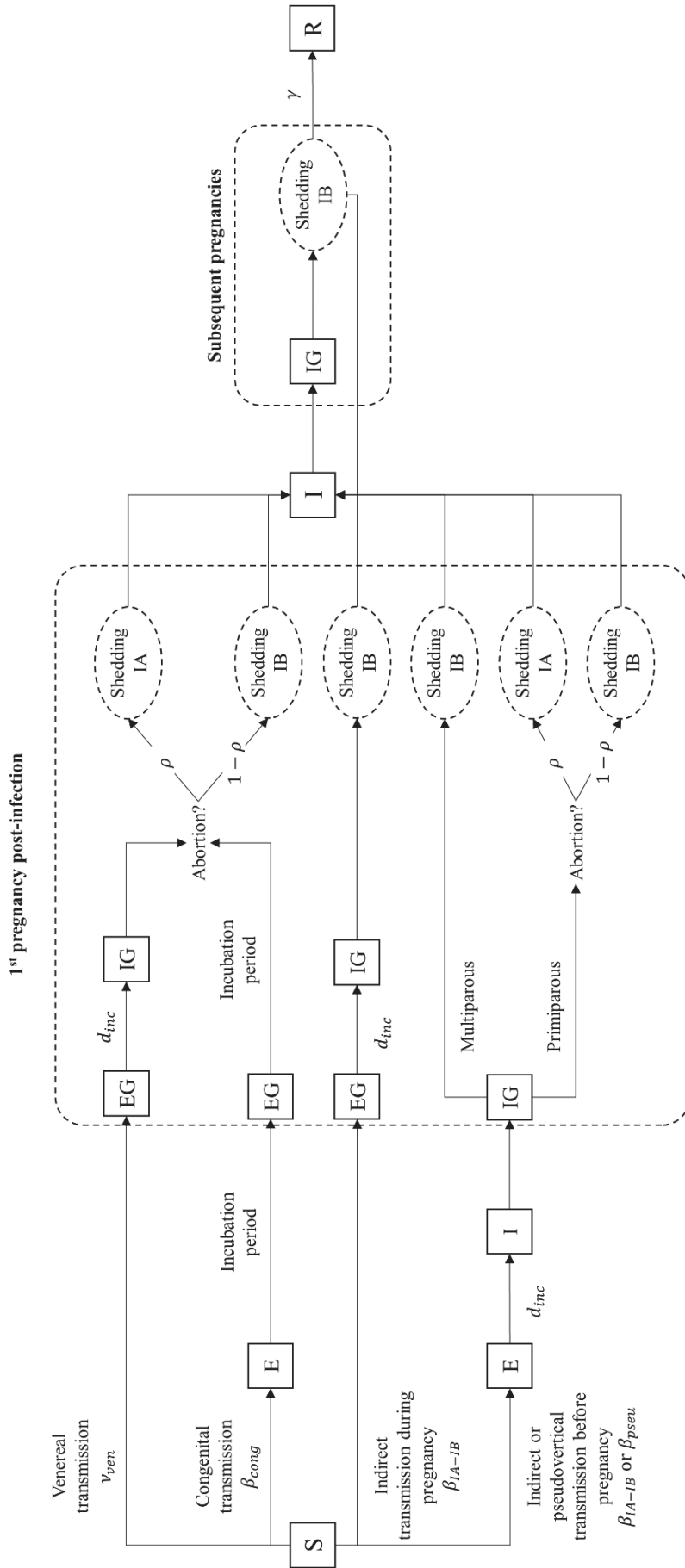


FIGURE 3.2A-4 | Changes in disease state and shedding status of females depending on the routes of transmission and parity. Squares represent health states: S, susceptible to infection, E, exposed, I, actively infected, R, non-actively infected (non-shedders), G, pregnant. Solid horizontal arrows represent flows between compartments. Dashed circles represent the consequences of *B. melitensis* infection on urogenital shedding through infectious abortion (IA) or through infectious birth (IB).

(18) For S and R pregnant females, giving birth was the outcome of a Bernoulli process with probability:

$$\begin{cases} p_i(t) = \eta_1, & \text{for primiparous females} \\ p_i(t) = \eta_2, & \text{for multiparous females} \end{cases}$$

with η_1 the probability of giving birth for primiparous females, and η_2 the probability of giving birth for multiparous females.

For these females, births were not associated with *Brucella* shedding.

The sex of each newborn was the outcome of a Bernoulli process with the probability δ , the proportion of female at birth.

Congenital/pseudo-vertical transmission

All newborns were S , except for newborns of I females who became E through vertical *in utero* transmission as a result of a Bernoulli process with probability β_{cong} . Remaining S newborns of I females were exposed to *Brucella* shedding in colostrum and milk, and became E through this pseudo-vertical route of transmission as a result of a Bernoulli process with probability β_{pseu} .

During the two weeks following parturition, we considered that an actively infected female that shed *Brucella* in genital fluids could only transmit infection to its newborn, which we considered as a special case of pseudo-vertical transmission because ibex females isolate themselves with their newborns during parturition time (Couturier, 1962; Villaret *et al.*, 1997; Bon *et al.*, 2001).

(19) For S kids born from an I female in the interval $[t - d_{isol} + 1; t]$, being infected with the Infectious Birth (IB) of their mother and becoming E was the outcome of a Bernoulli process with probability:

$$p_i(t) = \beta_{IB}$$

with β_{IB} the per capita probability of one female coming into effective contact with one infectious birth (per week), and d_{isol} the duration of postpartum isolation of the mother-offspring couple.

Horizontal transmission

(20) For S individuals in socio-spatial unit u at time t , being infected with Infectious Abortions (IA) of the same unit and becoming E was the outcome of a Bernoulli process with probability:

$$\begin{cases} p_i(t, u) = 1 - (1 - \beta_{IA}^{\circ})^{\Sigma IA(u, t)}, & \text{for females} \\ p_i(t, u) = 1 - (1 - \sigma \times \beta_{IA}^{\circ})^{\Sigma IA(u, t)}, & \text{for males } \leq 5 \text{ years old} \\ p_i(t, u) = 1 - (1 - \phi \times \beta_{IA}^{\circ})^{\Sigma IA(u, t)}, & \text{for males } > 5 \text{ years old} \end{cases}$$

with β_{IA}° the per capita probability of one female coming into effective contact with one infectious abortion (per week), σ the reduction in the number of contacts with infectious abortion for males 5 years of age and under, such as $\beta_{IA}^{\sigma \leq 5} = \sigma \times \beta_{IA}^{\circ}$, ϕ the reduction in the number of contacts with infectious abortion for males over 5 years of age such as $\beta_{IA}^{\sigma > 5} = \phi \times \beta_{IA}^{\circ}$, $IA(u, t)$ the number of females in socio-spatial unit u alive at time t which aborted in the interval $[t - d_{shed} + 1; t]$ and d_{shed} the duration of shedding of *Brucella* in genital fluids after infectious birth or abortion.

(21) For S adult females who gave birth before $t - d_{isol} + 1$, or for S newborn males or females born before $t - d_{isol} + 1$, being infected with Infectious Births (IB) and becoming E was the outcome of a Bernoulli process with probability:

$$p_i(t) = 1 - (1 - \beta_{IB})^{\Sigma IB(u, t)}$$

with β_{IB} the per capita probability of one female or newborn coming into effective contact with one infectious birth (per week), $IB(u, t)$ the number of females in socio-spatial unit u alive at time t which gave birth in the interval $[t - d_{shed} + 1; t - d_{isol}]$, d_{shed} the duration of shedding of *Brucella* in genital fluids after infectious birth or abortion, and d_{isol} the duration of postpartum isolation of the mother-offspring couple.

Incubation and recovery

After d_{inc} weeks of incubation, E individuals became I , except for E individuals infected through *in utero* transmission, as infection remained latent in these individuals and they stayed in state E up to the first abortion or birth for females, and up the age of sexual maturity in males (Plommet *et al.*, 1973).

(22) Then, at each time step, the probability for an I individual i to become R was:

$$p_i(t) = 1 - (1 - \gamma)^{\frac{1}{52}}$$

with γ the annual probability of recovery.

To estimate the probability of recovery γ , we used data on the proportion of actively infected individuals (defined as having at least one positive culture in any organ) by age among seropositive (ever-infected) individuals (Lambert *et al.*, 2018b). This proportion resulted from two processes: on the one hand, susceptible individuals entering the compartment of actively infected individuals, as a result of infection; on the other hand, infected individuals leaving the compartment of actively infected individuals, as a result of recovery.

We therefore developed a simple catalytic model (Muench, 1934), tracking individuals from birth, and following the evolution of the proportion of individuals that have ever been infected at different ages.

The corresponding differential equations (for proportions) are:

$$\frac{ds(a)}{da} = -\lambda s(a) \text{ and } \frac{dz(a)}{da} = \lambda s(a)$$

where $s(a)$ is the proportion of susceptible individuals at age a , $z(a)$ is the proportion of (ever)-infected individuals, and λ is the average force of infection (FOI). The force of infection represents the rate at which susceptible individuals become infected per unit time (Vynnycky and White, 2010).

The assumptions are that the average force of infection λ is independent of age and year, and that susceptible and infected animals have the same mortality rate (i.e., no brucellosis-related mortality).

If we consider that all individuals are susceptible at birth ($s(0) = 1$ when $a = 0$), then:

$$s(a) = e^{-\lambda a} \text{ and } z(a) = 1 - e^{-\lambda a}$$

We fitted this simple catalytic model to the observed age seroprevalence data from our population, using maximum likelihood estimation (MLE) procedure. The 95% profile

likelihood confidence interval (CI) was calculated based on the likelihood ratio statistic $2(L_g - L_r)$, where the general model g was the model which maximize the log-likelihood of the model, whereas the restricted model r was the model which considers $\hat{\lambda}$ as a known constant. The 95% profile likelihood confidence interval were the values of $\hat{\lambda}$ for which $3.84 > 2(L_g - L_r)$ (Heisey *et al.*, 2006).

The estimated force of infection was $\hat{\lambda} = 0.0782 [0.0654 - 0.0928] \text{ year}^{-1}$, with a log-likelihood of $L = -223.97$. The corresponding fitted model is illustrated in Figure 3.2A-5A.

We then divided the (ever)-infected compartment in two compartments, the ones with active infection $i(a)$ and the ones with no active infection $r(a)$, with $z(a) = i(a) + r(a)$. The corresponding differential equations (for proportions) are:

$$\frac{di(a)}{da} = \lambda s(a) - \gamma' \times i(a) \text{ and } \frac{dr(a)}{da} = \gamma' \times i(a)$$

where γ' is the recovery rate, assumed to be independent of age and time, and where the mortality rate of i and r are supposed to be similar. As $s(a) = e^{-\lambda a}$, we can write: $\frac{di(a)}{da} = \lambda e^{-\lambda a} - \gamma' \times i(a)$. Solutions of this differential equation are of the form: $i(a) = K(a) \times e^{-\gamma' a}$, where $K(a)$ is a function of age that we have to determine.

First, we calculated the derivative:

$$\frac{di(a)}{da} = \frac{dK(a)}{da} \times e^{-\gamma' a} - \gamma' K(a) \times e^{-\gamma' a} = \frac{dK(a)}{da} \times e^{-\gamma' a} - \gamma' \times i(a)$$

We also know that $\frac{di(a)}{da} = \lambda e^{-\lambda a} - \gamma' \times i(a)$, thus we get :

$$\frac{dK(a)}{da} \times e^{-\gamma' a} - \gamma' \times i(a) = \lambda e^{-\lambda a} - \gamma' \times i(a)$$

Finally, we obtain:

$$\frac{dK(a)}{da} \times e^{-\gamma' a} = \lambda e^{-\lambda a} \Leftrightarrow \frac{dK(a)}{da} = \lambda e^{(\gamma' - \lambda)a} \Leftrightarrow K(a) = \frac{\lambda}{\gamma' - \lambda} e^{(\gamma' - \lambda)a} + cte$$

Solutions are therefore: $i(a) = \left[\frac{\lambda}{\gamma' - \lambda} e^{(\gamma' - \lambda)a} + cte \right] \times e^{-\gamma' a}$

If we consider that no individual is infectious at birth ($i(0) = 0$ when $a = 0$ – which is the same assumption we made for the force of infection), *i.e.*, that all individuals are susceptible at birth ($s(0) = 1$), then we get $cte = -\frac{\lambda}{\gamma' - \lambda}$ and: $i(a) = \frac{\lambda}{\gamma' - \lambda}(e^{-\lambda a} - e^{-\gamma' a})$

Therefore, the proportion of actively infected individuals by age among seropositive (ever-infected) individuals is:

$$\frac{i(a)}{i(a) + r(a)} = \frac{i(a)}{1 - s(a)} = \frac{\frac{\lambda}{\gamma' - \lambda}(e^{-\lambda a} - e^{-\gamma' a})}{1 - e^{-\lambda a}} = \frac{\lambda(e^{-\lambda a} - e^{-\gamma' a})}{(\gamma' - \lambda)(1 - e^{-\lambda a})}$$

Using as previously a maximization function of the log-likelihood of the model and the estimated value $\hat{\lambda} = 0.0782$, the estimated recovery rate was $\hat{\gamma}' = 0.1700$ [0.1163 – 0.2436] $year^{-1}$, with a log-likelihood of $L = -56.24$. The corresponding fitted model is illustrated in Figure 3.2A-5B. To obtain the probability of recovery γ , *i.e.* the proportion of infectious that become non-infectious in one time step, we calculated: $\gamma = 1 - e^{-\hat{\gamma}'} = 0.1564$ [0.1098 – 0.2162].

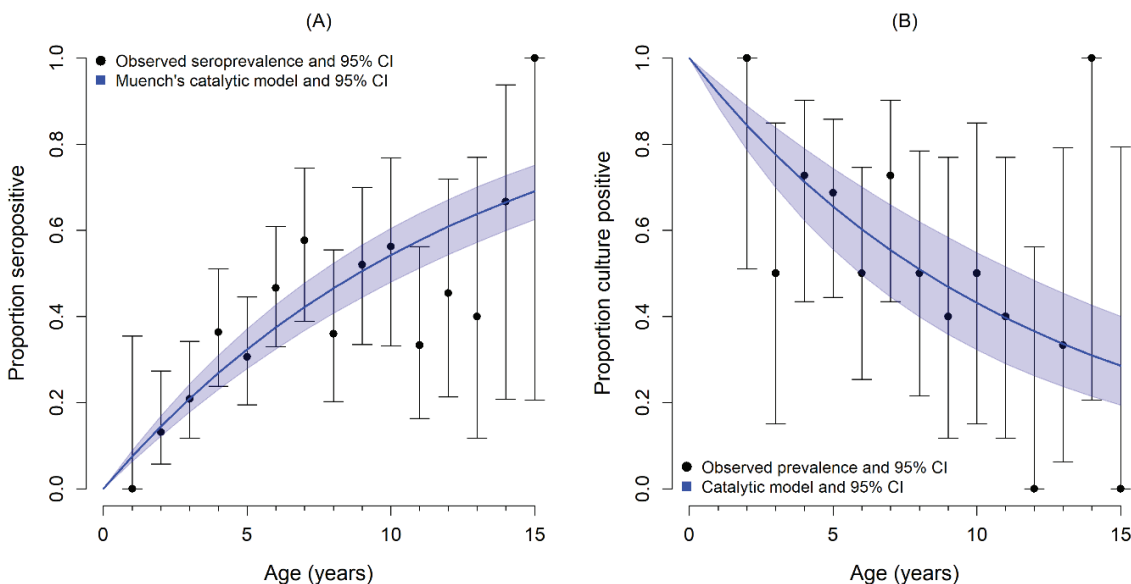


FIGURE 3.2A-5 | Fitting curves of the proportion of seropositive (ever-infected) individuals (A) and of the proportion of culture positive (actively infected) individuals among seropositive ones (B).

Blue lines represent fitted curves, and light blue areas represent the 95% confidence interval (CI) of the fitted model. Black dots represent observed proportions, and black arrows represent the associated 95% CI.

APPENDIX 3.2B: PARAMETER ESTIMATION

Due to the complexity of our stochastic individual-based simulation model and the inability to have a tractable likelihood function (Hartig *et al.*, 2011), we used an Approximate Bayesian Computation (ABC) algorithm (Beaumont *et al.*, 2002; Beaumont, 2010; Csilléry *et al.*, 2010). This approach was used before for complex infectious diseases models (Brooks-Pollock *et al.*, 2014; Kosmala *et al.*, 2016) and individual-based models (van der Vaart *et al.*, 2015). ABC has the advantage to approximate the likelihood by running the model a large number of times and comparing simulated outputs to the observed data (Toni *et al.*, 2009).

1. The ABC rejection algorithm

We used the ABC-rejection algorithm, based on the `abc` R package (Csilléry *et al.*, 2012), with the following steps:

1. Sample a set of parameter values θ_i from prior distributions.
2. Simulate a dataset y_i using the set of parameter values θ_i .
3. Compute the summary statistics for simulated outputs $S(y_i)$ and for observed data $S(y_0)$.
4. Compare the summary statistics computed from the simulated outputs $S(y_i)$ and the summary statistics obtained from the observed data $S(y_0)$ using the distance function d and a given (arbitrary) threshold ϵ . Accept the parameter value θ_i if the simulated summary statistic is close enough to the observed summary statistics, *i.e.* if $d(S(y_i), S(y_0)) \leq \epsilon$.
5. Return to 1.

We repeated the algorithm 10^6 times, *i.e.* we used simulations from 10^6 sets of parameter values from the prior distributions. In practice, the threshold is defined by using a tolerance rate, chosen as the proportion of accepted simulations (Csilléry *et al.*, 2012). For instance, if the tolerance rate is 0.001, then the number of accepted simulations will be 1000. The accepted θ_i then form a sample from an approximation of the posterior distribution.

2. Choice of summary statistics

In ABC, the dimensionality of the simulated outputs and observations is reduced through the use of summary statistics (Hartig *et al.*, 2011). We chose two types of summary statistics for use in the ABC approach, using two types of available data:

- The first concerned the population size over time. The simulated population size was compared to the estimated population size in 2013: 567 (95% Confidence Interval: [487-660]), 2014: 310 [275-352], 2015: 277 [220-351], 2016: 272 [241-312], and 2017: 291 [262-327].
- The second concerned the results of test-and-remove operations in autumn 2012, spring 2013, spring 2014, spring and autumn 2015, spring and autumn 2016, spring 2017 and spring 2018: number of captured and recaptured animals and expected number of seropositive individuals captured or recaptured were computed and compared to observed data in each sex and age classes and in each socio-spatial unit (Tables 3.2B-1 and 2).

The summary statistics for simulated data were two Euclidean distances between simulated outputs and observed data:

$$S(y_i^{pop}) = \sqrt{\sum_j (popsize_{sim}^j - popsize_{obs}^j)^2}$$

$$S(y_i^{capt}) = \sqrt{\sum_j (capt_{sim}^j - capt_{obs}^j)^2}$$

where $S(y_i^{pop})$ and $S(y_i^{capt})$ are the summary statistics for the simulated population size and test-and-remove operations results respectively for the i^{th} simulation; $popsize_{sim}^j$ and $popsize_{obs}^j$ are respectively the simulated and observed population size for data point j ; $capt_{sim}^j$ and $capt_{obs}^j$ are respectively the simulated and observed result of test-and-remove operation for data point j .

Because the number of data points between the two types of summary statistics were very different (5 and 266 respectively), we adopted a two-step approach: we performed the ABC rejection algorithm a first time, using data of population size only; then, we performed the ABC rejection algorithm a second time, using data of test-and-remove operations only, and using the posterior distribution of the first ABC as the prior distribution in the second.

TABLE 3.2B-1 | Number of animals captured + recaptured and seropositive in each age- and sex-classes aggregated by socio-spatial unit during capture operations in autumn (Aut.) and spring (Spr.) between 2012 and 2018.

	Aut. 2012		Spr. 2013		Spr. 2014		Spr. 2015		Aut. 2015		Spr. 2016		Aut. 2016		Spr. 2017		Spr. 2018		
	Sero+	Total	Sero+	Total	Sero+	Total	Sero+	Total	Sero+	Total	Sero+	Total	Sero+	Total	Sero+	Total	Sero+	Total	
♀																			
≤ 5y	0+0	2+0	2+0	16+0	11+0	19+2	7+0	20+1	0+0	6+0	1+0	3+0	0+0	0+2	3+0	9+1	1+0	12+0	
> 5y	9+0	12+0	9+0	13+0	8+0	13+0	13+1	24+2	0+0	8+0	2+1	5+2	0+0	0+4	4+0	6+1	2+0	5+5	
♂																			
≤ 5y	0+0	2+0	3+0	15+0	7+1	18+1	6+1	21+6	0+0	3+0	0+0	3+1	0+0	5+0	0+0	3+3	2+0	16+1	
> 5y	3+0	8+0	6+0	12+1	4+0	11+7	10+0	19+13	0+0	1+1	1+0	3+0	0+0	1+6	0+0	4+0	1+0	1+8	
TOTAL	12	24	20	57	31	71	38	106	0	19	5	17	0	18	7	27	6	48	

For animals captured several times, age was determined from the estimated age at first capture incremented by the time between first capture and time of recapture.

TABLE 3.2B-2 | Number of animals captured + recaptured and seropositive in each socio-spatial unit aggregated by age- and sex-classes during capture operations in autumn (Aut.) and spring (Spr.) between 2012 and 2018.

	Aut. 2012		Spr. 2013		Spr. 2014		Spr. 2015		Aut. 2015		Spr. 2016		Aut. 2016		Spr. 2017		Spr. 2018		
	Sero+	Total	Sero+	Total	Sero+	Total	Sero+	Total	Sero+	Total	Sero+	Total	Sero+	Total	Sero+	Total	Sero+	Total	
Unit 1	0+0	0+0	1+0	7+0	0+0	3+1	4+0	21+3	0+0	5+0	0+0	0+0	0+0	4+4	0+0	0+0	0+0	7+1	
Unit 2	0+0	0+0	1+0	8+0	0+0	0+1	0+0	8+2	0+0	13+0	0+0	0+0	0+0	1+1	0+0	3+1	1+0	11+0	
Unit 3	8+0	18+0	6+0	16+1	19+0	35+5	21+2	32+8	0+0	0+0	1+1	7+3	0+0	1+5	0+0	5+3	3+0	8+10	
Unit 4	4+0	6+0	4+0	8+0	6+0	6+0	5+0	6+1	0+0	0+0	3+0	7+0	0+0	0+1	5+0	7+1	0+0	3+0	
Unit 5	0+0	0+0	8+0	17+0	5+1	17+3	6+0	17+8	0+0	0+1	0+0	0+0	0+0	0+1	2+0	7+0	2+0	5+3	
TOTAL	12	24	20	57	31	71	38	106	0	19	5	17	0	18	7	27	6	48	

For animals captured several times, socio-spatial unit was determined from the socio-spatial unit at first capture.

3. Choice of parameters to estimate with ABC method

3.1 Uncertain parameters

To the exception of K (the carrying capacity), the demographic parameters (see Table 3.2-1 in the main text and Tables 3.2A-4 and 5 in Appendix 3.2A) were calibrated by integrating knowledge from published and unpublished data on ibex demography and behaviour in our study site or others, as well as from experts' knowledge (ANSES, 2015).

Some epidemiological parameters (see Table 3.2-2 in the main text) were calibrated using published data on brucellosis in domestic ruminant, experts' knowledge and unpublished data on brucellosis in ibex (ANSES, 2015), and published knowledge on ibex contact behaviour. The probability of recovery was also derived from data on bacteriological cultures in seropositive ibex of the study site (see Appendix 3.2A).

After examining literature and data, we were left with twelve parameters (eleven epidemiological parameters and the carrying capacity), that we considered as the most uncertain in our model (Table 3.2B-3 – see Table 3.2-1 and Table 3.2-2 in the main text for parameters definitions). We classified these twelve parameters in three categories, depending on the degree of available knowledge on these parameters (1: absence of knowledge, 2: derived from unpublished data on the study site, 3: published data or experts' knowledge).

3.2 Sensitivity analysis

In a second time, we performed a sensitivity analysis on these twelve parameters. The objective was twofold: identifying the parameters that influence the most the variability of the simulated outputs, which should be estimated in priority; and also identifying the parameters that influence the less the variability, which can be fixed without affecting model outputs (Cariboni et al., 2007). We indeed wanted to reduce the number of parameters so as to sufficiently explore the space of parameters and to get a realistic number of parameters to be fitted to the available data.

TABLE 3.2B-3 | Literature (when available) and range values of the twelve most uncertain parameters, categorised from 1 (most uncertain) to 3 (less uncertain).

	Literature value	Range	Range justification	Category
$\beta_{IA}^{\varnothing} = \beta_{IB}$	-	[0 - 1]	Probability	1
$\nu_{ven}^{T\varnothing}$	-	[0 - 1]	Probability	1
K	-	[400 - 800]	*	2
γ	-	[0.08 - 0.64]	From half to 4 times the nominal value	2
σ	-	[0.10 - 0.75]	**	2
ϕ	-	[0.03 - 0.13]	**	2
ζ_{τ}	-	[0.61 - 0.99]	***	2
ζ_{η}	-	[0.81 - 0.99]	***	2
κ	0.45 ^[1]	[0 - 1]	Reduction factor	3
ω	0.44 ^[2] , 0.53 ^[3] , 0.60 ^[4,5]	[0 - 1]	Reduction factor	3
$\beta_{cong} = \beta_{pseu}$	0.05 ^[6,7]	[0 - 0.10]	†	3
ρ	0.54 ^[8] , 0.72 ^[9] , 0.80 ^[10] , 0.85 ^[11] , 0.96 ^[12]	[0.50 - 1]	Probability and literature values	3

* Demographic data (C. Toïgo, pers. comm.)

** GPS data (P. Marchand, pers. comm.)

*** Data on reproductive success (C. Toïgo, pers. comm.)

† Experts knowledge (ANSES, 2015)

[1] Willisch and Neuhaus (2009)

[2] Nicolosi *et al.* (1994)

[3] European study group on heterosexual transmission of HIV (1992)

[4] Augustine (1998)

[5] Brunham *et al.* (1993)

[6] FAO and WHO (1986)

[7] Godfroid *et al.* (2013)

[8] Thorne *et al.* (1978)

[9] Plenderleith (1970)

[10] Godfroid *et al.* (2004)

[11] Manthei and Carter (1950)

[12] Ebinger *et al.* (2011)

3.2.1 Sensitivity analysis design

Sensitivity analysis is often restricted to a single output variable. Here, because we had numerous outputs and were interested in the overall impact of each parameters on model outputs, we performed instead a multivariate global sensitivity analysis on all simulated outputs, implemented in the multisensi R package (Lamboni *et al.*, 2009; Bidot *et al.*, 2017).

To simulate the outputs, we used a fractional factorial plan - FFP (Box *et al.*, 1978). Input parameters were the 12 parameters described above. We used 5 levels (values) per parameter: the median, the 2.5, 25, 75 and 97.5 percentiles of their range values (Table 3.2B-4).

TABLE 3.2B-4 | Parameter values used in the factorial design of the sensitivity analysis.

	Range	Values tested in the sensitivity analysis				
		410	500	600	700	790
K	[400 - 800]	410	500	600	700	790
ρ	[0.50 - 1]	0.5125	0.625	0.75	0.875	0.9875
γ	[0.08 - 0.64]	0.0940	0.220	0.36	0.500	0.6260
$\beta_{IA}^{\varphi} = \beta_{IB}$	[0 - 1]	0.0250	0.250	0.50	0.750	0.9750
σ	[0.10 - 0.75]	0.11625	0.2625	0.425	0.5875	0.73375
ϕ	[0.03 - 0.13]	0.0325	0.055	0.08	0.105	0.1275
$\nu_{ven}^{T\varphi}$	[0 - 1]	0.0250	0.250	0.50	0.750	0.9750
κ	[0 - 1]	0.0250	0.250	0.50	0.750	0.9750
ω	[0 - 1]	0.0250	0.250	0.50	0.750	0.9750
$\beta_{cong} = \beta_{pseu}$	[0 - 0.10]	0.0025	0.025	0.05	0.075	0.0975
ζ_{τ}	[0.61 - 0.99]	0.6195	0.705	0.80	0.895	0.9805
ζ_{η}	[0.81 - 0.99]	0.8145	0.855	0.90	0.945	0.9855

Using planor R package (Kobilinsky *et al.*, 2015), we generated a fractional factorial plan of resolution V, which allowed us to estimate all of the main effects and first order interactions assuming that higher order interactions are negligible (Kobilinsky, 1997; Bailey, 2008).

This design represented $5^5 = 3125$ scenarios (one scenario was a set of parameter values). As our model was stochastic, 400 iterations were performed per scenario. This number of iterations allowed us to obtain convergence of median and 2.5 and 97.5 percentiles of all simulated outputs (not shown).

We applied global sensitivity analysis after multivariate dimension reduction on the mean and variance of all simulated outputs using principal component analysis – PCA (Lamboni *et al.*, 2009; Bidot *et al.*, 2017). For each parameter, generalized sensitivity indexes *GSI* were calculated and were represented by Pareto plots.

3.2.2 Sensitivity analysis results

Three main parameters stood out with generalized sensitivity indices over 0.10:

1. K , the carrying capacity;
2. $\beta_{IA}^{\circ} = \beta_{IB}$, the per-capita probability of one female coming into effective contact with one infectious abortion or birth;
3. $\nu_{ven}^{T\circ}$, the probability of successful venereal transmission from tending males to females given contact (Figure 3.2B-1).

Results were similar when performing the sensitivity analysis on only the mean of the simulated outputs, only their variance, or both (not shown).

First-order interactions contributed significantly to global sensitivity indices for many parameters. For the three main parameters described above, the contributions to the interaction sensitivity indices are described in Table 3.2B-5. In each case, the two other main parameters were responsible for more than half of the contribution to the interaction sensitivity indices.

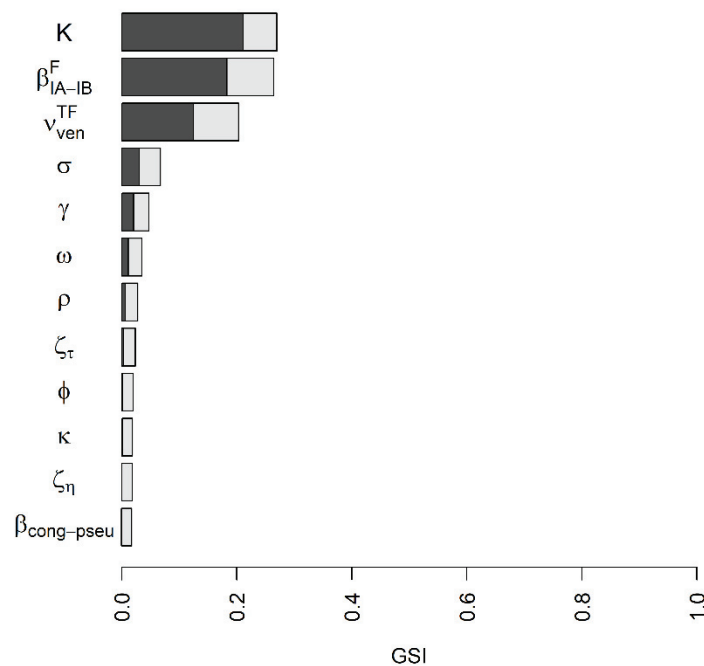


FIGURE 3.2B-1 | Generalised sensitivity indices for all simulated summary statistics. The main sensitivity indices are in dark bars and interaction ones are in pale bars. The total length represents the total sensitivity index.

TABLE 3.2B-5 | Contribution of model parameters to the interaction sensitivity indices of the three parameters with the highest global sensitivity indices.

	K	$\beta_{IA}^{\circ} = \beta_{IB}$	$\nu_{ven}^{T^{\circ}}$	Others
K	–	33.4%	28.7%	37.9%
$\beta_{IA}^{\circ} = \beta_{IB}$	24.1%	–	38.3%	37.6%
$\nu_{ven}^{T^{\circ}}$	21.4%	39.7%	–	38.9%

3.3 Parameters choice for ABC and prior distributions

Given the results of the sensitivity analysis above, we chose to perform the ABC estimation on the three parameters K , $\beta_{IA}^{\circ} = \beta_{IB}$ and $\nu_{ven}^{T^{\circ}}$. Fixed values were used for all other parameters (see Table 3.2-2 in the main text).

For the three parameters selected, we used a uniform prior distribution on their range value for the first ABC. The 10^6 sets of parameter values were sampled from the prior distributions using Latin hypercube sampling (LHS) available in the `tgpr` R package (Gramacy, 2007). For the second ABC, we used the posterior distribution of the first ABC as prior distribution in which 10^6 new sets of parameter values were sampled.

4. Numerical identifiability

We used a numerical identifiability procedure to know if ABC could accurately estimate the chosen parameters using the available data. We used model outputs as “pseudo-data” and then used ABC with these data to perform parameter estimation. Because the parameter values used for producing the “pseudo-data” were known, the result of the ABC could be compared with the real parameter value, and thus determine if ABC could accurately identify this value.

To do this, we used the cross-validation function from `abc` R package (Csilléry *et al.*, 2012). The function randomly chose a simulation among the 10^6 , and used the simulated data as “pseudo-data” to compute the summary statistics. It then performed the ABC rejection algorithm on the remaining simulations, accepting the θ_i closest to the “pseudo-data”.

The principle was then to compare the mean or the median of the posterior distribution obtained with the ABC, to the actual value used in the simulation picked to simulate the pseudo-data. This was repeated 100 times (Csilléry *et al.*, 2012).

We computed the prediction error:

$$E_{pred} = \frac{\sum_{i=1}^{100} (\hat{\theta}_i - \theta_i)}{100 \times \text{Var}(\{\theta_i | i = 1:100\})}$$

where θ_i was the true parameter value of the i^{th} randomly chosen simulation among the 100, $\hat{\theta}_i$ was the corresponding estimated parameter value (mean or median of the posterior distribution), and $\text{Var}(\{\theta_i | i = 1:100\})$ was the variance of the randomly chosen 100 true parameter values (Csilléry *et al.*, 2012).

We also looked at the proportion of cases for which the true parameter values were inside the 95% credible interval of the posterior distribution, and the proportion of cases for which each of the true parameter values were inside the 95% credible interval of their posterior distributions. Finally, we computed the correlation between estimated and true parameter values.

The prediction error was the best (lowest) for K , and between 0.2 and 0.5 for the other two parameters (Table 3.2B-6) when considering the mean of the posterior distribution as the estimated parameter value. The proportion of cases for which the true parameter value was inside the 95% credible interval of the posterior distribution was always above 94% for each of the parameters. Correlations between estimated and true parameter values were 0.78 for $v_{ven}^{\text{T}\varnothing}$, and more than 0.85 for K and $\beta_{IA}^{\varnothing} = \beta_{IB}$ (Figure 3.2B-2). The results of the cross-validation were qualitatively and quantitatively similar when considering the median of the posterior distribution as the estimated parameter value (Table 3.2B-6 and Figure 3.2B-2).

The cross-validation procedure allowed us to show that the mean and median of the accepted simulations were mostly accurate for our three parameters, K being the parameter that is the most accurate, followed by $\beta_{IA}^{\varnothing} = \beta_{IB}$ and then $v_{ven}^{\text{T}\varnothing}$. Figure 3.2B-2 also showed that the accuracy of $\beta_{IA}^{\varnothing} = \beta_{IB}$ seemed better for small values. Besides, the results seemed robust to the tolerance rate (not shown), and thus the larger tolerance rate of 0.001 was used for the parameter estimation, so as to obtain enough sets of parameter values in the posterior distribution.

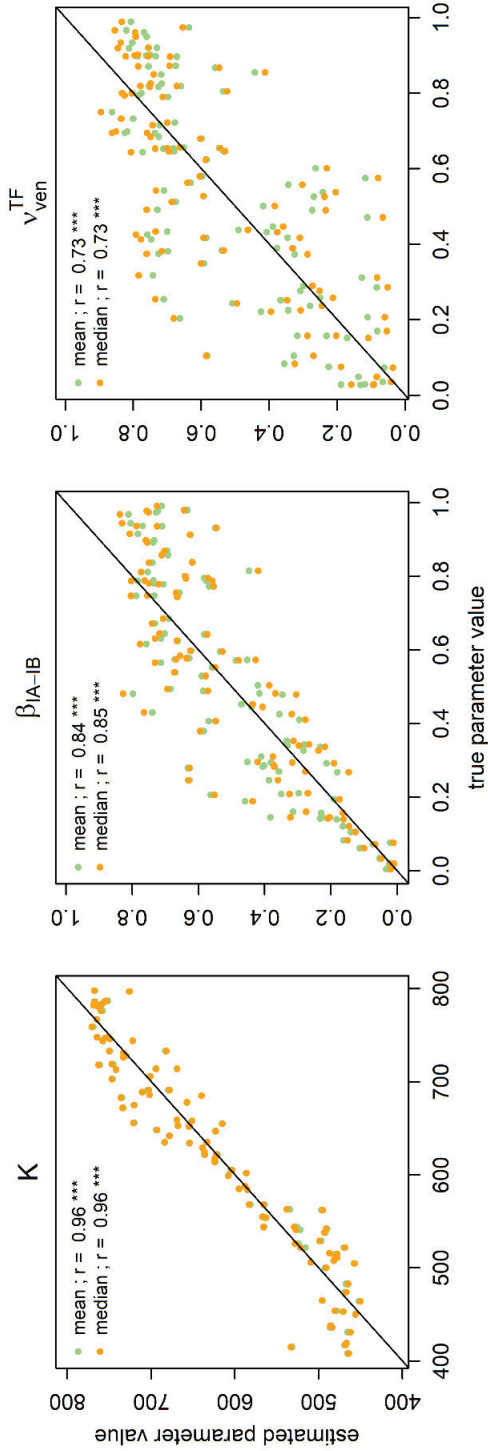


FIGURE 3.2B-2 | Relationship between true and estimated parameter values for the mean or median of the posterior distribution, with a tolerance rate of 0.001. Correlation coefficients r are shown, asterisks denote significance ($p < 0.001$, corrected for multiple testing using Holm's method).

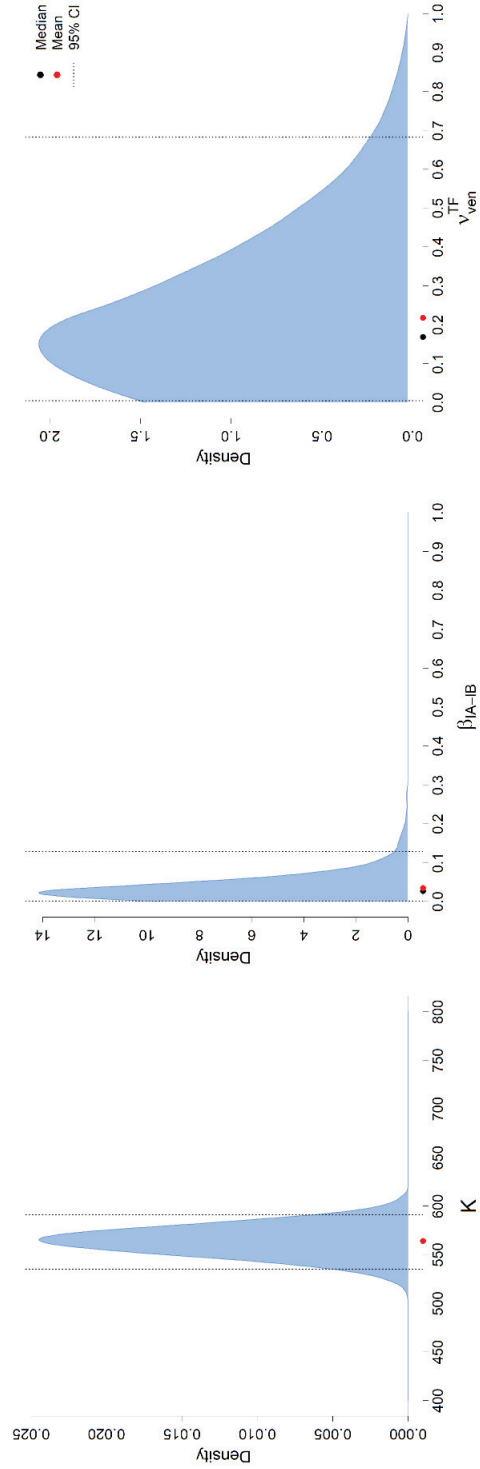


FIGURE 3.2B-3 | Posterior distributions of parameter values, with a tolerance rate of 0.001. The mean and median are respectively represented by a red and a black dot on the x axis. The 95% credible interval is delimited by black dotted lines.

TABLE 3.2B-6 | Prediction error and proportion of cases for which the true parameter value was inside the 95% credible interval of the posterior distribution for the three parameters.

	E_{pred}			p			
	K	$\beta_{IA}^{\varnothing} = \beta_{IB}$	$\nu_{ven}^{T\varnothing}$	K	$\beta_{IA}^{\varnothing} = \beta_{IB}$	$\nu_{ven}^{T\varnothing}$	All
Mean	0.08	0.29	0.48	0.94	0.96	0.94	0.85
Median	0.08	0.28	0.52	0.94	0.96	0.94	0.85

5. Results of the ABC estimation

We performed the rejection algorithm on the 10^6 simulations. The 1000 accepted θ_i , whose simulated summary statistics were closest to the summary statistics derived from the observed data, formed an approximation of the posterior distribution.

The three posterior distributions were significantly narrower than their prior (Levene's test, $p < 0.001$ after correcting for multiple testing using Holm's method). The summary statistics derived from the real data were therefore informative and allowed the ABC algorithm to narrow the parameters distribution, especially those of K and $\beta_{IA}^{\varnothing} = \beta_{IB}$ (Figure 3.2B-3).

The estimated parameter values were (median and 95% credible interval): $K = 564$ [535 – 591] for the carrying capacity; $\beta_{IA}^{\varnothing} = \beta_{IB} = 0.026$ [0.001 – 0.128] for the per-capita probability of one female to come into effective contact with one infectious abortion or birth per week; and $\nu_{ven}^{T\varnothing} = 0.168$ [0.005 – 0.682] for the probability of successful venereal transmission from tending males to females given contact.

We evaluated the correlations between the accepted parameter values (Figure 3.2B-4), and we found a correlation of 0.018 between K and $\nu_{ven}^{T\varnothing}$, of about -0.14 between $\beta_{IA}^{\varnothing} = \beta_{IB}$ and K and of -0.25 between $\nu_{ven}^{T\varnothing}$ and $\beta_{IA}^{\varnothing} = \beta_{IB}$.

The model was then run with 1000 iterations, each iteration corresponding to a set of parameter values drawn from the posterior distributions obtained with ABC. The weekly population size without newborns is shown in the main text in Figure 3.2-3A, with the population size estimates obtained in the field from 2013 to 2018. The number of captured and recaptured animals are detailed by age- and sex-classes and by socio-spatial units in Figures 3.2B-5 to 8, and the number of seropositive animals during captures and recaptures are detailed by age- and sex-classes and by socio-spatial units in Figures 3.2B-9 to 12.

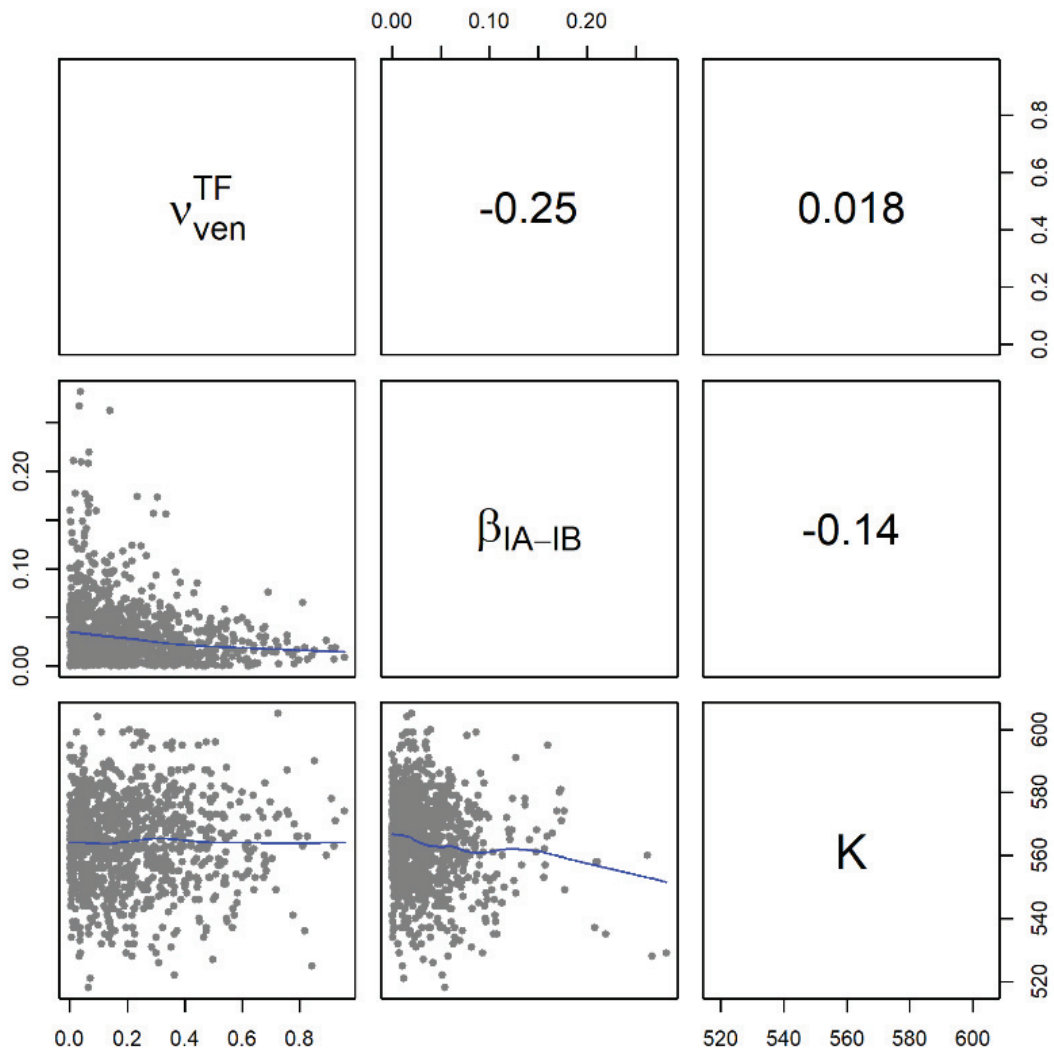


FIGURE 3.2B-4 | Correlations between accepted parameter values using ABC rejection algorithm and a tolerance rate of 0.001.

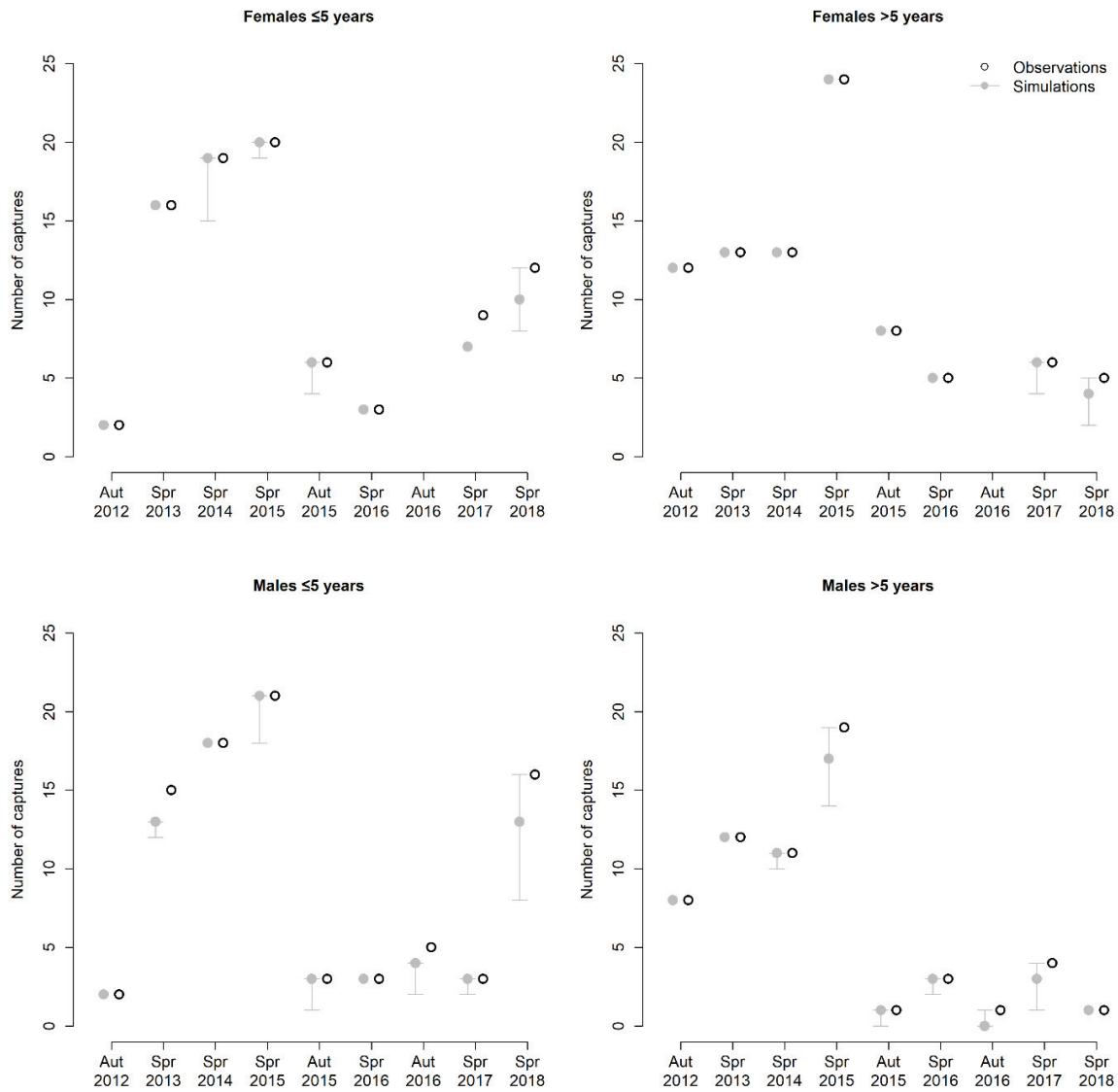


FIGURE 3.2B-5 | Fit of the model to the numbers of captured animals by age- and sex-classes, aggregated by socio-spatial units.

The grey points and solid grey lines are the median and 95% credible intervals (respectively) of the simulated numbers of captured animals over the 1000 iterations. The open black circles are the observed numbers of captured animals in the field.

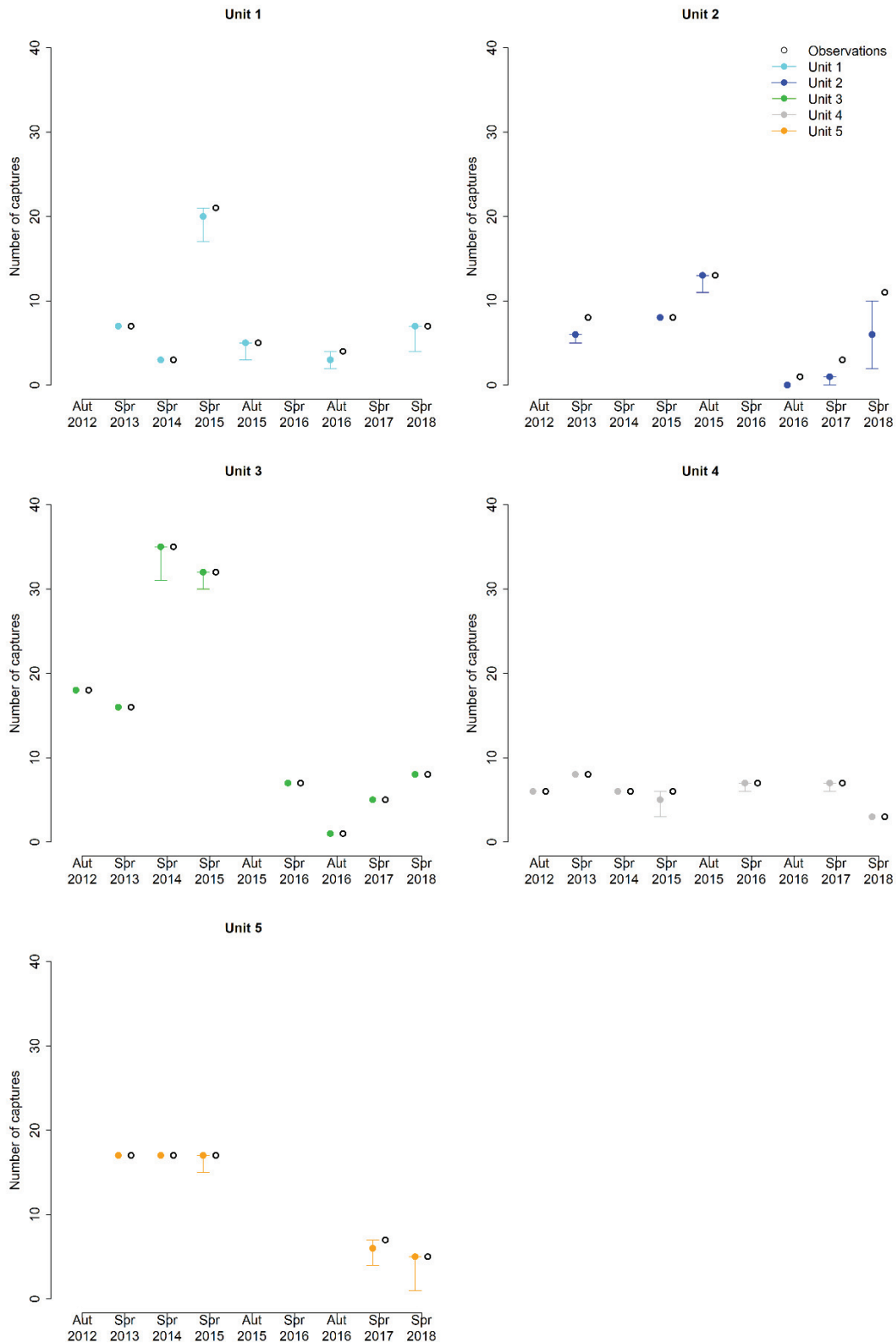


FIGURE 3.2B-6 | Fit of the model to the numbers of captured animals by socio-spatial units, aggregated by age- and sex-classes.

The coloured points and solid lines are the median and 95% credible intervals (respectively) of the simulated numbers of captured animals over the 1000 iterations. The open black circles are the observed numbers of captured animals in the field.

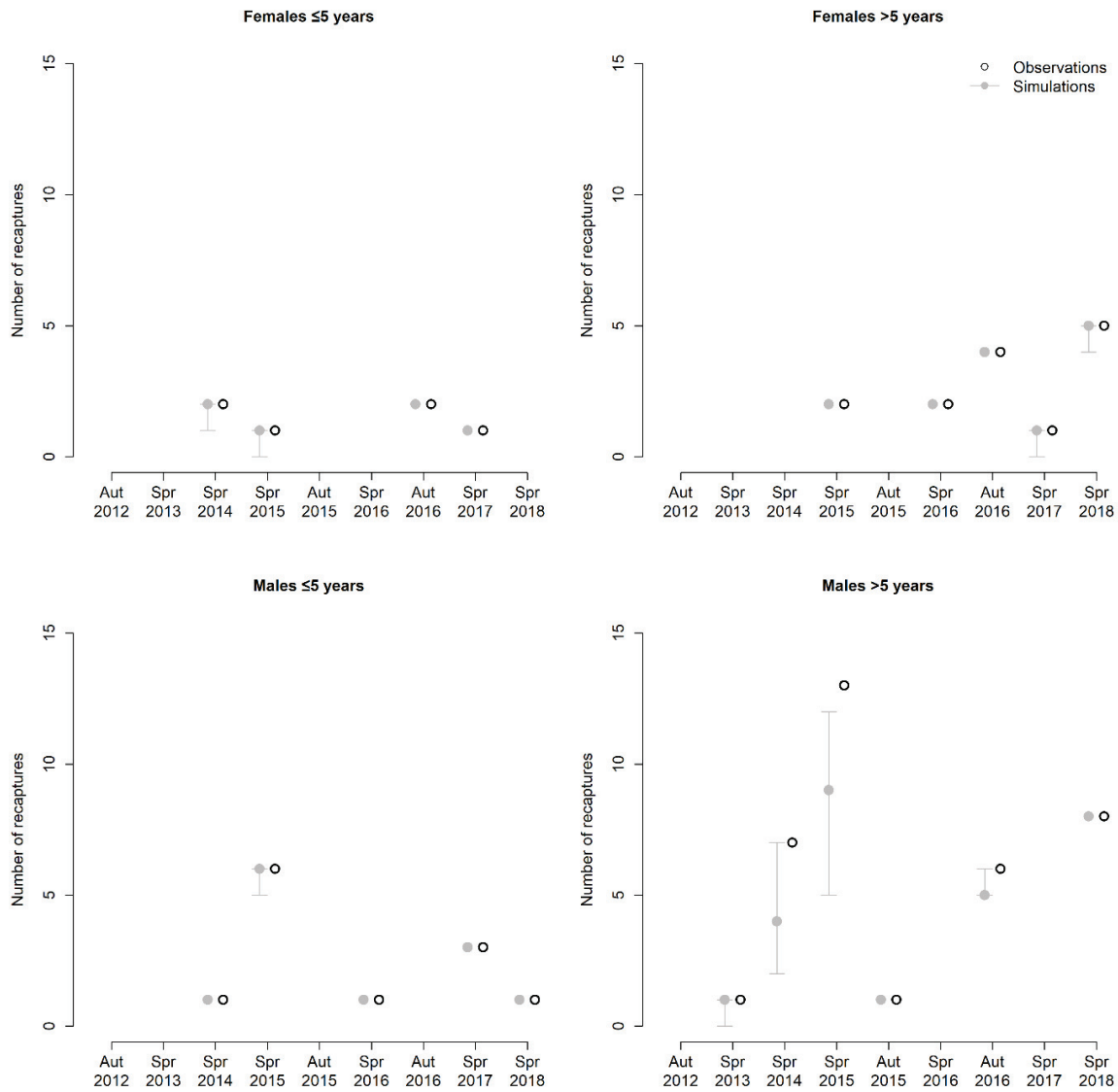


FIGURE 3.2B-7 | Fit of the model to the numbers of recaptured animals by age- and sex-classes, aggregated by socio-spatial units.

The grey points and solid grey lines are the median and 95% credible intervals (respectively) of the simulated numbers of captured animals over the 1000 iterations. The open black circles are the observed numbers of captured animals in the field.

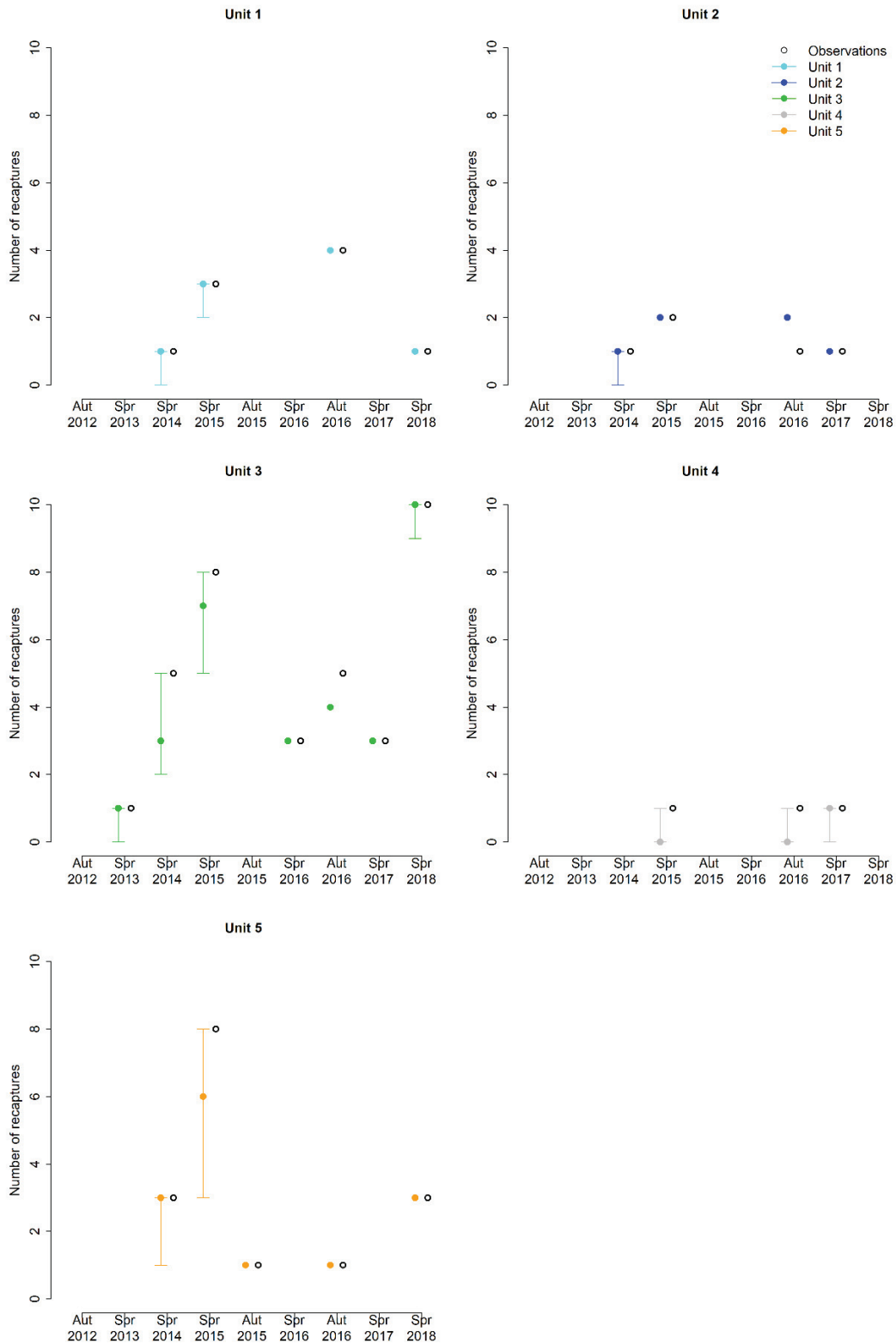


FIGURE 3.2B-8 | Fit of the model to the numbers of recaptured animals by socio-spatial units, aggregated by age- and sex-classes.

The coloured points and solid lines are the median and 95% credible intervals (respectively) of the simulated numbers of captured animals over the 1000 iterations. The open black circles are the observed numbers of captured animals in the field.

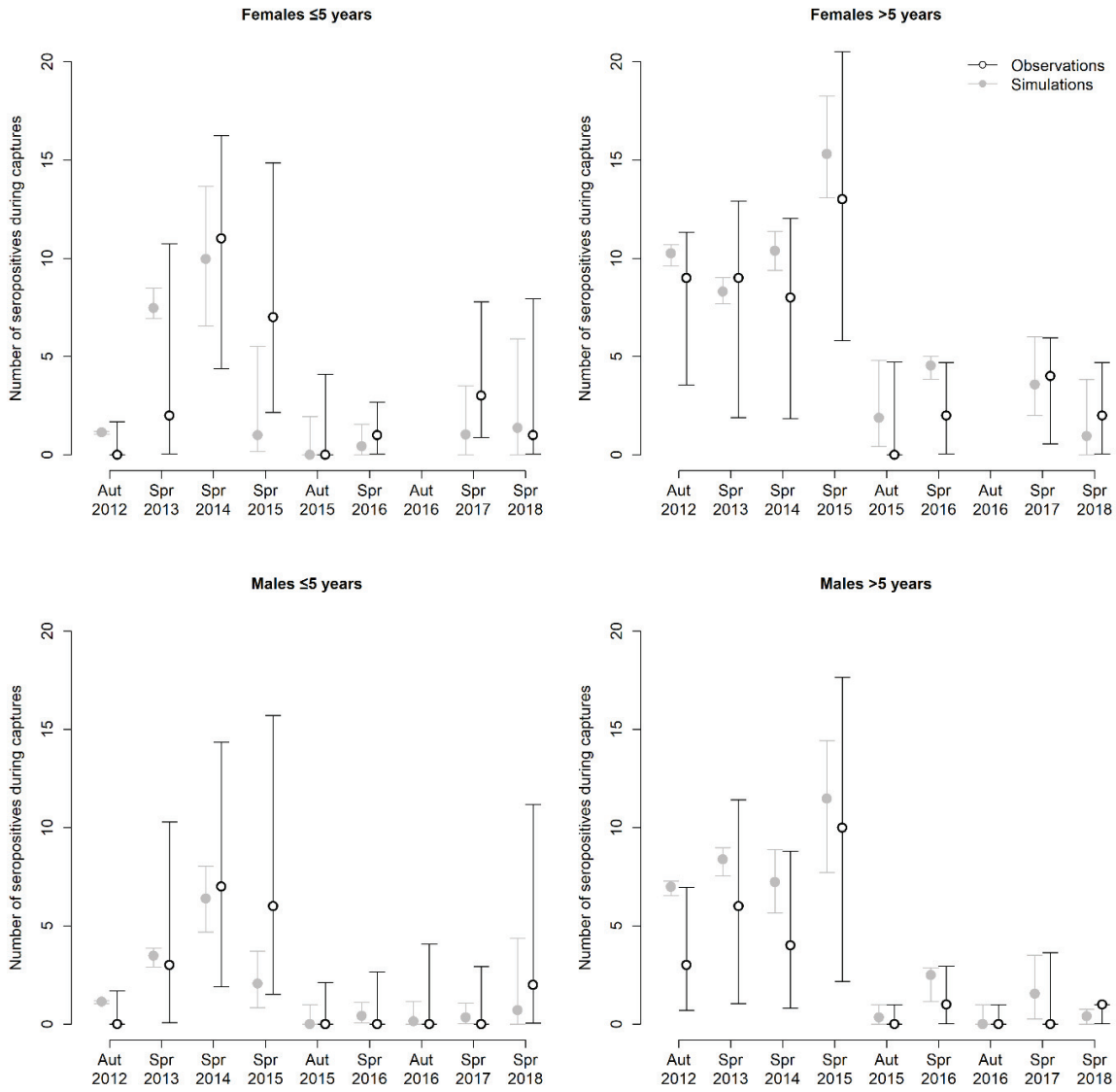


FIGURE 3.2B-9 | Fit of the model to the expected numbers of seropositive animals during captures by age- and sex-classes, aggregated by socio-spatial units.

The grey points and solid grey lines are the median and 95% credible intervals (respectively) of the simulated numbers of captured animals over the 1000 iterations. The open black circles and dotted black lines are the observed numbers of seropositive animals during captures in the field and the 95% confidence interval, respectively.

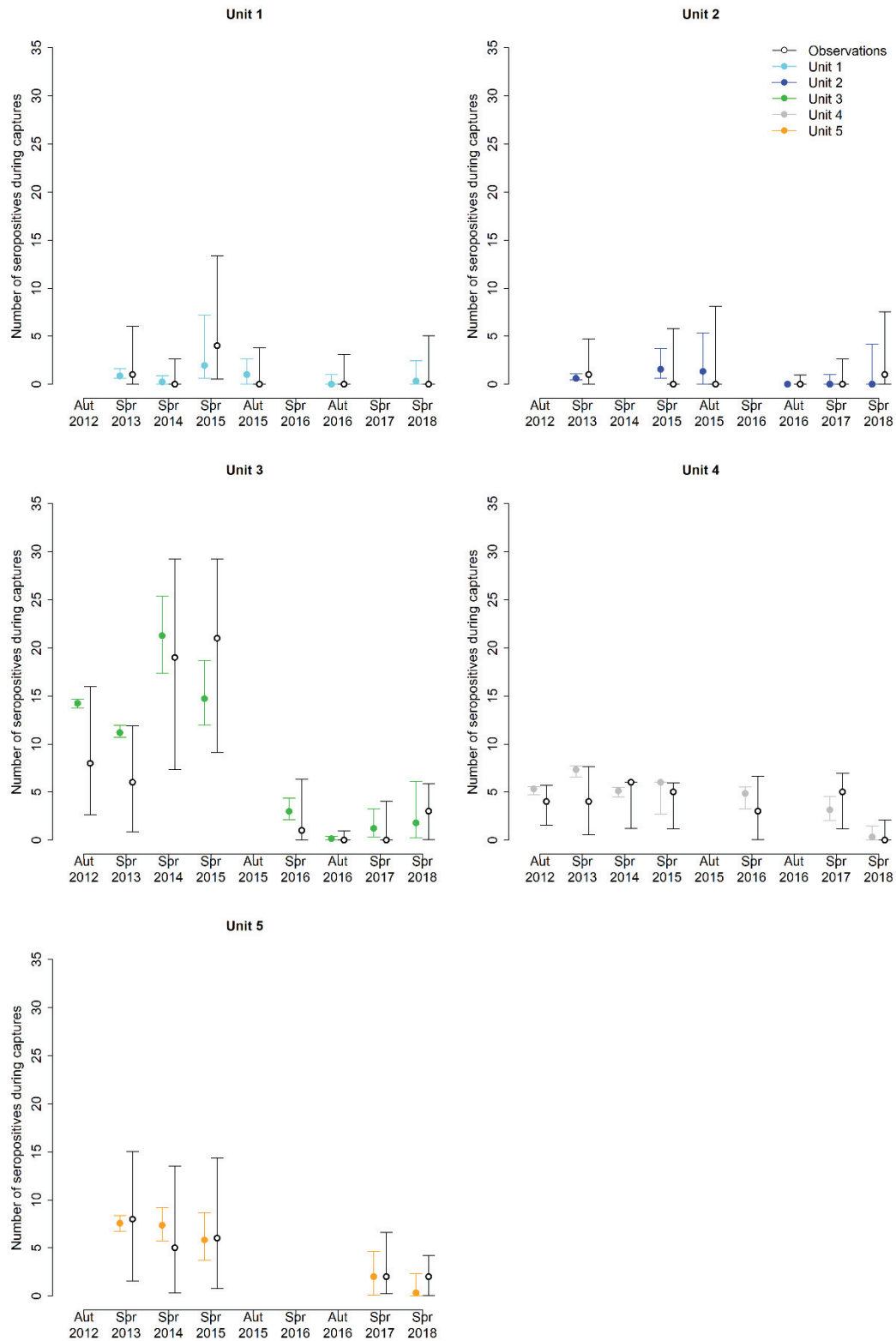


FIGURE 3.2B-10 | Fit of the model to the expected numbers of seropositive animals during captures by socio-spatial units, aggregated by age- and sex-classes.

The coloured points and solid lines are the median and 95% credible intervals (respectively) of the simulated numbers of captured animals over the 1000 iterations. The open black circles and dotted black lines are the observed numbers of seropositive animals during captures in the field and the 95% confidence interval, respectively.

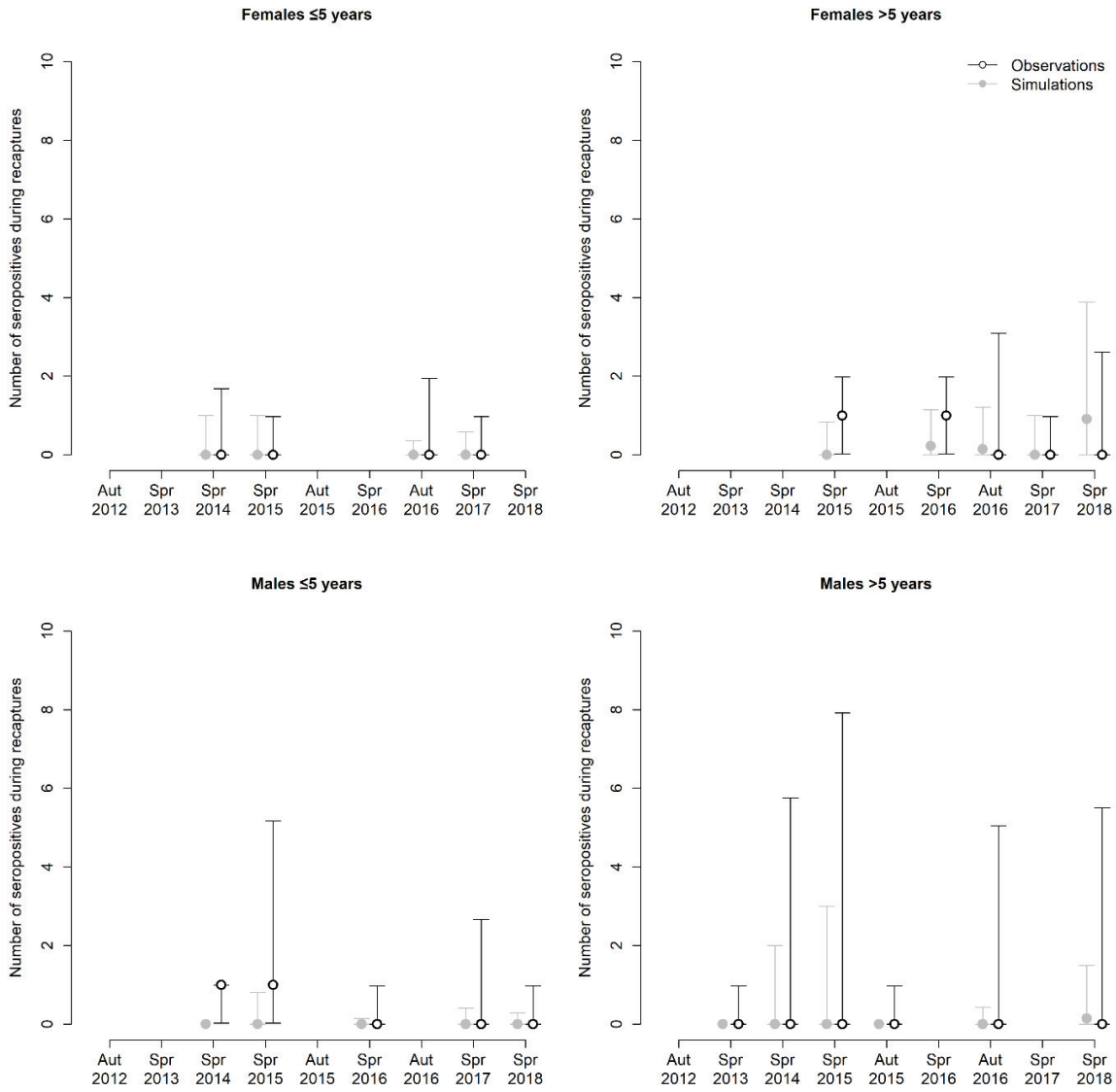


FIGURE 3.2B-11 | Fit of the model to the expected numbers of seropositive animals during recaptures by age- and sex-classes, aggregated by socio-spatial units.

The grey points and solid grey lines are the median and 95% credible intervals (respectively) of the simulated numbers of captured animals over the 1000 iterations. The open black circles and dotted black lines are the observed numbers of seropositive animals during captures in the field and the 95% confidence interval, respectively.

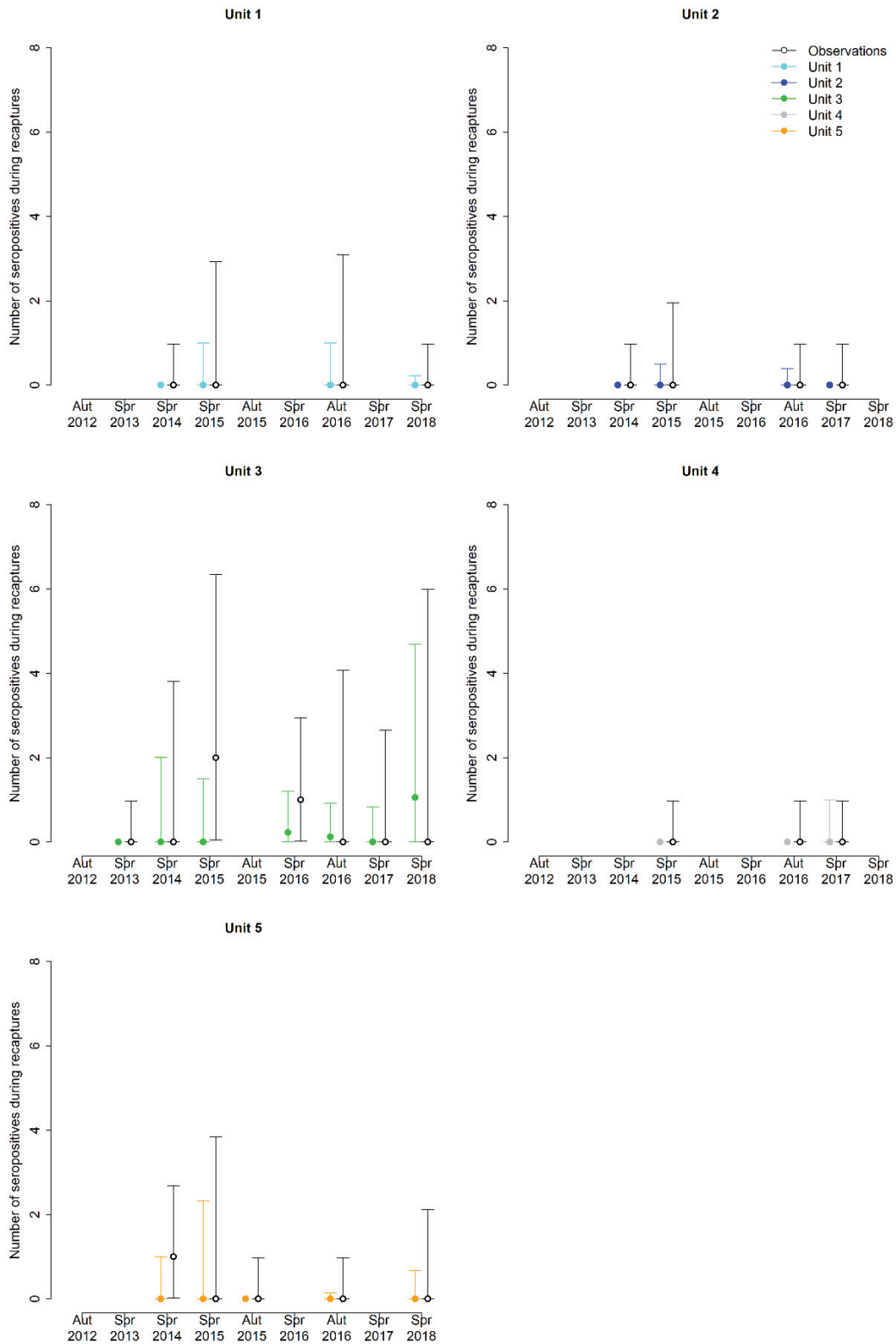


FIGURE 3.2B-12 | Fit of the model to the expected numbers of seropositive animals during recaptures by socio-spatial units, aggregated by age- and sex-classes.

The coloured points and solid lines are the median and 95% credible intervals (respectively) of the simulated numbers of captured animals over the 1000 iterations. The open black circles and dotted black lines are the observed numbers of seropositive animals during captures in the field and the 95% confidence interval, respectively.

APPENDIX 3.2C: SUPPLEMENTARY FIGURES

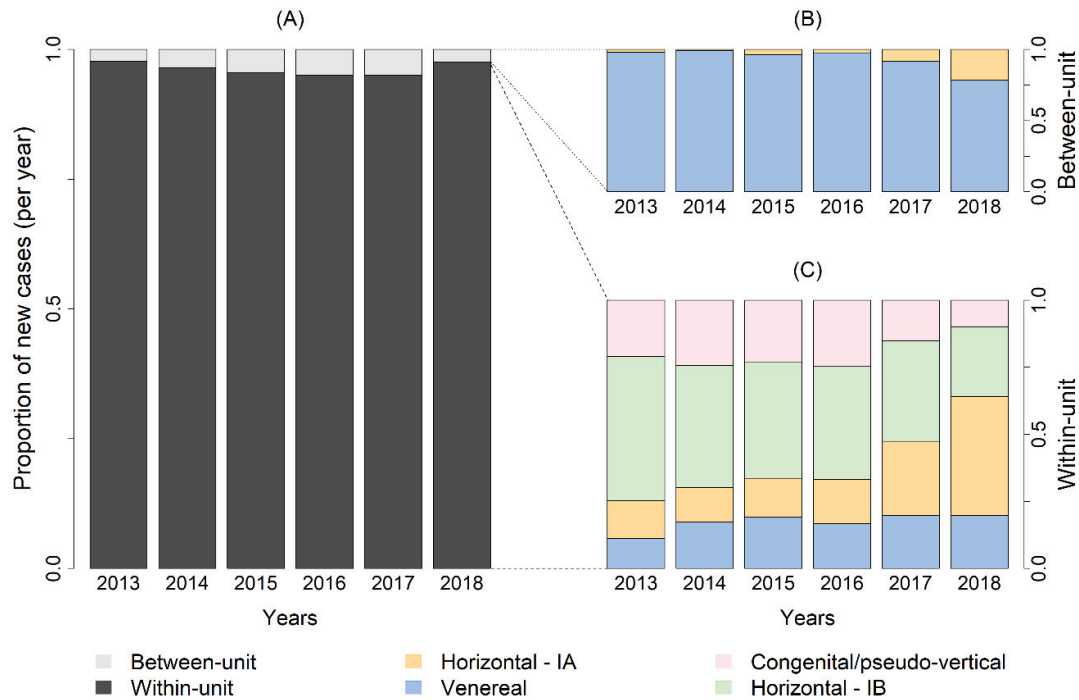


FIGURE 3.2C-1 | Evolution of the proportion of new cases caused by each transmission route. A: Average proportion of new cases caused by between-unit (light grey) or within-unit (dark grey) transmission. B: Average proportion of new cases caused by each transmission route for between-unit transmission. C: Average proportion of new cases caused by each transmission route for within-unit transmission. Vertical transmission (congenital and pseudo-vertical) is in light pink, horizontal transmission through infectious births (IB) is in green, horizontal transmission through infectious abortions (IA) is in orange, and venereal transmission is in blue.

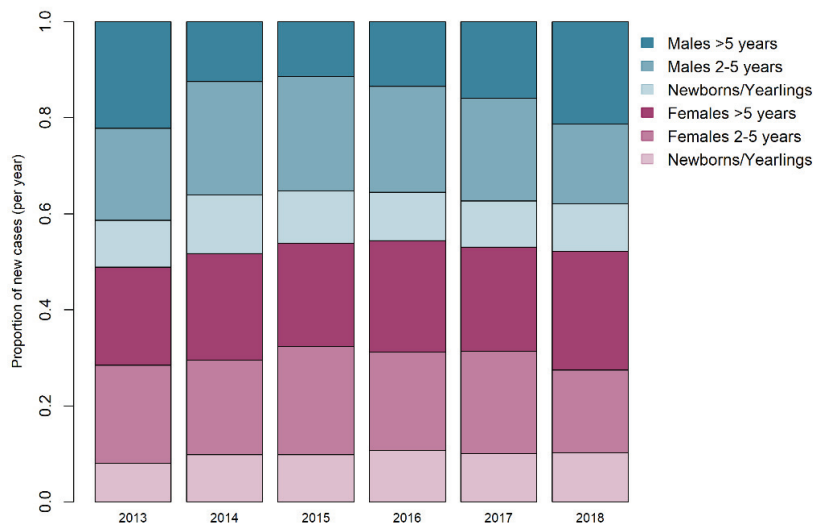


FIGURE 3.2C-2 | Evolution of the average proportion in each age- and sex-classes in the population.

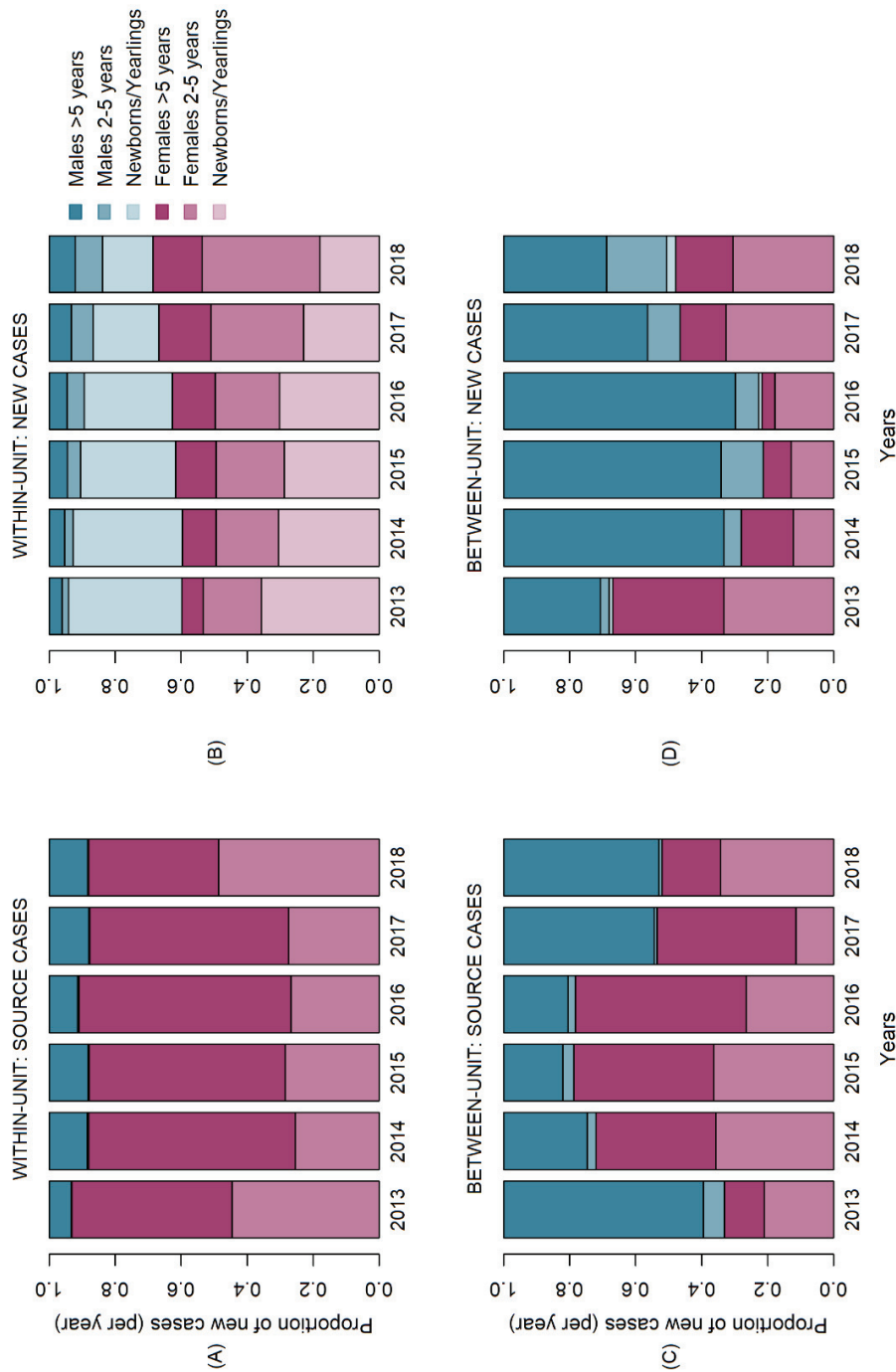


FIGURE 3.2C-3 | Distribution of secondary cases of brucellosis transmission according to their age- and sex-classes and the age- and sex-classes they originated from. A: average proportion of secondary cases that originated from primary cases of each age- and sex-class (“Source cases”) through within-unit transmission (>95% of new cases). B: average proportion of secondary cases that occurred in each age- and sex-class (“New cases”) through within-unit transmission (>95% of new cases). C: average proportion of secondary cases that originated from primary cases of each age- and sex-class (“Source cases”) through between-unit transmission (<5% of new cases). D: average proportion of secondary cases that occurred in each age- and sex-class (“New cases”) through between-unit transmission (<5% of new cases).

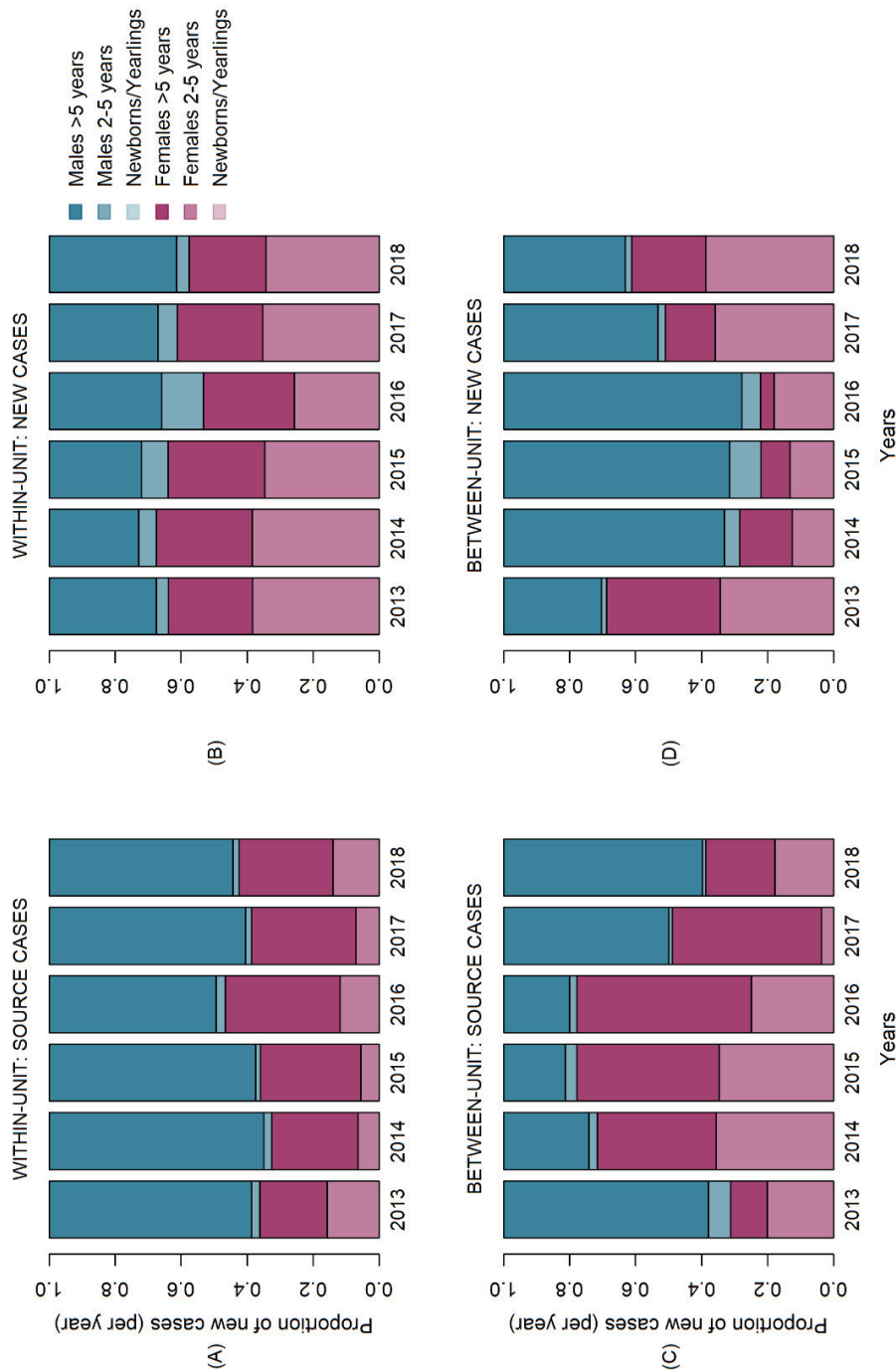


FIGURE 3.2C-4 | Distribution of secondary cases of brucellosis transmission through venereal transmission according to their age- and sex-classes and the age- and sex-classes they originated from. A: average proportion of secondary cases that originated from primary cases of each age- and sex-class (“Source cases”) through within-unit venereal transmission. B: average proportion of secondary cases that occurred in each age- and sex-class (“New cases”) through within-unit venereal transmission. C: average proportion of secondary cases that originated from primary cases of each age- and sex-class (“Source cases”) through between-unit venereal transmission. D: average proportion of secondary cases that occurred in each age- and sex-class (“New cases”) through between-unit venereal transmission.

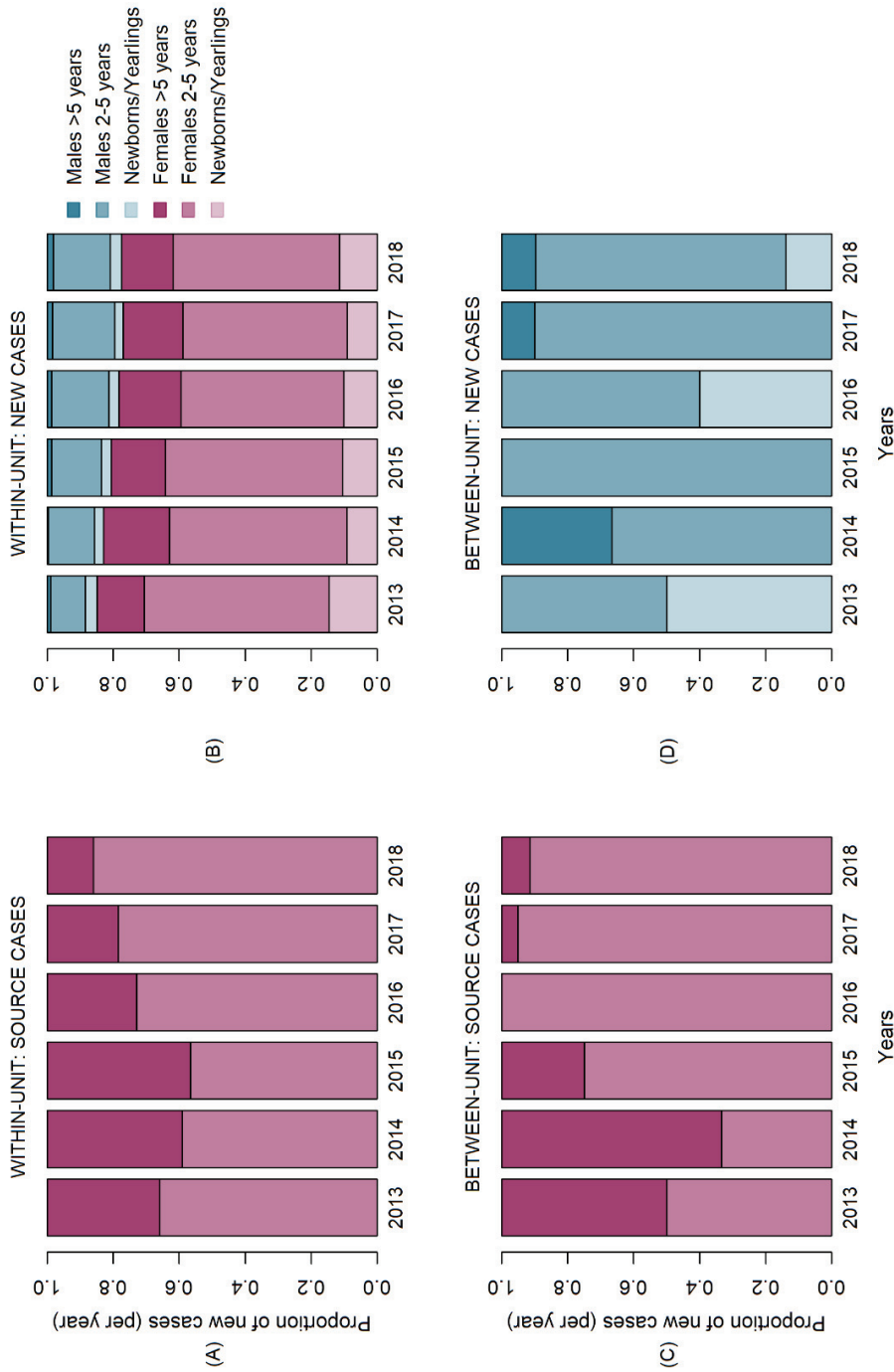


FIGURE 3.2C-5 | Distribution of secondary cases of brucellosis transmission through horizontal transmission by infectious abortions (IA) according to their age- and sex-classes and the age- and sex-classes they originated from. A: average proportion of secondary cases that originated from primary cases of each age- and sex-class (“Source cases”) through within-unit horizontal transmission by IA. B: average proportion of secondary cases that occurred in each age- and sex-class (“New cases”) through within-unit horizontal transmission by IA. C: average proportion of secondary cases that originated from primary cases of each age- and sex-class (“Source cases”) through between-unit horizontal transmission by IA. D: average proportion of secondary cases that occurred in each age- and sex-class (“New cases”) through between-unit horizontal transmission by IA.

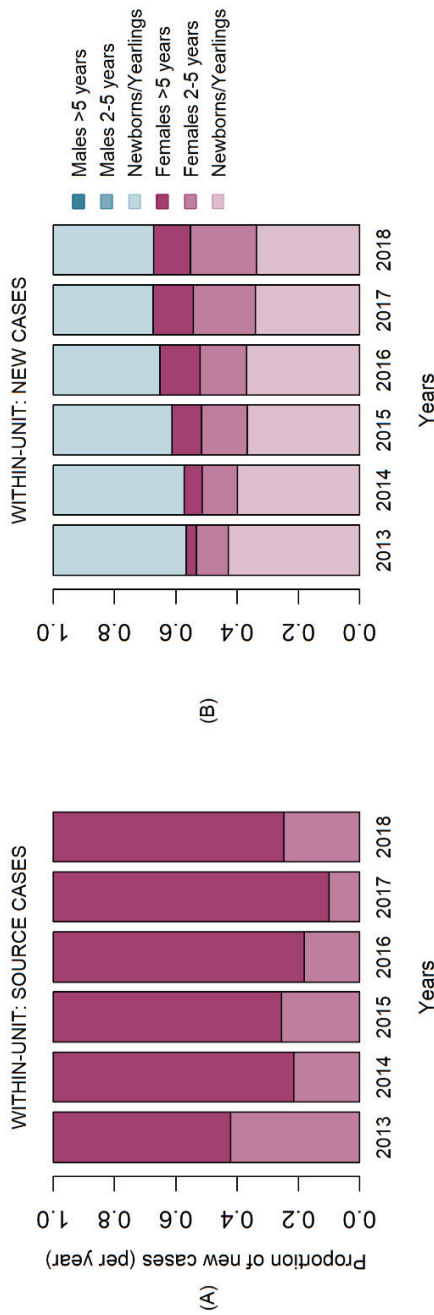


FIGURE 3.2C-6 | Distribution of secondary cases of brucellosis transmitted through horizontal transmission by infectious births (IB) according to their age- and sex-classes and the age- and sex-classes they originated from. A: average proportion of secondary cases that originated from primary cases of each age- and sex-class (“Source cases”) through within-unit horizontal transmission by IB. B: average proportion of secondary cases that occurred in each age- and sex-class (“New cases”) through within-unit horizontal transmission by IB.

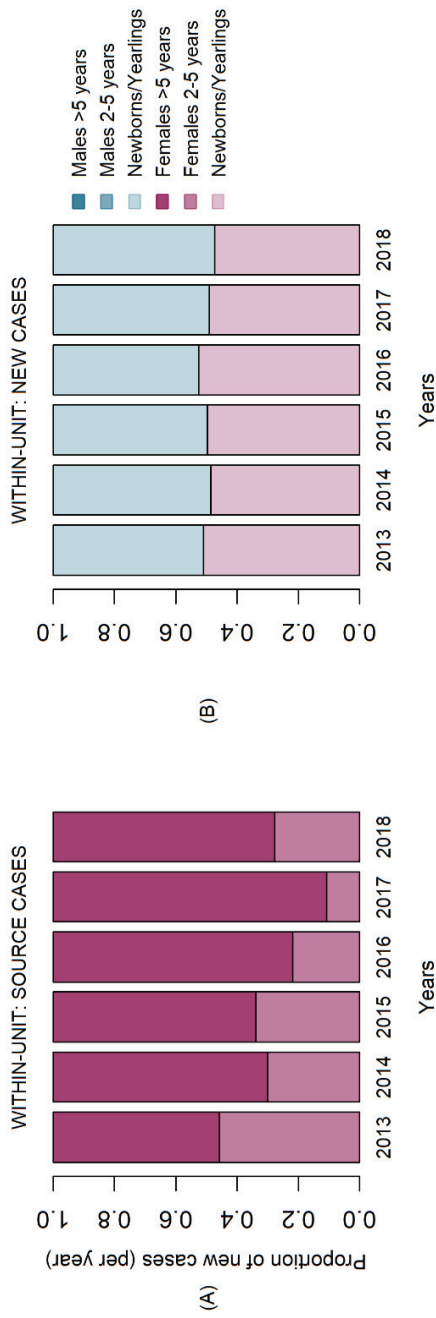


FIGURE 3.2C-7 | Distribution of the origins and characteristics of new cases of brucellosis through vertical (congenital and pseudo-vertical) transmission. A: average proportion of secondary cases that originated from each primary cases' age and sex class ("Origins") through within-unit vertical transmission. B: average proportion of the demographic characteristics of these secondary cases ("Characteristics") through within-unit vertical transmission.

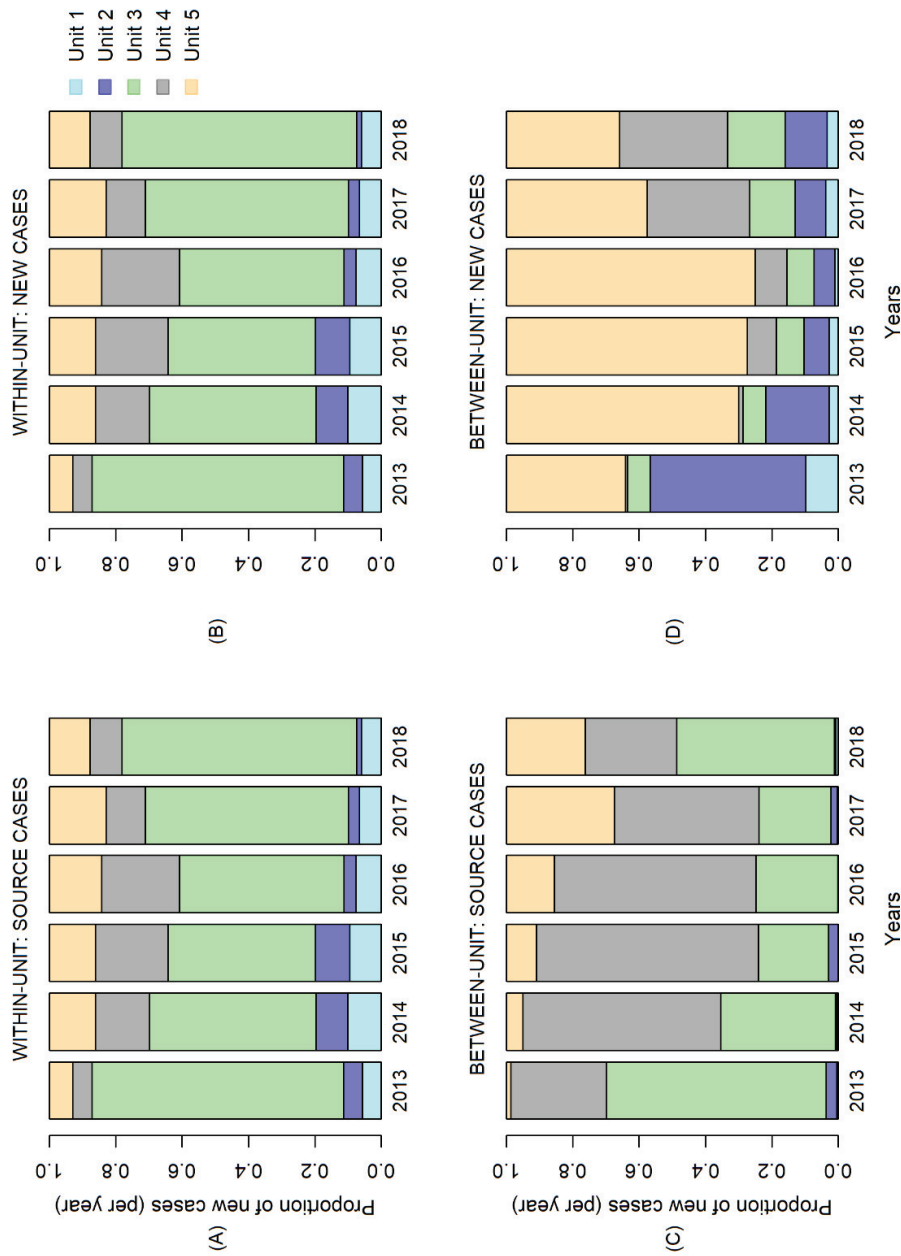


FIGURE 3.2C-8 | Distribution of secondary cases of brucellosis transmission according to their socio-spatial unit and the socio-spatial unit they originated from. A: average proportion of secondary cases that originated from primary cases of the five socio-spatial units of the population (“Source cases”) through within-unit transmission ($>95\%$ of new cases). B: average proportion of secondary cases that occurred in each socio-spatial unit (“New cases”) through within-unit transmission ($>95\%$ of new cases). C: average proportion of secondary cases that originated from primary cases of the five socio-spatial units of the population (“Source cases”) through between-unit transmission ($<5\%$ of new cases). D: average proportion of secondary cases that occurred in each socio-spatial unit (“New cases”) through between-unit transmission ($<5\%$ of new cases).

Addendum

The results of our model showed that the average proportion of new cases caused by bacterial shedding following abortion or birth was around 70%. This confirms that transmission following exposure to *Brucella* shed in genital fluids is the main transmission route, as in other wild and domestic ruminant species (Thorne *et al.*, 1978; European Commission, 2001; Rhyan *et al.*, 2009; Diaz-Aparicio, 2013; Rhyan, 2013).

Vertical transmission (pseudo-vertical or congenital) also played a significant role, and, overall, females were responsible for around 90% of new cases due to within-unit transmission, confirming our hypothesis that females maintain the infection inside each unit.

As regards between-unit transmission, venereal transmission was the predominant transmission route, but contrary to our expectation, transmission was caused by both males and females, which was counterintuitive given the sedentary behaviour of females. This is explained by movements of susceptible males which got infected from females of other units. Therefore, females represent also an important source of transmission between units. However, it is important to stress out that females are only playing a passive role in the spatial spread of brucellosis. Only males move between units in the ibex population and are thus essential for the spatial spread of brucellosis.

We also found that the socio-spatial units 3, 4 and 5 were hotspots of transmission, where more than 80% of within-unit new cases arose, with a large predominance of unit 3 over the others. Units 3 and 4 also were the main sources of between-unit transmission, while the units 2 and 5 received most of the new cases due to between-unit transmission and therefore acted as sinks.

Overall, our results from this chapter may offer first insights on how to improve disease management. For example, targeting the units acting as hotspots of transmission could be a possible way of improving the cost/benefit ratio of disease management. Management strategies should also probably focus on females, as they are responsible for the vast majority of new cases in the system. The relative efficacy of such targeted strategies were evaluated in chapter 5, using predictions from the individual-based model on the future dynamics of the population, with and without disease management.

Chapter 4

TEMPORAL DYNAMICS OF A MANAGED INFECTION



Personal picture

Foreword

In the previous chapter, we quantified transmission heterogeneity at the individual, demographic classes and spatial levels. These heterogeneities could provide a pathway towards better management, by allocating management resources on the effective sources of transmission instead of the whole population or all infected animals without distinction. Before evaluating the benefit of targeted strategies compared to untargeted strategies (chapter 5), the effects of past management interventions implemented in the population could provide guidance to the choice of possible management options in the future.

Since the infection was discovered in late 2012, the infected population of Alpine ibex was the subject of (i) a massive culling operation at the end of 2013, during which about one third of the animals were removed; (ii) a massive test-and-remove operation in spring 2014, with 71 captures and 31 seropositive individuals removed; (iii) a massive test-and-remove operation in spring 2015, with 106 captures and 38 seropositive individuals removed, followed in autumn 2015 by another round of 19 captures (zero removed) and the cull of 70 unmarked individuals; and (iv) lighter test-and-remove operations in 2016, 2017 and 2018 (less than 50 captures each year and removal of five to seven individuals), combined in 2017 and 2018 with the cull of 5 unmarked individuals.

The available data did not support any differences in brucellosis seroprevalence between the year before or the two years after the massive culling operation of 2013 (Marchand *et al.*, 2017). The aim of the first study of this chapter (4.1) was to estimate the temporal dynamics of the seroprevalence between 2013 and 2018, in order to evaluate the impact of these management strategies. The difficulty was to properly estimate the overall seroprevalence in marked and unmarked individuals, while the data on marked individuals is very limited (few recaptures and only four seropositive individuals recaptured between 2013 and 2018). In this study, we developed a Bayesian model to overcome this bias.

Because of potential biases due to the marked differences in seroprevalence between socio-spatial units (Marchand *et al.*, 2017), we focused on the core area of the massif, i.e., units 3, 4 and 5 that are in the central part of the massif and have the highest seroprevalence. We also focused on females, because they have different space use than males (Marchand *et al.*, 2017) and because it is possible to infer the infection status of seropositive females based on their antibody titres (Lambert *et al.*, 2018b; chapter 3.1), which was an integral part of the model.

4.1. Estimating disease prevalence and temporal dynamics using biased capture serological data in a wildlife reservoir: the example of brucellosis in Alpine ibex (*Capra ibex*)

CALENGE C., LAMBERT S., PETIT E., THÉBAULT A.,
GILOT-FROMONT E., TOÏGO C. and ROSSI S.

In prep.

ABSTRACT

The monitoring of the prevalence of a disease in a population is an essential component of its adaptive management. However, it is difficult to collect unbiased data for this estimation. This is the case for brucellosis infection of ibex in the Bargy massif (France). A test-and-cull program is carried out in this area to manage the infection: captured animals are euthanized when seropositive, and marked and released when seronegative. Because this mountainous species is difficult to capture, field workers tend to focus the capture effort on unmarked animals (as marked animals, controlled during previous years, are less likely to be infected). As the proportion of marked animals in the population becomes large, the captures can no longer be considered as a representative sample of the population to estimate the seroprevalence. We designed an integrated Bayesian model to correct this bias, by estimating the seroprevalence in the population as the combination of the separate estimates of the seroprevalence among unmarked animals (estimated directly from the data) and marked animals (estimated with a catalytic infection model, to circumvent the scarcity of the data). As seroprevalence may not be the most responsive parameter to management actions, we also estimated the proportion of animals in the population with an active infection by the bacteria. The actual infection status of captured animals was thus inferred as a function of their age and their level of antibodies, using a model based on bacterial cultures carried out for a sample of animals. Focusing on the population of adult females in the core area of the Massif, i.e. with the highest seroprevalence, we show that seroprevalence has been divided by two between 2013 (51%) and 2018 (21%). Moreover, the likely estimated proportion of actively infected females in the same population, though very imprecise, has decreased from a likely estimate of 34% to less than 15%, suggesting that the management actions have been effective in reducing the prevalence of the disease.

Keywords: wildlife disease, monitoring, capture-recapture, seroprevalence, management, brucellosis, Alpine ibex (*Capra ibex*)

4.1.1. INTRODUCTION

Health management is an essential component of wildlife management. Wildlife diseases can indeed infect humans and domestic animals (Daszak *et al.*, 2000; Joseph *et al.*, 2013), can be important drivers of population dynamics (Anderson and May, 1979), and may even cause the extinction of threatened species (De Castro and Bolker, 2005). Therefore, management actions may be carried out to reduce or eradicate most threatening diseases in wildlife, especially when wildlife constitute a reservoir and threat to human health or farming activities. However, such actions must be coupled with the monitoring of the disease in the population to assess their efficiency (Artois *et al.*, 2009), and thereby provide guidance on their possible adjustments over time.

Once the disease parameters to be monitored (e.g. seroprevalence) have been chosen, a sampling strategy must be designed to allow their estimation. Concerning seroprevalence, the most common approach consists in its estimation using hunting data (e.g. Staubach *et al.*, 2002; Wolfe *et al.*, 2002; Zanella *et al.*, 2008). Some or all of the hunted animals in the population of interest are analysed, and the proportion of infected or seropositive animals is used to infer the prevalence in the population. When the species is protected, monitoring often involves trapping of live animals (e.g. Beeton and McCallum, 2011). The proportion of infected captured animals is then used to infer the same proportion in the population. However, in the field, it is often not possible to collect the unbiased dataset that would be required for the monitoring of the disease. Both hunting and captures do select specific individuals based on their intrinsic characteristics (sex and age for hunting, personality for trapping) and on their accessibility due to spatial behaviour and landscape (e.g. see Réale *et al.*, 2000; Garel *et al.*, 2007; Bisi *et al.*, 2011). As a result, data obtained by hunting or capture are often not representative of the studied population (Leclerc *et al.*, 2016).

Such a biased data collection is observed in the brucellosis (*Brucella melitensis*) infected population of ibex (*Capra ibex* L.) in the Bargy massif (French Alps). The brucellosis outbreak in this area was revealed in 2012, after the report of two human cases due to the virulent *Brucella melitensis* biovar3. These two cases were related to the consumption of cheese made from fresh raw milk from an infected cattle herd grazing in this area (Mailles *et al.*, 2012). As other possible sources were excluded, wildlife was suspected of being the source of disease and field studies confirmed the high seroprevalence of this virulent zoonotic pathogen in the local population of Alpine ibex, a protected species of high

patrimonial value (38% were seropositive, see Hars *et al.*, 2013; Garin-Bastuji *et al.*, 2014). *B. melitensis* was isolated from several organs and lymph nodes of the first necropsied individuals (Garin-Bastuji *et al.*, 2014).

The public health and economic concerns (the concerned area is the core of the production of Reblochon, a famous cheese of high economical value), as well as the conservation issues for this recently restored and protected species, led to the implementation of several actions to eradicate this disease from the population. In parallel of two mass culling campaigns carried out in autumn 2013 and autumn 2015 (Marchand *et al.*, 2017), a capture program was designed (i) to implement a test-and-cull program and therefore reduce the prevalence of the disease in the population, and (ii) to allow the monitoring of the seroprevalence of the disease in the population. Every year since 2013, several animals are captured and a rapid serological test is performed on each individual to determine its serological status. Animals that are found to be seropositive are euthanized, whereas seronegative individuals are marked and released. Two important aspects of this program are that, since 2013, the population size has strongly decreased due to the two mass culling, and the number of marked animals have increased due to these captures. This led to a strong increase of the proportion of marked animals in the population over time.

Note that the capture of ibex is very time-consuming and costly: the capture of one animal requires safe working conditions for both the animal and the field workers, which may be difficult to achieve due to the steep slopes characterizing the habitat used by this species. This high cost constrains a lot the capture operations: as the main aim of the captures is to reduce the level of infection in the population, field workers increase the efficiency of these captures by focusing the capture effort on animals that are the most likely to be infected. In particular, animals that carry a mark have already been controlled as seronegative just a few years before, so that they are less probably infected than the unmarked animals; field workers therefore tend to avoid the recapture of already marked animals during capture operations. As a result, even if the proportion of marked animals can be important in the population, the proportion of already marked animals remains low in the sample of captured animals during the study period. In other words, the seroprevalence in the sample of captured animals is probably larger than the seroprevalence in the population. Therefore, the estimation of the seroprevalence in the population and its temporal variation with the data collected during the test-and-cull operations must account for this sampling bias and for the increasing proportion of marked animals in the population.

The test-and-cull operations implemented in this area rely on the test of the seropositivity of the animals. However, the presence of antibodies is not completely relevant to identify animals that are able to transmit bacterial infection at the time of sampling. Indeed, some seropositive animals may have been infected several years before sampling and may have cleared the infection from most of excreting organs (Lambert *et al.*, 2018b). Seroprevalence variation over time may thus be difficult to interpret in a long-lived animal species such as ibex, since it depends not only on immediate disease incidence but also on populations past exposure and turnover (National Academies of Sciences, Engineering, and Medicine, 2017). In that context, the relevant indicator for management would rather be the number of individuals that carry "active bacterial infection", i.e. an infection at levels and in organs that render plausible excretion of bacteria. Such individuals can in theory be identified with bacterial cultures of excreting organs (urogenital tracts, Lambert *et al.*, 2018b), but implementing bacterial cultures for all culled seropositive animals, or even for a large sample of them, would be too costly for this monitoring program. Fortunately, previous authors have demonstrated that positive bacterial culture is generally associated with higher antibody levels (Durán-Ferrer *et al.*, 2004; Treanor *et al.*, 2011), which is easier to measure. In particular, using data from post-mortem bacteriological investigations coupled to serological data, Lambert *et al.* (2018b) developed a model that relates the antibody level of a seropositive animal with the probability of active infection. Therefore, such studies demonstrate that it is in theory possible to use the easily measured antibody levels measured in captured animals to predict their infection status.

In this paper, our aim is to develop a statistical approach that allows to correct the bias caused by the non-random capture of animals in the estimation of the brucellosis seroprevalence in the Alpine ibex population. We also take into account individual antibody titres and the results of Lambert *et al.* (2018b) (detailing individual bacteria portage) to estimate the proportion of the population actively infected by the bacteria (Pepin *et al.*, 2017). We use an integrated Bayesian model to account for the different components of the seroprevalence and infection processes. We also discuss the possible causes of prevalence evolution over time in that population and management implications for this wild reservoir.

4.1.2. MATERIAL AND METHODS

4.1.2.1. Study area

The Bargy massif is a NE-SW oriented mountain covering about 7000 ha in the northern French Alps (46°N, 6.5°E), with an elevation ranging from 600 to 2348 m. The rainfall ranges from 1200 to 2000 mm, with snow falling from late October to early May. The ibex population originates from a reintroduction of 14 individuals captured in the Montpleureur population (Switzerland) carried out between 1974 and 1976 (Gauthier and Villaret, 1990). The ibex population has increased since then. Feeding mark-resight data in an immigration-emigration logit-normal model (Marchand *et al.*, 2017), the population size (without newborn) was estimated at about 550 animals in summer 2013, and decreased to about 300 animals in 2014; since 2014, the population size fluctuates around 300-350 animals (Figure 4.1-1).

As noted above, an outbreak of brucellosis caused by *B. melitensis* biovar3 occurred in 2012 in a dairy herd in this area. *Brucella* genotyping demonstrated that the strain infecting the dairy herd was the same as the one observed in the ibex population. Moreover, this strain was closely related to the strain observed in domestic livestock of this area in 1999, during the last outbreak reported in domestic cattle in the area, suggesting that a spillover probably occurred from domestic animals to ibex during the 1990's (Mick *et al.*, 2014).

Two indiscriminate mass culling were implemented during our study period. The first one was implemented in autumn 2013 (233 unmarked animals up to five years old were culled at this time), which led to the halving of the population size (Figure 4.1-1, Marchand *et al.*, 2017). The second one was implemented in 2015 (70 unmarked animals were culled), and also resulted into a reduction in population size, though to a lesser extent.

In parallel, a test-and-cull operation was implemented on the massif: a sample of animals was captured every year, and for each animal, a serological test (detailed below) led to the release after marking (seronegative) or the cull (seropositive) of the animal. Capture effort varied according to the year with a particularly high capture effort in 2015 when the strategy aimed at constituting a disease-free group (i.e. an intense capture period with 126 captured and tested individuals, followed by the mass culling described above, see Figure 4.1-1 and Marchand *et al.*, 2017).

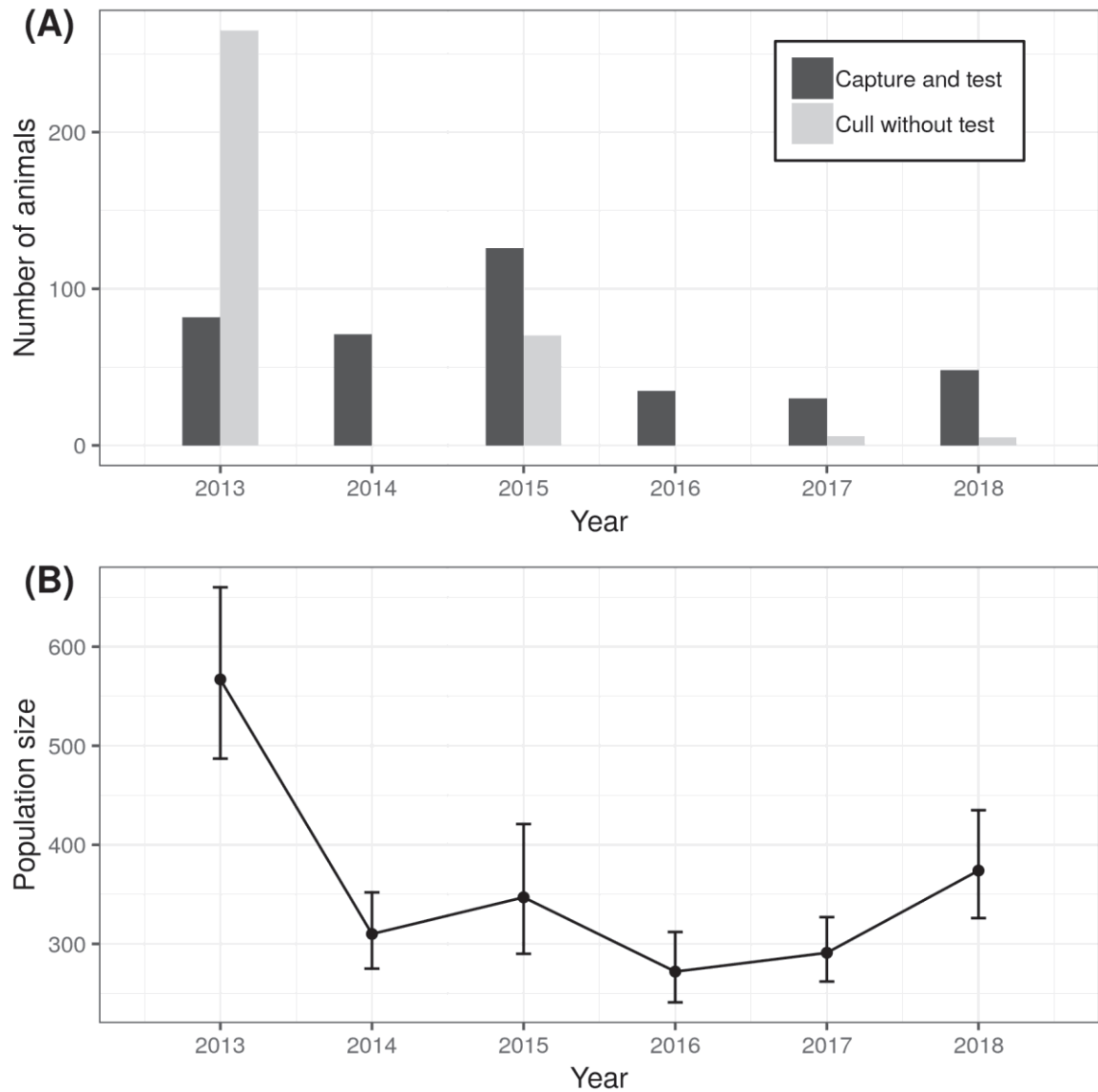


FIGURE 4.1-1 | (A) Number of ibex captured for brucellosis testing (followed either by further release or removal depending on the results of the test), and number of animals culled without test (mostly during mass culling) at the level of the whole Bargy massif. (B) estimated number of ibex (all zones and sexes, excepting newborn) per year using capture-mark-resight methods.

While extensive test-and-cull operations were performed at the scale of the whole massif from 2012 to 2015, lighter test-and-cull operations were performed after 2015 (i.e. a smaller number of animals were tested after 2015). These lighter operations were mostly focused on the core area of the massif. This core area of the massif corresponds to the central part of the massif (located between 45.98°N, 6.44°E and 46.02°N, 6.53°E) characterized by a connected high-elevation area with the highest disease prevalence.

4.1.2.2. *Datasets used in the model*

a. General modelling strategy

In this paper, we developed an integrated Bayesian model relying on three datasets. The general modelling strategy is described by the simplified graph in Figure 4.1-2; a formal directed acyclic graph (Spiegelhalter *et al.*, 1998) of the model is presented in the Appendix 4.1A, along with a table explaining all the mathematical notations. In this study, we focused on the females only, as they constitute the target of the capture effort by the field workers. Moreover, we limited our study area to the core area of the massif.

We fully describe the datasets and the model in further sections, but we first describe shortly the modelling strategy and the used datasets: (i) every year, we gathered the data concerning the females captured during the test-and-cull operation in the core area of the massif. We stored in the data table used for the model fit, for each tested female, its serological status, the titre of the complement fixation test (CFT) – one of the tests used to identify the seropositivity of the females, which is a good measure of the antibody level (see below) –, and whether the female was already marked or unmarked at the time of capture. This dataset (hereafter called "captures dataset", and noted Y_m) was used to estimate, for each year, the seroprevalence among marked and unmarked females separately; (ii) every year, field workers carry out censuses in spring and summer on the area, and record the number and composition (sex, age, number of marked animals) of ibex groups detected on the area. We used this dataset (hereafter called "censuses dataset" and noted Y_c) to estimate, for each year, the proportion of marked females in the population of females. This estimated proportion allowed to estimate the yearly seroprevalence in the total population from a weighted average of the separate seroprevalence estimates in the marked and unmarked groups of females; (iii) for a sample of 56 seropositive females culled during the test-and-cull operation, a bacterial culture of 1-15 tissues per animal allowed to model the relationship between the titre of the CFT and the actual presence of an active infection (i.e. detection of the bacteria, Lambert *et al.*, 2018b). We call this dataset "bacterial cultures dataset" in the rest of this paper, which we note Y_b .

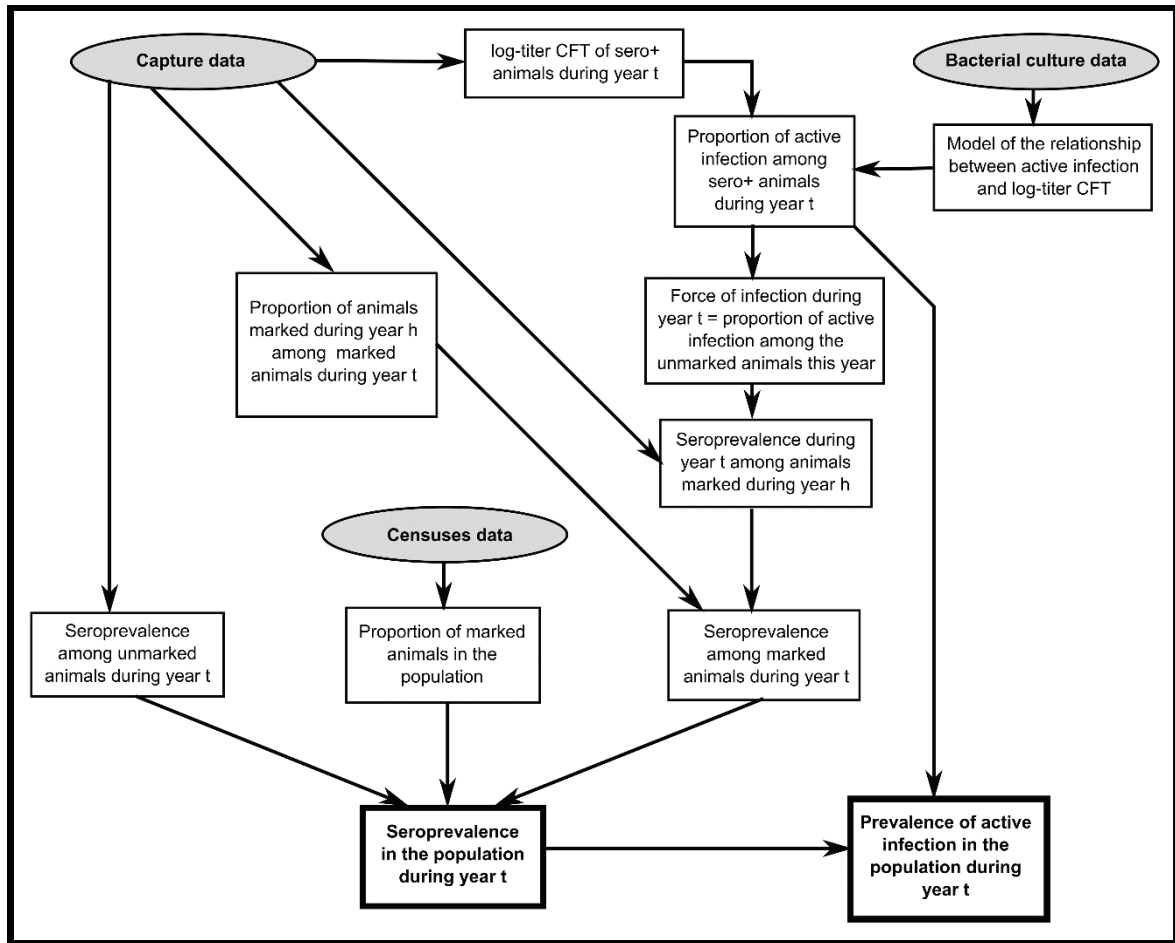


FIGURE 4.1-2 | Simplified graph describing the structure of the model used to estimate the seroprevalence and active infection by the brucellosis in the population of female ibex in the core area of the Bargy massif, France.

In this figure, the year *h* is the year of capture and marking of females, whereas year *t* is the year when an assessment of the seroprevalence and active infection is wanted.

Finally, we used this model relating titre of the CFT with active infection to estimate, for each year, the proportion of seropositive females with an active brucellosis infection, based on the titre of the CFT of seropositive females in the "captures dataset". Then, we could combine this proportion of infected females among seropositive females with the seroprevalence estimate in the population to estimate the prevalence of active infection in the population.

We now describe more precisely the three datasets used to fit this model.

b. Captures dataset Y_m

Between 2012 and 2018, 387 animals were captured and anaesthetised using a dart-gun xylazine/ketamine anaesthesia (Rompun, Bayer, Leverkusen, Germany and Imalgène, Merial, France; 100 mg/individual). For each animal, a blood sample was collected according to the requirements of the World Organisation for Animal Health in small ruminants, to test brucellosis seropositivity. Serological assays detected antibodies using the Rose Bengal test, the complement fixation test, indirect ELISA (IDEXX, Montpellier, France) and competitive ELISA (Ingenasa, Madrid, Spain), and since 2014 a rapid test in the field (Anigen Rapid G.S. Brucella Ab, Bionote, Gyeonggi-do, Republic of Korea, ANSES, 2014; Corde *et al.*, 2014). We consider here that the animal was seropositive when at least two tests gave positive results (ANSES, 2015). Seropositive individuals were shot in 2013-2014, and euthanized the following years by embutramide intravenous injection (T61, Intervet, Angers, France) whereas seronegative individuals were marked with ear tags and/or collars, to allow their visual identification. Seronegative individuals were then released. For each animal, we recorded the sex and the approximate capture location (i.e. capture site, based on topographical landmarks). We estimated the age of each captured individual by counting horn growth annuli (Michallet *et al.*, 1988). Marchand *et al.* (2017) showed with GPS data that the female ibex were structured in five socio-spatial units, and demonstrated that the seroprevalence was maximum in the core area of the massif. On the other hand, most adult males were roaming the entire massif without strict spatial sectors. This explains why we decided to focus our study on the female captured in the core area of the massif. Hereafter, the model and its parameters characterise only the population of females, and are therefore independent of the male population (in particular concerning disease transmission and population size). Thus, among the 387 animals of both sexes captured on the whole massif, 118 female ibex (65 seropositive + 53 seronegative) have been captured and marked on the core area of the massif from 2013 to 2018 (Table 4.1-1). As noted above, the field workers tended to focus the capture effort on unmarked animals, resulting in a small number of marked female ibex that were recaptured every year (in total 14 animals, among which 2 seropositive, see Table 4.1-1). We used these recaptured females to estimate seroprevalence for marked females.

TABLE 4.1-1 | Number of captured and recaptured female ibex each year in the core area of the Bargy massif from 2013 to 2018.

For each year, we also show the number of seropositive among first captured females (with the percentage of captured females in parentheses) and the number of seropositive females among already marked recaptured females (the numbers are too small to allow the calculation of a meaningful percentage).

Year	N captures	N capt. seropos.	N recaptures	N recapt. seropos.
2013	31	17 (55%)	0	0
2014	33	19 (58%)	1	0
2015	26	17 (65%)	2	1
2016	8	3 (38%)	2	1
2017	13	7 (54%)	4	0
2018	7	2 (29%)	5	0

All captured animals were handled by professionals from the French game and wildlife management agency (*Office national de la chasse et de la faune sauvage*) according to the ethical conditions detailed in the specific accreditations delivered by the Préfecture de Paris (prefectural decree 2009-014), by the French Minister of Ecology, Sustainable Development and Energy (Ministerial Orders of February 11, 2014) and by the Préfecture de la Haute-Savoie (prefectural decree 2015062-0018) in accordance with the French environmental code (Art. R421-15 to 421-31 and R422-92 to 422-94-1). Euthanasia was performed by veterinarians in accordance with the requirements of these accrediting authorities, and slaughtering operations were performed in accordance with accreditations delivered by the Préfecture de la Haute-Savoie (prefectural decrees 2013274-0001, DDT-2015-0513, DDT-2016-0933, DDT-2017-1003). We hence confirm that we treated animals in accordance with relevant guidelines and regulations and that our protocols were approved by these institutions.

c. Censuses dataset Y_c

Censuses were carried out once a month from May to September, every year from 2013 to 2018. Observers travelled on one of the nine defined transects in the morning, ideally the same day to avoid double counting. They recorded the detected groups of ibex and their

composition (number of animals and number of marked animals, and for each animal, the estimated age and sex). The number of detected animals per year was in average equal to 500 individuals (min: 114 animals in 2015; max: 813 animals in 2013). Note that field workers were not equipped with spotting scopes on stand during the first two years of the program. The estimation of the age and sex of animals was therefore probably noisier in 2013 and 2014 when workers only worked with naked eyes, than during the following years, when workers were better equipped.

d. Bacterial cultures dataset Y_b

A subset of 56 culled seropositive females have been collected for necropsy (animals where necropsied when possible). For each animal, bacterial cultures were performed on 1 to 15 organs, focusing on organs having shedding potential, such as genital tract, supramammary lymph node, etc. Tissues were sampled during necropsy, and either directly analysed by culture, or frozen for delayed culture. Cultures were performed by a local laboratory officially authorized to provide brucellosis diagnoses (Biosafety level 3 facilities): the veterinary laboratory of the Savoie department (LDAV 73). Isolated *Brucella* strains were further bio-typed by the National Reference Laboratory for animal brucellosis (ANSES). All methods were performed according to OIE standards as described previously (Alton *et al.*, 1988; Mick *et al.*, 2014). Further details on the collection of this dataset can be found in Lambert *et al.* (2018b).

4.1.2.3. *Statistical model*

a. Estimation of the seroprevalence in the population

Our integrated model was built by several components. We first describe the component that allowed the estimation of the seroprevalence of brucellosis in the population. First, we estimated this overall seroprevalence π_t during year t by: (i) estimating the proportion γ_t of marked females in the population, (ii) estimating the seroprevalence $\pi_t^{(u)}$ among unmarked females, (iii) estimating the seroprevalence $\pi_t^{(m)}$ among marked females, and (iv) combining these estimations together with:

$$\pi_t = \pi_t^{(m)} \times \gamma_t + \pi_t^{(u)} \times (1 - \gamma_t) \quad (1)$$

We describe these steps below.

First, we used the censuses dataset to estimate the proportion γ_t of marked females in the population during year t . We supposed that the number $m_{j(t)}$ of marked females detected by the observers during census day $j(t)$ of the year t could be described by a binomial distribution:

$$m_{j(t)} \sim \mathcal{B}(n_{j(t)}, \gamma_t)$$

where $n_{j(t)}$ is the total number of detected females (marked+unmarked) this day.

Then, we used the captures dataset to estimate seroprevalence in the population. We first estimated the seroprevalence $\pi_t^{(u)}$ among unmarked females during year t , by supposing that the number ℓ_t of seropositive unmarked females captured this year was drawn from a binomial distribution:

$$\ell_t \sim \mathcal{B}(L_t, \pi_t^{(u)})$$

where L_t is the total number of unmarked females (seropositive + seronegative) captured for the test-and-cull operation of the year t .

Finally, we estimated the seroprevalence $\pi_t^{(m)}$ among marked females during year t . The sample size of marked females was too small to allow a separate estimation of these annual seroprevalences. We used a more complex model for this part of the population. We supposed that the number z_t^h of females first captured and seronegative during year h , and recaptured and seropositive during year t was drawn from a binomial distribution:

$$z_t^h \sim \mathcal{B}(Z_t^h, \zeta_t^h)$$

where Z_t^h is the total number of females first captured and seronegative during year h , and recaptured (whatever their serological status) during year t . The unknown parameter ζ_t^h is the probability that a female captured and seronegative during year h was seropositive when recaptured during year t .

As the sample size of marked females was also too small to allow the separate estimation of all the unknown parameters ζ_t^h , we modelled these parameters thanks to the adaptation of a catalytic infection model (Heisey *et al.*, 2006; Hens *et al.*, 2010) to our case, allowing the force of infection (i.e., the rate at which susceptible individuals become infected per unit of time, Vynnycky and White, 2010) to vary from year to year. More precisely, we supposed

that the probability that a female controlled seronegative during year h was seropositive during year t was equal to:

$$\zeta_t^h = 1 - e^{-\sum_{s=h}^{t-1} \lambda_s}$$

where λ_s is the unknown force of infection of brucellosis for the year s (see Appendix 4.1A for a full mathematical derivation of this expression).

Again, the sample size of marked females was too small to allow the separate estimation of the six forces of infection λ_t . We therefore supposed that, for a given year t , the unknown force of infection λ_t of marked females depended on the estimated infection level among unmarked females (i.e., we supposed that only infected unmarked females could infect the marked females). More precisely, we supposed:

$$\lambda_t = \eta \times u_t$$

where η is an unknown parameter that can be considered as an effective contact rate, to be estimated from the data, and assumed to be constant with time and age, and u_t is the proportion of the population corresponding to unmarked females with an active infection (estimated in a later section). Thus, given u_t , the calculation of the forces of infection λ_t , and therefore of the probabilities ζ_t^h only depends on the estimation of the unknown parameter η .

Then, we could use these probabilities ζ_t^h to estimate the seroprevalence $\pi_t^{(m)}$ among marked females at time t . We had to account for the mortality that occurred between the first capture during year h and the recapture during year t in this calculation. Thus, we first predicted the unknown number N_t^h of females that were first marked during year h and that were still alive during year t . We supposed that the annual survival rate is known and equal to $\phi = 0.95$ (Gaillard and Yoccoz, 2003; Toigo *et al.*, 2007), so that N_t^h follows a binomial distribution:

$$N_t^h \sim \mathcal{B}(L_h - \ell_h, \phi^{(t-h)})$$

Using this number, we could calculate the proportion ψ_t^h , among the marked females recaptured at time t (seropositive + seronegative), of females that were first captured and seronegative at time h , by:

$$\psi_t^h = \frac{N_t^h}{\sum_{t'=1}^t N_t^{t'}}$$

So that the seroprevalence among marked females was calculated by:

$$\pi_t^{(m)} = \sum_{h=1}^t \zeta_t^h \times \psi_t^h$$

i.e. by summing the probabilities over all years h that a marked female captured at time t was first captured at time h and became seropositive between h and t .

We finally estimated the overall seroprevalence π_t every year, from the values of γ_t , $\pi_t^{(u)}$ and $\pi_t^{(m)}$, using equation (1). The complete model thus requires the estimation of the vector of unknown parameters $\theta_1 = \left(\eta, \{\gamma_t\}_{t=1}^6, \{\pi_t^{(u)}\}_{t=1}^6 \right)$.

Note that we tried to fit other models to describe the infection process among marked females before choosing the catalytic model described above: one model supposed a constant seroprevalence among marked females through time, one model allowed a variation in seroprevalence (a female newly marked having more chances to become infected as time passes), but supposed a constant risk of infection through time, and the model described above allowed a variation in the risk of infection through time. Our analyses suggested that the choice of the model did not affect the estimation of the overall seroprevalence or prevalence of active infection. We chose to work with this latter model, because it was more realistic and it returned an interesting parameter for the monitoring of the disease (the force of infection due to unmarked females on marked, healthy females). However, the other models are described in the Appendix 4.1A.

b. Relationship between the logarithm of CFT titre and active infection

We modelled the relationship between the antibody level (measured by the titre of the CFT) and the probability of active infection as a component of our integrated model, to allow the estimation of the prevalence of active infection in the population. This component can be decomposed in two parts: (i) a submodel describing the detectability of active infection of an animal with bacterial cultures on its organs, and (ii) a submodel describing the relationship between CFT titre and active infection, accounting for the age of the animal.

We used the bacterial cultures dataset \mathbf{Y}_b for this component. Consider the female r of this dataset. A bacterial culture was carried out on o_r organs of this female. We define the

hidden state variable $b_r = 1$ if the female r of the bacterial cultures dataset is actively infected and $b_r = 0$ otherwise (unknown to us and to be estimated). We suppose that this variable is drawn from a Bernoulli distribution characterized by the unknown probability $w^{(b)}$ that a female of this dataset is actively infected (to be estimated). We suppose that the number q_r of organs for which the bacterial culture indicated an active infection is described by a binomial distribution:

$$q_r \sim \mathcal{B}(o_r, p_d \times b_r)$$

where p_d is the probability of detection of the bacteria by a bacterial culture when it is present. Note that we suppose here that a non-infected animal always returns negative cultures. This submodel allowed the estimation of the parameters p_d , b_r , and $w^{(b)}$.

Moreover, we supposed the following submodel to describe the CFT titre f_r for female r as a function of active infection b_r and age a_r . Preliminary data exploration suggested that the distribution of the logarithm of the titer for female r of the bacterial cultures dataset could be described by a Gaussian distribution. We therefore supposed the following model:

$$\log f_r \sim \mathcal{N}(\mu_r, \sigma_f)$$

where σ_f is an unknown standard deviation to be estimated and μ_r is modelled by:

$$\mu_r = \beta_0 + \beta_1 \times b_r + \beta_2 \times a_r + \beta_3 \times b_r \times a_r$$

This model was based on the results of Lambert et al. (2018b): we modelled the CFT level as the result of an interaction between the presence of an active infection b_r and the age a_r of the female.

Eventually, this component of our integrated model requires the estimation of the vector of unknown parameters $\theta_2 = (w^{(b)}, v\{b_r\}, p_d, \sigma_f, \beta_0, \beta_1, \beta_2, \beta_3)$.

c. Proportion of the population with active infection

We used the model described in the previous section to predict the proportion of seropositive females in the capture dataset Y_c that were actively infected by the bacteria.

For a given year t , and for each seropositive female i of the capture dataset, we represented the active infection by the bacteria by a hidden state variable b_{it} taking the value

1 if the female is actively infected and 0 otherwise. We supposed that the distribution of the infection states is represented by a Bernoulli variable characterized by a probability w_t :

$$b_{it} \sim \mathcal{B}(1, w_t)$$

The parameter w_t represent the target parameter of this submodel: it describes the proportion of the population of seropositive females with an active infection during year t .

We supposed the same model as for the previous section to describe the relationship between the infection state b_{it} of a captured seropositive female and the log-titer of its CFT:

$$\log f_{it} \sim \mathcal{N}(\mu_{it}, \sigma_f)$$

where μ_{it} is modelled by:

$$\mu_{it} = \beta_0 + \beta_1 \times b_{it} + \beta_2 \times a_{it} + \beta_3 \times b_{it} \times a_{it}$$

The values of β_0, \dots, β_3 have been estimated in the previous section. Therefore, the present model allows the estimation of $\theta_3 = (\{b_{it}\}, \{w_t\}_{t=1}^6)$.

Once these parameters are estimated, it is straightforward to calculate the proportion ω_t of females with active infection in the population, based on the seroprevalence estimates π_t and on the parameters w_t : this proportion is simply equal to $\omega_t = \pi_t \times w_t$.

Moreover, with a similar approach, it is also easy to estimate the proportion u_t of the population corresponding to unmarked females with active infection during year t , which was used for the estimation of the seroprevalence among marked females:

$$u_t = (1 - \gamma_t) \times \pi_t^{(u)} \times w_t$$

d. Model fit

We used a Bayesian approach to estimate the vector $\theta = (\theta_1, \theta_2, \theta_3)$. Note that the three submodels composing our model are not independent. We need the proportion of actively infected seropositive females w_t every year, estimated with θ_3 to calculate the force of infection of marked females in θ_1 . And we need to know the value of the coefficients $\beta_0 \dots \beta_3$ as well as σ_f , estimated in θ_2 , to calculate the probability of active infection estimated with the elements in θ_3 . Consequently, we decompose the posterior distribution of θ by:

$$[\theta | Y_m, Y_c, Y_b] \propto [\theta_1 | \theta_3, Y_m, Y_c] \times [\theta_3 | \theta_2, Y_m] \times [\theta_2 | Y_b]$$

where $[A|B]$ describes the probability of A given B. We defined noninformative priors on all the elements of θ , i.e. a uniform prior on $[0,1]$ for the parameters on a probability scale (e.g. $\pi_t^{(u)}$), a uniform prior on $[0,100]$ for the parameter η , a Gaussian prior with a standard deviation equal to 10 for the parameters β_k , and a gamma prior $\mathcal{G}(0.001,0.001)$ for the parameter σ_f . We fitted our model by MCMC with the JAGS software (Plummer, 2010), using 5 chains of 5000 iterations. We checked the mixing properties of the chains both visually and using the diagnostic of Gelman & Rubin (1992).

We checked the goodness of fit of our model, using the approach recommended by Gelman & Meng (1996). Each MCMC iteration generated a sampled value θ^r of the vector of parameters θ . For each simulated value θ^r , we simulated a replication of the captures, bacterial cultures and censuses datasets. We then compared summary statistics calculated on the observed datasets to the distribution of these summary statistics calculated on the simulated datasets. More specifically, we used as summary statistics: (i) the number of marked females detected during each day of census in the censuses dataset, (ii) the proportion of unmarked females detected as seropositive each year in the seroprevalence dataset, (iii) the same proportion calculated only on marked females, (iv) the log-titre of the CFT for each seropositive female. All these criteria indicated a correct fit of our model (see Appendix 4.1A).

We also used this type of approach to assess the assumption of constant capture effort over the core area of the massif (i.e., no spatial variation, see Appendix 4.1A for a complete description of this analysis): we divided our study area into two halves: a northeastern part and a southwestern part. We focused on the censuses dataset, and we used our model to test whether the proportion of marked animals observed in these two areas could have been expected under our non-spatialized model. This approach revealed that the capture effort was larger in the southwestern part than in the northeastern part, though the difference was small: the largest observed difference between the observed proportion of marked animals and the same proportion expected under a non-spatialized model occurred in 2018, when the observed proportion of marked animals in the southwestern part was equal to 56%, whereas a model ignoring this spatial variation predicted a maximum proportion of 45% of marked females at this place. However, we did not account for this spatial structure of the capture effort in our model, because (i) the differences between the model and the observed data were small, and (ii) this spatial structure would have made our model far more complex than it already is, probably too complex for such a small dataset.

All our analyses were carried out with the R software. We used the packages *rjags* for model fit (Plummer, 2016), *coda* (Plummer *et al.*, 2006) and *bayesplot* (Gabry and Mahr, 2018) for the analysis of the fit, and *tidyverse* (Wickham, 2017) and *ggplot2* (Wickham, 2016) for data manipulation and graphical display respectively.

4.1.3. RESULTS

The posterior distributions of the parameters of interest are displayed in Figure 4.1-3. We also present the 90% credible intervals on the seroprevalence and active infection parameters in Table 4.1-2 and Table 4.1-3 respectively.

The seroprevalence (Figure 4.1-3A) was roughly constant from 2013 to 2015, with approximately 45-50% of seropositive females in the population of interest. This proportion suddenly decreased between 2015 and 2016 (a calculation based on the MCMC samples indicated that the probability of a decrease of the seroprevalence between these two years was equal to 0.96), reaching about 20-30% in 2016-2018. Note that the proportion of marked females increased linearly from 2013 to 2016 (Figure 4.1-3B), but reached an asymptote after this date, with a proportion of 35-40% of marked females in the core area of the population.

The most striking feature in the plot of the posterior distribution of the proportion of actively infected females in the population of seropositive females (Figure 4.1-3C) in the core area of the massif is the uncertainty of our estimation. For example, in 2013, a 90% credible interval on this proportion indicated that this proportion was comprised between 22% and 73%. This uncertainty was even larger in 2017, where this credible interval covered all values between 35% and 94% (Table 4.1-3). However, despite this uncertainty, several patterns can be identified: a comparison of the posterior distributions for 2013 and 2014 indicated that there was a probability of 0.9 that the active infection in the whole population increased suddenly between these two years. Inversely, there was a probability of 0.94 that a decrease occurred between 2017 and 2018. This decrease seems to have started at the end of 2016, although the large uncertainty in 2017 precludes any firm conclusions on this aspect (the probability of a decrease between 2016 and 2017 was equal to 0.73).

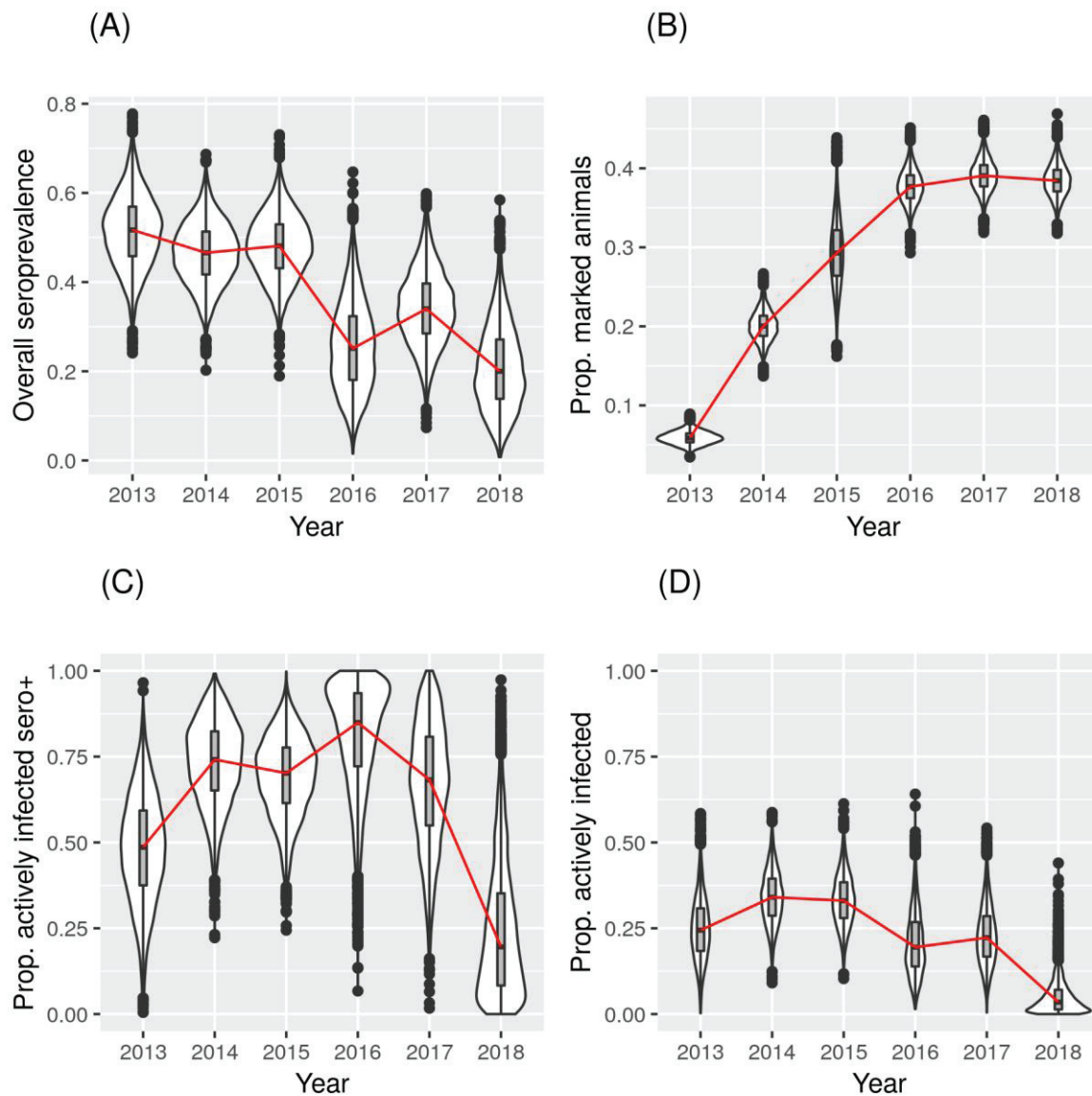


FIGURE 4.1-3 | Posterior distribution of the main parameters describing brucellosis in the population of female ibex of the core area of the Bargy massif, for each year between 2013 and 2018.

For each parameter, the posterior distribution is displayed by both a boxplot and a violin plot (i.e. a kernel smoothing of the distribution rotated and put on both sides of the boxplot, mirroring each other). (A) Posterior distribution of the seroprevalence in the population of females of the core area of the massif. (B) Posterior distribution of the proportion of marked females among females of the core area of the massif. (C) Proportion of actively infected females among seropositive females in the core area of the massif. (D) Proportion of the females in the core area of the massif actively infected.

TABLE 4.1-2 | Posterior mode and 90% credible intervals on the parameters of the model predicting the seroprevalence of brucellosis in the population of female ibex of the core area of the Bargy massif, for each year between 2013 and 2018. The seroprevalence is estimated for the marked females, the unmarked females, and globally. The estimated proportion of marked females in this population of females, used for the estimation of the overall seroprevalence, is also displayed.

Year	Overall seroprev.	Seroprev. Marked	Seroprev. unmarked	Prop. marked among all anim.
2013	0.51 [0.38-0.65]	0 [0-0]	0.55 [0.4-0.69]	0.06 [0.05-0.07]
2014	0.46 [0.35-0.57]	0.05 [0-0.15]	0.57 [0.43-0.7]	0.2 [0.17-0.23]
2015	0.48 [0.36-0.59]	0.08 [0-0.2]	0.65 [0.5-0.78]	0.29 [0.23-0.36]
2016	0.26 [0.11-0.43]	0.05 [0-0.16]	0.38 [0.16-0.64]	0.38 [0.34-0.41]
2017	0.34 [0.21-0.48]	0.06 [0-0.16]	0.52 [0.32-0.73]	0.39 [0.36-0.43]
2018	0.21 [0.07-0.38]	0.01 [0-0.07]	0.33 [0.11-0.59]	0.38 [0.35-0.42]

TABLE 4.1-3 | Posterior mode and 90% credible intervals on the parameters of the model predicting the active infection by brucellosis in the population of female ibex of the core area of the Bargy massif, for each year between 2013 and 2018. We present the proportion of seropositive females with an active infection, and the overall proportion of females with an active infection.

Year	Prop. seropos. active inf.	Overall prop. active inf.
2013	0.48 [0.22-0.73]	0.25 [0.1-0.41]
2014	0.73 [0.52-0.91]	0.34 [0.22-0.47]
2015	0.69 [0.48-0.87]	0.33 [0.21-0.46]
2016	0.81 [0.48-0.99]	0.21 [0.07-0.38]
2017	0.67 [0.35-0.94]	0.23 [0.1-0.38]
2018	0.24 [0.02-0.62]	0.05 [0-0.15]

The temporal changes of the proportion of actively infected females among all females (Figure 4.1-3D) reflect those of both the overall seroprevalence and the proportion of seropositive females with active infection (Table 4.1-3). Thus, this overall proportion of actively infected females probably increased from 2013 to 2014 (a calculation based on the MCMC samples indicated that there was a probability of 0.8 for this increase), reaching a maximum proportion of about 34% of actively infected females in the population. It decreased after 2015 (with a probability of 0.85) to reach about 20% in 2016 and 2017, and then decreased again between 2017 and 2018 (with a probability of 0.97) to reach 5% of the population in 2018.

The temporal changes in the force of infection (expressed as the proportion $1 - e(-\lambda_t)$ of animals infected during a year) estimated by our model (Figure 4.1-4) followed closely the changes in the proportion of actively infected females, with a maximum of 11% of newly infected marked females per year in 2014 (90% CI: 3%-24%), and a minimum of 1.7% in 2018 (90% CI: 0.06%-5.5%).

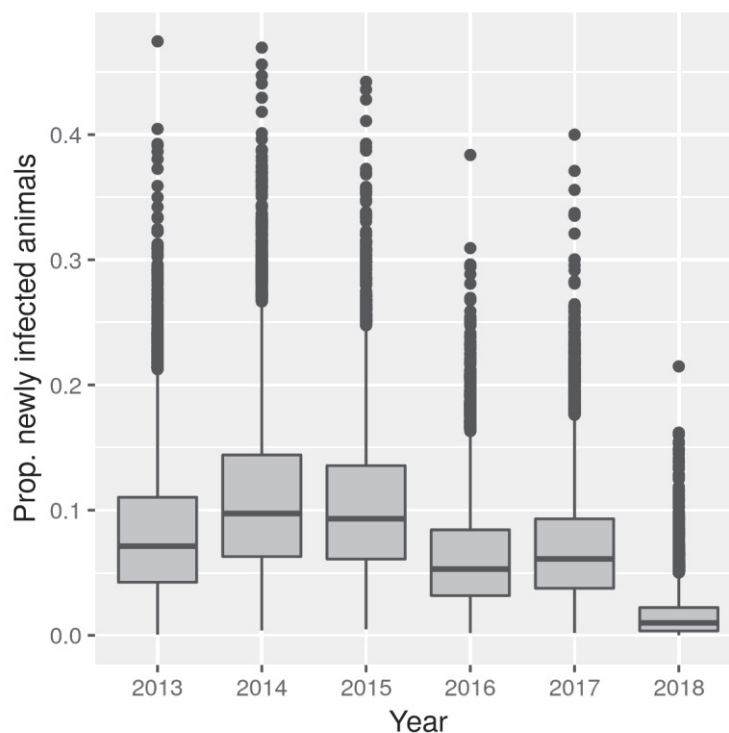


FIGURE 4.1-4 | Boxplot showing the posterior distribution of the force of infection by brucellosis for each year between 2013 and 2018. This force of infection is here represented as the proportion $1 - e(-\lambda_t)$ of marked females of the core area of the ibex population of the Bargy massif that are seronegative at the beginning of a year, and which become seropositive at the end of the year.

4.1.4. DISCUSSION

We have developed a new model for the estimation of key parameters for the monitoring of brucellosis in the ibex population of the Bargy massif. This model allowed to correct the data collection bias caused by a capture program designed to implement an efficient test-and-cull strategy, an aim incompatible with the unbiased monitoring of the disease on this difficult-to-catch species in this difficult-to-access mountainous area. Moreover, we estimated the proportion of the population actively infected by the bacteria, and not only seroprevalence. Indeed, seroprevalence may not be the most reactive parameter to a change of the disease prevalence in the population, as already highlighted in other brucellosis wildlife reservoirs (National Academies of Sciences, Engineering, and Medicine, 2017).

We corrected the data collection bias by modelling separately the subset of marked and unmarked females in the population. The models used for these two subsets were very different: we had enough data to estimate the seroprevalence of the disease every year for unmarked females, but the number of recaptured females was too small to allow the same strategy for marked females. We therefore developed a model of the disease transmission to estimate the seroprevalence among marked females. This model relied on the assumption that the force of infection of marked females was proportional to the proportion of the population of females corresponding to unmarked females with an active infection. This is valid under three assumptions: (i) that the marked females are mostly infected by the unmarked females, (ii) that the infection from males is negligible, or follows a similar pattern as the infection from unmarked females, and (iii) that the infection is frequency dependent (i.e. depends on the proportion of infected females in the population).

We supposed that the force of infection of marked females was only determined by actively infected unmarked females, and therefore ignored the possibility of transmission among marked females. This is a reasonable hypothesis, as our model suggests that the number of newly infected marked females is very small (Table 4.1-2), so that most actively infected females are actually unmarked females. However, we must keep in mind that this might not be the case in the future, if the disease prevalence were to increase among marked females. Our model also ignored the possible transmission of the disease from males to females. However, the similarity of seroprevalence patterns between males and females (e.g. see Figure 4 in Marchand *et al.*, 2017) suggests that we would not have obtained different results if we had estimated the proportion of actively infected animals for the two sexes.

The exact form of the disease transmission has not been assessed in the Bargy massif, but this type of mechanism is generally highly complex, implying a large number of factors acting across several scales (Pepin *et al.*, 2017), and probably includes both frequency-dependent and density-dependent components. The sexual route, a typically frequency-dependent transmission route (Ryder *et al.*, 2007), is one possible way of transmission of the disease in domestic ruminants and is probably largely involved in the large spatial scale transmission over the massif (Marchand *et al.*, 2017). On the other hand, recent results suggest that the environment (i.e. through infectious abortions for example) is the main cause of transmission of the disease at a small scale in the ibex population of the Bargy (Marchand *et al.*, 2017), also suggesting a density-dependent transmission. The halving of the population size resulting from the mass culling probably led to a decrease of the density-dependent component of the force of infection, and therefore of the force of infection itself. Therefore, in theory, if our model had accounted for density dependence, it would have estimated an even larger decrease with time of the infection and seroprevalence levels. In practice however, our results proved very robust to the violation of this assumption: the three very different models fit to describe the infection process among marked females indeed returned similar estimates of the overall seroprevalence (see Appendix 4.1A). We even tried to fit a fourth model supposing that the force of infection was proportional to a very approximate estimate of the density of infected unmarked females (i.e. modelling a possible density dependence of the infection, and therefore accounting for the large changes of density observed on the massif). This model – presented in the Appendix 4.1A – also resulted in estimates nearly identical to the ones given in this paper. Therefore, our integrated model is very robust to the form of the model supposed for the infection process.

Our model relies on the assumption that the sample of captured females is representative of the population. In particular, the spatial distribution of the captured females must reflect the spatial distribution of the population, and the age distribution in our sample must be similar to the age distribution in the population.

The approach of Gelman & Meng (1996) used to assess the goodness of fit of our model revealed that, at the scale of the whole year, the proportion of marked females was slightly larger in the southwestern part of the core area of the massif than in the northeastern part. This southwestern part corresponds to the very core of the massif, where the prevalence of the disease was the largest at the beginning of the study period (Marchand *et al.*, 2017). The higher effort of the test-and-cull operations at this place resulted in a slightly larger

proportion of animals captured in this highly infected area. However, we do not think that this led to a discernible bias in our estimates of seroprevalence and infection level, as: (i) the difference of capture rates between the two parts of the massif was not very large, (ii) the difference in seroprevalence between the two areas was not large either (compare "Grand Bargy/Jallouvre-Peyre" – corresponding to the southwestern part – vs. "Petit Bargy" – corresponding to the northeastern part – in Figure 4 of Marchand *et al.*, 2017), and (iii) the uncertainty of our estimates of seroprevalence and infection level was very large, probably far larger than the possible resulting bias. Moreover, note that, as the proportion of animals controlled by these test-and-cull operations increased with time, this higher disease control effort in the more highly infected area probably resulted into a more homogeneous spatial distribution of prevalence over the massif at the end of the study period, thereby reducing the already small impact of this spatial distribution of captures. In fact, by focusing our analysis on the sedentary females inhabiting the more homogeneous and more infected core area of this ibex population, we accounted for most of the strong spatial structure of the disease.

The age distribution of the captured females must be similar to the age distribution in the population. This is an important assumption, as the probability of active infection is generally strongly related to the age of the females for brucellosis (Treanor *et al.*, 2011; Lambert *et al.*, 2018b). Actually, although the available data on these two distributions are rather imprecise, it seems that the age distribution of the captured females is similar to the age distribution in the population. Based on a comparison between the censuses dataset and capture dataset (see Appendix 4.1A) we could not find any significant difference between the proportion of female aged 6 years old observed in the sample and in the population from 2015 to 2018. Small significant differences were however observed in 2013 and 2014: the females were in average older in the sample than in the population in 2013, and the reverse was true in 2014. This may explain the sudden increase in the prevalence of active infection among seropositive females observed between these two years (Figure 4.1-3A): as older females are generally less prone to active infection (Lambert *et al.*, 2018), this prevalence may be overestimated (resp. underestimated) when the sample is composed of younger (resp. older) females than the population. It is therefore possible that the actual prevalence of active infection was more constant than predicted by the model at the beginning of the study period. On the other hand, we must remain cautious concerning any conclusions implying the estimated age of detected animals: as noted previously, the field workers were not equipped

with telescopes before 2015, so that the estimation of the age structure of the population based on the censuses is probably very noisy (see below). It is therefore also possible that the age structure of our sample of captured animals was similar to the age structure in the population, and that this increase of active infection was real at the beginning of our study period.

Our model revealed that the seroprevalence of brucellosis suddenly decreased between 2015 and 2016. This is probably the result of both the extensive test-and-cull operations and the mass culling carried out in 2015. Indeed, this year, 125 animals were captured and controlled over the whole massif, and 70 additional unmarked animals were indiscriminately culled, from a population of about 300 animals at that time (Figure 4.1-1). On the one hand, the test-and-cull process directly affects the proportion of seropositive individuals in the population by the selective elimination of those seropositive animals. On the other hand, the mass culling operation targeted unmarked individuals, which are more at risk of being infected. When combined, these two operations led to a significant change in the dynamic of the disease, and in particular to a decrease in the force of infection after 2015 (Figure 4.1-4).

Although seroprevalence did not vary significantly from 2016 to 2018, the proportion of actively infected ibex decreased drastically and significantly after 2015, from about 30% in 2015 to about 5% in 2018. This result confirms our assumption that seroprevalence is not a very reactive parameter to management measures, certainly due to the long lasting of antibodies and the long lifespan of adult ibex (16 years for males, 20 years for females, Toïgo *et al.*, 2007). We also observed a significant drop of the proportion of actively infected ibex from 2017 to 2018, even though we cannot exclude an undetectable sampling bias due to the small number of seropositive ibex from 2018 ($n=2$). Indeed, with such small sample sizes the resulting estimates must be considered with great caution, i.e. as indicative cues rather than as a definite proof of an ongoing extinction of the disease.

Nevertheless, this estimated decrease of active infection in 2018 suggests that the lighter test-and-cull measures targeting the core area implemented from 2016 to 2018 also contributed to decrease the disease prevalence within the core area. In parallel, raw disease prevalence decreased in the periphery of the Bargy massif (S. Rossi, unpublished data), which suggests that the management measures implemented in 2015 (mass test-and-cull and mass culling) and the following years (targeted test-and-cull within the core area) contributed to contain brucellosis transmission at the scale of the whole massif. This result

is important since test-and-cull or mass culling campaigns brings moderate and highly variable disease control efficacy in free ranging wildlife reservoirs (Gortázar *et al.*, 2015). Its success may be partly due to the relative sedentary behaviour of ibex and the fact that management mainly targeted the core area over the past 3 years.

Note that the mass culling performed in 2013, which resulted in the halving of the population size, was not immediately followed by a decrease of seroprevalence. Actually, preliminary results based on the comparison for marked animals in 2013 between the remote age estimation and the age estimation at the time of capture (with the animal in hand), showed that classification error by field workers was very large this year, due to the absence of telescopes: about half of females actually > 6 years old were reported as < 6 years old by field workers (C. Calenge, unpublished data). Thus, field workers did not necessarily succeed in culling preferentially older females, explaining this lack of effect of this major mass culling on the seroprevalence. On the other hand, the observed increase in the proportion of actively infected ibex between 2013 and 2014, if not an artefact caused by a biased age structure in the sample (see above), could correspond for example to social rearrangements consecutive to the mass culling performed in autumn 2013, causing a short incidence increase within the core area in naive individuals.

Our approach thus provides a first appreciation of the current epidemiological situation on the Bargy massif, and allows a preliminary assessment of management measures efficacy, with a strong effect of intensive test-and-cull operations combined to mass culling (in 2015), but also of lighter targeted test-and-cull within the core area (2016-2018). However, data analysis is obviously not sufficient to predict the future evolution of the outbreak and a sound assessment of management measure efficacy, which requires the development of dynamic models able to reproduce population and bacteria dynamics (Lambert *et al.*, 2018b).

Nevertheless, this success is partial since brucellosis is still observed in the Bargy population by 2018 (i.e. 6 years after the discovery of the outbreak). Furthermore, the limited number of captures currently performed in the core area of the massif and the likely decrease of the ability of the rapid test to diagnose seropositivity (due to the decrease of antibody titres in animals), might both contribute to a limited efficacy of the test-and-cull strategy in the future.

4.1.5. CONCLUSION

Overall, here a combination of management operations was associated to a decrease in seroprevalence and in the force of infection. However, the exact consequences of each applied measure (indiscriminate versus selective culling) cannot be disentangled here. In other host-pathogens systems, indiscriminate culling was often associated to unwanted side effects due to the socio-spatial disorganization of the population and/or the density-dependent population processes. This has been largely studied in the case of tuberculosis in badgers *Meles meles* (McDonald *et al.*, 2008) but has also been demonstrated in other varied situations (Choisy and Rohani, 2006; Prentice *et al.*, 2014; Lee *et al.*, 2018).

The case of brucellosis in ibex may be one of the first examples of a management program entailing more positive than negative effects, possibly due to the specific features of this system. These include a host-pathogen system that is possibly intrinsically unstable due to moderate basic reproductive number (the Bargy population is the only case of persistence of brucellosis in this species – sporadic cases are generally described elsewhere), and drastic management options with a major population size reduction and a strong capture effort. Even when transmission is enhanced after culling, this effect may be masked by drastic management measures as long as these measures are maintained (Prentice *et al.*, 2014). Here, a better understanding of the mechanisms underlying the observed decreases in prevalence and force of infection would be helpful to infer the future evolution of the situation after 3 years of stability and with management measures that involve smaller numbers of individuals.

This model provided an interesting approach to the issue of the brucellosis monitoring when the disease prevalence was high in the population, and when the capture effort was high enough to allow a precise estimation of the proportion of actively infected females. However, it seems that, for several years, we have been reaching a point where the disease is so rare that such a statistical monitoring of this disease is difficult. A power study is now required to assess the required number of captures under different scenarios (i.e. for different values of seroprevalence and prevalence of active infection) to reach a given precision of our estimates. On the other hand, mathematical epidemiological models, such as SEIR models (Keeling and Rohani, 2008), could provide an interesting alternative to statistical models, in the case where not enough data can be collected for this monitoring. Actually, such a model is currently being developed based on what is known of the ibex population

dynamics on the massif, on the social organisation of this species, and on what is known of the history of the disease in this area (Lambert *et al.*, in prep. – chapter 3.2 of this thesis). This model will be used to investigate how the disease parameters will change under different management scenarios, and hopefully eradicate the brucellosis from the area.

APPENDIX 4.1A

1. Notations used in the paper

We present, in the table below, the notations used in this paper. We distinguish several types of notations:

- D: data used to fit the model
- P: stochastic parameter estimated with MCMC
- N: other notations

In the table below, we give:

- the notation
- the type of notation
- a description of the variable or parameter

We recall that the population of interest here is the population of females of the core area of the massif. The parameters are given by order of appearance in the main text.

Notation	Type	Description
Y_m	D	Captures dataset
Y_c	D	Censuses dataset
Y_b	D	Bacterial cultures dataset
π_t	P	Overall seroprevalence during year t in the population of interest
$\pi_t^{(u)}$	P	Seroprevalence during year t in the population of unmarked females
$\pi_t^{(m)}$	P	Seroprevalence during year t in the population of marked females
γ_t	P	Proportion of marked females during year t in the population of interest
$n_{j(t)}$	D	Total number of detected females during day j of the year t
$m_{j(t)}$	D	Number of detected marked females during day j of the year t
L_t	D	Number of unmarked females captured during the year t
ℓ_t	D	Number of seropositive females among the unmarked females captured during the year t
Z_t^h	D	Number of females first captured during year h and recaptured during year t
z_t^h	D	Number of females seropositive at time t among the females first captured during year h and recaptured during year t

(continued)

Notation	Type	Description
ζ_t^h	P	Probability that a female first captured and seronegative during year h was seropositive when recaptured during year t
$v^+(x)$	N	Proportion of marked females seropositive at time x (notation only used in the Appendix)
$v^-(x)$	N	Proportion of marked females seronegative at time x (notation only used in the Appendix)
λ_t	P	Force of infection during the year t
u_t	P	Proportion of the unmarked females characterised by active infection at time t
η	P	Parameter controlling the relationship between u_t and λ_t
N_t^h	P	Number of females that were first marked during year h and that were still alive during year t
ϕ	P	Annual survival rate, supposed to be equal to 0.95
ψ_t^h	P	Proportion, among the marked females recaptured at time t , of females that were first captured and seronegative at time h
b_r	P	Hidden state variable taking the value 1 if the r -th female of \mathbf{Y}_b is actively infected and 0 otherwise
$w^{(b)}$	P	Probability that a female of \mathbf{Y}_b is actively infected
o_r	D	Number of organs for which a bacterial culture has been carried out for the female r of \mathbf{Y}_b
q_r	D	Number of organs where the culture indicates an active infection for the female r of \mathbf{Y}_b
p_d	P	Probability to detect the bacteria with a bacterial culture of an organ, given that the female is actively infected
f_r	D	Titre of the complement fixation test (CFT) for the female r of \mathbf{Y}_b
a_r	D	Age of the female r of \mathbf{Y}_b
σ_f	P	Standard deviation of the Gaussian distribution of the log-titre of the CFT
$\beta_0 \dots \beta_3$	P	Coefficients of the relationship relating age and active infection on the one hand, and log-titre of the CFT on the other hand
w_t	P	Proportion of animals with active infection among seropositive females during year t
b_{it}	P	Hidden state variable taking the value 1 if the i -th female during year t of \mathbf{Y}_m is actively infected and 0 otherwise
f_{it}	D	Titre of the CFT for the female i of year t in \mathbf{Y}_m
a_{it}	D	Age of the female i of year t in \mathbf{Y}_m
ω_t	P	Proportion of the females with an active infection during year t
$c_{j(t)}$	D	Number of females older than 6 years old detected during day j of the year t (notation only used in the Appendix)
ξ_t	P	Probability that a female on the core area of the massif is older than 6 years old during year t (notation only used in the Appendix)
g_t	D	Total number of females captured during year t (notation only used in the Appendix)

(continued)

Notation	Type	Description
d_t	D	Number of captured females older than 6 years old during year t (notation only used in the Appendix)
τ_t	P	Probability that a captured female is older than 6 years old during year t (notation only used in the Appendix)

2. Dynamic model of the infection of the marked females

In this appendix, we develop the catalytic model of infection that we used in the paper to describe the infection of marked females.

The force of infection represents the rate at which susceptible individuals become infected by unit of time, at time t (Vynnycky and White, 2010). The force of infection depends of two parameters: the rate at which two specific individuals come into effective contact (i.e. come into contact and the contact leads to infection) per unit time, and the number of infectious individuals at time t (density-dependent model) or the frequency of infectious individuals in the population at time t (frequency-dependent model, Begon *et al.*, 2002). Given the mathematical relationship between risks and rates, the incidence risk can be derived from the force of infection following: $1 - e^{-\lambda}$.

If we were to follow over time a cohort of individuals born the same year, we would observe that, as the cohort ages, the proportion of individuals who have ever been infected increases. Catalytic models therefore track individuals from birth, to estimate the average force of infection from the proportion of individuals who have ever been infected at different ages (i.e. who experienced different exposure times).

We adapted a catalytic model (Heisey *et al.*, 2006; Hens *et al.*, 2010) of reinfection of marked females for the estimation of the force of infection of marked females. The assumptions of a catalytic model are, classically, that the average force of infection λ is independent of age and year, that susceptible and infected females have the same mortality rate (i.e., no infection-related mortality experienced by infected females), and that all individuals are susceptible at birth (or when they are marked, after checking for seronegativity). However, those assumptions are not necessarily true, and here we relaxed the first assumption, setting that the force of infection can change with time, for example if infectious individuals or their prevalence is not constant with time.

We use the same notations as in the paper. Consider a given year t . Let x be the time spent since the start of the year t , expressed in years (for example $x = 0.5$ indicate 6 months after the start of the year). Let $v^+(x)$ be the proportion of marked females seropositive at time x , and $v^-(x)$ the proportion of marked females that are seronegative at time x . We suppose the following catalytic model to describe the infection of seronegative marked individuals during a given year:

$$\frac{dv^+(x)}{dx} = \lambda_t \times v^-(x)$$

with λ_t the force of infection of the disease during year t . Similarly, the equation describing the changes in the number of seronegative females is:

$$\frac{dv^-(x)}{dx} = -\lambda_t \times v^-(x)$$

The solution to this equation is: $v^-(x) = v_t^- \times e^{-\lambda_t x}$, where v_t^- is the proportion of females seronegative at the beginning of year t . Consider a given year t . The proportion of seronegative females at the beginning of the year $t + 1$ can be calculated by setting $x = 1$:

$$v_{t+1}^- = v_t^- \times e^{-\lambda_t}$$

The proportion of seronegative females at the beginning of the year $t + 2$ will therefore be equal to:

$$v_{t+2}^- = v_t^- \times e^{-\lambda_t - \lambda_{t+1}}$$

Therefore, if v_1^- is the proportion of seronegative females at the start of year 1, then:

$$v_t^- = v_1^- \times e^{-\sum_{h=1}^{t-1} \lambda_h}$$

with $\lambda_0 = 0$. Remember that we here only focus on marked females. Year 1 corresponds here to the year of capture and release. By definition, since animals are only released if they are seronegative, $v_1^- = 1$.

Now consider a female captured and marked during year h and recaptured during year t . Then, since the animal was seronegative at time h , it follows that the probability ζ_t^h that a female seronegative at time h and still alive at time t was seropositive is equal to:

$$\zeta_t^h = 1 - e^{-\sum_{s=h}^{t-1} \lambda_s}$$

which corresponds to the equation given in the paper.

3. Directed acyclic graph

We present the directed acyclic graph of the model fitted in the paper in Figure 4.1A-1 of this appendix.

4. Comparison of different models for the seroprevalence of marked females

The main difficulty of the model presented in the paper was to estimate the seroprevalence every year among marked females. We tried three different solutions before choosing the solution presented in the paper (and developed in section 2 above). The three compared models were the following:

- *Variable force of infection*: the model used in the paper, where the force of infection of marked females changes from one year to the next, and is supposed to be proportional to the proportion of unmarked females with an active infection.

- *Constant force of infection*: in this model, we supposed that the force of infection of marked females was constant through time, i.e. $\lambda_t = \lambda \forall t$. We did not suppose that this force of infection was proportional to the proportion of active infection among unmarked females, but we just supposed that the force of infection stays the same throughout the whole period (λ was therefore the only parameter to be estimated in the submodel describing seroprevalence in marked females).

- *Constant seroprevalence*: in this model, we supposed that the seroprevalence of marked females was constant throughout time. Therefore, we supposed that the females captured in 2013 and recaptured in 2018 had the same probability to be seropositive as the females captured in 2017 and recaptured in 2018 (this seroprevalence was therefore the only parameter to be estimated in the submodel describing the seroprevalence in marked females).

Note that the first of these models, which was used in our paper, supposed that the force of infection was proportional to the **proportion** of the unmarked females actively infected. In other words, we supposed that the infection was frequency dependent. We tried to fit a fourth model supposing that the force of infection was proportional to the **number** of unmarked females actively infected. However, since we did not estimate this number in our paper, we relied on the point estimates of the population size obtained by capture-recapture (see Figure 4.1-1 of the main paper). We supposed that the force of infection was equal to:

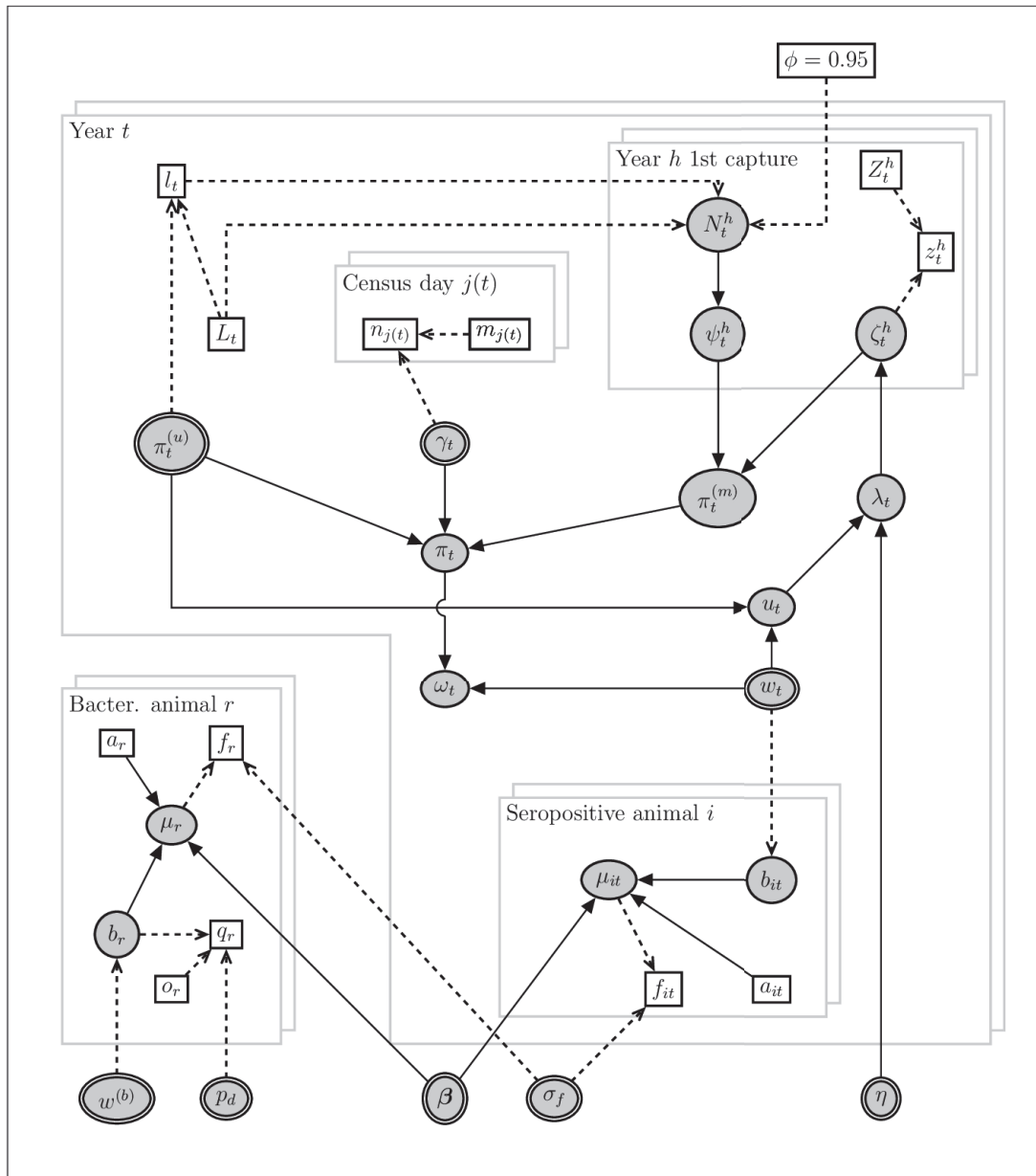


FIGURE 4.1A-1 | Directed acyclic graph describing the model used to estimate the seroprevalence and prevalence of active infection by the brucellosis among females of the ibex population of the core area of the Bargy mountains, France. Mathematical notations are listed in appendix 1. On this graph, each parameter is represented in an elliptic grey box. Parameters at the top of the hierarchy (i.e. those for which we defined a prior distribution with fixed hyperparameters) are characterized by a double border. Data are represented by squared white boxes. Parameters and data are located in frames with grey borders, describing the level of their definition (e.g. there is one value of w_t to be estimated by value of Year t , so that w_t is located in the frame "Year t "). Arrows connecting two boxes describe a relationship between two parameters or a parameter and a data point. The arrow is dashed when the relationship between the two elements is stochastic, and plain when the relationship between the two elements is deterministic.

$$\lambda_t = \eta \times u_t \times E_t$$

where η, u_t have the same meaning as in the model used in the paper, and E_t is the point estimate of the population size over the whole Bargy massif. Note that this is a very rough approach, as we do not account for the imprecision of the estimation of E_t , and as the population size estimate targeted the whole massif and the two sexes (and not only the females of the core area). For this reason, this model was not a good candidate to achieve our aim, but it was nevertheless useful, as it allowed to assess the effects of the large changes of the population size on our estimates.

We fitted these four models and compared the estimates of seroprevalence in the whole population under these four models in Figure 4.1A-2 of this appendix. These four very different models do not result into very different estimates of the overall seroprevalence. The most important difference was between the estimates of the model supposing a constant seroprevalence among marked females and the estimates of the other three models. Indeed, the force of infection seems to have decreased with time as a result of the management operations (test-and-cull), which explains why the difference between the estimates increase through time.

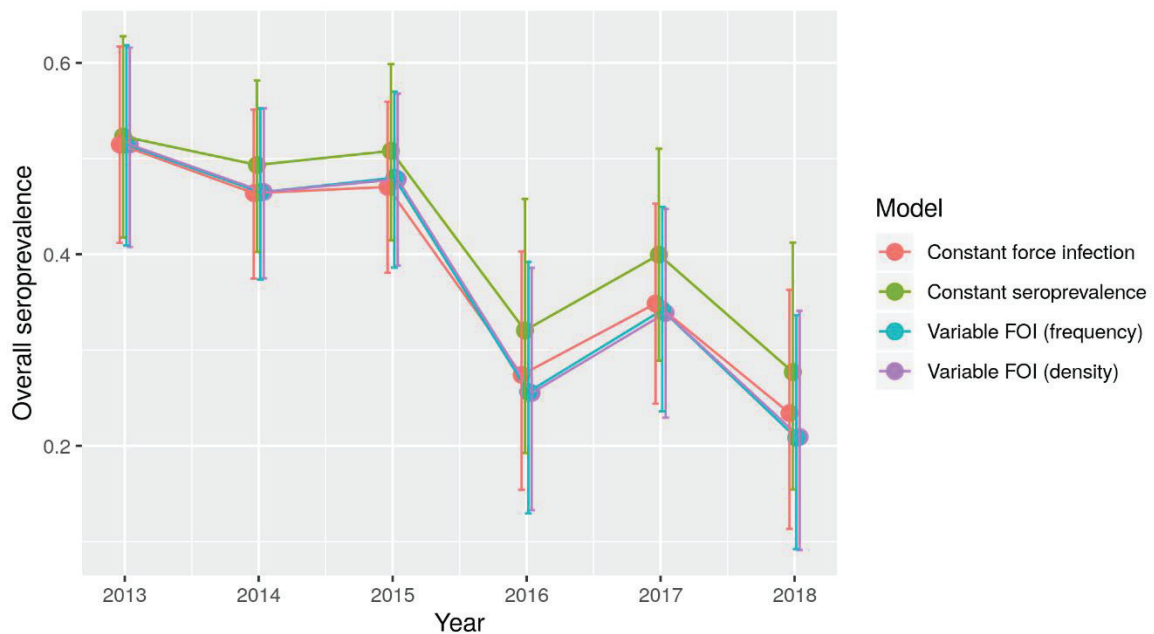


FIGURE 4.1A-2 | Estimates of the overall seroprevalence obtained under four models supposed for the seroprevalence of marked females. The error bars correspond to 80% credible intervals on estimate.

We chose to model the seroprevalence of marked females with the model of variable force of infection, because this model was more realistic than the two other candidate models (i.e. constant prevalence and constant force of infection), and because it was not more complex than these two models: given the rest of the model, this submodel relies on only one unknown parameter (η), similarly to the alternative two submodels (constant force of infection: λ ; constant seroprevalence: the seroprevalence among unmarked females).

Note that accounting for large changes in population size had nearly no effect on the seroprevalence estimates, indicating the robustness of our estimates to the violation of the frequency-dependence assumption.

5. Goodness of fit of our models

In this section, we illustrate the goodness of fit of our model. We first present in Figure 4.1A-3 a traceplot of the MCMC chains for all parameters of the model. As clearly illustrated on this figure, the mixing of the MCMC chains was excellent for all parameters.

We also tested the goodness of fit of our model by simulating a replication of our datasets for each MCMC iteration (see main paper). We then compared summary statistics calculated on the observed datasets to the distribution of these summary statistics calculated on the simulated datasets. More specifically:

- The number of marked females among all females detected during a given census day is in the 80% credible intervals in 81% of the cases.
- The log-titres of the CFT generated were in the 80% credible intervals in 88% of the cases.

The percentage of unmarked females detected as seropositive is in the centre of the 80% credible interval for all years of the study period, as shown in the table below:

Year	Observation	Simulated 80% CI
2013	55	[39-71]
2014	58	[42-73]
2015	65	[46-81]
2016	38	[12-75]
2017	54	[31-77]
2018	29	[0-71]



FIGURE 4.1A-3 | Traceplot of the MCMC chains for all parameters of our model. The name of the parameter generally corresponds to its notation (e.g. β_0 is named *beta0*). For other parameters: *pbaet* corresponds to w_t , *ppor* corresponds to ω_t , *ppret* corresponds to π_t , *pprev* corresponds to σ_f . $\pi_t^{(u)}$, *prevre* corresponds to $\pi_t^{(m)}$, *probad* corresponds to p_d , *probai* corresponds to w^b , *probatq* corresponds to γ_t , *sigmafc* corresponds to σ_f . The index within brackets indicate the year (e.g. lambda[1] corresponds to λ_1 for year 1 of the study, i.e. 2013).

Note that this is also the case for the overall seroprevalence of marked females (calculated over all years; observation: 14%, simulation: [0-36]).

Our model did not account for any spatial variation in the capture effort. We assessed the validity of this assumption using the censuses dataset. We distinguished two areas in our study area: the northeastern part (Petit Bargy, located in the rectangle delimited by the points with coordinates [46°N, 6.48°E] and [46.02°N,6.53°E]) and the southwestern part (Jallouvre-Peyre and Grand Bargy, rest of the core area). Our model therefore supposes that the proportion of marked females was the same among females detected in the northeastern part and in the southwestern part. We assessed this assumption by simulating a predictive distribution of the proportion of marked females among all detected females for each year and each area.

We present below, for each year and each area, the total number of females detected during the censuses ("Total") and the number of marked females detected during these censuses ("Marked"). We present in parentheses the percentage of marked females among detected females. Finally, we present the limits of the credible intervals on this percentage, as simulated by our model ignoring spatial variation of capture effort.

Year	Area	Total	Marked (%)	Simulated 95% CI
2013	Southwest	499	24 (5%)	[3%-9%]
2014	Southwest	249	72 (29%)	[14%-27%]
2015	Southwest	61	28 (46%)	[16%-44%]
2016	Southwest	285	152 (53%)	[31%-45%]
2017	Southwest	303	163 (54%)	[32%-46%]
2018	Southwest	326	184 (56%)	[32%-45%]
2013	Northeast	299	8 (3%)	[3%-9%]
2014	Northeast	187	15 (8%)	[13%-27%]
2015	Northeast	54	6 (11%)	[15%-44%]
2016	Northeast	218	36 (17%)	[30%-45%]
2017	Northeast	266	62 (23%)	[32%-46%]
2018	Northeast	217	46 (21%)	[31%-46%]

The proportion of marked females among all detected females was underestimated by the model in the southwestern part, and overestimated by the model in the northeastern part (especially at the end of the study period – compare the percentage given in the column "Marked" with the limits of the credible intervals). This is likely due to a stronger capture effort in the southwestern part than in the rest of the study area (see main text).

6. Age structure

In this section, we explored the available data to check the assumption that the age structure of our sample of captured females is similar to the age structure of the population of female. We used the censuses dataset as a validation dataset, as the observers defined the age class of the detected females during the censuses. We focus here only on the females detected on the core area of the massif. We believe that classification error may have been frequent in this process (age determination by remote visual observation is less precise than when the observer has the females at hand during the captures), but this can yet give cues on the respect of this assumption.

As the observers often classify the detected females into two age classes (6 years old or younger, and > 6 years old), we dichotomized the age of captured females and all females observed during the censuses into two age classes ≤ 6 years old and > 6 years old. We fitted a Bayesian model to estimate the proportion of > 6 years old in each group (captures and censuses), for each year. Remember that $n_{j(t)}$ is the number of detected females during day j of the year t . Let $c_{j(t)}$ be the number of females older than 6 years old detected this day. We suppose that $c_{j(t)}$ follows a binomial distribution:

$$c_{j(t)} \sim \mathcal{B}(n_{j(t)}, \xi_t)$$

with ξ_t the probability that a female on the core area is older than 6 years old during year t .

Similarly, let g_t be the total number of females captured during year t (marked + unmarked) and let d_t be the number of females older than 6 years old in this group. We suppose that d_t also follows a binomial distribution:

$$d_t \sim \mathcal{B}(g_t, \tau_t)$$

with τ_t the probability that a captured female is older than 6 years old during year t .

We used the JAGS software to estimate ξ_t and τ_t (defining uniform prior bounded by 0 and 1 on these parameters). The estimates for these parameters are displayed on Figure 4.1A-4.

Significant differences are observed for 2013 (captured females are in average older than the population) and 2014 (captured females are in average younger than the population), but no differences are highlighted for the other years.

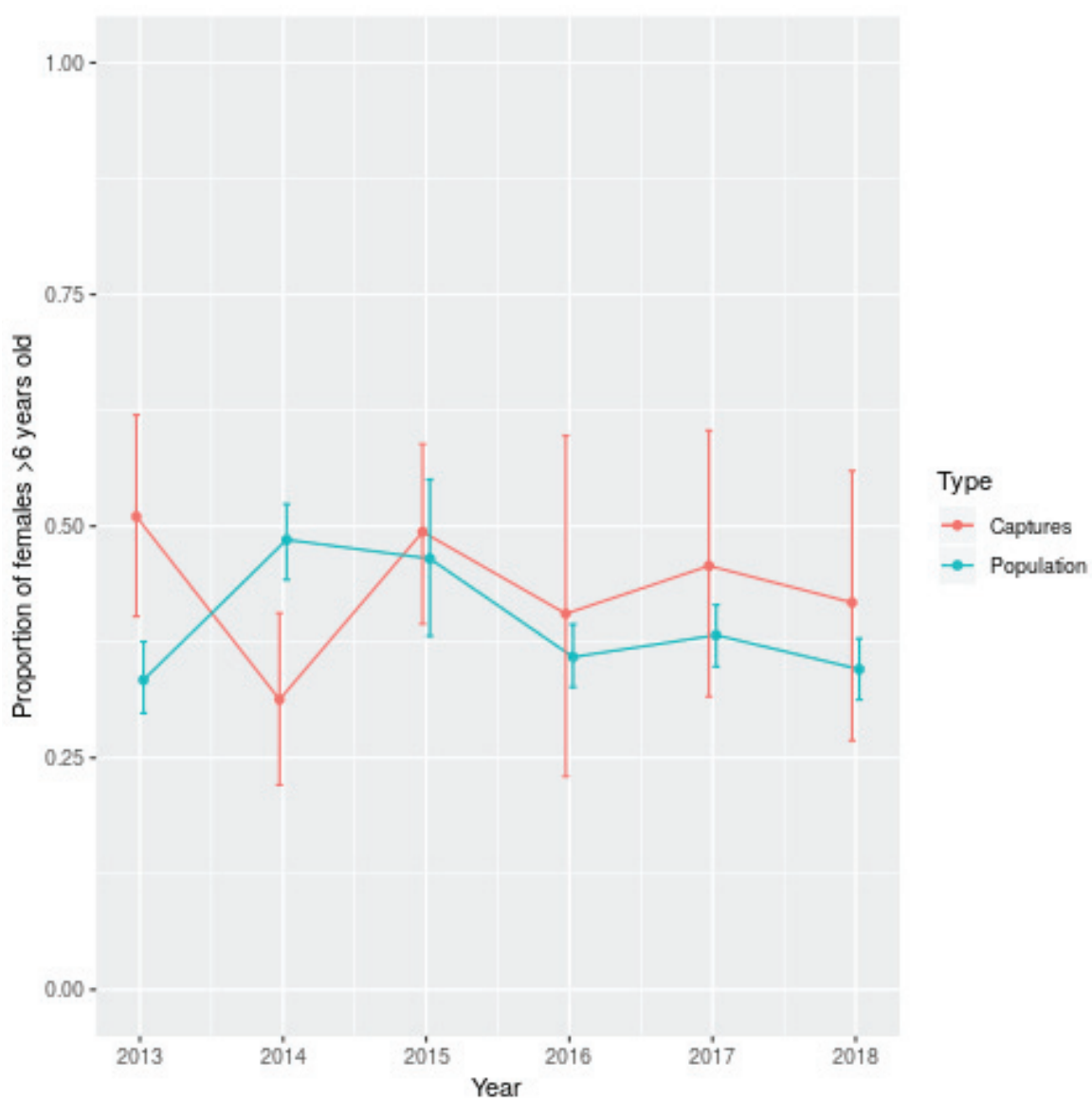


FIGURE 4.1A-4 | Estimates of the probability that a female is older than 6 years old in the population and the captures. Error bars correspond to the 90% credible intervals on the estimates.

Interlude

Focusing on the population of adult females in the core area of the massif, we demonstrated in this first study (4.1) that the seroprevalence has decreased between 2015 and 2016. Moreover, the predictions of the proportion of actively infected females, based on the relationship between active infection and CFT titre (3.1 – Lambert *et al.*, 2018b), also showed a decrease during the study period, from a likely estimate of 34% to less than 15%. Overall, these results suggest that the management interventions have been effective in reducing both the seroprevalence and the prevalence of the infection. The strong decrease between 2015-2016 especially suggest that the extensive test-and-remove operation carried out in 2015, combined to the culling of 70 unmarked individuals, was especially effective.

In the Bayesian model used in 4.1, the force of infection, i.e. the rate at which susceptible individuals become infected per unit time, was used to estimate the seroprevalence in marked females. This force of infection, which was allowed to vary from year to year, was not directly estimated from data because of the small sample size of marked females. Instead, we made the assumption that the force of infection was directly proportional to the proportion of unmarked females with an active infection in the population. The temporal changes of the force of infection therefore followed closely the changes in the proportion of actively infected females.

In the following study (4.2), we were interested in estimating the force of infection directly from the available data, instead of assuming a particular mechanism. A successful disease management is expected to reduce the force of infection, which secondary results in decreasing infection prevalence (proportion of infected at a given time) and/or spatial distribution. Therefore, we predicted that the FOI should decrease between 2012 and 2018, if the decrease in the seroprevalence and the prevalence of active infection estimated in the previous study were really an indication that management interventions were effective. We used two distinct methods for unmarked and marked individuals, adapted to the type of dataset available in each case, in order to compare the FOI between both groups.

4.2. Effects of disease management interventions on the force of infection of brucellosis in an Alpine ibex (*Capra ibex*) population

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In prep.

ABSTRACT

Disease management in wildlife requires a sound evaluation of the epidemiological situation and of the effects of management interventions on the infection and the population. In this study, we assessed the effects of management interventions on the force of infection of brucellosis in an Alpine ibex (*Capra ibex*) population. This population was the object of several culling operations and test-and-remove during captures, where seronegative individuals were marked and released and seropositive individuals were removed. We fitted a general force of infection model to current status data obtained from unmarked animals, and a survival model to event time data obtained from recapture of marked animals. Despite the low number of recaptured animals and the few events of seroconversion that were observed, we found no difference in infection hazards between unmarked and marked individuals. We found no evidence of the effectiveness of the massive culling operation that occurred in 2013, while both models showed a strong decrease in the force of infection in spring 2015, when a large test-and-remove operation was conducted. This suggests that this operation or the combination of both measures were effective. Our results illustrate that estimating the force of infection may help in assessing the success of disease management in wildlife populations rather than relying only on the surveillance of the seroprevalence.

Keywords: wildlife diseases; disease management; infection hazard; force of infection; seroprevalence; current status data; catalytic model; survival analysis

4.2.1. INTRODUCTION

Recent outbreaks of wildlife diseases in Europe such as Chronic Wasting Disease (CWD) or African Swine Fever (ASF) have brought to light the need for effective disease management strategies in the wild (Mysterud and Rolandsen, 2018; Vicente *et al.*, 2019). Depending on the host species and infectious disease considered, interventions such as culling, fencing, test-and-remove or vaccination have been implemented with various success in wild populations (e.g., McDonald *et al.*, 2008; le Roex *et al.*, 2015; Müller *et al.*, 2015; Mysterud and Rolandsen, 2019). Ideally, the first step in the choice of an appropriate management intervention is a sound evaluation of the epidemiological situation, in order to understand the epidemiological dynamics before any action is implemented (Artois *et al.*, 2009). Then, ongoing management interventions should be accompanied by a close monitoring of the infection and the population to assess their effectiveness (Vicente *et al.*, 2019) and to evaluate management success (Joseph *et al.*, 2013). A successful disease management is expected to reduce infection hazard for susceptible hosts, which secondary results in decreasing infection prevalence (proportion of infected at a given time) and/or spatial distribution (Artois *et al.*, 2009). In practice however, management actions are often implemented before a full picture of the situation has been drawn. Monitoring begins, or is reinforced, at the same time as management actions are introduced, and may even depend on them, for example if the pathogen is searched from carcasses of culled animals. It is therefore of particular importance to use monitoring, in order (i) to improve understanding of the epidemiological dynamics, and (ii) to detect changes, and not only to estimate the epidemiological parameters after a given intervention.

Moreover, the parameter to be monitored has to be chosen carefully, in order to depict actual changes in epidemiological dynamics. In wildlife populations, the collection of epidemiological data is often performed using serological tests (Gilbert *et al.*, 2013). At the population level, seroprevalence, the proportion of animals positive to serological tests, represents a useful measure to evaluate the instantaneous situation of a population and compare it to previous or other cases. However, seroprevalence does not necessarily represent the level of infection hazard, for example when animals recover from the infection but maintain detectable levels of antibodies over long periods. A decreasing level of seroprevalence can be obtained for example by removing seropositive individuals that have been infected earlier but are no longer infectious to others, thus letting the infection hazard unchanged. The infection hazard, also referred to as the force of infection (FOI), is the rate

at which susceptible individuals become infected and is another useful measure that can provide information on the infection dynamics in the population (Heisey *et al.*, 2006; Hens *et al.*, 2010; Pepin *et al.*, 2017). Methods for estimating the FOI are based on the fact that seroprevalence increases with age as a result of increased time of exposure to the infection (Heisey *et al.*, 2006; Hens *et al.*, 2010). These methods can bring insights into whether there is evidence for infection-associated mortality, or on comparisons of the infection hazard over different groups, e.g. sex- and age-classes (Heisey *et al.*, 2006). However, these methods usually assume that the FOI is constant over time, or at least constant on average (Hens *et al.*, 2010), which is probably not the case when there are management interventions going on.

Here, we were interested in estimating the force of infection of brucellosis (*Brucella melitensis*) in Alpine ibex (*Capra ibex*) in the Bargy Massif (French Alps). This is the first reported example of self-sustained brucellosis infection in European ungulates, with seroprevalences as high as 38% in the population (Hars *et al.*, 2013; Garin-Bastuji *et al.*, 2014). As France is officially free of *Brucella melitensis* and *Brucella abortus* in domestic ruminants, the persistence of *B. melitensis* in a wildlife population raises serious public health and economic concerns. The risk that ibex transmit the infection to domestic livestock in shared areas led the French authorities to undertake several disease management interventions since the discovery of the outbreak in 2012 (Marchand *et al.*, 2017; Lambert *et al.*, 2018b). Among these interventions, two massive culling operations were carried out in autumn 2013 (n=233 individuals culled, estimated population size before culling: 567, 95% CI [487-660]) and in autumn 2015 (n=70, estimated population size before culling: 277 [220-351]). In parallel, the population was monitored by Capture-Mark-Recapture (CMR) to collect demographic and epidemiological data every year (2012-2013: 81 captures, 2014: 71, 2015: 125, 2016: 35, 2017: 27, 2018: 48). During captures, a test-and-removal program was implemented where seropositive individuals were removed, while seronegative individuals were marked and released.

This study first aimed at evaluating temporal changes of the force of infection, in order to evaluate the efficacy of these management interventions. The rationale behind this analysis is that effective management interventions should decrease the FOI. On the contrary, a constant or increasing FOI would reveal that interventions had no effect or even enhanced disease transmission, which is not desirable. Here, we took advantage of repeated

punctual interventions which permitted to compare the FOI before and after interventions, which would not have been possible if interventions were implemented continuously.

Recent developments have proposed solutions to infer time-varying forces of infection (e.g., Heisey *et al.*, 2010), but they usually require long temporal datasets with large sample sizes (Pepin *et al.*, 2017). Pepin *et al.* (2017) offered an alternative by integrating individual data on antibody titres to estimate the time since infection of each individual and then derive the FOI as a function of time. However, this method requires knowledge on the kinetics of antibody in the species of interest or an immunologically similar species. In ibex, quantitative antibody data were available for the Complement Fixation Test (CFT – Lambert *et al.*, 2018b). However, in the case of brucellosis, serologic titres may increase as a response to recent exposure but also to reactivation of chronic infection that occurs in favourable occasions such as parturition (Treanor *et al.*, 2011). Therefore, it would be particularly difficult to infer the time since infection. Because of this difficulty and given the relatively small sample sizes in the available data, we looked for simple ways to circumvent the assumption that the FOI is constant over time.

In addition to the temporal trends of the FOI, we aimed at comparing the infection hazard between different groups, in order to better analyse transmission patterns. Although the Alpine ibex is a gregarious species, males and females segregate outside the mating period (Villaret and Bon, 1995; Bon *et al.*, 2001), leading to variations in contact rates and therefore potential differences of the FOI between age- and sex-classes. We also expected differences between the five socio-spatial units identified in the population, as seroprevalence showed a strong spatial variability (Marchand *et al.*, 2017). The three central spatial units of the massif (n°3, 4 and 5) displayed high seroprevalence, up to 70%, while the two units in the periphery (n°1 and 2) showed seroprevalence <15% (Marchand *et al.*, 2017). We were also interested in comparing the FOI between marked and unmarked animals. Indeed, the number of marked animals has increased in the population over the years due to the ongoing CMR program. However, most captures were targeted towards unmarked animals, in an attempt to test new individuals rather than marked individuals that were tested as seronegative in a more or less recent past (Calenge *et al.*, in prep.). As a result, only a few seropositive cases were found among the few recaptured marked animals. Whether marked and unmarked animals differ in terms of risk of infection has not been tested, but can be plausible if we consider that the captures are directed toward individuals easiest to catch, and that these animals may differ from others for other traits such as exposure and susceptibility to infection.

Finally, our last aim was to look for evidence of infection-related mortality, which can be assessed with methods for estimating the FOI (Heisey *et al.*, 2006). Indeed, methods for estimating the FOI are based on the fact that seroprevalence increases with age as a result of increased time of exposure to the infection. However, infection-related mortality can decrease the apparent seroprevalence data, the proportion of seropositive animals in the sample, because infected individuals have more chance of dying. As a result, the FOI would be underestimated, and therefore the existence of infection-related mortality should be assessed (Heisey *et al.*, 2006). There is currently no evidence for brucellosis-related mortality in the Alpine ibex population of the Bargy massif (no massive mortality detected and no seropositive case detected in found-dead animals), but it remains a possibility as there is evidence for brucellosis-related mortality in other wild species (e.g., Forbes *et al.*, 1996; Gorsich *et al.*, 2015).

4.2.2. MATERIAL AND METHODS

4.2.2.1. *Current status and event time datasets*

In this study, we used two different kinds of seroprevalence data collected from the Alpine ibex population of the Bargy Massif (French Alps). The population dynamics, the epidemiological context and the methods of population follow-up have been described previously (Marchand *et al.*, 2017; Lambert *et al.*, 2018b). Since 2012, this population has been monitored through Capture-Mark-Recapture (CMR). Moreover, disease management consisted of several culling operations and a test-and-removal program during captures.

The first dataset we used was age-seroprevalence (also called “current status”) data in unmarked individuals (Figure 4.2-1 and Figure 4.2-2A), extracted from the culling operations and the first capture of individuals. In total, serological data from 362 individuals were available (319 individuals captured and 43 individuals culled for which results of serological tests were available – 129 seropositive and 233 seronegative). The serological tests were performed according to requirements of the European Union for diagnosis of brucellosis in small ruminants and following standards of the World Organization of Animal Health (OIE) – see Marchand *et al.* (2017) and Lambert *et al.* (2018b) for more details. For each individual, information on age (by counting horn growth annuli, Michallet *et al.*, 1988), sex, date of sampling and socio-spatial unit were also recorded.

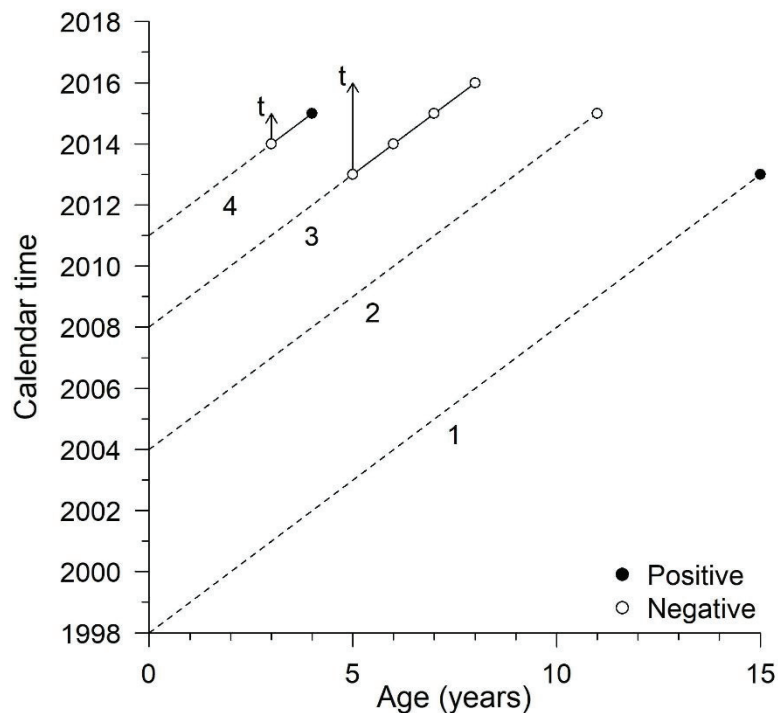


FIGURE 4.2-1 | Examples of seroprevalence data for brucellosis in ibex in the Bargy Massif (France) on the age/time plane.

The dotted diagonal lines represent the life of the animals before they were culled or captured for the first time, where they tested either seropositive (black dots) or seronegative (white dots). The solid diagonal lines represent the life of marked animals between their first capture and their recapture(s). Seropositive individuals were euthanized, while seronegative individuals were marked and released. Data from culling or capture constituted the age-seroprevalence (“current status”) data, while data from recaptures constituted the event time data. For example, ibex 1 was captured in 2013 at the age of 15 and tested seropositive, and ibex 2 was captured in 2015 at the age of 11 and tested seronegative. These animals were included in the age-seroprevalence data set, but they were never recaptured and were therefore not included in the event time dataset. Data from recaptures constituted the event time data, where the event of interest is the seroconversion of seronegative individuals. For example, ibex 3 was first captured in 2013 at the age of five, and was recaptured for the last time in 2016, more precisely $t=1240$ days after the first capture, when it was still seronegative. In this case, the event of interest has not yet occurred and the data is “right-censored”. On the other hand, ibex 4 was first captured in 2014 at the age of three, and was recaptured only once in 2015, $t=363$ days after the first capture, when it was seropositive. In this case, the event occurred somewhere between $t=0$ and $t=363$, but the exact time is not known, the data is “interval-censored”. Note that the time between the first capture and the infection event was not equivalent to the calendar time as it started at different dates for each individual (represented by vertical arrows for $n^{\circ}3$ and $n^{\circ}4$).

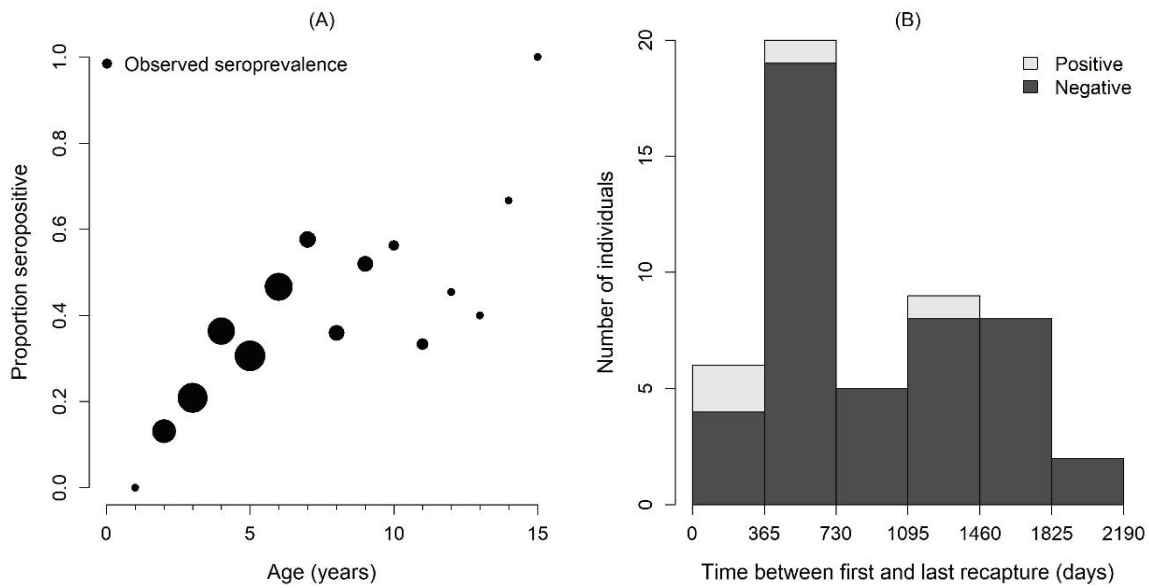


FIGURE 4.2-2 | (A) Age-seroprevalence data, or “current status” data, for brucellosis in ibex captured for the first time or culled between 2012 and 2018 in the Bargy massif (France), with black points representing the observed seroprevalence for each age, with the size of the points proportional to the sample taken ($n=362$). (B) Distribution of the duration between the first capture (where the individuals were seronegative, marked and released) and the last recapture, where the ibex either tested seronegative, or converted to seropositive (the event of interest), for ibex that were recaptured at least once ($n=50$).

Captured individuals were either euthanized when they were seropositive, or marked and released if they were seronegative at the time of sampling. Among the 204 seronegative ibex that were marked and released, 50 individuals were recaptured at least once during the study period and were retested for antibodies against brucellosis. The second dataset we used was therefore the time (in days) between the first capture and the last recapture of marked animals. Because these animals were seronegative at the first capture, they either remained seronegative ($n=46$) or became seropositive ($n=4$ – Figure 4.2-1 and Figure 4.2-2B). This type of data is also called “event time” data, where the event here would be the seroconversion from negative to positive. Individuals that seroconverted are “interval censored”, i.e., the event of interest occurred between the last negative test and the positive test, but the exact time is not known (Kleinbaum and Klein, 2005). Conversely, individuals that remained seronegative at the last recapture are “right censored”, i.e., the event of interest did not occur yet and may or may not occur in the future (Kleinbaum and Klein, 2005). In this dataset, age was determined from age at first capture incremented by the time between first capture and last recapture.

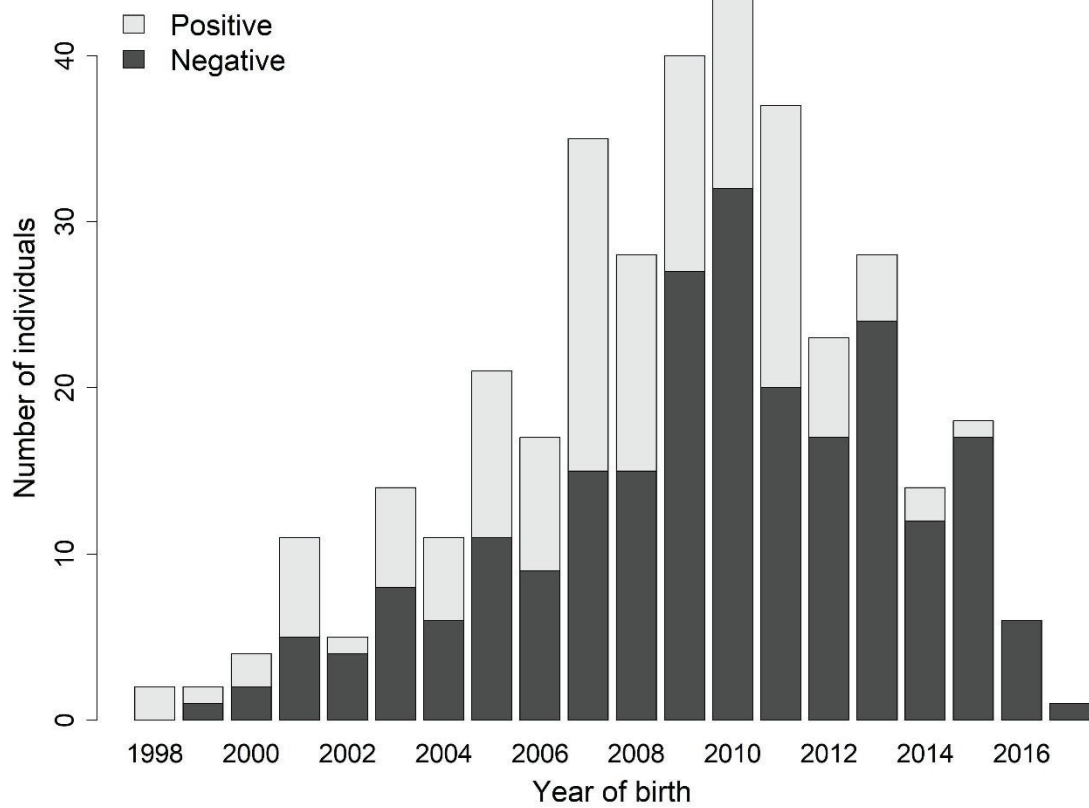


FIGURE 4.2-3 | Distribution of serological results (seropositive: light grey, seronegative: dark grey) for brucellosis in ibex captured or culled between 2012 and 2018 in the Bargy massif (France), according to their year of birth.

Serological results according to the year of birth seemed to indicate a decline in the proportion of seropositive results since the 2012 cohort, when brucellosis was discovered in the population and management interventions started (Figure 4.2-3). Moreover, in a previous study, we estimated that the seroprevalence decreased between 2015 and 2016 (Calenge *et al.*, in prep.). Given this information, we expected that the FOI would change between 2012 and 2018. In both our datasets, we assessed for the existence of varying values of the FOI by dividing our data in several periods, and comparing the goodness of fit between models considering constant or periodic FOI.

4.2.2.2. *Estimating force of infection using FOI models of current status data*

The force of infection $\lambda(t)$ (FOI) represents the rate at which susceptible individuals become infected per unit of time, at time t (Vynnycky and White, 2010). The FOI can be calculated using the equation: $\lambda(t) = cv \frac{I(t)}{N(t)}$, where c is the contact rate, v is the probability that the contact leads to infection, and $\frac{I(t)}{N(t)}$ is the proportion of infectious individuals in the population at time t (Begon *et al.*, 2002). The FOI changes over time, although its average value remains unchanged over time in endemic situations.

If we were to follow over time a cohort of individuals born the same year, we would observe that, as the cohort ages, the proportion of individuals who have ever been infected increases. Similarly, a cross-sectional sample would produce a similar pattern, as animals would be of different ages and therefore would have experienced various exposure times (Heisey *et al.*, 2006). This has led to the development of the ‘catalytic model’ (Muench, 1934), that can be written as:

$$z(a) = 1 - e^{-\lambda a}$$

where $z(a)$ is the proportion of seropositive at age a , and λ is the average FOI. The main assumptions are that the average FOI λ is independent of age and year, that all individuals are born without infection, and that susceptible and infected animals have the same mortality rate (i.e., no infection-related mortality experienced by infected animals).

This model can be fitted to observed age-seroprevalence data to estimate the average FOI. However, the limitations related to the model assumptions led to the development of new models over the years, dealing for instance with age-dependent forces of infection (see Hens *et al.*, 2010 for a review).

In this study, we used the general force of infection model developed by Heisey *et al.* (2006) that allows for three main improvements of Muench’s catalytic model. The model allows for the existence of infection-related mortality in infected animals, leading to the new equation:

$$z(a) = \frac{1 - e^{-(\lambda-\mu)a}}{1 - \frac{\mu}{\lambda} e^{-(\lambda-\mu)a}}$$

where $z(a)$ is the proportion of seropositive at age a , λ is the average FOI and μ is the infection-related mortality (infection being brucellosis in our case). Note that when $\mu = 0$, the equation simplifies to Muench's model: $z(a) = 1 - e^{-\lambda a}$. The model still assumes that all animals are born without infection, but animals can immediately start to leave this state and become positive (Heisey *et al.*, 2006).

The second improvement is the use of logistic regression to test the influence of covariates X_1, \dots, X_n on the FOI λ , by using $\lambda = \lambda_0 e^{\beta_1 X_1 + \dots + \beta_n X_n}$, where λ_0 is the baseline infection hazard, which is constant over age and time. Among possible covariates, the age a can also be tested, even though in this case the function for $z(a)$ would be incorrect, as it was obtained under the hypothesis that λ was independent of age. However, this is very useful as a diagnostic tool, as if age or a function of age as covariate improves the model fit, it means that this assumption should be revised and functions other than constant with age should be explored for the baseline infection hazard (Heisey *et al.*, 2006).

Finally, Heisey *et al.* (2006) used a piecewise constant hazards approach to enable the use of age-varying infection hazard models for the baseline infection hazard λ_0 such as the Weibull, the Gompertz or the log-logistic models.

We fitted this general force of infection model to our age-seroprevalence data using a maximum likelihood approach and the R function *currentstatus.1* provided in the Supplementary material of Heisey *et al.* (2006). In our case, besides age and year for diagnostic purposes, we explored the effects of sex and socio-spatial unit, which were demonstrated to play a major role in explaining variation in brucellosis seroprevalence in the ibex population (Marchand *et al.*, 2017). In particular, males were less often found seropositive than females, and seroprevalence was highest in the two central areas (named 3 and 4 in Marchand *et al.*, 2017), lowest in the most western ones (1 and 2) and intermediate in the eastern area (5). For each type of model (constant FOI, diagnostic model with covariate age – AGE, diagnostic model with covariate quadratic age – AGE²), we fitted all possible submodels of additional mortality (μ), socio-spatial unit (UNIT) and sex (SEX). For each model, the number of parameters (degrees of freedom), the log-likelihood, the Akaike Information Criterion corrected for small sample sizes (AICc), and the difference between the AICc and the lowest AICc value (Δ AICc) were computed. The Akaike Weights, which are relative model likelihoods normalized over the likelihoods of all possible submodels, were computed using the function *aictabCustom* (*AICcmodavg* R package – Mazerolle, 2019). The weight can be regarded as the conditional probability given the data of being the

best model among the set of possible sub-models (Wagenmakers and Farrell, 2004). We selected a set of models for which the ΔAICc was below two (Burnham and Anderson, 2002), and then we chose the model with the fewest parameters as the final model, following the principle of parsimony.

For each explanatory variable, results were expressed as Hazard Ratios (HR). The hazard function is expressed as $\lambda = \lambda_0 e^{\beta_1 X_1 + \dots + \beta_n X_n}$. For a single covariate X that is either present ($X = 1$) or absent ($X = 0$), the hazard (force of infection) is $\lambda = \lambda_0 e^\beta$ for $X = 1$, and $\lambda = \lambda_0$ for $X = 0$. Therefore, the hazard ratio is $HR = \lambda_0 e^\beta / \lambda_0 = e^\beta$. Hazard ratios have a similar interpretation as odds ratios (Dohoo *et al.*, 2009). Profile likelihood 95% confidence interval around each estimated coefficient were computed (Therneau and Grambsch, 2000).

If the transmission of brucellosis was reduced, the age-seroprevalence relationship would be expected to be modified (Gilot-Fromont *et al.*, 2018b). In particular, cohorts after the management period would have lowered slopes of the age-seroprevalence relationship than cohorts born during the period when brucellosis was naturally circulating in the population. Therefore, we compared the FOI between cohorts born since 2012 and cohorts born beforehand, which represented the reference of the period of endemic brucellosis in the population. We therefore assumed that the FOI could be considered as time-independent on average once an individual was born. This remains an approximation, but it allowed us to assess for the existence of temporal changes in the FOI while staying in the framework of the general force of infection model.

4.2.2.3. *Estimating FOI using survival analysis of event time data*

As we were interested in the time until the occurrence of infection (“survival time”), we analysed our event time dataset using survival models. We note T the random variable representing the time between the first capture and the infection event, in days. The survival function is the probability that infection has not yet occurred by time t : $Surv(t) = P(T \geq t)$. It can be related to the hazard function $\lambda(t)$, i.e., the rate at which the event occurs per unit time, at time t , using the following equation (Kleinbaum and Klein, 2005):

$$Surv(t) = e^{-\int_0^t \lambda(u) du}$$

In our case, the event of interest is infection, and therefore the hazard function is the force of infection. Note that if we assume a constant hazard risk (FOI) with time, the hazard

function is $\lambda(t) = \lambda$, and the corresponding survival function is $Surv(t) = e^{-\lambda t}$ (exponential model).

The proportional hazards model, also known as the Cox regression model, is a multivariate analysis for survival data. It is based on the assumption that the hazard $\lambda(t)$ for an individual is a product of a baseline hazard $\lambda_0(t)$ and an exponential function of covariates X_1, \dots, X_n : $\lambda(t) = \lambda_0(t) e^{\beta_1 X_1 + \dots + \beta_n X_n}$. More precisely, Cox proportional hazards does not assume any specific functional form for the baseline hazard, and model the hazard ratio: $HR = \frac{\lambda(t)}{\lambda_0(t)} = e^{\beta_1 X_1 + \dots + \beta_n X_n}$. The assumption is therefore that the hazard ratio is constant over time, hence the denomination proportional hazards. We used the function `coxph` of the *survival* R package to fit Cox proportional hazards model to our data (Therneau and Grambsch, 2000; Therneau, 2015). We tested for the effects of the age at first capture AGE and the SEX. We were not able to test for the effect of the socio-spatial unit, as the model did not converge when we used this covariate because of small sample sizes. For each variable, the proportional hazards assumption was tested using the function `cox.zph` of the *survival* R package.

Then, we explored several time-varying forms of the baseline hazard, using parametric models from the function `flexsurvreg` (*flexsurv* R package – Jackson, 2016). In our data, animals were captured for the first time at different dates, meaning that the time between the first capture and the infection event was not equivalent to the calendar time (Figure 4.2-1). In these models, the baseline hazard was allowed to vary with time (i.e., time since first capture), using for example Weibull, Gompertz or log-logistic distributions.

Finally, we also explored the existence of variations with calendar time, using a model assuming two periods during the study characterised by two different forces of infection (independent of age and time since first capture – Figure 4.2-4). This model was based on the hypothesis that the FOI would change at least once during the study period as a result of management interventions. We fitted models considering all possible threshold dates between November 2012 and June 2018, to find when this change of FOI most likely happened, and compared them to the model considering a constant FOI. We did not look for the existence of three or more periods to keep the model simple given the paucity of available data.

Models were fitted to our data using maximum likelihood approach and were selected based on the AICc.

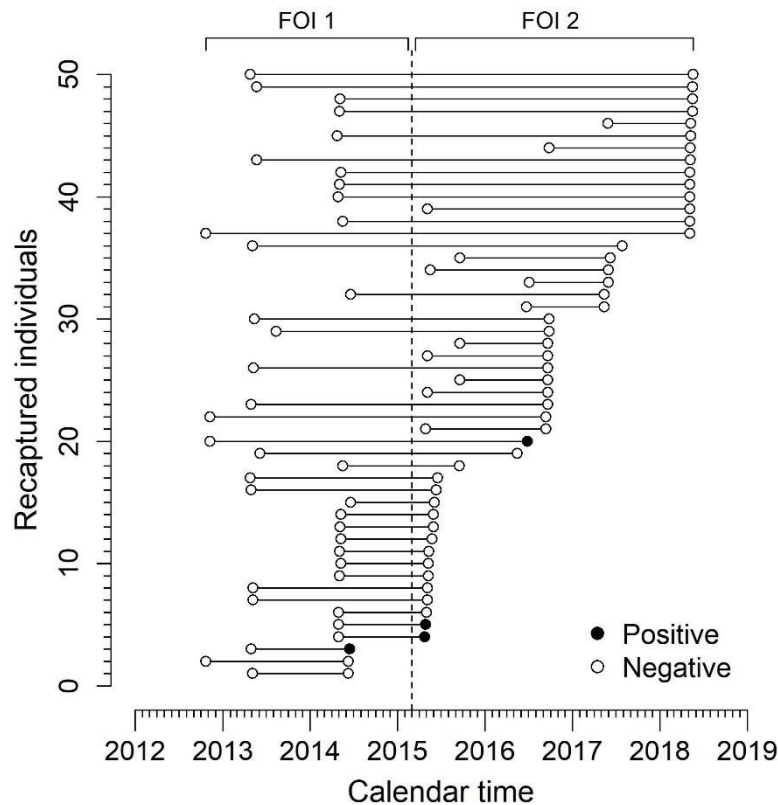


FIGURE 4.2-4 | Dates of first capture and last recapture of marked individuals and threshold date between the two periods characterised by two different forces of infection.

The solid horizontal lines represent the life of marked animals between the date of their first capture, where they tested seronegative (white dots), and their last recapture, where they either tested seronegative or seropositive (black dots). The dashed vertical line represents a possible threshold date that separates the first period where individuals are exposed to a certain force of infection (FOI 1) from the second period where individuals are exposed to a different force of infection (FOI 2).

4.2.3. RESULTS

4.2.3.1. Force of infection estimated from current status data

We fitted general force of infection models to our age-seroprevalence data, using either a constant FOI with calendar time and age, a FOI depending on the covariate age, or a FOI depending on the covariate quadratic age. For each of these types of model, eight submodels were evaluated using the covariates socio-spatial unit and sex, and including infection-related mortality or not.

Among the 24 models (Table 4.2A-1 in Appendix 4.2A), all models with $\Delta AICc < 2$ included the variables UNIT and SEX (Table 4.2-1). The most parsimonious (final) model used a constant force of infection with age and calendar time and included only these two variables. The FOI was lower for males than for females (hazard ratio $HR_{\text{males vs. females}} = 0.6653$, 95% Confidence Interval: [0.4906 – 0.8786]). The forces of infection were similar in units 3 and 4 (core area), but were significantly lower in units 1, 2 (periphery) and 5 (core area) compared to unit 3 (Table 4.2-2). The FOI was significantly lower in units 1 and 2 compared to unit 5 ($HR_{\text{unit 1 vs. unit 5}} = 0.3592$, 95% CI [0.1967 – 0.5319]; $HR_{\text{unit 2 vs. unit 5}} = 0.1111$, 95% CI [0.0282 – 0.2363]).

Based on confidence intervals, the corresponding estimated forces of infection for the selected model (model N°1) were 0.0273 [0.0098 – 0.0588] for females of unit 1, 0.0085 [0.0014 – 0.0261] for females of unit 2, 0.1617 [0.1344 – 0.1928] for females of unit 3, 0.1618 [0.1091 – 0.2314] for females of unit 4 and 0.0761 [0.0498 – 0.1105] for females of unit 5. Adding infection-related mortality μ to this model did not significantly improve model fit (Table 4.2-1, likelihood ratio test: $p = 0.16$).

TABLE 4.2-1 | Model selection table to analyse the age-seroprevalence data using a constant force of infection with age and time, and testing for the effect of infection-related mortality μ , socio-spatial unit, sex, year of sampling, and age (linear or quadratic).

N°	AGE	AGE ²	μ	UNIT	SEX	DF	LL	AICc	$\Delta AICc$	W
17		+		+	+	7	-183.08	380.48	0.00	0.25
9	+			+	+	7	-183.59	381.50	1.02	0.15
1				+	+	6	-184.74	381.72	1.24	0.13
5			+	+	+	7	-183.75	381.82	1.34	0.13
21		+	+	+	+	8	-183.01	382.44	1.96	0.09

Only models with $\Delta AICc < 2$ are presented here (see the complete table in Appendix 4.2A). For each model, the table gives the number of Degrees of Freedom (DF), the Log-Likelihood (LL), the Akaike Information Criterion corrected for small sample sizes (AICc), the difference between their AICc and the lowest AICc value ($\Delta AICc$), and the Akaike Weights (W), which are relative model likelihoods normalized over the likelihoods of all possible submodels (Table 4.2A-1 in Appendix 4.2A). The model selected is in bold.

TABLE 4.2-2 | Parameters of the selected model (N°1 in Table 4.2-1) with hazard ratios (HR) and profile-likelihood 95% confidence interval (CI) for each explanatory variable.

Model	Explanatory variables	HR and 95% CI
$z(a) = 1 - e^{-\lambda a}$ with $\lambda = \lambda_0 e^{\beta_1 X_1 + \dots + \beta_n X_n}$	UNIT (1)	0.1690 [0.0606 – 0.3634]
	UNIT (2)	0.0523 [0.0087 – 0.1615]
	UNIT (4)	1.0003 [0.6748 – 1.4306]
	UNIT (5)	0.4705 [0.3081 – 0.6833]
	SEX (males)	0.6653 [0.4906 – 0.8786]

The reference levels are 3 (for the variable UNIT) and females (for the variable SEX).

TABLE 4.2-3 | Comparison of models considering two or three groups of cohorts with the model previously selected in Table 4.2-1, considering only one cohort.

Cohorts born before 2012 represented the reference of the period of endemic brucellosis in the population, and were therefore always gathered in the same group. When considering two groups, we tested all possible combinations for the cohorts born since 2012. Then, we tested all possible combinations when considering three groups, starting from the best model considering two groups.

Groups	Cohorts	DF	LL	AICc	$\Delta AICc$
1	1998-2017	6	-184.74	381.72	0.00
	1998-2011 and 2012-2017	7	-184.11	382.54	0.82
2	1998-2012 and 2013-2017	7	-183.80	381.92	0.20
	1998-2013 and 2014-2017	7	-183.37	381.06	-0.66
	1998-2014 and 2015-2017	7	-182.50	379.32	-2.40
	1998-2015 and 2016-2017	7	-183.67	381.66	-0.06
	1998-2016 and 2017	7	-184.73	383.78	2.06
	1998-2011 and 2012-2014 and 2015-2017	8	-182.47	381.36	-0.36
3	1998-2012 and 2013-2014 and 2015-2017	8	-182.48	381.38	-0.34
	1998-2013 and 2014 and 2015-2017	8	-182.50	381.42	-0.30
	1998-2014 and 2015 and 2016-2017	8	-182.24	380.90	-0.82
	1998-2014 and 2015-2016 and 2017	8	-184.74	385.90	4.18

For each model, the table gives the number of Degrees of Freedom (DF), the Log-Likelihood (LL), the Akaike Information Criterion corrected for small sample sizes (AICc), and the difference between their AICc and the AICc value of the model previously selected in Table 4.2-1 considering only one group ($\Delta AICc$). The best model is in bold.

The model with the lowest AICc (N°17 in Table 4.2-1) included a quadratic function of age ($HR_{\text{per year}} = 0.9870$, 95% CI [0.9747 – 0.9980]), which could be an indication that the assumption of an age-independent FOI is not appropriate. To further explore this, we fitted several models of age-varying FOI (Table 4.2A-2 in Appendix 4.2A). None of these model had a better AICc, which does not provide strong evidence for variation of the force of infection with age.

Starting from the model selected in the previous step, we compared the FOI between cohorts born since 2012 and cohorts born beforehand that represented the reference of the period of endemic brucellosis in the population. Among models considering two or three groups of cohorts, the best model was obtained when considering two groups of cohorts, 1998-2014 and 2015-2017 (Table 4.2-3), and improved the model fit (AICc = 379.32, $\Delta AICc = -2.40$ with the model 1 selected in Table 4.2-1). The FOI was lower for the cohort 2015-2017 than for the cohort 1998-2014 ($HR_{2015-2017 \text{ vs. } 1998-2014} = 0.2043$ [0.0117 – 0.8998] – Figure 4.2-5). The average FOI over the whole population was estimated to be 0.0804 [0.0672 – 0.0954] for the cohort 1998-2014, and 0.0179 [0.0010 – 0.0786] for the cohort 2015-2017.

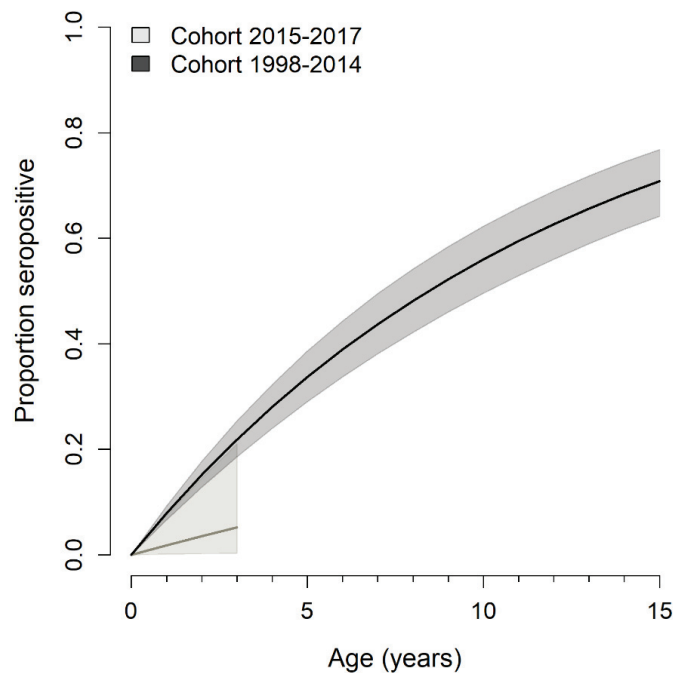


FIGURE 4.2-5 | Predictions of the proportion of seropositive results with age using the general force of infection model considering two groups of cohorts, 1998-2014 (dark grey) and 2015-2017 (light grey). The lines represent the means, and the areas represent the 95% confidence intervals.

4.2.3.2. Force of infection estimated from survival analysis of event time data

Using Cox proportional hazards model to fit our event time data collected on marked animals, we found no significant effect of the variables AGE or SEX with this model (Table 4.2-4). The proportional hazards assumption held for the two variables ($p=0.786$ and 0.913 respectively).

We fitted several parametric models with time-varying forms of the baseline hazard, without any explanatory variable. The exponential model (constant baseline hazard) was the best in term of AICc (Table 4.2-5), and although almost all the other models had a $\Delta AICc < 2$, it seems that there is no strong evidence that the FOI depends on the time since first capture. When fitting an exponential model to our interval-censored data, the estimated value for the annual average force of infection λ was 0.0334 year^{-1} [$0.0105 - 0.0793$].

TABLE 4.2-4 | Parameters of the Cox proportional hazards model, with hazard ratios (HR) and 95% confidence interval (CI) for each explanatory variable and p-value of the Wald test.

Model	Explanatory variables	HR and 95% CI	p-value
$HR = \frac{\lambda(t)}{\lambda_0(t)} = e^{\beta_1 X_1 + \dots + \beta_n X_n}$	AGE	0.914 [0.630 – 1.324]	0.634
	SEX (Females)	1.532 [0.193 – 12.16]	0.687

The reference level is males (for the variable SEX).

TABLE 4.2-5 | Model selection table of survival parametric models, with various time since first capture distributions of the baseline hazard (force of infection).

Model	DF	LL	AICc	$\Delta AICc$
Exponential	1	-41.22	84.52	0
Log-normal	2	-40.75	85.75	1.23
Log-logistic	2	-41.02	86.30	1.78
Gamma	2	-41.03	86.32	1.80
Weibull	2	-41.06	86.38	1.86
Gompertz	2	-41.21	86.68	2.16

For each model, the table gives the number of Degrees of Freedom (DF), the Log-Likelihood (LL), the Akaike Information Criterion corrected for small sample sizes (AICc), the difference between their AICc and the lowest AICc value ($\Delta AICc$). The model selected is in bold.

Finally, we fitted a model with two different periods on the calendar time, where individuals were submitted to two different constant forces of infection before and after a threshold date. We indeed expected that the force of infection could change because of the effects of management interventions. Among models considering all possible threshold dates between November 2012 and June 2018, the best model was obtained when considering a threshold date in early spring 2015 (Figure 4.2-6), and improved the model fit compared to the simple exponential model ($\Delta AICc = -3.56$). The FOI was estimated to be higher for the first period (0.0854 year^{-1} [$0.0265 - 0.1985$]) than for the second period (0 [$0 - 0.0321$]).

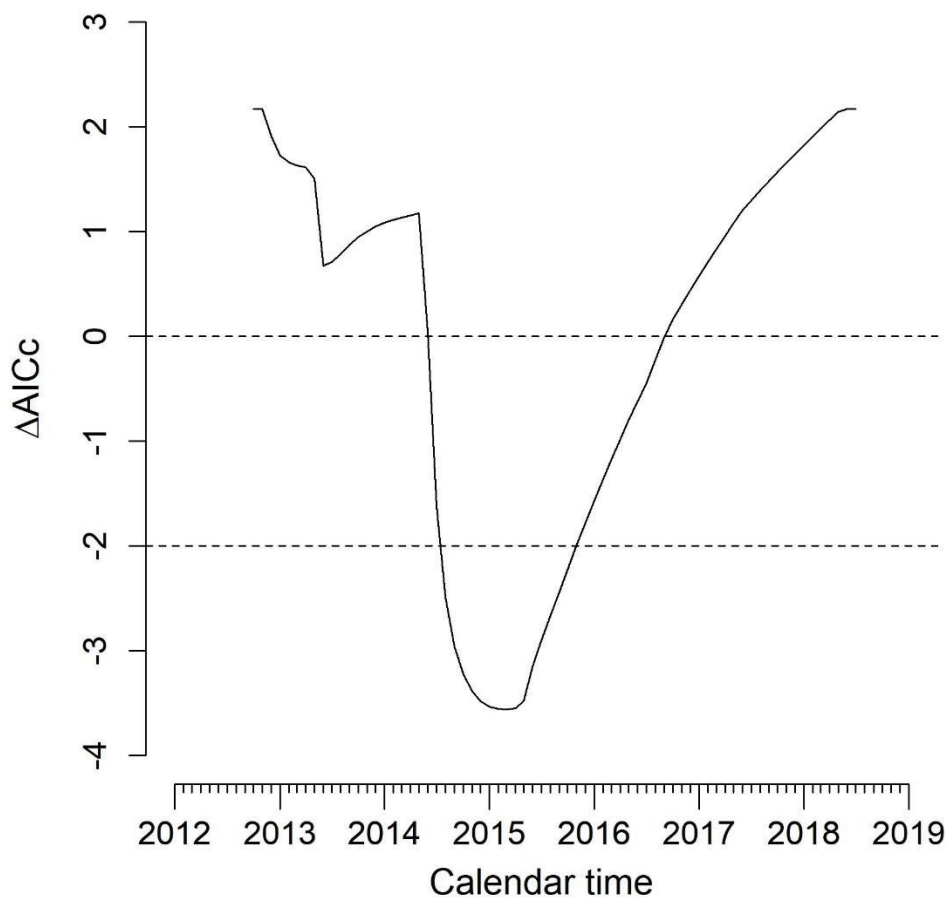


FIGURE 4.2-6 | Difference $\Delta AICc$ between the $AICc$ of the model considering two different FOI for two different periods on the calendar time, for all possible threshold dates between November 2012 and June 2018, and the $AICc$ value of the simple exponential model considering a constant FOI.

4.2.4. DISCUSSION

In this study we estimated the force of infection using two different datasets: a dataset of current status data on individuals sampled during capture or culling operations, and a dataset of event time data, taking advantage of marked animals resampled during recaptures. The first dataset estimated higher FOI in females than males, and higher FOI in the two central units of the massif than in the three units in the periphery, with an intermediate FOI for the northeastern unit (unit 5). However, there were no strong evidence that the FOI varied with age (although not tested for all possible polynomials). The second dataset did not find any differences between sexes and we were not able to test for the effect of the socio-spatial unit. This can be explained by the small sample size of this second dataset. For both datasets, however, there was evidence for a strong decrease in the FOI since spring/summer 2015.

Here, the current status analysis, considering the first capture of each individual, appeared more powerful to detect variations in the FOI than the survival analysis of marked animals. However, this does not mean that the analysis of recaptures is not relevant, as this result is partly due to the captures targeting non-marked individuals. Although a higher number of recaptures may have provided a more complete dataset, in terms of management the captures would have led to less animals being removed from the population. When management actions and disease surveillance both rely on the same field actions, a compromise has to be found between both objectives that may lead to gather less relevant surveillance data. In this case, the development of appropriate analysis methods is particularly relevant.

4.2.4.1. *Model assumptions*

In both FOI models of current status data and survival analysis of event time data, the main assumption is the independence of the FOI with age. We did not find strong evidence for age-dependency in our datasets, although it could not completely be excluded for the current status data (Table 4.2-1).

For the FOI model considering several cohorts, we made the assumption that one cohort was submitted to the same FOI throughout their life. This remains an approximation, because if the FOI changed at some point in time, it most likely affected all individuals and not only those born after the change. However, this approximation allowed us to assess for the existence of temporal changes in the FOI using simple general force of infection models.

Finally, the survival analysis model made the additional assumption that the FOI did not change as the time since first capture increased. We did not find evidence for such variations either, which allowed us to use the simple constant FOI (exponential) model.

4.2.4.2. *Differences between sex- and age-classes*

The FOI analysis confirmed the difference between sexes already observed in Marchand *et al.* (2017) on a dataset up to 2015. Males and females are not exposed to brucellosis in the same way. In adult ibex, the main transmission routes are through the venereal route and the shedding of *Brucella* in genital fluids at the time of abortion or parturition (Lambert *et al.*, 2018b), as in domestic ruminants (Diaz-Aparicio, 2013). The lower infection hazard in males can be accounted for by the phenomenon of sexual segregation. In ibex, males and females segregate and live in separate groups in distinct areas outside the mating period (Villaret and Bon, 1995; Bon *et al.*, 2001). This behaviour explains that susceptible males are less exposed than susceptible females to infectious females that shed the bacteria in genital fluids, and hence the differences in the force of infection between sexes that were found in this study.

In a former study, seroprevalence was found to decrease in old individuals, especially in males (Marchand *et al.*, 2017). It was hypothesized that this phenomenon could be explained either by a lower survival rates or by lower probabilities to test positive in older individuals infected by brucellosis (Marchand *et al.*, 2017). In our study, we did not find strong evidence for infection-related mortality, although it could not completely be excluded (Table 4.2-1). Although strong effects of brucellosis were found on the survival of moose (*Alces alces* – Forbes *et al.*, 1996) and African buffalo (*Syncerus caffer* – Gorsich *et al.*, 2015), no effects were found in bison (*Bison bison* – Joly and Messier, 2005; Fuller *et al.*, 2007), similarly as our findings. However, we cannot exclude age-specific infection-related mortality nor a decrease of antibody levels under detectable levels as the animals age, which are difficult to reveal using seroprevalence data (Heisey *et al.*, 2006 – but see Benavides *et al.*, 2017).

4.2.4.3. *Spatial and temporal variations of the force of infection*

In the current status dataset, we found evidence that the force of infection decreased for individuals born in 2015 and after. This was further confirmed by the event time dataset, showing a much lower FOI after than before the beginning of spring 2015. In Marchand *et al.* (2017), analysis of seroprevalence data from 2012 to 2015 showed no evidence of seroprevalences differences before and after the mass culling of autumn 2013. Similarly, we did not find any differences in the force of infection between individuals born in 2012, 2013 or 2014, and, interestingly, the force of infection in the first years after the discovery of the outbreak was similar to the years before, when the infection was naturally circulating in the population. Evidence for a detectable efficacy of the mass culling operation of autumn 2013 therefore remains scarce given the available data, whereas the capture-test-removal operations of 2015 were associated to a decreased FOI. Using recent seroprevalence data, Calenge *et al.* (in prep.) also showed a strong decrease in seroprevalence between animals captured before and after September 2015. This decrease could therefore be the result of the decreased force of infection that we evidenced here as early as spring 2015. This provides further support to the hypothesis that the intensive test-and-remove operation conducted in spring 2015 was effective. Because the infection hazard was lowered in marked individuals including in spring 2015, when the abortion period occurs, the test-and-remove operations conducted in 2014 could also have been beneficial, and the 2015 intervention could have benefited from cumulative effects with the previous one.

We found marked differences in the force of infection between socio-spatial units, in accordance with the results of Marchand *et al.* (2017) on seroprevalence. The central units (3 and 4) with the highest seroprevalences had also the highest FOI, while the units in the periphery (1 and 2) had the lowest seroprevalences and FOI, with intermediate results for unit 5. Unfortunately, we could not test for differences in temporal variations in the FOI between socio-spatial units (interactions between calendar time and spatial unit), due to low sample sizes. In a context where the infection hazard increases, such interactions can distinguish whether spatial variations of the FOI are related to hotspots of infection, i.e., spatial units where the infection hazard increases faster (strong interactions), or simply to different arrival times in the different spatial units (Heisey *et al.*, 2010). Conversely, in a context of decreasing infection hazard such as ours, interactions between calendar time and spatial unit could reveal socio-spatial units where the management interventions are the most effective.

4.2.4.4. *Infection hazard in marked and unmarked animals*

Finally, we were interested in comparing the infection hazard between marked and unmarked animals. As stated in the introduction, most captures targeted unmarked individuals, in an attempt to test new individuals and probably have more chances to remove infected individuals than by capturing marked individuals that have more chances to be seronegative as they already were during their last capture in a more or less recent past (Calenge *et al.*, in prep.). As a result of ongoing capture operations and decreased population size due to culling operations, the proportion of marked animals strongly increased in the population over time (Calenge *et al.*, in prep.). Only a few seropositive cases were found among the few recaptured marked animals. Marked and unmarked animals may differ in terms of risk of infection. Indeed, captures are directed toward individuals easiest to catch, and these animals may differ from others for other traits such as exposure and susceptibility to infection. Interestingly, when looking at the force of infection before the decrease related to management interventions, the estimates were very similar for marked: 0.0854 year⁻¹ [0.0265 – 0.1985] and unmarked individuals: 0.0804 [0.0672 – 0.0954]. In the second period, estimates of the FOI were very low in both cases (0 [0 – 0.0321] and 0.0179 [0.0010 – 0.0786] respectively). These results rather indicate that the infection hazard is very similar in susceptible individuals from both groups, and therefore that marked individuals have similar patterns of exposure as unmarked individuals.

4.2.5. CONCLUSION

In conclusion, using two distinct methods, estimates of the force of infection revealed a decrease in the infection hazard in 2015, probably as a result of the successive management operations and especially of the massive operation implemented in spring 2015. Although we used a small dataset on recaptured ibex, we found here similar infection hazards between marked and unmarked individuals, we thus have no strong indication that marked individuals are less at risk of getting infection than unmarked individuals. Analysing the force of infection could therefore provide valuable insights in the context of disease management interventions in wildlife populations. Interesting results can be obtained using rather simple models as we did here, provided that there is no strong evidence that the basic assumptions are violated. In the future, a framework unifying both types of analysis and using a unique dataset for both marked and unmarked animals, could be interesting.

APPENDIX 4.2A: MODEL SELECTION TABLES

TABLE 4.2A-1 | Complete model selection table to analyse the current status data using a constant force of infection with age and time, and testing for the effect of infection-related mortality μ , socio-spatial unit, sex, and age (linear or quadratic).

	N°	μ	Unit	Sex	DF	LL	AIC	Δ AIC	W
Constant	1		+	+	6	-184.74	381.72	1.24	0.13
	2		+		5	-187.10	384.37	3.89	0.04
	3			+	2	-217.55	439.13	58.65	0.00
	4				1	-218.89	439.79	59.31	0.00
	5	+	+	+	7	-183.75	381.82	1.34	0.13
	6	+	+		6	-186.34	384.92	4.44	0.03
	7	+		+	3	-216.33	438.73	58.24	0.00
	8	+			2	-217.96	439.95	59.47	0.00
Linear age	9		+	+	7	-183.59	381.50	1.02	0.15
	10		+		6	-185.93	384.10	3.62	0.04
	11			+	3	-216.12	438.31	57.82	0.00
	12				2	-217.79	439.61	59.13	0.00
	13	+	+	+	8	-183.59	383.60	3.11	0.05
	14	+	+		7	-185.72	385.76	5.28	0.02
	15	+		+	4	-216.11	440.33	59.85	0.00
	16	+			3	-217.77	441.61	61.12	0.00
Quadratic age	17		+	+	7	-183.08	380.48	0.00	0.25
	18		+		6	-185.56	383.36	2.88	0.06
	19			+	3	-215.38	436.83	56.34	0.00
	20				2	-217.12	438.27	57.79	0.00
	21	+	+	+	8	-183.01	382.44	1.95	0.09
	22	+	+		7	-185.54	385.40	4.92	0.02
	23	+		+	4	-215.31	438.73	58.25	0.00
	24	+			3	-217.09	440.25	59.76	0.00

For each model, the table gives the number of Degrees of Freedom (DF), the Log-Likelihood (LL), the Akaike Information Criterion corrected for small sample sizes (AICc), the difference between their AICc and the lowest AICc value (Δ AICc), and the Akaike Weights (W), which are relative model likelihoods normalized over the likelihoods of all possible submodels (1-24; models 25-56 of Table 4.2A-2 were not included in the calculation of W). The model selected is in bold.

TABLE 4.2A-2 | Complete model selection table to analyse the age-seroprevalence data using age-varying forces of infection– Weibull $\lambda_0(a) = \alpha\beta(\alpha a)^{\beta-1}$, log-logistic $\lambda_0(a) = \alpha\beta(\alpha a)^{\beta-1}/[1 + (\alpha a)^\beta]$, Pareto $\lambda_0(a) = \alpha\beta/(1 + \beta a)$, Gompertz $\lambda_0(a) = e^{\alpha+\beta a}$, and testing for the effect of infection-related mortality, socio-spatial unit and sex.

	N°	μ	Unit	Sex	DF	LL	AIC	ΔAIC
Weibull	25		+	+	7	-184.21	382.74	2.26
	26		+		6	-186.52	385.28	4.80
	27			+	3	-216.86	439.79	59.30
	28				2	-218.40	440.83	60.35
	29	+	+	+	8	-183.71	383.84	3.35
	30	+	+		7	-186.33	386.98	6.50
	31	+		+	4	-216.13	440.37	59.89
	32	+			3	-217.73	441.53	61.04
Log-logistic	33		+	+	7	-183.44	381.20	0.72
	34		+		6	-185.90	384.04	3.56
	35			+	3	-216.34	438.75	58.26
	36				2	-217.93	439.89	59.41
	37	+	+	+	8	-182.05	380.52	0.03
	38	+	+		7	-185.08	384.48	4.00
	39	+		+	4	-214.47	437.05	56.57
	40	+			3	-216.35	438.77	58.28
Pareto	41		+	+	7	-183.80	381.92	1.44
	42		+		6	-186.12	384.48	4.00
	43			+	3	-216.38	438.83	58.34
	44				2	-217.98	439.99	59.51
	45	+	+	+	8	-183.66	383.74	3.25
	46	+	+		7	-186.11	386.54	6.06
	47	+		+	4	-216.23	440.57	60.09
	48	+			3	-217.88	441.83	61.34
Gompertz	49		+	+	7	-183.65	381.62	1.14
	50		+		6	-185.98	384.20	3.72
	51			+	3	-216.19	438.45	57.96
	52				2	-217.84	439.71	59.23
	53	+	+	+	8	-183.61	383.64	3.15
	54	+	+		7	-185.96	386.24	5.76
	55	+		+	4	-216.16	440.43	59.95
	56	+			3	-217.83	441.73	61.24

For each model, the table gives the number of Degrees of Freedom (DF), the Log-Likelihood (LL), the Akaike Information Criterion corrected for small sample sizes (AICc), the difference between their AICc and the lowest AICc value ($\Delta AICc$), which is the AICc value of model 17 of Table 4.2A-1).

Addendum

Estimates of the force of infection revealed a decrease in the force of infection in 2015 in both marked and unmarked individuals, probably as a result of the successive management operations and especially of the massive operation implemented in spring 2015. Because the force of infection was lowered in marked individuals including in spring 2015, when the abortion period occurs, the operation conducted in 2014 could also have been beneficial, and the 2015 intervention could have benefited from cumulative effects with the previous one.

Calenge *et al.* (in prep. – chapter 4.2) also showed a strong decrease in seroprevalence between animals captured before and after September 2015. This decrease could therefore be the result of the decreased force of infection that we evidenced here as early as spring 2015.

Overall, our results from this chapter suggest that disease management interventions in the population implemented between the mass culling of 2013 and 2015 were effective, as indicated by a decrease in three major disease parameters: seroprevalence, prevalence of active infection and force of infection.

The effectiveness of the lighter test-and-remove operations from 2016 to 2018, combined in 2017 and 2018 with the cull of 5 unmarked individuals, remains unclear. Indeed, between 2016 and 2018, the seroprevalence did not vary significantly (4.1) and the FOI did not seem to change (4.2). However, the proportion of actively infected female in the core area population decreased between 2017 and 2018 (4.1), which could indicate that the measures contributed to decrease the disease prevalence within the core area. However, this result must be considered with great caution due to the small number of seropositive ibex from 2018 ($n=2$).

In the next chapter, we evaluated the efficacy of several disease management strategies using predictions from the model developed in chapter 3.2. The choice of these strategies was based on the results from both chapter 3 and chapter 4. In particular, we evaluated the benefit of strategies targeting specific classes of individuals and/or specific areas compared to untargeted strategies, which could improve management according to the results of chapter 3. We also focused on test-and-remove operations, more or less combined with other measures such as culling of unmarked individuals, in accordance with the results from chapter 4.

Chapter 5

DISEASE MANAGEMENT IN A HETEROGENEOUS POPULATION



Personal picture

Foreword

In this last chapter, we expanded the model developed in chapter 3.2 for predictions on the future dynamics of the population, with and without disease management, so as to evaluate the relative efficacy of various strategies.

In accordance with the results from chapter 4, we focused on test-and-remove operations (TR), i.e., serological testing of individuals during capture followed by removal of seropositive individuals, which appeared to be the most effective in reducing brucellosis transmission in the population. The simulated strategies were elaborated in close connection with field monitoring in order to simulate realistic schemes and parameters. For example, the objective levels for the number of capture were either 15 or 30 animals each year, as those were the levels of capture that were achieved in the last years in the population among an overall population of around 300 individuals.

We also combined culling of unmarked individuals to the test-and-remove strategy. Indeed, this combined strategy was used several times in the population, with high number of captures and culls in 2015 and lower numbers in 2017 and 2018. The objective levels for the number of unmarked individuals culled were 5 or 20 animals each year, to be compatible with the numbers that were allowed recently in this population.

Finally, the last strategy we evaluated was a combination of test-and-remove with vaccination (TVR). Indeed, a preliminary work, based on a non-spatial individual-based model, suggested a higher efficacy of TVR than TR (ANSES, 2015; Thébault *et al.*, 2015). Vaccination also appeared to be a promising strategy for bison brucellosis in the Yellowstone (Treanor *et al.*, 2010; Ebinger *et al.*, 2011).

Based on the results from chapter 3, we formulated the following predictions:

- First, management strategies targeting the core area of the Massif should be more effective than management strategies applied at the scale of the whole Massif.
- Second, targeting specifically females, regardless of their age, should be more effective than management applied to both males and females, because the latter play a predominant role in the transmission of brucellosis.
- Finally, we also predicted that combining both approaches (targeting females in the core area) should be even more effective than either one alone.

**Multilevel targeted strategies for the
management of wildlife diseases: the case of
brucellosis in Alpine ibex (*Capra ibex*)**

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In prep.

ABSTRACT

The management of infectious diseases in wildlife reservoirs is particularly challenging and face several limitations. Strategies targeting specific classes of individuals and/or areas, having particular role in transmission, could be more effective and represent a more acceptable alternative than population-wide interventions. Transmission of *Brucella melitensis* in the wild population of Alpine ibex (*Capra ibex*) of the Bargy massif in the French Alps is highly heterogeneous both at the individual and spatial levels. Indeed, females transmit the infection in 90% of cases. The spatial units in the centre of the massif (“core area”) are occupied by the subpopulations with the highest seroprevalence and abundance, and act as sources of transmission for peripheral areas. We tested whether targeting females and/or the core area for management would be more effective than untargeted strategies. For this purpose, we expanded an individual-based model that was developed and parameterized in a previous study, to predict the outcome of various disease management strategies. The simulated strategies were based on feasible options given field characteristics and combined test-and-removal of captured animals, vaccination of seronegative captured individuals and culling of unmarked animals. Our results confirmed that targeting females or the core area is more effective than untargeted management, and also that targeting both is even more effective. Although there was no silver bullet for the management of brucellosis in the population of study, targeted strategies offered a wide range of promising refinements to classical sanitary measures. We therefore encourage to look for heterogeneity in other wildlife diseases and to evaluate potential strategies for improving management in terms of efficiency but also acceptability.

Keywords: mathematical modelling; vaccination; culling; spatial heterogeneity; superspreading; fadeout; targeting

5.1.1. INTRODUCTION

Wildlife populations can act as reservoirs of multi-host infections shared with domestic livestock that have an impact on human health or veterinary health, such as classical swine fever, tuberculosis or brucellosis (Rhyan and Spraker, 2010). This role of reservoir may become apparent when disease management programs entail massive decrease of incidence and prevalence in domestic animals, up to a level when the eradication efforts are hampered by the wildlife reservoir as observed for example for bovine tuberculosis in several areas in the world (Corner, 2006). Wildlife reservoirs can also trigger a re-emergence of the infection after eradication was obtained in the domestic compartment, and thus jeopardize the infection-free status. Such situations have raised a growing interest in disease management in wildlife reservoirs in the last decades (Gortázar *et al.*, 2015).

Mathematical modelling has been used to evaluate disease management strategies in wildlife (e.g., Cowled *et al.*, 2012; Zanella *et al.*, 2012; Anderson *et al.*, 2013). One major advantage of mathematical models is the possibility to compare the relative efficacy of several strategies through simulations, which is often impractical in real life conditions (Smith *et al.*, 2009). To evaluate the effectiveness of management as accurately as possible, a detailed understanding of infection dynamics and the integration of biological characteristics relevant for the persistence of infectious agent are required.

Spatial structure of host populations is among the characteristics that can most impact the persistence of infectious agents and the outcome of management (Grenfell and Harwood, 1997; Eisinger and Thulke, 2008). Whether spatial structure increases or decreases persistence is dependent on population factors such as density or movement rates, and the relationship between these factors and persistence cannot be predicted easily (Swinton *et al.*, 1998; Jesse *et al.*, 2008; Jesse and Heesterbeek, 2011). Individual heterogeneity is another example of characteristic that impacts the persistence of infections, and is receiving increasing attention (Galvani and May, 2005; Lloyd-Smith *et al.*, 2005b). Accounting for individual heterogeneity in models makes infection invasion less likely when introduced into a fully susceptible population but with greater epidemic outbreaks (Lloyd-Smith *et al.*, 2005b).

Besides their relevance for the accurate estimation of infection persistence, considering spatial and individual heterogeneity also offer the possibility to evaluate management strategies targeting the areas or the individuals responsible for most transmissions. Such

targeted strategies have proven useful for increasing the efficacy of management interventions. For instance, instead of applying management to the entire population, specific subpopulations can be targeted depending on their spatial situation and their role in transmission (Hess, 1996; Fulford *et al.*, 2002; Haydon *et al.*, 2006). Similarly, targeting the most infectious individuals can provide better results than population-wide management (Woolhouse *et al.*, 1997; Lloyd-Smith *et al.*, 2005b; Matthews *et al.*, 2006). In the case of wildlife diseases, targeted management strategies may represent an opportunity for eradication schemes which are more cost-effective and more acceptable for wildlife conservation (Fenichel and Horan, 2007; Ebinger *et al.*, 2011).

In the present study, we focused on the management of *Brucella melitensis* infection in the wild population of Alpine ibex (*Capra ibex*) of the Bargy massif in the French Alps. This is an example of multi-host infection shared between wildlife, livestock and humans, that causes economic and public health issues. In this specific case study, the population of ibex acted as a reservoir of brucellosis, which was revealed when the infection reemerged in livestock and humans in 2012, while France has been officially free of Brucellosis in domestic ruminants for several years (Mailles *et al.*, 2012; Garin-Bastuji *et al.*, 2014). Effective disease management for reducing the infection level or even eradicating brucellosis in the ibex population would be desirable to mitigate the risk to livestock and humans. Targeted management strategies could be relevant in this case to address both public health concerns and ethical and conservation issues, as ibex have been restored in the Alps during the last decades and is now protected.

We expanded the model developed in a previous study (Lambert *et al.*, in prep. – chapter 3.2 of this thesis) for predictions on the future dynamics of the population, with and without disease management, so as to evaluate the relative efficacy of various strategies. By taking advantage of detailed information available on ibex population dynamics, behaviour, and habitat use, and on epidemiological surveys, the previous model revealed a strong heterogeneity for brucellosis transmission in the study population. More specifically, females transmitted the infection in 90% of cases, and the subpopulations with the highest seroprevalence and abundance, that also occupy a central position in the Massif, were hotspots of transmission and acted as sources for the subpopulations in the periphery. Therefore, we were interested in evaluating disease management strategies targeting specific age- and sex-classes, specific subpopulations, or both. We evaluated several management strategies, classically applied in wildlife populations: (i) “do nothing”, without any capture

or cull, which corresponds to the reference to which the other strategies will be compared to, (ii) serological testing of individuals during capture followed by removal of seropositive individuals (“test-and-remove”), (iii) test-and-remove combined with vaccination of seronegative individuals (“test and vaccinate or remove”) and (iv) test-and-remove combined to culling. The simulated strategies were also elaborated in close connection with field monitoring in order to simulate realistic schemes and parameters.

We formulated the following predictions:

- First, management strategies targeting the core area of the Massif, i.e., the subpopulations at the center of the Massif where most transmission occurs and that act as sources of infection for the subpopulations in the periphery, should be more effective than management strategies applied at the scale of the whole Massif.
- Second, targeting specifically females, regardless of their age, should be more effective than management applied to both males and females, because the latter play a predominant role in the transmission of brucellosis.
- Finally, we also predicted that combining both approaches (targeting females in the core area) should be even more effective than either one alone.

5.1.2. MATERIAL AND METHODS

5.1.2.1. Study site

Our model was built to predict the future dynamics of the Alpine ibex population of the Bargy massif in the French Alps (46°N, 6.5°E; elevation: 600-2348 m; area: *ca.* 7000 ha) from 2019 onwards, with various predicted disease management strategies. Brucellosis was detected in the ibex population in 2012, with a seroprevalence over 40% (Hars *et al.*, 2013; Garin-Bastuji *et al.*, 2014), and a model developed in a previous study (Lambert *et al.*, in prep. – chapter 3.2 of this thesis) was built on the kinetics of this population between 2012 and 2018. The current study thus started in the same situation as when the previous one stopped. More precisely, the pre-breeding population size was 567 individuals (95% confidence interval: [487-660]) in 2013, and decreased by half after massive test-and-remove and culling operations conducted in 2013, 2014 and 2015 (Marchand *et al.*, 2017). After 2015, lighter test-and-remove operations were implemented and the population size stabilized (C. Toigo, unpubl. Data). In parallel, the seroprevalence of brucellosis decreased

in the population: in females in the core area of the Bargy massif for example, the seroprevalence decreased from 51% in 2013 to 21% in 2018 (Calenge *et al.*, in prep. – chapter 4.1 of this thesis). The context in which we placed our study was therefore a context of decreased abundance and decreased levels of transmission because of the management strategies that already took place in the population.

5.1.2.2. *Individual-based model*

We describe the individual-based model we built following the updated ODD protocol (“Overview”, “Design concepts”, “Details”) of Grimm *et al.* (2006, 2010). The third part, “Details”, is described concisely in the main text below. For a complete description of this part and the complete system of mathematical equations, see Lambert *et al.* (in prep.).

a. Purpose

We expanded the stochastic individual-based, spatially implicit SEIR model developed in a previous study to represent the ibex brucellosis dynamics in the Bargy massif (Lambert *et al.*, in prep. – chapter 3.2 of this thesis). The purpose of the present study was to predict the relative efficacy of various disease management strategies that could be used in the field in the future. In our previous model, we demonstrated that high levels of heterogeneity of transmission arose from differences among individuals in terms of social behaviour, space use, mating behaviour, transmission routes and active infection. For this reason, we used an individual-based model to provide a picture of the transmission and the persistence of brucellosis dynamics in ibex as accurate as possible.

b. Entities, state variables, and scales

To track information on each individual at each time step, the model included several state variables: individual ibex identity, sex, age in weeks, and socio-spatial unit, health status (susceptible: S ; exposed: E ; actively infected: I ; non-actively infected, i.e., non-shedder: R ; or vaccinated: V), year of infection, year of recovery, year of vaccination and reproductive status (sexually receptive, pregnant, abortion, birth, parity). During captures, seropositive individuals were removed, whereas seronegative ones were marked and released. Therefore, the model also included if the individual was marked or not.

To account for the structuration into five socio-spatial units in the population, we used a spatial metapopulation model. Contacts were homogeneous within each unit, and we used explicit movements of animals, quantified through the use of GPS data, to model contacts between units. Females usually stay in their spatial unit, and we therefore assumed that females were faithful to their unit and did not engage in any movements between units (Marchand *et al.*, 2017). Males, however, were found to move between units during the periods when they can transmit or acquire infection (Marchand *et al.*, 2017). Therefore, the socio-spatial unit occupied was included as an individual state variable in the model. At birth, each individual was attributed the socio-spatial unit of its mother (permanent socio-spatial unit). All individuals living in the same socio-spatial unit constituted a subpopulation. Each subpopulation was characterised by its name and its own relative carrying capacity, defined as a proportion of the carrying capacity of the whole population. The five subpopulations constituted the overall metapopulation.

The model was developed to represent the evolution of the population dynamics between December 1, 2012 and November 30, 2028 with a discrete weekly time step. The model first ran for six years following the recorded kinetics of the population and considering the management measures that were actually applied, as in Lambert *et al.* (in prep. – chapter 3.2 of this thesis), and then we applied predicted management strategies each year starting at year 7 and for 10 consecutive years.

c. Process overview and scheduling

Brucellosis can be transmitted through four transmission routes: horizontal transmission after abortion or parturition when *Brucella* is shed in genital fluids, venereal transmission, congenital transmission and pseudo-vertical transmission (Diaz-Aparicio, 2013). All routes relate to reproduction, which is strongly seasonal in ibex. Thus, by extension, transmission of brucellosis in this species also follows a seasonal pattern.

Simulated years began on December 1st, and were split into two periods: the mating period, which lasts from early December to mid-January in ibex (Couturier, 1962; Gauthier *et al.*, 1991; Willisch and Neuhaus, 2009) and was therefore fixed at 7 weeks in the model; and the non-mating period for the remaining 45 weeks. During the mating period, the events occurring successively at each discrete time step were mortality, spatial movements, reproduction, venereal transmission, incubation and recovery. During the non-mating

period, the events occurring successively at each discrete time step were surveillance and management strategies, mortality, spatial movements, abortions or births, congenital/pseudo-vertical transmission, horizontal transmission, incubation and recovery. Such an order is artificial but is required to apply probabilities of dying or getting infected to the correct numbers of individuals. For each event, individuals were processed simultaneously. Based on the mating period, horizontal transmission of *Brucella* through abortions was possible from early April to the end of May and horizontal transmission following birth was possible from early June to the end of July. To represent how future management interventions would be conducted in the field, predicted test-and-remove operations were implemented in May and predicted culling operations were implemented in June.

d. Design concepts

Our model included several concepts of disease ecology that are critical for wildlife disease management (Joseph *et al.*, 2013). In the case study, the invasion of the population by the infectious agent was clearly successful when the outbreak was discovered. The aim of management is therefore to obtain the endemic fade-out, by depleting the number of susceptible and/or infectious individuals to decrease transmission (Lloyd-Smith *et al.*, 2005a).

By including random events, our model accounted for the effects of demographic stochasticity, defined as the variation in dynamics of small populations owing to the probabilistic nature of individual processes such as transmission of infectious agents (Lloyd-Smith *et al.*, 2005a). Demographic stochasticity is particularly important when these processes are related to a small number of individuals (e.g., Lambert *et al.*, 2018a). In the case study, random fluctuations leading to stochastic fadeout of *Brucella* are likely as only a small proportion of infectious individuals transmit the infection each year (Lambert *et al.*, in prep.) and because the number of infected individuals is decreasing as a result of management interventions.

The effects of reducing the numbers of susceptible hosts through management interventions such as culling depend on how transmission relates to host density. In the case of ibex brucellosis, it may be effective in reducing horizontal transmission of *Brucella* through infectious abortions or births, because the infection hazard for susceptible

individuals exposed to this route increased with the number of infectious females (positive density-dependence). However, it may not be as effective in reducing venereal transmission, because for this route the infection hazard for susceptible individuals depends on the proportion rather than the number of infectious individuals (frequency-dependent transmission).

Demographic processes that play a role in the replenishment of susceptible individuals and therefore in infection persistence are also fundamental (Lloyd-Smith *et al.*, 2005a). Our model included seasonality of the reproductive cycle of ibex, which notably leads to a seasonal peak of births (i.e., susceptible individuals) in late May and early June. In addition, survival and reproduction parameters decreased as the abundance got close to the carrying capacity, which is a common process in populations of large herbivores (Bonenfant *et al.*, 2009). Such density-dependent processes play a crucial role in disease ecology because it can interact with transmission and persistence, for example by increasing the number of births and therefore the pool of susceptible individuals when management interventions reduce the size of the population (Choisy and Rohani, 2006). The massive culling operation (233 individuals) that was conducted in autumn 2013 did not induce any increase in female reproductive success, despite the drop in population size (C. Toïgo, pers. comm.). This could be explained by a delay on density-dependent responses, that need several years before they can occur (ANSES, 2015). Therefore, in the model, density-dependent responses of population parameters began several years after the massive culling operation in 2013, to reproduce this phenomenon. The delay between culling operations and density-dependent response of the population is unknown, however no increase in reproduction parameter was observed up to 2018 (C. Toïgo, pers. comm.), we thus considered this delay to be at least 5 years. We considered two possible delays in the model: (i) a five-years delay (“short-delay hypothesis”), i.e., between 2014 and 2018, which means that the population growth rate increased in 2019 when the predicted management strategies started (Figure 5-1A); (ii) a ten-years delay (“long-delay hypothesis”), i.e., between 2014 and 2023, thus the management strategies were implemented for five years before the population growth rate increased (Figure 5-1B).

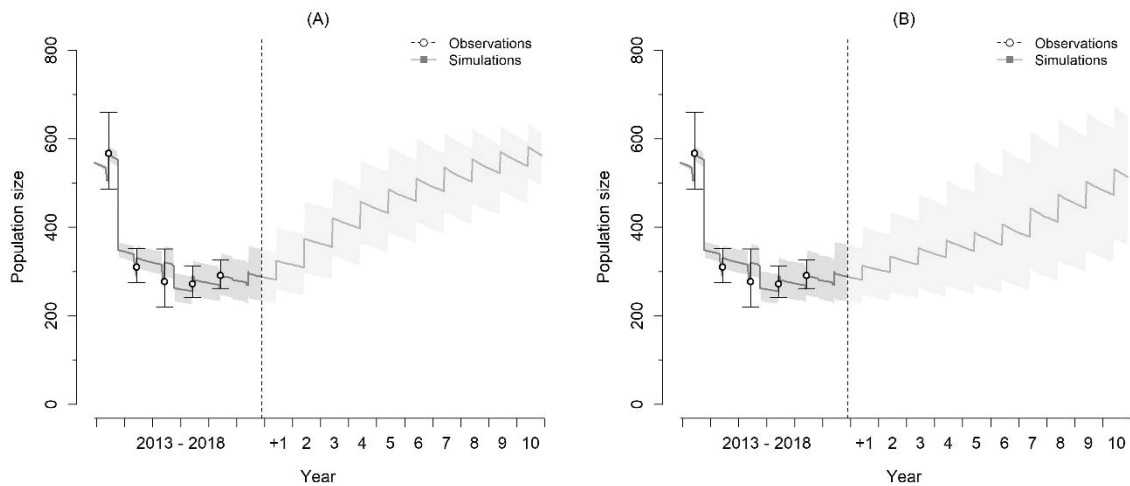


FIGURE 5-1 | Simulated variation of the pre-breeding population size for the “do nothing” scenario.

(A) Short-delay hypothesis: the density-dependent responses of demographic parameters are delayed by 5 years after the massive culling operation in 2013, i.e., between 2014 and 2018. (B) Long-delay hypothesis: the density-dependent responses of demographic parameters are delayed by 10 years after the massive culling operation in 2013. The open black circles and black lines are the observed data and the 95% confidence interval, respectively. The grey line represents the median of the simulated outputs over the 1000 iterations, while the grey area represents the 95% credible interval. The vertical dashed line represents the separation between the 2013-2018 period that followed the recorded dynamics of the population, as in Lambert *et al.* (in prep.), and the 10 consecutive years of predicted management scenarios.

e. Initialisation

The initial conditions for the first time step of the model (December 1, 2012) were the same as in Lambert *et al.* (in prep.). Regardless of the management scenarios that were evaluated, the model first ran for six years following the recorded dynamics of the population, as in Lambert *et al.* (in prep.). Then, we applied the simulated management scenarios for the 10 following years, i.e., from 2019 to 2028. Therefore, all scenarios started in the same conditions, i.e., the conditions obtained at the last time step of the first six years (end of 2018).

f. Input data

The model does not use input data or an external model to represent time series of driving environmental variables.

g. Submodels

Surveillance and management strategies: Test-and-remove and culling operations implemented between 2012 and 2018 on the Alpine ibex population of the Bargy massif were integrated in the model – see Lambert *et al.* (in prep.) for further details. Predicted management strategies (do nothing: NO, Test-and-Remove: TR, Test and Vaccinate or Remove: TVR, or Test-and-Remove and Culling: TRC) were implemented in the model between 2019 and 2028.

To simulate the capture process (TR, TVR and TRC strategies), several levels of number of captures were tested in the model, that represented the number of individuals that field agents capture each year. The model randomly allocated a proportion of this number in each age- and sex-classes and each socio-spatial unit that were targeted, and sampled individuals at random in each category among those available (if the number of individuals to be captured was greater than the number available in a given age- and sex-class and socio-spatial unit, all available individuals with those characteristics were captured). Seropositive individuals were removed, whereas seronegative ones were marked and released (and vaccinated in the case of TVR). In the model, *I* and *R* individuals were considered seropositive, but some were mistakenly detected as seronegative, as the sensitivity of serological tests was assumed to be 95% (ANSES, 2015). On the contrary, *S* and *E* individuals were considered seronegative and specificity of the serological tests was assumed to be 100% (ANSES, 2015). Although values of sensitivity and specificity of the serological tests that were used in ibex are unknown in this species, high values were demonstrated when applied to the diagnosis of brucellosis in domestic ruminants (e.g., Ferreira *et al.*, 2003; Nielsen *et al.*, 2004; Ducrottoy *et al.*, 2018). Moreover, the serological status in the data of the ibex Bargy population was based on several serological tests performed in parallel, which improves the accuracy of the diagnosis (Godfroid *et al.*, 2004). Additionally, we considered that vaccinated individuals could be distinguished by a particular marking, and were therefore not removed if captured nor included in the calculation of the seroprevalence in the population.

Several levels of culling were also tested in the model, that represented the number of unmarked individuals that field agent attempt to cull each year. The model randomly allocated a proportion of this number in unmarked individuals of each age- and sex-classes and each socio-spatial unit that were targeted, and sampled individuals for removal at random inside each category.

Mortality: We used a complete age-dependent model, with a mortality estimate at each age and for each sex (Toïgo *et al.*, 2007). We assumed no brucellosis-related mortality. The density-dependent response of winter juvenile survival was determined using a function that related survival to the carrying capacity and abundance of the subpopulation (Lambert *et al.*, in prep.) The carrying capacity was estimated by Approximate Bayesian Computation (ABC) in a previous study (Lambert *et al.*, in prep. – chapter 3.2 of this thesis).

Spatial movements: Movements of males between units during the mating period and the abortion period, when they can transmit or acquire infection, were derived from data on space use collected by GPS collars. We considered all movements to be temporary, as the model randomly sampled a new temporary socio-spatial unit at each time step based on the movement probabilities (Lambert *et al.*, in prep.).

Reproduction: In the model, reproductive success of females was the outcome of two processes: sexual receptivity during mating, and the probability to give birth to a living newborn. We considered that a mature female could become sexually receptive during mating only once every year, with probabilities depending on age (starting at 1.5 years old – Couturier, 1962; Gauthier *et al.*, 1991). The density-dependent response of female receptivity was determined using a function that related receptivity to the carrying capacity and the abundance of the subpopulation (Lambert *et al.*, in prep.).

Reproductive success of males was the outcome of two processes: association of several males to a receptive female, and selection of the male which succeeds in engaging sexual intercourse with the female. These processes were mainly driven by males age, and resulted in a small fraction of males that engaged in sexual intercourse, mainly older (dominant) males (Willisch and Neuhaus, 2009; Willisch *et al.*, 2012; Tettamanti, 2015).

Every female who engaged in sexual intercourse with a male became pregnant. In our model, we assumed that *Brucella* infection decreased sexual receptivity during mating, thus leading to a lower probability of gestation (C. Toïgo, pers. comm.).

Venereal transmission: The model assumed the existence of this transmission route for ibex, and the corresponding transmission probability was estimated using ABC in a previous study (Lambert *et al.*, in prep. – chapter 3.2). Venereal transmission was possible only between individuals that engaged in sexual intercourse.

Abortions or births: In domestic ruminants, 80% of infectious females abort and most of them do so only once, during the first pregnancy post-infection (FAO and WHO, 1986; Godfroid *et al.*, 2004). If abortion does not occur, invasion of the uterus leads to *Brucella* shedding in genital fluids at the time of parturition (European Commission, 2001). In the model, we assumed that pregnant females could abort only during their first pregnancy following infection with a probability of 80%. When abortion does not occur, females give birth to a live newborn. In both cases, females shed *Brucella* in genital fluids for *ca.* three weeks.

For the other pregnant females, and for actively infected females that already went through their first pregnancy following infection, the probability to give birth to a live newborn that survives until winter depends on their parity. We also assumed that active *Brucella* infection decreases the probability to give birth to a live newborn (C. Toïgo, pers. comm.). Actively infected females that give birth to a live newborn shed *Brucella* in genital fluids for three weeks. The sex-ratio at birth is expected to be 1.

Congenital and pseudo-vertical transmission: Congenital transmission from infected mothers to their offspring *in utero* is demonstrated in domestic ruminants (Renoux, 1962; Plommet *et al.*, 1973; Lapraik *et al.*, 1975) and happens in *ca.* 5% of kids born from infectious mothers (FAO and WHO, 1986; Godfroid *et al.*, 2013). Pseudo-vertical transmission from infectious mothers to kids can also happen through colostrum or milk consumption in all gestation events (Philippon *et al.*, 1971; European Commission, 2001), but this transmission only concerns a small proportion of kids (Grilló *et al.*, 1997). Both transmission routes are suspected in Alpine ibex (Lambert *et al.*, 2018b). Therefore, we included both transmission routes in the model, with fixed low probabilities of 5% (ANSES, 2015; Lambert *et al.*, in prep.).

Horizontal transmission: In domestic ruminants, excretion of the *Brucella* in female genital discharges through infectious abortions or births is considered as the main route of transmission to other individuals (European Commission, 2001). For this transmission route, contacts were not modelled explicitly. A susceptible individual was exposed to all abortions or births caused by brucellosis within the same socio-spatial unit. We assumed that at a given time step, the probability of an individual coming into effective contact (*i.e.*, coming into a contact that leads to infection) with one infectious abortion or with one infectious birth was the same. This probability was estimated using Approximate Bayesian Computation in a previous study (Lambert *et al.*, in prep.).

In ibex, males over 5 years of age segregate from females both socially and spatially soon after the rut, whereas segregation is more gradual for males 5 years of age and under, which are still associated to females during spring (Villaret and Bon, 1995; Bon *et al.*, 2001). Thus, we assumed in the model that males under 5 were less exposed than females to *Brucella* shed following infectious abortions, and males over 6 even less (Lambert *et al.*, in prep.). After being isolated for two weeks following parturition, females and kids gather to form nurseries (Gauthier *et al.*, 1991), and have different space use than males older than 1 year old and females without kids (Grignolio *et al.*, 2007a,b). Thus, we considered that mothers began to gather in nurseries three weeks after parturition. Horizontal transmission through infectious births was assumed to occur only in nurseries, from infected mothers to non-infected mothers and susceptible newborns.

Incubation and recovery: Transmission of *Brucella* to a susceptible ibex S led to an incubation (state E) of three weeks, which is the duration of the incubation period after which seroconversion generally occurs in domestic ruminants (European Commission, 2001). For kids born after congenital transmission, the incubation ends only at abortion or parturition after their first pregnancy for females or at the age of sexual maturity for males (Plommet *et al.*, 1973). After incubation, we considered that infected individuals first entered the I class, characterised by an active infection and therefore the ability to shed the bacteria.

We assumed that I individuals could transit to the R class, *i.e.* infected but without active infection and unable to shed the bacteria, based on the probability of recovery that we derived from bacteriological data in a previous study (Lambert *et al.*, in prep. – chapter 3.2 of this thesis).

The complete system of mathematical equations is provided in Lambert *et al.* (in prep.). All simulations and further analyses were performed using R 3.4.1 (R Core Development Team, 2017).

5.1.2.3. *Management strategies*

We evaluated several management strategies, classically applied in wildlife populations, that we refined for our case study by targeting specific age- and sex-classes or specific socio-spatial units depending on their role in transmission. The first strategy was doing nothing (NO), which corresponded to the reference to which the other strategies were compared to.

The second strategy was serological testing of individuals during capture followed by removal of seropositive individuals (TR). Only individuals that were never captured before (i.e., unmarked) were targeted for capture. Ibex under 2 years of age were not targeted, as those are seldom captured in the population of study. The objective levels for the number of capture were either 15 or 30 animals each year, as those were the levels of capture that were achieved in the last years in the population among an overall population of around 300 individuals. Four versions of the TR strategy were evaluated: (i) TR on the whole metapopulation regardless of age and sex (TRall), (ii) TR on the socio-spatial units of the core area of the massif, regardless of age and sex (TRcore), (iii) TR on the whole metapopulation focusing only on females, regardless of age (TRfemale) and (iv) TR on females in the core area exclusively, regardless of age (TRcorefemale). The rationale behind these versions was that most transmission occurs in the core area, and that females play a predominant role in the transmission of brucellosis (Lambert *et al.*, in prep. – chapter 3.2). Therefore, targeting these specific areas and gender should be more effective than population-wide interventions.

The third strategy was to combine vaccination of seronegative individuals with the previous strategy (TVR), with the same objective levels. Because the majority of new cases occurred in young individuals (5 years old and under – Lambert *et al.*, in prep.), we predicted that TVR would be more effective by targeting individuals 2-5 years of age than older ones. Four versions were therefore evaluated: (i) TVR on the whole metapopulation regardless of age and sex (TVRall), (ii) TVR on the socio-spatial units of the core area of the massif, regardless of age and sex (TVRcore), (iii) TVR on the whole metapopulation focusing only on individuals 2-5 years of age, regardless of gender (TVRyoung) and (iv) TVR on

individuals 2-5 years of age in the core area exclusively, regardless of gender (TVRcoreyoung). We made several assumptions about the vaccine. The live *B. melitensis* Rev. 1 strain is considered the only vaccine available for brucellosis management in small domestic ruminants (Blasco, 1997). Indeed, the efficacy of killed vaccines is not high enough (Elberg and Faunce, 1957) and only live vaccines are considered suitable for brucellosis management (Blasco, 1997). Moreover, alternative live vaccines are less efficient than Rev. 1 (Morgan *et al.*, 1966; Verger *et al.*, 1995; Blasco, 1997; El-Idrissi *et al.*, 2001; Barrio *et al.*, 2009). We considered that this vaccine could also be used in Alpine ibex, although no data on the efficacy of the vaccine in this species are currently available. To avoid adding too much complexity to an already detailed model, we made several simplifying assumptions. (i) Although the Rev. 1 vaccine does not confer 100% protection against *B. melitensis* infection in domestic ruminants (Elberg and Faunce, 1957; Elberg, 1959), we assumed 100% efficacy of vaccination, meaning that all susceptible individuals that received vaccination could not become infected as long as vaccination conferred protection. (ii) We also assumed that the vaccine had no detrimental effects on pregnant females, despite the fact that vaccine-induced abortions are possible (Blasco, 2010). (iii) The duration of protective immunity was supposed to last 4.5 years, based on experiments on small domestic ruminants (Alton, 1966, 1968). (iv) Vaccination of infected individuals that were mistakenly detected as seronegative during capture was supposed to have no effect, as there is no evidence that the vaccine confers protection in previously infected animals (Blasco, 2010). (v) Finally, we made the assumption that vaccinated individuals did not shed the vaccine strain and transmit it to other individuals. This major drawback unfortunately occurred during an experiment that was carried out to evaluate post-vaccination responses of Alpine ibex (Ponsart *et al.*, 2017; Ponsart *et al.*, in prep.). This represents a serious limitation to the use of the vaccine in the field, as individuals exposed to the vaccine strain can develop strong serological responses that cannot be distinguished from the serological responses of naturally-infected animals (ANSES, 2019). Nonetheless, for the sake of simplicity, we did not consider this process in the model, but we kept in mind that it could limit the use of vaccination if this strategy turned out to be promising according to the results of the model.

The last strategy was to combine culling of unmarked individuals with the second strategy (TRC), with the same objective levels. The idea behind this strategy is that unmarked individuals are expected to have a higher risk of being infected than marked individuals (which were seronegative when released). The combination of TR with culling of unmarked

individuals should therefore decrease the number of infected individuals more effectively, while maintaining a proportion of marked individuals that are not submitted to culling (and supposedly healthy). This strategy was only applied to the core area (TRCcore), regardless of the age- and sex-classes of the individuals. Only newborns were not targeted. The objective levels for the number of unmarked individuals culled were either 5 animals each year, as this was the level of culling that was achieved in the last years in the population, or four times this level, i.e., 20 animals each year. We also evaluated a third level, 90% of the total number of unmarked individuals in the core area each year, to evaluate the effects of a massive culling repeated in the population. This last strategy was proposed by local authorities as a mean to create a group of healthy ibex (marked individuals) to maintain the population and then eradicate brucellosis by culling all individuals with unknown health status (unmarked individuals).

In order to compare the effects and cost of each strategy, we simulated the evolution of the population between 2012 and 2028 ($n = 1000$ simulations). For each scenario, we retrieved (i) the proportion of simulations where *Brucella* was no longer present at the end of the simulation, (ii) the seroprevalence at the end of the simulations (median and 95% credible interval) and (iii) the numbers of individuals captured and culled, in order to approach the cost of field operations (median and 95% credible interval).

5.1.3. RESULTS

5.1.3.1. *Test-and-remove*

In all scenarios, the seroprevalence at the end of the simulations and the proportion of simulations where *Brucella* was still persistent at the last time step were higher in the situation where the response of density-dependent parameters was delayed by 5 years than in the situation where the delay was 10 years.

Compared to the scenario with no management (e.g., 23.5% [0.15-59.1] in the situation where the response of density-dependent parameters was delayed by 10 years), the seroprevalence at the end of the simulations decreased when test-and-remove was applied, with a stronger impact when the objective level was 30 captures every year (16.2% [0-48.1]) compared to 15 (21.9% [0-56.3] – Figure 5-2A). Similar results were obtained when targeting only the core area, targeting only females or targeting both (Figure 5-2B-D).

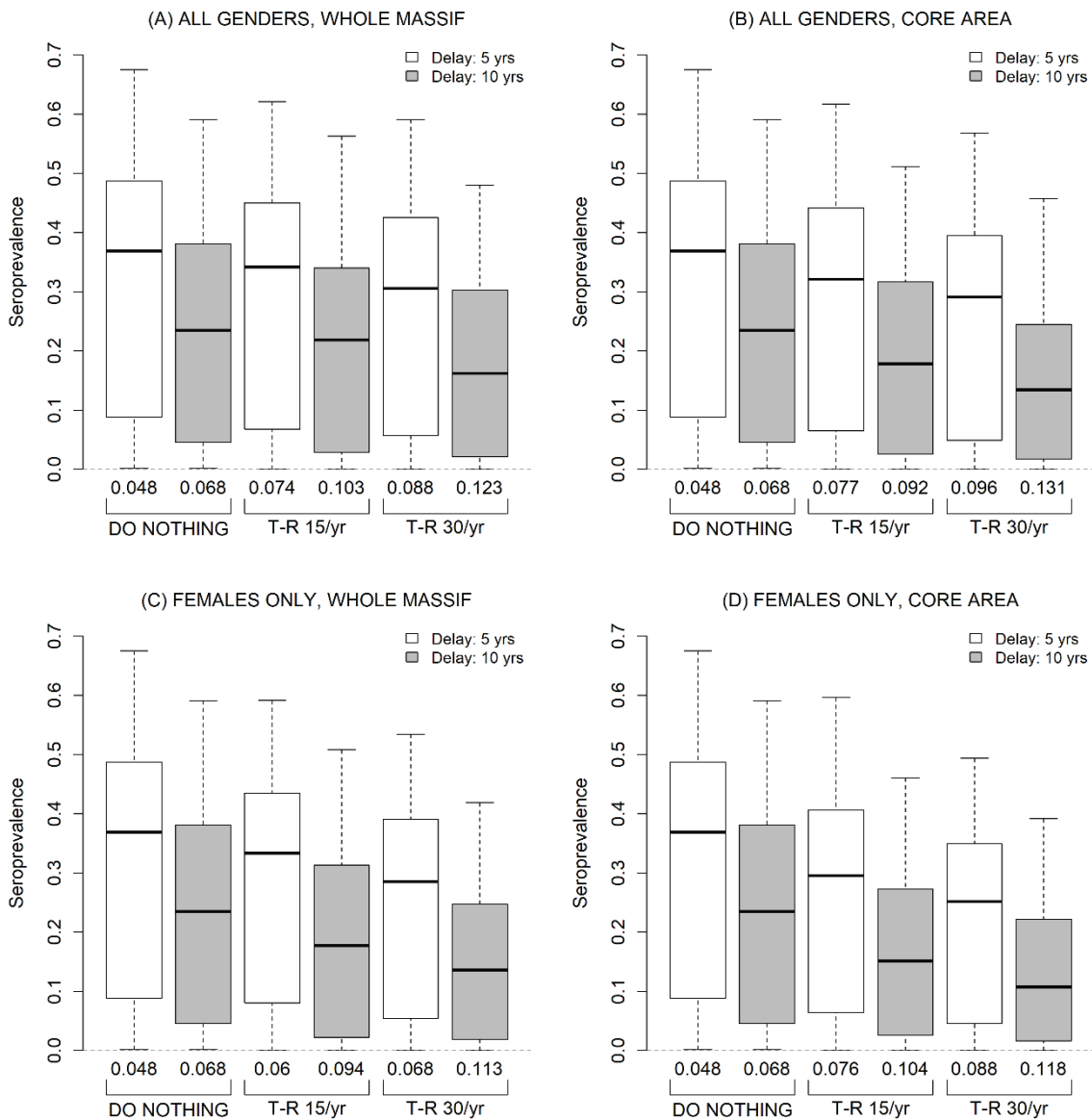


FIGURE 5-2 | Seroprevalence at the end of the simulations for the scenario “Test-and-Remove” (TR) compared to the reference scenario with no management (“Do nothing”). (A) TR on the whole metapopulation, regardless of age and sex. (B) TR on the socio-spatial units of the core area of the massif, regardless of age and sex. (C) TR on the whole metapopulation focusing only on females, regardless of age. (D) TR on females in the core area exclusively, regardless of age. The objective levels were either 15 or 30 captures every year. Boxplots represent the interquartile range and the median of the outputs of the 1000 simulations, and whiskers indicating the 95% credible interval. The density-dependent responses of demographic parameters were delayed after the massive culling operation in 2013, either by 5 years (white boxplots) or by 10 years (grey boxplots). Numbers below each boxplot indicate the proportion of simulations (n=1000) where *Brucella* was no longer persistent at the end of the simulations.

Compared to the TRall strategy (16.2% [0-48.1]), the seroprevalence was only slightly smaller when targeting only the core area compared to targeting the whole massif (e.g., 13.4% [0-45.7] for 30 captures/year and a 10 years delay – Figure 5-2A and B), or when targeting only females compared to targeting both genders (e.g., 13.6% [0-41.8] – Figure 5-2A and C). When targeting the core area, the total numbers of captures after 10 years were roughly the same as when targeting the whole massif, but more individuals were removed as a result of these captures (Table 5-1). When targeting only females in the whole massif, the total number of captures dropped especially when the objective was 30 captures/year, which reflects the fact that there were less females available than the numbers that were targeted (Table 5-1). Nonetheless, the number of individuals removed was similar to that when targeting individuals regardless of gender (Table 5-1).

TABLE 5-1 | Total number of capture and removals after ten years of test-and-remove, with objective levels of either 15 or 30 captures every year, and a delay in density-dependent responses of demographic parameters by either 5 or 10 years.

Scenario	Objective	Delay	Number of capture (over 10 years)	Number of removals (over 10 years)
All genders, whole massif	15/yr	5	132 [118-142]	21 [2-61]
	15/yr	10	126 [109-140]	17 [2-56]
	30/yr	5	232 [205-255]	35 [5-114]
	30/yr	10	221 [178-253]	27 [5-90]
All genders, core area	15/yr	5	136 [122-147]	29 [4-88]
	15/yr	10	132 [107-145]	23 [4-74]
	30/yr	5	223 [196-246]	50 [7-137]
	30/yr	10	213 [167-250]	34 [7-110]
Females only, whole massif	15/yr	5	121 [101-137]	24 [3-69]
	15/yr	10	115 [89-135]	18 [4-62]
	30/yr	5	173 [144-204]	40 [6-96]
	30/yr	10	168 [119-209]	27 [5-82]
Females only, core area	15/yr	5	113 [93-132]	33 [5-84]
	15/yr	10	108 [79-132]	24 [5-72]
	30/yr	5	157 [126-186]	49 [7-106]
	30/yr	10	140 [95-190]	25 [6-81]

Finally, targeting both the core areas and the females always allowed better result compared to untargeted intervention or strategies targeting either one (e.g., 10.7% [0-39.1] for 30 captures/year and a 10 years delay – Figure 5-2D). In this last scenario, the total numbers of capture were the smallest, but the number of individuals removed was similar to what was obtained when targeting individuals in the core area regardless of gender (Table 5-1).

5.1.3.2. *Test-and-vaccinate-or-remove*

The seroprevalence (vaccinated individuals excluded) at the end of the simulations was smaller when test-and-vaccinate-or-remove was applied (e.g., 19.6% [0-51.9] for 15 captures/year and in the situation where the response of density-dependent parameters was delayed by 10 years) compared to the scenario with no management (23.5% [0.15-59.1] – Figure 5-3A). The decrease was more marked when the objective level was 30 captures every year (14.4% [0-45.6]).

Seroprevalence was lower when targeting only the core area compared to targeting the whole massif (e.g., 9.7% [0-42.3] for 30 captures/year and a 10 years delay – Figure 5-3A and B). The total numbers of capture after 10 years were roughly the same as when targeting the whole massif, but more individuals were removed (Table 5-2).

The results also indicate a slightly greater efficacy of TVRall (14.4% [0-45.6]) compared to TRall only (16.2% [0-48.1], see 5.1.3.1 and Figure 5-2A), and of TVRcore (9.7% [0-42.3]) compared to TRcore only (13.4% [0-45.7]).

Seroprevalence also decreased when targeting only individuals 2-5 years old compared to targeting all age-classes (e.g., 12.6% [0-42.3] for 30 captures/year and a 10 years delay – Figure 5-3A and C), but not as much as when targeting the core area. The total numbers of capture after 10 years were roughly the same as when targeting all age-classes, but less individuals were removed and hence more individuals were vaccinated (Table 5-2).

Finally, targeting only young individuals of the core area was only slightly more efficient in reducing seroprevalence (e.g., 8.5% [0-36.5] for 30 captures/year and a 10 years delay – Figure 5-3D). The total number of captures was similar or even slightly higher than when targeting individuals in the core area regardless of age, but the number of individuals removed was lower, meaning that the number of individuals that were vaccinated (i.e., not removed) was higher (Table 5-2).

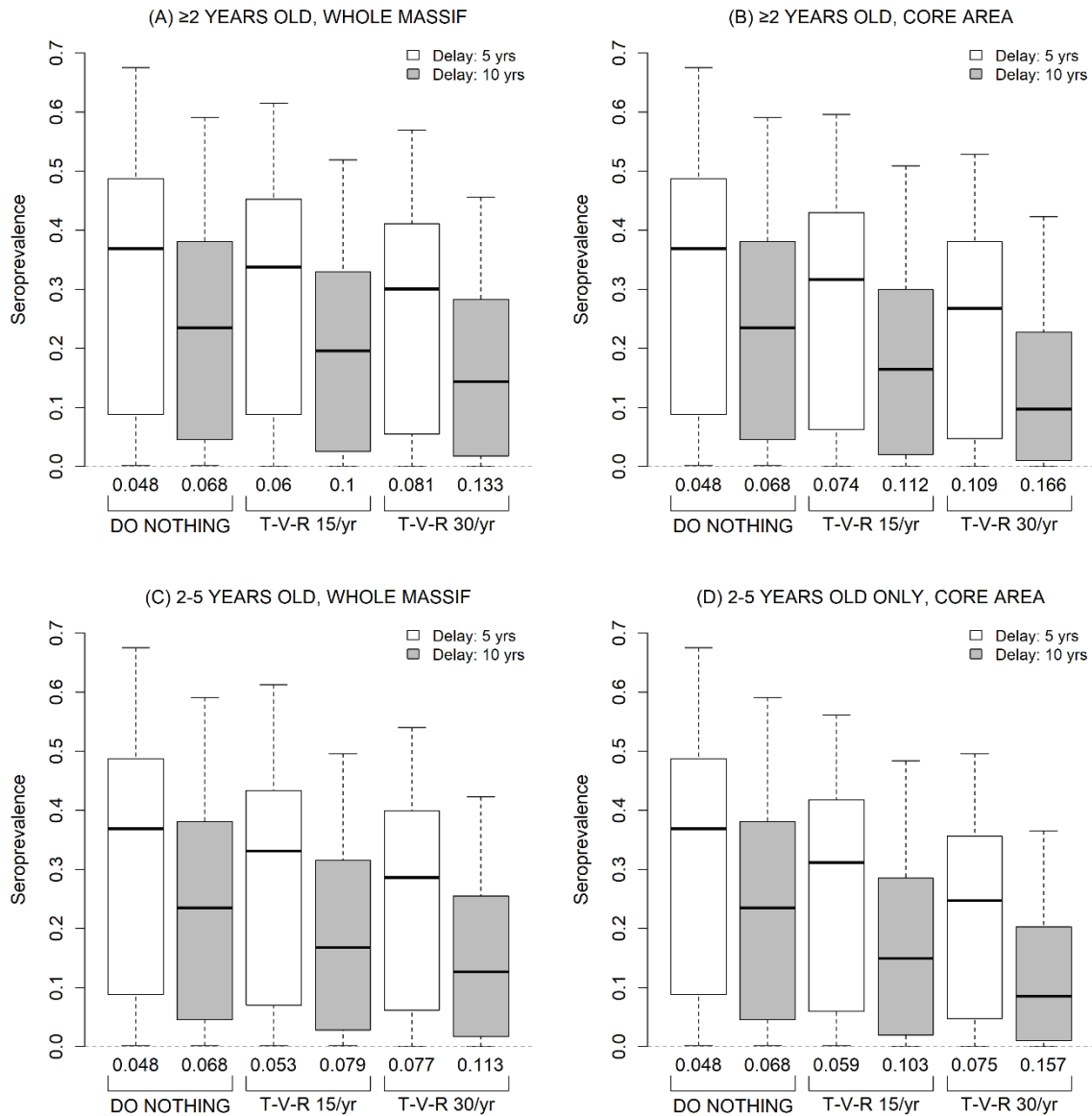


FIGURE 5-3 | Seroprevalence at the end of the simulations for the scenario “Test-and-Vaccinate-or-Remove” (TVR) compared to the reference scenario with no management (“Do nothing”).

(A) TVR on the whole metapopulation, regardless of age and sex. (B) TVR on the socio-spatial units of the core area of the massif, regardless of age and sex. (C) TVR on the whole metapopulation focusing only on individuals 2-5 years of age, regardless of gender. (D) TVR on individuals 2-5 years of age in the core area exclusively, regardless of gender. The objective levels were either 15 or 30 captures every year. Boxplots represent the interquartile range and the median of the outputs of the 1000 simulations, and whiskers indicating the 95% credible interval. The density-dependent responses of demographic parameters were delayed after the massive culling operation in 2013, either by 5 years (white boxplots) or by 10 years (grey boxplots). Numbers below each boxplot indicate the proportion of simulations (n=1000) where *Brucella* was no longer persistent at the end of the simulations.

TABLE 5-2 | Total number of capture and removals after ten years of test-and-vaccinate-or-remove, with objective levels of either 15 or 30 captures every year, and a delay in density-dependent responses of demographic parameters by either 5 or 10 years.

Scenario	Objective	Delay	Number of capture (over 10 years)	Number of removals (over 10 years)
All ages (≥ 2 years), whole massif	15/yr	5	132 [119-141]	20 [3-62]
	15/yr	10	127 [110-140]	16 [3-58]
	30/yr	5	231 [203-257]	34 [5-108]
	30/yr	10	220 [178-253]	26 [5-94]
All ages (≥ 2 years), core area	15/yr	5	136 [120-146]	29 [4-82]
	15/yr	10	133 [108-146]	22 [4-74]
	30/yr	5	225 [196-248]	47 [8-135]
	30/yr	10	214 [165-250]	34 [7-112]
2-5 years old only, whole massif	15/yr	5	143 [133-149]	14 [0-59]
	15/yr	10	139 [122-149]	9 [0-48]
	30/yr	5	248 [221-271]	25 [0-90]
	30/yr	10	242 [197-277]	16 [0-86]
2-5 years old only, core area	15/yr	5	143 [131-150]	21 [0-80]
	15/yr	10	141 [116-150]	14 [0-63]
	30/yr	5	231 [208-253]	37 [0-127]
	30/yr	10	225 [173-266]	19 [0-96]

5.1.3.3. *Test-and-remove combined with culling*

TRCcore was compared to the TRcore strategy. Culling 5 unmarked animals in the core area each year in addition to TR was not determinant in improving the effectiveness of the strategy (e.g., 12.6% [0-41.8] with culling vs. 13.4% [0-45.7] for TRcore only, for 30 captures/year and a 10 years delay – Figure 5-4). With an objective of 20 unmarked animals culled every year, seroprevalence decreased more markedly, especially when the response of density-dependent parameters was delayed by 10 years (8.45% [0-40.2] – Figure 5-4). Culling 5 or 20 unmarked individuals each year decreased the total number of capture and removals, because it reduced the number of unmarked individuals available for capture (Table 5-3). Conversely, compared to 15 captures/year, capturing 30 individuals each year decreased the number of culls when the objective levels were 5 or 20 culls/year. Indeed, a higher number of individuals were marked and released, and were therefore unavailable for culling (Table 5-3).

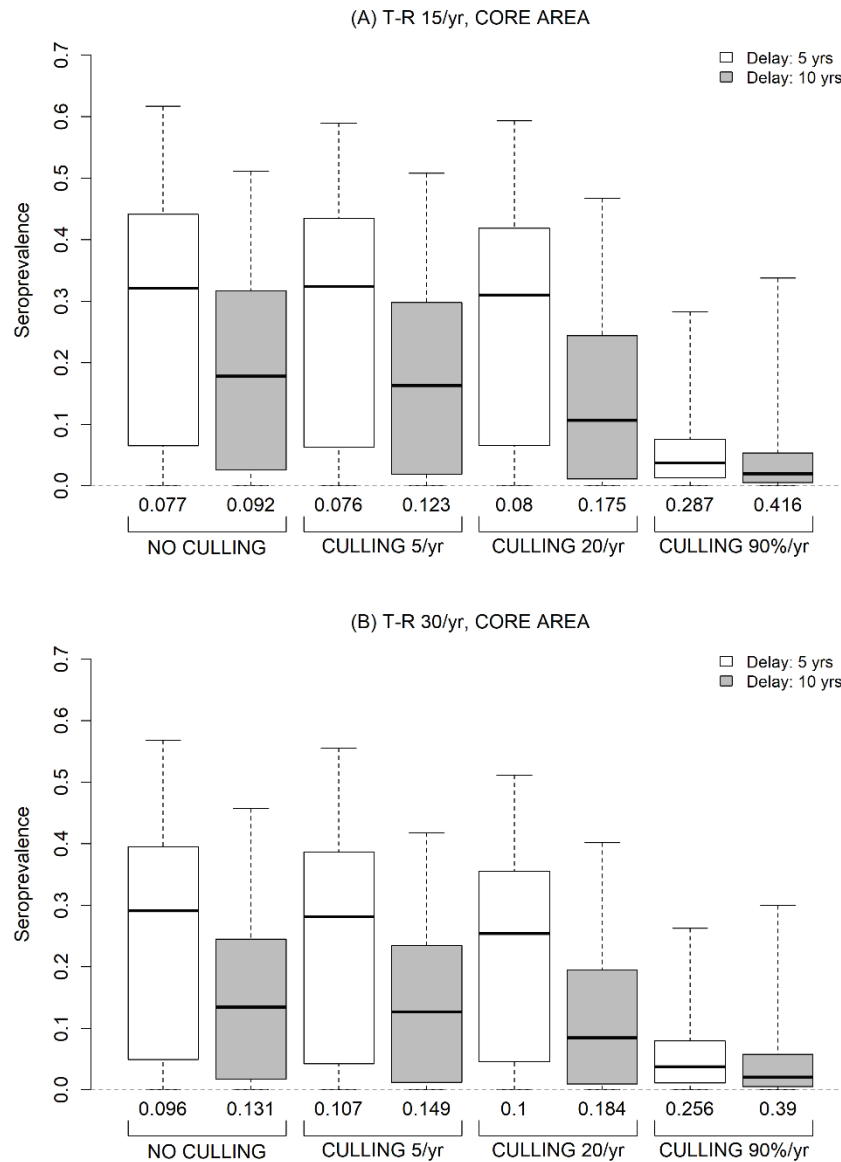


FIGURE 5-4 | Seroprevalence at the end of the simulations for the scenario Test-and-Remove (TR) associated to culling of unmarked individuals, compared to TR without culling. This strategy was only applied to the core area, regardless of the age- and sex-classes of the individuals. (A) Objective level of 15 captures every year. (B) Objective level of 30 captures every year. The objective levels for culling were either 5 or 20 unmarked individuals culled every year, or 90% of the total number of unmarked individuals in the core area each year. Boxplots represent the interquartile range and the median of the outputs of the 1000 simulations, and whiskers indicating the 95% credible interval. The density-dependent responses of demographic parameters were delayed after the massive culling operation in 2013, either by 5 years (white boxplots) or by 10 years (grey boxplots). Numbers below each boxplot indicate the proportion of simulations (n=1000) where *Brucella* was no longer persistent at the end of the simulations.

TABLE 5-3 | Total number of capture, removals and culls after ten years of a combination of test-and-remove (15 or 30 captures every year) and culling of unmarked individuals (none, 5 or 20 culls every year, or 90% of unmarked individuals), and a delay in density-dependent responses of demographic parameters by either 5 or 10 years.

Scenario	Objective (culling)	Delay	Number of capture (over 10 years)	Number of removals (over 10 years)	Number of culls (over 10 years)
TR 15/yr + culling	No culling	5	136 [122-147]	29 [4-88]	-
	No culling	10	132 [107-145]	23 [4-74]	-
	5/yr	5	130 [112-141]	28 [4-81]	46 [40-50]
	5/yr	10	122 [99-142]	20 [4-68]	44 [36-50]
	20/yr	5	109 [89-124]	26 [3-65]	159 [139-177]
	20/yr	10	96 [67-120]	16 [3-52]	146 [116-173]
	90%/yr	5	28 [22-34]	5 [1-14]	385 [257-489]
	90%/yr	10	22 [16-28]	4 [0-11]	274 [181-373]
TR 30/yr + culling	No culling	5	223 [196-246]	50 [7-137]	-
	No culling	10	213 [167-250]	34 [7-110]	-
	5/yr	5	213 [186-235]	47 [7-124]	40 [33-46]
	5/yr	10	199 [149-236]	31 [7-97]	38 [29-45]
	20/yr	5	188 [150-212]	43 [5-109]	142 [125-158]
	20/yr	10	156 [99-204]	24 [6-76]	130 [99-153]
	90%/yr	5	56 [46-65]	9 [2-26]	384 [242-501]
	90%/yr	10	46 [37-55]	7 [2-19]	270 [172-377]

TR associated to culling 90% of unmarked animals in the core area each year resulted in more determinant decreases of the seroprevalence compared to TR alone (e.g., 2.04% [0-30.0] for 30 captures/year and a 10 years delay – Figure 5-4). For this scenario, the number of culls was about twice the number of culls obtained when the objective was 20 culls/year, and was similar whether the objective was 15 or 30 captures/year (Table 5-3). The decrease in seroprevalence was very similar whether the objective was 15 or 30 captures/year (e.g., 1.96% [0-33.8] for 15 captures/year and a 10 years delay – Figure 5-4). However, contrary to the scenarios with 5 or 20 unmarked individuals culled each year, the strategy where the objective was to cull 90% of unmarked animals was less effective for eliminating *Brucella* when the objective was to capture 30 individuals each year instead of 15 (Figure 5-4).

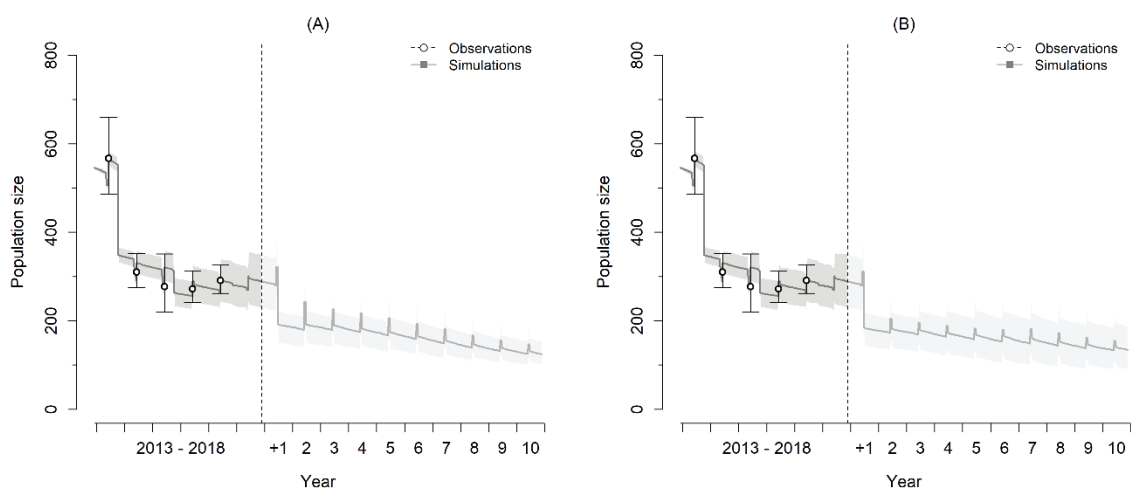


FIGURE 5-5 | Simulation of the evolution of the pre-breeding population size for the scenario combining test-and-remove (15 captures/year) and culling (90% of unmarked individuals each year).

(A) Situation where the density-dependent responses of demographic parameters are delayed by 5 years after the massive culling operation in 2013. (B) Situation where the density-dependent responses of demographic parameters are delayed by 10 years after the massive culling operation in 2013. The open black circles and black lines are the observed data and the 95% confidence interval, respectively. The grey line represents the median of the simulated outputs over the 1000 iterations, while the grey area represents the 95% credible interval. The vertical dashed line represents the separation between the 2013-2018 period that followed the recorded dynamics of the population, as in Lambert *et al.* (in prep.), and the 10 consecutive years of predicted management scenarios.

This last scenario was by far the most detrimental on the demography of the population. The population size at the end of the simulations for 15 captures/year were 124 [101-153] for a 5 years delay and 134 [91-188] for a 10 years delay (Figure 5-5), which represents a two-fold decrease compared to the population size before the implementation of predicted interventions and a four-fold decrease compared to the population size when the outbreak was discovered. For 30 captures/year, the population sizes were slightly higher: 146 [118-177] for a 5 years delay and 153 [112-212] for a 10 years delay.

5.1.4. DISCUSSION

The management of infectious diseases in wildlife is particularly challenging compared to their counterpart in domestic animals. Numerous tools are available to mitigate or eradicate infectious diseases in wildlife, but they all face important limitations. Among them, logistical and financial constraints, preservation of genetic variability of the host population, ethical and acceptability issues, or low efficacy due to interactions with population demography or social structure are just a few examples (Artois *et al.*, 2001, 2011; Wobeser, 2002, 2007; Prentice *et al.*, 2014). Strategies targeting more precisely the entities (e.g., individuals, areas) responsible for most transmissions could be more effective because they focus on the real sources of infection. They could also represent a more acceptable alternative than population-wide interventions, because they concern a smaller proportion of individuals.

Using brucellosis in Alpine ibex as a case study, we evaluated several targeted disease management strategies. In this highly heterogeneous system, females are responsible for the majority of transmission, that occur mostly in young individuals, mainly newborns and yearlings followed by individuals between 2 and 5 years old (Lambert *et al.*, in prep.). Moreover, the population of study is structured in five socio-spatial units, that display very different levels of seroprevalence (Marchand *et al.*, 2017). The units with the highest seroprevalence, that also occupy central positions in the Massif (“core area”), are hotspots of transmission and act as sources of transmission for the other units (Lambert *et al.*, in prep.). Therefore, we could evaluate the effectiveness of targeting the individuals responsible for most transmissions, the spatial units responsible for most transmission, and additionally, the combination of both approaches. We performed this evaluation for test-and-remove and test-and-vaccinate-or-remove, two management methods that are available in wildlife. We also evaluated the use of culling in combination to test-and-remove.

5.1.4.1. *Brucellosis persistence and efficacy of management*

In the absence of management after 2018, brucellosis faded-out only in a small proportion of the simulations during the study period of ten years. As for any infection in finite populations, extinction of the infectious agent is inevitable (Lloyd-Smith *et al.*, 2005a), but, given the results of our model, spontaneous extinction (for the do nothing strategy) should

not be expected in a near future. This would be even more the case if the demographic parameters should happen to increase in the next few years as a result of the massive culling operation conducted in autumn 2013. Indeed, no increase in female reproductive success was yet detected five years after the drop in population size induced by this operation, but such an increase could occur after a delay of several years. Our results showed that the sooner the demographic parameters increased, the longer brucellosis persisted and the higher the seroprevalence was in the population. This result is in accordance with the theoretical model of Lloyd-Smith *et al.* (2005a), where faster demographic turnover favours longer persistence, because a higher number of susceptible individuals are born each year.

Test-and-remove decreased brucellosis persistence and seroprevalence in the population when the objective was to capture 30 individuals each year, although not by much (16.2% [0-48.1] vs. 23.5% [0.15-59.1] in the best case scenario – Figure 5-2A). However, the efficacy was even less for 15 captures each year, which only provided slightly better results than the reference scenario without management actions. This could be explained by a lower probability of capturing and removing infectious individuals, and as a result a lower effect on transmission. In a model of brucellosis in bison (*Bison bison*) in the Greater Yellowstone Area (GYA), test-and-remove achieved eradication of brucellosis in 20% of simulations when the objective was to remove 100 seropositive individuals each year (around 5% of the population) after 35 years of management (Ebinger *et al.*, 2011). The comparison is of course not straightforward, because the ecology of ibex differs from the ecology of bison and the factors affecting brucellosis persistence in both systems are not exactly the same. However, there are common characteristics about brucellosis biology. For instance, only a proportion of seropositive individuals are infectious (Roffè *et al.*, 1999; Lambert *et al.*, 2018b) and females play a predominant role in transmission (Ebinger *et al.*, 2011; Lambert *et al.*, in prep.). Therefore, these results indicate that higher levels of capture effort, or alternatively refinements of the strategy, may be required to achieve the removal of a higher number of seropositive infectious individuals in ibex. The outcome of past interventions in the population of study also supports this, because there is evidence that the massive test-and-remove operation conducted in 2015, with more than 100 captures, may have corresponded to the only period of clear decrease in seroprevalence (Calenge *et al.*, in prep.).

The addition of vaccination compared to test-and-remove alone was only slightly more efficient in reducing seroprevalence, all else being equal (14.4% [0-45.6] vs. 16.2% [0-48.1] – Figure 5-2A and Figure 5-3A). The outcomes in terms of brucellosis persistence are

however not obvious, and it does not appear from our results that vaccination helps much to obtain the fade-out of the infection. Although we used a 100% efficacy of the vaccine in our study, this result could be explained by a small coverage of vaccination in the population. Indeed, around 200 animals detected as seronegative were vaccinated in ten years when the objective was 30 captures each year (Table 5-2), but it represented only a very small proportion of the vaccination each year (only 30 animals, and only some of them can be vaccinated). Moreover, we assumed that protective immunity induced by the vaccine was not lifelong. Therefore, vaccinated individuals who lost their protection after some time returned to the susceptible state and could get the infection. Because vaccinated animals were marked and no recapture were implemented in our scenarios, these individuals who lost their protection could neither be vaccinated again or be removed if they got infected, which probably hampered the efficacy of vaccination. In bison in the GYA, the most effective vaccination strategy evaluated by modelling allowed for repeated vaccinations of animals, thus extending the duration of vaccine protection (Treanor *et al.*, 2010). Although the duration of vaccine protection may be longer than the 4.5 years that were used here, there are currently no data to validate or invalidate this in ibex. If the vaccine should be used in the population of study, it would therefore be recommended to recapture vaccinated individuals after some time to extend the duration of vaccine protection. A scenario where recaptures are allowed in our model could also be evaluated to confirm the additional benefit of this strategy. A previous work, based on a non-spatial individual-based model, roughly fitted to the data of 2014, showed a higher efficacy of TVR than TR with high number of capture each year (150-200 animals – ANSES, 2015; Thébault *et al.*, 2015). Results cannot be directly compared, because several years of management strategies occurred after 2014, and because the IBM analysed here was spatially structured and more precisely fitted with ABC. Nonetheless, we could expect a better efficacy of the TVR strategy if the number of capture could be increased.

Culling five unmarked individuals after the test-and-remove operation each year was not much more effective in reducing seroprevalence than test-and-remove alone, all else being equal (e.g., 12.6% [0-41.8] vs 13.4% [0-45.7] – Figure 5-4). However, culling 20 unmarked individuals appeared more interesting (e.g., 8.45% [0-40.2]), especially if the response of density-dependent parameters was delayed by 10 years (Figure 5-4). When the response was delayed by 5 years, the additional benefit of culling 20 unmarked individuals each year in addition to test-and-remove seemed less marked (e.g., 31% [0-59.3] vs. 32.1% [0-61.7] for

15 captures/year – Figure 5-4). This is probably because the number of individuals culled represented a lower proportion of the overall population when the response was delayed by 5 years, because the growth rate of the population rapidly increased in this situation. Indeed, for this scenario, the pre-breeding population size at the end of the simulations was 473 individuals [261-572] for a 5 years delay, against only 353 individuals [171-539] for a 10 years delay.

Interestingly, the combination of test-and-remove with the culling of 20 individuals each year gave very similar or even slightly better results in terms of seroprevalence and brucellosis persistence than the combination of test-and-remove with vaccination (e.g., 8.45% [0-40.2] vs. 9.7% [0-42.3] for 30 captures/year and a 10 years delay – Figure 5-4 and Figure 5-3B). This may be explained by the fact that culling should have more lasting effects on the number of susceptible individuals compared to vaccination. As explained above, vaccination only had a temporary effect in our model, whereas culling is by definition permanent.

Another interesting result is provided by the comparison between the objective level for capture. When combined with the culling of 5 or 20 individuals, test-and-remove with an objective level of 30 captures/year always performed better than with an objective of 15 captures/year (Figure 5-4). Conversely, because there was a competition between test-and-remove and culling for access to unmarked individuals, the number of culls was always lower with an objective level of 30 captures/year compared to 15 captures/year (Table 5-3). Therefore, in this situation, it seems that increasing the annual number of captures is relatively more interesting than increasing the annual number of culls.

However, this may not be true for higher objective levels of culling. In the last strategy, where the objective level was to cull 90% of unmarked individuals in the population, the scenarios with an objective level of 15 captures/year performed slightly better than those with 30 captures/year (Figure 5-4). In this particular case, most culls happened the first year, when the number of unmarked individuals in the population was high, and resulted in a sharp population decline (Figure 5-5). The following years, culling interventions maintained a strong pressure on the population, that decreased slowly as a result. The number of individuals culled was approximately the same between the scenarios with 15 or 30 captures/year (Table 5-3). Indeed, the massive culling on the first year removed most of unmarked individuals, leaving very few of them for capture in the subsequent years. Nonetheless, the number of captures, and therefore the number of individuals marked and

released, were approximately twice when the objective level was 30/year than when the objective was 15/year (Table 5-3). This is the reason why the scenarios with an objective level of 15 captures/year performed slightly better, because there were less marked individuals, and therefore less transmission in this compartment. On the contrary, brucellosis persisted more easily when the level was 30 captures/year, because there were more marked individuals that could get the infection, and remained unaffected by management interventions. Therefore, if such large scale interventions should be implemented, it would be strongly recommended to also implement recaptures with test-and-remove to eradicate the infection in marked animals.

The strategy where the objective level was to cull 90% of unmarked individuals in the population appeared to be the most effective scenario among those that we evaluated with our model, both in terms of seroprevalence and brucellosis persistence. However, despite these drastic interventions for a very long period, brucellosis remained persistent in more than half the simulations (Figure 5-4). In comparison to the severe consequences in terms of ibex demography (up to 500 ibex culled, and a population size half as much as the current situation), this result remains unsatisfying. Moreover, we must remain cautious as regards these results. First, we did not include any perturbation effect, i.e., an increase in transmission as a consequence of this strong population reduction. Such an increase could be the result of a disruption of social structures leading to increased numbers of contacts, or of a change in susceptibility of hosts due to stress (Prentice *et al.*, 2014). If population reductions led to such effects in the population of study, then this strategy would be less effective, or might even promote persistence (Prentice *et al.*, 2014). Second, in our scenario, the maintenance of culling over the duration of the study prevented any increase of the population due to density dependent reproduction. However, we would expect a sharp increase of the population when the culling interventions are stopped, which could result in strong increase in transmission and seroprevalence in the simulations where brucellosis persisted (Choisy and Rohani, 2006).

5.1.4.2. *Targeting individuals or areas*

Strategies that targeted the core area of the massif, where most transmission happened (Lambert *et al.*, in prep.), were more effective than their counterparts that were applied to the whole massif. For instance, seroprevalence was 13.4% [0-45.7] for test-and-remove, vs.

16.2% [0-48.1] when applied indistinctly in the population (Figure 5-2A and B). For similar levels of capture, more seropositive individuals were removed when the core area was specifically targeted (Table 5-1), which explains why it was more effective. This is also true for test-and-vaccinate-or-remove (Table 5-2). On the other hand, for this strategy, it also means that less (seronegative) individuals were vaccinated. However, the decrease that resulted from targeting the core area was more pronounced than for test-and-remove alone, especially when the objective was 30 captures/year: 9.7% [0-42.3] for TVR in the core area, vs. 14.4% [0-45.6] when applied to the whole population (Figure 5-3A and B). This can probably be explained by the fact that individuals in the spatial units of the periphery have a low risk of getting transmission, compared to individuals in the core area (Lambert *et al.*, in prep.). As a consequence, vaccination of individuals in the peripheral units make little or no difference in terms of transmission, and vaccinating only in the core area is therefore more beneficial, by protecting individuals where most transmission actually happens.

For TR, targeting females decreased the seroprevalence compared to untargeted interventions (13.6% [0-41.8] vs. 16.2% [0-48.1] – Figure 5-2A and C), despite the fact that the number of individuals removed was similar in both scenarios (Table 5-1). This confirms the prediction that removing females is more effective than removing both genders at random, because females are responsible for the majority of transmission in the system (Lambert *et al.*, in prep.). One possible limitation to this refinement is the amount of effort required, which is partially reflected by the smaller number of captures achieved when targeting only females (Table 5-1). Although this should be taken into account by managers, it should also be noted that field agents are more flexible than our model. For example, if females are not available during one capture interventions, agents could target males instead, which was not permitted in our model. Therefore, such a flexible strategy could result in higher numbers of capture and removals, and therefore even higher efficacy, than the results we provided here.

In bison, a selective test-and-remove focusing on pre-reproductive females was more effective compared to the same strategy in females regardless of age (Ebinger *et al.*, 2011). The rationale behind this result was that those females were removed before they could shed *Brucella* during their first pregnancy, and was more effective than removing females that already shed the bacteria in previous pregnancies (Ebinger *et al.*, 2011). Unfortunately, ibex under 2 years of age (the age of sexual maturity) are seldom captured in the population of study. However, females most often reproduce at later ages, and targeting younger females

could represent a relevant option for evaluation by our model in the future. A possible effect of TR is the reduction of herd immunity, by removing seropositive females that already recovered from the infection (Ebinger *et al.*, 2011). Another possible refinement could therefore be to specifically target infectious females, if there is a way to distinguish them from recovered individuals. For instance, actively infected females have higher complement fixation titres, which could be the basis for the development of a diagnostic test separating infectious from recovered individuals (Lambert *et al.*, 2018b). The interest of such a development could be theoretically evaluated in our model, by evaluating interventions that specifically target infectious rather than seropositive females.

For TVR, we evaluated the benefit of targeting individuals 2-5 years of age rather than all individuals regardless of age. Indeed, a high proportion of individuals from this age class are seronegative and at risk of getting the infection. This is reflected by the lower number of individuals removed for similar numbers of captures (Table 5-2). As a result, more seronegative individuals were captured and vaccinated, and it decreased the seroprevalence compared to untargeted TVR (12.6% [0-42.3] vs. 14.4% [0-45.6] – Figure 5-3A and C). However, it did not decrease seroprevalence as much as when TVR targeted the core area regardless of age (9.7% [0-42.3]). This is probably due to two factors: (i) the vaccination of individuals from the peripheral units, which as explained above is not very useful; and (ii) the lower number of seropositive individuals that were removed.

Finally, targeting both individuals and spatial units was always more effective than strategies targeting either one (Figure 5-2D and Figure 5-3D). For TR, targeting only females in the core area decreased the seroprevalence compared to individuals of all genders in the core area (10.7% [0-39.1] vs. 13.4% [0-45.7]). For TVR, seroprevalence decreased from 9.7% ([0-42.3]) when targeting the core area regardless of age to 8.5% ([0-36.5]) when targeting individuals 2-5 years old from the same area. We can however observe that the benefit of targeting young individuals in addition to the core area for TVR is a bit less interesting than the benefit of targeting females in addition to the core area for TR. It could be interesting in the future to evaluate TVR targeting at females of the core area, regardless of age.

5.1.5. CONCLUSION

Our results confirmed our predictions that targeting specific classes of individuals or specific areas that play a major role in transmission is more effective than untargeted management, and also that targeting both is more effective than targeting either one, which is an original contribution of our study. However, there is no silver bullet for the management of brucellosis in the ibex population of the Bargy massif, and all management strategies that we evaluated had limitations, with brucellosis fade-out not predicted to happen in a near future. Combination of tools appeared to be better than a single strategy. Scenarios removing less infected individuals but more susceptible individuals (either by culling or vaccination) were not as effective, which suggests that the primary strategy should be to remove as many infected individuals as possible. In the study population, this is achieved by targeting the central units of the population, that are transmission hotspots, and females, that play a predominant role in transmission compared to males. Vaccinating the seronegative individuals, or alternatively, culling at least 20 individuals each year, could represent valuable complementary interventions. Targeted management represents a valuable option for managing wildlife infectious diseases, and offers a wide range of possible refinements to classical sanitary measures. We therefore encourage to look for heterogeneity in other systems and to evaluate potential strategies for improving management in terms of efficiency but also acceptability.

Addendum

The results from this chapter confirmed our predictions that targeting specific classes of individuals or specific areas that play a major role in transmission is more effective than untargeted management, and also that targeting both is more effective than targeting either one, which is an original contribution of our study.

However, there is no silver bullet for the management of brucellosis in the ibex population of the Bargy massif, and all management strategies that we evaluated had limitations, with brucellosis fade-out not predicted to happen in a near future. Combination of tools appeared to be better than a single strategy. However, scenarios removing less infected individuals but more susceptible individuals (either by culling or vaccination) were not as effective, which suggests that the primary strategy should be to remove as many infected individuals as possible. This is consistent with the findings from chapter 4.

In chapter 4, the effectiveness of the lighter test-and-remove operations from 2016 to 2018 (less than 50 individuals captured each year), combined in 2017 and 2018 with the cull of 5 unmarked individuals, remained unclear. The results from chapter 5 seems to indicate that such light operations may indeed decrease brucellosis seroprevalence. However, there was no additional benefit of culling 5 unmarked individuals. Culling 20 unmarked individuals appeared more interesting, and could represent a valuable complementary intervention. Moreover, in this framework of feasible interventions in the field, the efficacy of test-and-remove combined to culling 20 unmarked individuals was very similar or even slightly better than test-and-vaccinate-or-remove.

Chapter 6

GENERAL DISCUSSION AND PERSPECTIVES



Personal picture

The aim of this thesis was to provide insights on the monitoring and management of infectious diseases in heterogeneous wild populations, using *Brucella melitensis* infection in the wild population of Alpine ibex of the Bargy Massif in the French Alps as a case study. Our work contributed to (i) a better understanding of how brucellosis is transmitted in this population; (ii) determining the effects of past management interventions on brucellosis transmission; and (iii) predicting the efficacy of possible management interventions that could be used in the future to eradicate brucellosis in ibex or at least mitigate the risk to livestock and humans by reducing the level of infection.

Throughout the thesis, several models were used to explore the temporal dynamics of brucellosis transmission and the evolution of seroprevalence in the population. In Chapter 3, we developed an individual-based, mechanistic model of brucellosis transmission in ibex, which represented the majority of the PhD work, and was also used in Chapter 5 to evaluate predicted management strategies. We also developed force of infection models and an integrated Bayesian model of the seroprevalence, that were used in Chapter 4 to assess the impact of past management strategies. These models made different assumptions, which I will discuss in the first part of this chapter, and I will compare the results obtained using each method. In the second part of this chapter, I will summarize and discuss the original results obtained in the PhD, and how it contributed to expand the understanding of the system. Finally, I will propose some remaining unanswered questions about the system of study, that could be addressed in the future, and also some larger perspectives about heterogeneity of transmission and management that aroused from this specific case of study.

6.1. COMPARISONS BETWEEN MODELS

6.1.1. TEMPORAL DYNAMICS OF PREVALENCE

The aim of Chapter 4.1 was to estimate seroprevalence in the population using a Bayesian model to correct for biased capture data that were mainly composed of unmarked animals (i.e., animals captured for the first time – Calenge *et al.*, in prep.). In Chapter 3.2, transmission parameters of the individual-based model were fitted using the same capture data, and these parameters allowed to predict the seroprevalence over time from 2012 to 2018 in the population (Lambert *et al.*, in prep.). Therefore, it was possible to compare the outputs of the Bayesian and the individual-based model and their interpretation. As a

reminder, Chapter 4.1 focused on females in the core area of the massif, and therefore the outputs of the individual-based model that we will present thereafter will represent the same compartment of the population to allow for a better comparison between the results.

In the Bayesian model, the overall seroprevalence on females in the core area of the massif was roughly constant from 2013 to 2015, followed by a sudden decrease between 2015 and 2016 and again a constant level in 2016-2018 (Figure 6-1A – Calenge *et al.*, in prep.). In the mechanistic model, the seroprevalence decreased more gradually from 2013 to 2015, and then was roughly constant in the last years (Figure 6-2A). This gradual decrease in seroprevalence between 2013 and 2015 was mainly due to the test-and-remove (TR) operations in spring 2014 and spring 2015. On the contrary, the massive culling operation of autumn 2013 had no effect on the seroprevalence (constant at the end of 2013 – Figure 6-2A), which is consistent with the findings of Marchand *et al.* (2017). The differences between the Bayesian and the individual-based model could be explained by an overestimation of the seroprevalence in females in the core area by the individual-based model in 2013 and an underestimation in 2015 (Figures B.9 and B.10 in Appendix 3.2B). This could have led to overestimate the effect of the TR operations in 2013 and 2014 by removing more seropositive individuals in the model than what was achieved in real life, and vice versa for the TR operation in 2015. Alternatively, the effect of TR operations could have been more gradual than estimated by the Bayesian model, but the differences may have been too small to be detected in the available capture data from 2013 to 2015.

The proportion of marked individuals among females in the core area followed a similar pattern in both models (Figure 6-1B and Figure 6-2B). The proportion increased from 2013 to 2016, and then stabilised around 40%. Therefore, it seems that censuses data provide accurate estimations of the proportion of marked individuals in the population.

Results for the proportion of active infection among seropositive females of the core area were more contrasted between the two models (Figure 6-1C and Figure 6-2C). In the Bayesian model, despite the uncertainty in estimation, this proportion seemed to increase between 2013 and 2014, and to decrease between 2017 and 2018 (Calenge *et al.*, in prep. – chapter 4.1). However, age-biases could be accounted for the increase between 2013 and 2014, and it is possible that the proportion of active infection was more constant in the early years (Calenge *et al.*, in prep. – chapter 4.1). In the individual-based model, this proportion appeared to be almost stable from 2013 to 2018 without the sharp decrease between 2017 and 2018, although uncertainty increased during the last years because of the uncertainty

around parameter estimation and stochasticity (Figure 6-2C). In the individual-based model, the transition of seropositive individuals from actively infected to non-actively infected was governed by the probability of recovery, which was estimated using the data on bacterial cultures of Lambert *et al.* (2018b) to fit the decrease of active infection with increasing age (Chapter 3.2 Appendix 3.2A). In the Bayesian model, the proportion of active infection among seropositive females was fitted using age, but also the titre of the complement fixation test (CFT – Chapter 4.1). Indeed, the proportion of active infection in females decreased with decreasing CFT titre (Lambert *et al.*, 2018b – Chapter 3.1). This was however not included in the individual-based model, because fitting the model to such data would have required to model the kinetics of antibodies in ibex. This would have added complexity and would have required more knowledge on antibody kinetics in ibex than currently available. This difference could account for the contrasted results obtained between the two models, especially the drop between 2017 and 2018 estimated in the Bayesian model. Indeed, only two seropositive females were captured in the core area in 2018, with low levels of CFT titre (Calenge *et al.*, in prep. – Chapter 4.1). This could have happened by chance because of the small sample size, rather than reflect an actual decrease in the proportion of active infection among seropositive females in the core area.

Finally, the proportion of actively infected among all females in the core area was the combination of the overall seroprevalence and of the proportion of actively infected among seropositive females. In the Bayesian model, it was roughly constant from 2013 to 2015, then dropped in 2016 because the seroprevalence dropped, and then dropped again in 2018 because the proportion of actively infected among seropositive females dropped (but we saw above that this should be considered with great caution because of the small sample size – Figure 6-1D). In the individual-based model, the overall proportion of actively infected females decreased more gradually in 2013-2015, and then was roughly constant in 2016-2018 (Figure 6-2D). Despite the differences we discussed above, the two models therefore agree on the reduction of the overall proportion of actively infected females in the core area.

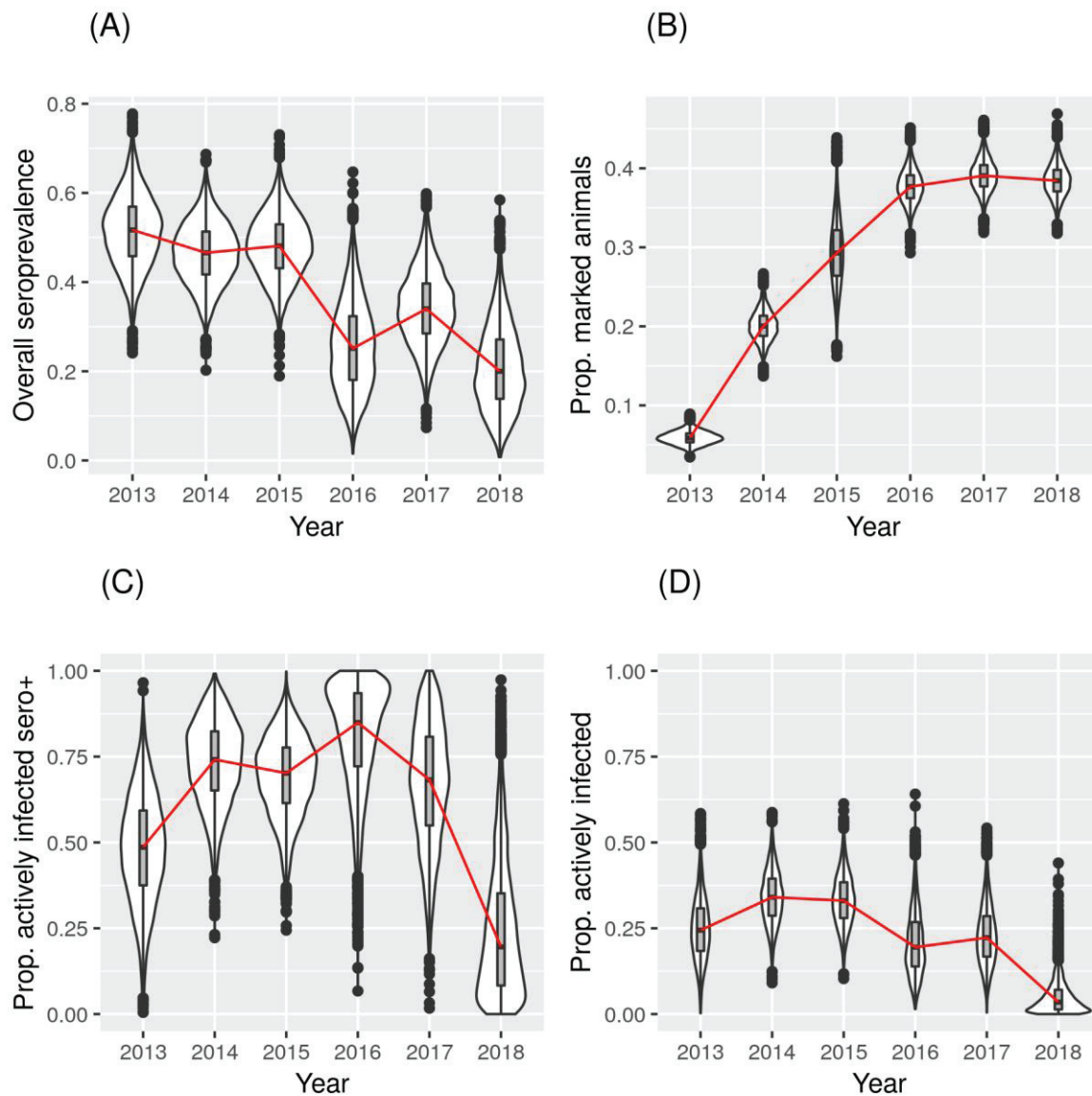


FIGURE 6-1 | Posterior distribution of the main parameters describing brucellosis in the population of female ibex of the core area of the Bargy massif, for each year between 2013 and 2018, in the Bayesian model.

For each parameter, the posterior distribution is displayed by both a boxplot and a violin plot (i.e. a kernel smoothing of the distribution rotated and put on both sides of the boxplot, mirroring each other). (A) Posterior distribution of the seroprevalence in the population of females of the core area of the massif. (B) Posterior distribution of the proportion of marked females among females of the core area of the massif. (C) Proportion of actively infected females among seropositive females in the core area of the massif. (D) Proportion of the females in the core area of the massif actively infected. This is a reproduction of Figure 4.1-3.

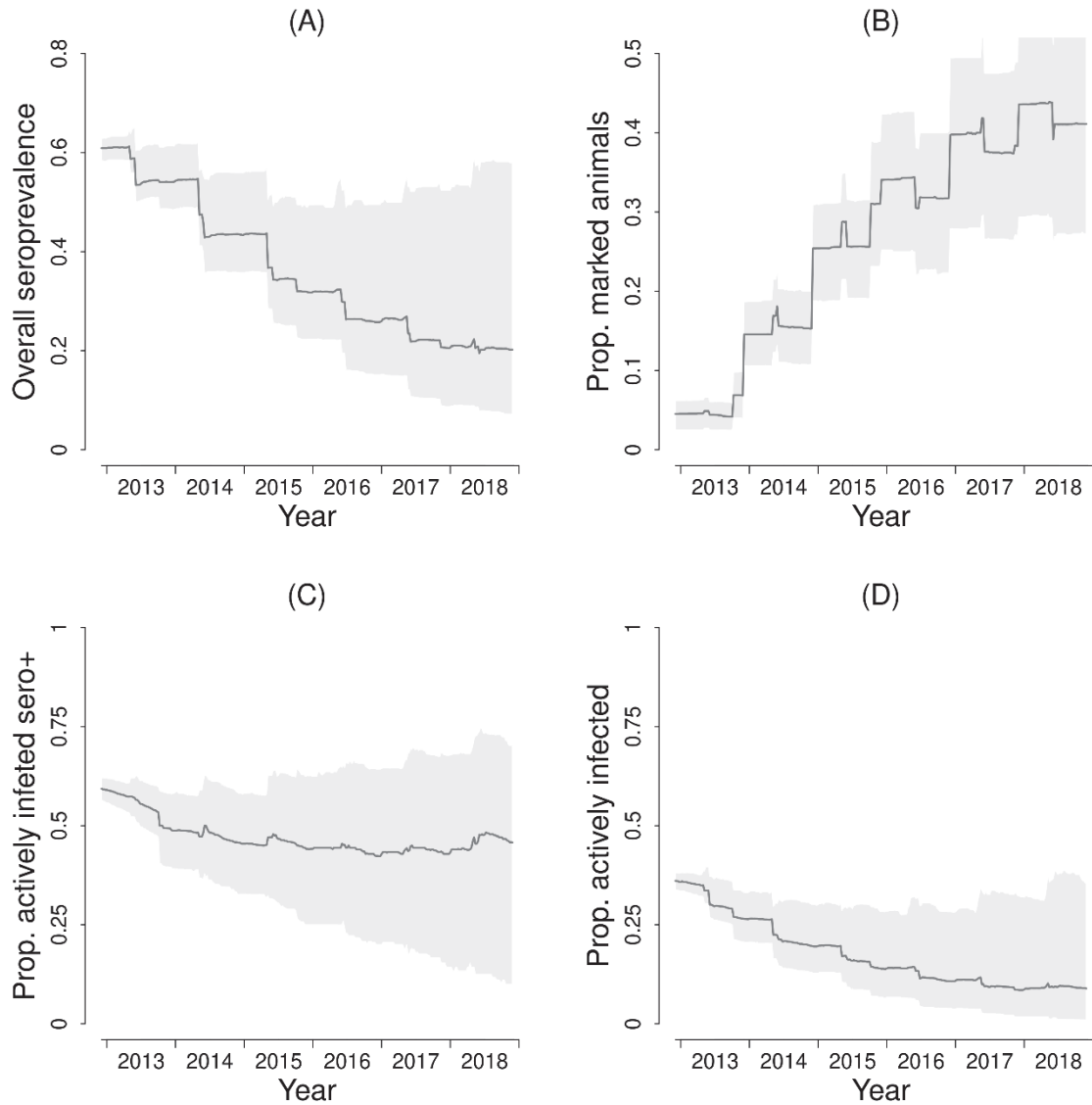


FIGURE 6-2 | Median and 95% credible interval of the outputs describing brucellosis in the population of female ibex of the core area of the Bargy massif, for each year between 2013 and 2018, in the individual-based model.

For each output, the median is displayed by a grey line and the 95% credible interval by the grey area. (A) Seroprevalence in the population of females of the core area of the massif. (B) Proportion of marked females among females of the core area of the massif. (C) Proportion of actively infected females among seropositive females in the core area of the massif. (D) Proportion of the females in the core area of the massif actively infected. This figure should be compared to Figure 6-1.

6.1.2. DYNAMICS OF THE FORCE OF INFECTION

In the Bayesian model, the seroprevalence in marked animals was estimated using a force of infection model (Calenge *et al.*, in prep. – Chapter 4.1). The force of infection is the rate at which susceptible individuals become infected per unit time (Chapter 2 section 2.2.2.1). We assumed in the Bayesian model that, for a given year, the force of infection for a susceptible marked female was proportional to the proportion of the population corresponding to unmarked females with an active infection. The estimates of the annual force of infection were then used to derive the proportion of marked females that were susceptibles at the beginning of the year, and became seropositive at the end of the year (Figure 6-3). Because the force of infection was by definition proportional to the proportion of actively infected females, their evolution matched closely (compare Figure 6-1D with Figure 6-3).

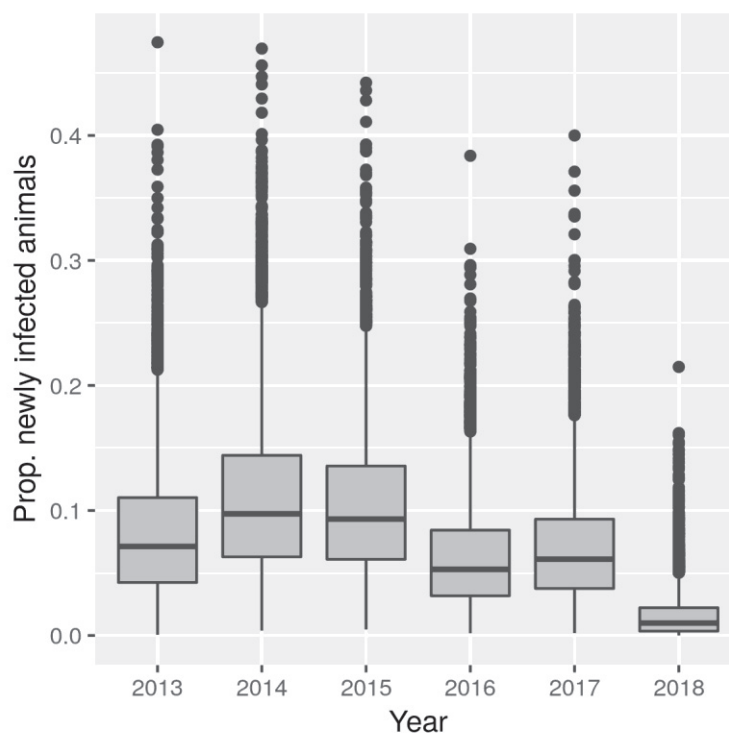


FIGURE 6-3 | Boxplot showing the posterior distribution of the force of infection by brucellosis for each year between 2013 and 2018. This force of infection is here represented as the proportion of marked females of the core area of the ibex population of the Bargy massif that are seronegative at the beginning of a year, and which become seropositive at the end of the year.

Thus, the limitations discussed in section 6.1.1 about the proportion of actively infected females also apply to the FOI, i.e., the increase between 2013 and 2014 could be due to age-biases and the FOI was probably more constant in the early years, and the decrease between 2017 and 2018 is driven by a small sample size ($n=2$) and should be considered with great caution. The FOI was therefore probably constant between 2013 and 2015, and then decreased in 2016 before stabilising again.

The same pattern was found for the force of infection for susceptible marked individuals in Chapter 4.2 using event time data and a survival model. Indeed, a model considering two periods with two different forces of infection, before and after 2015, improved the model fit compared to a constant force of infection during the study period (4.2). Although we were not able to look for more variations of the FOI in this framework, the decrease between 2015 and 2016 appears as a robust result. Moreover, it was also found when analysing current status data from unmarked individuals, because the FOI was lower for the cohort 2015-2017 than for the previous ones.

As for the seroprevalence and the prevalence of active infection (6.1.1), we could wonder whether the decrease of the FOI was really as sudden or whether it decreased more gradually as management interventions occurred one after another. Because the numbers of seropositive females captured and removed in the core area were very similar in 2014 ($n=33$, 19 removed) and 2015 ($n=26$, 17 removed – Table 4.1-1) while the population size was roughly constant (310 [275-352] in 2014 and 277 [220-351] in 2015), there was no obvious differences that could have explained why test-and-remove would cause a sudden drop in FOI after 2015 and not after 2014, except that the TR operation from spring 2015 was combined with a culling operation of unmarked individuals in autumn 2015.

The evolution of the FOI following interventions such as culling or test-and-remove depends on how transmission relates to the size of the population. As a reminder, the FOI can be viewed as the product:

$$\text{FOI} = cv \frac{I}{N}$$

where (i) c is the contact rate, i.e., the number of individuals that each (susceptible) individual contacts per unit time, where contacts are of an appropriate type for transmission to be possible if one of the hosts is infectious, (ii) v is the probability of successful transmission, the probability that an appropriate contact between an infectious and a susceptible host does in fact lead to transmission, and (iii) I/N is the probability that the

contact is indeed with an infectious host (Begon *et al.*, 2002). If the contact rate is independent of the population size N ($c = c^{te}$), then the FOI is proportional to I/N and transmission is said to be frequency-dependent (FD) thus $FOI = \beta \frac{I}{N}$ where $\beta = cv$. On the other hand, density-dependent (DD) transmission corresponds to the case where the contact rate is proportional to N ($c = \kappa N$) and therefore the FOI is proportional to I : $FOI = \beta I$ where $\beta = \frac{cv}{N} = \kappa v$.

Although transmission of brucellosis probably follows a pattern between those two extremes (see 6.1.3), FD versus DD transmission creates a theoretical context that can help us understand the effects of management interventions on the FOI. In the case of culling, both I and N would be similarly reduced, which should conduct to a decrease in the FOI in the case of DD transmission and to a constant FOI for FD transmission. For test-and-remove, N could be considered as a constant and only I would be reduced, leading to a decrease in the FOI in both cases.

The apparent absence of effect of the massive culling operation of 2013 on the seroprevalence, prevalence of active infection and force of infection is an argument in favour of a contact rate closer to the FD than the DD. However, even if this transmission was completely frequency-dependent, it would not explain the apparent differences between the TR operations of 2014 and of 2015. Instead of directly depending on the population size N , transmission of brucellosis in ibex could be related to other measures such as density or local group size (Cross *et al.*, 2013), and our results call for further studies to better understand the patterns of contact in this species in relation to brucellosis (see 6.1.3).

Another possible explanation for the difference between the TR operations of 2014 and of 2015 is the timing of intervention. The transmission of brucellosis is highly seasonal (Chapter 3.2) and the timing of intervention could therefore have great implications on their efficacy. The distribution of the capture dates was very similar in 2013, 2014 and 2015 (not shown). However, in 2013 and 2014, the rapid serological test was not yet validated and all individuals were released during capture after blood samples were taken. Therefore, seropositive individuals had to be tracked down and shot after laboratory testing, which necessarily delayed the removal of those individuals by several weeks. Because capture occurred at the period of abortion and birth, they could shed the bacteria in genital fluids during the interval between capture and removal. In contrast, in 2015, seropositive individuals were euthanized directly after the result of the rapid test during capture, which

could explain a higher level of efficacy. These differences were not implemented in our individual-based model, but the effects of timing on the efficacy of interventions could be tested with this model in the future.

6.1.3.FREQUENCY- OR DENSITY-DEPENDENT CONTACTS?

The form of the contact rates depends on the transmission routes of the infection and the ecology and behaviour of the host species. Frequency- and density-dependent models of transmission may not represent the reality or may depend on the scale considered, and intermediate forms of contact rates between these two extremes may exist (McCallum *et al.*, 2001). For brucellosis in ibex, it is probable that the form of transmission may lie between FD and DD transmission, and that the form varies according to the transmission route considered.

For the venereal transmission, contacts depend on the mating system of the host and not on density, and it is how it was represented in the individual-based model. This is typically a case of FD transmission (McCallum *et al.*, 2001). However, for horizontal transmission following contact with *Brucella* shed in genital fluids, the form of transmission is probably more complex. In the individual-based model, this transmission was not density-dependent *stricto sensu*, because it was not dependent on the whole population size. Instead, it depended on the size of the socio-spatial unit for infectious abortion events, and on the size of the nurseries gathering mothers and kids inside each socio-spatial unit for infectious birth events. This explains why in the model both culling and test-and-remove operations resulted in a decrease in incidence to reach a minimum in 2016 (Figure 3.2-4B). It also explains the increase in incidence that followed in 2017 and 2018, because the population size started to slowly increase again in some simulations as the pressure of management interventions decreased (Figure 3.2-3A).

One of the major limitation of this representation is that it does not account for the similar seroprevalence observed in socio-spatial units 3 and 4, that had the highest levels in the population when the outbreak was discovered (Marchand *et al.*, 2017). Indeed, according to our estimation of the initial population size in 2012 (Appendix 3.2A), the population size of unit 3 was around 6 times higher than all other units, including unit 4. Therefore, it should have conducted to higher seroprevalence in the socio-spatial unit 3. Transmission could depend on density instead of numbers, but in our case this does not account either for the

similar seroprevalence in unit 3 and 4, because the area occupied by unit 3 is also the largest (P. Marchand, unpubl. data).

Other forms of contact rates happening at smaller scales could therefore be involved, that limit transmission even in socio-spatial units with large sizes. In the individual-based model, we considered that all individuals in the same socio-spatial unit mixed homogeneously (to the exception of males that had smaller contact rates because of sexual segregation). Indeed, ibex form groups inside spatial units, but they experience very loose bonds (Villaret and Bon, 1998) and groups are not stable and split and merge rapidly (fission-fusion dynamics; C. Calenge, pers. comm.). This result was based on repeated visual observations between April and June, but it is possible that at smaller temporal scales, such as the week or the duration of *Brucellosis* shedding in genital fluids (*ca.* three weeks), only individuals in the same group at that time may be exposed. In elk from the Greater Yellowstone Area (GYA) infected with *Brucella abortus*, female contact rates were neither frequency- nor density-dependent, but instead were positively correlated to the group size (Cross *et al.*, 2013). It is also possible that only a fraction of ibex in the same group would be exposed to infectious events (abortions or births), as it was demonstrated for bison in the GYA (Treanor *et al.*, 2010; Ebinger *et al.*, 2011). It could be interesting to collect more data on ibex contact rates around abortion or birth, but this would be difficult because of the meteorological conditions at the time of abortion (snow cover) and because of ibex behaviour around birth, that tend to isolate themselves in cliffs. Alternatively, several model formulations could be tested and evaluated against available data.

In our individual-based model, we therefore represented contact rates to the best of current knowledge. Our main results should however be robust to changes in the form of contact rates. Indeed, even if transmission following exposure to infectious abortions or births is proportional to smaller sizes than the size of the socio-spatial unit, it would still represent higher transmission rates than the venereal transmission. Therefore, the predominant role of females would still be maintained. It would also lead to fewer differences between the socio-spatial unit 3 and the other socio-spatial units from the core area (4 and 5), but the core area would still represent a transmission hotspot because of the larger number and proportion of actively infected individuals. Finally, it would reduce even further the efficacy of culling, while having low or no impact on test-and-remove, which goes on the same direction as our conclusions in Chapter 5. Moreover, in our predictive scenarios, the increase of the population growth rate led to increasing sizes inside each socio-

spatial unit, which in turn led to increasing transmission rates. If transmission was for example proportional to group size instead of unit size, then transmission rates would increase slower and therefore the model would predict higher probabilities of *Brucella* extinction. Beside being in accordance with the best available knowledge, using the form of density-dependence that we chose in our model was conservative concerning management, because we placed ourselves in the “worst case” scenario, where the efficacy of the predictive scenarios (including without management) could not be better in the model than it would be in reality.

6.2. HOW TO MANAGE BRUCELLOSIS?

6.2.1. BRUCELLOSIS IN IBEX AND HETEROGENEITY

Because the Alpine ibex population of the Bargy Massif was the very first case of self-sustained infection with *B. melitensis* in this species, our knowledge of *Brucella* infection and pathogenesis was scant in ibex when the outbreak was discovered. Gaining knowledge on brucellosis transmission was therefore a necessary first step towards the development of effective management strategies.

Using data on the distribution of *Brucella* from bacterial cultures in seropositive individuals, we found evidence that the two main transmission routes for brucellosis in other ruminant species may also exist in ibex (Chapter 3.1). Transmission following exposure to *Brucella* shed in genital fluids is the main transmission route in other wild and domestic ruminant species (Thorne *et al.*, 1978; European Commission, 2001; Rhyan *et al.*, 2009; Diaz-Aparicio, 2013; Rhyan, 2013). The quantification of this transmission route in the individual-based model allowed us to determine that it was also the case for brucellosis in ibex (Chapter 3.2). More controversial is the role of venereal transmission: this transmission route is possible in domestic ruminants, but is not believed to be a major mode of transmission (King, 1940; Thomsen, 1943; FAO and WHO, 1986). In bison from the GYA, further studies are required to determine if venereal transmission is possible (Frey *et al.*, 2013; Uhrig *et al.*, 2013). In our individual-based model (Chapter 3.2, we assumed that this transmission route existed in ibex, which was supported by the results of bacterial cultures (Chapter 3.1). The posterior distribution of the probability of venereal transmission was narrower than its prior distribution, which indicates that the available data was informative

for this parameter, although uncertainty remained high. In the model, this transmission route was needed to explain the spatial spread of brucellosis in the population because we made the assumption that only males move between units (Chapter 3.2). Nonetheless, venereal transmission was quantitatively less important than all other transmission routes (less than 20% of new cases were due to venereal transmission), especially when compared to transmission following *Brucella* shedding in genital fluids, as observed in other species. Our results suggest that venereal transmission should not be discarded in wildlife populations, because it could play a qualitatively essential role on the spatial spread of the infection.

As a consequence of these findings on transmission routes, we found that females are the most frequent source of brucellosis infection in ibex (Chapter 3.2). This result challenges the view that males play a critical role in diseases transmission in mammals synthesised by Skorping and Jensen (2004), but is in accordance with brucellosis dynamics in other species of domestic and wild ruminants (European Commission, 2001; Rhyan, 2013). This highlights the critical need to determine the existence of sex-related differences in transmission routes, in addition to differences in behaviour and susceptibility, to explain heterogeneity of transmission between males and females. In the case study, this marked difference between males and females was not clearly reflected on the data on seroprevalence, although the existence of a spatial heterogeneity suggested that transmission by males played a less important role (Marchand *et al.*, 2017). This is the reason why seroprevalence studies are not sufficient in wildlife disease systems, and should be completed by studies of within-individual pathogen distribution when possible (González-Barrio *et al.*, 2015; Lambert *et al.*, 2018b).

In addition to the quantification of the predominant role of females, the individual-based model allowed to determine the contribution of each socio-spatial unit to brucellosis transmission (Chapter 3.2). We revealed a strong heterogeneity at the spatial level, with the three units of the core area of the population acting as major transmission hotspots.

By integrating of all available data on the individual-based model, we demonstrated heterogeneity of transmission between units, between genders and between infectious individuals. The study system follows the '20-80' pattern, where 20% of infectious individuals contribute to the majority of transmission (Figure 3.2-6). Because only a minority of individuals are responsible for new cases of infection, management should probably target this minority to be effective (Woolhouse *et al.*, 1997; Lloyd-Smith *et al.*, 2005b; Matthews *et al.*, 2006).

6.2.2. EFFICACY OF MANAGEMENT STRATEGIES

Since the discovery of the outbreak, disease management was implemented in the population of Alpine ibex. In particular, the population was subject to a massive culling operation at the end of 2013, during which about one third of the animals were removed. However, the available data did not support any variation in brucellosis seroprevalence between the year before or the two years after this culling operation (Marchand *et al.*, 2017). The results of the analysis that were conducted in Chapter 4 did not bring further support to a detectable efficacy of the mass culling operation of autumn 2013 alone.

As a prerequisite to the evaluation of predicted management strategies, we were also interested in the effects of the other disease management methods implemented in the population, especially test-and-remove during captures, sometimes associated with culling of unmarked individuals. The two studies in Chapter 4 suggest that both the seroprevalence and the force of infection decreased in the population after 2015. This result is in favour of an efficacy of the massive test-and-remove operations conducted in 2015, after the massive culling operation of 2013. Results from the individual-based model provided additional support to this conclusion, although there were some discrepancies between the different studies on the efficacy of test-and-remove operations conducted the first two years (see 6.1).

Based on these results, we developed several management scenarios to be evaluated by the individual-based model (Chapter 5). Given the results of past management interventions, these scenarios were all based on test-and-remove. This strategy was refined by: (i) targeting specific individuals or socio-spatial units based on the new knowledge on transmission heterogeneity; (ii) adding vaccination, which is extensively used for brucellosis eradication schemes in domestic ruminants (Blasco, 2010) and could represent a valid option in wildlife; and (iii) adding culling of unmarked individuals, which was already applied in the population in the last years, and could help further reduce the number of infected individuals; moreover, the massive test-and-remove operation of 2015 was followed by culling of 70 unmarked individuals in autumn 2015, which could have contributed to increasing the efficacy of the intervention (Calenge *et al.*, in prep. – Chapter 4.1).

The results of our simulations indicated that:

- Test-and-remove is more effective than doing nothing, and effectiveness increases with the number of captures performed;

- Adding vaccination or even more so culling of unmarked individuals to test-and-remove can increase efficacy.

Moreover, we demonstrated that targeting females for test-and-remove is more effective than capturing both genders because of the predominant role of females in transmission. Therefore, targeted strategies based on individual heterogeneity were more effective, which was already demonstrated for infections in humans and animals (e.g., Woolhouse *et al.*, 1997; Lloyd-Smith *et al.*, 2005b; Matthews *et al.*, 2006; Fenichel and Horan, 2007). Previous studies also suggested that disease management targeted towards a subset of units in a metapopulation, based on their position in the configuration of the metapopulation and their contribution to transmission, could be more effective (Hess, 1996; Fulford *et al.*, 2002; Haydon *et al.*, 2006). It was indeed the case here, where focusing efforts on transmission hotspots, the core area in the case study, was more effective than population-wide interventions. Finally, and perhaps most importantly, we proved that targeting both females and the core area was more effective than either one separately, which was an original development arising from the characteristics of our case study, that could be of interest for other infections in wildlife populations.

The management of brucellosis in the ibex population of the Bargy massif should primarily aim at removing as many seropositive as possible through test-and-remove operations, which would be better achieved by targeting the central units of the population and females. Vaccinating the seronegative individuals, or alternatively, culling at least 20 unmarked individuals each year, could represent valuable complementary interventions.

However, the efficacy of vaccination was not as strong as culling when both measures were considered in the framework of feasible interventions in the field. This included relatively low number of captures (15-30) due to the difficulty of approaching animals, and number of animals culled compatible with the numbers that were allowed recently in this population. In this scheme, vaccination did not increase the probability of success of the management by much.

Moreover, the use of vaccination could make the monitoring of the population even more complex or costly than it already is. Indeed, the vaccine induces long-lasting serological responses in vaccinated animals, which cannot be distinguished from naturally-infected animals (except at necropsy with brucellosis identification and/or genotyping of strain). In domestic animals, this difficulty can be circumvented by vaccinating only animals 3-4

months of age, which develop only a transient serological response (Fensterbank *et al.*, 1985, 1987). However, in ibex, individuals under 2 years are only seldom captured which prevents from using a similar approach. Using specific marking of vaccinated animals could represent another solution. However, in the eventuality that the vaccine would not confer a complete protection against infection, it would still be impossible to distinguish uninfected from infected individuals on the basis of serological tests among those that received the vaccine.

Even more detrimental for monitoring, the possibility that vaccinated individuals shed the vaccine strain and transmit it to other individuals cannot be ruled out (Zundel *et al.*, 1992; Ponsart *et al.*, 2017; Ponsart *et al.*, in prep.). Such phenomenon would make the interpretation of seroprevalence results more difficult even among unmarked-unvaccinated individuals (ANSES, 2019). Finally, in agreement with recent report (ANSES, 2019), we conclude that combining test-and-remove with culling of unmarked individuals should be preferred to test-and-vaccinate-or-remove.

6.3. PERSPECTIVES FOR FUTURE RESEARCH

6.3.1. OPEN QUESTIONS ABOUT IBEX BRUCELLOSIS

The situation of the Alpine ibex population of the Bargy Massif is unique, because it constitutes the first reported case of persistence of *B. melitensis* in this species, and in European wild ungulates in general. Previous cases of *B. melitensis* in chamois and Alpine ibex were localized events and outbreaks spontaneously faded out (Garin-Bastuji *et al.*, 1990; Ferroglio *et al.*, 1998; Hars and Garin-Bastuji, 2013). In the Bargy Massif area, no other individual from other species was found infected among 129 tested, to the exception of one chamois (Garin-Bastuji *et al.*, 2014).

Differences in susceptibility to infection between species, as observed for example for elk and bison in the GYA (Thorne *et al.*, 1978; Olsen and Johnson, 2011), could explain that the high levels of infection found in ibex would not be found in other wild ruminant species. Although we did not explore ibex susceptibility to brucellosis in the thesis, we demonstrated in our first study a high shedding potential of *B. melitensis* in ibex and a higher proportion of active infection among seropositive individuals than in domestic ruminants (Lambert *et*

al., 2018b). This result could be explained by a higher susceptibility of ibex to brucellosis compared to domestic ruminants, which was also suggested by the results of a study comparing post-vaccination responses in Alpine ibex and in domestic goats (Ponsart *et al.*, in prep.). In the future, experimental studies aiming at determining the relative susceptibility of ibex as compared to domestic species and other wild species such as Alpine chamois would be interesting. Other factors could explain the absence of brucellosis in other ruminant species in the same area, such as differences in contact rates that make within- or between-species transmission more difficult than it is in ibex.

Why brucellosis was maintained in this specific population and not in others is another question that was not addressed in this thesis. Specific genetic or immune characteristics of this population compared to others, that would account for a higher susceptibility to infection, could offer a valuable explanation. A first analysis of the genetic structure of the Bargy population suggested that, although this population shows a low neutral genetic diversity, its level of variability lies in the range of previously studied populations. In contrast, immunity-related genes have maintained some polymorphism and may have contributed to transmission heterogeneity in this population (Quéméré *et al.*, in prep.). The success of invasion by an infectious agent in a population is also determined by other factors, such as population density or stochastic fluctuations (Lloyd-Smith *et al.*, 2005a), which could explain why brucellosis successfully invaded the Bargy massif population and not other areas. Further research, simulating various hypotheses for disease emergence for example, could provide valuable insights on the determinants of brucellosis invasion in Alpine ibex populations.

In our individual-based model, the simulations started after the discovery of the outbreak, because no data on the population was available beforehand. In particular, the date of the introduction of brucellosis in the population was unknown, although it most probably started before 1999 (last observation of brucellosis in local domestic cattle and sheep herds). It could have been possible to estimate this date by testing several hypotheses with the model (e.g., Beaunée *et al.*, 2015), but it would also have required data on population dynamics that were only partially known. In particular, a detailed understanding of the evolution of the population structure while the population was expanding would be necessary to retrace the colonisation of the various socio-spatial units, because it may have major consequences on the success and timing of brucellosis invasion in each socio-spatial unit. For the same reasons as the introduction date, we were not able to determine with our model the factors that gave

rise to the spatial differences in seroprevalence that were observed in the early years of the outbreak (Marchand *et al.*, 2017). Higher seroprevalences could either be the result of higher transmission rates, or simply the sign that the infection was introduced sooner (Heisey *et al.*, 2010). Each hypothesis could have been evaluated in the model if we had been able to model the population before 2012.

Alternatively, the date of brucellosis introduction in ibex could be inferred from genomic data on the bacteria, which could also provide insights on the frequency and timing of interspecies transmission and of the spatial spread of the bacteria in the massif (e.g., Kamath *et al.*, 2016). This would be a promising approach to develop in the future, that may allow (i) to determine when brucellosis was introduced in ibex compared to its eradication in the domestic population; (ii) to identify the location and the domestic species that were responsible for the spill-over, which could provide further understanding on the risk associated with inter-species transmission and help prevent spillback from ibex to domestic ruminants; and (iii) to estimate the date of introduction of brucellosis in each socio-spatial unit, which could bring valuable insights on the origins of the spatial heterogeneity. However, it would require the whole-genome sequencing of many *Brucella* isolates from both domestic and wildlife species.

Several other aspects were not studied here. The impact of heterogeneity of immune genes was not included in our model, although a recent study found significant associations between the polymorphism of some immunity-related genes such as *Slc11A1* and *Brucella* infection status (Quéméré *et al.*, in prep.). The risk of transmission to other ibex populations (like the population of the adjacent area of the Aravis massif) was not estimated here, nor the risk of spreading to domestic animals and to humans. These elements could represent future developments of the model we developed in this thesis.

Finally, the model could also be used to guide the monitoring of the population and the assessment of the efficacy of management strategies in the field. Indeed, we could simulate several levels of sampling (e.g., captures) to evaluate how many animals would be needed to detect variations of infection parameters (e.g., seroprevalence or force of infection) in the population. This could help provide relevant monitoring protocols to detect the moment when the epidemiological situation has improved enough to stop, lighten or change management options.

6.3.2. INVASION, PERSISTENCE AND HETEROGENEITY

One of the main characteristics of our case study was the simultaneous existence of spatial and individual heterogeneity. Such heterogeneities had necessary repercussions on the persistence of brucellosis in the population, but we could not assess their relative impact. Because of the repercussions on disease management, understanding the effects of several levels of heterogeneity on the invasion and persistence of infections in a more general framework could be very useful.

Lloyd-Smith *et al.* (2005b) demonstrated that accounting for individual heterogeneity decreased the success of invasion of infectious agents. This study did not assess the impact of individual heterogeneity on persistence, but showed that heterogeneity increased the growth rate of outbreaks when those occurred (Lloyd-Smith *et al.*, 2005b), which should decrease the time to extinction owing to a quick depletion of susceptible individuals (epidemic fade-out – Lloyd-Smith *et al.*, 2005a). Similarly, because individual heterogeneity increases the variability of the dynamics (McCaig *et al.*, 2011), stochastic variations in the number of infected individuals around the equilibrium in endemic situations should decrease the time to extinction, especially if this number is low (endemic fade-out – Lloyd-Smith *et al.*, 2005a).

Accounting for spatial heterogeneity in a metapopulation framework can lead to complex behaviours in relation to infection invasion and persistence, depending on how subpopulations are connected to each other. If the subpopulations are not connected to each other, then the probability that the infection goes extinct in the metapopulation is much higher than in a homogeneous population (Keeling and Rohani, 2008). When they are connected, the infectious agent can reinvade a subpopulation from which it faded out (a process termed “rescue event”), which increases the persistence in the overall metapopulation (Keeling and Rohani, 2008). These rescue events are more probable as the connection (e.g., movement rates of animals) between subpopulation increases. However, it also requires that the subpopulations are not all in the same state at the same time (i.e., there are infected and susceptible subpopulations), otherwise reinvansion cannot occur. This is possible only when the level of connection between subpopulation is relatively low, because at high levels of connection the infection spreads almost simultaneously in all subpopulations (synchrony) and the metapopulation can be considered as homogeneous (Keeling and Rohani, 2008). Therefore, persistence can peak at intermediate levels of

connection that favours both rescue events and asynchrony between subpopulations (e.g., Swinton *et al.*, 1998; Jesse *et al.*, 2008).

To our knowledge, the consequences of including individual heterogeneity (superspreading) into a metapopulation framework has not yet been studied. As a purely theoretical prediction, we could expect two effects of accounting for individual heterogeneity in a metapopulation. First, it could decrease the probability of invasion, because there is a greater chance that infected individuals that move to a susceptible subpopulation will not transmit the disease because they have a low expected number of secondary cases. Therefore, increasing connection would be necessary to increase persistence by increasing the probability that a superspreader would move to a susceptible subpopulation. On the contrary, individual heterogeneity could also increase asynchrony. Indeed, the infection is expected to spread faster in each subpopulation once invasion succeeded, because superspreaders would cause explosive outbreaks (Lloyd-Smith *et al.*, 2005b). This would therefore leave more time to the subpopulation to become susceptible again by population turnover, and increase the chances of rescue events. Overall, we would expect that persistence would also peak at intermediate levels of connection, but this peak could occur at higher levels than in a metapopulation without individual heterogeneity. Testing these simple predictions in a theoretical model would be an interesting development, and we cannot exclude that unanticipated dynamics could emerge from this interaction between individual and spatial heterogeneity.

References



Personal picture

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Appendices



Personal picture

Appendix 1: L'apport de la modélisation dans la compréhension du système brucellose-bouquetin du Bargy

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RESUME

La modélisation dynamique est un outil complémentaire aux études d'observation et d'expérimentation sur les systèmes complexes, tels que celui de la brucellose des bouquetins du Barga. Dans ce cas, la première étape de la modélisation était de représenter le système et de faire le point sur les connaissances actuelles. Cette étape permet déjà de fournir des conclusions qualitatives sur les mécanismes clés et leur comportement. La deuxième étape est analytique : il s'agit d'identifier les paramètres les plus influents et de les estimer, afin d'identifier les processus les plus importants. Ici, les paramètres liés aux voies de transmission majeures (avortement, vénérien) et la capacité d'accueil du milieu, qui influence la dynamique de population, semblent être les facteurs clés dans la transmission de la brucellose. Enfin, la dernière étape est de prédire l'effet de différents scénarios de gestion du foyer sauvage. Evaluer l'efficacité de plusieurs scénarios de lutte est rendu possible par le biais de simulations, ce qui est pratiquement impossible à mettre en place sur le terrain. La modélisation est alimentée par les données de terrain et d'expérimentation, et permet de prédire la dynamique du système dans des situations qui ne se sont pas ou pas encore produites, et ainsi de conduire à de nouvelles questions de recherche en retour. Malgré sa complexité et le fait que le système n'ait jamais été décrit auparavant, l'état des connaissances sur l'écologie du bouquetin et sur la pathogénie et l'épidémiologie de la brucellose chez les ruminants domestiques a permis d'avoir une représentation fine du système et de mieux comprendre les possibles interactions entre l'hôte et l'agent pathogène dans la dynamique épidémiologique.

Mots-clés : modèle épidémiologique, transmission, gestion sanitaire

ABSTRACT

Dynamic modelling is a complementary tool to observational and experimental data on complex systems, such as *Brucella melitensis* infection in Alpine ibex in the Bargy Massif (French Alps). In such a case, the first step of the modelling approach was to formally represent the system and summarize the available knowledge. This step can already provide valuable insights and qualitative conclusions on key mechanisms of the system. The second step is analytical. The aim is to identify key parameters and to estimate them, so as to reduce uncertainty and assess the most important processes. Here, parameters related to major transmission routes of brucellosis (abortion and venereal) as well as the capacity of the environment to affect the Ibex population dynamics, appear to be key factors in brucellosis transmission. Finally, the last step consists in predicting the effects of wild outbreak management scenarios. Numerical simulations allow comparing several scenarios, which is not feasible in field or laboratory experiments. An important aspect of the modelling approach is that field data and biological knowledge are both used to formulate model assumptions and to estimate parameters. In return, model results can predict dynamics of future situations, and therefore lead to new research questions and can help to prioritize future experimental or field research. Despite the complexity of the system, available knowledge on ibex ecology and on brucellosis epidemiology of domestic ruminants allowed us to accurately model this unique situation and to better understand the possible interactions between the host and the pathogen.

Keywords: epidemiological model, transmission, disease management

I – INTRODUCTION

La gestion des maladies de la faune sauvage transmissibles à la faune domestique et/ou à l'Homme diffère de manière significative de celle dans la faune domestique. En effet, les animaux sauvages n'évoluent pas dans un environnement contrôlé comme en élevage, et il est par conséquent à la fois plus difficile de mettre en place les mesures et d'en prévoir les effets sur l'espèce cible comme sur son environnement (Artois *et al.*, 2001). Les populations sauvages ont une dynamique de population et une structuration sociale et spatiale complexes, qui peuvent influencer la propagation d'une maladie mais aussi interagir avec les mesures de gestion mises en place (Choisy and Rohani, 2006; Prentice *et al.*, 2014). Par exemple, une réduction d'effectif peut conduire à une surcompensation de la fécondité, à une augmentation des naissances et donc à un afflux d'individus sensibles, qui peuvent au final se traduire par une augmentation de la transmission de la maladie (Choisy and Rohani, 2006). Les mesures de gestion peuvent aussi interagir avec la dynamique de transmission, par exemple en modifiant les taux de contacts entre les individus, ce qui peut dans certains cas conduire à un effet de perturbation et à une augmentation de la transmission (Prentice *et al.*, 2014). L'intérêt porté à la gestion de ces maladies est relativement récent comparé à la gestion des maladies chez l'Homme ou dans la faune domestique (Wobeser, 2002), et peu de mesures ont démontré leur efficacité dans les populations sauvages (Wobeser, 2007; Artois *et al.*, 2011). L'absence de mesures efficaces peut d'ailleurs conduire à la décision de ne rien faire, qui est un choix fréquent dans la gestion des maladies de la faune sauvage (Gortázar *et al.*, 2015).

Pour cette raison, évaluer l'efficacité des mesures de gestion et leurs effets à la fois sur l'agent pathogène et sur la population d'hôtes est primordial. Dans ce but, la modélisation peut permettre de déterminer les mesures ou combinaisons de mesures qui ont les meilleures chances de fonctionner, en comparant divers scénarios et leurs effets (Smith *et al.*, 2009), ce qui est pratiquement impossible à mettre en place sur le terrain. Elle permet aussi d'optimiser les stratégies, par exemple en ciblant plus efficacement les individus qui font l'objet des mesures (Keeling and Rohani, 2008). Il s'agit donc d'un outil d'aide à la décision pertinent, en complément de l'évaluation d'autres aspects tels que la faisabilité des mesures, le rapport coût/bénéfice ou encore l'acceptabilité des mesures par les acteurs impliqués.

C'est dans cette optique que la modélisation a été utilisée dans le cas de la brucellose du bouquetin des Alpes (*Capra ibex*) dans le massif du Bargy en Haute-Savoie (ANSES, 2015).

Ce foyer a été identifié à partir d'un cas humain dans la commune du Grand Bornand en 2012, qui a conduit à l'identification d'un élevage bovin infecté dans la même commune (Mailles *et al.*, 2012). L'origine du foyer étant restée inconnue malgré les enquêtes épidémiologiques, une surveillance a été mise en place dans la faune sauvage qui a conduit à l'identification de séroprévalences élevées dans la population locale de bouquetins (Hars *et al.*, 2013; Garin-Bastuji *et al.*, 2014). Il s'agit du premier cas décrit de persistance de *Brucella melitensis* dans une population de ruminants sauvages en Europe (Hars and Garin-Bastuji, 2013), avec transmission retour aux ruminants domestiques (Mick *et al.*, 2014). Les enjeux en termes de santé publique, de santé vétérinaire et de conservation (le bouquetin étant une espèce protégée en France) ont conduit à l'évaluation des stratégies de gestion envisageables dans cette population sauvage, en partie par une approche de modélisation (ANSES, 2015; Thébault *et al.*, 2015).

L'évaluation des mesures de gestion n'est cependant pas le seul apport possible de la modélisation. Classiquement, trois grandes étapes du processus de modélisation se distinguent, avec leurs apports respectifs (Ezanno *et al.*, 2012) :

- La première étape est la représentation formelle du système sous une forme simplifiée, ce qui permet de faire le point sur les connaissances disponibles, de mieux comprendre les processus impliqués et de fournir des conclusions qualitatives sur le comportement du système ;
- La deuxième étape consiste à évaluer les paramètres les plus influents et estimer les valeurs des paramètres, et ainsi identifier les processus les plus importants pour le système ;
- Enfin, la troisième et dernière étape porte sur la prédiction de l'évolution du système par le modèle, dont fait partie l'évaluation de l'efficacité relative des mesures de gestion.

Ce sont ces trois étapes que nous allons développer et expliciter dans le cadre du système brucellose-bouquetin du Bargy, en nous attachant à chaque fois à dégager les apports passés ou à venir de la modélisation dans cette situation précise.

II – DECRIRE ET REPRESENTER UN SYSTEME COMPLEXE

Un modèle est une représentation simplifiée de la réalité, fondée sur une description des processus que l'on suppose être impliqués dans le système étudié. Par extension, un modèle mathématique traduit cette représentation en équations qui traduisent l'évolution du système au cours du temps. Ainsi, à partir d'un état initial donné, le modèle permet de prédire l'état du système à chaque pas de temps.

La construction d'un modèle mathématique nécessite donc une étude approfondie du système représenté, ce qui permet de faire un bilan exhaustif des connaissances actuelles sur l'agent pathogène, la population hôte et sur les interactions entre les deux. De plus, la prise en compte des mécanismes essentiels permet également d'aboutir à des conclusions qualitatives.

II-1. FLUX ENTRE ETATS DE SANTE

1.1 Voies de transmission de la brucellose

Le premier temps de construction du modèle consiste à déterminer quelles voies de transmission sont impliquées dans le système. En amont de la découverte du foyer sauvage du Bargy, seuls quelques cas sporadiques de brucellose ont été décrits chez des ruminants sauvages européens (Garin-Bastuji *et al.*, 1990; Ferroglia *et al.*, 1998; Hars and Garin-Bastuji, 2013). Par conséquent, la pathogénie et les voies de transmission chez le bouquetin des Alpes sont très peu connues. Cependant, il est le plus souvent admis qu'une partie des connaissances sur l'infection par *Brucella abortus* ou *B. melitensis* chez les ruminants domestiques peut être transposée aux ruminants sauvages (Godfroid *et al.*, 2013).

Quatre voies de transmission majoritaires sont décrites chez les ruminants domestiques, en lien avec le tropisme de la bactérie pour les organes génitaux et la mamelle. Tout d'abord, l'excrétion de la bactérie dans les produits d'avortement ou de mise bas chez les femelles infectées est considérée comme la principale voie de transmission chez les ruminants domestiques (European Commission, 2001).

La transmission vénérienne est la deuxième voie possible chez les ruminants domestiques, mais elle n'est pas considérée comme jouant un rôle majeur (King, 1940; FAO and WHO, 1986). Cependant, la reproduction chez les ruminants domestiques passe souvent par l'insémination artificielle, ce qui pourrait expliquer le faible rôle de cette voie. Chez les

espèces sauvages, l'importance de cette voie pourrait être plus élevée que chez les espèces domestiques, étant donné que la reproduction se fait naturellement et que la proportion de mâles est bien plus élevée. Chez le bison (*Bison bison*), des quantités de *B. abortus* en dessous de la dose infectante ont été trouvées dans la semence des mâles en dehors de la saison de reproduction, ce qui semble indiquer un rôle mineur de cette voie de transmission (Frey *et al.*, 2013). Chez les bouquetins du Bargy, la semence n'a pas pu être analysée en culture, mais la présence de bactéries en grand nombre a été mise en évidence dans les testicules en dehors de la saison de reproduction (Freycon, 2015). On peut donc supposer qu'autant de bactéries, voire plus, puissent être retrouvées dans les testicules pendant le rut, et par conséquent dans la semence des mâles.

Enfin, la transmission de la mère à son petit a été démontrée chez les bovins (Plommet *et al.*, 1973, les chèvres Renoux, 1962 et les moutons Grilló *et al.*, 1997). La transmission congénitale de la mère au petit *in utero* ou au moment de la mise bas survient dans environ 5 % des cas (FAO and WHO, 1986; Godfroid *et al.*, 2013) et les petits infectés ont la particularité de ne développer une réponse sérologique qu'au moment de leur première gestation pour les femelles ou de la maturité sexuelle pour les mâles (Plommet *et al.*, 1973). De plus, la transmission pseudo-verticale par la consommation de lait ou de colostrum est une autre voie possible (Philippon *et al.*, 1971; European Commission, 2001), même si elle n'est pas très fréquente (Grilló *et al.*, 1997).

1.2 Pathogénie et états de santé

Une fois les voies de transmission déterminées, il faut s'intéresser à la pathogénie de la maladie, afin de déterminer l'ensemble des états possibles pour les individus. Dans le cas de la brucellose, un individu sensible noté *S* exposé aux excréments d'individus infectieux développe à son tour l'infection avec une certaine probabilité que l'on notera β , qui dépend de la voie de transmission concernée. Si l'individu sensible est infecté, il passe à l'état d'incubation noté *E* pendant 3 semaines (European Commission, 2001), sauf s'il s'agit d'une transmission congénitale auquel cas l'incubation dure jusqu'à la première gestation pour les femelles ou l'âge à la maturité sexuelle pour les mâles (Plommet *et al.*, 1973). Ensuite, il développe une réaction immunitaire et passe à l'état infecté noté *I*.

Dans le cas de la brucellose, cet état infecté *I* n'est pas homogène ; il regroupe différents états d'excrétion qui dépendent notamment du sexe de l'individu, de son état physiologique

ou de la période de l'année. Par exemple, une femelle sensible non gestante au moment où elle a acquis l'infection pourra selon les cas soit ne pas excréter si l'infection est non active (Freycon, 2015; Lambert *et al.*, 2018b), soit excréter au moment de l'avortement brucellique lors de la première gestation post-infection brucellique (Godfroid *et al.*, 2004) ou de la mise bas lors des gestations suivantes (European Commission, 2001). D'autres états sont possibles en fonction de la voie de transmission, de l'état des individus et du moment de l'infection ... L'ensemble des combinaisons ont pu être décrites et représentées dans le modèle construit (ANSES, 2015; Thébault *et al.*, 2015).

Au final, notre modèle peut se résumer à un schéma à trois compartiments principaux *SEI* (Figure 1), en gardant à l'esprit qu'il s'agit d'une représentation simplifiée, l'état *I* regroupant plusieurs possibilités et les paramètres de probabilité de transmission β et de durée d'incubation $d_{incubation}$ dépendant de la voie de transmission impliquée.

II-2. DYNAMIQUE DE POPULATION ET PATRONS DE CONTACT

En plus des transitions entre les différents états de santé, il est nécessaire de représenter dans le modèle la dynamique de la population de bouquetins qui fait partie du système qui nous intéresse. En particulier, les naissances et les mortalités doivent être décrites (Figure 1). Dans le cas de la population du Bargy, la dynamique de population n'a pas été suivie entre 1999 - date du dernier foyer local de brucellose chez les animaux domestiques - et 2012 - date de découverte du foyer actuel (ANSES, 2015). Face à une telle absence de suivi, il est possible d'utiliser les paramètres d'autres populations connues, à condition de rester prudent sur les résultats du modèle en termes quantitatifs. En l'occurrence, les paramètres démographiques utilisés dans le modèle ont été tirés du suivi de la population de Belledonne Sept Laux, en faisant l'hypothèse que la dynamique de ces deux populations est proche.

Certains paramètres démographiques sont densité-dépendants. En particulier, la survie des juvéniles et la fécondité des femelles diminuent dans les situations de forte densité (Bonenfant *et al.*, 2009). Ces paramètres s'expriment en fonction de la taille de population par rapport à la capacité d'accueil de la zone. Or, cette capacité d'accueil de la zone notée K n'est pas un paramètre directement mesurable sur le terrain, et reste mal connue. Etant donné l'impact que ces mécanismes de densité-dépendances peuvent avoir sur les mesures de gestion mises en place, comme expliqué en introduction (Choisy and Rohani, 2006), ce

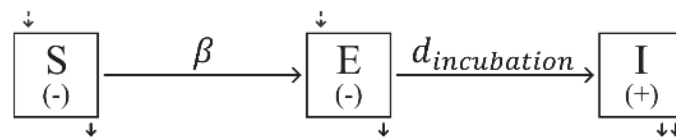


FIGURE 1 | Représentation schématique du modèle de transmission de la brucellose.

Les carrés représentent les différents états de santé : S , sensible ; E , en incubation ; I , infecté. Les flèches horizontales représentent les flux entre états de santé, avec les paramètres correspondants : β , la probabilité de transmission ; $d_{incubation}$, la durée d'incubation. Les flèches pointillées verticales représentent les naissances. Les flèches continues verticales représentent les mortalités.

paramètre de capacité d'accueil fera partie des éléments clés du modèle qu'il faudra chercher à mieux connaître et estimer dans un second temps (*cf.* partie III).

Enfin, la transmission de la brucellose étant liée au fonctionnement de l'appareil génital, c'est le cycle de reproduction de l'espèce qui entraîne la dynamique de transmission de l'agent pathogène. Chez les ongulés de montagne, la reproduction est fortement saisonnière, avec des périodes de rut et de naissance courtes. Dans le cas du bouquetin, trois périodes principales se distinguent : la période de transmission vénérienne pendant le rut, du mois de décembre à la mi-janvier (Couturier, 1962; Gauthier *et al.*, 1991; Willisich and Neuhaus, 2009) ; la période de transmission par les avortements au cours du troisième tiers de gestation (Diaz-Aparicio, 2013), de début avril à mi-mai ; et la période de transmission par les mises bas et par la voie verticale au moment des naissances, de début juin à mi-juillet (Gauthier *et al.*, 1991). A chacune de ces périodes, les contacts entre individus infectieux et sensibles sont différents.

Au moment du rut, mâles et femelles sont regroupés et ce sont les appariements entre mâles et femelles pour la reproduction qui déterminent les contacts infectieux. Au cours de ces appariements, une femelle est en contact en moyenne avec quatre mâles, un mâle dominant, entre 8 et 14 ans, et trois mâles subordonnés, entre 2 et 7 ans (Willisich and Neuhaus, 2009; Willisich *et al.*, 2012). Les mâles dominants ont un succès reproducteur plus élevé que les mâles subordonnés (Willisich *et al.*, 2012) ; on peut donc en déduire que les contacts infectieux liés à la voie vénérienne sont davantage dus aux vieux mâles qu'aux jeunes mâles.

Après la période de rut, une ségrégation sexuelle à la fois sociale et spatiale (séparation entre mâles et femelles avec utilisation d'habitats différents) se met progressivement en

place (Villaret and Bon, 1995; Bon *et al.*, 2001), ce qui conditionne les contacts infectieux pour la transmission indirecte par les produits d'avortements, puis par les produits de mise bas. Au printemps, pendant la période des avortements, les vieux mâles de plus de 6 ans se séparent des groupes de femelles, tandis que les jeunes mâles leur restent associés (Villaret and Bon, 1995; Bon *et al.*, 2001). Par conséquent, les mâles les plus exposés aux excréments des femelles ayant avorté sont ces jeunes mâles de moins de 5 ans. A la mise bas, les femelles s'isolent dans les falaises et les zones rocheuses avec leur petit (Couturier, 1962; Villaret *et al.*, 1997; Bon *et al.*, 2001). Lors de la troisième semaine du cabri, les femelles ayant mis bas se rassemblent en nurseries (Gauthier *et al.*, 1991) et utilisent un habitat différent des femelles sans cabri (Grignolio *et al.*, 2007b). Par conséquent, la transmission issue des mises bas concerne uniquement les femelles ayant mis bas et leurs cabris, et la durée d'exposition est faible du fait de l'isolement des femelles pendant deux semaines.

Tous ces éléments complexes de l'écologie des bouquetins sont déterminants dans la transmission de la brucellose. Les connaissances disponibles ont permis une description fine des patrons de contact, qui peut être ensuite représentée dans le modèle.

II-3. SIMULATIONS ET CONCORDANCE AVEC LES OBSERVATIONS

Pour construire le modèle, nous avons vu qu'il était nécessaire de décrire l'ensemble des processus impliqués, d'une part, dans la transmission et la pathogénie de la brucellose (partie II-1.) et, d'autre part, dans la dynamique de la population des bouquetins (partie II-2.). La description fine de ce système a été rendue possible grâce à la connaissance des experts, en particulier des pathologistes, écologues, épidémiologistes et spécialistes de la brucellose réunis dans le groupe de travail de l'Anses en 2015 (ANSES, 2015; Thébaud *et al.*, 2015), et grâce à une revue de la bibliographie. Ces deux sources d'information ont permis de lister l'ensemble des processus impliqués, et de faire le point sur les connaissances disponibles, en particulier sur les valeurs des différents paramètres (probabilités de transmission, capacité d'accueil, ...).

L'ensemble de ces processus doit ensuite être transcrit en équations, puis traduit numériquement par l'étape de programmation informatique. Concrètement, le modèle représente l'état de chaque individu (identifié individuellement avec ses caractéristiques de date de naissance, sexe...) pour chaque semaine écoulée. Cette échelle de représentation individuelle par semaine était nécessaire du fait des variations interindividuelles et

temporelles décrites ci-dessus. A partir d'un état initial donné de la population de bouquetins dans lequel on introduit un certain nombre d'individus infectés noté n_{intro} , on suit l'état de chaque individu, et donc de la population, à chaque pas de temps. Le nouvel état d'un individu résulte de tirages aléatoires qui dépendent de son état précédent. Par exemple, le devenir d'un individu S en contact avec un individu infectieux est représenté par un tirage aléatoire dans une loi de Bernoulli avec la probabilité de transmission β correspondante. Si le résultat du tirage est 1, alors l'individu devient E pendant la durée d'incubation, puis I . En revanche, si le résultat du tirage est 0, l'individu reste S .

Grâce à ces opérations, on obtient des simulations au cours du temps où l'on peut suivre chaque individu, et par conséquent suivre l'état global de la population. On peut ainsi prédire l'évolution de la taille de la population, de sa composition démographique, de la séroprévalence... au cours du temps.

A ce stade de la démarche de modélisation, il est alors particulièrement intéressant de vérifier que la prise en compte des processus listés précédemment permet au modèle de reproduire une dynamique qui soit concordante avec les données observées. Par exemple, on peut vérifier que les prédictions en termes de taille de population sont cohérentes avec les estimations de taille de population obtenues sur le terrain depuis 2013.

En conclusion de cette première étape d'élaboration du modèle, la démarche de modélisation nous a permis à ce stade :

- De lister l'ensemble des processus qui peuvent intervenir dans la dynamique épidémiologique et démographique ;
- De faire le bilan des connaissances à leur propos ;
- De vérifier que prendre en compte ces processus permet d'obtenir une prédiction cohérente avec les observations de terrain ;

De disposer de schémas représentatifs qui permettent d'échanger avec l'ensemble des acteurs concernés par la situation.

III – VALIDER ET HIERARCHISER LES MECANISMES CLES

La deuxième étape de la démarche de modélisation est une étape analytique. Parmi tous les paramètres du modèle, certains sont documentés dans la littérature, d'autres peuvent être estimés à l'aide des données de terrain. Certains paramètres jamais estimés précédemment font l'objet d'hypothèses ou sont estimés à dire d'experts, mais peuvent aussi être estimés par la modélisation (voir plus bas). L'objectif de cette étape est d'identifier les paramètres qui font le plus varier les sorties du modèle, afin de focaliser les recherches en vue de l'estimation sur ces paramètres clés. Cette étape est essentielle pour l'étude des mécanismes en jeu dans le modèle, et pour pouvoir réaliser des prédictions fiables en particulier dans la troisième étape.

III-1. IDENTIFICATION PAR ANALYSE DE SENSIBILITÉ

L'analyse de sensibilité d'un modèle consiste à faire varier les paramètres en entrée du modèle dans leur intervalle de valeurs possibles (par exemple, les probabilités de transmission entre 0 et 1) et à observer l'impact de ces variations sur la variation des sorties du modèle (par exemple, la taille de la population ou la séroprévalence). Cet outil permet notamment d'identifier les paramètres qui influencent le plus les sorties du modèle, et qui donc sont à estimer en priorité pour réduire l'incertitude, et au contraire identifier les paramètres sans influence dont la valeur peut être fixée sans que cela ait un impact sur les prédictions du modèle (Cariboni *et al.*, 2007).

L'identification des paramètres clés influençant le plus le système permet donc :

- De hiérarchiser les futures recherches de terrain afin de mieux connaître ces paramètres, lorsque cela est possible, et ainsi de réduire l'incertitude sur les sorties du modèle ;
- Ou de cibler les paramètres à estimer à l'aide du modèle et des données de terrain déjà disponibles (voir paragraphe suivant).

Dans le cas de la brucellose des bouquetins du Bargy, les principales données de terrain sont les estimations de taille de population annuelles et la composition (âge, sexe et statut sérologique) des individus capturés dans le cadre de la surveillance et de la gestion du foyer. Parmi les sorties du modèle figurent la taille de population et la séroprévalence au cours du

temps. Les opérations de capture sont reproduites dans le modèle, ce qui permet d'obtenir dans les sorties le nombre d'individus séropositifs à chaque saison de capture.

Ainsi, le modèle nous a permis de montrer que quatre paramètres principaux influencent ces sorties (taille de population et nombre d'individus séropositifs capturés), qui sont dans l'ordre décroissant : la probabilité de transmission par une femelle infectée et excrétrice au moment d'un avortement ou d'une mise bas (notée $\beta_{indirecte}$); la capacité d'accueil du milieu (notée K); le nombre d'individus infectés dans la situation initiale (noté n_{intro}); et la probabilité de transmission par la voie vénérienne (notée $\beta_{vénérienne}$ - Figure 2). Au contraire, d'autres paramètres n'influencent quasiment pas la variation des sorties et leurs valeurs peuvent donc être fixées sans modifier les résultats du modèle, comme par exemple l'impact de la brucellose sur la reproduction ou sur la survie, ou les paramètres de transmission verticale et pseudo-verticale.

Il ressort de cette première analyse que c'est la transmission indirecte par le biais des avortements et des mises bas qui a le plus d'impact sur la variation des sorties du modèle, tandis que la voie vénérienne a une influence plus modérée.

Le deuxième paramètre en termes d'influence est la capacité d'accueil du milieu, ce qui souligne l'importance des effets de densité-dépendance dans la population. On peut donc s'attendre à des effets d'augmentation de la fécondité en cas de diminution forte de la taille de population, avec les potentiels effets sur la dynamique épidémiologique. Par conséquent, il est important d'essayer de déterminer au mieux ce paramètre, pour évaluer avec le moins d'incertitude possible les effets des mesures de gestion.

Enfin, le nombre d'individus infectés dans l'état initial influence aussi fortement les sorties du modèle. En l'occurrence, l'état initial choisi est 1999, année du dernier foyer domestique de brucellose dans la région (ANSES, 2015). Il a donc été fait l'hypothèse que la transmission des ruminants domestiques aux bouquetins datait au plus tard de cette année, ou était antérieure. Cependant, la date exacte de la transmission et le nombre d'individus infectés en 1999 restent inconnus, et ce dernier peut varier d'un individu à plusieurs dizaines, la seule donnée disponible étant les 31 analyses sérologiques réalisées entre 1987 et 1996 qui se sont toutes révélées négatives (D. Gauthier, communication personnelle).

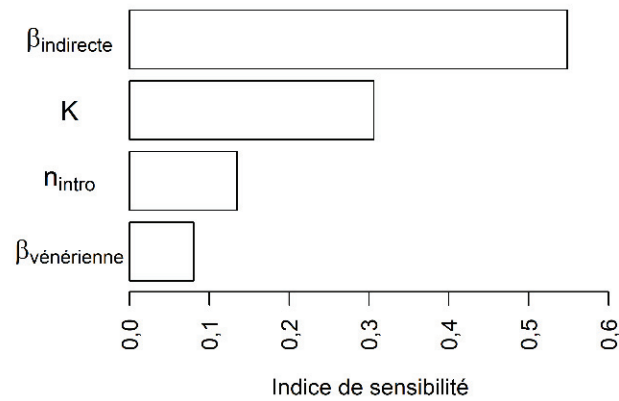


FIGURE 2 | Sensibilité des sorties du modèle à la variation des paramètres, pour les quatre paramètres les plus influents : $\beta_{indirecte}$: la probabilité de transmission indirecte par avortement/mise bas ; K : la capacité d'accueil du milieu ; n_{intro} : le nombre d'individus infectés dans l'état initial ; et $\beta_{vénérienne}$: la probabilité de transmission par la voie vénérienne.

III-2. ESTIMATION DES PARAMÈTRES CLÉS

Après avoir déterminé les paramètres clés à l'aide de l'analyse de sensibilité, l'étape suivante consiste à en estimer certains en utilisant le modèle et les données de terrain disponibles. Le principe consiste à réaliser un grand nombre de simulations du modèle avec des valeurs de paramètres variées, et de sélectionner les simulations ayant produit des résultats proches de ceux observés en réalité sur le terrain. Les valeurs de paramètres qui ont servi à produire ces simulations proches sont alors utilisées pour déterminer la valeur la plus probable des paramètres clés. Cette approche a été utilisée à de nombreuses reprises dans des modèles épidémiologiques (Bekara *et al.*, 2014; Beaunée *et al.*, 2015; Kosmala *et al.*, 2016).

Dans le cas de la brucellose des bouquetins du Bargy, une approche possible est la technique d'*Approximate Bayesian Computation* (ABC) (Beaumont *et al.*, 2002). Les paramètres clés identifiés (*cf.* paragraphe précédent) sont difficiles à mesurer directement sur le terrain. Les probabilités de transmission par exemple ne sont pas directement observables et ne pourraient pas faire l'objet d'études de terrain pour les déterminer. Par conséquent, l'utilisation d'une méthode d'estimation de paramètres est utile, et pourrait permettre à partir de données mesurées sur le terrain comme la séroprévalence d'inférer les valeurs des paramètres associés aux mécanismes sous-jacents.

Cette étape est déterminante pour quantifier l'importance relative des différentes voies de transmission par exemple, ou permettre de réaliser les étapes suivantes telles que la comparaison de modèles ou la prédiction des effets des scénarios de gestion.

En conclusion de cette deuxième étape, l'étude analytique du modèle permet :

- De hiérarchiser les mécanismes impliqués dans le système et d'identifier les mécanismes clés ;
- D'orienter les futures recherches de terrain ;
- De valider et de quantifier les différents processus, grâce à l'estimation des paramètres ;
- De tester des hypothèses biologiques, en comparant les résultats de différents sous-modèles, qui incluent ou non ces hypothèses (Ezanno *et al.*, 2012).

Par exemple, dans le cas du Bargy, cette étape pourrait permettre de conclure sur l'importance des différentes voies de transmission, en quantifiant chacune de ces voies grâce à l'estimation de paramètres, et en comparant par exemple un modèle comprenant toutes les voies de transmission à un modèle sans transmission vénérienne, afin de confirmer ou non la coexistence des deux types de transmission.

C'est uniquement une fois ces étapes analytiques réalisées, permettant la compréhension du système et sa description la plus correcte possible, qu'il est possible de réaliser l'autre volet de la modélisation, à savoir la prédiction d'un état futur, et en particulier évaluer les effets de mesures de gestion dans la population.

IV – EVALUER LES SCENARIOS DE GESTION

Le dernier apport possible de la modélisation est l'évaluation des scénarios de gestion et de leur efficacité relative. Les modèles sont en effet un outil d'aide à la décision en comparant les scénarios et en intégrant l'incertitude de leurs effets (Smith *et al.*, 2009).

La mesure de l'efficacité passe par l'étude de différentes sorties du modèle, telles que l'évolution de la séroprévalence au cours du temps ou encore la probabilité d'extinction de l'agent pathogène. L'impact démographique des mesures est aussi important à prendre en compte : la viabilité de la population restante doit être assurée, d'autant plus dans le cas du bouquetin où la consanguinité est importante.

L'évaluation des mesures de gestion par la modélisation entreprise en 2015 a comparé plusieurs scénarios (ANSES, 2015; Thébault *et al.*, 2015) avec la prise en compte de la faisabilité, et donc de différents efforts de capture/abattage :

- Suivi de la population sans mesures de gestion, qui est le scénario de référence pour évaluer l'efficacité des autres scénarios ;
- Captures avec euthanasie des séropositifs et marquage des séronégatifs ;
- Captures avec euthanasie des séropositifs, marquage et vaccination des séronégatifs ;
- Captures avec euthanasie des séropositifs et marquage des séronégatifs, suivies d'un abattage massif des non marqués ;
- Abattage massif.

Cette évaluation a déjà permis l'obtention de premiers résultats, pouvant aider la stratégie de gestion, compte tenu des hypothèses rappelées plus hauts (ANSES, 2015; Thébault *et al.*, 2015) :

- L'extinction de l'agent pathogène en l'absence de mesures de gestion est très peu probable dans la quinzaine d'années à venir ;
- L'extinction de *B. melitensis* à la suite d'un abattage massif sur une année est aussi très peu probable ;
- La combinaison de mesures de gestion comme la vaccination et l'abattage sélectif a de meilleures chances de succès ;
- L'effort de gestion doit être important et répété sur plusieurs années pour espérer obtenir une extinction de la brucellose dans la population.

La faisabilité d'un modèle sur une situation émergente pouvant aider à la stratégie de gestion, tout autant que ses premiers résultats, ont encouragé la poursuite du travail de modélisation sur ce sujet complexe. Le premier modèle bâti en 2015 sur moins de données et davantage d'hypothèses avait un certain nombre de limites : hypothèse d'un faible nombre d'individus infectés en 1999 ce qui majore la probabilité de transmission, calibrage et non estimation des paramètres, faible nombre d'itérations, hypothèses d'homogénéité spatiale de la population de Bargy, transposition des résultats obtenus sur d'autres espèces.... Ce

modèle est actuellement amélioré en utilisant les données actuelles, ce qui permettra une comparaison de scénarios de gestion fondée sur une connaissance actualisée du système.

La modélisation permet de comparer différentes mesures ou combinaison de mesures, mais aussi d'autres aspects comme la durée d'application des mesures, le nombre d'individus concernés, la saisonnalité des mesures... La modélisation permet aussi de tester des scénarios différents qui n'ont pas nécessairement été observés et ainsi s'intéresser à des situations qui pourraient émerger par exemple dans d'autres populations. Par exemple, il est possible de tester des scénarios de gestion dans des populations de taille différente, pour lesquelles la bactérie aurait été introduite à d'autres périodes, ou pour des structures de population différentes...

V – CONCLUSION

Pour conclure sur l'apport de la modélisation dans le système brucellose-bouquetin du Bargy, il est important de souligner l'interconnexion entre l'approche de modélisation et la collecte et l'analyse des données sur le terrain. En effet, ce sont les connaissances du système et les données disponibles qui permettent de correctement représenter le système et sa complexité, d'estimer les valeurs des paramètres et de valider la dynamique du modèle. Inversement, le modèle permet de tester des hypothèses, de comparer des scénarios et de hiérarchiser les futures recherches afin de réduire l'incertitude liée à certains paramètres.

De plus, il s'agit d'une démarche itérative, les résultats du modèle alimentant les recherches sur le terrain en posant de nouvelles questions, tandis que les nouvelles données viennent modifier les hypothèses du modèle, qui conduisent à de nouveaux résultats et ainsi de suite.

Par exemple, dans le cas de la brucellose des bouquetins du Bargy, les nouvelles données de terrain issues de la surveillance de 2015 à nos jours permettent d'affiner l'estimation des paramètres et donc de réévaluer les scénarios de gestion. Par ailleurs, les nouvelles connaissances sur la spatialisation de l'infection dans le massif (Marchand *et al.*, 2017) vont par la suite être intégrées dans la représentation du système. Ainsi, de nouvelles questions en termes de gestion de risque, cette fois spatialisées dans le massif, pourront être testées à l'aide de la modélisation.

Appendix 2: Hétérogénéité de l'excrétion de *Brucella melitensis* chez les bouquetins

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RESUME

La dynamique de transmission de la brucellose du bouquetin était pour l'essentiel inconnue en 2012. La découverte du réservoir constitué par la population de bouquetins du massif du Bargy a soulevé la nécessité d'identifier les facteurs qui permettent la persistance de l'infection à un fort niveau de prévalence dans cette population. Pour identifier les modes et voies de transmission ainsi que les classes d'animaux les plus susceptibles de constituer des sources de bactéries, 88 bouquetins séropositifs ont été autopsiés entre 2012 et 2017. Des recherches bactériologiques ont été menées sur 1 à 15 organes pour chaque animal. La bactérie a été détectée chez 51 (58%) des animaux autopsiés, et chez 45 animaux elle était détectée dans au moins un échantillon du tractus urogénital ou un nœud lymphatique de la région pelvienne, ces animaux étant donc excréteurs potentiels. Parmi ces excréteurs potentiels, 26 (58%, soit 30% de tous les animaux autopsiés) avaient au moins une culture positive dans un organe du système urogénital et ont donc été considérés comme excréteur effectivement la bactérie au moment de la capture. Une hétérogénéité était présente entre les classes d'âge et de sexe : la bactérie était plus souvent détectée chez les animaux séropositifs avant l'âge de 5 ans, possiblement en lien avec une infection primaire, impliquant un avortement possible à la première gestation chez les femelles, tel qu'il est connu chez les ruminants domestiques. Enfin, l'excrétion bactérienne était liée à la réponse sérologique chez les femelles, suggérant que cette réponse pourrait permettre d'identifier les femelles présentant un risque plus élevé d'excrétion. Le fort potentiel d'excrétion chez les femelles jeunes peut avoir contribué au maintien de l'infection à long terme dans cette population. Les mâles et les femelles pourraient donc jouer des rôles épidémiologiques distincts, les femelles contribuant à la pérennisation de l'infection dans un secteur et les mâles à la transmission entre secteurs. Cette hétérogénéité doit être prise en compte pour évaluer des scénarios de gestion, dans cette situation comme dans d'autres cas.

Mots-clés : *Brucella melitensis*, bactériologie, sérologie, transmission

ABSTRACT

The transmission dynamics of *Brucella melitensis* in the alpine ibex was mostly unknown in 2012. The discovery of a reservoir in the population of the Bargy massif raised the need to identify the factors that allowed the persistence of infection at a high level in this population. In order to determine the transmission routes and to identify which classes of individuals were more at risk to excrete bacteria, we necropsied 88 seropositive ibexes between 2012 and 2017. Bacteriological cultures were undertaken in 1 to 15 samples for each individual. The bacteria was detected in 51 (58%) of the necropsied ibexes. In 45 individuals, the bacteria was detected in at least one sample issued from the genital tract or a lymph node from the pelvic area, thus these individuals were potentially at risk of excreting the bacteria in the future. Among these, 26 (58%, or 30% of all necropsied animals) had at least one positive culture from an organ of the urogenital sphere and were thus considered as actually at risk of excreting the bacteria at the time of capture. The bacteriological results were heterogeneous among age and sex classes: the bacteria was most often detected in seropositive females aged less than 5 years old. This was possibly due to a primary infection that would include a possible abortion event during the first pregnancy, as is described in domestic ruminants. Moreover, the bacteriological result was related to the serological response in females, suggesting that the serological response may help identify females having a high risk of excretion. The high shedding potential in young females may have contributed to the long-term maintenance of infection in this population. This heterogeneity should be considered to evaluate management scenarios, in this and other situations.

Keywords: *Brucella melitensis*, bacteriology, serology, transmission

I – INTRODUCTION

Lors de la découverte du foyer du Bargy en 2012, la dynamique de transmission de la brucellose chez le bouquetin était pour l'essentiel inconnue. En effet, avant ce foyer, seuls des cas sporadiques de brucellose à *Brucella melitensis* avaient été décrits, et la possibilité d'une persistance à long terme dans une population de bouquetins n'était pas considérée comme probable. En 2012 et 2013, après la détection de l'infection chez des personnes et dans un troupeau bovin, les enquêtes réalisées ont indiqué comme source la plus probable la population de bouquetins, chez laquelle l'infection aurait persisté pendant au moins quinze ans depuis la disparition des derniers foyers bovins. Il s'agissait du premier cas de persistance à long terme chez des ongulés sauvages en Europe [Garin-Bastuji et al. 2014].

Compte-tenu des enjeux de santé animale et de santé publique, il était nécessaire de proposer des actions de lutte, et pour ce faire d'identifier les facteurs qui permettent la persistance de l'infection à un fort niveau de prévalence dans cette population.

Plusieurs questions doivent être abordées pour mieux comprendre la transmission de l'infection. Tout d'abord, il est nécessaire d'identifier les voies de transmission possibles entre animaux infectés et sains, afin de savoir dans quelles circonstances se produit la transmission et de la prévenir. La deuxième question est de savoir dans quelle mesure les animaux porteurs d'anticorps peuvent excréter la bactérie ou du moins porter des bactéries détectables. En effet, la sérologie est souvent utilisée pour diagnostiquer l'infection, mais dans le cas de la brucellose des bouquetins la proportion d'animaux séropositifs réellement à même d'excréter des bactéries n'était pas connue. Enfin, les individus peuvent être hétérogènes en termes de présence et de capacité d'excrétion bactérienne. En particulier, la présence de la bactérie peut être corrélée au niveau de réponse immunitaire des individus comme cela a été montré pour d'autres infections [Gonzalez-Barrio et al. 2015]. Or, une telle hétérogénéité est connue pour avoir des conséquences épidémiologiques majeures sur la dynamique des épidémies, leur probabilité d'extinction mais aussi sur l'efficacité des mesures de gestion mises en place [Lloyd-Smith et al. 2005]. Pour répondre à ces trois questions, l'étude bactériologique détaillée d'animaux considérés comme infectés était nécessaire.

II – MATERIEL ET METHODES

Entre 2012 et 2017, des bouquetins ont été capturés ou abattus dans le cadre des opérations de lutte et de surveillance menées dans le massif du Bargy. En tout, les captures concernaient 339 individus différents (certains capturés plusieurs fois), qui faisaient l'objet d'un test sérologique lors de la capture, avec euthanasie des animaux séropositifs. Par ailleurs, les opérations d'abattage concernaient 326 individus dont 33 ont été analysés pour la sérologie. Les tests sérologiques combinaient le test au Rose Bengale, le test de Fixation du Complément, un test ELISA indirect, un test ELISA par compétition, et depuis 2014 un test immunochromatographique rapide utilisable sur le terrain, les résultats des tests étant généralement concordants [Corde et al. 2014, Lambert et al. 2018]. Au total, les opérations de capture et d'abattage ont permis d'identifier 127 bouquetins séropositifs abattus ou euthanasiés. Pour les autopsier, il était nécessaire de transporter les carcasses dans des conditions de biosécurité appropriées et dans un temps compatible avec de bonnes conditions d'autopsies jusqu'au Laboratoire Départemental d'Analyse Vétérinaires (LDAV) de la Savoie. Seuls les animaux pour lesquels toutes les informations étaient disponibles (bactériologie, mais aussi âge, sexe, localisation de capture ou d'abattage, date et titre anticorps par le test de fixation du complément) ont été considérés dans ce travail, soit 88 bouquetins séropositifs.

Les bouquetins ont fait l'objet d'une autopsie détaillée et de prélèvements sur 1 à 15 organes. Les organes ciblés étaient ceux habituellement atteints dans les infections brucelliques ou ceux pouvant présenter un potentiel d'excrétion et de transmission important (articulations, tractus génital, mamelles, testicules, nœuds lymphatiques de la sphère génitale, fœtus) ainsi que les organes présentant des lésions. Les cultures bactériennes sur milieu sélectif ont été réalisées au LDAV de la Savoie à l'aide des méthodes standard de l'OIE [Organisation Mondiale de la Santé Animale 2016, Freycon et al. 2017]. Les cultures positives ont fait l'objet d'une énumération des colonies bactériennes et ont été classées de 1 à 4 selon qu'elles présentaient moins de 10, 10 à 50, 50 à 100 ou plus de 100 colonies.

III – VOIES DE TRANSMISSION

Pour tester l'existence de différentes voies de transmission, la bactérie a été cherchée dans les organes excréteurs correspondants.

D'après les connaissances dont nous disposions sur la brucellose dans d'autres espèces, quatre voies de transmission pouvaient être supposées. La transmission peut tout d'abord être horizontale (entre individus présents dans la même population au même moment) par voie indirecte via les excréments génitaux (avortement et mises-bas compris), ou par voie vénérienne lors des accouplements (dans un sens mâle-femelle ou femelle-mâle). Pour tester ces deux hypothèses, des cultures bactériennes ont été réalisées sur les excréments génitaux, les écouillons vaginaux et prépuçiaux, les testicules, le tractus génital et le placenta des animaux autopsiés. La transmission pouvait également être verticale (d'une génération à la suivante), soit par voie congénitale à travers le placenta soit par voie pseudo-verticale via le lait. Pour tester ces deux hypothèses, des cultures bactériennes ont été pratiquées sur différents organes de fœtus (cœur, reins, foie, rate, caillette, testicules) ainsi que sur les mamelles chez les femelles.

Au total, 516 résultats bactériologiques ont été obtenus à partir des 88 animaux autopsiés. Les organes infectés ont confirmé la possibilité des quatre voies de transmission envisagées. En effet, 11 des 59 écouillons génitaux et 14 des 52 tractus génitaux, ainsi qu'un des 7 placentas testés ont permis d'isoler des bactéries, confirmant la possibilité d'une transmission indirecte liée à la contamination de l'environnement par les excréments génitaux, notamment par les produits de mise-bas ou d'avortement. Chez les mâles, 12 des 32 prélèvements de testicules ont donné un résultat positif, suggérant une transmission vénérienne possible. Il faut noter cependant que les prélèvements ont été faits entre le printemps et l'automne et non en période de rut hivernal, pendant laquelle la physiologie des testicules peut être modifiée. Parmi les trois fœtus testés, l'un a permis la culture de bactéries à partir des cinq organes testés (cœur, reins, foie, rate, caillette) et avait donc été infecté à travers le placenta. Enfin, cinq des sept prélèvements de mamelles ont permis d'isoler la bactérie, ce qui laisse envisager une transmission possible par le lait. Toutefois, pour la voie vénérienne comme pour la voie pseudo-verticale, ce ne sont pas le sperme ni le lait qui ont été testés mais les organes producteurs, l'excrétion bactérienne peut donc seulement être présumée. De plus, les prélèvements ayant été faits entre le printemps et l'automne, les résultats obtenus pourraient être modulés en fonction de la saison et de la physiologie des organes reproducteurs à cette période.

En plus de ces organes susceptibles d'excréter directement la bactérie, l'infection était fréquemment détectée dans les nœuds lymphatiques supramammaires (17 des 48 femelles analysées), inguinaux (7/20 bouquetins) et iliaques internes (30/79 bouquetins). L'excrétion

potentielle, possiblement par recirculation des bactéries à partir de ces organes, était donc elle aussi fréquente.

Les résultats bactériologiques permettent de confirmer que les quatre voies de transmission envisagées sont possibles, ce qui confirme un schéma de transmission complexe : si la transmission verticale et pseudo verticale peut expliquer la persistance dans un groupe social, la transmission liée à la contamination environnementale pourrait permettre une transmission vers tous les individus présents localement, et la transmission vénérienne combinée à l'occupation socio-spatiale des deux sexes pourrait permettre une extension spatiale de l'infection.

IV – RELATION ENTRE STATUT SEROLOGIQUE ET STATUT INFECTIEUX

Le lien entre séropositivité et présence de bactéries détectables a été analysé en mesurant la proportion d'animaux autopsiés chez lesquels la bactérie était détectable. Dans de nombreuses espèces, on rencontre des individus sérologiquement positifs ne permettant pas l'isolement bactérien, la bactérie étant probablement contenue en faible densité dans un nombre restreint d'organes [Huber et Nicoletti 1986, Roffé et al. 1999]. Chez le bouquetin, compte-tenu de la persistance à long terme dans la population, nous nous attendions à une fréquence élevée d'infection détectable et donc active, qui est une condition à la persistance à long terme dans la population.

Au total, la bactérie a été détectée chez 51 (58%) des animaux autopsiés. En moyenne, sur 5,9 organes analysés par individu, 2,0 cultures étaient positives. Parmi ces 58 bouquetins porteurs d'une infection active, 45 permettaient l'isolement bactérien à partir d'un échantillon du tractus urogénital ou un nœud lymphatique de la région pelvienne. Cette présence pouvait refléter une infection latente comprenant un risque d'excrétion suite à une recirculation de la bactérie, les animaux correspondants ont donc été considérés comme excréteurs potentiels. Parmi ces excréteurs potentiels, 26 (58%, soit 30% de tous les animaux autopsiés) avaient au moins une culture positive dans un organe du système urogénital et ont donc été considérés comme excréteurs effectivement la bactérie au moment de la capture.

V – CARACTERISTIQUES DES ANIMAUX INFECTIEUX

Nous supposons que la probabilité de trouver des cultures positives pouvait varier avec l'âge. En effet, dans d'autres espèces, l'excrétion bactérienne peut soit se poursuivre au cours du temps, comme chez la chèvre, soit diminuer avec l'âge comme chez le bison [Treanor et al. 2011]. Nous avons également formulé l'hypothèse que le niveau de réaction immunitaire, mesuré par la concentration en anticorps évaluée par le test de fixation du complément, pouvait être corrélé à la présence d'une infection active, comme cela a été montré précédemment chez des ovins [Durán-Ferrer et al. 2004].

Enfin, pour tester ces hypothèses et identifier les caractéristiques des individus les plus souvent infectieux, nous avons analysé la probabilité d'obtenir un résultat positif en culture bactérienne pour un individu séropositif, à l'aide de modèles linéaires généralisés mixtes de type binomial. Pour prendre en compte le fait que le nombre de cultures réalisées était différent d'un individu à l'autre, le nombre de cultures réalisées pondérait les données pour chaque individu. Nous avons testé les effets de l'âge, du sexe, du titre en anticorps mesuré par fixation du complément, de la période (avant/après les mesures d'abattage massif de fin 2013), du traitement des échantillons (culture effectuée après congélation ou non) et des interactions deux à deux entre les variables âge, sexe et titre, la dynamique d'infection pouvant être variable entre classes d'âge et de sexe. Les modèles ajustés prenaient aussi en compte deux variables aléatoires, l'année et le secteur, la dynamique d'infection étant variable entre les cinq secteurs du massif [Marchand et al. 2017]. A partir du modèle incluant tous les effets des variables à tester, tous les sous-modèles ont été ajustés et comparés en utilisant le critère d'Akaike corrigé pour les petits effectifs (AICc), une faible valeur d'AICc indiquant un bon compromis entre qualité d'ajustement et simplicité du modèle. Dans le modèle sélectionné, les paramètres représentent les logarithmes des odds-ratios (OR), qui sont des estimateurs du risque relatif associé à chaque modalité comparée. La significativité de chaque paramètre inclus dans le modèle sélectionné a été testée à l'aide du test de Wald. Nous avons également vérifié la distribution normale des effets aléatoires et les hypothèses du modèle à l'aide de représentations graphiques. Les analyses statistiques ont été effectuées avec le logiciel R [R Development Core Team 2017] et la fonction dredge de la librairie MuMIn [Kamil 2016] pour la sélection de modèle.

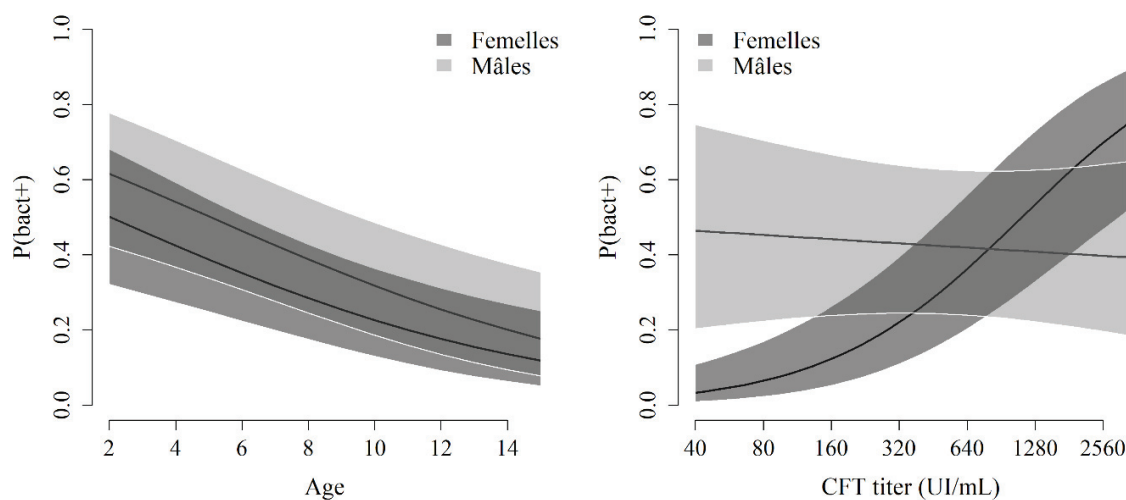


FIGURE 1 | Probabilité d'obtention d'une culture bactérienne positive modélisée en fonction du sexe et de l'âge (gauche) et en fonction du sexe et du titre sérologique mesuré par fixation du complément (droite), à partir des données obtenues sur 88 bouquetins *Capra ibex* issus du massif du Bargy (France) autopsiés entre 2012 et 2017.

Le modèle retenu pour expliquer la probabilité de culture positive incluait les effets de l'âge, du sexe, du titre sérologique et l'interaction entre le sexe et le titre sérologique (Figure 1).

Le risque d'excréter la bactérie diminue avec l'âge : il est multiplié par un OR = 0,85 pour chaque année de vie, avec un intervalle de confiance à 95%, IC95, de [0.77–0.93]. L'excrétion bactérienne est donc plus probable chez les jeunes animaux infectés, possiblement en lien avec une infection primaire, impliquant un avortement possible à la première gestation pour les femelles, tel qu'il est connu chez les ruminants domestiques. Les animaux jeunes sont donc moins souvent infectés que les plus âgés [Marchand et al. 2017] mais parmi les infectés, plus souvent excréteurs. Ce schéma ne concerne cependant que les animaux âgés d'au moins deux ans, aucune information n'ayant été recueillie chez des bouquetins plus jeunes.

La probabilité de culture positive était également légèrement plus élevée chez les mâles par rapport aux femelles : OR = 1,55 [0,97 – 2,47] (Figure 1). Cela ne signifie pas nécessairement un rôle épidémiologique plus important pour les mâles que pour les femelles. Tout d'abord des organes différents ont été testés, ce qui rend la comparaison peu évidente. D'autre part, le potentiel d'excrétion et de contact avec d'autres bouquetins est différent

entre les sexes : pour les mâles, il concerne essentiellement le rut, une femelle étant exposé à chaque accouplement ; tandis que les femelles peuvent infecter leur partenaire au moment du rut, mais aussi tous les congénères en contact avec l'environnement infecté lors d'avortement ou de mise-bas. Le rôle respectif des mâles et des femelles pourrait donc être qualitativement distinct : l'excrétion par les femelles pourrait contribuer à la contamination environnementale et donc au maintien de l'infection dans un secteur, tandis que les mâles pourraient être impliqués dans la transmission entre secteurs.

Enfin, l'excrétion bactérienne était liée à la réponse sérologique : chez les femelles, ce risque était multiplié par $OR = 2.71 [1.84-3.97]$ par unité de $\log(\text{titre})$, tandis que cette relation n'existait pas chez les mâles ($= 0.83 [0.45-1.52]$, Figure 1). Bien que le titre en anticorps diminue au fil de l'âge, cette relation était présente une fois pris en compte l'âge des femelles, ce qui suggère que le niveau de réponse sérologique est un facteur supplémentaire d'hétérogénéité d'excrétion en plus de l'âge chez les femelles. Ce résultat suggère que l'examen du niveau de réponse sérologique pourrait permettre d'identifier, au moins a posteriori, les femelles présentant un risque élevé d'excrétion. L'identification de ces femelles pourrait permettre de mieux comprendre la persistance de l'infection dans certains secteurs géographiques. En particulier, chez les femelles âgées, on observe simultanément une diminution du titre sérologique et une diminution du risque d'excrétion liée à l'âge. Ces deux observations laissent penser que la réaction immunitaire conduit progressivement à une diminution du risque d'excrétion, possiblement liée à une séquestration des bactéries dans des organes non ouverts.

VI – CONCLUSION

Ce résultat illustre l'hétérogénéité du risque d'infection par la brucellose, qui pourrait être largement lié aux quelques individus jeunes, notamment femelles. Les mâles et les femelles pourraient donc jouer des rôles épidémiologiques distincts, avec des femelles impliquées dans le maintien de l'infection dans un secteur, et des mâles dans la transmission entre secteurs. Le fort potentiel d'excrétion chez les femelles jeunes peut avoir contribué au maintien de l'infection à long terme dans cette population

Cette hétérogénéité doit être prise en compte pour évaluer des scénarios de gestion, dans cette situation comme dans d'autres cas.

TRANSMISSION ET GESTION SANITAIRE DE LA BRUCELLOSE DANS UNE POPULATION SAUVAGE HETEROGENE DE BOUQUETINS DES ALPES (*CAPRA IBEX*)

Résumé

La gestion des maladies infectieuses dans la faune sauvage se heurte à de nombreuses limites, et le développement de stratégies efficaces représente un défi de taille. Pour atteindre cet objectif, une compréhension fine des facteurs influençant la transmission et la persistance de l'infection est nécessaire. Parmi ces facteurs, l'hétérogénéité de transmission est une caractéristique importante des populations sauvages. En effet, la diversité des comportements, des structures sociales et spatiales, ou encore des espèces peut conduire à des contributions très variables au nombre de nouvelles infections. Par conséquent, quantifier l'hétérogénéité de transmission pourrait permettre d'améliorer l'efficacité des mesures de gestion sanitaire dans la faune sauvage, en ciblant les individus ou les unités de population qui sont responsables de la majorité des événements de transmission.

L'objectif de cette thèse était d'améliorer les connaissances sur la gestion des maladies infectieuses dans des populations sauvages hétérogènes, en utilisant la brucellose à *Brucella melitensis* dans une population de bouquetin des Alpes (*Capra ibex*) comme modèle d'étude. En effet, la biologie de la brucellose et l'écologie de l'espèce hôte se prêtent bien à l'existence et donc à l'étude d'une hétérogénéité de transmission à différentes échelles.

A l'aide de cultures bactériennes, nous avons tout d'abord montré que seulement 58 % des individus séropositifs sont à risque d'excréter la brucellose, et que ce risque diminue avec l'âge. Ensuite, mettant à profit l'existence d'informations détaillées sur la dynamique de population et le comportement du bouquetin, et de données épidémiologiques dans la population d'étude, nous avons développé un modèle individu-centré afin de quantifier l'hétérogénéité individuelle et spatiale de la transmission. Nous avons démontré que la transmission de la brucellose était hétérogène entre individus, les femelles provoquant environ 90% des nouvelles infections, et entre unités spatiales, plus de 80% des cas de transmission ayant lieu dans les trois sous-unités socio-spatiales qui forment la zone cœur du massif. Nous avons également estimé l'évolution temporelle de la séroprévalence et de la force d'infection, en utilisant différents modèles statistiques. Les résultats suggèrent que l'importante opération de capture menée en 2015, avec test systématique et élimination des individus séropositifs, a permis de diminuer la transmission de la brucellose dans la population. Sur la base de l'ensemble de ces résultats, nous avons évalué une série de stratégies de gestion sanitaire qui pourraient être utilisées à l'avenir dans la population. Les résultats, issus du modèle individu-centré, confirment que la stratégie prioritaire devrait être d'éliminer le plus d'individus infectés possible par test-et-élimination, et que cibler les femelles et/ou la zone cœur permet d'améliorer l'efficacité des mesures.

Bien qu'il n'y ait pas de solution évidente pour la gestion de la brucellose dans notre cas d'étude, les stratégies de gestion ciblées sont très prometteuses et permettent de raffiner les mesures sanitaires classiquement utilisées. Il est donc primordial de bien comprendre l'hétérogénéité de transmission dans les populations sauvages infectées, et de rechercher des stratégies ciblées qui peuvent permettre d'améliorer la gestion en termes d'efficacité et d'acceptabilité.

Mots-clés : épidémiologie, maladies infectieuses, réservoirs sauvages, modélisation, hétérogénéité, force d'infection, gestion ciblée, *Capra ibex*

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TRANSMISSION AND MANAGEMENT OF BRUCELLOSIS IN A HETEROGENEOUS WILD POPULATION OF ALPINE IBEX (*CAPRA IBEX*)

Summary

The management of infectious diseases in wildlife reservoirs is particularly challenging and faces several limitations. The development of appropriate management strategies requires a detailed understanding of the factors affecting the transmission and persistence of the infectious agent in the population. Among these factors, heterogeneity of transmission is a common characteristic in natural host-pathogen systems. Indeed, wild animals express a broad range of behaviours, are organised in a variety of social and spatial structures, occupy many areas with very different characteristics and belong to a large diversity of species. Such heterogeneities, from between-individuals to between-species, may result in different contributions to the overall number of new cases of infections. Thus, understanding transmission heterogeneity could provide valuable insights on how to effectively manage these systems, by targeting the individuals or areas that are responsible for most transmissions.

The aim of this thesis was to provide insights on the monitoring and management of infectious diseases in heterogeneous wild populations, using *Brucella melitensis* infection in a French population of wild Alpine ibex (*Capra ibex*) as a case study. The biology of brucellosis and the ecology of Alpine ibex makes this case study a good candidate for transmission heterogeneity at several levels.

Using bacterial examinations, we first established that only 58% of seropositive individuals were at risk to excrete *Brucella*, and that this risk decreased with increasing age. Then, we took advantage of detailed information available on ibex population dynamics, behaviour, and habitat use, and on epidemiological surveys, to build an individual-based model in order to quantify heterogeneity at the individual and spatial levels. The transmission is extremely heterogeneous between individuals, with females generating around 90% of the new cases of brucellosis infection, and between spatial units, three of the five socio-spatial units (the core area) accounting for more than 80% of brucellosis transmission. Using statistical models to estimate the temporal dynamics of the seroprevalence and of the force of infection in the population, we found evidence that the massive captures with test-and-remove operations that were conducted in 2015 managed to reduce brucellosis transmission in the population. Based on these results, we evaluated several predictive disease management strategies in the individual-based model. Our results confirmed that the primary strategy should be to remove as many infected individuals as possible through test-and-remove, and that strategies targeting females and/or the core area are more effective than untargeted management.

Although there is no silver bullet for the management of brucellosis in the population of study, targeted strategies offer a wide range of promising refinements to classical sanitary measures. We therefore encourage to look for heterogeneity in other infection-wildlife systems and to evaluate potential targeted strategies for improving management schemes in terms of efficiency and acceptability.

Keywords: epidemiology, infectious diseases, wildlife reservoirs, modelling, heterogeneity, force of infection, targeted management, *Capra ibex*

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