l'université nantes angers le mans
PÔLE DE RECHERCHE ET D'ENSEIGNEMENT SUPÉRIEUR

Oniris
École Nationale Vétérinaire, Agroalimentaire et de l'Alimentation — Nantes Atlantique
Santé et alimentation au cœur de la vie

Thèse de Doctorat

Pranav Pandit

Mémoire présenté en vue de l'obtention du

grade de Docteur d'Oniris - l'École Nationale Vétérinaire Agroalimentaire et de l'Alimentation Nantes-Atlantique

sous le label de L'Université Nantes Angers Le Mans

École doctorale : *Biologie - Santé*

Discipline : *Epidemiology*

Spécialité : *Infectious disease modelling*

Unité de recherche : *UMR 1300 ONIRIS-INRA Biologie, Epidémiologie et Analyse de risque en santé animale (BioEpAR)*

Soutenue le 26 novembre 2015

Regional spread and control of Q fever in dairy cattle herds:

A multiscale modelling approach

JURY

Rapporteurs:	**Simon MORE**, Professor, University College Dublin, School of Veterinary Medicine
	Emmanuelle GILOT-FROMONT, Professeur, VetAgro Sup Campus Vétérinaire de Lyon
Examinateurs:	**Egil. A. J. FISCHER,** Assistant Professor, Universiteit Utrecht
	Aurélie COURCOUL, Chercheur, ANSES, Laboratoire de santé animale
Directeur de Thèse :	**François BEAUDEAU**, Professeur, *UMR 1300 ONIRIS-INRA*
Co-directeur de Thèse :	**Pauline EZANNO**, Directeur de Recherche, *UMR 1300 ONIRIS-INRA*
Co-Encadrant :	**Thierry HOCH**, Ingénieur de Recherche, *UMR 1300 ONIRIS-INRA*

Regional spread and control of Q fever in dairy cattle herds: a multiscale modelling approach.

by

Pranav Sudhir Pandit
B.V.Sc & A.H., MPVM

Mémoire présenté en vue de l'obtention du
grade de Docteur d'Oniris - l'École Nationale Vétérinaire Agroalimentaire et de l'Alimentation Nantes-Atlantique

L'Université Nantes Angers Le Mans

2015

Dedication

Left intentionally blank

Acknowledgements

Left intentionally blank

Abstract

Regional spread and control of Q fever in dairy cattle herds: a multiscale modelling approach.

Q fever, a worldwide zoonotic disease caused by the bacterium *Coxiella burnetii*, is a looming concern for livestock and public health. Epidemiological features of transmission of *C. burnetii* between cattle herds by wind and trade of cows are poorly understood. We developed a novel dynamic spatial model describing the inter-herd regional spread of the *C. burnetii* in dairy herds, quantifying the ability of windborne transmission and animal trade in *C. burnetii* spread in an enzootic region. Spread of *C. burnetii* between dairy herds of Finistère department (France) was simulated and compared with observed spread of the infection. Our model predictions indicated that the majority of infections in disease-free herds occur due to windborne transmission. Infections acquired through this pathway are shown to cause relatively small and ephemeral intra-herd outbreaks. On the other hand, disease-free herd purchasing an infectious cow could experience higher intra-herd prevalence. Results also indicated that, both transmission routes are independent from each other without any synergistic effect. Lastly, effects of implementation of vaccination on regional spread were assessed by comparing different strategies to select herds for vaccination. Vaccinating cows and heifers of 70% of herds using Phase I vaccine over 10 years resulted in a large simulated reduction in the prevalence of *C. burnetii* positive herds. Vaccinating already infected herds was found to be most effective strategy. Targeting highly connected herds or herds situated in a dense area would also represent a valuable alternative. Besides providing better understanding of *C. burnetii* infection dynamics at regional scale, this work also gives important insights to control the infection in animal populations.

Contents

List of Figures

List of Tables

Chapter 1

Introduction

1.1 History and Background of *Coxiella burnetii*

In early 1933, numbers of cases of febrile illness among abattoir workers in Brisbane were reported. Patients suffered acute onset of the disease along with varied symptoms including fever, headache, shivers and sweats, rash and jaundice. The pathogen was inoculated in Guinea pigs which also developed clinical symptoms [1]. After ruling out common abattoir related diseases such as typhus, undulant fever, paratyphoid and leptospirosis, authorities were convinced that they were dealing a with disease which was not described previously. An investigation of this mysterious feverish ailment was the beginning of the studies on Q fever [1]. Initially, the agent was thought to be a 'filterable virus'[2]. Few decades later the pathogen was classified as rickettsia and the disease was called as 'Queensland rickettsia fever', later was rechristened as 'Q fever', where 'Q' stands for 'Query'[3]. The scientific binomial was decided to be *Rickettsia burnetii* to honour Sir Frank Burnet's contribution in the initial identification of the pathogen. The works of Dr Herald Cox from Montana, USA showed that the pathogen is different enough, deserving its own generic name and was re-named as *Coxiella burnetii*. The bacterium *C. burnetii* is an obligate intra-cellular Gram negative organism. It is now classified under Phylum *Proteobacteria*, Class *Gammaproteobacteria*, Order *Legionellales*, Family *Coxiellaceae* [4, 5]. It occurs in two variants, small cell variant (SCV) and large cell variant (LCV). Metabolically active LCV are intra-cellular forms and they undergo sporogenic differentiation to produce spore like forms called SCV. These SCV are released when infected cell lyse and they can survive for long period in the environment [6]. The bacteria also show phase variation phenomenon (phase I and phase II). This is lipopolysaccharide transition similar to the one seen in Enterobacteriaceae family and is associated with the resistance to complement mediated immunity. Bacterium expressing complete lipopolysaccharides is virulent Phase I [7].

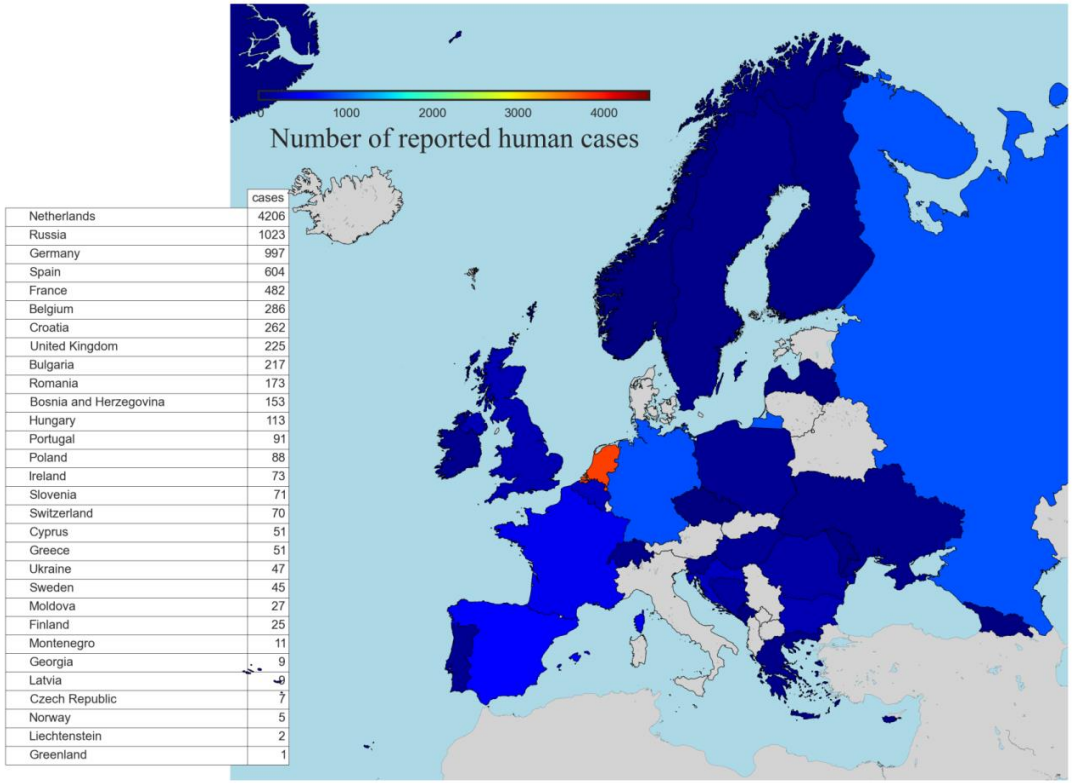
Until now, the pathogen has been isolated from several mammals, birds and arthropods (mainly ticks) [8]. Our understanding about the disease and the bacterium has grown tremendously since its discovery, but 'Queries' about Q fever are still baffling infectious disease specialists. 'There is no disease to match the Q fever for queer stories' [9] as quoted by Dr MacFarlane Burnete himself still holds true after more than eighty years since its discovery. The bacterium is known for its extraordinary versatility as a parasite and the reservoirs of *C. burnetti* are extensive. It is found throughout the world except New Zealand. In 1942, USA listed the bacterium among the agents that can be used as weapon during offensive biological program

and significant studies about bacteriological, clinical, ecological and epidemiological features of the bacterium and the disease were conducted during this period [10-12].

1.2 Impact and global relevance

Q fever is one of the important zoonotic diseases of rising concern. Aerosols generated from infected placentas, faeces and contaminated dust cause infection after inhalation [13, 14]. It is essentially an airborne disease. People who are in close association with animals are known to be at higher risk of getting infected. Zoonotic transmission is mostly associated with abortions in domestic ruminants. The seasonal variation in human cases has been attributed to environmental contamination because of lambing and searing in spring and early summer [15, 16]. Along with direct contact with aborted animals and their birth products, indirect transmission by contaminated wool has been seen on multiple occasions [17]. Raw milk consumption, contaminated clothing and sexual contact are considered as rare routes of transmission in humans [18]. Along with livestock, pets such as cats, rabbits, dogs and sometimes pigeons have been shown to be a potential source for urban outbreaks [19, 20].

The Netherlands suffered one of the largest community outbreaks ever described, which started in 2007. Cases were reported from the provinces of Noord-Brabant and Gelderland [21, 22]. The outbreak which began in 2007 continued till 2009. Cases were attributed to the infected dairy goat farms within the radius of 5 km [23]. Airborne transmission of these contaminated particles excreted during abortions in those farms was facilitated by dry and hot weather during that time [25, 26]. Further studies also indicated that land applied goat manures from Q fever positive goat farms might have also played its role in the sustaining the outbreak during the years of 2007-2010 [27, 28]. Similarly, human cases were reported from Cheltenham, England where sheep farms were identified as source [29].



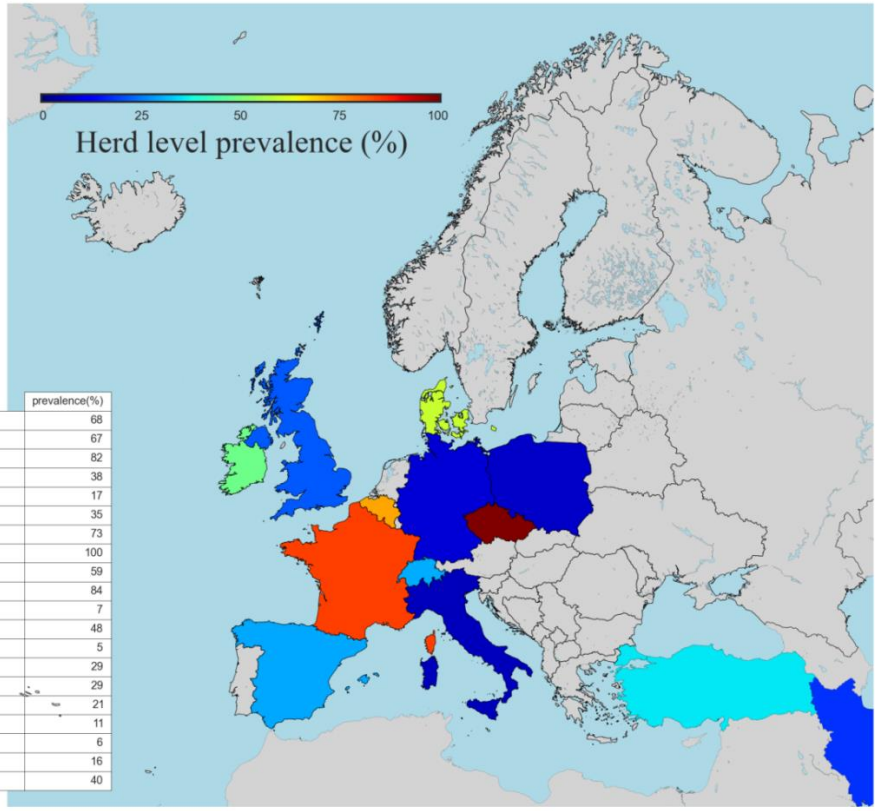
	cases
Netherlands	4206
Russia	1023
Germany	997
Spain	604
France	482
Belgium	286
Croatia	262
United Kingdom	225
Bulgaria	217
Romania	173
Bosnia and Herzegovina	153
Hungary	113
Portugal	91
Poland	88
Ireland	73
Slovenia	71
Switzerland	70
Cyprus	51
Greece	51
Ukraine	47
Sweden	45
Moldova	27
Finland	25
Montenegro	11
Georgia	9
Latvia	9
Czech Republic	7
Norway	5
Liechtenstein	2
Greenland	1

Figure 1 Number of reported Q fever human cases in Europe 2005-14. Data taken from World Animal Health Information System (WAHIS) [24]

In France, between 1990 and 1995, near Marseille and Aix-en-Provence, 289 Q fever patients were reported. Sheep densities were statistically and graphically correlated with these cases and strong mistral winds were thought to be responsible for the transmission of the pathogen from source to humans [16, 30]. A smaller outbreak in the town of Florac, France in 2007 was explained by aerial transmission from ovine flocks in the nearby villages [31]. Since 2005, Europe has reported 9461 human cases of Q fever [24] (Figure 1), with exceptionally high reporting of cases from the Netherlands (4206 cases), Russia (1023 cases) and Germany (997 cases). In these last ten years France reported 482 human cases.

About 60% human patients are asymptomatic seroconversions. In acute infections people mainly show flu like symptoms which include fever, atypical pneumonia and hepatitis. The febrile illness is characterized by sudden onset with temperature reaching up to 40 °C with severe headache, weight loss, myalgy and cough. Chronic Q fever develops invariably in patients with predisposing conditions such as heart valve lesion, vascular abnormalities and immunosuppression. *C. burnetii* infection to pregnant mother poses risks both to mother and foetus, as the bacteria settles in the uterus. Infection in first trimester leads to abortion most of the time and may also lead to foetal death, premature delivery, intra-uterine foetal death, and intra-uterine growth retardation [32].

Interest in Q fever studies is growing worldwide as increasing number of human cases are being seen and is reflected by numerous reviews published recently [8, 33-43]. The disease is now considered as re-emerging zoonotic disease in many countries. A report published in 2012 identifies Q fever as one of the most important thirteen zoonotic diseases to poor livestock keepers, based on its impact on human health and livestock health [44]. Recent events, especially unprecedented number of human cases in the Netherlands, prompted European Union Commission to form a scientific committee at the European Food Safety Authority which reviewed the risk posed by Q fever for humans and animals and advised EU about the scale and distribution of the infection, risk factors for its occurrence and persistence and assessed the effective disease control options. The report states that further investigations are needed to identify the factors influencing the maintenance of the infection along with improved understanding of the transmission pathways in animals, both at intra-herd and inter-herd levels, and clarify the role of environmental conditions and climatic factors in the transmission of bacteria in animal populations and its spillover to humans [45]. The report also reinforces



	prevalence(%)
Cameroon	68
Canada	67
Mexico	82
United States	38
Iran	17
Turkey	35
Belgium	73
Czech Rep.	100
Denmark	59
France	84
Germany	7
Ireland	48
Italy	5
Spain	29
Switzerland	29
United Kingdom	21
Australia	11
Poland	6
Lao PDR	16
Sudan	40

Figure 2 Prevalence of *Coxiella burnetii* in cattle herds in European Countries. Table shows weighted average herd level prevalence in different countries across the World presented in Guatteo et al [39].

focusing on the assessment of different control options in animal populations, subsequently reducing the spillover in humans.

1.3 Q Fever in ruminants

Most of the infected animals remain asymptomatic, so much so that many prefer the term coxiellosis as an appropriate designation [46]. In acute phase the bacteria can be found in blood, lungs, spleen and liver, where as in chronic phase it can be seen persistently shedding in urine and faeces. The infection in mammals can lead to abortion, stillbirth or delivery of weak lamb, calf or kid. In most of the cases, the abortion occurs at the end of gestation without any imminent clinical sign. Aborted foetuses usually appear normal and infected placentas exhibit exudate and intracotyledonary fibrous thickening. In goats the myometrium shows inflammation and in cattle metritis is a very unique manifestation of the disease. Abortion rate can vary from 3% to 80% [47, 48]. Generally, high rates of abortion are seen in caprine herds [47]. Aborting females recover rapidly and do not abort again in successive pregnancies.

The prevalence of the disease is widely studies across the world in different species. Small ruminants and cattle are the most frequently surveyed animals. A systematic review published in 2011, on the prevalence of the *C. burnetii* in the period of 1960 to 2010 [39] re-states the pan-global prevalence of the infection in animals, with high animal level prevalence as well as high prevalence at herd level. The estimates of given in the study are based on a metanalysis of multiple studies having different methodologies, tests and sampling frames. Prevalence of infected cattle herds varied considerably across the world, from 4.4% to 100% (median 37.7%) as shown in Figure 2, while intra-herd prevalence varied from 0 to 47% (median 26.3%). Similarly, in sheep, the herd level prevalence varied from 0 to 89% (median 25%) and in goats the range is 0-100% (median 26%) [39].

1.4 Transmission of *C. burnetii* in animals

There are two distinct transmission patterns of *C. burnetii*. Sylvatic cycle and transmission cycle in livestock. In sylvatic cycle, infection circulates amongst wild animals and in their ecto-parasites especially ticks. Ticks play important role in transmission and maintenance of the infection in wild vertebrates especially in rodents, lagomorphs and wild birds [46]. However, they do not play a significant role in transmission in livestock [41]. In livestock populations the infection is maintained by windborne transmission and purchasing of animals from infected regions. Purchasing infected animals is known to cause abortion storms in goat and cow herds [49-52].

Infected ruminants shed the bacteria through milk, faeces, birth products and vaginal mucus [53, 54]. Chronically infected cows can shed the bacteria for several months [54], and goats shed at successive parturitions [55]. These shedding animals are of importance in transmission of the disease, as the contamination by these animals will lead to infection of susceptible animals as well as humans. The route and the quantity of bacteria shed show great heterogeneity between animals, which can depend on the age, lactation stage and infection state of the animal [53, 54, 56, 57]. In asymptomatic herds cows shed more in milk than in vaginal mucus or faeces [53, 58]. Ewes are generally found shedding via faeces and vaginal mucus. On the other hand goats generally are reported shedding in milk and mucus [53, 57, 59]. Animals get infected by inhalation of the shed bacteria. Mice are very less susceptible orally when compared for their susceptibility to intra-peritoneal inoculation [56], but cats and dogs may be infected by the consumption of placentas [35]. *C. burnetii* is also found in the semen of bull, indicating the possibility of sexual transmission [60].

In cattle the transmission of infection can be divided into two separate components. The maintenance and circulation of the infection within a herd, where infection can pass from one cow to another and second being the circulation of the pathogen between different herds.

Multiple studies have shown the heterogeneity in bacterial shedding by cows and its influence on the transmission of *C. burnetii* within the herd. Earlier study, observing the shedding through different routes (milk, faces, vaginal mucus) by cows in naturally infected herds showed that there is no predominant route of excretion. 65% of the positive cows were shedding only via one route, while only 7% cows were shedding by all the three observed routes [54]. Concentrations of bacteria shed in vaginal mucus or milk can also vary

tremendously (100bacteria/gm to 1,000,000 bacteria/gm) [58]. A longitudinal study following naturally infected cows in herds showed that faecal shedding by cows was very sporadic (>90% of times), shedding in vaginal mucus occurred frequently but for limited time and shedding in milk was persistent [58]. More recent study, focusing on the shedding of bacteria after confirmed abortion because of *C. burnetii*, showed that shedding in vaginal mucus after abortion occur for a very short duration after abortion. Only 8 out of 24 aborted cows were shedding in vaginal mucus after 14 days of abortion [61]. Shedding levels of bacteria in seronegative and seropositive shedding cows in faeces and vaginal mucus just after calving is an important parameter in the transmission of infection within a herd [62].

Predominantly two pathways are known to be responsible for the circulation of *C. burnetii* between different cattle herds. Multiple studies have shown airborne dispersal of the bacterium [29, 63-65], indicating that windborne transmission is one of the routes of inter-herd transmission in cattle herds. Along with this, large number of cattle trade exchanges taking place between herds can introduce the infection to a naïve herd. Very few studies which try to address transmission of the infections in cattle herds at regional scale are available. High regional animal density (odds ratio = 2.34) and in-degree (number of cattle purchasing partners) (odds ratio = 2.31) of herds both were found to be risk factors for the dairy cattle herds in Finistère department, France [51]. Moreover, higher proportion of cases were attributed to windborne transmission than to animal movements in areas with higher cattle density [51]. Studies have also shown that dairy cattle herds in Sweden having environmental conditions conducive for windborne transmission (i.e. strong winds, open landscape, high animal density and high temperature) show very high risk of becoming infected [52]. Besides these few studies, there is a scarcity of studies quantifying the effect of windborne transmission and trade of cows on the transmission of *C. burnetii* within a region.

Quantitative impacts of these transmission routes in a geographical region still need to be estimated. Because of our lack of knowledge about how disease circulates, we lack comprehensive strategy to control the infection which can be implemented in an enzootic region. Controlling infections generally transmitted because of trade of animals between farms is studied for multiple diseases and even is addressed using network graph theory [66-68]. But the disease under question here provides more complex interplay of windborne transmission and cattle trade and strengths of these two routes presumptively depend on the density of animals, geographical conditions, contact network of trading between herds, prevalence of the

infection in the region and few other underlying factors. Hence, to have a global understanding and subsequent control of the infection in cattle herds in a given region we must first:

Problem Statement 1

Quantify the relative contributions of the windborne transmission and cattle trade in the regional spread of C. burnetii between cattle herds

---

C. burnetii infection in cattle is a disease of lesser concern than its implications in small ruminants. Even though, nearly all the recent human outbreaks are linked with small ruminants and no information is currently available with respect to spillover of infection from cattle [45, 65], intensive cattle farming always remains a looming concern for public health. Hence, investigation of infection dynamics in cattle herds at the first sign of its emergence is essential in the emergence-to-control continuum [69]. Moreover, infection in cows being mostly asymptomatic, generating longitudinal databases of the disease prevalence becomes easy as collected data is not sensitive from farmer's perspective. For cattle farms, data on cattle trade and movements is available because of European regulations, which is not easily available for small ruminants. Many regions of the world such as Brittany in France are known for their predominance in cattle farming. Hence these cattle farming regions become very good study cases to understand the transmission of *C. burnetii* in cattle herds via windborne transmission and trade, especially when the epidemiological and trade data are easily available.

1.5 Control of *C. burnetii* in cattle populations

Control of the *C. burnetii* within a dairy cattle herd depends on our understanding of the intra-herd infection dynamics and generally aims at reducing the environmental contamination by bacteria in the long run. The environmental contamination can be reduced by regular cleaning and disinfection of the farm, especially the parturition area. Pregnant animals should be kept in separate pens and their reproductive products should be removed as soon as possible to avoid contamination. In order to achieve *C. burnetii* free herd regrouping of the flocks should be avoided; also contact with wildlife and ticks should be minimized [70].

Previously, antibiotics especially tetracyclines were routinely used in infected animals to reduce the shedding. They were used in two different regimes: at drying off, to prevent late abortion and around calving time to reduce the shedding peak [57]. Few experiments assessing the effectiveness of antimicrobial treatment in reducing the bacterial shedding in cows [71] and in sheep [72, 73] have been performed.

Vaccination is also known as an efficient way of reducing the bacterial shedding through milk, placenta and colostrum [74, 75] and hence to control the disease in a herd [76]. Arricau-Bouvery et al. [77] compared the efficiency of phase I and phase II vaccines in goats: the phase I vaccine prevented abortions and dramatically reduced the frequency of bacteria shedding in the milk, vaginal mucus and faeces, while the phase II vaccine did not affect the course of infection indicating that phase I vaccines are much more effective. Non pregnant susceptible cows when vaccinated with a phase I vaccine are known to have lesser probability of becoming a shedder, hence effectively reducing the bacterial contamination [78]. Many other observational and experimental studies have confirmed the reduction in infection within a herd after vaccination with phase I vaccine [79-83]. For instance, experimental field trial in naturally infected herds with random allotment of chemotherapy and vaccination also showed that vaccinated cows show reduced shedding of bacteria [79]. Modelling study predicting the effects of the vaccination within a naturally infected herd showed that vaccinating only for first three years will not stabilise the infection dynamics and also showed that vaccinating both heifers and cows is slightly more efficient than vaccinating only cows [84]. Other control options can be used in emergency situations when public health is at risk. Culling of pregnant animals, temporary breeding ban or control of animal movements are some of the measures implemented in the Netherlands during the outbreak in 2007 [45, 65].

Vaccination is a prospective technique which can be applied on regional scale. For effective implementation of vaccination in a region, field experiments, optimisation based on data is essential [45, 65]. In a given region of study, identifying dominant route of transmission between herds aids in tremendous manner to formulise a control strategy. Implementation of prospective control strategy should account for the relative contributions of transmission routes in the regional spread. Specific herds, identified as important in the chain of transmission for each of the transmission route, can be targeted for the implementation of control strategy. This leads us to the second problem statement of the thesis.

Problem Statement 2

Assessment of the effectiveness of vaccination in controlling the regional spread of C. burnetii in cattle farms of an enzootic region.

1.6 Modelling approach

The spread of the *C. burnetii* is a complex phenomenon poorly understood. The infection is known to spread by wind and the bacteria travel to newer places through diffusion of contaminated aerosols. These processes are difficult to observe on the field. Moreover, large number of dairy animal herds in the study region and their intricate network of contacts (animal trade) contributing to the spread of pathogen, it is very convenient and reliable to use modelling approach to understand the spread of the disease. Use of generic epidemiological models is also considered as a robust framework to assess control options for Q fever in domestic ruminant populations [45].

A mathematical model of a biological phenomenon is an abstract representation of the system, formulised by systems of equations. The core of the models lies in the underlying assumptions made on biological mechanisms while structuring the model. The models help us to come to logical conclusions based on the assumptions of the model. The framework of the model helps modellers to reach conclusions which perhaps can be non-intuitive, keeping in mind that the certainty about conclusions always remains relative to assumptions [85]. Studies that use mathematical models generally have objectives which can be broadly classified into two groups: understanding (characteristics and mechanisms of biological system) and prediction (speculating the future progression of the system). These models are applied on various scales of biology: ranging from modelling of gene and cellular structures in systemic biology, evolutionary models describing trends in evolution to modelling phenomena at the scale of large ecosystems (ecological modelling).

From mathematical point of view, different formalisms and technical choices are available: stochastic or deterministic models according to if demographic and environmental stochasticity of modelled phenomena are taken into account or not; in discrete or continuous time; with

various structuring variables (such as age); at various granularities and scales (individual based, in population, metapopulation,). The dynamic character of these types of models allows us to follow the evolution of variables of interest (such as number of infected individuals) over time and also in space (if spatial component is involved in the model). The appropriate choice is made for each specific biological system under study [86].

Classically health states of individuals considered in a simple modelling framework are S (susceptible) and I (infectious). These as they are called as SI models, are suitable for very few diseases such as HIV where transition stage before becoming infectious are not seen and individuals remain infectious throughout life if not treated. In a case of disease where an individual recovers and become susceptible to infection, the model becomes SIS. Though in reality such simplistic transitions are rarely seen, commonly used simple formulations of a compartmental mathematical model of diseases are SIR and $SEIR$ as shown in figure 3.E stands for exposed and represents latent stage of infection status when individuals are not infectious and might or not show clinical signs. R stands for recovered individuals which are immune to the disease.

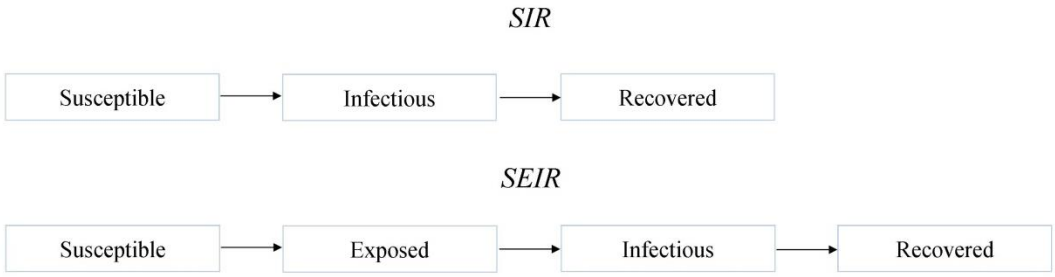


Figure 3 Structure of SIR and SEIR models

Modelling as a tool to understand the basic epidemiological process of disease dynamics in populations is now a well-established technique [86, 87]. Insights gained from such models are robust and generic and hence help in collecting epidemiologically more relevant data, and in deciding important elements in the system [86]. In recent years, use of such epidemiological models for animal diseases is increasing with more focus on diseases of higher concern such as FMD [88-93], avian influenza [94-100]. Modelling of enzootic diseases such as Bovine Viral Diarrhoea Virus [101-103], paratuberculosis [104-106], salmonella [107-109] is also becoming common. These studies aim either to understand the disease transmission (pathogen/ hosts

interactions, contact structure, transmission routes etc.) or to identify the most efficient prevention and control strategy at group/farm level or at larger scale of a specific region, country.

Since it is easy to numerically simulate large number of experiments, which are otherwise not-possible to conduct in field; large epidemiological studies can be conducted using simulation models. When large population is studied, scenarios which are improbable to occur and observe naturally can be simulated easily to thoroughly explore the behaviour and reach a theoretical conclusion.

Given the advantages of modelling approach, multiscale mechanistic modelling of *C. burnetii* in a cattle metapopulation integrating cattle trade and meteorological data becomes a very promising scientific endeavour. Multiscale models have been frequently implemented to describe spread of the infections in metapopulations [102, 110-115]. Here, two scales which are under consideration are herd scale (intra-herd dynamics) and regional scale (inter-herd dynamics). One of the main advantages of using mechanistic models is that they allow determining the causes of infection, and subsequently help assessing targeted interventions [114] with easy integration of data with model.

1.7 Objectives and outline of the thesis

The two main objectives of the thesis are: (i) the assessment of relative role of airborne transmission and transmission because of cattle trade in the regional dynamics of *C. burnetii* and (ii) the assessment of the effectiveness of vaccination strategy to reduce the spread of infection within a region. These questions were tackled in three distinct stages presented here as separate chapters.

First, a mathematical model describing the spread of *C. burnetii* between cattle herds over a region via windborne transmission and cattle trade was conceptualised. This model is presented in chapter 2. The chapter explains the framework of the metapopulation model. We detail the intra-herd model adapted from Courcoul et al [62] along with the changes done in the original intra-herd model to couple it with Gaussian dispersion model and cattle trade model. Following that, concept of dispersion model, different types of dispersion models which are generally utilized are described. Then, we briefly present the framework of Gaussian dispersion model which is implemented here. At the end of the chapter, we detail part of the model which simulates the cattle trade in a region. The chapter also focuses on how all these different model

sections are integrated with each other to generate a meta-population model, its use in identifying the causes of disease introduction in disease free herds and its ability to describe the disease progression at regional scale and at individual herd level.

Chapter 3 is an example of the case study where we apply the conceptualized model. We use the model to simulate the spread of *C. burnetii* in dairy herds of Finistère department, France. First we describe the data available for the region: epidemiological data on the heard infection status, cattle trade data and wind velocity data. We predict the spread of infection in the dairy cattle herds for the period 2012-2013 and compare the prediction results with the observed data using receiver operating characteristic analysis. Along with the relative contributions of windborne dispersion and cattle trade in the spread of *C. burnetii* in the region, we also study in detail the differences in the intra-herd dynamics of newly infected herds because of windborne transmission and cattle trade. We finally simulate the model over a period of ten years while addressing the contributions of routes. Methods developed in Chapter 2 and results of Chapter 3 constituted the first research paper which is currently under revision in Journal *Veterinary Research* (see appendix II).

As we proceed toward addressing the second goal, while contemplating about possible control strategies which can be implemented, we explain the selection of vaccination as prospective control strategy over other possible control techniques. We present the assumptions underlying approach of modelling the vaccination. Secondly, we briefly describe the intra-herd dynamics of an isolated vaccinated herd. Then we generate different vaccination scenarios in metapopulation where we implement this strategy in targeted herds based on their epidemiologically relevant features and assess the effectiveness of vaccination strategies.

Chapter 5 finally provides the general discussion on the whole project. It discusses the main results with respect to both objectives and their implications in the field. It also discusses the modelling methodology used here along with its limitations. Final thoughts and prospects are also mentioned.

Chapter 2

Conceptualization of the metapopulation model

Chapter 2 and 3 constitutes the first research paper which is under revision in journal Veterinary Research

Pandit P, Hoch T, Ezanno P, Beaudeau F, Vergu E: **Q fever spread between dairy cattle herds in an enzootic region: modelling contributions of windborne transmission and trade.** *Veterinary Research*, under review

Manuscript submitted to Journal Veterinary Research

Initial Submission: 13th July 2015

Editor Decision: 10th August 2015

First Author Revision: 27th August 2015

This chapter focuses on describing the generic model developed for representing the spread of *Coxiella burnetii* between dairy cattle herds. We will first describe the overall concept of the model followed by detailed description of the assumptions made and equations for each section of the model.

2.1. Overview of the metapopulation model

Metapopulation consists of a group of spatially separated populations of individuals with their own distinct dynamics which interact with each other through various dynamics processes [116]. Metapopulations can occur naturally as well as are created as a result of human actions. Natural ponds in forests, mixed forests with grasslands, islands archipelagos are examples of habitats which are naturally found metapopulations [117, 118].

Livestock farming in a geographical area is a very good example of metapopulation system formed due to human activities. Subpopulations, i.e. the farms, are spatially constrained and specified. In feedlot beef production farms and dairy farms where pasturing is limited, the animals in subpopulations remain constrained to a very specific geographical region and have controlled interactions with other subpopulations. These interactions mostly can be in the form of trade of animals or sharing of environmental resources. To model spread of a pathogen between dairy herds, metapopulation framework of model becomes a suitable option [86, 102, 114, 115, 119-123].

C. burnetii is an enzootic pathogen and the natural progression of the disease in an infected cow is slow. Results of multiple studies also indicate that the transmission of *C. burnetii* within a herd is a slower process [62, 124-128] when compared with other fast spreading highly infectious diseases which cause epizootic outbreaks in animal populations such as Foot and Mouth Disease (FMD) and Highly Pathogenic Avian Influenza (HPAI). In mathematical models of highly contagious viruses, infection dynamics within a subpopulation is simplified and its representation in the metapopulation model is minimalistic [97, 100, 129, 130]. Here, for modelling *C.burnetii* transmission, the dynamics of the infection within a herd and the herd management techniques occur at the same time scale and cannot be ignored as they are known to be very influential in the infection transmission process. Hence, a detailed intra-herd model is essential in the metapopulation framework when modelling the spread of pathogens such as *C.burnetii*. Such multiscale models where the dynamics is modelled at both the scales of herd and region are already used for models of diseases such as paratuberculosis, bovine tuberculosis and bovine viral diarrhoea [102, 114, 131, 132].

The model for *C.burnetii* transmission over a region can be conceptualised by dividing the processes modelled into two distinct parts. The first part describes the infection spread within an infected dairy

herd. The infection dynamics within a herd is represented using a stochastic model adapted from Courcoul at al [62]. The second part of the model connects such local models of intra-herd dynamics to describe regional propagation leading to the between herd transmission section of the model. Regional spread of the infection is described by two processes, namely the dispersion of the infectious particles by wind and the introduction of infected animals through cattle trade. As described earlier in Chapter 1, other pathways of transmission such as tick vectors do not play significant role in the transmission of the infection in livestock [41, 65].

Hence, in brief, the regional spread of *C. burnetii* was conceptualised by a multiscale model (inter-herd and intra-herd scales), with spatially separated herds having their own infectious and demographic dynamics and interacting with each other via cattle trade and windborne dispersion (Figure 4).

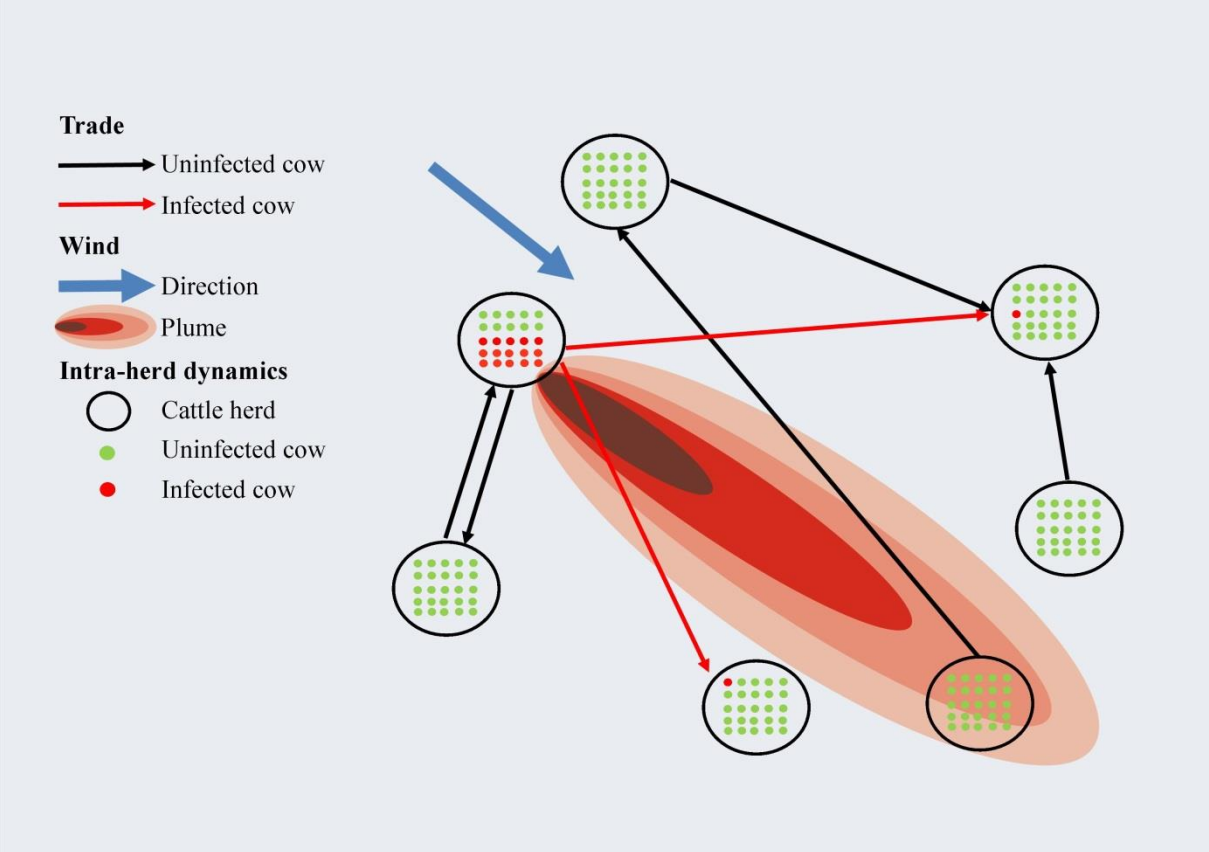


Figure 4 Metapopulation framework of the model. A hypothetical representation of a cattle herd metapopulation with infection-free herds (only green points – susceptible animals) and one infected herd (containing red points – infected animals). The inter-herd

dynamics is governed by cattle trade (arrows) and windborne dispersion of pathogen (red plume).

2.2. Intra-herd dynamics of infection and demographics

The intra-herd model used in the study is adapted from a stochastic discrete-time individual-based model introduced earlier by Courcoul et al. [62]. This is a data driven model where the parameters describing the transition from one health state to another are estimated from longitudinal observational study using Bayesian estimation methods [124, 133]. Similarly the herd dynamics of within a dairy farm is based on the observational data and management practices followed in a typical dairy herd of Western France.

2.2.1. Intra- herd infection dynamics

The model of infection dynamics is conceptualised using standard compartmental structure (Figure 5) with added complexity concerning shedding routes, sero-positivity and intermittency in shedding. Cows of the herd undergo different transformations in their health states as shown in Figure 5, with parameters defined in Table 1. A susceptible, non-shedder, sero-negative cow (*S*) becomes infected and changes its health state to shedder sero-negative cow (I^-). The probability of infection for S cows (transmission through the environment) depends on the bacterial load present in the herd environment (equation 1). I^- cows then become sero-positive, either I^+ (shedder, with antibodies) or $I^{+\text{milk pers}}$ (shedder with antibodies, permanently shedding in milk at higher levels) or return to S state. Shedder sero-positive (I^+) cows then can become carriers which do not shed, C^+ (with antibodies) and subsequently C^- (without antibodies). C^+ cows can restart shedding. Shedding cows can shed the bacteria through milk, mucus/ faeces or through all the three routes with distributions α, β, β_{calv}, γ and γ_{calv} depending on the health-states $I^-, I^+, I^{+\text{milk pers}}$ respectively. Low, medium and high levels of shedding, corresponding respectively to a quantities (Qty) shed equal to 1/3000, 1/30 and 1 unit of environment per week were considered with distributions of Q1 to Q5 according to the type of shedder and lactation stage (Table 2). The proportions of cows shedding through different routes and at different levels change according to whether cows are in early lactating stage ($\leq$ 4 weeks post calving) or not. The quantities ε1, ε2 and ε3 are the amounts of bacteria shed during a time step by an individual I^-, I^+ and $I^{\text{milk pers}}$, respectively, and contaminating the environment. These are the sum of quantities shed by all the shedders through all the shedding routes times the fraction ($\rho^{m/f}$and ρ^m) of these quantities reaching the environment of the herd.All the parameters related to heterogeneity in shedding are presented in Table 2. The probability $p_i(t)$ of a susceptible

cow of herd i to acquire infection at time t depends upon the environmental compartment ($E_i(t-1)$) of the herd:

$$p_i(t) = 1 - e^{-(E_i(t-1))} \quad \textbf{(1)}$$

$E_i(t)$ [number of bacteria / time step] is equivalent to the force of infection related to the bacterial contamination of the environment (for simplicity of writing the time step multiplying $E_i(t-1)$ was omitted in equation 1). It corresponds to the bacterial load at time t (expressed in infectious doses) shed by shedding animals (according to their infection status and the shedding route), times the contact rate between animals and the environment, times the probability that a contact of a susceptible animal with an environment contaminated by one infectious dose leads to a successful infection event. Similar formulation of probability of infection has been proposed previously for aerosol infection of *C. burnetii* [134].

Equations (2-7) describe the updating (between time steps *(t-1)* and *t*) of variables corresponding number of cows of a health state in herd *i*:

Susceptible cows

$$S_i(t) = S_i(t-1) - NI_i^-(t) + NS_i(t) \quad \textbf{(2)}$$

$$I_i^-(t) = I_i^-(t-1) + NI_i^-(t) - NS_i(t) - NI_i^+ - NI_i^{+milk\ pers} \quad \textbf{(3)}$$

$$I_i^+(t) = I_i^+(t-1) + NI_i^+(t) - NI^+C_i^+(t) + NC^+I_i^+(t) + NC^-I_i^+(t) \quad \textbf{(4)}$$

$$I_i^{+\ milk\ pers}(t) = I_i^{+milk\ pers}(t-1) + NI_i^{+milk\ pers}(t) - NI^{+milk\ pers}C_i^+(t) \quad \textbf{(5)}$$

$$C_i^+(t) = C_i^+(t-1) + NI^+C_i^+(t) + NI^{+milk\ pers}C_i^+(t) - NC^+I_i^+(t) - NC^+C_i^-(t) \quad \textbf{(6)}$$

$$C_i^-(t) = C_i^-(t-1) + NC^+C_i^-(t) - NC^-I_i^+(t) \quad \textbf{(7)}$$

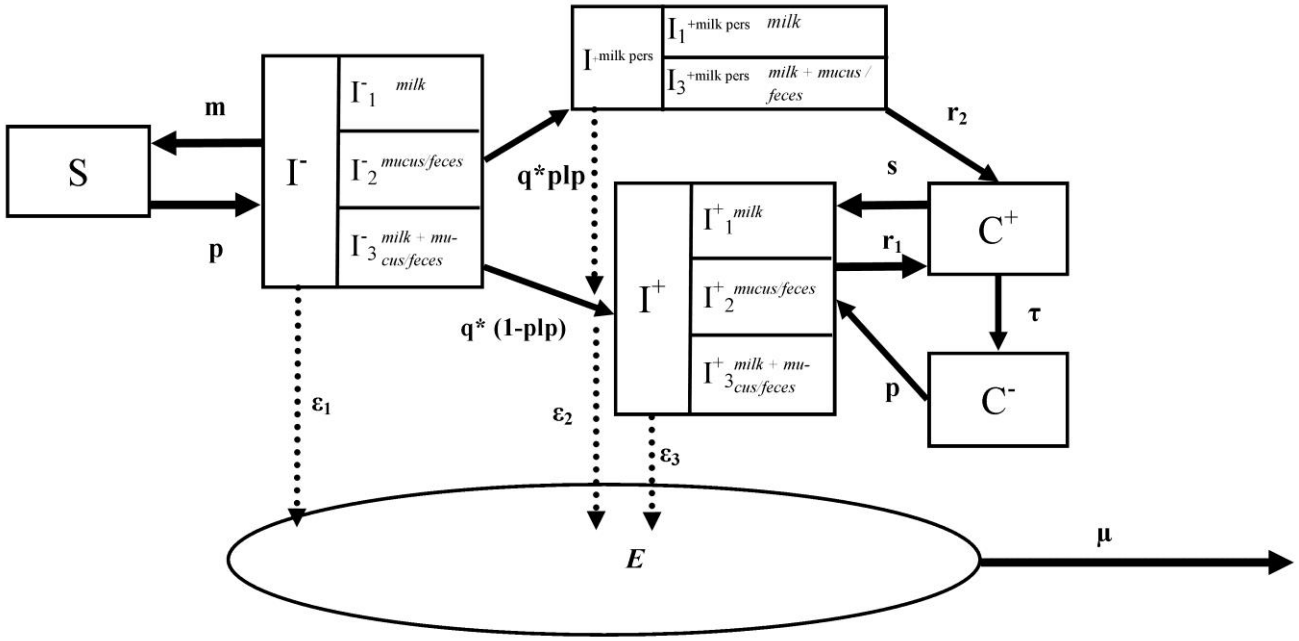


Figure 5 Schematic presentation of the model describing health states of cows and transitions between these states, and environmental bacterial load of the herd (adapted from [14]). Variables and parameters are defined in the text here above and Table 1.

This updating is written using new variables NS_i $NI_i^-, NI_i^+, NI^{+milk\ pers}C_i^+(t), NI^+C_i^+,$ $NI^{+milk\ pers}C_i^+(t), NC^+C_i^-(t), NC^+I_i^+$ and $NC^-I_i^+$; describing (new) flows entering or going out from a given health state compartment . Dynamics of these variables are given by equations (8-13).

$$NI_i^-(t) \sim Bin\ (S_i(t-1), p_i(t)),\ where\ p_i(t) = 1 - e^{-(E_i(t-1))} \quad \textbf{(8)}$$

$$(NS_i(t), NI_i^+, NI_i^{+\ milk\ pers}) \sim Multinom(I_i^-(t-1), (\frac{m}{m+q}, \frac{qpIp}{m+q}, \frac{q(1-pIP)}{m+q})) \quad \textbf{(9)}$$

$$NI^+C_i^+(t) \sim Bin\ (I_i^+(t-1), r_1\ ) \quad \textbf{(10)}$$

$$NI^{+milk\ pers}C_i^+(t) \sim Bin\ (I_i^{+milk\ pers}(t-1), r_2\ ) \quad \textbf{(11)}$$

$$(NC^+C_i^-(t), NC^+I_i^+(t)) \sim Multinom(C_i^+(t-1), (\frac{\tau}{\tau+s}, \frac{s}{\tau+s})) \quad \textbf{(12)}$$

$$NC^-I_i^+(t) \sim Bin\ (C_i^-(t-1), p_i(t)) \quad \textbf{(13)}$$

Based on equations (2-7), it is possible to define two main outputs of the model at the herd level as:

$$Seroprevalence_i(t) = I_i^+(t) + I_i^{+\ milk\ pers}(t) + C_i^+(t) \quad \textbf{(14)}$$

$$Shedders_i(t) = I_i^-(t) + I_i^+(t) + I_i^{+\ milk\ pers}(t) \quad \textbf{(15)}$$

The overall dynamics of the environmental infection force is governed by animals shedding through different routes at different stages of their reproductive cycle and at different levels of shedding. According to [62], this hence can be summarized in the following equation:

$$E_i(t) = E_i(t-1)(1-\mu) + \sum_{k,l}\left(\rho^k Qty_l \sum_{i,x,w} n_{t,xwkl}\right) \text{ [62]} \quad \textbf{(16)}$$

where, $x \in \{I^-, I^+, I^{+\ milkp\ pers}\}$ are the different health states of cows which can shed the bacteria, $w \in \{\leq 4\ weeks\ post\ calving\ , > 4\ weeks\ post\ calving\}$ is the state of reproductive cycle of the cow, $k \in \{milk, mucus/feaces\}$ is the route by which bacteria are shed, $l \in \{low, medium, high\}$ is the level of bacterial shedding,

$n_{t,xwkl} \sim Multinomial\left(N_{t,xwk}, Q_{c,(x,w,k)}\right)$ with $N_{t,xwk}$ the number of animals in corresponding health state and shedding route at time *t* and $Q_{c,(x,w,k)}$ (*Q1 to Q5*) the probability distributions governing shedding levels. The remaining parameters are defined in Tables 1 and 2.

Table 1 Parameters of infection dynamics within a dairy herd (adapted from [13, 14])

Parameter	Definition	Value
m	Transition probability $I^- \Rightarrow S$	0.7
q	Transition probability $I^- \Rightarrow I^+$	0.02
pIp	Proportion of cows going from I^- to I^+ and becoming $I^{+milk\ pers}$	0.5
$r1$	Transition probability $I^+ \Rightarrow C^+$	0.2
$r2$	Transition probability $I^{+milk\ pers} \Rightarrow C^+$	0.02
s	Transition probability $C^+ \Rightarrow I^+$	0.15
τ	Transition probability $C^+ \Rightarrow C^-$	0.0096
μ	Proportion of bacteria eliminated due to death and to plume generation (can be written as $\mu_{death} + \mu_{plume\ source}$)	0.2
p	Infection probability	$1 - e^{-E}$
$\rho^{m/f}$	Proportion of bacteria shed through mucus/faeces filling the environment compartment	0.28
ρ^{m}	Proportion of bacteria shed through milk filling the environment compartment	$0.125\rho^{m/f}$

2.2.2. Herd demographics and interplay between demography and infection

The intra-herd model also captures the herd demography and for this component it completely follows the assumptions presented in previous intra-herd model study [62]. The model incorporates only cows. Heifers, calves and bulls are not represented. Purchase of cows by farmer is modelled according to the data and is explained in detail in the following section. In a herd, only susceptible primiparous cows, which have just calved (i.e. heifer becoming lactating cows) enter the herd at any time of the year. Introduction of such new heifer ceases if the herd size is above 1.15 times the initial herd size, a value in agreement with field practices. Recruiting of heifers at a given time step is implemented using a binomial draw with probability of occurrence of $^{\text{Replacement rate(year}^{-1})}/_{52}$. The replacement rate is given in Table 3.

Table 2 Description and probability distributions used for different shedding routes and levels.

Parameter		Definition	Value
α	α_1, milk	Probability distribution of the shedding routes for the I^- cows	0.31
	α_2, mucus/faeces		0.62
	α_3, milk+mucus/faeces		0.07
β	β_1, milk	Probability distribution of the shedding routes for the I^+ cows after 4 weeks post-calving	0.61
	β_2, mucus/faeces		0.33
	β_3, milk+mucus/faeces		0.06
β_{calv}	β_{calv1}, milk	Probability distribution of the shedding routes for the I^+ cows in the 4 first weeks post-calving	0.14
	β_{calv3}, mucus/faeces		0.5
	β_{calv3}, milk+mucus/faeces		0.36
γ	γ_1, milk	Probability distribution of the shedding routes for the $I^{+milk\ pers}$ cows after 4 weeks post-calving	0.83
	γ_3, milk+mucus/faeces		0.17
γ_{calv}	γ_{calv1}, milk	Probability distribution of the shedding routes for the $I^{+milk\ pers}$ cows in the 4 first weeks post-calving	0.25
	γ_{calv3}, milk+mucus/faeces		0.75
Q1	Low level	Probability distribution of the shedding levels for all the I^- and for the I^+ shedding in mucus/faeces after 4 weeks post-calving	0.85
	Mid-level		0.15
	High level		0
Q2	Low level	Probability distribution of the shedding levels for the I^+ shedding in milk after 4 weeks post-calving	0.4
	Mid-level		0.5
	High level		0.1
Q3	Low level	Probability distribution of the shedding levels for all the I^+ in the 4 first weeks post-calving	0.25
	Mid-level		0.25
	High level		0.5
Q4	Low level	Probability distribution of the shedding levels for the $I^{+milk\ pers}$ shedding in mucus/faeces after 4 weeks post-calving	0.6
	Mid-level		0.4
	High level		0
Q5	Low level	Probability distribution of the shedding levels for all the $I^{+milk\ pers}$ shedding in milk and for the $I^{+milk\ pers}$ in the 4 first weeks post-calving	0.15
	Mid-level		0.6
	High level		0.25

The probability of a cow to get culled depends on its lactation year. The culling can also occur at any time step of the simulation and culling stops if the initial herd size is below 0.85 times of the initial herd size. The probabilities of culling according to the age are given in Table 3.

For each cow, we represent the lactation/gestation cycle. We consider inter calving interval of 55 weeks. The lactation cycle is composed of 47 weeks of lactation starting at calving followed by 8 weeks of dry period. The gestation cycle is composed of a non-gestation period of 15 weeks starting at calving followed by a gestation of 40 weeks. The lactation cycle of a cow is represented in illustration shown in Figure 6.

Table 3 Description of the parameters of the herd demography model and their standard value

Description	Standard Value
Replacement rate (year^{-1})	0.355
Culling rate (week^{-1})	
Lactation 1	0.0057
Lactation 2	0.0052
Lactation 3	0.0065
Lactation 4	0.0067
Lactations 5 & 6	0.0161

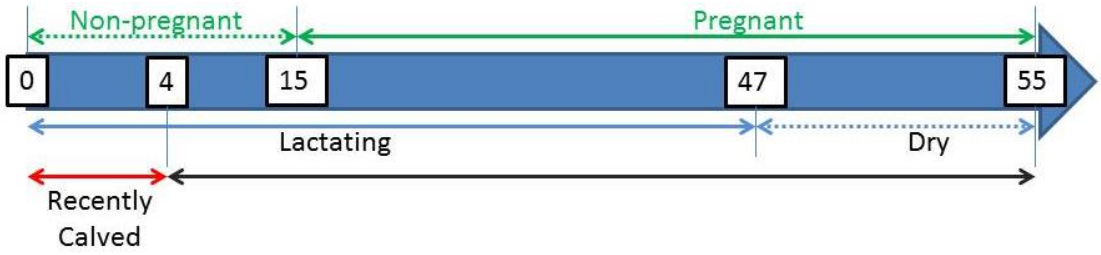


Figure 6 Illustration of lactation cycle of a cow (in weeks)

An infected cow can abort at any time after 3 weeks following its resumption of shedding (which can occur during a transition from S to I^- from C^+ to I^+ or from C^- to I^+). It is also assumed that a cow can abort only once during its lifetime. The amount of bacteria shed at the time of abortion depends on the trimester in which it aborts. If it aborts in the first two trimesters, it sheds moderate quantity of bacteria, else in the last trimester it sheds high quantity of bacteria through route 'faeces/mucus'. If cow aborts during the first two trimesters the dry period is reduced to 8 weeks (instead of 15), and if a cow aborts after week 22 of gestation, it starts a new lactation. If it aborts before, its current lactation continues for a maximum of 50 weeks of lactation. Afterwards, it is dried off.

We also assume that from spring to the beginning of winter (mid-March to mid-November) dry cows are kept on pastures and they shed bacteria in $E_{pasture}$. The probability of infection is

thus lower than for lactating cows and based on $E_{pasture}$ for dry cows during this period. Outside this period, there is only one compartment for the environment in which all the cows (lactating and dry) shed bacteria. This environment is $E_{building}$ (Figure 7).

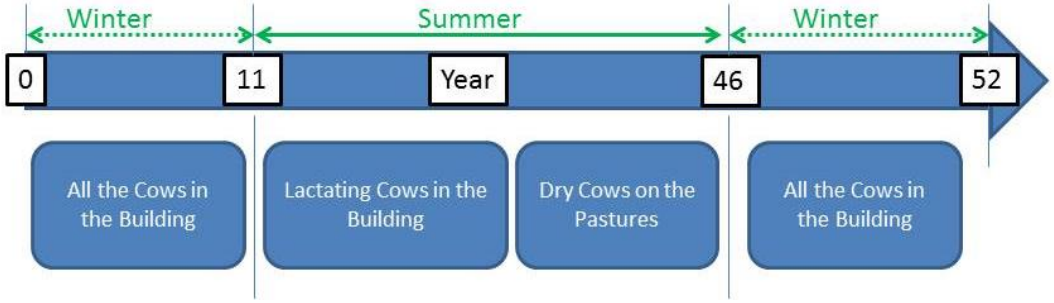


Figure 7 Yearly cycle of use of pastures by cows according to their lactation cycle (in weeks)

The shedding of bacteria by an infected cow thus depends on its status in gestation cycle, subsequently its status in lactation cycle. As shown in Table 4, recently calved (weeks 0-4) cow will shed higher quantities of bacteria governed by distributions Q3 for I^{+} through routes governed by β_{calv} distribution and Q5 for $I^{+milk\,pers}$ through routes governed by γ_{calv} distribution.

Table 4 Table describing the gestation, lactation status and corresponding bacterial excretion status for infected cows

Weeks in cycle	Gestation	Lactation	Excretion Status
0-4	Non-pregnant	Lactating	Higher Shedding Parameters
4-15	Non-pregnant	Lactating	Normal Shedding Parameters
16-47	Pregnant	Lactating	Normal Shedding Parameters
47-55	Pregnant	Dry	Normal Shedding (Faeces and Mucus)

2.3. Inter-herd windborne transmission of *C. burnetii*

Atmospheric dispersion modelling refers to mathematical description of contaminant transport in the atmosphere. Generally, the term *dispersion* describes the combination of advection (due

to wind) and diffusion (because of eddy diffusion) [135]. These models have been used in various fields ranging from studying emissions from large industrial operations, volcanic eruptions [136], seed pollen and insect dispersal [137-139], odour propagation from livestock facilities [140], nuclear contamination [141], and pathogen dispersal [97, 130, 142].

2.3.1. Types of dispersion models

Gaussian dispersion models: These are the earliest developed dispersion models, and consider normal probability distribution for diffusion within the atmosphere. They are used to describe for both non-continuous (puff models) and continuous (plume) air pollution dispersion. In puff models, two important assumptions are made: (i) wind speed and direction to determine variations in the position of the centre of each puff and (ii) decrease in the concentration around the centre of the puff to determine the age of the puff [143].

In plume model assumptions are that the wind speed is over 1 ms^{-1} and transfer time is long enough for pollutant to reach long distance (but still less than 10 km) [143].

Lagrangian dispersion models: These models consider mass conservation equation by following each and every particle. Advantages associated with these models include simplicity, flexibility and relatively accurate results in turbulent atmospheric conditions and complex field terrain conditions [130, 143].

Two studies one about FMD dispersion [130] and the other about dispersion of radionuclides [144] comparing Lagrangian and Gaussian models showed that both the models predicted similar directions of infection and differences were attributed to the way atmospheric conditions were inferred in these models. Lagrangian models were found applicable to nearly all real life situations with atmospheric conditions with vertical wind shear and wind fields modified by topography [145]. On the other hand, Gaussian dispersion models are useful for temporally and spatially constant and isotropic wind and turbulent conditions. They are useful only when the topography is moderately plain. Commercially and established algorithms already developed and available are given in Table 5. They are used in multiples epidemiological studies to study the dispersion of pathogens due to wind [29, 64, 130, 143, 146, 147].

Use of dispersion models to model the windborne dispersion of *C.burnetii* can be seen in following two studies. Wallensten et al used Lagrangian model "NAME" to both identify the risk for people to get infected from suspected farms and to identify source herds based on the air quality in Cheltenham, UK [29]. A recent study correlates human cases in the Netherlands

using Operations Priority Substance Short Term model [64]. Both studies concern the zoonotic spillover of the infection from livestock to humans with a more or less similar aim of identifying source livestock. None of the study as per our knowledge uses dispersion model in the context of transmission between livestock farms.

Table 5 Atmospheric dispersion models available and developed

Model types & developers	
Gaussian Models	**Lagrangian Models**
PLUME (UK National Radiological Protection Board)	NAME (UK Met Department)
ADMS 3 (Cambridge Environmental Research Consultants)	NARAC (National Atmospheric Release Advisory Center, US)
ICAIR 3V (Maisons-Alfort, France)	MLD0P (Canadian Meteorological Center)
VetMet (Danish Meteorological Institute): Local scale Rimpuff	
PDEMS: Plume Dispersion Emergency Management System, Calpuff	
AIWM: Australian Department of Agriculture, Fisheries and Forestry	
Operations Priority Substance Short term Model (RIVM, Netherlands)	

2.3.2. Gaussian dispersion model describing airborne transmission of C.burnetii

As Gaussian plume models have shown significantly good accuracy and are known to be less intensive for implementation, we used this tye of model with deposition to describe the airborne dispersion of bacteria from one herd to another. Gaussian dispersion model describes the concentration profiles of particles over three axes in the direction of the wind from a continuous source (Figure 8). Basic two dimensional Gaussian dispersion model equation for the calculation of plume concentration *C* at *x, y* location at time *t* is

$$C_{(x,y,0,t)} = \frac{Q_{(t-x/u)}}{2\pi\sigma_x(x)\sigma_y(x)} e^{\left[-\frac{y^2}{2\sigma^2{}_y(x)}\right]} \quad \textbf{(17)}$$

Where standard deviation of Gaussian dispersion function is $\sigma_y^2 = \frac{2K_y(x)}{u}$ *and* $\sigma_x^2 = \frac{2K_x(x)}{u}$, K_x and K_y (eddy diffusivity coefficients) = 0.03(m^2/s), u is wind velocity (m/s), x and y are differences (meters) between coordinates of source and destination on each

axis. $Q_{(t-x/u)}$(bacteria/s) is the bacterial source at time $t - x/u$. Different Gaussian dispersion equations used for different scenarios are reviewed by Stockie [135].

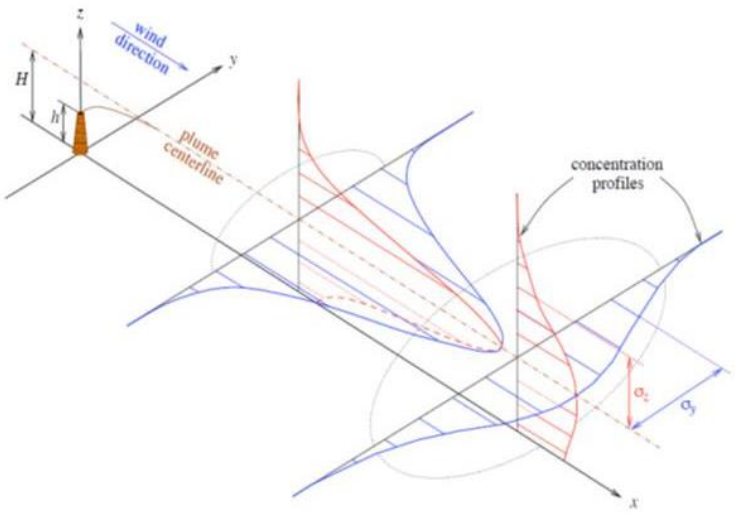


Figure 8 A contaminant plume emitted from a continuous point source, with wind direction aligned with the x–axis. Profiles of concentration are given at two downwind locations, and the Gaussian shape of the plume cross-sections are shown relative to the plume centreline (taken from Stockie J. M., 2011 [135])

Bacterial loss in the environmental compartment can result from various mechanisms such as natural death and from dispersion due to wind. The bacterial source for the plume is therefore a proportion of lost environmental infection force which was estimated from Courcoul et al [62]. The small cell variant (SCV) of bacteria shed is very resistant to the environmental conditions and can survive well in the environment [19]. Plume transportation takes place with simultaneous deposition and settling of particles. Indeed, the small droplets generated by sneezing, coughing, splashing and other activities remain suspended in the air and dry fast enough to produce small particles called droplet nuclei which can remain suspended in the air for long duration and can be transported along with the wind to distant places, unlike larger particles. Hence, the inherent capacity of windborne transmission of any infectious agent depends on the production of the appropriate range of droplet particle sizes with viable pathogens [148]. Multiple studies have suggested a higher risk of windborne transmission of Q fever within the radius of 5 km from the source in moderate environmental conditions [23, 29]. Hence we restrict our dispersion model to a radius of 5 km from the source herd.

Plume transportation takes place with simultaneous deposition and settling of particles. The transport and deposition of infectious particles were modelled by underlying Equation (18), to account for phenomena such as settling and gravitation [135, 149]. The concentration (*C)* of bacteria reaching herd *i* from source herd *j* (where *x*, *y* are differences in respective coordinates of herds *i* and *j*) was calculated using the following equation:

$$C_{i,j,(x,y,z)} = \frac{Q_j}{2\pi U\sigma_y\sigma_z} e^{\left(\frac{-y^2}{2{\sigma_y}^2}\right)} e^{\left(\frac{-W_{set}(z-h)}{2K_z} - \frac{{W_{set}}^2{\sigma_z}^2}{8{K_z}^2}\right)} \left[e^{\left(\frac{-(z-h)^2}{2{\sigma_z}^2}\right)} + e^{\left(\frac{-(z+h)^2}{2{\sigma_z}^2}\right)} - \frac{\sqrt{2\pi}W_o\sigma_z}{K_z} e^{\left(\frac{W_o(z+h)}{K_z} + \frac{{W_o}^2{\sigma_z}^2}{2K_z^2}\right)} \mathrm{erfc}\left(\frac{W_o\sigma_z}{\sqrt{2}K_z} + \frac{(z+h)}{\sqrt{2}\sigma_z}\right) \right] \quad \textbf{(18)}$$

Equation (**18**) is the solution of an atmospheric advection-diffusion equation accounting for particle dispersion and deposition as developed in Ermak 1967 [20]. Quantities forming the different terms are the following: Q_j [number of bacteria / time step] is the force of infection in source herd *j*; *U* [m/s] is the wind velocity; σ_y [m] and σ_z [m] are the standard deviation for dispersion coefficients, taking the form $\sigma_y(\mathrm{x}) = a_y x^{b_y}$ and $\sigma_z(\mathrm{x}) = a_z x^{b_z}$ with a_y, a_z, b_y, b_z corresponding to the atmospheric stability class C (3-5 m/s wind velocity, slightly unstable environment); W_0 [m/s] writes as $W_0 = W - 0.5W_{set}$, where *W* [m/s] is the deposition velocity due to gravitation and W_{set} [m/s] is the settling velocity, fixed to $\frac{2\varphi gr^2}{9\eta}\frac{2\varphi \mathrm{gr}^2}{9\eta}$, with ϕ [kg/m^3] the particle density, *r* [m] the particle radius, η [kg/m s] the dynamic viscosity of air, and g [m/s^2] the gravitational acceleration; *h* [m] is the height of reception at destination herd*;* and K_z [m^2/s] is the coefficient of eddy diffusivity set to $K_z = 0.5a_z b_z U x^{(b_z-1)}$. In the last term of (**18**), *erfc* is the complementary error function ($erfc\ (x) = 1 -\ \mathrm{erf}(x)$) resulting from the approximation of the solution of the partial differential equation of advection-diffusion. Parameters were taken from the standard model presented in Stockie et al. (2011) [21]. Additional details on dispersion related parameters are given in Table 6. The relationships between $C_{i,j,(x,y,z)}$, Q_j, and the intra-herd infection dynamics at source and destination herds are presented in the next subsection.

Table 6 Parameters of dispersion model

Parameter	Definition	Estimation	Unit
g	Gravitational acceleration	9.8	m s^{-2}
z	Height of plume generation	4	m
h	Height of plume reception	4	m
η	Dynamic viscosity of air	$1.8*10^{-5}$	Kg $m^{-1}s^{-1}$
φ	Density of particles	1150 [7]	Kg m^{-3}
r	Radius of particle	10^{-6}	m^{*}
W	Deposition velocity	0.01 [8]	m s^{-1}
$a_{y,}\ a_z$	Guifford-Pasquill stability	0.34, 0.27[3]	$m^{(1-b)}$
$b_{y,}\ b_z$	class 'C' stability parameters	0.82, 0.82 [3]	

The dispersion model uses the vector of wind velocity reaching herd *i* from source herd *j*. The data generally available for wind velocity consists of northward wind component (v_j) and eastward wind component (u_j). Based on it, the wind speed was estimated as $windspeed = \sqrt{northward\ wind\ component^2 + eastward\ wind\ component^2}$, and the direction of the wind flow was estimated through its angle ϕ_{POLAR} with the original x-axis, where $\phi_{POLAR} = \tan^{-1}\frac{northward\ wind\ component}{eastward\ wind\ component}$. Diagrammatic representation of $northward\ wind\ component$ (v_j) and $eastward\ wind\ component$ (u_i) is shown in Figure 9, where ϕ_{MET} is the directional angle of wind.

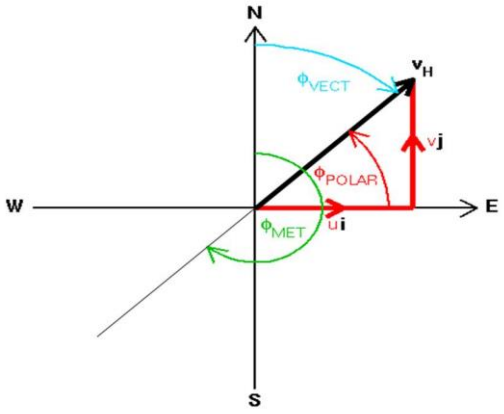


Figure 9 Description of wind components taken from the Natural Environment Research Council (NERC)

Weekly averages of wind direction and speed (a unique value for the whole area under study, as very little spatial variation in wind velocity and direction was observed) were used in the Gaussian dispersion model. Adjustment of the frame of the receiving herd *i* and source herd *j* coordinates according to the direction of wind flow was done based on the distance between the two herds ($Distance_{ij}$), direction of the wind angle between the line linking the two herds and the x-axis ($angle_{ij}$) as described in Stockie [135] (Figure 10).

$$x_{adjusted} = Distance_{ij} * \cos(angle_{ij} - \phi) \quad \textbf{(19)}$$

$$y_{adjusted} = Distance_{ij} * \sin(angle_{ij} - \phi) \quad \textbf{(20)}$$

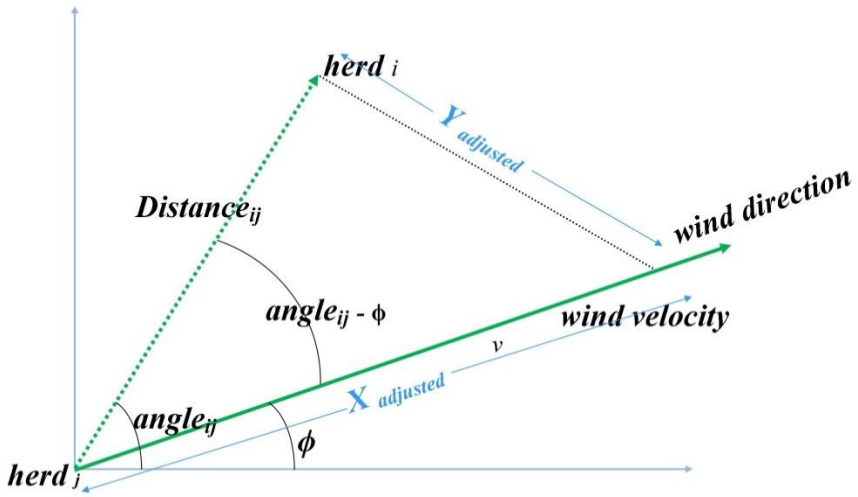


Figure 10 Adjustment of frame of the receiving herd

2.3.3. Cattle trade

Modelling of cattle trade was completely based on data. As the intra-herd model includes only cows, cattle movements were also restricted to the trade of dairy cows, excluding nulliparous heifers, bulls and calves. The source and destination herds, the date of the movement and the age of the animal were based on the observed data and therefore deterministically implemented. An animal of the same lactation number was randomly chosen from the selling herd to move to the destination herd. The probability of trading an infectious cow was based on the proportion of infectious animals in the given lactation age in the source herd. Because of the comparatively low time spent by cows in markets during trading, it was assumed that there was no transmission between cows following any possible interaction between them in markets. Animal purchases from herds outside the study region were modelled slightly differently. A random herd from the metapopulation was selected to copy an animal based on the lactation number. This was done to ensure that the probability of purchasing an infectious cow from outside the study region is same as buying an infectious animal from the metapopulation. In the case of buying an animal from a herd within the metapopulation, animal was 'moved' while in the latter case it was just copied. Matrix Ω was defined as describing the incoming (purchase) and outgoing (sell) trade of cattle for a herd *i*.

$$\Omega[S, I^-, I^+, I^{milk\ pers}, C^+, C^-]_i(t) = \sum_{j=1}^{Nb} Multinomial\ (n_{ji}(t), P_{X,ji}(t)) - \sum_{j=1}^{Nb} Multinomial\ (n_{ij}(t), P_{X,ij}(t)) \quad \textbf{(21)}$$

Where,

$$P_{X,ji}(t) = \left[\frac{S_{j,l}(t)}{N_{j,l}(t)}, \frac{I^-_{j,l}(t)}{N_{j,l}(t)}, \frac{I^+_{j,l}(t)}{N_{j,l}(t)}, \frac{I^{milk\ pers}_{j,l}(t)}{N_{j,l}(t)}, \frac{C^+_{j,l}(t)}{N_{j,l}(t)}, \frac{C^-_{j,l}(t)}{N_{j,l}(t)}\right] \quad \textbf{(22)}$$

is the probability of purchasing a cow with specific health state from herd j in the lactation year l.

$$P_{X,ij}(t) = \left[\frac{S_{i,l}(t)}{N_{i,l}(t)}, \frac{I^-_{i,l}(t)}{N_{i,l}(t)}, \frac{I^+_{i,l}(t)}{N_{i,l}(t)}, \frac{I^{milk\ pers}_{i,l}(t)}{N_{i,l}(t)}, \frac{C^+_{i,l}(t)}{N_{i,l}(t)}, \frac{C^-_{i,l}(t)}{N_{i,l}(t)}\right] \quad \textbf{(23)}$$

is the probability of selling a cow with specific health state from herd i to herd j in the lactation year l. The lactation year l, n_{ji} number of purchases made by herd i from j, and n_{ij} number of cows sold to herd j at time step t and is based on the data. Nb is number of purchasing or selling neighbours of herd i.

2.4. Coupling of cattle trade and wind dispersion model with intra-herd dynamics and identification of cause of infection

Coupling of intra-herd model with cattle trade was done by characterizing each cow in a herd, based on its origin and health status. Cows which are born in the same herd or when susceptible *(S)* at purchase were called *internal animals.* Cows which were infected outside the herd and that were shedders (I^-, I^+ or $I^{+\,milk\ pers}$) or carriers (C^+) at the time when they were bought were called *external animals*. The infection dynamics of the *internal animals* and *external animals* were assumed to be identical, the first contributing to the local subsection of the environmental compartment, $E_{i,internal}$, while the second contributing to the external subsection, $E_{i,external}$. After coupling of cattle trade with the intra-herd model, equations (2 - 7) change to the following equations.

$$S_i(t) = S_i(t-1) - NI^-_i(t) + NS_i(t) + \Omega S_i(t-1) \quad \textbf{(24)}$$

$$I_i^-(t) = I_i^-(t-1) + NI_i^-(t) - NS_i(t) - NI_i^+ - NI_i^{+milk\ pers} + \Omega I_i^-(t-1) \quad \textbf{(25)}$$

$$I_i^+(t) = I_i^+(t-1) + NI_i^+(t) - NI^+C_i^+(t) + NC^+I_i^+(t) + NC^-I_i^+(t) + \Omega I_i^+(t-1) \quad \textbf{(26)}$$

$$I_i^{+\,milk\ pers}(t) = I_i^{+milk\ pers}(t-1) + NI_i^{+milk\ pers}(t) - NI^{+milk\ pers}C_i^+(t) + \Omega I_i^{+milk\ pers}(t-1) \quad \textbf{(27)}$$

$$C_i^+(t) = C_i^+(t-1) + NI^+C_i^+(t) + NI^{+milk\ pers}C_i^+(t) - NC^+I_i^+(t) - NC^+C_i^-(t) + \Omega C_i^+(t-1) \quad \textbf{(28)}$$

$$C_i^-(t) = C_i^-(t-1) + NC^+C_i^-(t) - NC^-I_i^+(t) + \Omega C_i^-(t-1) \quad \textbf{(29)}$$

$\Omega X_i(t)$ for each health state is calculated using equation (**21**).

Coupling of the intra-herd model with windborne transmission of infectious particles was also done, through the environmental compartment. Bacteria arriving from a neighbouring herd *j* through windborne transmission accumulate in compartment $E_{i,j,dep}$. This writes as $E_{i,j,dep}$ = $area_i$ W $C_{i,j,(x,y,z)}$, where the area for each herd ($area_i$) was approximated using average space recommendation for a cow and the number of cows in a given herd. W and $C_{i,j,(x,y,z)}$ are presented in equation (**18**). Similarly, a fraction κ of the bacteria leaving the environmental compartment (due to the various mechanisms encompassed in term μ, Table 2) was assumed to become the source for generation of the plume and was defined as $Q_i = E_i \mu_{plume\ source}$, with Q_j defined in equation (**18**) and $\mu_{plume\ source} = \kappa\,\mu$.

Hence, after accounting for inter-herd processes in the intra-herd model, the environmental force of infection for each herd can be decomposed into two terms related to the origin of shedders ($E_{i,internal}$) and ($E_{i,external}$) and one term related to deposited bacteria due to windborne transmission from all possible source herds j ($\sum_j E_{j,dep}(t-1)$). The general formulation of the environmental force of infection due to bacteria in herd *i* was represented as follows:

$$E_i(t) = E_i(t-1)(1-\mu) + E_{i,internal}(t-1) + E_{i,external}(t-1) + \sum_j E_{i,j,dep}(t-1) \quad \textbf{(30)}$$

$$E_{x,origin}(t) = \sum_{k,l}\left(\rho^k Q t y_l \sum_{i,j} n_{t,xwkl}\right)_{origin} \quad \textbf{(31)}$$

Or in other words $E_{origin} = E_{building} + E_{pasture}$ where $origin \in \{internal, external\}$ and the loss of bacteria from the environment, μ, encompassing death and plume generation, was defined as $\mu = \mu_{death} + \mu_{plume\ source}$.

Figure 11 shows a diagrammatic representation of intra-herd infection dynamics coupled with the model describing cattle trade and Gaussian dispersion model of a single herd in a metapopulation of cattle herds.

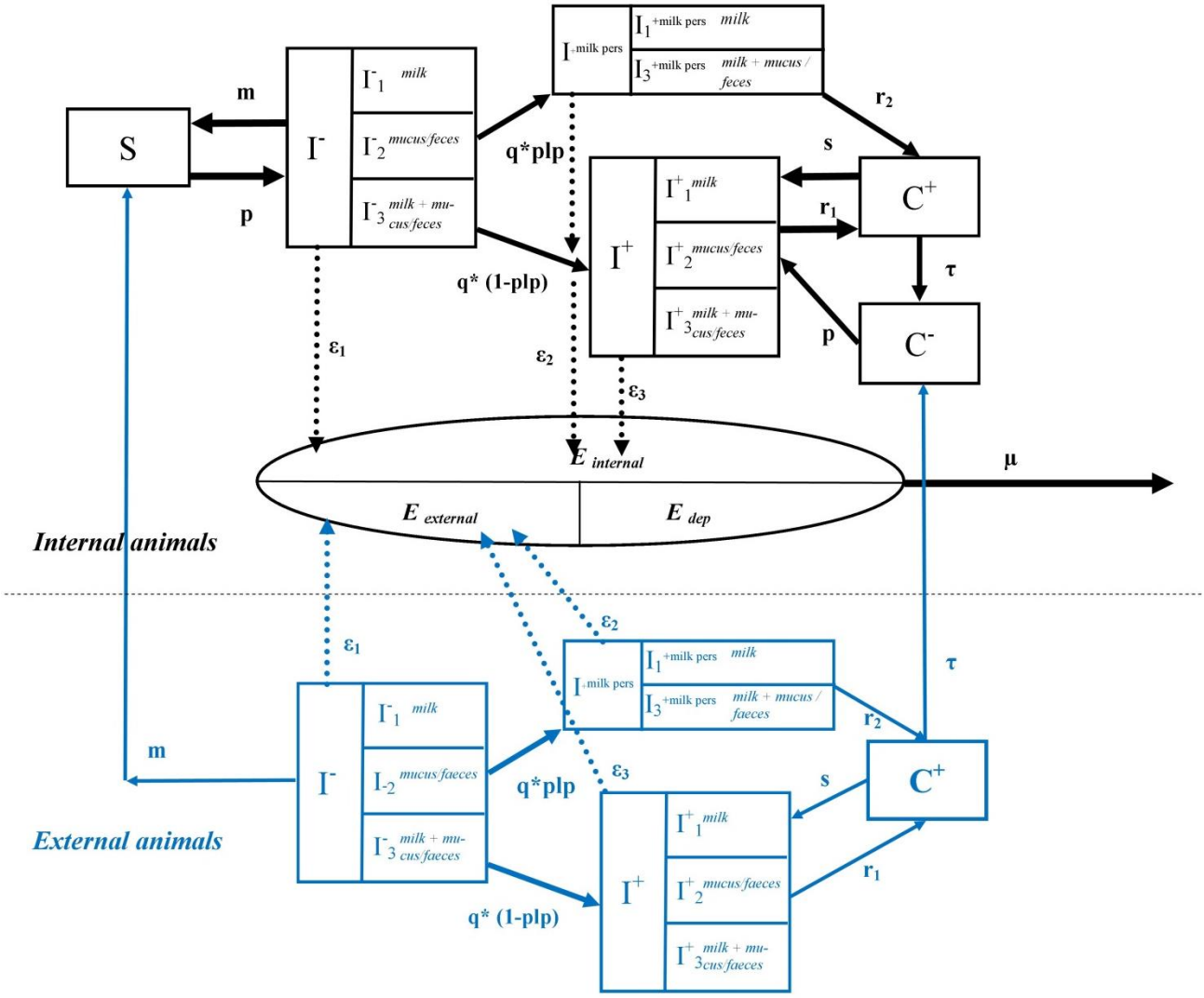


Figure 11 Flow diagram describing the intra-herd spread of *C. burnetii* in a dairy cattle herd. The diagram describes the health statuses of cows and transitions between these statuses, and environmental bacterial load of the herd (adapted from [62]). The blue section represents the infection dynamics of external animals, while the black section corresponds to internal animals.

2.5. Re-coding and implementation in Python

The intra-herd model of Courcoul et al [62] was originally implemented in R. As the aim was to develop a more complex and efficient metapopulation model using this model as a building block, the model was recoded in Python 2.7.6. We used standard package list available in *conda* 3.16.0. Plots were generated using *matplotlib* 1.4.3., *pandas* 0.15.2 and additionally installed *seaborn* 0.6 visualisation packages. Maps were based on the *basemap* 1.0.7.

The R model was not translated line by line, but two Python based objects (*Python class*) representing a *Cow* and a *Herd* were created contrary to R code. These objects had their own variables (categorical and numerical values) as well as functions which defined and manipulated their states and values in the model. All the model outputs results were stored again as *Python class* and were saved as text files after serialisation with the help of package '*pickle'*, for reanalysis later.

Outputs of the model coded by Courcoul et al in 'R' were compared with the ones obtained with the model coded in Python developed here. In both models, same initial conditions at time *t=0* were created. Infection in a herd of 50 susceptible cows was initiated with introduction of a primiparous I^- cow which has just calved. Parameters of infection dynamics and demographics were set to their standard values (Tables 1-4) and for each model 200 stochastic simulations of the '*intra-herd model*' were run to see the dynamics of infection over the period of five years. Four important outputs from both models, proportion of susceptible cows (equation 2), seroprevalence (equation 14), proportion of shedders (equation 15) and force of infection represented by environmental compartment of the herd were compared with each other.

The model coded in the Python was significantly faster than the one coded in R. Average time taken by the model in R was about 3 hours for 200 stochastic simulations, while it took less than 20 seconds in Python. Visual comparison of the model outputs from the re-coded model in Python and the model outputs from the model coded by Courcoul et al [62] showed good agreement (Figure 12). Minor deviation of the outputs was seen in the 95^{th} quartile of the outputs after two years. These deviations are due to the stochastic nature of models and different random number generators in Python and R.

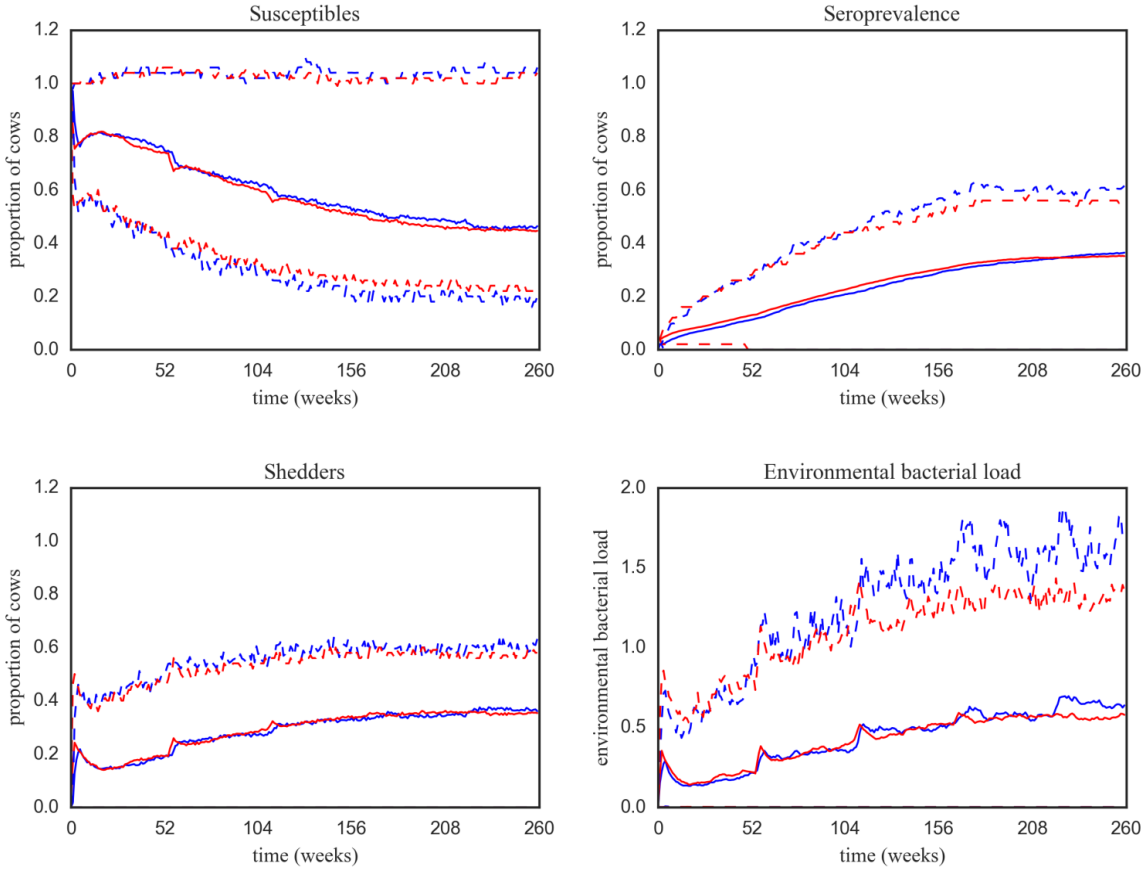


Figure 12 Comparing simulation outputs of model in R (blue) and model in Python (red) based on 200 simulations. For each model mean (plain line) and 2.5th and 97.5th percentiles (dashed lines) are represented.

2.6. Discussion

Modelling of transmission of pathogens through environment, as for *C. burnetii*, requires information about parameters such as elimination rate of the pathogen, pick up rate of the pathogen by susceptible individuals (contact parameter), the quantity of pathogen in the environment and the infectivity of the pathogen in the given environment [150]. These models of environmental transmission were applied to other airborne pathogens such as influenza viruses [150]. Similar model is proposed for smallpox infection of a susceptible cell denotes the probability of infection (Pr) of a cell as $\Pr = 1 - e^{(-\rho \times \mu_n)}$, where ρ is the probability of successful infection per virus particle and μ_n is the average number of virus particles encountered by the cell [151].

Concerning the expression of the probability of infection, such '*one hit*' model is also presented for *C. burnetii* where the probability of airborne infection from the environmental contamination of the bacteria is modelled as $p = 1 - e^{-\lambda\kappa}$ [134]. The authors define λ as the average number of pathogens received by the host and κ is the probability of infection for each received organisms via inhalation [134]. The formulation presented by Courcoul et al [62] for probability of infection ($p = 1 - e^{-E}$) does not differentiate between number of viable pathogens available (λ), and the contact term (κ). Instead, it considers the force of infection in the environment equivalent to the *E* compartment. Shedding by each individual presented in the model can be interpreted as the corresponding contributions to this force of infection. Another novelty of the metapopulation model proposed here lies in the way dispersion model is utilized. It is a common practice to model the transport of pathogens and to calculate the concentration of pathogens at a place of concern [97, 130, 144, 152]. Contrary to this, we calculate the force of infection at a given place following transport of pathogens due to wind.

The translated *intra-herd model* from R into the new coding environment of Python resulted into a computationally more efficient model, which was essential as the subsequent aim was to couple such *intra-herd models* to each other via windborne dispersion and cattle trade. The comparison of the model outputs showed no significant change in the model outputs.

The individual based nature of the original intra-herd model developed by Courcoul et al [62]; which was recoded here, aided in formalizing this system by identifying each animal based on its origin, and hence subsequently helping in identifying the cause of infection. Hence, the spatio-temporal dynamic model presented here describing the spread of *C.burnetii* between

dairy herds, via windborne transmission and cattle trade, provides a good platform to address our objectives of thesis. The multiscale model can be applied to different geographical regions with the availability of geo-coordinates and population estimates of dairy cattle herds, meteorological parameters and cattle trade data.

In the following chapters, we will use this model to a specific dataset related to Finistère department, France to also test the reliability of the model, by assessing its ability of predicting similar spread of infection as observed in 2012-2013 in the same geographic region and to identify the contributions of the routes in the regional spread of infection.

Chapter 3

Assessing the relative role of windborne transmission and cattle trade in the transmission of Q fever in dairy herds of Finistère Department (France)

In previous chapter, we discussed the overall structure of the mathematical model which describes the spread of Q fever in dairy cattle herds on a regional scale. We also discussed the ability of the model developed in identifying the route responsible for the introduction of *Coxiella burnetii* in an infection-free herd. In this chapter, we apply this model to a case study in Finistère department in France.

Important objectives being addressed here are (i) assess the reliability of the model predictions by comparing them with the available data of infection spread in Finistère department, (ii) to identify the relative impact of transmission routes on the regional spread and on the intra-herd infection dynamics of the *C. burnetii* in dairy herds. Along with this, we also did a preliminary sensitivity analysis of the model parameters. The third objective was to understand the persistence of infection over a longer duration in a metapopulation in absence of any control strategy along with a sub-objective to see if the contributions of the routes are similar even on longer duration as seen or shorter duration.

To address these objectives in this chapter we describe two simulation experiments concerning the spread of the *C. burnetii* among the dairy herds of Finistère. The first simulation predicts the spread of infection over one year period (2012-13). We first assess the ability of the model in predicting the spread of infection as observed during the same time period. Along with that, we quantify the contribution of transmission routes in the regional spread, then impact on the intra-herd dynamics, and performed a preliminary sensitivity analysis. The second experiment describes the spread of infection over longer duration of 10 years identifying the cause of infection in infection free herds.

3.1. Finistère Department

Finistère is a department of Brittany region in France. It is located in the north western corner of metropolitan France and is surrounded by English Channel to its north, and Atlantic Ocean to east west and south (Figure 13, inset). The climate of the region is oceanic characterized by presence of wind which flows mostly toward south east direction from the sea. The overall terrain of the region is flat with a small range of hillocks. The highest elevation in the region is not more than 400 meters.

The region is known for predominant dairy farming and in 2014, Finistère was the 4th department in France in terms of milk production. More than 80% farms in the Brittany region are dairy farms (www.finistere-economie.fr). In 2014 the department had 2179 pure dairy, 436

mixed and 1528 beef herds registered with the Groupements de Défense Sanitaire (GDS) de Bretagne, (France) and has seen a steady annual decrease of 10% in number of number of herds.

The department becomes a very good case study with the availability of meteorological data, data related to cattle trade, and detailed data about the dairy cattle herds, including their demographics, geolocations and epidemiological data on Q fever prevalence. The intra-herd model developed earlier and incorporated into the metapopulation here is also based on the data collected from the herds in the same region. It also models the management practices prevalent in the region. Keeping this in mind, the intra-herd model can be used here as it is, without any changes in assumptions.

3.2. Data

The data used for these simulations are of three types. 1. Data related to herds (epidemiological, demographic and geographical), 2. Wind velocity data, and 3. Cattle trade data. Before explaining the details of the methodology used in the simulation experiments we briefly describe these three data sets

3.2.1. Epidemiological and demographic data

In May 2012, a survey was conducted in the dairy cattle farms of the region to estimate the sero-prevalence of *C. burnetii*. Bulk Tank Milk (BTM) samples were collected from dairy herds at two time points and ELISA test was used to detect antibodies against *C. burnetti*. In May 2012, 1,963 herds were found positive for the presence of antibodies in BTM. Out of the 866 negative herds in 2012, 828 herds were retested in May 2013, and 307 were found positive (epidemiological: dataset 1).

The data related to dairy herds in the department of Finistère was procured from GDS Bretagne. The data included details with respect to number of total cattle present, number of cows, latitude and longitude of 2883 dairy cattle herds in the department. Along with that, the data also included the herd unique identification number, name of the proprietor, and postal address of the farm office (demographical: dataset 2).

For the purpose of simulation of the model based on these two datasets (dataset 1 and 2) we used a subset of 2,799 (shown under Model column of Table 7) herds (69% of all the cattle

herds in Finistère) for which we had information from both datasets in 2012 (Table 7 and Figure 13).

Table 7 Sero-prevalent herds for *C. burnetii* in Finistère department in 2012 and 2013 based on presence of antibodies in BTM. Observation columns give number of herds tested in the field and Model columns give number of herds which had data from both datasets (1 and 2) and were used in the model for simulation.

		May 2012		May 2013	
Interpretation of ELISA	**Classes of S/P* ratio of BTM**	**Observation**	**Model**	**Observation**	**Model**
Number of herds tested		2829	2799	828	823
-	≤ 30	866 (30.6%)	858	521 (62.9%)	520
+	30 < S/P ratio ≤ 100	822 (29.1%)	809	129 (15.6%)	128
++	100 < S/P ratio ≤ 200	1108 (39.2%)	1099	133 (16.1%)	130
+++	> 200	33 (1.1%)	33	45 (5.4%)	45

*** sample to positive ratio**

3.2.2. Cattle trade data

The French Ministry of Agriculture (*Ministère de l'agriculture, de l'Agroalimentaire et de la Forêt*) maintains pioneering database about life histories of each individual livestock animal. Tracing individual animal started way back in 1969 in France, but it was generalised in 1978 by making it mandatory to tag and trace all the cattle. France was the first country to do so in the World. Since then, each cow, bull in the country has received a unique identification number and a passport. This system provided the efficient backbone to the French national herd disease prevention and epidemiological surveillance system, and was a step ahead of the subsequent OIE (World Organization for Animal Health) and WHO (World Health Organization) guidelines. This framework and long experience of this tracing system has served as a template for the series of EU regulations (EC 1760/2000, EC 1825/2000). System provided a significant ease in tracing the source of packed meat or its products from 'farm to fork' which is evident in the labelling seen in the meat products available across Europe. The computerisation of this database has resulted into more accessible database called as "Base de Données Nationale d'Identification" ('BDNI').

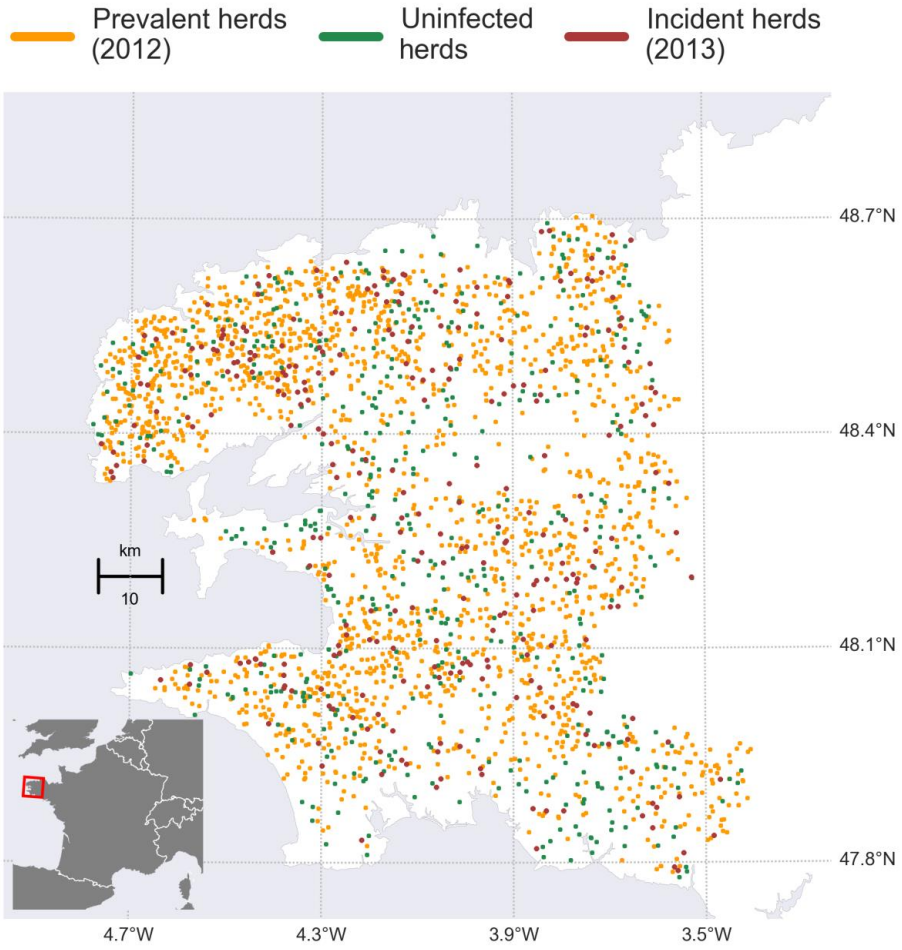


Figure 13 Sero-prevalent herds as observed in May 2012 and May 2013 in the Finistère department, France. Inset map shows location of Finistère department in France.

The data is generated since the birth of a calf. Farmer should tag animal within 20 days after its birth, by fitting it two ear tags carrying a unique identification number 'FRxxxxxxxxxx', where FR stands for France and 10 digit code. Since 2010, farmers can opt to upgrade one of these ear tags to an electronic ID ear tag fitted with a transponder. The farmer has one week after ID-tagging their animal in which to provide the national computerized information database [the 'BDNI'] with the compulsory set of animal data, which they can do either via internet or by sending in a certified form: ID number, date of birth, farm herd number, the number of calf's mother, breed of the calf and of its mother and parent bull, and so on. Each successive owner

who purchases this animal is required to notify the BDNI system within 7 days of animal transfer (purchase or sell) or slaughter (http://en.france-genetique-elevage.org/). Detailed country wide analysis of this database and its implication on the ability to spread the disease were presented by Rautureau et al [153] and Dutta et al [66].

For the purpose of the study, data for the individual movements of cows from one dairy herd to another only within the Finistère department, for the time period 2005 to 2014, were extracted from BDNI. Data were filtered out according to the requirement of the model. First cattle exchanges involving any herd within the Finistère department were separated from the global database. This involved cattle trade within the herds of the department and purchase of cattle from herds outside the department and sell of cattle to herds outside the department. As described earlier in the Chapter 2 (section 2.4) the model accounts only for cows and excludes heifers, all the movements of cattle less than 730 days of age were excluded which further shortened the database to 66,448 cow trade events. A directed static network of this dataset was generated to analyse the structure and nature of the cattle movements in the region. Centrality measures of this network were estimated as they allow ranking of nodes according to their importance in the network.

Out of 2799 herds under question, 2603 herds participated in cattle exchanges during the period of 2005- 2014, with mean degree (number of trade partners) of 7.01 (±7.6). 1,925 herds participated in selling cows to herds within the department and 1988 herds bought cows from herds within the department or from outside of the department. The number of animal transactions shown in the data per week is shown in Figure 14(a). Red highlighted data is the data used for the first experiment, while Figure 14(b), (c), (d) shows the typical power law distribution of degrees, in-degrees and out-degrees respectively.

3.2.3. Wind velocity data

Wind velocity data required for dispersion modelling were taken from publically available European Centre for Medium Range Weather Forecasts database [154]. Northward and eastward wind component data for Finistère department for the periods of the experiments were procured. The dataset used for these experiments come from dataset ‘ERA Interim Daily’ [155] (http://apps.ecmwf.int/datasets/data/interim-full-daily/) and two variables namely ‘10 metre U wind component’ (Eastward wind component) and ‘10 metre V wind component’ (Northward wind component) were downloaded for the given time period of the study. Data was utilised in

the model by converting daily averages into weekly averages as mentioned in Chapter 2, section 2.3.2.

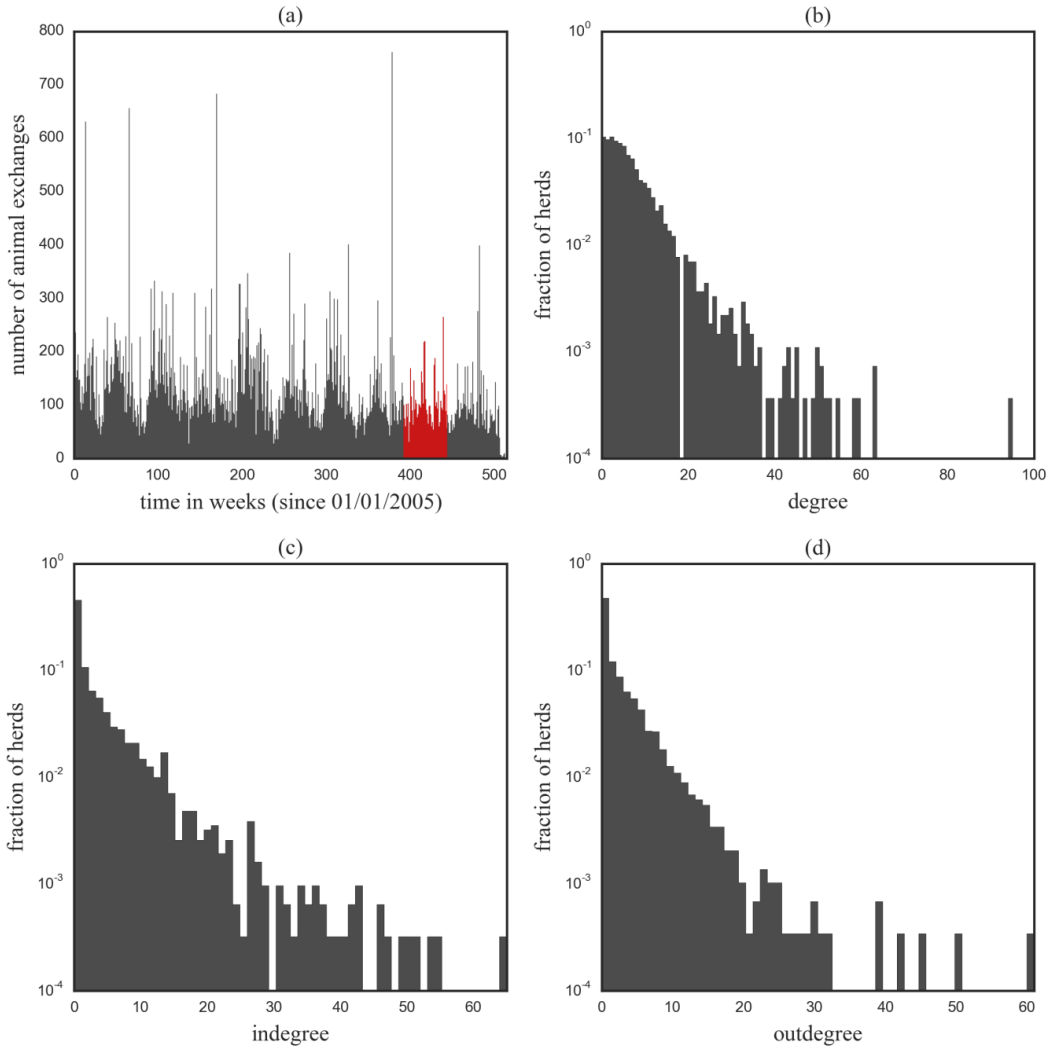


Figure 14: Trade of cows involving dairy herds of Finistère department from 2005-2014.

3.3. Simulation experiments

3.3.1. Initial conditions and simulation set up

For both experiments the same initial conditions were considered to generate a metapopulation with enzootically established infection of *C. burnetii*. Initial conditions were simulated for prevalent herds in agreement with field observations in May 2012. The ELISA in BTM classifies the S/P ratio (sample to positive ratio) in three different ranges. For these three

different interpretations of ELISA results, estimates of sero-prevalence were taken from a study by Taurel et al (Table 8) [127]. For all positive herds in May 2012 as listed in Table 7, the intra-herd model was run, with forced introduction of 1 infected cow until its sero-prevalence reached a value in the interval between the expected mean ± sd of sero-prevalence as given in Table 8 [127]. This simulation was done independently without considering transmission between herds. The distribution of simulated sero-prevalence in prevalent herds at the beginning of the simulation is show in Figure 15.

Table 8 S/P ratio of ELISA of BTM associated with within-herd sero-prevalence of *C. burnetii* in milking dairy herd (Taken directly from AF Taurel et al, 2012 [127])

Interpretation of ELISA	Variables	Estimate of serprevalence (sd)	95% CI
	Classes of S/P ratio of BTM:		
-	≤ 30	-	-
+	30 < S/P ratio ≤ 100	0.20 (0.09)	(0.02 ; 0.38)
++	100 < S/P ratio ≤ 200	0.40 (0.07)	(0.29 ; 0.54)
+++	> 200	0.37(0.07)	(0.23 ; 0.51)

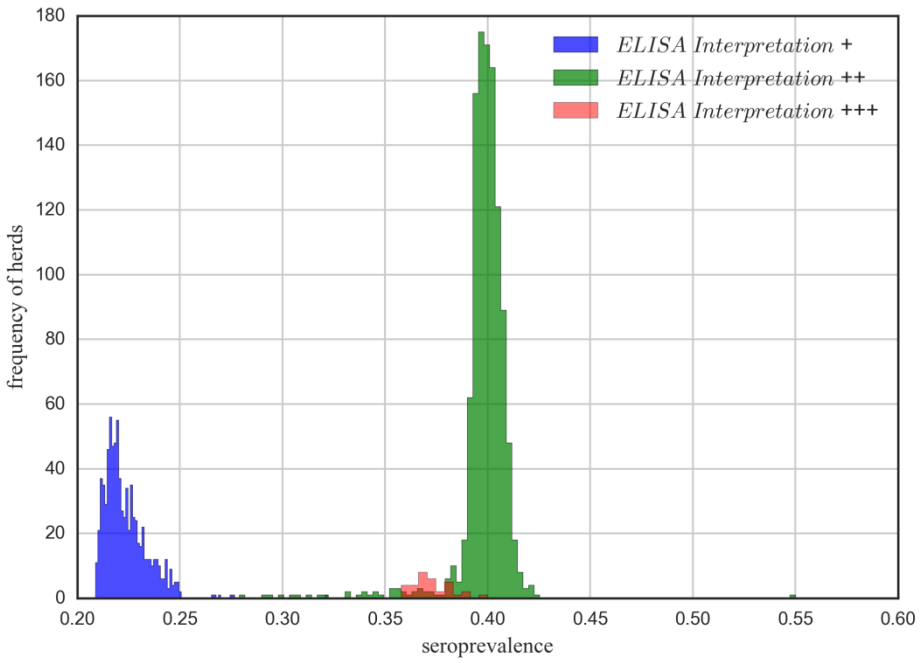


Figure 15: Distribution of sero-prevalence simulated for the initial conditions of prevalent herds based on the ELISA for BTM results.

Once the initial conditions were obtained, the between-herd spread of the infection was simulated over the duration of one year. 100 stochastic simulations were run for this scenario as comparison of outputs at 100, 200 and 300 stochastic simulations showed similar results. Hence for computational efficiency 100 stochastic simulations were run for each scenario of one year duration. During this experiment, the parameter values for the intra-herd model were taken from a previous study where they were estimated based on field data [62, 124] (Table 1-4). For the dispersion model, parameters were taken from standard dispersion model presented in Stockie (2011) [135] (Table 6).

For the second experiment, simulation was done for the duration of 10 years from 2005 to 2014. Even though the initial conditions of the metapopulation in Finistère department in 2005 were completely different and unknown, we generated the enzootically infected metapopulation of herds as seen in May 2012 as we did for the first experiment assuming a stable distribution of seroprevalence between years. Here in this experiment external risk of introduction of infected animals through trade from outside the population was considered as was implemented as described in Chapter 2 section 2.4.Because of computational and time constrains, 50 stochastic simulations of the model were run with same parameter values as described earlier.

3.3.2. Model outputs

In the first experiment, the spatial dynamic model was used to predict the status (in May 2013) of initially (in May 2012) susceptible herds. Introduction of infection in herds was defined as the generation of the first case among *internal animals* (Cows which are born in the same herd or when susceptible *(S)* at purchase). Identifying contamination sources allowed us to allocate a cause to the primary local case and therefore to assess the relative contribution of each of the two transmission routes considered for each incident herd. Herds receiving infectious animals previous to the generation of the first local case were designated as being infected by cattle trade, the rest of the incident herds were attributed to windborne transmission. In addition to the cause of infection, the *probability of infection* (*PI*) was also estimated for each incident herd based on the proportion of runs it experienced infection: *PI = (number of runs with at least one local case) / (total number of runs).* Herds were predicted positive by the model if their predicted *PI* was higher than a threshold, which was calibrated according to the available data, as described in the next subsection (Assessment of model predictions). Concerning the intra-herd dynamics in incident herds, four model outputs were considered: *seroprevalent animals, proportion of shedders* (Equation 15), *extinction rate* (equal to the proportion of runs with no

shedding and no seropositive cow at the end of the simulation among those runs where the herd was infected) and *herd incubation period* (calculated as the time elapsed between exposure to the identified cause and generation of the first local case). Descriptive statistical measures (mean, median, standard deviation and percentiles) of seroprevalence and proportion of shedders in incident herds were calculated only over runs in which herds experienced an infection.

In the second experiment, an important output of the model was temporal dynamics of *prevalent herds* in the metapopulation and was defined as number of herds with at least one shedding animal at given time point. Over the long duration of simulation *incident herds* can lose the infection to become susceptible again, and can get infected again either because of wind transmission, purchase of infectious cow, or because of environmental contamination from previous outbreak. Hence, in this experiment, we followed the causes of not just preliminary outbreak, but also successive outbreaks of *C. burnetii* infections. Three possible causes of infection were defined based on the amount of environmental contamination in the sub-compartments of environment: wind, trade and old. When the environmental contamination of $E_{internal}$ was found to be greater than both E_{dep} and $E_{external}$ it was assumed that the re-infection is due to the contamination caused by previous outbreak and was defined as *old* infection. For the other two transmission routes the identification as a cause was decided as for the first experiment.

3.3.3. Assessment of model predictions

In the first experiment, to assess the accuracy of the model in predicting the binary outcome (infected / non infected) for all the initially susceptible herds, as observed at the end of the study period, we performed receiver operating characteristics (ROC) analysis, based on *Sensitivity (Se)* (or true positive rate of detection) and *Specificity (Sp)* (more precisely 1-Sp, representing the false positive rate). ROC analysis consists in evaluating the performance of a classifier in detecting binary behaviour for different discrimination thresholds. More specifically, for each initially susceptible herd, the predicted infectious status was compared against the observed one (the reference) at the end of the study period. Each point of the ROC curve corresponds here to a different threshold to which the PI for each initially susceptible herd is compared in order to be classified as infected or not. To assess possible improvements in prediction, we also relaxed the spatial precision in the ROC analysis and compared the output for a neighbourhood around an expected incident herd (neighbourhood level analysis).

The comparison was done for neighbourhood distances of multiple radii (1, 2, 3 or 4 km). Sp for the neighbourhood level analysis was considered equal to that of herd level analysis. AUC (Area Under the Curve) was used for assessment of model performance.

The cut-off PI was used to classify herds into two categories: herds with a PI larger than the cut-off were considered as positive (infected), the others as negative (uninfected). This categorisation concerns the simulated herd status at one year after the onset of pathogen spread into the metapopulation. The optimum cut-off is chosen based on comparison of simulation to data concerning herd status at the end of one year. In general, the optimum cut-off value is chosen based on the epidemiological situation of the case concerned, such as prevalence in the population and consequences of false positive and false negative results [156]. In the literature, prevalence dependent (Sensitivity (*Se*), Specificity (*Sp*), Youden index (*J*), odds ratio etc.) and independent criteria (Efficiency, kappa), both are used to come up to a decision.

The optimum *cut-off* (threshold) values for PI to classify herds as positive or negative were selected based on three criteria: equality of Se and Sp, (*Se =Sp*); *maximum accuracy (*Acc_{max}*)*, where *Acc = (true positive herds + true negative herds) / (total population)* or, equivalently, *Acc = Se* x *prevalence* + *Sp* x *(1-prevalence);* $\mathrm{Acc} = (\mathrm{truepositiveherds} + \mathrm{truenegativeherds})/(\mathrm{totalpopulation})$, or, equivalently, $\mathrm{Acc} = \mathrm{Se} \times \mathrm{Prevalence} + \mathrm{Sp} \times (1\text{-}\mathrm{Prevalence})$ and *maximum Youden index* (J_{max}), where $J = Se + Sp - 1$ [156].

Figure 16 shows the basic idea and differentiation of the herd level ROC analysis and the neighbourhood analysis. We show a hypothetical situation of observed data of positive herds (in red) and hypothetical model predictions of *PI* and classification of herds positive (red) and negative (grey) based on a *PI* cut-off of 0.2. On the right side, calculation of *Se* and *Sp* is shown using standard 2×2 contingency table for herd level and neighbourhood analysis.

At herd level the comparison is straightforward, by comparing herd by herd level model results. In neighbourhood level, we considered a predetermined radius only around expected incident herd (here in the example around herd 'A') and that too only if the model fails to predict the herd as positive. Rest of the comparison remains same for neighbourhood level.

3.3.4. Spatial cluster analysis

For the first experiment, spatial cluster analysis for predicted incident herds or herd cases was done using a Poisson model (SatScan®) with a null hypothesis of expected number of cases in

each area proportional to its population size, hence adjusting the model for the density of cows. Definition of a case herd was based on the optimum PI cut-off suggested by the ROC analysis.

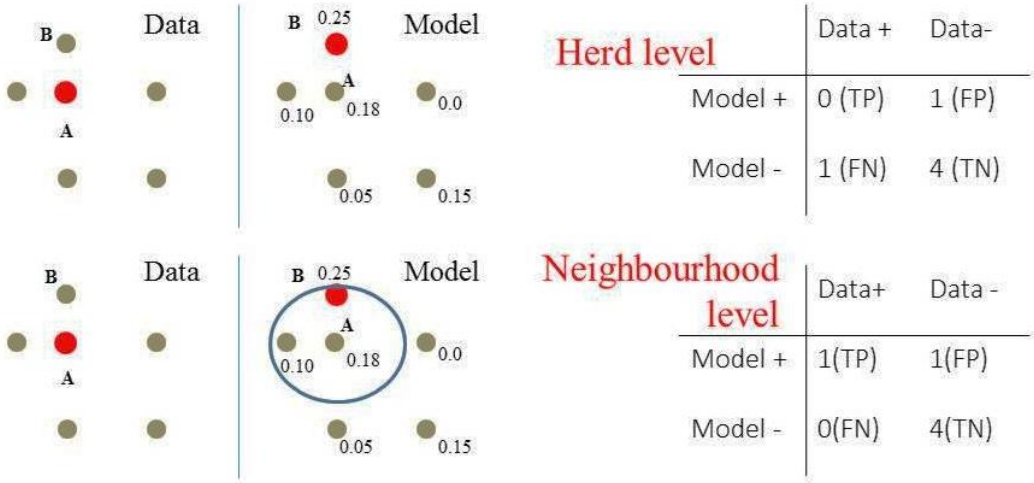


Figure 16: Hypothetical example of ROC analysis at herd level and neighbourhood level to compare model outputs and observed data. Red: positive herds, grey: negative herds. Sensitivity and specificity is calculated using TP (true positive), FP (False positive), FN (False negative) and TN (True negative) *Sensitivity analysis*

A preliminary sensitivity analysis was done in order to assess the robustness of the model predictions with respect to parameter variations. In a detailed sensitivity analysis conducted on the intra-herd infection dynamics model by Courcoul *et al*. [62], three significantly sensitive parameters were found: $Q1$, ρ and μ (Table 9). Along with these three parameters, three more parameters from the dispersion model κ (fraction of μ becoming plume source), r (radius of fomite particle), W (deposition velocity) were tested in the analysis. The values chosen to be tested in the sensitivity analysis were those used in [62] for $Q1$, ρ and μ. For κ,r, W the standard value was varied by fifty percent, in the limits of biological plausibility. Each parameter was varied independently of other parameters (univariate sensitivity analysis) the effect of these variations on three model outputs (relative contribution of windborne transmission to new infections of herds, number of incident herds and proportion of shedders in incident herds) was evaluated.

Table 9 Parameters considered for the sensitivity analysis of the model.

Parameter	Definition	Standard value	Values tested in sensitivity analysis			
$Q1$	Probability distribution of the shedding	Distributio	Distributio	Distribution		
Low-level	levels of all the $I^-$and for the I^+	n I	n II	III	)istribution	
Mid-level	shedding in mucus/faeces after 4 weeks	0.85	0.6	0.25	IV	
High-level	post calving	0.15	0.4	0.25	0.15	
		0.0	0	0.5	0.6	
					0.25	
ρ	Proportion of bacteria shed through mucus and faeces filling the compartment	0.28	0.05	0.15	0.35	0.5
μ	Elimination rate of *C. burnetii* from the herd environment	0.2	0.1	0.5	0.8	
κ	Ratio between $\mu_{plume\ source.}$ and μ	0.5	0.25	0.75		
r	Radius of a fomite particle	1e-6	0.5e-6	1.5e-6		
W	Deposition velocity due to gravitation	0.01	0.005	0.015		

3.3.5. Relative impact of transmission routes on the regional spread and intra-herd dynamics of the infection

In the first experiment, to identify the contribution of cattle trade and wind dispersion as routes of transmission, we used two complementary approaches. First, we tested four scenarios to understand the role of each transmission route both independently and in association with one another: absence of between herd transmissions (Scenario A), transmission only by movement of animals (scenario B), transmission only by windborne dispersion of the pathogen (scenario C), both routes of transmission (scenario D). Temporal dynamics of incidence at herd level, total number of incident herds and temporal dynamics of shedder cows in incident herds were compared to assess the impact of presence and absence of the transmission routes on regional spread. The second approach focused on identifying the relative roles of the two transmission pathways in introducing the infection in incident herds, by further evaluation of scenario D. To investigate differences in the intra-herd dynamics of the incident herds concerning the causative transmission route, *PI*, *extinction rate* and *time after exposure* were compared using Mann-Whitney U test. A similar analysis was done on a subset of herds at risk of acquiring infection through both routes, i.e. those herds that purchased animals and were exposed to *C. burnetii* due to windborne transmission.

3.4. Results

3.4.1. Experiment 1: Spread of C. burnetii over one year period

3.4.1.1. Incidence prediction and agreement with the observed data

Out of 823 susceptible herds at the beginning of the simulation, 768 got infected at least once over the total number of runs. The PI predicted for incident herds showed spatial heterogeneity (Figure 17a). Most of the incident herds showed low values of PI. Out of 768 herds, 38.8 % herds showed PI < 0.1, while only 1.5% herds showed PI ≥ 0.9 (Figure 17b).

For different decreasing cut-offs of *PI*, the number of predicted positive herds decreased drastically (Figure 18). Simultaneously, the incidence attributed to airborne dispersion decreased similarly and the incidence attributed to trade of animals decreased gradually. The highest relative contribution of animal trade in the total incidence was 46.1% and was observed at 0.63 infection probability cut off.

The model had moderate agreement with data at herd level. It performed better for predictions at the neighbourhood level (Figure 19a). In the radius of 2, 3, 4 km there were on average 1.7, 3.8 and 6.6 initially susceptible neighbour herds around an expected incident herd respectively in the Finistère department. The gain in the model's predictive ability in terms of AUC with the increase in neighbourhood radius was weighed against the loss in the precision of model predictions arising because of an increase in the number of initially susceptible herds, the calculations rely on, resulting into a subjective compromise for a neighbourhood of 3 km for further analyses of model results.

At herd level, the model was found performing better at PI=0.11 for the first and the third criteria ($se = 0.57, sp = 0.59$), J_{max}=0.15) and at PI=0.61 for the second one (Se=0.1, Sp=0.95, Acc_{max}=0.64). For a neighbourhood of 3 km, the optimal cut-off was found to be equal to 0.21 based on the first criterion($se = 0.76, sp = 0.75$)0.25 based on the second one (Se=0.71, Sp=0.80, Acc_{max}=0.76), and 0.15 according to the third one (Se=0.86, Sp=0.66) (Figure 19b and 19c). Details of the Se, Sp, Acc, J, predicted incidence, contribution of windborne transmission to the incidence and the spatial distribution of incident herds at these cut-offs are given in Table 10 and Figure 20. The subsequent clustering analyses were performed using a cut-off value of 0.25 (i.e. herds were declared positive if their PI > 0.25) as this value provided the uniformly best results with respect to the three criteria at the neighbourhood level.

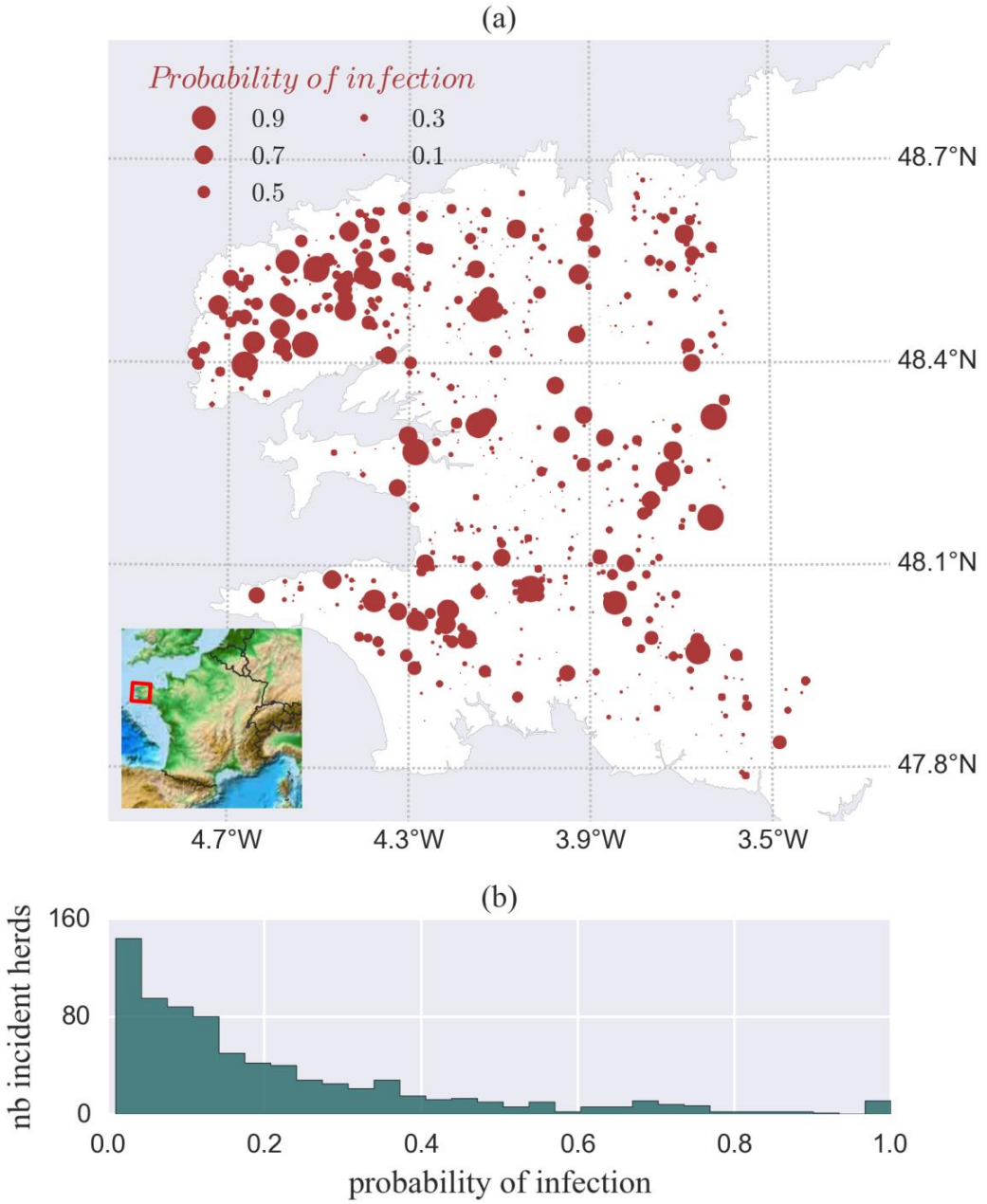


Figure 17 Simulated probability of infection (PI) by *C. burnetii* one year after its spread between herds, for herds initially susceptible (observed to be infection-free in May 2012). (a) Map of Finistère department in North-Western France with the locations of incident herds (bubbles sizes are proportional to PI). (b) Distribution of PI.

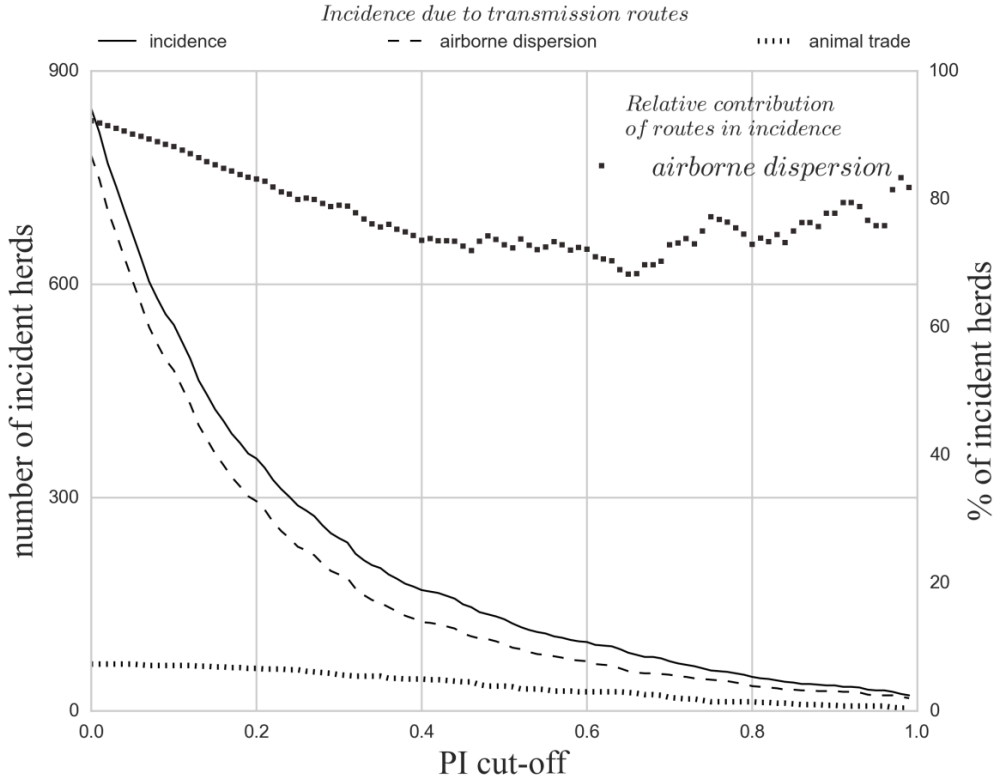


Figure 18 Overall incidence and incidence attributed to infection causes at different cut-offs of the PI. Y axis on the right hand side shows % of herds infected because of airborne dispersion.

Table 10 Performance of the model concerning the choice of PI cut-off optimal values at herd and neighbourhood levels. (Values in bold are values at which the thresholds of PI were seen at)

	Herd Level			**Neighbourhood (3km)**		
Criteria	$Se \approx Sp$	Acc_{max}	J_{max}	$Se \approx Sp$	Acc_{max}	J_{max}
PI cut-off	**0.11**	**0.61**	**0.11**	**0.21**	**0.25**	**0.15**
Sensitivity	**0.58**	0.10	0.58	**0.75**	0.71	0.86
Specificity	**0.58**	0.95	0.58	**0.75**	0.80	0.66
Accuracy	0.58	**0.64**	0.58	0.75	**0.76**	0.73
Youden index (J)	0.15	0.06	**0.15**	0.51	0.51	**0.53**
Incidence	419	58	419	259	219	346
% airborne transmission	86	57	86	78	75	83

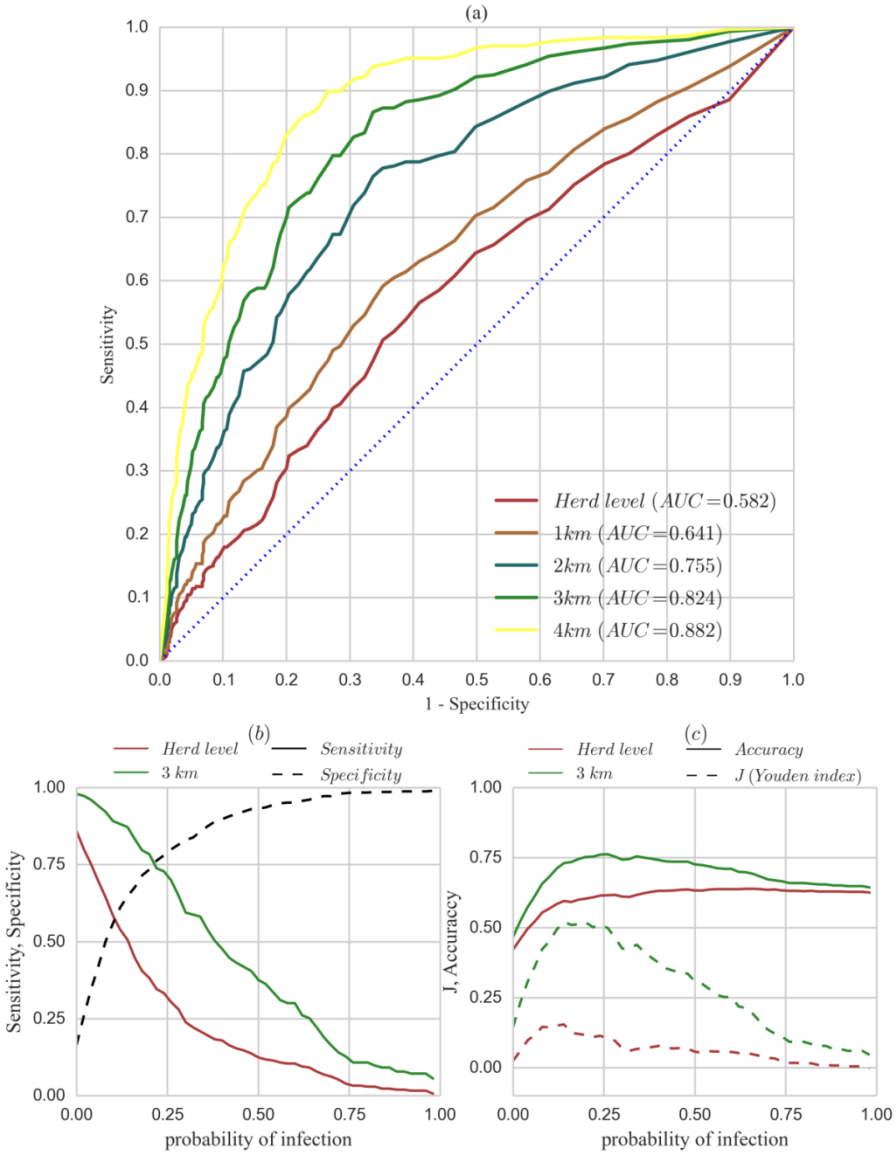


Figure 19 Receiver operating characteristics (ROC) analysis of model output. ROC analysis (data are the reference) for the simulated probability of infection (PI) by *C. burnetii* one year after its spread between herds, for herds initially susceptible. (a) ROC curves for herd level analysis and neighbourhoods of 1,2,3,4 km. (b) and (c) Variation of the four indicators (Sensitivity – Se, Specificity – Sp, Accuracy – Acc, Youden Index – J) used for building the three criteria (Se = Sp, max(Acc), max(J)) to optimise the cut-off of PI for the classification of herds as positive and negative. Calculations were performed at herd level and for a neighbourhood of 3 km. The Sp of the model is considered identical over different neighbourhoods and hence is shown by a single line

3.4.1.2. Cluster analysis

According to the clustering analysis, herds predicted as positive by the model at the cut-off of 0.25 showed seven non-overlapping statistical clusters, three in the north and four in the south of the Finistère department (Figure 21). A small cluster (Cluster 1, Figure 21) in northern Finistère department showed the highest relative risk of 7.7 and a slightly bigger cluster with same relative risk was also seen in the south-eastern corner for the department.

3.4.1.3. Sensitivity analysis

Model outputs were sensitive (Figure 22) to **Q1**, **ρ**, μ, and **κ**, whereas very little perturbations were induced by variations in particle size, **r** and deposition velocity, **W** (except for number of incident herds). Results showed that, despite a considerable sensitivity of the model to the parameters tested (except for **r**), the relative contribution of windborne transmission in the simulated incidence mostly remained higher than the contributions of cattle trade, regardless the parameter values tested, except for some values of κ and μ for which this trend was reversed in the last six months of simulation duration.

3.4.1.4. Contribution of transmission pathways to the regional spread

Windborne transmission was responsible for the infection of the majority of incident herds predicted by the model at all the optimum PI cut-offs derived in the ROC analysis. The contribution over these cut-offs varied from 57 % to 86 % at herd level and from 75 % to 83 % at neighbourhood of 3 km. The sensitivity analyses showed that windborne dispersion contributed to more than 50% and 70% of the new herd infections in 88 % and 63 % of the tested situations, respectively (Figure 22).

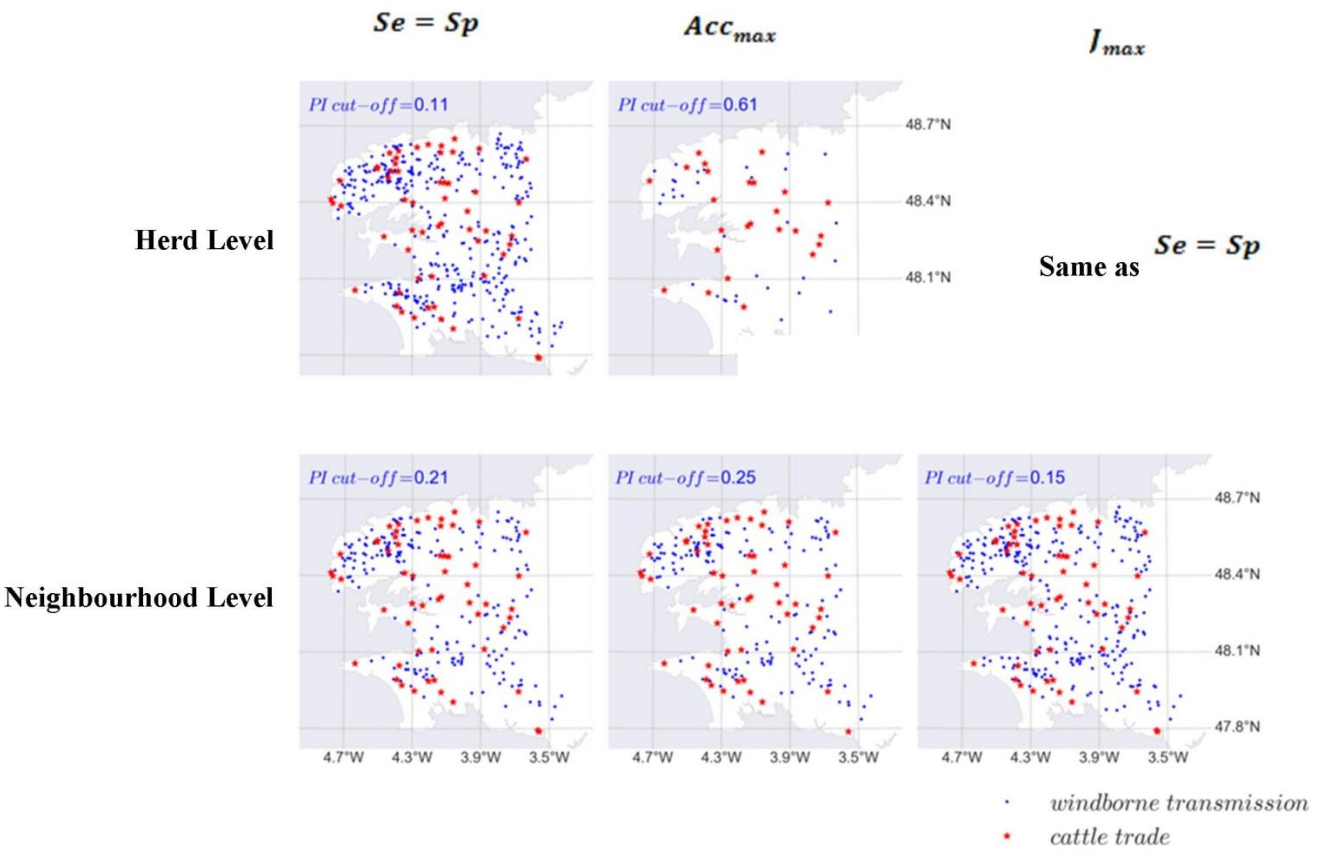


Figure 20 Incidence predicted at cut-offs of 0.11 and 0.61 (optimum PI values for herd level analysis) and at 0.21, 0.22 and, 0.15 (optimum PI values for neighbourhood of 3 km).

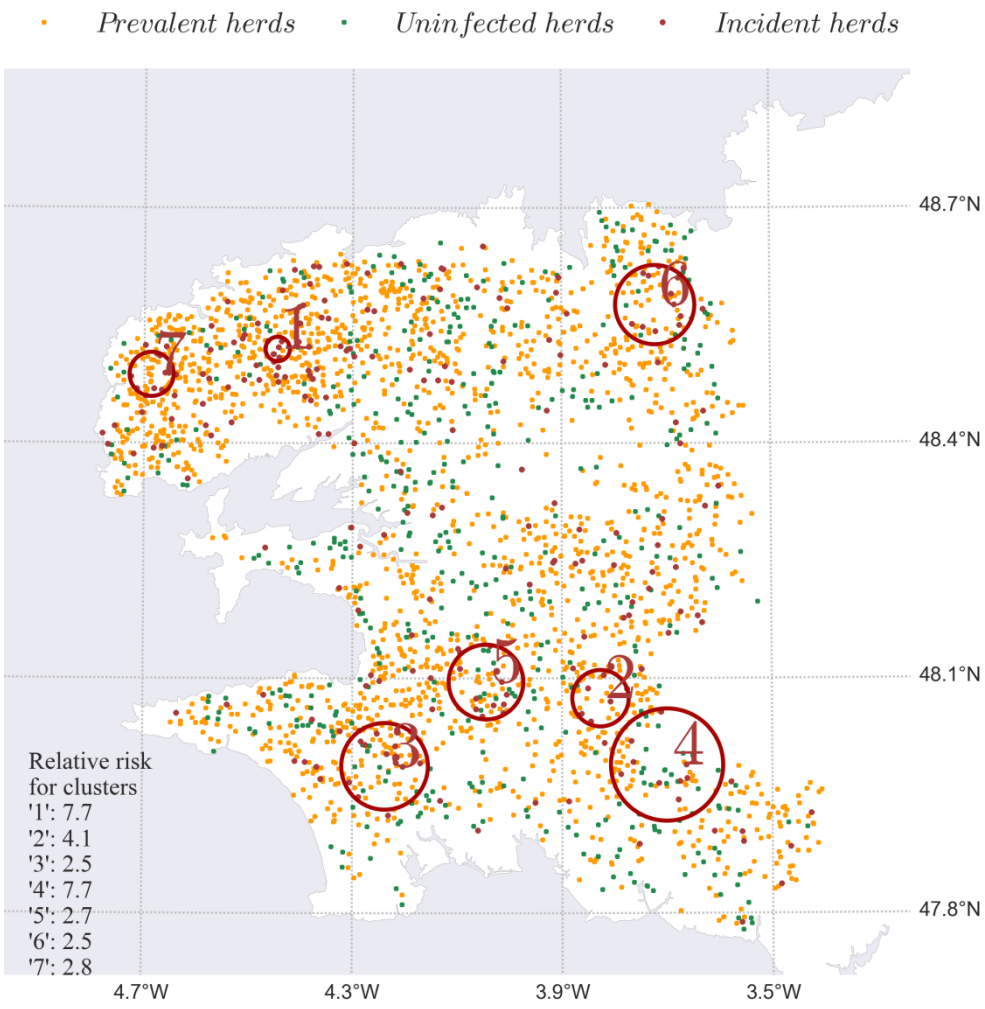


Figure 21 Spatial clustering of infection probability in Finistère department. Statistically significant spatial clusters (circled in red) with high relative risk (RR) of presence of simulated positive herds (red dots), initially susceptible, infected by *C. burnetii* one year after its spread between herds. The positive herd is defined based on a cut-off value of 0.25 for the probability of infection (PI). Herds initially seroprevalent according to the data (orange dots) and herds which remain uninfected (green dots) are also represented.

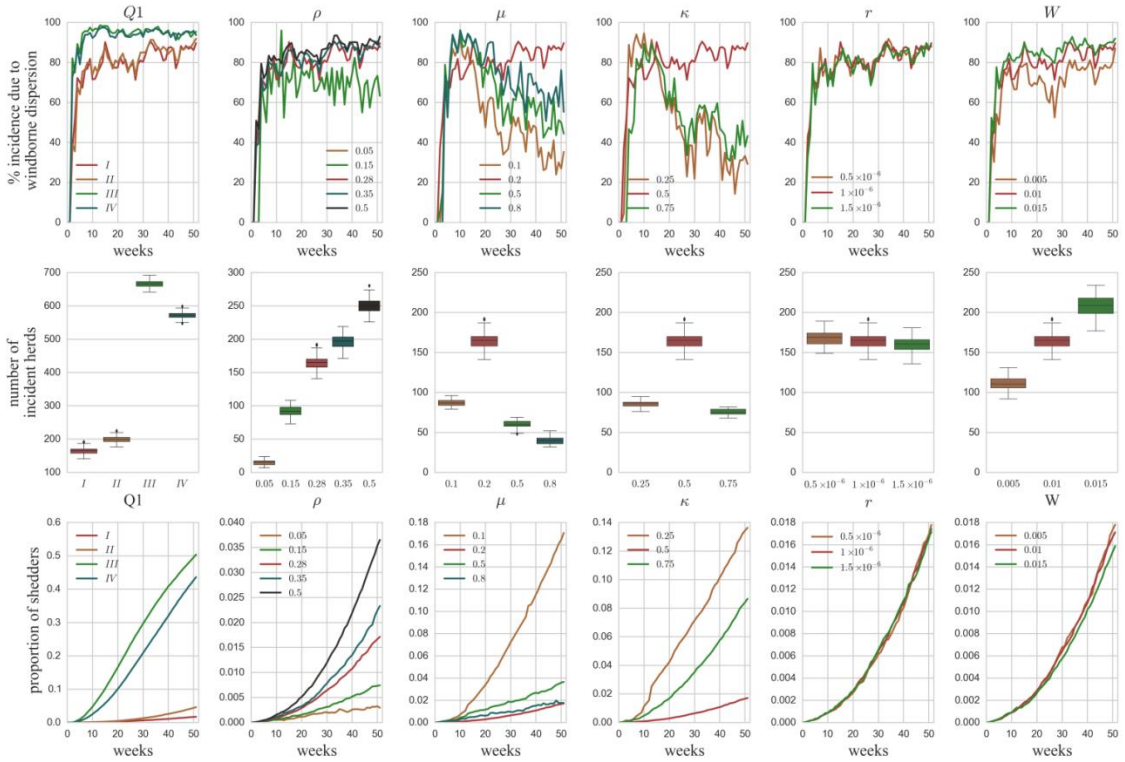


Figure 22: Sensitivity analysis of three dynamical outputs of the model with respect to the variation in six parameters. The outputs considered are: the proportion of newly infections of herds due to wind dispersion (top line; mean), the number of incident herds (middle line), and the mean proportion of shedders in incident herds (bottom line) over 100 stochastic iterations of the model. The six parameters, which were varied, are, from the left to the right: Q1, ρ, μ, κ, r and W.

Figure 23 illustrates the effect of windborne dispersion and cow trade on the regional spread of infection. More incident herds were seen in scenarios comprising windborne transmission (C and D, at least five times more incident herds on average than in scenario B), as depicted in Figure 23(a) and 23(b). Further analysis of scenario D carried out in the second approach provided similar results for the predicted incidence. In all 100 iterations of the standard stochastic model, 92 % of all the introductions of infections were attributed to windborne transmission, while the rest (8%) to cattle trade. The incidence dynamics over the time period attributed to these two transmission routes, when acting simultaneously, showed close coherence with the incidence predicted in scenarios B and C, where each transmission route was considered separately (Figure 23b). Incidence attributed to windborne transmission (scenario C and deconvolution of scenario D) showed an initial rapid increase followed by steady growth, while the incidence attributed to cattle trade was comparatively low and constant throughout the simulation period (scenario B and deconvolution of scenario D). The analysis performed on the subset of herds at risk from getting infected through both routes, with parameter values corresponding to the standard scenario, led to results consistent to those obtained for the whole population of initially susceptible herds. On average, the majority of the introductions were due to windborne transmission (65%).

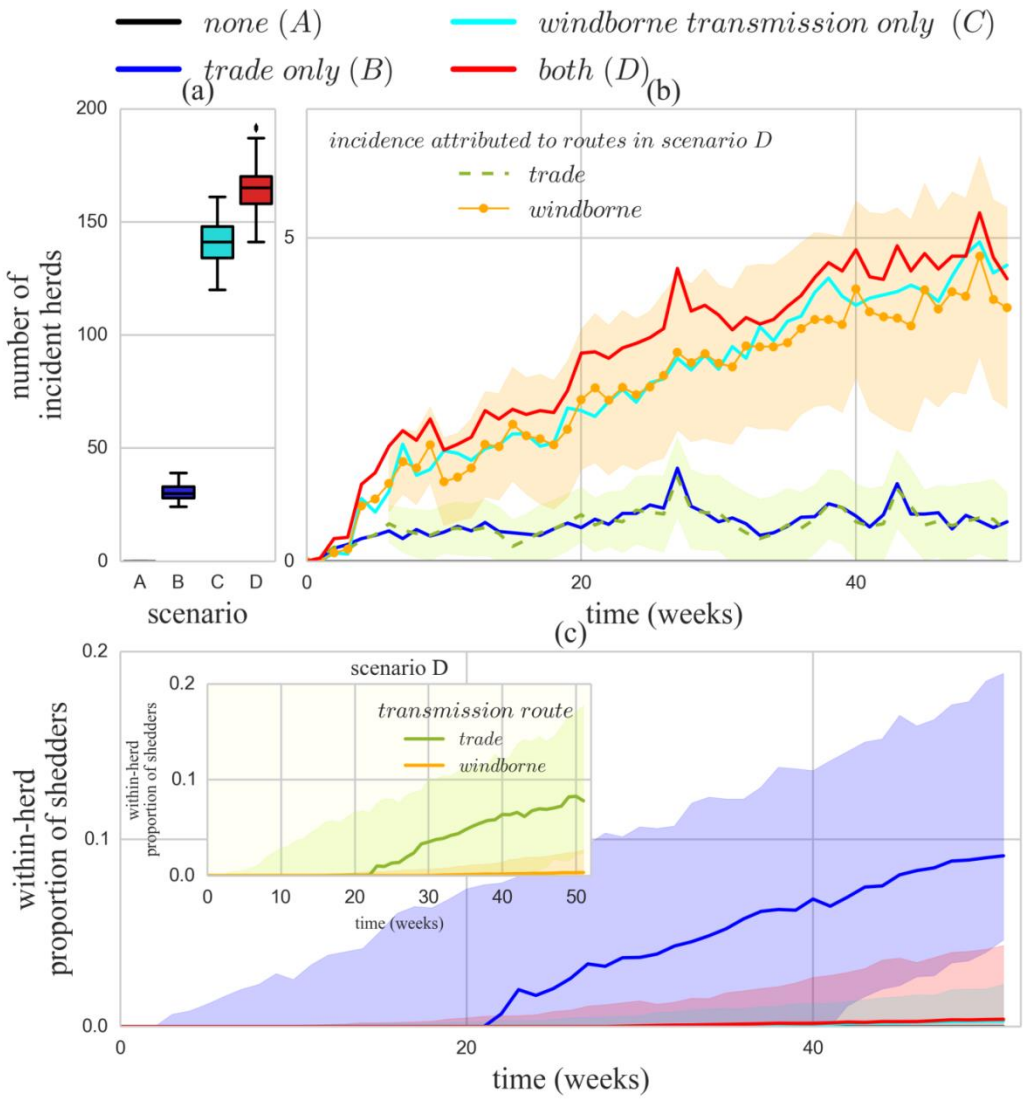


Figure 23 Infection dynamics of *C. burnetii* spread over one year in four simulated scenarios. Absence of between-herd transmission (A, black), transmission by cattle trade only, (B, blue), transmission by wind dispersion, (C, cyan) and presence of both transmission routes, (D, red). The subdivision of scenario D based on the identified cause of herd infection is also represented (due to animal trade – orange; by wind dispersion – green). (a) Distribution of the total number of predicted incident herds. (b) Dynamics of incidence (mean over 100 runs). Shaded regions for the subdivisions of scenario D represent 95% empirical confidence intervals. (c) Median proportion of within-herd shedders and 10-90th percentile (represented by shaded area) for all the scenarios. Inset figure shows the proportion of shedders (median and 80th percentile) for subdivisions of scenario D. Median and percentiles are calculated for runs where herds experienced infection (sample sizes are 16,733 for D, 13,814 for C and 3,617 for B).

3.4.1.5. Impact of transmission pathways on the intra-herd dynamics

The impact of presence and absence of a transmission route on the intra-herd infection dynamics was highlighted in the four scenarios. Scenario involving only trade (B) showed higher proportion of shedders (Figure 23(c)) and seroprevalence within incident herds, than scenarios involving windborne transmission only (C) or both transmission pathways (D). When both transmission routes were accounted for, herds infected due to windborne transmission showed significantly lower levels of shedding animals than those infected after purchasing an infectious cow (Figure 23(c) inset). Other representative parameters of the infection dynamics also were found statistically significantly different ($p < 0.05$, Figure 24). PI was higher for herds infected by cattle trade, while extinction rate was higher in windborne infected herds. These latter also took significantly longer time to generate the first local case after exposure to the respective cause than herds infected by cattle trade.

Variation in the intra-herd dynamics (proportion of shedders) followed similar trends when performed on the subset of herds exposed to both transmission routes, as seen in the analysis done on all susceptible herds. Also, for all outputs considered (*PI, herd incubation period* and *extinction rate)* statistically significant difference in herds infected by windborne transmission and herds infected by cattle trade was found.

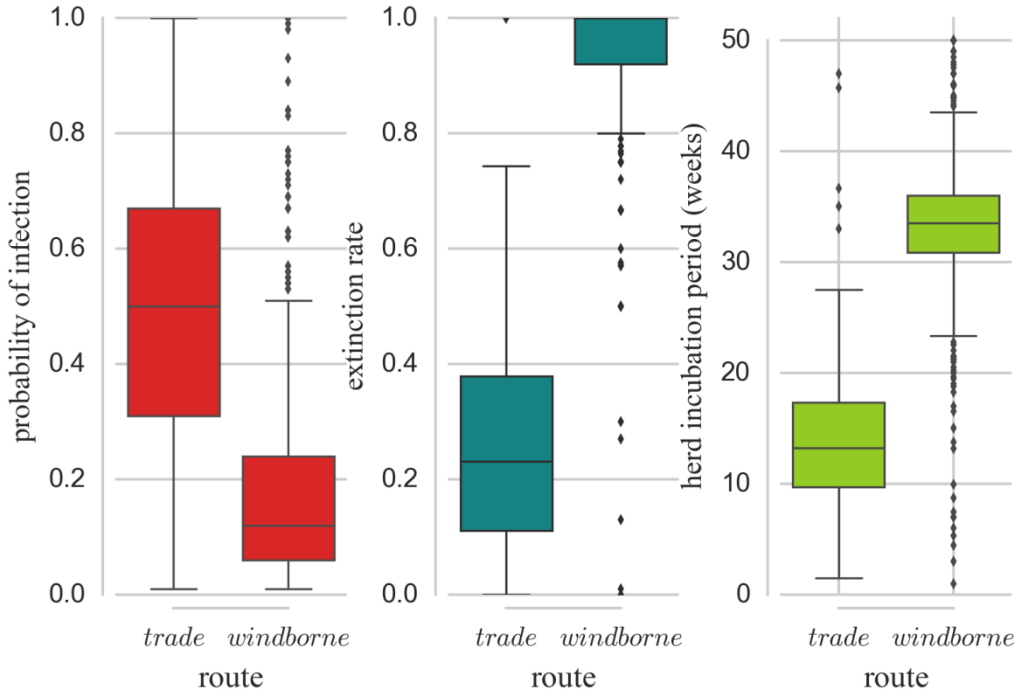


Figure 24 Distribution of the simulated probability of infection (PI), extinction rate and herd incubation period after exposure to the cause of infection, in *C. burnetii* infected herds (one year of simulated infection dynamics) by windborne transmission and by cattle trade.

3.4.2. Experiment 2

3.4.2.1. Spread of infection over long duration.

Over the period of 10 years, majority of introductions of *C. burnetii* infection in infection free dairy herds were also due to windborne transmission (Figure 25). Overall incidence in the metapopulation stabilised after initial increase of two years. While the incidence because of contamination from previous outbreak quickly stabilised within two years and contribution of trade in incidence diminishes steadily after two years.

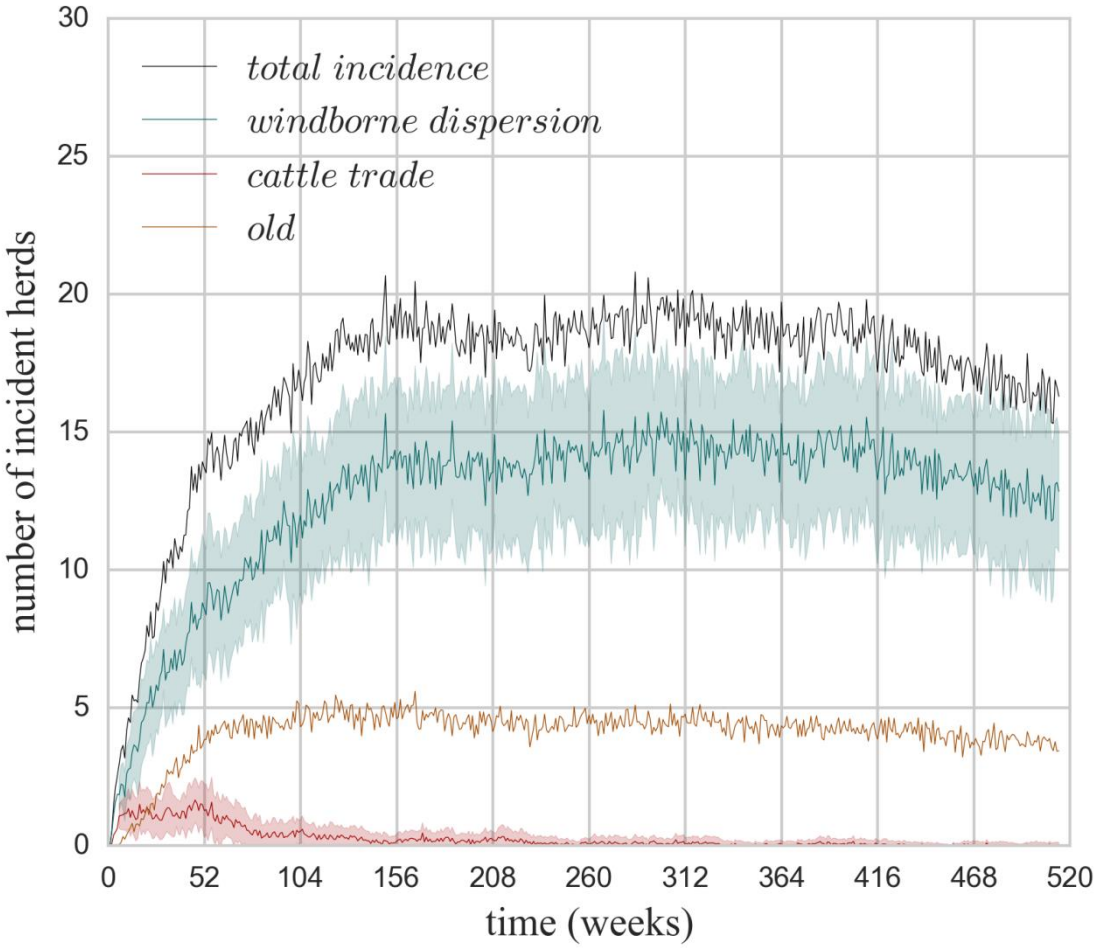


Figure 25 Overall incidence (black line) and incidence as per causes of *C. burnetii* infection in susceptible herds over the period of 10 years (2005-2014).

The overall prevalence of infected herds in the metapopulation also shows steady increase over the period of 10 years in the metapopulation (Figure 26), with a maxium number of prevalent herds reaching upto 2,475 (85.8%) in average over 50 stochastic simulations.

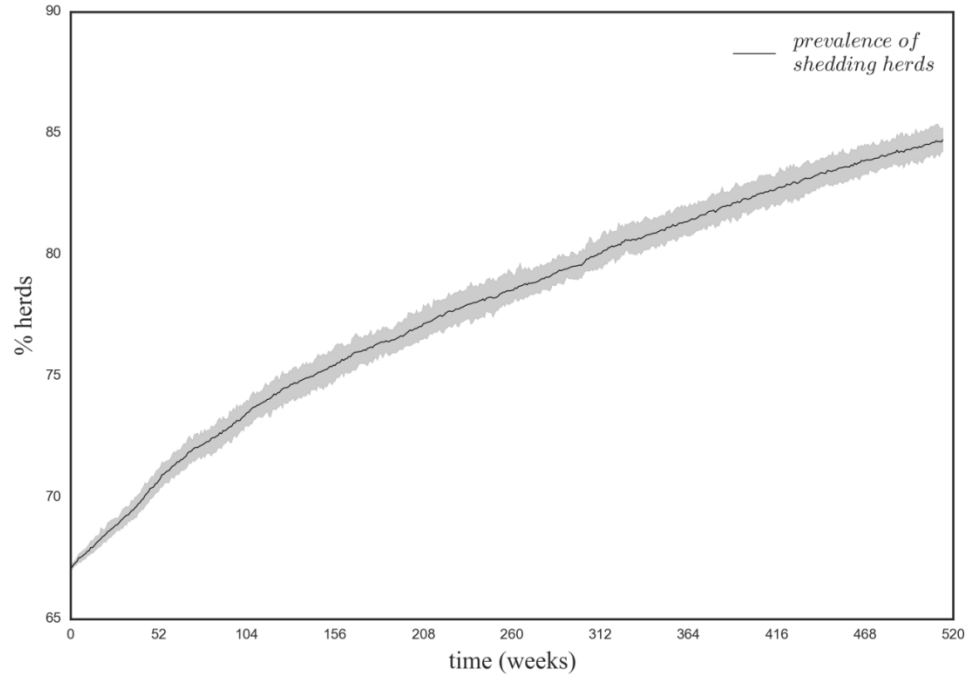


Figure 26: Prevalence of infected herds over a period of 10 years. Line shows median of 50 simulations while shaded region shows 5th and 95th percentile

3.5. Discussion

Our findings show that windborne transmission and movement of cows both affect the regional spread of *C. burnetii* but with different capacities. On the one hand, in both experiments windborne transmission has the ability to introduce the pathogen in a large number of herds if the generation of plume occurs at high enough rates, but the generated outbreaks are generally ephemeral and small. On the other hand, animal trade results in a limited number of incident herds, but purchasing an infectious cow can instigate comparatively larger outbreaks. The differences in the impact of each transmission route on the intra-herd infection dynamics arise from the intrinsic nature of these transmission routes in spreading the infection. Regardless the route, the first generated local case is always a cow with health status I^- as shown in Figure11. Such a seronegative shedding cow is a transient shedder, which can become susceptible again. Therefore, in herds infected by windborne transmission, infection can easily go extinct if the transient first local case does not shed enough to generate secondary cases, which are essential for infection persistence. In herds introducing infectious cows by trade, the animal purchased

can be either a transient shedder (I^-) or a permanent shedder (I^+ or $I^{milk\ pers}$). Hence, after the generation of the first local case, there are at least two shedding cows in herds purchasing infectious animals, leading to potential higher bacterial contamination and increasing the probability of intra-herd infection persistence.

Our results, based on a mechanistic dynamical model of infection spread at different scales, are consistent with a previous study from the same group [51] based on a statistical regression model, which indicated that windborne transmission and cattle trade are both risk factors for the dairy cattle herds in Finistère department. The study [51] also attributed higher proportion of cases to the neighbourhood (which authors consider a proxy representation of windborne transmission) than to animal movements in areas with high cattle density. A cluster analysis performed for the 2012 seroprevalence in dairy herds showed a high-risk cluster in North-western corner of the Finistère department. Clusters for the predicted probabilities of herd infection in 2013 showed two high-risk clusters in the same area, known to have a high density in cattle.

The contribution of animal trade in transmitting livestock diseases is known to vary considerably according to the disease under study. For Q fever, cattle trade seems to explain quite a low proportion of incidence (compared to wind), at least in areas with high cattle density. It is known to play an important role in the regional spread of other infectious diseases, such as foot-and-mouth disease (FMD) and bovine viral diarrhoea virus [92, 102]. For bovine tuberculosis - as here for Q fever -, trade is correlated to a low number of infections [132] compared to other transmission routes. While these studies focus on the regional contribution of transmission pathways, here we also highlighted differences in intra-herd infection dynamics depending on these pathways. The simulated differences in the intensity of intra-herd outbreaks experienced by herds acquiring infection by cattle trade and by windborne transmission, and the capacities of these routes to affect infection-free herds provide valuable insights for risk assessment. Even if cattle trade seems not to generate large proportion of newly infected herds in certain conditions, preventing the purchase of infected animals is still a relevant measure to limit infection spread at the intra-herd scale.

From the model perspective, it is the first time, to our knowledge, that a Gaussian dispersion model for infectious particles is coupled with an intra-herd infection dynamics model to describe the spread of an enzootic livestock disease. Gaussian dispersion models previously have been employed in the description of the spread of viral diseases of livestock and poultry

such as FMD and avian influenza [97, 152]. A dispersion model also has been used to detect the possible risk of Q fever occurrence in human communities from nearby sheep farms [29].

One of the main advantages of using mechanistic models is that they allow determining the causes of infection, and subsequently help assessing targeted interventions [114]. For a given scenario (characterized by a set of fixed parameter values), the mechanistic model presented here identifies the cause of infection of susceptible herds based on the dominant contributory route, at the time of generation of the first local case, and also provides very similar results with the two scenarios assuming single transmission route. Moreover, according to our investigations, the combined effect of the two processes (windborne transmission and animal trade) at a regional scale is additive and not synergistic.

Performance measures of the model at the neighbourhood level can be interpreted as the model ability to predict an observed herd case within a given area. The increase in the AUC for the comparisons done at different neighbourhood radii also indicates the model ability to capture the spatial nature of the dispersion. Assuming that the neighbourhood range and the accuracy of the model depend on the herd density and the clustering of the infection in the study region, selection of a neighbourhood range becomes case-specific. The ROC analysis performed for different neighbourhoods is an effort to increase the sensitivity of the model without altering its specificity, with more weightage given to the capacity of the model of identifying positive herds. The sensitivity of the model hence increases with the decreasing spatial granularity.

Irrespective of the benefits, mechanistic models are generally difficult to be consistent with data. Spatio-temporal outcome of FMD models, when tested against the 2001 outbreak data, have shown about 10-15% accuracy [157]. In the current Q fever model, high accuracy of the model is probably due to the high prevalence and the enzootic nature of the infection in the study region. Models are generally used to simulate the overall spread of an infection to produce expected epidemic curve, and are often difficult to judge for their relevance, especially in the absence of detailed and accurate data. Since many models are increasingly depicting the spatial spread of infections in livestock in enzootic regions, more refined evaluation of their ability to produce spatial patterns in agreement with field observation needs to be addressed. Analysis based on ROC spatial analysis, like the one used here, can be useful in understanding the complex spatial behaviours of such models.

Although we cannot deny the possible existence of interactions between the tested parameters with potential impact on model outputs, the one-at-a-time sensitivity analysis performed supports the relative robustness of model predictions at elementary level. The main output of

the model concerning the relative contributions of the transmission routes in the regional spread of *C. burnetii* showed moderate perturbations to parameter variations, especially when the plume was generated at rates high enough (allowing windborne transmission) compared to death rate of bacteria (κ related to the ratio between these two rates). To reduce the uncertainty on these parameters and hence on their effect on the infection dynamics, more data collection is essential to estimate the bacterial quantities generally found in and leaving farm buildings. The possible effects of super shedders were indirectly assessed using sensitivity analysis of the model to Q1, which is the probability distribution of the shedding levels for all the I^- and for the I^+ shedding in mucus/faeces after 4 weeks post-calving. Two of the probability distributions tested (described in Table 2) assumed proportions of high shedders of 0.25 (distribution IV) and 0.5 (distribution III), whereas the reference scenario assumed no high shedders in these classes. It seems that in scenarios corresponding to distributions III and IV for *Q1* the contribution of trade was diminished, but this needs to be confirmed in further refined analysis.

The model is expected to underestimate the spread of the infection as we ignore beef herds in the study region, which can transmit infection to dairy herds by windborne transmission, and also as we consider cattle trade within the concerned department only for the first experiment. Indeed, according to the analysis of a larger database over the period 2005-2009, 22% of all the concerned transactions of cows involving dairy herds located in Finistère department corresponded to purchases from outside the department. However, no epidemiological information was available for these herds; in second experiment suitable, assumptions were made to model purchase of animals from outside the department and to have same probability of purchasing and infected cow as within the department. Similarly, the impact of small ruminant flocks also was neglected as very few small ruminant flocks are present in the region. Accuracy of the model could be further improved if epidemiological data about beef herds and other livestock flocks in and around the region were available.

The time-varying nature of the network describing cattle trade, in particular the large variability in the trade relationships between herds from one year to the next (as described in France by Dutta *et al*. [66]), suggests that the transmission route due to trade could have a larger impact on the regional dynamics over a longer duration. Indeed, new susceptible target herds could be linked to the network of herds by enlarging (more than one year) the time window of the study. The capacity of windborne transmission of the bacteria is relatively unhindered and all herds get exposed in a very densely populated region without any geographical barriers such as

Finistère. Hence, the regional spread and corresponding control strategies predominantly depend on the prevalence of infection, characteristics of the cattle trade network, and cattle density. On the backdrop of these, the model presented here can become a useful tool to assess the impact of relevant interventions such as vaccination of cows [79][79] and testing of cows for the presence of the pathogen before trading, on the control of the regional spread of infection.

Chapter 4

Modelling the impact of vaccination strategies in an enzootic region

Methods to control *Coxiella burnetii* transmission between livestock farms are limited. They have varying efficacy in controlling the infection within a herd and between herds. In general these techniques can be divided into three groups of measures (i) Hygienic and disinfection methods: proper disposal of manure with heat treatment [27, 28, 158-160], disinfection and removal of aborted placenta and regular cleaning of faeces [45] can reduce intra-herd and subsequently inter-herd transmission of *C. burnetii*. (ii) Testing of infected animals: Identified infected animals can be culled and therefore removed from the transmission cycle. Similarly, trade of infected animals can be restricted to reduce inter-herd transmission of the pathogen [66, 131, 161, 162]. (iii) Medical interventions: These include chemotherapy with the use of tetracyclines, even though it is very much effective in human cases to reduce the symptoms but is known to be moderately effective in reducing the bacteria shedding and the duration of shedding in infected animals [72, 163]. Vaccination of animals using a vaccine composed of inactivated whole Phase I bacteria is known to be much more effective than Phase II vaccine [77], but the role of Phase I vaccine in cattle and small ruminants is considerably different in separate conditions such as *preventive vaccination* (immunization precedes infection) and *outbreak vaccination* (immunization after infection). *Preventive vaccination* of non-pregnant cows substantially reduces their chances to become shedder while pregnancy is thought to affect the immunity adversely rendering the vaccine impotent [78]. Other experimental and field trials also have indicated that the vaccination of non-infected goats and cows reduces shedding after infection. Details of studies regarding vaccination in cattle herds are presented in Chapter 1: section 1.2.3.

In all the available control options which can be implemented to reduce the prevalence of *C. burnetii* in cattle herds of a region, vaccination of animals with Phase I vaccine is professed as a strong candidate for implementation [65]. Vaccination is known a long term strategy particularly in enzootically infected areas and in heavily infected herds as it is expected to reduce the environmental contamination by reducing the shedding by infected animals over long periods, subsequently reducing the transmission within and between animal stocks and to humans. Culling of pregnant animals is generally considered as very effective in reducing the spread, but is extremely difficult to implement because of the high cost of animals, political and ethical issues. Identifying shedders and culling those animals generally is considered ineffective, especially because of the low specificity of currently available tests [161]. Similarly, controlling trade of shedding animals also becomes difficult. Moreover, identifying

infected herds requires mass testing and can be financially difficult. The use of antibiotics is known to be ineffective in substantially reducing the level and duration of shedding in domestic ruminants [65]. Moreover, in the context of the development of antimicrobial resistance in pathogens, it is advisable to avoid long term use of such antibiotics.

This chapter assesses the efficacy of implementation of vaccination in reducing the spread of *C. burnetii* in dairy cattle herds at a regional scale. The further part of this chapter is written in the format of a scientific manuscript. In this chapter we modify the model developed in Chapter 2 to accommodate vaccination and model the effects of vaccination in the metapopulation of dairy herds in Finistère department (France).

4.1. Introduction

Q fever is a worldwide zoonotic disease caused by *Coxiella burnetii* which is found in wide range of animals including ruminants [41]. Ruminants are known as important source of infection to humans [45, 70]. In livestock the infection causes reproductive problems, abortions which result into non-negligible economic losses. Recent years have seen large outbreaks in humans especially in the Netherlands and all these outbreaks were caused by spillovers of the pathogen from livestock [159, 164, 165]. Therefore, the control of the infection because of its consequences for animal as well as human health is crucial [45, 65]. Multiple control options including wide use of antibiotics, trade restrictions and vaccination are possible, which can be implemented on large scale in livestock. Except vaccination of animals with a vaccine composed of Phase I bacteria, other options such as wide use of antibiotics and testing of animals to curtail the trade of infected animals is not-recommended [45]. This is mainly due to rising concerns about possibilities of development of antibiotic resistance and poor performance of diagnostic tests to detect infected animals [161]. Vaccination is considered as an important technique to control the spread of *C. burnetii* in livestock population.

As a prospective strategy to be implemented in a region, vaccination of cattle herds against *Coxiella burnetii* on regional scale provides a few challenges. Implementation of vaccination on a large number of herds is difficult and costly and in many times logistically not feasible. The question under study is a complex scenario of *C. burnetii* transmission between cattle herds. Transmission is influenced by windborne dispersion of the pathogen and cattle trade [45, 51, 52]. Herds which are already infected, herds within high cattle density areas and herds trading large number of cows are known to be significant contributors to the transmission cycle

in a region [51, 65]. Various epidemiological risk factors of herds which positively influence the circulation of the bacteria within a livestock metapopulation can be used as guiding principles in formalising different vaccination strategies. A vaccination strategy could aim to target such specific herds which play an important role in the circulation of the infection in the metapopulaion. Identifying such herds this is essential, as well as assessment of the efficacy of targeted vaccination schemes in reducing the prevalence in a region. Along with that the vaccination threshold required to interrupt the transmission cycle in a region needs to be assessed.

Efficacy of vaccination strategies, as related to the duration of immunity induced by the vaccine and the effect on the intra-herd prevalence of *C. burnetii* is studied by Courcoul et al [84] but the efficacy of vaccination to control *C. burnetii* in livestock populations in a wider geographical area still remains unexplored. Efficacy of vaccination strategy depends on various factors related to the vaccine, coverage of the vaccine in the population, and targeting herds which are significantly contributing to the transmission of infection in the region.

The objective of the study is to formalise an effective strategy which can be implemented to reduce the prevalence of *C. burnetii* in a metapopulation of dairy cattle herds. First we delve into the effects of varying the duration of immunity a vaccine induce on an intra-herd dynamics of infection in an isolated dairy herd. Second we identify the important characteristics of a cattle herd which can be used as directive to implement vaccination program leading to effective reduction in the regional spread of *C. burnetii*, and third we identify the minimum coverage a vaccination should reach in an enzootically infected region to significantly reduce the regional prevalence in dairy cattle herds.

4.2. Methods

4.2.1. Metapopulation model of transmission of C. burnetii, with implementation of vaccination in dairy cattle herds

The metapopulation model developed earlier Pandit et al [166] is used in this study and is adapted to include the vaccination at herd level. In this section we briefly describe the metapopulation model starting with infection dynamics at herd level (intra-herd infection dynamics) along with details of modelling vaccination. Inter herd dynamics is then explained which is modelled using Gaussian dispersion model and observed data on cattle trade.

The model is an individual-based stochastic model in discrete time with one week simulation time step. Cows of the herd undergo different transformations in their health states as shown in Figure 27, with parameters defined in Table 1.

For non-vaccinated cows, susceptible, non-shedder, sero-negative cows (S) become infected and change their health states to shedder sero-negative cows (I^-). The probability of infection for S cows (transmission through the environment) depends on the force of infection present in the herd environment due to bacterial shedding by infectious cows and deposition of bacteria received due to windborne transmission.

$$p_i(t) = 1 - e^{-(E_i(t-1))} \quad \textbf{(32)}$$

I^- cows then become sero-positive, either I^+ (shedder, with antibodies) or $I^{+milk\ pers}$ (shedder with antibodies, permanently shedding in milk at higher levels) or return to S state. I^+ cows then can become carriers which do not shed, C^+ (with antibodies) and subsequently C^- (without antibodies). C^+ cows can restart shedding by returning to I^+ health state.

Modelling of vaccination in a dairy cattle herd is based on earlier work presented by Courcoul et al [84]. We assumed that the vaccine is effective only when applied to non-pregnant uninfected animals [78]. Hence, in the epidemic model (Figure 1), in the intra-herd subsection of the model, only non-pregnant susceptible (S) and sero-negative recovered (C^-) cows get *effectively vaccinated* (S_{ev} and C_{ev}^- respectively). These effectively vaccinated animals undergo alternative epidemic model with complementary health state transitions as for the ones which are not effectively vaccinated, with parameters defined in Table 11. In an experimental field study conducted by Guatteo et al, cows vaccinated when non pregnant had five time lower chance of becoming a shedder than placebo [78]. Hence S_{ev} and C_{ev}^- cows were assumed to get infected with reduced probability of p_v (where $p_v/p = 0.21$ and p is the infection probability of S and C^- cows, based on Guatteo et al [78]). Except for this difference, effectively vaccinated animals go through all the alternative vaccinated health states ($I_{ev}^-, I_{ev}^+, I_{ev}^{milk\ pers}, C_{ev}^+$) with identical transmission rates as for non-vaccinated cows.

Shedding cows can shed the bacteria through milk, mucus/ faeces or through both routes (distributions α, β and γ) at low, medium and high levels of shedding (distributions $Q1$ to $Q5$

for non-vaccinated cows and $Q1_{ev}$ to $Q5_{ev}$ for effectively vaccinated cows). Assumption considered regarding the shedding levels of *effectively vaccinated* cows were based on the quantification presented in the study by Gautteo et al [78] and Rousset et al [59]. As presented in $Q1_{ev}$ to $Q5_{ev}$ shedding distributions, it was assumed that no high level shedding is possible when the cow is *effectively vaccinated* and the probability to shed at low level is increased. The proportions of cows shedding through different routes and at different levels also change according to whether the cow is in early lactating stage ($\leq 4\ weeks\ post\ calving$) or not. All the parameters related to heterogeneity in shedding are presented in Table 12. The final assumption was that *effectively vaccinated* cows cannot abort based on Arricau-Bouvery et al, 2005 [77]. We considered that *effectively vaccinated* cows would lose their immunity after three years of vaccination as assumed by Courcoul et al [84] and made the transitions into the corresponding non-vaccinated health states.

Inter-herd transmission of *C. burnetii* due to windborne transmission is modelled using Gaussian dispersion model which incorporated deposition due to gravity and settling and modelling of cattle trade is based on the cattle trade data available for the study region. Dispersion of pathogen and cattle trade directly and indirectly affect the dynamics of environmental bacterial load of a herd and were modelled according to the study presented in Pandit et al. [166]. The intra-herd model also described the herd demographics and modelled lactation cycle, culling and recruitment of heifers.

In a herd where vaccination strategy is implemented, we presumed that all of the cows irrespective of their pregnancy status are injected the prescribed dose of vaccine at the same time. According the health state and pregnancy status, they get classified into *effectively vaccinated* and non-vaccinated. At the end of the immunity period, all *effectively vaccinated* cows lose their immunity and again booster dose is administered to all of the cows of the herd. All heifers during the recruitment in the herd are assumed to be susceptible and *effectively vaccinated*.

Table 11 Definitions of the epidemiological model parameters and their values used for simulations for non-vaccinated and effectively vaccinated cows

Parameter	Definition	Value
M	Transition probability $I^- \Rightarrow S$ and $I_{ev}^- \Rightarrow S_{ev}$	0.7
Q	Transition probability $I^- \Rightarrow I^+$ and $I_{ev}^- \Rightarrow I_{ev}^+$	0.02
pIp	Proportion of cows going from I^- to I^+ and becoming $I^{milk\ pers}$, similarly Proportion of cows going from I_{ev}^- to I_{ev}^+ and becoming $I_{ev}^{milk\ pers}$	0.5
$r1$	Transition probability $I^+ \Rightarrow C^+$ and $I_{ev}^+ \Rightarrow C_{ev}^+$	0.2
$r2$	Transition probability $I^{milk\ pers} \Rightarrow C^+$ and $I_{ev}^{milk\ pers} \Rightarrow C_{ev}^+$	0.02
S	Transition probability $C^+ \Rightarrow I^+$ and $C_{ev}^+ \Rightarrow I_{ev}^+$	0.15
T	Transition probability $C^+ \Rightarrow C^-$ and $C_{ev}^+ \Rightarrow C_{ev}^-$	0.0096
M	Proportion of bacteria eliminated due to death and to plume generation (can be written as $\mu_{death} + \mu_{plume\ source}$)	0.2
P	Infection probability of S and C^- cows	$1 - e^{-E}$
p_v	Infection probability of S_{ev} and C_{ev}^-cows	$p \times 0.21$
$\rho^{m/f}$	Proportion of bacteria shed through mucus/faeces filling the environment compartment	0.28
ρ^m	Proportion of bacteria shed through milk filling the environment compartment	$0.125\rho^{m/f}$

Table 12 Description and probability distributions used for different shedding routes and levels for non-vaccinated and effectively vaccinated cows

Parameter		Definition	Value
α	α_1, milk	Probability distribution of the shedding routes for the I^- and I_{ev}^-cows	0.31
	α_2, mucus/faeces		0.62
	α_3, milk+mucus/faeces		0.07
β	β_1, milk	Probability distribution of the shedding routes for the I^+ and I_{ev}^+cows after 4 weeks post-calving	0.61
	β_2, mucus/faeces		0.33
	β_3, milk+mucus/faeces		0.06
β_{calv}	β_{calv1}, milk	Probability distribution of the shedding routes for the I^+ and I_{ev}^+cows in the 4 first weeks post-calving	0.14
	β_{calv3}, mucus/faeces		0.5
	β_{calv3}, milk+mucus/faeces		0.36
γ	γ_1, milk	Probability distribution of the shedding routes for the $I^{milk\ pers}$ $I_{ev}^{milk\ pers}$cows after 4 weeks post-calving	0.83
	γ_3, milk+mucus/faeces		0.17
γ_{calv}	γ_{calv1}, milk	Probability distribution of the shedding routes for the $I^{milk\ pers}$ and $I_{ev}^{milk\ pers}$cows in the 4 first weeks post-calving	0.25
	γ_{calv3}, milk+mucus/faeces		0.75
$Q1$	Low level	Probability distribution of the shedding levels for all the I^- and for the I^+ shedding in mucus/faeces after 4 weeks post-calving	0.85
	Mid-level		0.15
	High level		0

Q2	Low level	Probability distribution of the shedding levels for the I^+	0.4
	Mid-level	shedding in milk after 4 weeks post-calving	0.5
	High level		0.1
Q3	Low level	Probability distribution of the shedding levels for all	0.25
	Mid-level	the I^+ in the 4 first weeks post-calving	0.25
	High level		0.5
Q4	Low level	Probability distribution of the shedding levels for the	0.6
	Mid-level	$I^{milk\ pers}$ shedding in mucus/faeces after 4 weeks post-	0.4
	High level	calving	0
Q5	Low level	Probability distribution of the shedding levels for all	0.15
	Mid-level	the $I^{milk\ pers}$ shedding in milk and for the $I^{milk\ pers}$ in	0.6
	High level	the 4 first weeks post-calving	0.25
$Q1_{ev}$	Low level	Probability distribution of the shedding levels for all	1
	Mid-level	the I_{ev}^- and for the I_{ev}^+ shedding in mucus/faeces after 4	0
	High level	weeks post-calving	0
$Q2_{ev}$	Low level	Probability distribution of the shedding levels for the I_{ev}^+	0.9
	Mid-level	shedding in milk after 4 weeks post-calving	0.1
	High level		0
$Q3_{ev}$	Low level	Probability distribution of the shedding levels for all	0.5
	Mid-level	the I_{ev}^+ in the 4 first weeks post-calving	0.5
	High level		0
$Q4_{ev}$	Low level	Probability distribution of the shedding levels for the	1
	Mid-level	$I_{ev}^{milk\ pers}$ shedding in mucus/faeces after 4 weeks post-	0
	High level	calving	0
$Q5_{ev}$	Low level	Probability distribution of the shedding levels for all	0.75
	Mid-level	the $I_{ev}^{milk\ pers}$ shedding in milk and for the $I_{ev}^{milk\ pers}$ in	0.25
	High level	the 4 first weeks post-calving	0

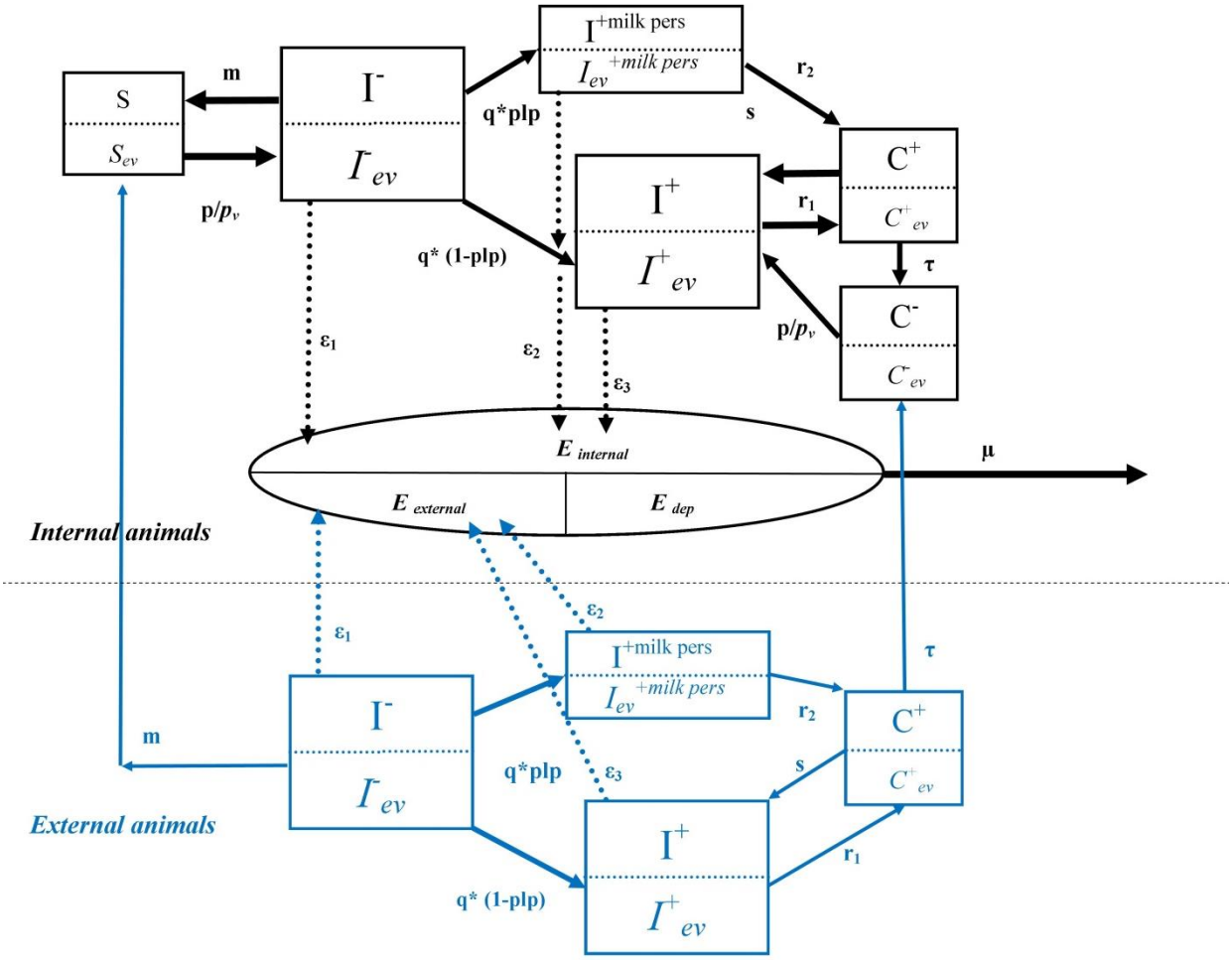


Figure 27 The diagram describes the health statuses of effectively vaccinated and non-vaccinated cows and transitions between these statuses, and environmental bacterial load of the herd. The blue section represents the infection dynamics of external animals, while the black section corresponds to internal animals. Dotted line between effectively vaccinate and non-vaccinated health states indicate that effectively vaccinated cows will become respective non-vaccinated health state after the end of immunity duration

4.2.2. Modelling the effect of duration of immunity on intra-herd infection dynamics

A hypothetical dairy cattle herd of 50 cows completely isolated was considered for these simulations. To generate a sustained intra-herd outbreak, we introduced an infected cow and simulated the intra-herd model until it generated a prevalence of shedders between 10 to 20% to mimic a mildly but endemically infected herd as shown in observational study [127]. After generation of initial conditions, a vaccination strategy was implemented for 10 years to observe the effect of vaccination in reducing the overall transmission of *C. burnetii* within the dairy herd.

The mean number of shedders $\left(I^{-} + I^{+} + I^{milk\ pers} + I_{ev}^{-} + I_{ev}^{+} + I_{ev}^{milk\ pers}\right)$, number of susceptible cows ($S + S_{ev}$), environmental bacterial load, *effectively vaccinated* cows and number of cows eligible for acquiring immunity if vaccinated (non-pregnant susceptible but not vaccinated) were the outputs of interest.

To assess the effect of duration of immunity vaccine can induce in an effectively vaccinated cow on the intra-herd infection dynamics; we systematically varied duration of immunity. Duration of implementation of booster vaccination also varied which was equal to the duration of immunity. Values tested for the duration of immunity in years were 1, 2, 3, 5, and 10 (5 scenarios). For comparison a negative control of absence of vaccination was also simulated. We simulated 100 stochastic simulations for each scenario.

4.2.3. Efficacy of vaccination strategies in Finistère department, France

The Finistère department located in North-Western France and is characterized by a high density of dairy cattle. It is an ideal case study to understand the long term effects of vaccination. The infection of *C. burnetii* is known to be enzootic in the cattle population of this region. In May 2012, 2,799 dairy herds (69% of all the cattle herds in Finistère) were individually and spatially identified, and were also tested for the antibodies against *C. burnetii* in bulk tank milk (BTM) using ELISA, and 1,941 were found seropositive (referred hereafter as *prevalent herds*) as shown in Pandit et al [166]. For the purpose of the study, movement data related to the herds in Finistère department for the time period 2005- 2015 (515 weeks), were extracted from the national register (source: Groupements de Défense Sanitaire de Bretagne, France). Wind velocity data required for dispersion modelling were procured from publically available European Centre for Medium Range Weather Forecasts database [154]. Northward

and eastward wind components data were procured for Finistère department for period 2005-2015. The details of incorporation of the data are in Pandit et al [166].

4.2.4. Initial conditions and model outputs

The initial conditions were mimicking the seroprevalence observed in May 2012 in the department based on the BTM samples conducted and were generated by simulating the isolated intra-herd infection model without vaccination with a forced introduction of an infected cow until the desired (observed) sero-prevalence was reached. We simulated the implementation of different vaccination strategies over the course of ten years. 50 stochastic iterations of the model were run for each scenario. Herd level *Prevalence* was defined as the proportion of herds with at least one shedding cow. Two additional model outputs of total number of shedders in the metapopulation and total number of *effectively vaccinated* cows in the metapopulation were also compared for different strategies. Implementation of vaccination was done in selected herds, and targeting those herds was done by multiple ways and is explained in the next section.

4.2.5. Modelling regional vaccination strategies in dairy cattle herds

Four criteria for targeting herds were tested. Criteria notwithstanding the infection status of herds were (I) animal density in the vicinity of the herd, (II) total degree of the herd (number of trade partners) and (III) the initial size of the herd in terms of number of cows. A criterion regarding the infection status was (IV) prevalence of infection based on the survey conducted in 2012. To compare these strategies, constant vaccination coverage at herd level of 70% was used. Herds in the last 70 percentile for the value of characteristics under consideration in the scenario were chosen as *target herds* for implementation of vaccination. For the scenario where only prevalent herds were targeted, which are 67.2% of the total number of herds, additional 2.8% herds were randomly chosen from susceptible herds to reach 70% of vaccination target. Along with these strategies we also created a scenario with a strategy where herds were randomly (V) chosen for vaccination. A positive control strategy (VI) of vaccinating all the herds and a negative control reference strategy of absence of vaccination (ref) were used also simulated. As shown in table 13, different vaccination coverages were tested for strategies I, II and V.

For each strategy, after generating initial conditions as explained earlier we implemented vaccination in predetermined *target herds*. In a herd where vaccination strategy was

implemented, we assumed that all the animals get vaccinated, generating *effectively vaccinated* animals in it. *Target herds* also received booster vaccinations after every 3 years.

Penetration of different vaccination strategies in the metapopulation was assessed by the proportion of *effectively vaccinated animals* in the metapopulation each strategy could produce over the period of 10 years. Reduction in the spread of *C. burnetii* was observed by comparing the dynamics of the proportion of prevalent herds and shedders in the metapopulation. Efficacy of a strategy at a given time step VE_t, was estimated by comparing the attack rate (AR) in the strategy with vaccination (VS) verses in the reference strategy (RS).

$$VE(t) = 1 - ({}^{AR(t)_{VS}}\big/_{AR(t)_{RS}}) \quad \textbf{(33)}$$

Where, $AR(t)_s$ for strategy s was calculated as

$$AR(t)_S = \frac{number\ of\ newly\ infected\ herds(t)}{number\ of\ infection\ free\ herds\ (t-1)} \quad \textbf{(34)}$$

Table 13 Description of different strategies, estimation of the criteria and coverage of herds tested

Strategy	Target criteria	Definition	Coverage (%)
I	Animal density	density of animals within 5km radius	0,10, 25,40, 55, **70**, 85, 100
II	Total degree	Total number of trade partners	0,10, 25,40, 55, **70**, 85, 100
III	Size	initial herd size	**70**
IV	Prevalent	positive herds (ELISA in BTM in May 2012)	**70**
V	Random	randomly selected herds	0,10, 25,40, 55, **70**, 85, 100
VI	All	all the herds of metapopulation	**100**
Ref	Null	Absence of vaccination	**0**

4.2.6. Characteristics of targeted herds and distributions of risk factors

Distribution of animal density showed a narrow range with 98.3% of the herds having animal density less than 2 cows/km^2 (Figure 28a). Distribution of degree (Figure 28b) of herds showed typical power law distribution and initial herd sizes of herds showed a median of 62 cows and herds with initial herd size greater than 51 cows were classified as *target herds* for

implementation of vaccination (Figure 3c). The spatial positions of herds vaccinated for strategies I (density), II (degree), III (size) and IV (Prevalent) are shown in Figure 29. Figure 30, the venn diagram represents the number of exclusive and common herds vaccinated in mentioned four strategies. Strategy I and strategy II had 28.2% difference in *targeted herds*. Similarly, strategy III had 28.1 % and 28.4% differences with strategies I and II, respectively. Differences in the *targeted herds* in strategy I and II at given vaccination coverage were calculated as the proportion of herds which were exclusively targeted in each strategy. The Pearson's correlation coefficient between animal densities around herds and total degree of herds was 0.02 (p=0.13), between densities and herd size was 0.01 (p=0.5), and between size and degree was 0.15 (p<0.05) and indicated a very low correlation between herd density, size and degree.

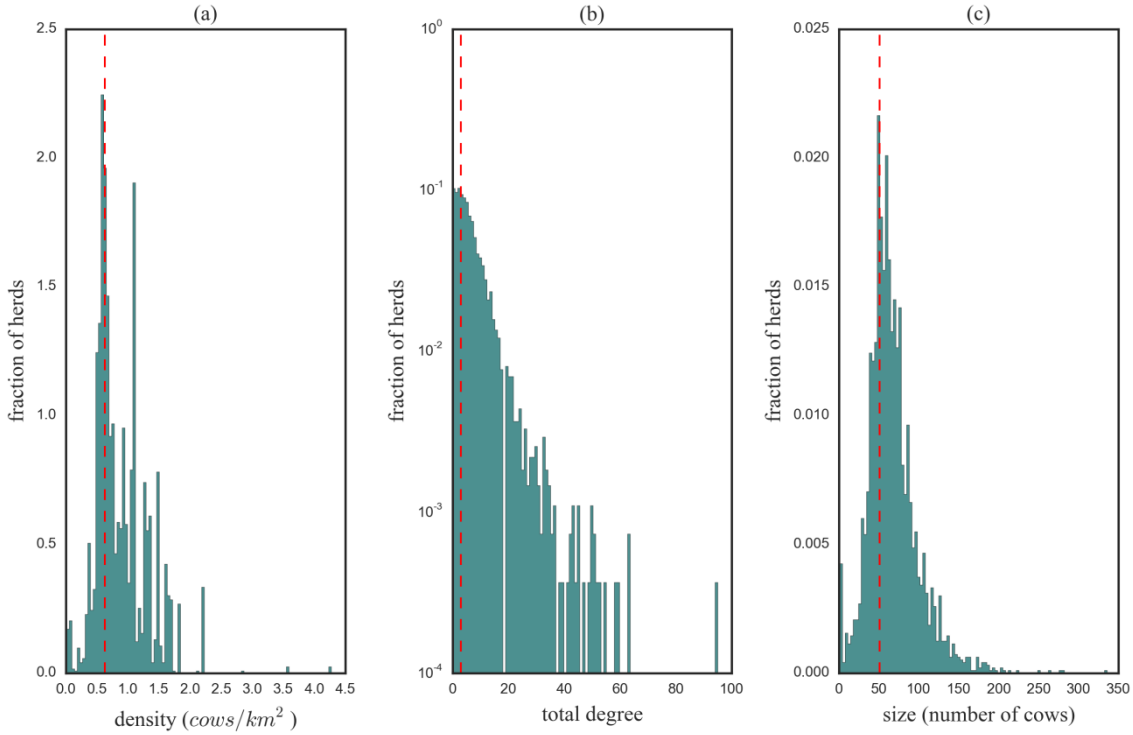


Figure 28: Distributions of animal density (a), degree (b) and size of herds (c) in the Finistère department. Red dotted line denotes the 30th percentile; herds with higher values than the line were vaccinated in strategy I, II and III, respectively.

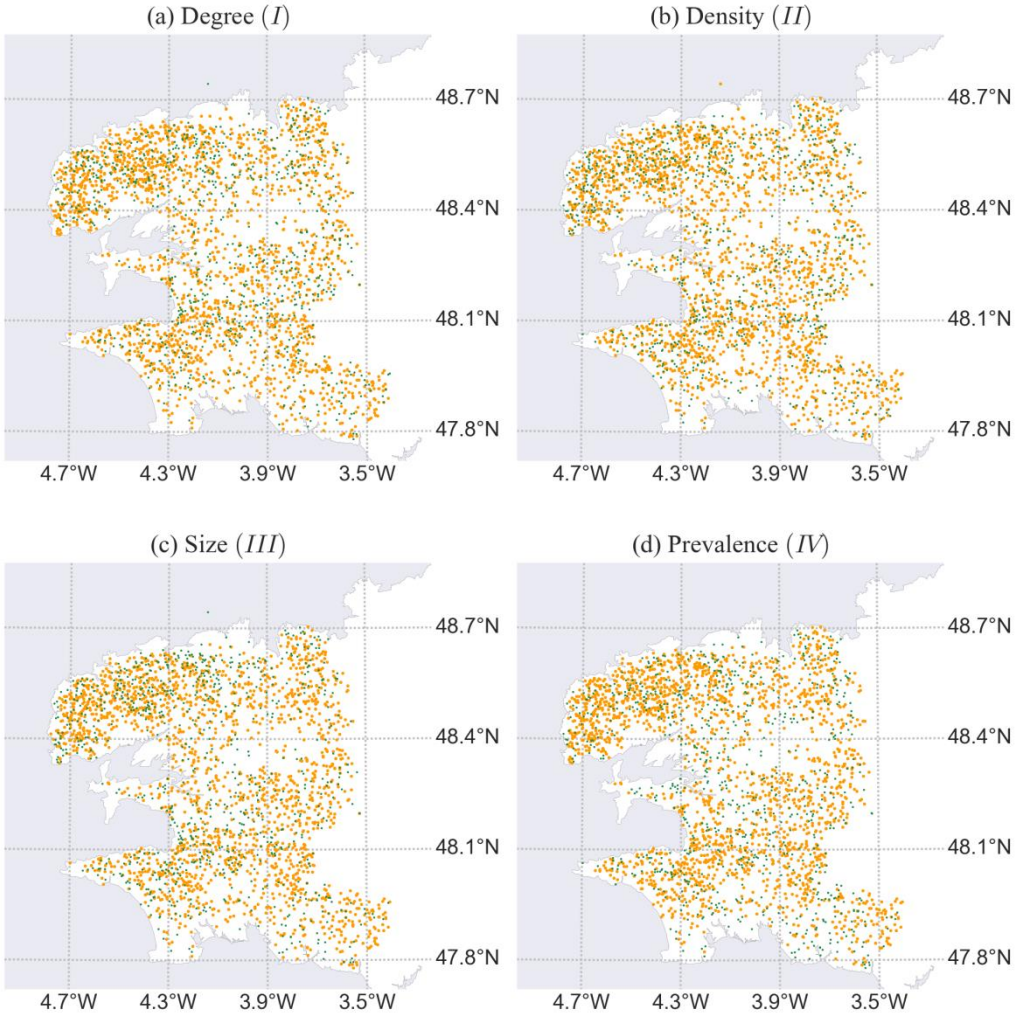


Figure 29 Spatial positions of herds vaccinated (yellow) in scenario I, II and III.

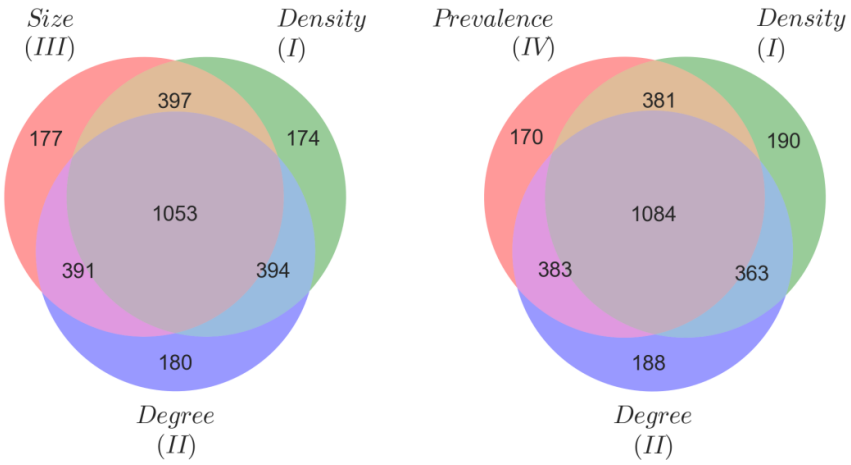


Figure 30 Venn diagram representing common and exclusive target herds vaccinated in strategies I (density), II (degree), III (size) and IV (Prevalence).

4.3. Results

4.3.1. Influence of duration of immunity on model outputs in a herd

In all 600 (100 × 6) stochastic simulations (including the one with absence of vaccination), had on average 28.7 % (±2.24) of shedders at the time of vaccination (6 scenarios shown here Figure 31). Average environmental bacterial load at that time was 0.39 (± 0.02). In negative control (absence of vaccination), the number of susceptible cows, shedders and environmental bacterial load in the herd quickly reached a steady of state of 32%, 36% and 0.6 units respectively. On the contrary, shedders and environmental load for any vaccination scenario, irrespective of the duration of immunity decreased drastically. This decrease is supported by a corresponding increase in the accumulation of the number of vaccinated cows in the vaccination scenarios. As expected, all the scenarios showed similar trends until the end of the first year. After that, the number of vaccinated animals stabilised in the year of duration of immunity of the scenario and diverged from the remaining scenarios at that time. Despite that, all the scenarios with duration of immunity higher than three years showed similar trends for the environmental bacterial load.

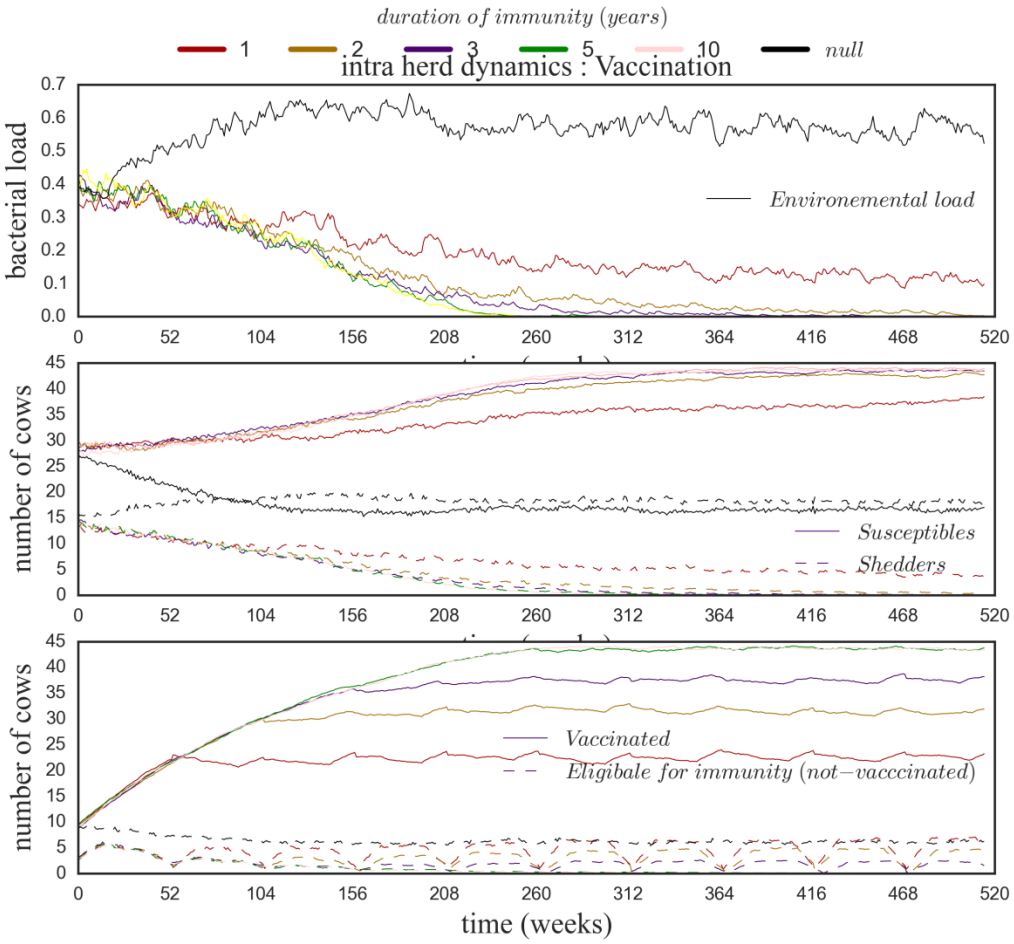


Figure 31 Effect of variation of duration of immunity on the intra-herd infection dynamics of *Coxiella burnetii*.

4.3.2. Effect of vaccination on the regional spread of C. burnetti

In strategy VI (all herds were vaccinated) the proportion of *effectively vaccinated* cows in the metapopulation increased sharply over the first 5 years and stabilised at 0.85 (Figure 32). Similarly, for all the other vaccination strategies with a vaccination coverage of 70%, except for strategy III, the proportion of *effectively vaccinated* cows stabilised at 0.6 after the first five years. For strategy III (size) it stabilised slightly higher near 0.68.

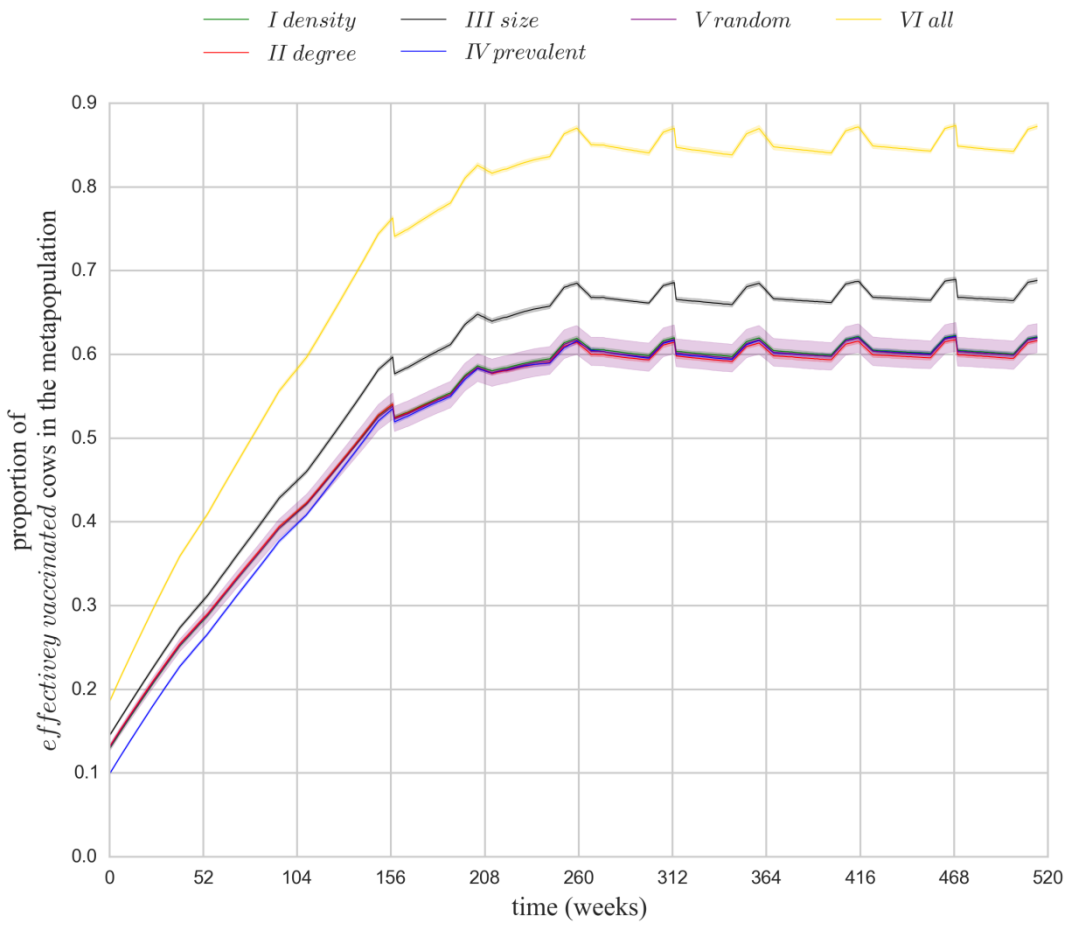


Figure 32 : Mean proportion of effectively vaccinated cows in the metapopulation over the period of ten years with different vaccination scenarios.

Figure 33 shows the temporal dynamics of the prevalence in the metapopulation of dairy cattle herds in the Finistère department. If vaccination was not implemented, the herd level prevalence in the metapopulation increased steadily reaching 85% in ten years. All the strategies with vaccination resulted in decrease in the herd level prevalence after an initial temporary increase, and went below the initial prevalence within the first four years of implementation of vaccination. The mean prevalence in strategy VI went below the initial prevalence the earliest in the 142nd week after vaccination. Similarly, for strategies I, II III and V it took 187, 185, 223, and 189 weeks respectively to reduce the prevalence below the initial prevalence (Figure 33 inset). In strategy IV, despite a higher initial increase in the prevalence than other strategies, it took only 191 weeks to reduce the prevalence below the enzootic initial prevalence. The strategy where all the herds were vaccinated (VI) showed an effective

reduction in the prevalence with a maximum mean prevalence of 68.6% and reducing it to 18.8 at the end of the ten years. In strategies with vaccination of targeted 70% of herds, strategy IV was the most effective with a mean prevalence reaching 29.1%. In strategy II the prevalence reached 36.6% at the end slightly outperforming strategies I and V with mean prevalence of 38.9% and 39.5%, respectively. Strategy I and V showed similar temporal dynamics of prevalence.

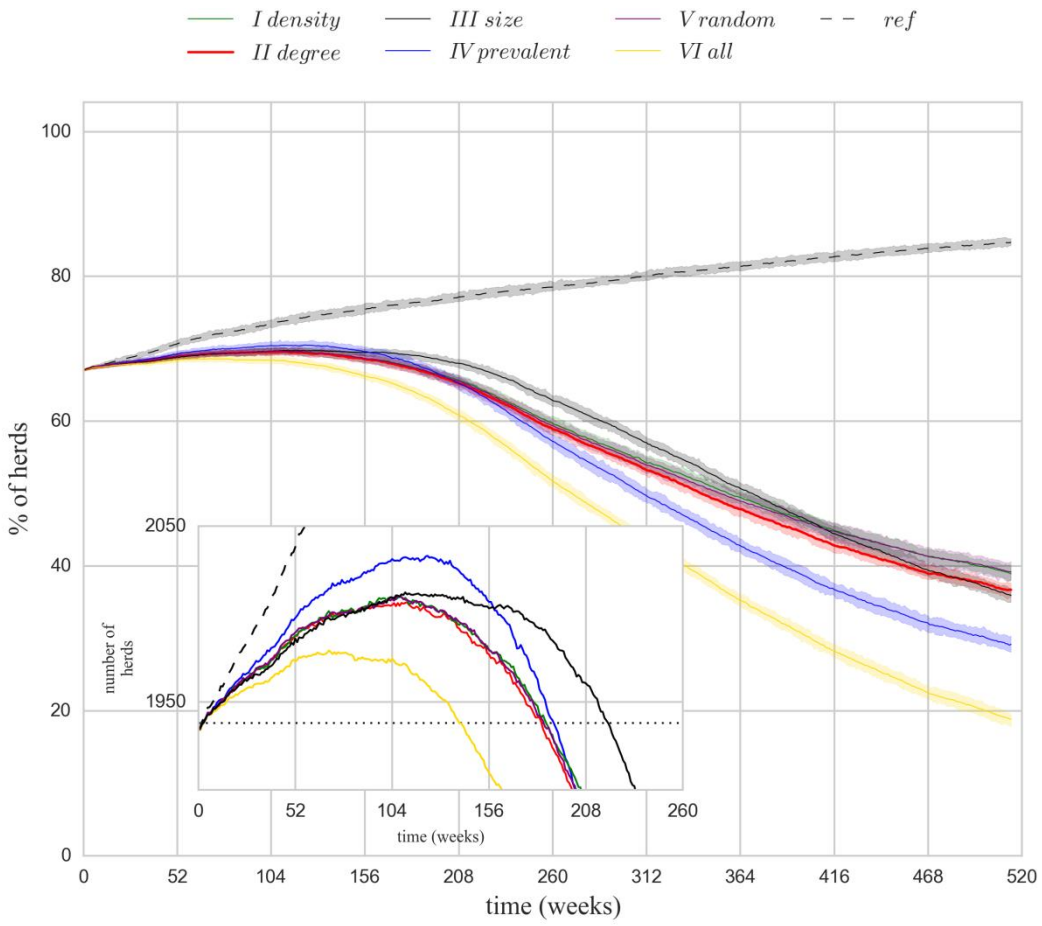


Figure 33 Mean prevalence (lines) and 95% CI of herds with shedding cows in the metapopulation over the period of ten years with different vaccination scenarios. Legends show the criteria used in the strategy implemented (inset: shows mean number of prevalent herds in first five years for all strategies)

Similar results were seen for the temporal dynamics of proportion of shedders in the metapopulation (Figure 34). The proportion of shedders in the metapopulation for all the tested strategies reduced rapidly during the first four years and then decreased gradually. In strategy VI, the proportion reached 0.02. Strategies based on degree (II) and animal density (I) were

slightly better than the random (V) strategy and showed slightly lower prevalence at the end of the ten years. The proportion of shedders was lower in strategies IV and III than in other strategies with identical herd coverage (70%).

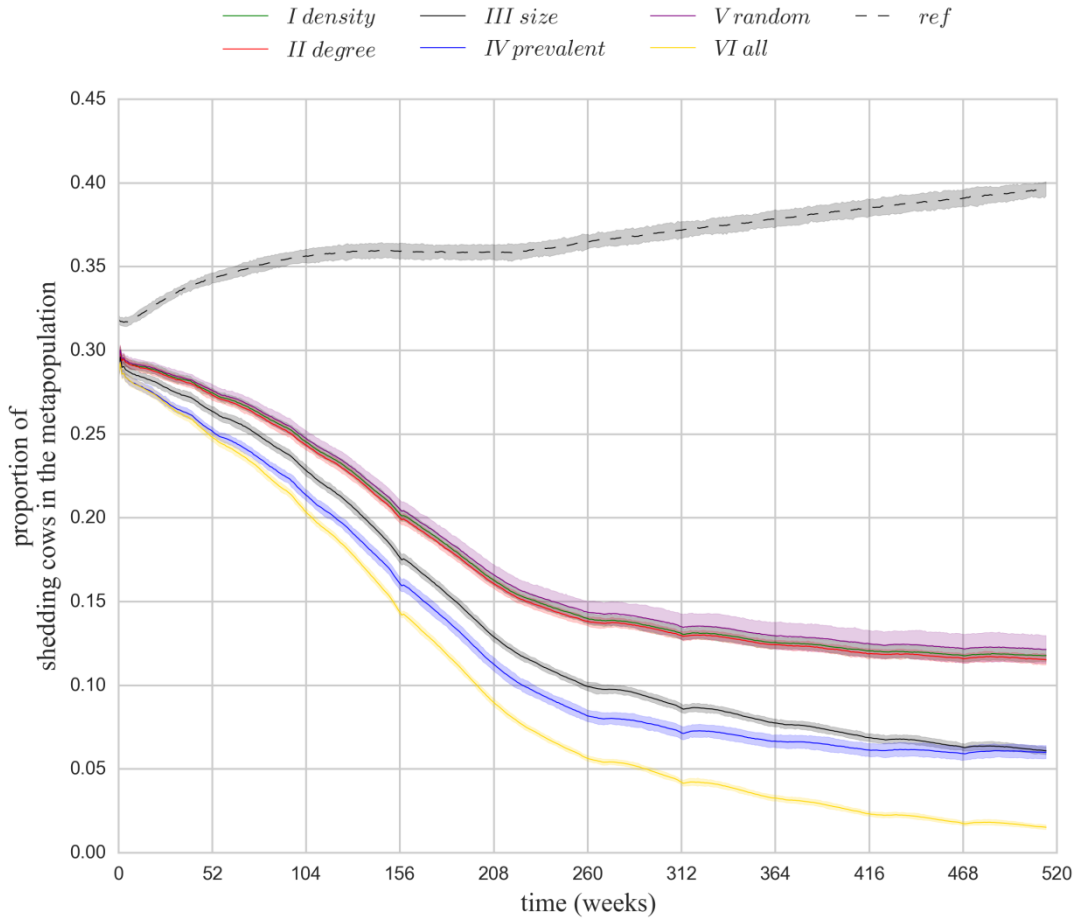


Figure 34 Temporal dynamics (means and 95% CI over 50 simulations) of proportion of shedders in the metapopulation for different tested vaccination strategies.

The incidence (newly infected herds) was curtailed in different capacities for all the vaccination strategies. In absence of vaccination (reference, Figure 35) the incidence at herd level stabilised after first four years near the value of 18 new infected herds/ week. All the tested vaccination strategies showed initial stable incidence and a gradual increase in the incidence after first five years. Strategies I, II and V showed similar temporal dynamics over the period of 10 years. For strategy IV, the incidence was slightly higher than for all the other vaccination strategies but later on converged to incidence rate at the end of 10 years. Strategy VI showed the least

incidence in first five years while ending at a lower incidence rate than the other vaccination strategies.

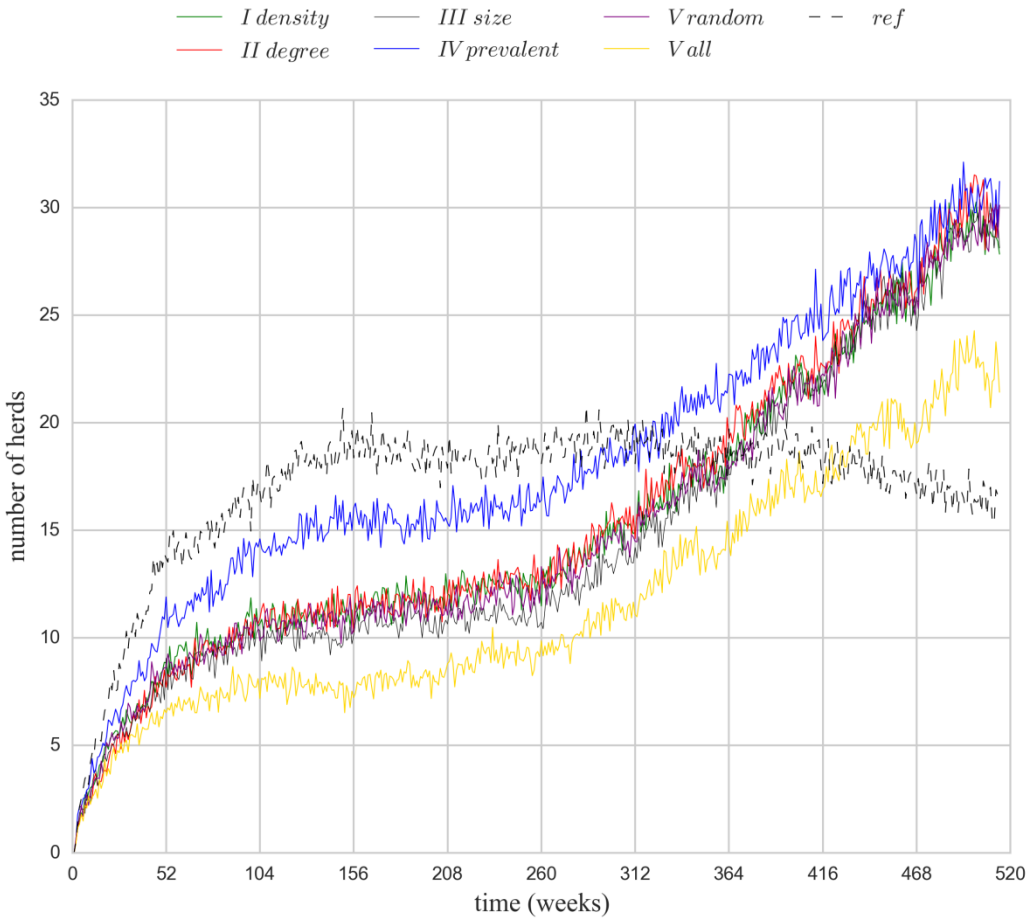


Figure 35 Temporal dynamics of overall incidence (mean over 50 simulations) for tested vaccination scenarios.

Efficacy of each strategy when compared with the reference strategy of absence of vaccination showed that all the strategies with 70% coverage converge to similar efficacy between 0.5 and 0.6. Strategies I, II, III, and V showed similar efficacies throughout the ten year period (Figure 36). Strategy IV initially performed poor with respect to other strategies but the efficacy increased later and it slightly performed better than other strategies at the end of simulations. As expected the efficacy of strategy VI was higher, closer to 0.7 at the end of the simulation.

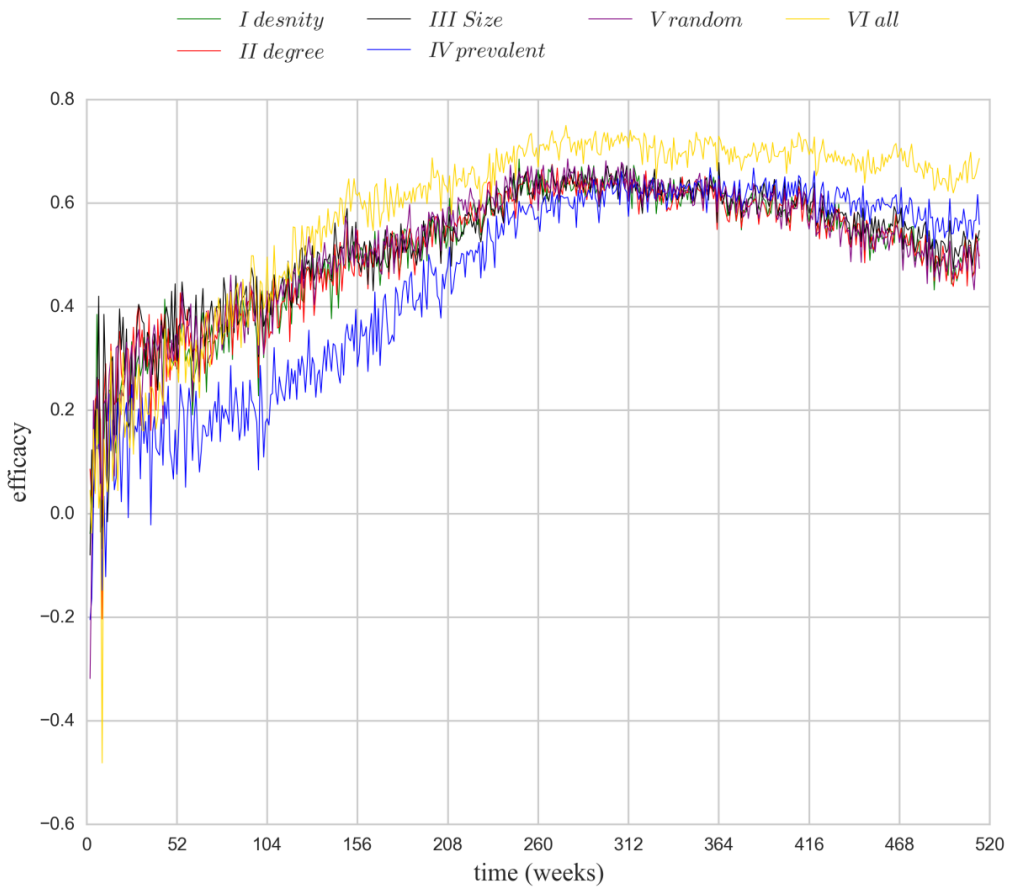


Figure 36 Variation of efficacy of vaccination strategies at the metapopulation level when compared with the reference strategy

4.3.3. Effect of vaccination coverage on the effectiveness of vaccination strategies

Difference in the herds vaccinated at different coverages for strategy I (density dependent vaccination) and II (degree dependent vaccination) decreased linearly with the increase in the coverage as shown in Figure 37. The prevalence of infected herds at the end of the simulation also reduced linearly for both strategies I and II with increase in the vaccination coverage. At all the different coverages, strategy II showed lower prevalence than strategy I and strategy showed similar prevalence at the end of the simulations.

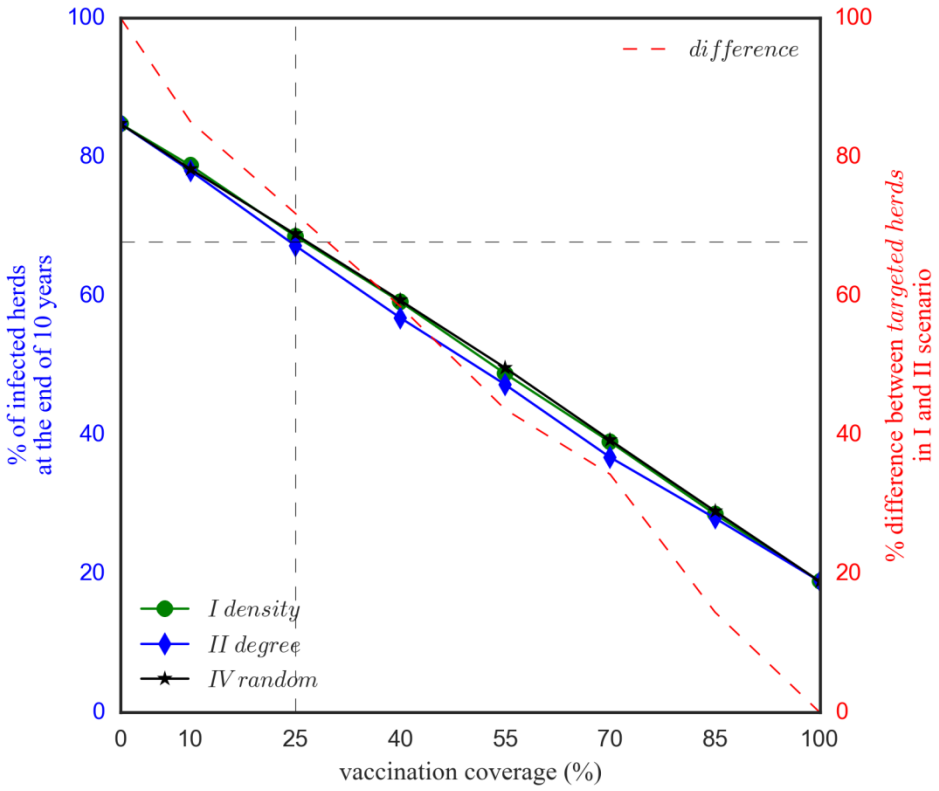


Figure 37 Proportion of infected herds at the end of simulation at different vaccination coverage and difference in targeted herds in scenarios I,II, and IV. Intersection of grey line indicates minimum vaccination coverage required to reduce prevalence below the initial herd level prevalence.

4.4. Discussion

4.4.1. Implications of variation of the duration of immunity on the infection dynamics of C. burnetii within a herd

Results of the numerical experiments conducted to comprehend effects of the duration of immunity following vaccination can provide insights on the intra-herd persistence of infection and indicate that duration of immunity induced by vaccine is an important parameter and considerably affect the infection dynamics of *C. burnetii* within a dairy cattle herd. In general, vaccination of cows and recruiting heifers and their subsequent boosting regularly reduced the overall environmental contamination of *C. burnetii* and subsequently reduced the prevalence of shedders. The results shown here are in agreement with more detailed modelling study

conducted previously to test wider varieties of vaccination scenarios [84]. The reduction is more efficient if the duration of immunity is more than 3 years. The average lifespan of a cow being less than three lactations cycles and hence vaccinating before their first insemination results into practically lifelong immunity. Hence, similarly all the scenarios with more than 3 years of duration of immunity show similar trends throughout. Hence for the metapopulation experiments we choose the duration of immunity of three years as it is effectively reducing the bacterial load within 5 years of simulation and it is also corresponds to the recommended booster time by the vaccine manufacturers.

4.4.2. Vaccination strategies and regional dynamics of C. burnetii in dairy cattle herds

Results indicate that vaccination of cows and recruited heifers with a vaccine which provides three years of duration of immunity and subsequent implementation of booster vaccination on large scale in an enzootically infected cattle metapopulation of Finistère will significantly reduce the prevalence both at herd level and animal level over a period of ten years.

Vaccinating all the herds (Strategy VI) as a best possible scenario was used to compare the efficiency of other strategies with 70% coverage in the Finistère. In all of the tested strategies, targeting herds based on their infection status was the most efficient strategy (Strategy IV) for the Finistère department. After ten years, both the proportion of prevalent herds and the proportion of shedding cows were significantly lower in strategy IV than in the other strategies tested. On the contrary, in the first four years, the number of prevalent herds in this strategy showed a sharp rise, probably because of rapid introduction of infection in non-vaccinated susceptible herds. We believe that in a metapopulation with a high proportion of prevalent herds such as in Finistère, vaccinating prevalent herds is the most efficient strategy. We also think that in areas with low prevalence and dense animal population the strategy might not be as efficient, as the initial few years of rapid spread might create large infected subpopulation of newly infected non-vaccinated herds within initial years of implementation which will continue the transmission cycle within the region. In such areas, other strategies might be equally efficient and should be tested. In the case of the Finistère department, in absence of any information about prevalence or infection status of herds in the region, strategy based on number of trade partners a herd has, is more effective than a strategy based on the density, as indicated by the dynamics of prevalence and proportion of shedder cows. The scenario based on animal density and scenario with random selection of herds showed similar effectiveness,

which might be due to the fact that the distribution of animal densities of herds did not show large variations leading to the selection of similar herds in both the scenarios.

Previous results indicate that windborne transmission plays an important role in the spread of *C. burnetii* between dairy herds of Finistère department [166]. Vaccination of animals as a preventive tool is generally regarded as an important strategy in windborne spread diseases [96, 167, 168]. Here, vaccination is shown to reduce the incidence at herd level for a few years, which increases steadily later as number of susceptible herds increase. Similarly, targeting herds based on cattle trade network characteristics aims to curtail the spread of the disease due to cattle trade. Counterintuitively, strategy I based on animal density (a risk factor for windborne dispersion) performs poor than strategy II. This could be due to the fact that the infections related due to animal trade are long lasting and produce larger number of animal cases within infected herds [166]. Moreover, both strategies share 69.2% of herds, hence vaccinating the rest of the 30.8 % herds based on trade characteristics is more rewarding than targeting them on animal density. Even at multiple vaccination coverage values tested here, when the difference in the *targeted herds* between strategy I and II varied considerably from each other, strategy II based on the degree was always performing slightly better than the strategy based on density, which showed similar results to strategy V (random) at all the coverages.

For strategy II, we used the estimates of degree (number of trade partners) for each herd based on 10 years of accumulated data. The cattle trade networks are known to be dynamic and herds can exchange animals with different herds with different strengths in different years, despite the fact that overall distributions of network characteristics remain similar after period of 6 months [66].

It should be noted that these results are highly influenced by the conditions of the case study presented here. Finistère department is a region with high density of dairy cattle farms with nearly 67% of herds enzootically infected with *C. burnetii*. Though similar risk factors have been identified in sparsely populated region of Gotland, Sweden [51, 52], efficacies of these vaccination strategies should be tested before extrapolating these conclusions to other metapopulations. Vaccination programs in livestock are highly influenced by cost of implementation of the program [169-172] and such cost-benefit studies are essential before

implementation of a vaccination program against control of *C. burnetii* in cattle at regional scale.

Chapter 5

General discussion

5.1. Significance of the study objectives

In my PhD project, I have focused on the regional spread of *Coxiella burnetii* in livestock population and with emphasis on dairy cattle system, to understand important transmission pathways involved in the regional spread of the pathogen. Further, assessment of effectiveness of implementation of vaccination at a regional scale also has been done to inform policy makers and farmers about possible control strategies they can adopt to reduce the regional spread and prevalence of *C. burnetii* infection in dairy cattle herds. European Food Safety Authority's 'Scientific opinion on Q fever', explicitly mentions the significance of studying potentials of transmission pathways and identifying effective control strategies to control the infection in livestock populations [1]. Similarly, as one of the foremost zoonotic diseases, Q fever features in the list of diseases of common interest of animal and human health in an agreement of OIE with FAO and WHO in the development of Global Early Warning and Response System [2]. In the context of One Health, for the development of any early prediction system at human and animal levels, results of the thesis contribute to first steps. They can indirectly contribute to reduce human outbreaks by reducing the circulation of the pathogen among livestock populations.

5.2. Comments of result highlights

5.2.1. Relative contributions of two main routes in the transmission of C. burnetii in Finistère department

Our results indicated that in dairy cattle herds of Finistère department, *C. burnetii* is essentially transmitted by windborne dispersion. Indeed, the initiation of infection in disease-free herds has been shown to be more likely due to windborne dispersion than to the purchase of infected cows. We also observed that both routes affect the newly infected herds differently. Infections initiated by windborne dispersion in dairy cattle herds cause smaller outbreaks and do not last long with high probability of extinction. Contrary to this, purchasing an infected cow leads to larger intra-herd outbreaks with lower chances of extinction. Over longer time period, as herds experience multiple outbreaks, bacterial contamination in the herd due to previous outbreaks also contributes significantly in initiating infections in herds, along with windborne transmission. Windborne dispersion and cattle trade have been shown to act independently of each other on regional infection dynamics of *C. burnetii*, at least over short periods (e.g. one year, as studied here). In a herd which sells a large number of cows, infection due to windborne

transmission was expected to synergistically act with the transmission due to trade, by infecting an important node herd in the cattle trade network. Such interaction between the two transmission routes was not observed. Considering the fact that the two routes have different and separate impacts on transmission, to achieve complete eradication in livestock a control strategy which hampers transmission by both routes is essential.

Due to the ubiquitous nature of *C. burnetii*, which is found in multiple animal taxa in wildlife and ectoparasites like ticks [3], on rare occasions, transmission of *C. burnetii* due to these animals is possible, especially when they are known to be reservoirs of the pathogen [4-9]. Evaluation of the roles of these pathways in the dynamics of infection in livestock is difficult. This is mainly because knowledge about the prevalence of *C. burnetii* in these animals and their interaction with livestock is lacking. Moreover, as the infection dynamics is dominated by windborne dispersion and cattle trade, the role of synanthropic and sylvatic animals is considered as negligible [1, 10].

5.2.2. Effectiveness of vaccination

Vaccination of cows in dairy herds and vaccinating heifers before their first pregnancy with Phase I vaccine at large scale in a region have been shown to significantly reduce the prevalence of *C. burnetii* in the region. In our exploration of scenarios, herds can be chosen for implementation of vaccination based on their infection status, animal density, number of animals or the number of trade partners. These strategies have different efficacies in preventing the spread. For the case of Finistère department, a strategy of vaccinating herds which are already infected was the most efficient at a given coverage. This might be due to high herd prevalence in the studied region. However, prevalence might be lower in other areas or herd infection status might be unknown. We have shown that other strategies give almost as effective results without requiring information on herd infection status. A strategy of targeting larger herds was better at reducing the infection at animal level, but the overall reduction in prevalence at herd level was similar with strategies of targeting herds surrounded by higher animal density and important number of trading partners. Vaccination is a long term control option and its effects are reflected after around three and half years to five years (depending on the strategy and coverage). During the first few years of its implementation, we can still see an increase in the prevalence. A variation in the vaccination coverage (in animal density and degree dependent strategies) showed linear relationship with the reduction of prevalence. Targeting larger herds will imply vaccinating more animals and hence might be more costly

due to requirement of more vaccination doses in spite being more effective in reducing animal level prevalence which will increase the cost of implementation. Similarly, decisions with respect to vaccination coverage should be undertaken keeping in mind the reduction goals desirable to policy makers along with the cost of the cost of the program.

5.2.3. External validity of the results

The abilities of windborne dispersion and of cattle trade to spread *C. burnetti* from one cattle herd to another, and the ability of vaccination programs to reduce the prevalence of the pathogen at a regional scale are very well established in the thesis, but their capacities in doing so depend on characteristics of the population under question. In regions with different animal density, cattle trade network structure or prevalence of infection than Finistère, the contributions of the two routes can vary, and hence the effectiveness of a vaccination strategy. For example, the distribution of cattle density in Finistère is completely different from the one on the Gotland Island in Sweden, where cattle herds are sparsely located. Finistère has shown a statistical cluster of positive herds in its north-western corner while similar analysis on the Gotland population showed none [11], indicating that routes might have slightly different quantitative contributions in these two populations. However, the model presented here provides a very good framework to evaluate the spread of infection in such regions.

5.3. Relevance of the modelling framework and methods used

I have developed an original multiscale model of the regional spread and control of *C. burnetii* in dairy cattle herds. It is the first model for the infection dynamics of this pathogen combining windborne dispersion and cattle trade between herds, and within herd and between herd infection and population dynamics. It constitutes a framework to evaluate control strategies by targeting herds according to their characteristics (trade pattern, size, location, prevalence of infection) to implement vaccination. Such formalisation was convenient to address the issue of quantifying the relative contributions of transmission routes in the regional spread of *C. burnetii*. Data available for the Finistère department was used to validate the model at a preliminary level by comparing the model outputs with observed data. To further understand the model behaviour, we did a sensitivity analysis of model outputs to input parameter variation.

Multiscale metapopulation studies, coupling within-herd dynamics and between-herd dynamics in livestock populations have previously investigated the roles of infection transmission

mechanisms. Bovine tuberculosis spread in Great Britain is explicitly modelled through movements of cows and environment [12]. Regional spread of paratuberculosis in cattle metapopulation is described using cattle trade data [13]. Models for FMD and HPAI have tackled the problem modelling airborne dispersion [14, 15] and trade of livestock separately [16]. According to our knowledge this is the first study comprising of a detailed intra-herd model coupled with model describing regional spread of a disease via windborne dispersion and trade of animals. Statistical approach applied by Nusinovici et al has been able to quantify the capacities of transmission routes and identify the factors contributing to the transmission of *C. burnetii* between dairy herds [11, 17, 18] and results from these studies are complementary to the one presented here.

5.3.1. Model structure

We use an individual based, stochastic, discrete time framework to formalise the model of the regional spread of *C. burnetii* between dairy cattle herds, with a time step of one week. Almost 30% of the herds in the Finistère department have less than 50 cows. Hence, since we dealt with small populations, it was appropriate to consider stochastic transitions between health states and probability distributions for shedding routes and shedding levels. One of the important advantages of using an individual based model was that it facilitated the integration of the cattle trade data into the metapopulation model. The cattle trade data '*BDNI*' (the abbreviation of the French name of the database) tracks transfers between farms of each individual French cattle based on its identification number and incorporates animal characteristics. Hence, with an individual based model structure, we could use the data as it is, making the model more realistic. Furthermore, since we can track an individual cow in the model we can also track the source of infection in the case the cow is infected. The intra-herd model is parameterised on the dataset presented in a longitudinal study by Guatteo et al [19]. In this study the samplings of individuals in herds was done every week and considering the slow progression of the disease in an animal, lead to the choice of one week as a time step for the intra-herd model presented in previous studies [20-22].

The genotype diversity of *C. burnetii* in cattle is explored in some studies [26, 27]. As the genetic diversity among *C. burnetii* infecting cattle in Europe is considered as low [27] and the knowledge about the virulence in these strains is lacking, we did not consider the possible variation in the spread of pathogen because of different genotypes in the model.

Another important structural characteristic of the model is the explicit representation of the infection through an environmental compartment, which represents the infection force in the environment due to the bacterial shedding by infected cows. Modelling studies accounting for environmental transmission generally model infection probability as $p = 1 - e^{-\lambda\kappa}$, where exponential term $(-\lambda\kappa)$ is generally a product of terms representing number of pathogens available and probability of successful contact [23, 24]. When bacterial are shed through different routes such as vaginal mucus, faeces, urine, placenta, milk, they are not readily available for infection through inhalation. Smaller droplets of these shed *C. burnetii* in its small scale variant, generated by sneezing, coughing, splashing, and other activities and form droplet nuclei after drying [25]. These droplet nuclei are readily available for inhalation for animal in the immediate surroundings or can be transported along with the wind to distant places, unlike larger particles. Many bacterial shed by cows do not become available for inhalation either due to natural death or just because they do not form inhalable particles. The model incorporates this loss, and only fraction (ρ) of the shed bacterial reaches a stage where it is available for transmission, which is represented as the environmental compartment. For each cow, we assume that it is exposed to bacteria present (deposited due to windborne transmission and shed by animals in the herd) in the area within its vicinity ($3m^2$ per cow). Hence, in the dispersion model, the overall infection force due to deposited bacteria in the environmental compartment of a herd is based on number of cows rather than the actual area the barn building and available pasture can have and we consider a farm as a single co-ordinate in the spatial mesh of the model.

5.3.2. Dispersion model

Gaussian dispersion model used here to model the windborne dispersion of *C. burnetii* from one herd to another describes the transport of a part of the environmental compartment from the source herd (infection force due to bacterial contamination of the environment), to the destination herd. We treat a herd, including its barn building and pasture as a single co-ordinate in the spatial mesh. Since we are concerned about dispersion of the environmental contamination and not actual number of particles of pathogen as done in conventional dispersion studies of pathogens [14, 15, 28-35], it is convenient to use the Gaussian dispersion model rather than the Lagrangian model. The Lagrangian model tracks the dispersion of each particle on a random walk principle and thus it was not possible to couple it with the current intra-herd model framework available as we here model do not model spread of infectious

particles but the infection force due to windborne dispersion. Besides, given the relatively flat terrain of the Finistère department, we decided to use a Gaussian dispersion model, which accounts for the deposition due to gravity and settling. Utilisation of Lagrangian model would have increased the accuracy of the dispersion prediction and should be utilised in complex terrain conditions [14, 36]. We believe that better estimates of the concentrations of viable *C. burnetii* organisms inside and outside the herd buildings are required to implement Lagrangian model along with their association with the proportion of shedders within the herd. Studies can be found which try to estimate the bacterial concentration in the air and the dust of barn buildings [37]. Currently efforts are more focused on longitudinal sampling of air within barns using PM 10 pumps [38, 39].

5.3.3. Cattle trade

We modelled the cattle trade in the current study based on the raw data. Modelling of cattle trade can be also achieved by utilising the trade network characteristics for each herd, but using raw data allows us to incorporate temporal variation in the trade very easily. Moreover, the data mentions cow specific information such as its age at the time of trade, which is one of the influential parameters for the disease dynamics within herd [22]. Hence, utilising raw data makes model more realistic in representing cattle trade and spread of *C. burnetii* because of it.

Since these data are not available for future years (by definition), it is important to have long time series in the present to infer assumptions for future cattle trade, to be incorporated in transmission models. Generally, availability of such extensive data for trade or movements for long duration of livestock between holdings is infrequent. Currently, very few regions in the World have mandatory recoding of such data for cattle trade. Sometimes, data might be available for shorter durations such as one year. In such cases, repeating the same data in the model for multiple years might not represent the complete dynamic nature of trading network. In such cases, generation of networks can be generated based on the topography of the network studied from the available data. In absence of any information about trading pattern, studies have used random network structure [40].

5.3.4. Identifying the cause of infection

Dividing the environmental compartment according to the source of contamination was done in order to be able to identify the relative contributions of routes at the time of generation of first case amongst *internal animals*. We also assumed that a herd that has received an infected

animal and in which initiation of infection is observed after the purchase is infected due to cattle trade. Otherwise, we considered it to be infected by windborne dispersion. This was due to the fact that, in pilot simulations, we observed that in all runs, when a herd had bought an infected animal, the contribution of the corresponding sub-compartment always had higher value of infection force, when compared with the one corresponding to windborne dispersion. This was especially due to the fact that the amount of bacteria a cow can shed is very high compared to bacterial deposition.

In the first experiment presented in Chapter 3, we quantify the ability of transmission routes to initiate the infection in infection free herds. Comparison of further contributions in number of cases at animal level attributed to each of the route was not done as it might not be possible to attribute cause to each individual case in the current model structure. This is mainly due to the fact that the probability of infection ($p = 1 - e^{-E}$) comprises combined contributions of three sources: shedding cows which are purchased from other herds, shedding cows of the same herd which got infected during the intra-herd outbreak, and bacteria deposited due to windborne transmission. What could be interesting is to follow the temporal contributions of each of these routes and to relate there contributions with the intra-herd prevalence, to comprehend the impact each route have on the intra-herd infection dynamics. Even though this would be an interesting complementary approach, initial results indicated that after successful initiation of infection in a herd, multiple cows start shedding, which ultimately leads to its high relative contribution in the overall environmental compartment. Hence, we hypothesise that, on the long term, cattle trade and windborne dispersion might not play a significant role in maintaining the infection in an already infected herd, and their role might only be restricted to instigate infections in disease-free herds in the metapopulation of livestock herds.

5.3.5. ROC analysis

The model showed satisfactory capability in correctly predicting infection status of a herd in a year. Keeping in mind the spatial nature of the transmission, we are more interested in knowing if herds within a small area will suffer an outbreak. Model was substantially better in predicting the outcome for small zones. This interpretation of model result for vicinity is practical for farmers as they can pre-empt by implementing some measures such as vaccination or by following strict hygienic practices, in their community.

Comparison of the observed data and model outputs was done using ROC analysis. ROC analysis is generally used to validate a new diagnostic test by comparing it with results of a gold standard test [41]. The accuracy or validity of any test is generally defined as the closeness of agreement between the results of the measurement and the true value of the measured characteristic. It should not be confused with the precision of the test which is the closeness between repeated measures under prescribed conditions [42]. Here we used the presented model as a new test able to predict / detect the health status of a herd, to be compared with the observed data in the ROC analysis (which plays the role of the gold standard). This analysis was used for estimation of sensitivity and specificity of the model as a prediction tool. One important assumption which should not be ignored here is that the observed data cannot be considered as a gold standard. The exact sensitivity and specificity of the ELISA test used to detect antibodies in the BTM are not well established but some estimates suggest that the test is moderately sensitive and specific [43]. Hence, the accuracy of the present model was impacted by the quite low performances (sensitivity and specificity) of this ELISA test. We believe that, along with the reason mentioned above, further reasons of disagreement between data and model outputs might be related to unaccounted processes, such as un-modelled beef cattle herds, and also related to methodological limits of the Gaussian model and of the intra-herd model used here. Along with dry deposition, wet deposition of infectious particles (due to rain and humidity in the atmosphere), use of spatially and temporally more precise meteorological data should be used to increase the prediction accuracy. The parameterisation of the intra-herd model is done on prior estimates based on observations from five herds. Observations on larger sample of herds in the region can provide better estimates of the health transmission parameters. Along with that, frequently sampled longitudinal data about prevalence of infection in the herds of a given region is very essential for better parameter estimation. This will enable more detail comparison of data and model outputs by other means such as inference of key model parameters from data.

5.3.6. Sensitivity analysis

The sensitivity analysis was conducted for a limited number of parameters of the model. Intra-herd parameters of the transitions between animal health states have been estimated in previous study from data collected in the field, using Bayesian statistical methods [20]. A sensitivity analysis study conducted by Courcoul et al [22] using a fractional factorial design showed that the most influential parameters were the probability distributions governing the levels of

bacterial shedding through vaginal mucus and faeces, and parameters governing the dynamics of the bacteria in the environment of the herd. We tested sensitivity of the regional model outputs to variation of these parameters, which were found influential also at regional scale. The relative contribution of windborne transmission in the regional spread of the infection was higher than the one of cattle trade in most of the scenarios tested. Other parameters which we tested were the parameters of the dispersion model, whose values were approximations based on literature. Compared to the set of parameters discussed above, outputs of interests showed little sensitivity to the parameters of dispersion model. The aim of this sensitivity analysis was to test the robustness of the model in the back drop of uncertain and known sensitive parameters. Ideally, a larger and multivariate experimental design for sensitivity analysis would have been preferable, but we stick to limited number of parameters with one at a time approach because of the intense computational and time efforts required otherwise.

5.3.7. Vaccination scenarios and effectiveness of strategies tested

Additional vaccination scenarios could have been evaluated based on the infection status of herds. Herds could have been be targeted if they were initially infection-free and get infected during the simulation, or if they suffered abortions. As the model is stochastic and dynamic, such scenarios would involve variable numbers of vaccinated herds. Currently in the Finistère department, vaccination in cattle is implemented only when a confirmed identified abortion due to *C. burnetii* is reported. Hence, such strategies are close to reality, easier for implementation, and would be welcomed by farmers. Since our aim was to compare different strategies, keeping similar vaccination coverage among scenarios was essential in the current experimental setup, we stick with predetermined vaccination coverage instead of variable vaccination coverage.

Generally, vaccine efficacy is measured for its ability to avoid infection in vaccinated individual, and is calculated from by comparing attack rates in vaccinated and non-vaccinated individuals [44]. The present model and case study provide interesting multiple levels where such comparison of attack rates can be calculated. First, the strategies can be compared with each other. Second, within each strategy, the attack rate in vaccinated herds can be compared with the attack rate in non-vaccinated herds. At animal level within each strategy the attack rates of vaccinated and non-vaccinated animals can be compared. Such multiscale comparison of vaccine efficacy highlights the differences in disease prevention capacity of vaccination at these scales, if any. Such analysis is essential as in case of variation of efficacy at different scales, decision to choose a strategy might depend on the epidemiological unit of concern.

5.4. Perspectives

Future perspectives of the model presented here can take the studies into different directions to delve into the questions regarding transmission and control of Q fever in animal populations. The model could be utilised for cattle metapopulations of other geographic regions to be compared with the results obtained in the Finistère department. Moreover, in complex scenarios where beef farming, small ruminant herds are also present, the model can be modified by incorporating separate intra-herd models for each of those herds. For example, a dairy goat intra-herd model already has been developed [45], which can be integrated into the metapopulation model. Data about trade of small ruminants, longitudinal studies about beef cattle herds to estimate intra-herd model parameters, and information about herd locations then would be essential to extend this model to a more complex multispecies situation. Moreover, in multispecies model, consideration of genotypes predominantly circulating in each species is essential as genotypes prevalent in small ruminants and cattle are known to be different [46, 47]. Interaction of these genotypes with other species also needs to be studied and included in the model. Economical cost benefit analysis of vaccination strategies, as well as other possible control strategies such as restriction of cattle trade infected herds to infection-free herds, is also an important question which needs to be and can be addressed with the help of the model. Finally, we can also predict the risk of human spillovers using the multispecies model framework to pre-empt possible human outbreaks.

References

1. Derrick EH: **"Q" fever, a new fever entity: clinical features, diagnosis and laboratory investigation.** *Rev Infect Dis* 1983, **5:**790-800.
2. Davis GE, Cox HR, Parker R, Dyer R: **A filter-passing infectious agent isolated from ticks.** *Public Health Rep* 1938, **53:**2259-2267.
3. Burnet FM, Freeman M: **Experimental studies on the virus of "Q" fever.** *Rev Infect Dis* 1983, **5:**800-808.
4. Masuzawa T, Sawaki K, Nagaoka H, Akiyama M, Hirai K, Yanagihara Y: **Identification of rickettsiae isolated in Japan as Coxiella burnetii by 16S rRNA sequencing.** *Int J Syst Bacteriol* 1997, **47:**883-884.
5. Skerman V, McGowan V, Sneath P, Moore W, Moore LV: **Approved Lists.** *International journal of systematic bacteriology* 1980, **30:**225-420.
6. McCaul TF, Williams JC: **Developmental cycle of Coxiella burnetii: structure and morphogenesis of vegetative and sporogenic differentiations.** *J Bacteriol* 1981, **147:**1063-1076.
7. Baca OG, Paretsky D: **Q fever and Coxiella burnetii: a model for host-parasite interactions.** *Microbiol Rev* 1983, **47:**127-149.
8. Sawyer LA, Fishbein DB, McDade JE: **Q fever: current concepts.** *Rev Infect Dis* 1987, **9:**935-946.
9. Burnet M: **Derrick and the story of Q fever.** *Med J Aust* 1967, **2:**1067-1068.
10. Bossi P, Tegnell A, Baka A, Van Loock F, Hendriks J, Werner A, Maidhof H, Gouvras G, Task Force on Biological and Chemical Agent Threats PHD, European Commission, L.xembourg: **Bichat guidelines for the clinical management of Q fever and bioterrorism-related Q fever.** *Euro Surveill* 2004, **9:**E19-20.
11. Kagawa FT, Wehner JH, Mohindra V: **Q fever as a biological weapon.** *Semin Respir Infect* 2003, **18:**183-195.
12. Christopher GW, Agan MB, Cieslak TJ, Olson PE: **History of U.S. military contributions to the study of bacterial zoonoses.** *Mil Med* 2005, **170:**39-48.
13. BENENSON AS, TIGERTT WD: **Studies on Q fever in man.** *Trans Assoc Am Physicians* 1956, **69:**98-104.
14. Gonder JC, Kishimoto RA, Kastello MD, Pedersen CE, Larson EW: **Cynomolgus monkey model for experimental Q fever infection.** *J Infect Dis* 1979, **139:**191-196.
15. Hellenbrand W, Breuer T, Petersen L: **Changing epidemiology of Q fever in Germany, 1947-1999.** *Emerg Infect Dis* 2001, **7:**789-796.
16. Tissot-Dupont H, Torres S, Nezri M, Raoult D: **Hyperendemic focus of Q fever related to sheep and wind.** *Am J Epidemiol* 1999, **150:**67-74.
17. Johnson JE, Kadull PJ: **Laboratory-acquired Q fever. A report of fifty cases.** *Am J Med* 1966, **41:**391-403.
18. Anderson A, Bijlmer H, Fournier PE, Graves S, Hartzell J, Kersh GJ, Limonard G, Marrie TJ, Massung RF, McQuiston JH, et al: **Diagnosis and management of Q fever--United States,**

2013: recommendations from CDC and the Q Fever Working Group. *MMWR Recomm Rep* 2013, **62:**1-30.
19. Marrie TJ, Raoult D: **Update on Q fever, including Q fever endocarditis.** *Curr Clin Top Infect Dis* 2002, **22:**97-124.
20. Stein A, Raoult D: **Pigeon pneumonia in provence: a bird-borne Q fever outbreak.** *Clin Infect Dis* 1999, **29:**617-620.
21. Schimmer B, Morroy G, Dijkstra F, Schneeberger PM, Weers-Pothoff G, Timen A, Wijkmans C, van der Hoek W: **Large ongoing Q fever outbreak in the south of The Netherlands, 2008.** *Euro Surveill* 2008, **13**.
22. Delsing CE, Kullberg BJ: **Q fever in the Netherlands: a concise overview and implications of the largest ongoing outbreak.** *Neth J Med* 2008, **66:**365-367.
23. Schimmer B, Ter Schegget R, Wegdam M, Züchner L, de Bruin A, Schneeberger PM, Veenstra T, Vellema P, van der Hoek W: **The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak.** *BMC Infect Dis* 2010, **10:**69.
24. WAHID O: **Office International des Epizooties–World Animal Health Information Database (WAHID) Interface.** *See http://www oie int/wahis/public php* 2009.
25. Roest HI, Tilburg JJ, van der Hoek W, Vellema P, van Zijderveld FG, Klaassen CH, Raoult D: **The Q fever epidemic in The Netherlands: history, onset, response and reflection.** *Epidemiol Infect* 2011, **139:**1-12.
26. Karagiannis I, Schimmer B, Van Lier A, Timen A, Schneeberger P, Van Rotterdam B, De Bruin A, Wijkmans C, Rietveld A, Van Duynhoven Y: **Investigation of a Q fever outbreak in a rural area of The Netherlands.** *Epidemiol Infect* 2009, **137:**1283-1294.
27. Hermans T, Jeurissen L, Hackert V, Hoebe C: **Land-applied goat manure as a source of human Q-fever in the Netherlands, 2006-2010.** *PLoS One* 2014, **9:**e96607.
28. van den Brom R, Roest HJ, de Bruin A, Dercksen D, Santman-Berends I, van der Hoek W, Dinkla A, Vellema J, Vellema P: **A probably minor role for land-applied goat manure in the transmission of Coxiella burnetii to humans in the 2007-2010 Dutch Q fever outbreak.** *PLoS One* 2015, **10:**e0121355.
29. Wallensten A, Moore P, Webster H, Johnson C, van der Burgt G, Pritchard G, Ellis-Iversen J, Oliver I: **Q fever outbreak in Cheltenham, United Kingdom, in 2007 and the use of dispersion modelling to investigate the possibility of airborne spread.** *Euro Surveill* 2010, **15**.
30. Tissot-Dupont H, Amadei MA, Nezri M, Raoult D: **Wind in November, Q fever in December.** *Emerg Infect Dis* 2004, **10:**1264-1269.
31. King LA, Goirand L, Tissot-Dupont H, Giunta B, Giraud C, Colardelle C, Duquesne V, Rousset E, Aubert M, Thiéry R, et al: **Outbreak of Q fever, Florac, Southern France, Spring 2007.** *Vector Borne Zoonotic Dis* 2011, **11:**341-347.
32. Carcopino X, Raoult D, Bretelle F, Boubli L, Stein A: **Managing Q fever during pregnancy: the benefits of long-term cotrimoxazole therapy.** *Clin Infect Dis* 2007, **45:**548-555.
33. Fournier PE, Marrie TJ, Raoult D: **Diagnosis of Q fever.** *J Clin Microbiol* 1998, **36:**1823-1834.
34. Kovácová E, Kazár J: **Q fever--still a query and underestimated infectious disease.** *Acta Virol* 2002, **46:**193-210.
35. Maurin M, Raoult D: **Q fever.** *Clin Microbiol Rev* 1999, **12:**518-553.
36. Norlander L: **Q fever epidemiology and pathogenesis.** *Microbes Infect* 2000, **2:**417-424.

37. Georgiev M, Afonso A, Neubauer H, Needham H, Thiery R, Rodolakis A, Roest H, Stark K, Stegeman J, Vellema P, et al: **Q fever in humans and farm animals in four European countries, 1982 to 2010.** *Euro Surveill* 2013, **18**.
38. Serbezov VS, Kazár J, Novkirishki V, Gatcheva N, Kovácová E, Voynova V: **Q fever in Bulgaria and Slovakia.** *Emerg Infect Dis* 1999, **5:**388-394.
39. Guatteo R, Seegers H, Taurel AF, Joly A, Beaudeau F: **Prevalence of Coxiella burnetii infection in domestic ruminants: a critical review.** *Vet Microbiol* 2011, **149:**1-16.
40. Woldehiwet Z: **Q fever (coxiellosis): epidemiology and pathogenesis.** *Res Vet Sci* 2004, **77:**93-100.
41. Angelakis E, Raoult D: **Q Fever.** *Vet Microbiol* 2010, **140:**297-309.
42. Agerholm JS: **Coxiella burnetii associated reproductive disorders in domestic animals--a critical review.** *Acta Vet Scand* 2013, **55:**13.
43. Garcia-Ispierto I, Tutusaus J, López-Gatius F: **Does Coxiella burnetii affect reproduction in cattle? A clinical update.** *Reprod Domest Anim* 2014, **49:**529-535.
44. Grace D, Mutua F, Ochungo P, Kruska R, Jones K, Brierley L, Lapar L, Said M, Herrero M, Phuc P: **Mapping of poverty and likely zoonoses hotspots.** 2012.
45. **EFSA Panel on Animal Health and Welfare (AHAW); Scientific Opinion on Q Fever.** *EFSA Journal* 2010, **8:**114.
46. Lang GH: **Coxiellosis (Q fever) in animals.** *Q fever* 1990, **1:**23-48.
47. Palmer NC, Kierstead M, Key DW, Williams JC, Peacock MG, Vellend H: **Placentitis and Abortion in Goats and Sheep in Ontario Caused by Coxiella burnetii.** *Can Vet J* 1983, **24:**60-61.
48. Zeman DH, Kirkbride CA, Leslie-Steen P, Duimstra JR: **Ovine abortion due to Coxiella burnetti infection.** *J Vet Diagn Invest* 1989, **1:**178-180.
49. Sanford SE, Josephson GK, MacDonald A: **Coxiella burnetii (Q fever) abortion storms in goat herds after attendance at an annual fair.** *Can Vet J* 1994, **35:**376-378.
50. HUEBNER RJ, BELL JA: **Q fever studies in Southern California; summary of current results and a discussion of possible control measures.** *J Am Med Assoc* 1951, **145:**301-305; passim.
51. Nusinovici S, Hoch T, Widgren S, Joly A, Lindberg A, Beaudeau F: **Relative contributions of neighbourhood and animal movements to Coxiella burnetii infection in dairy cattle herds.** *Geospat Health* 2014, **8:**471-477.
52. Nusinovici S, Frössling J, Widgren S, Beaudeau F, Lindberg A: **Q fever infection in dairy cattle herds: increased risk with high wind speed and low precipitation.** *Epidemiol Infect* 2015**:**1-11.
53. Rodolakis A, Berri M, Héchard C, Caudron C, Souriau A, Bodier CC, Blanchard B, Camuset P, Devillechaise P, Natorp JC, et al: **Comparison of Coxiella burnetii shedding in milk of dairy bovine, caprine, and ovine herds.** *J Dairy Sci* 2007, **90:**5352-5360.
54. Guatteo R, Beaudeau F, Berri M, Rodolakis A, Joly A, Seegers H: **Shedding routes of Coxiella burnetii in dairy cows: implications for detection and control.** *Vet Res* 2006, **37:**827-833.
55. Berri M, Rousset E, Champion JL, Russo P, Rodolakis A: **Goats may experience reproductive failures and shed Coxiella burnetii at two successive parturitions after a Q fever infection.** *Res Vet Sci* 2007, **83:**47-52.
56. Durand MP: **[Lacteal and placental excretion of Coxiella burnetti, agent of Q fever, in the cow. Importance and prevention].** *Bull Acad Natl Med* 1993, **177:**935-945; discussion 945-936.

57. Arricau Bouvery N, Souriau A, Lechopier P, Rodolakis A: **Experimental Coxiella burnetii infection in pregnant goats: excretion routes.** *Vet Res* 2003, **34:**423-433.
58. Guatteo R, Beaudeau F, Joly A, Seegers H: **Coxiella burnetii shedding by dairy cows.** *Vet Res* 2007, **38:**849-860.
59. Rousset E, Berri M, Durand B, Dufour P, Prigent M, Delcroix T, Touratier A, Rodolakis A: **Coxiella burnetii shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds.** *Appl Environ Microbiol* 2009, **75:**428-433.
60. Kruszewska D, Tylewska-Wierzbanowska S: **Isolation of Coxiella burnetii from bull semen.** *Res Vet Sci* 1997, **62:**299-300.
61. Guatteo R, Joly A, Beaudeau F: **Shedding and serological patterns of dairy cows following abortions associated with Coxiella burnetii DNA detection.** *Vet Microbiol* 2012, **155:**430-433.
62. Courcoul A, Monod H, Nielen M, Klinkenberg D, Hogerwerf L, Beaudeau F, Vergu E: **Modelling the effect of heterogeneity of shedding on the within herd Coxiella burnetii spread and identification of key parameters by sensitivity analysis.** *J Theor Biol* 2011, **284:**130-141.
63. van der Hoek W, Hunink J, Vellema P, Droogers P: **Q fever in The Netherlands: the role of local environmental conditions.** *Int J Environ Health Res* 2011, **21:**441-451.
64. van Leuken JP, van de Kassteele J, Sauter FJ, van der Hoek W, Heederik D, Havelaar AH, Swart AN: **Improved correlation of human Q fever incidence to modelled C. burnetii concentrations by means of an atmospheric dispersion model.** *Int J Health Geogr* 2015, **14:**14.
65. Forland F, Jansen A, de Carvalho Gomes H, Nøkleby H, Escriva A, Coulombier D: **Risk assessment on Q fever.** *European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden* 2010.
66. Dutta BL, Ezanno P, Vergu E: **Characteristics of the spatio-temporal network of cattle movements in France over a 5-year period.** *Prev Vet Med* 2014, **117:**79-94.
67. Kleczkowski A, Oleś K, Gudowska-Nowak E, Gilligan CA: **Searching for the most cost-effective strategy for controlling epidemics spreading on regular and small-world networks.** *J R Soc Interface* 2012, **9:**158-169.
68. Mweu MM, Fournié G, Halasa T, Toft N, Nielsen SS: **Temporal characterisation of the network of Danish cattle movements and its implication for disease control: 2000-2009.** *Prev Vet Med* 2013, **110:**379-387.
69. Murphy FA: **Emerging zoonoses.** *Emerg Infect Dis* 1998, **4:**429-435.
70. Arricau-Bouvery N, Rodolakis A: **Is Q fever an emerging or re-emerging zoonosis?** *Vet Res* 2005, **36:**327-349.
71. Behymer D, Ruppanner R, Riemann HP, Biberstein EL, Franti CE: **Observation on chemotherapy in cows chronically infected with Coxiella burnetii (Q fever).** *Folia Vet Lat* 1977, **7:**64-70.
72. Astobiza I, Barandika JF, Hurtado A, Juste RA, García-Pérez AL: **Kinetics of Coxiella burnetii excretion in a commercial dairy sheep flock after treatment with oxytetracycline.** *Vet J* 2010, **184:**172-175.
73. Astobiza I, Barandika JF, Juste RA, Hurtado A, García-Pérez AL: **Evaluation of the efficacy of oxytetracycline treatment followed by vaccination against Q fever in a highly infected sheep flock.** *Vet J* 2013, **196:**81-85.

74. Biberstein EL, Riemann HP, Franti CE, Behymer DE, Ruppanner R, Bushnell R, Crenshaw G: **Vaccination of dairy cattle against Q fever (Coxiella burneti): results of field trials.** *Am J Vet Res* 1977, **38:**189-193.
75. Sádecký E, Brezina R, Kazár J, Urvölgyi J: **Immunization against Q-fever of naturally infected dairy cows.** *Acta Virol* 1975, **19:**486-488.
76. Rodolakis A: **Q Fever in dairy animals.** *Ann N Y Acad Sci* 2009, **1166:**90-93.
77. Arricau-Bouvery N, Souriau A, Bodier C, Dufour P, Rousset E, Rodolakis A: **Effect of vaccination with phase I and phase II Coxiella burnetii vaccines in pregnant goats.** *Vaccine* 2005, **23:**4392-4402.
78. Guatteo R, Seegers H, Joly A, Beaudeau F: **Prevention of Coxiella burnetii shedding in infected dairy herds using a phase I C. burnetii inactivated vaccine.** *Vaccine* 2008, **26:**4320-4328.
79. Taurel AF, Guatteo R, Joly A, Beaudeau F: **Effectiveness of vaccination and antibiotics to control Coxiella burnetii shedding around calving in dairy cows.** *Vet Microbiol* 2012, **159:**432-437.
80. Taurel AF, Guatteo R, Lehebel A, Joly A, Beaudeau F: **Vaccination using phase I vaccine is effective to control Coxiella burnetii shedding in infected dairy cattle herds.** *Comp Immunol Microbiol Infect Dis* 2014, **37:**1-9.
81. Astobiza I, Barandika JF, Ruiz-Fons F, Hurtado A, Povedano I, Juste RA, García-Pérez AL: **Coxiella burnetii shedding and environmental contamination at lambing in two highly naturally-infected dairy sheep flocks after vaccination.** *Res Vet Sci* 2011, **91:**e58-63.
82. Astobiza I, Barandika JF, Ruiz-Fons F, Hurtado A, Povedano I, Juste RA, García-Pérez AL: **Four-year evaluation of the effect of vaccination against Coxiella burnetii on reduction of animal infection and environmental contamination in a naturally infected dairy sheep flock.** *Appl Environ Microbiol* 2011, **77:**7405-7407.
83. Astobiza I, Barandika JF, Juste RA, Hurtado A, García-Pérez AL: **Evaluation of the efficacy of oxytetracycline treatment followed by vaccination against Q fever in a highly infected sheep flock.** *Vet J* 2012.
84. Courcoul A, Hogerwerf L, Klinkenberg D, Nielen M, Vergu E, Beaudeau F: **Modelling effectiveness of herd level vaccination against Q fever in dairy cattle.** *Vet Res* 2011, **42:**68.
85. Gunawardena J: **Models in biology: 'accurate descriptions of our pathetic thinking'.** *BMC Biol* 2014, **12:**29.
86. Keeling MJ, Rohani P: *Modeling infectious diseases in humans and animals.* Princeton University Press; 2008.
87. Dorjee S, Poljak Z, Revie CW, Bridgland J, McNab B, Leger E, Sanchez J: **A review of simulation modelling approaches used for the spread of zoonotic influenza viruses in animal and human populations.** *Zoonoses Public Health* 2013, **60:**383-411.
88. Dubé C, Ribble C, Kelton D, McNab B: **A review of network analysis terminology and its application to foot-and-mouth disease modelling and policy development.** *Transbound Emerg Dis* 2009, **56:**73-85.
89. Keeling MJ: **Models of foot-and-mouth disease.** *Proc Biol Sci* 2005, **272:**1195-1202.
90. Kitching RP, Hutber AM, Thrusfield MV: **A review of foot-and-mouth disease with special consideration for the clinical and epidemiological factors relevant to predictive modelling of the disease.** *Vet J* 2005, **169:**197-209.
91. Green LE, Medley GF: **Mathematical modelling of the foot and mouth disease epidemic of 2001: strengths and weaknesses.** *Res Vet Sci* 2002, **73:**201-205.

92. Green DM, Kiss IZ, Kao RR: **Modelling the initial spread of foot-and-mouth disease through animal movements.** *Proc Biol Sci* 2006, **273:**2729-2735.
93. Kao RR: **The role of mathematical modelling in the control of the 2001 FMD epidemic in the UK.** *Trends Microbiol* 2002, **10:**279-286.
94. Tian H, Zhou S, Dong L, Van Boeckel TP, Cui Y, Wu Y, Cazelles B, Huang S, Yang R, Grenfell BT, Xu B: **Avian influenza H5N1 viral and bird migration networks in Asia.** *Proc Natl Acad Sci U S A* 2015, **112:**172-177.
95. Nickbakhsh S, Matthews L, Reid SW, Kao RR: **A metapopulation model for highly pathogenic avian influenza: implications for compartmentalization as a control measure.** *Epidemiol Infect* 2014, **142:**1813-1825.
96. El Masry I, Rijks J, Peyre M, Taylor N, Lubroth J, Jobre Y: **Modelling influenza A H5N1 vaccination strategy scenarios in the household poultry sector in Egypt.** *Trop Anim Health Prod* 2014, **46:**57-63.
97. Ssematimba A, Hagenaars TJ, de Jong MC: **Modelling the wind-borne spread of highly pathogenic avian influenza virus between farms.** *PLoS One* 2012, **7:**e31114.
98. Van Kerkhove MD, Mumford E, Mounts AW, Bresee J, Ly S, Bridges CB, Otte J: **Highly pathogenic avian influenza (H5N1): pathways of exposure at the animal-human interface, a systematic review.** *PLoS One* 2011, **6:**e14582.
99. Bourouiba L, Teslya A, Wu J: **Highly pathogenic avian influenza outbreak mitigated by seasonal low pathogenic strains: insights from dynamic modeling.** *J Theor Biol* 2011, **271:**181-201.
100. Pandit PS, Bunn DA, Pande SA, Aly SS: **Modeling highly pathogenic avian influenza transmission in wild birds and poultry in West Bengal, India.** *Sci Rep* 2013, **3:**2175.
101. Damman A, Viet AF, Arnoux S, Guerrier-Chatellet MC, Petit E, Ezanno P: **Modelling the spread of bovine viral diarrhea virus (BVDV) in a beef cattle herd and its impact on herd productivity.** *Vet Res* 2015, **46:**12.
102. Courcoul A, Ezanno P: **Modelling the spread of Bovine Viral Diarrhoea Virus (BVDV) in a managed metapopulation of cattle herds.** *Vet Microbiol* 2010, **142:**119-128.
103. Ezanno P, Fourichon C, Viet AF, Seegers H: **Sensitivity analysis to identify key-parameters in modelling the spread of bovine viral diarrhoea virus in a dairy herd.** *Prev Vet Med* 2007, **80:**49-64.
104. Ezanno P, van Schaik G, Weber MF, Heesterbeek JA: **A modeling study on the sustainability of a certification-and-monitoring program for paratuberculosis in cattle.** *Vet Res* 2005, **36:**811-826.
105. Marcé C, Ezanno P, Weber MF, Seegers H, Pfeiffer DU, Fourichon C: **Invited review: modeling within-herd transmission of Mycobacterium avium subspecies paratuberculosis in dairy cattle: a review.** *J Dairy Sci* 2010, **93:**4455-4470.
106. Marcé C, Ezanno P, Seegers H, Pfeiffer DU, Fourichon C: **Within-herd contact structure and transmission of Mycobacterium avium subspecies paratuberculosis in a persistently infected dairy cattle herd.** *Prev Vet Med* 2011, **100:**116-125.
107. Lanzas C, Warnick LD, Ivanek R, Ayscue P, Nydam DV, Gröhn YT: **The risk and control of Salmonella outbreaks in calf-raising operations: a mathematical modeling approach.** *Vet Res* 2008, **39:**61.
108. Lurette A, Belloc C, Touzeau S, Hoch T, Ezanno P, Seegers H, Fourichon C: **Modelling Salmonella spread within a farrow-to-finish pig herd.** *Vet Res* 2008, **39:**49.

109. Lurette A, Touzeau S, Ezanno P, Hoch T, Seegers H, Fourichon C, Belloc C: **Within-herd biosecurity and Salmonella seroprevalence in slaughter pigs: a simulation study.** *J Anim Sci* 2011, **89:**2210-2219.
110. Balcan D, Colizza V, Gonçalves B, Hu H, Ramasco JJ, Vespignani A: **Multiscale mobility networks and the spatial spreading of infectious diseases.** *Proceedings of the National Academy of Sciences* 2009, **106:**21484-21489.
111. Durand B, Dubois M, Sabatier P, Calavas D, Ducrot C, Van de Wielle A: **Multiscale modelling of scrapie epidemiology: II. geographical level: hierarchical transfer of the herd model to the regional disease spread.** *Ecological modelling* 2004, **179:**515-531.
112. Giraudoux P, Delattre P, Takahashi K, Raoul F, Quéré J, Craig P, Vuitton D, Pawlowski Z: **Transmission ecology of Echinococcus multilocularis in wildlife: what can be learned from comparative studies and multiscale approaches?** In *Proceedings of the NATO Advanced Research Workshop on cestode zoonoses: echinococcosis and cysticercosis: an emergent and global problem, Poznan, Poland, 10-13 September 2000*. IOS Press; 2002: 251-266.
113. Vespignani A: **Multiscale mobility networks and the large scale spreading of infectious diseases.** In *APS March Meeting Abstracts*. 2010: 4002.
114. Brooks-Pollock E, Roberts GO, Keeling MJ: **A dynamic model of bovine tuberculosis spread and control in Great Britain.** *Nature* 2014, **511:**228-231.
115. Brooks-Pollock E, Wood JL: **Eliminating bovine tuberculosis in cattle and badgers: insight from a dynamic model.** *Proc Biol Sci* 2015, **282:**20150374.
116. Hanski I: **Metapopulation dynamics.** 1997.
117. MacArthur RH, Wilson EO: *The theory of island biogeography.* Princeton University Press; 1967.
118. Akçakaya HR, Mills G, Doncaster CP: **The role of metapopulations in conservation.** *Key topics in conservation biology* 2007**:**64-84.
119. Grenfell B, Harwood J: **(Meta)population dynamics of infectious diseases.** *Trends Ecol Evol* 1997, **12:**395-399.
120. Brooks-Pollock E, de Jong MC, Keeling MJ, Klinkenberg D, Wood JL: **Eight challenges in modelling infectious livestock diseases.** *Epidemics* 2015, **10:**1-5.
121. Ezanno P, Lesnoff M: **A metapopulation model for the spread and persistence of contagious bovine pleuropneumonia (CBPP) in African sedentary mixed crop-livestock systems.** *J Theor Biol* 2009, **256:**493-503.
122. Jesse M, Ezanno P, Davis S, Heesterbeek JA: **A fully coupled, mechanistic model for infectious disease dynamics in a metapopulation: movement and epidemic duration.** *J Theor Biol* 2008, **254:**331-338.
123. Vergu E, Busson H, Ezanno P: **Impact of the infection period distribution on the epidemic spread in a metapopulation model.** *PLoS One* 2010, **5:**e9371.
124. Courcoul A, Vergu E, Denis JB, Beaudeau F: **Spread of Q fever within dairy cattle herds: key parameters inferred using a Bayesian approach.** *Proc Biol Sci* 2010, **277:**2857-2865.
125. Hogerwerf L, Courcoul A, Klinkenberg D, Beaudeau F, Vergu E, Nielen M: **Dairy goat demography and Q fever infection dynamics.** *Vet Res* 2013, **44:**28.
126. Guatteo R, Beaudeau F, Joly A, Seegers H: **Assessing the within-herd prevalence of Coxiella burnetii milk-shedder cows using a real-time PCR applied to bulk tank milk.** *Zoonoses Public Health* 2007, **54:**191-194.

127. Taurel AF, Guatteo R, Joly A, Beaudeau F: **Relationship between the level of antibodies in bulk tank milk and the within-herd seroprevalence of Coxiella burnetii in cows.** *Epidemiol Infect* 2012, **140:**1710-1713.
128. Taurel AF, Guatteo R, Joly A, Seegers H, Beaudeau F: **Seroprevalence of Q fever in naturally infected dairy cattle herds.** *Prev Vet Med* 2011, **101:**51-57.
129. Hagenaars TJ, Dekker A, de Jong MC, Eblé PL: **Estimation of foot and mouth disease transmission parameters, using outbreak data and transmission experiments.** *Rev Sci Tech* 2011, **30:**467-477.
130. Gloster J, Jones A, Redington A, Burgin L, Sørensen JH, Turner R, Dillon M, Hullinger P, Simpson M, Astrup P, et al: **Airborne spread of foot-and-mouth disease--model intercomparison.** *Vet J* 2010, **183:**278-286.
131. Gates MC, Humphry RW, Gunn GJ, Woolhouse ME: **Not all cows are epidemiologically equal: quantifying the risks of bovine viral diarrhoea virus (BVDV) transmission through cattle movements.** *Vet Res* 2014, **45:**110.
132. Green DM, Kiss IZ, Mitchell AP, Kao RR: **Estimates for local and movement-based transmission of bovine tuberculosis in British cattle.** *Proc Biol Sci* 2008, **275:**1001-1005.
133. Guatteo R, Beaudeau F, Joly A, Seegers H: **Performances of an ELISA applied to serum and milk for the detection of antibodies to Coxiella burnetii in dairy cattle.** *Revue de médecine vétérinaire* 2007, **158:**250-252.
134. Jones RM, Nicas M, Hubbard AE, Reingold AL: **The infectious dose of Coxiella burnetii (Q fever).** *Applied Biosafety* 2006, **11:**32.
135. Stockie JM: **The mathematics of atmospheric dispersion modeling.** *Siam Review* 2011, **53:**349-372.
136. Turner R, Hurst T: **Factors influencing volcanic ash dispersal from the 1995 and 1996 eruptions of Mount Ruapehu, New Zealand.** *Journal of Applied Meteorology* 2001, **40:**56-69.
137. Levin SA, Muller-Landau HC, Nathan R, Chave J: **The ecology and evolution of seed dispersal: a theoretical perspective.** *Annual Review of Ecology, Evolution, and Systematics* 2003**:**575-604.
138. Loos C, Seppelt R, Meier-Bethke S, Schiemann J, Richter O: **Spatially explicit modelling of transgenic maize pollen dispersal and cross-pollination.** *Journal of Theoretical Biology* 2003, **225:**241-255.
139. Yang Y, Wilson L, Makela M, Marchetti M: **Accuracy of numerical methods for solving the advection–diffusion equation as applied to spore and insect dispersal.** *Ecological modelling* 1998, **109:**1-24.
140. Smith R: **Dispersion of odours from ground level agricultural sources.** *Journal of Agricultural Engineering Research* 1993, **54:**187-200.
141. Baklanov A, Sørensen JH, Hoe SC, Amstrup B: **Urban meteorological modelling for nuclear emergency preparedness.** *J Environ Radioact* 2006, **85:**154-170.
142. Sørensen JH, Mackay DK, Jensen CO, Donaldson AI: **An integrated model to predict the atmospheric spread of foot-and-mouth disease virus.** *Epidemiol Infect* 2000, **124:**577-590.
143. Kritana P, Taehyeung K, Soyoung K, Hyeontae K, Ki Youn K, Wongeun S, Chatchawan V: **Review of Air Dispersion Modelling Approaches to Assess the Risk of Wind-Borne Spread of Foot-and-Mouth Disease Virus.** *Journal of Environmental Protection* 2012, **2012**.
144. Lutman E, Jones S, Hill R, McDonald P, Lambers B: **Comparison between the predictions of a Gaussian plume model and a Lagrangian particle dispersion model for annual average**

calculations of long-range dispersion of radionuclides. *Journal of environmental radioactivity* 2004, **75:**339-355.
145. Mayer D, Reiczigel J, Rubel F: **A Lagrangian particle model to predict the airborne spread of foot-and-mouth disease virus.** *Atmospheric Environment* 2008, **42:**466-479.
146. Pedersen UB, Hansen JE: **Assessment tools in support of epidemiological investigation of airborne dispersion of pathogens.** *Am J Disaster Med* 2008, **3:**327-333.
147. Sørensen JH, Baklanov A, Hoe S: **The Danish emergency response model of the atmosphere (DERMA).** *J Environ Radioact* 2007, **96:**122-129.
148. Organization WH: **Infection prevention and control of epidemic-and pandemic-prone acute respiratory diseases in health care: WHO interim guidelines.** 2007.
149. Ermak DL: **An analytical model for air pollutant transport and deposition from a point source.** *Atmospheric Environment (1967)* 1977, **11:**231-237.
150. Li S, Eisenberg JN, Spicknall IH, Koopman JS: **Dynamics and control of infections transmitted from person to person through the environment.** *American journal of epidemiology* 2009, **170:**257-265.
151. Nicas M, Hubbard AE, Jones RM, Reingold AL: **The Infectious Dose of Variola (Smallpox) Virus.** *Journal of the American Biological Safety Association* 2004, **9:**118.
152. Gloster J, Champion HJ, Mansley LM, Romero P, Brough T, Ramirez A: **The 2001 epidemic of foot-and-mouth disease in the United Kingdom: epidemiological and meteorological case studies.** *Vet Rec* 2005, **156:**793-803.
153. Rautureau S, Dufour B, Durand B: **Vulnerability of animal trade networks to the spread of infectious diseases: a methodological approach applied to evaluation and emergency control strategies in cattle, France, 2005.** *Transboundary and emerging diseases* 2011, **58:**110-120.
154. Forecasts ECfM-RW: **ECMWF.** 2013.
155. Balsamo G, Albergel C, Beljaars A, Boussetta S, Brun E, Cloke H, Dee D, Dutra E, Pappenberger F, de Rosnay P: **ERA-Interim/Land: A global land-surface reanalysis based on ERA-Interim meteorological forcing, ERA Report Series, ECMWF, Shinfield Park.** *Reading* 2012.
156. Greiner M, Pfeiffer D, Smith RD: **Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests.** *Prev Vet Med* 2000, **45:**23-41.
157. Tildesley MJ, Deardon R, Savill NJ, Bessell PR, Brooks SP, Woolhouse ME, Grenfell BT, Keeling MJ: **Accuracy of models for the 2001 foot-and-mouth epidemic.** *Proc Biol Sci* 2008, **275:**1459-1468.
158. Berri M, Rousset E, Champion JL, Arricau-Bouvery N, Russo P, Pepin M, Rodolakis A: **Ovine manure used a a garden fertiliser as a suspected source of human Q fever.** *Vet Rec* 2003, **153:**269-270.
159. de Bruin A, van der Plaats RQ, de Heer L, Paauwe R, Schimmer B, Vellema P, van Rotterdam BJ, van Duynhoven YT: **Detection of Coxiella burnetii DNA on small-ruminant farms during a Q fever outbreak in the Netherlands.** *Appl Environ Microbiol* 2012, **78:**1652-1657.
160. Arricau-Bouvery N, Souriau A, Moutoussamy A, Ladenise K, Rodolakis A: **Etude de l'excrétion de Coxiella burnetii dans un modèle expérimental caprin et décontamination des lisiers par la cyanamide calcique.** *Rencontres autour des recherches sur les ruminants* 2001**:**153-156.

161. Hogerwerf L, Koop G, Klinkenberg D, Roest HI, Vellema P, Nielen M: **Test and cull of high risk Coxiella burnetii infected pregnant dairy goats is not feasible due to poor test performance.** *Vet J* 2014, **200:**343-345.
162. Fèvre EM, Bronsvoort BMdC, Hamilton KA, Cleaveland S: **Animal movements and the spread of infectious diseases.** *Trends in microbiology* 2006, **14:**125-131.
163. Cremoux R, Rousset E, Touratier A, Audusseau G, Nicollet P, Ribaud D, David V, Pape M: **Assessment of vaccination by a phase I Coxiella burnetii-inactivated vaccine in goat herds in clinical Q fever situation.** *FEMS Immunology & Medical Microbiology* 2012, **64:**104-106.
164. Commandeur M, Jeurissen L, van der Hoek W, Roest HJ, Hermans TC: **Spatial relationships in the Q fever outbreaks 2007-2010 in the Netherlands.** *Int J Environ Health Res* 2014, **24:**137-157.
165. Dijkstra F, van der Hoek W, Wijers N, Schimmer B, Rietveld A, Wijkmans CJ, Vellema P, Schneeberger PM: **The 2007–2010 Q fever epidemic in The Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming.** *FEMS Immunol Med Microbiol* 2012, **64:**3-12.
166. Pranav P, Thierry H, Pauline E, François B, Elisabeta V: **Q fever spread between dairy cattle herds in an enzootic region: modelling contributions of windborne transmission and trade.** *Veterinary Research* Under review.
167. McReynolds SW, Sanderson MW, Reeves A, Hill AE: **Modeling the impact of vaccination control strategies on a foot and mouth disease outbreak in the Central United States.** *Prev Vet Med* 2014, **117:**487-504.
168. Wooldridge M: **Risk modelling for vaccination: a risk assessment perspective.** *Dev Biol (Basel)* 2007, **130:**87-97.
169. TAKADA M, ITOH K, YASUI Y, MITANI A, NOMURA S, MIZUNO N, TRONTELJ M, TRONTELJ J, ofNeurology N: **Cost-benefit analysis of vaccination against paratuberculosis in dairy cattle.** *The Veterinary Record* 1996, **139:**624-627.
170. De La Fuente J, Rodríguez M, Redondo M, Montero C, García-García J, Méndez L, Serrano E, Valdés M, Enriquez A, Canales M: **Field studies and cost-effectiveness analysis of vaccination with Gavac™ against the cattle tick Boophilus microplus.** *Vaccine* 1998, **16:**366-373.
171. Berentsen P, Dijkhuizen A, Oskam A: **A dynamic model for cost-benefit analyses of foot-and-mouth disease control strategies.** *Preventive Veterinary Medicine* 1992, **12:**229-243.
172. Bates TW, Carpenter TE, Thurmond MC: **Benefit-cost analysis of vaccination and preemptive slaughter as a means of eradicating foot-and-mouth disease.** *Am J Vet Res* 2003, **64:**805-812.
173. FAO O, WHO U, UNICEF: **The World Bank (2008) Contributing to One World, One Health: A Strategic Framework for Reducing Risks of Infectious Diseases at the Animal–Human–Ecosystems Interface.** Rome: FAO; 2012.
174. Gardon J, Héraud JM, Laventure S, Ladam A, Capot P, Fouquet E, Favre J, Weber S, Hommel D, Hulin A, et al: **Suburban transmission of Q fever in French Guiana: evidence of a wild reservoir.** *J Infect Dis* 2001, **184:**278-284.
175. Cooper A, Stephens J, Ketheesan N, Govan B: **Detection of Coxiella burnetii DNA in Wildlife and Ticks in Northern Queensland, Australia.** *Vector Borne Zoonotic Dis* 2012.
176. Kirchgessner MS, Dubovi EJ, Whipps CM: **Disease Risk Surface for Coxiella burnetii Seroprevalence in White-Tailed Deer.** *Zoonoses Public Health* 2012.

177. Marreros N, Hüssy D, Albini S, Frey CF, Abril C, Vogt HR, Holzwarth N, Wirz-Dittus S, Friess M, Engels M, et al: **Epizootiologic investigations of selected abortive agents in free-ranging Alpine ibex (Capra ibex ibex) in Switzerland.** *J Wildl Dis* 2011, **47:**530-543.
178. Reusken C, van der Plaats R, Opsteegh M, de Bruin A, Swart A: **Coxiella burnetii (Q fever) in Rattus norvegicus and Rattus rattus at livestock farms and urban locations in the Netherlands; could Rattus spp. represent reservoirs for (re)introduction?** *Prev Vet Med* 2011, **101:**124-130.
179. González-Barrio D, Maio E, Vieira-Pinto M, Ruiz-Fons F: **European Rabbits as Reservoir for Coxiella burnetii.** *Emerg Infect Dis* 2015, **21:**1055-1058.
180. Beaunée G, Vergu E, Ezanno P: **Modelling of paratuberculosis spread between dairy cattle farms at a regional scale.** *Veterinary Research* 2015, **46:**111.
181. de Bruin A, van Alphen PT, van der Plaats RQ, de Heer LN, Reusken CB, van Rotterdam BJ, Janse I: **Molecular typing of Coxiella burnetii from animal and environmental matrices during Q fever epidemics in the Netherlands.** *BMC Vet Res* 2012, **8:**165.
182. Nusinovici S, Hoch T, Brahim M, Joly A, Beaudeau F: **The Effect of Wind on Coxiella burnetii Transmission Between Cattle Herds: a Mechanistic Approach.** *Transboundary and Emerging Diseases* 2015.
183. Astobiza I, Tilburg JJ, Piñero A, Hurtado A, García-Pérez AL, Nabuurs-Franssen MH, Klaassen CH: **Genotyping of Coxiella burnetii from domestic ruminants in northern Spain.** *BMC Vet Res* 2012, **8:**241.
184. Roest HI, van Solt CB, Tilburg JJ, Klaassen CH, Hovius EK, Roest FT, Vellema P, van den Brom R, van Zijderveld FG: **Search for possible additional reservoirs for human Q fever, The Netherlands.** *Emerg Infect Dis* 2013, **19:**834-835.
185. Thornley JH, France J: **Modelling foot and mouth disease.** *Prev Vet Med* 2009, **89:**139-154.
186. Boni MF, Manh BH, Thai PQ, Farrar J, Hien TT, Hien NT, Van Kinh N, Horby P: **Modelling the progression of pandemic influenza A (H1N1) in Vietnam and the opportunities for reassortment with other influenza viruses.** *BMC Med* 2009, **7:**43.
187. Yanase T, Muramatsu Y, Inouye I, Okabayashi T, Ueno H, Morita C: **Detection of Coxiella burnetii from dust in a barn housing dairy cattle.** *Microbiol Immunol* 1998, **42:**51-53.
188. Hogerwerf L, Borlée F, Still K, Heederik D, van Rotterdam B, de Bruin A, Nielen M, Wouters IM: **Detection of Coxiella burnetii DNA in inhalable airborne dust samples from goat farms after mandatory culling.** *Appl Environ Microbiol* 2012, **78:**5410-5412.
189. Dybkaer R: **Result, error and uncertainty.** *Scandinavian journal of clinical & laboratory investigation* 1995, **55:**97-118.
190. Paul S, Toft N, Agerholm JS, Christoffersen AB, Agger JF: **Bayesian estimation of sensitivity and specificity of Coxiella burnetii antibody ELISA tests in bovine blood and milk.** *Prev Vet Med* 2012.
191. Weinberg GA, Szilagyi PG: **Vaccine epidemiology: efficacy, effectiveness, and the translational research roadmap.** *Journal of Infectious Diseases* 2010, **201:**1607-1610.
192. Tilburg JJ, Rossen JW, van Hannen EJ, Melchers WJ, Hermans MH, van de Bovenkamp J, Roest HJ, de Bruin A, Nabuurs-Franssen MH, Horrevorts AM, Klaassen CH: **Genotypic diversity of Coxiella burnetii in the 2007-2010 Q fever outbreak episodes in The Netherlands.** *J Clin Microbiol* 2012, **50:**1076-1078.
193. Tilburg JJ, Roest HJ, Buffet S, Nabuurs-Franssen MH, Horrevorts AM, Raoult D, Klaassen CH: **Epidemic genotype of Coxiella burnetii among goats, sheep, and humans in the Netherlands.** *Emerg Infect Dis* 2012, **18:**887-889.

Appendix I: Summary of the thesis in French

Résumé long de la thèse en français

Propagation et contrôle de la fièvre Q dans les troupeaux bovins laitiers à l'échelle régionale : une approche par modélisation multi-échelles

1. Introduction

La fièvre Q, causée par la bactérie *Coxiella burnetii*, est une zoonose présente au niveau mondial. Un rapport publié en 2012 identifie cette maladie comme l'une des treize plus importantes maladies zoonotiques, en se basant sur son impact sur la santé humaine et animale [1]. Largement présente en élevage de ruminants, cette infection constitue un risque majeur de santé publique vétérinaire, notamment pour les professionnels de l'élevage mais également pour les populations proches de zones à fortes densités animales, comme l'a montré la récente et importante épidémie dans des populations humaines aux Pays-Bas en 2007-2009 [2]. La maîtrise de la fièvre Q en élevage de ruminants est donc cruciale pour réduire ce risque, induisant un intérêt croissant pour une meilleure compréhension des modalités de transmission de la bactérie à une échelle régionale et des leviers de maîtrise disponibles. Ainsi, l'Union Européenne (UE) a mis en place un comité scientifique au sein de l'Autorité Européenne de Sécurité des Aliments. Ce comité a étudié les risques posés par la fièvre Q et a conseillé l'UE concernant le niveau et la distribution de l'infection, les facteurs de risque de son apparition et de sa persistance, et les options pour une maîtrise efficace de la maladie. Le rapport produit insiste sur la nécessité d'évaluer les différentes options de maîtrise en populations animales, en vue de réduire les épidémies chez l'homme.

Les bovins constituent une population d'étude d'intérêt en Bretagne (France) car la densité animale y est très élevée et très peu de petits ruminants y sont présents, les populations bovines constituant donc une source majeure potentielle. De plus, des données de terrain y sont disponibles, permettant d'envisager une étude à large échelle.

Les vaches acquièrent l'infection par inhalation de bactéries présentes dans l'environnement, qui ont été excrétées par les animaux infectieux, ceux-ci présentant une hétérogénéité des niveaux et voies d'excrétion. Il a été démontré qu'à l'échelle du troupeau bovin laitier la dynamique d'infection dépend fortement de cette hétérogénéité d'excrétion [3]. A une échelle régionale inter-troupeaux, l'infection se propage via les mouvements commerciaux d'animaux et une dispersion de la bactérie par voie aérienne. La transmission de *C. burnetii* par voie aérienne est un phénomène bien documenté [1, 4, 5]. Par ailleurs, aucun test n'est généralement effectué pour déterminer le statut infectieux des vaches avant achat. Par conséquent, le commerce des animaux en tant que voie de transmission directe peut aussi jouer un rôle dans la propagation de l'infection au sein d'une région. Des études récentes ont identifié un risque d'infection dans les troupeaux laitiers via la dispersion de bactéries et le commerce d'animaux [6, 7], mais la contribution relative de ces deux routes de transmission de l'agent pathogène entre troupeaux reste mal connue.

Une maîtrise efficace de la propagation de *C. burnetii* entre troupeaux bovins laitiers requiert une bonne connaissance des dynamiques d'infection intra- et inter-troupeaux, et la prise en compte des niveaux de contamination environnementale. Les stratégies utilisées visent généralement à réduire cette dernière. Parmi elles, la vaccination à l'aide d'un vaccin de Phase I est reconnue comme un moyen efficace de réduire l'excrétion de la bactérie par le lait, le placenta et le colostrum, et ainsi de maîtriser la maladie dans un troupeau [8]. Il serait intéressant d'évaluer maintenant les troupeaux à cibler prioritairement pour la mise en œuvre d'une vaccination permettant une maîtrise efficace de la propagation régionale de *C. burnetii*.

Dans ce contexte, nous avons assigné deux objectifs à la thèse :

1. Quantifier la contribution respective de la transmission par le vent et via le commerce des animaux dans la propagation de *C. burnetii* entre troupeaux à l'échelle régionale ;

2. Evaluer l'efficacité de la vaccination pour la maîtrise de la propagation de *C. burnetii* entre les troupeaux bovins laitiers d'une région en conditions enzootiques.

Pour répondre à ces deux objectifs, nous avons utilisé une approche de modélisation. Une modélisation mécaniste multi-échelles de la propagation de *C. burnetii* dans une métapopulation bovine intégrant le commerce des animaux et les données météorologiques constitue une entreprise scientifique prometteuse. Deux échelles sont à considérer : le troupeau (dynamique intra-troupeau) et la région (dynamique inter-troupeaux). L'un des principaux

avantages des modèles mécanistes provient du fait qu'ils permettent de déterminer les causes de l'infection, et en conséquence aident à évaluer des interventions ciblées.

2. Conceptualisation du modèle de métapopulation

Ce chapitre est centré sur la description du modèle générique développé pour représenter la propagation de *Coxiella burnetii* entre troupeaux bovins laitiers. Nous décrivons dans un premier temps le concept général du modèle avant de détailler les hypothèses sous-jacentes et les équations pour chaque partie du modèle.

Le modèle de la transmission de *C. burnetii* à l'échelle d'une région peut être conceptualisé en divisant les processus modélisés en deux parties distinctes. La première partie décrit la propagation de l'infection au sein d'un troupeau bovin laitier infecté. La dynamique d'infection au sein d'un troupeau est représentée à l'aide d'un modèle stochastique individu-centré adapté de Courcoul et al. 2011 [3] . La seconde partie du modèle établit des connections entre des modèles locaux de dynamique intra-troupeau afin de décrire la propagation à une échelle régionale, constituant la section du modèle qui représente la transmission entre troupeaux. La propagation de l'infection au sein d'une région est décrite par deux processus : la dispersion des particules infectieuses par le vent et l'introduction d'animaux infectés via le commerce du bétail.

Le modèle intra-troupeau utilisé dans cette étude est un modèle stochastique individu-centré en temps discret qui représente la transmission de l'infection entre vaches (et exclut les génisses nullipares et les veaux). Le modèle prend en compte l'hétérogénéité des voies et niveaux d'excrétion. De plus, la dynamique de population est incluse à travers la réforme et le cycle de lactation des vaches.

Le transport par le vent et le dépôt de *C. burnetii* dans un nouvel environnement a été modélisé par une équation de dispersion gaussienne, qui prend en compte les phénomènes de gravitation et de tassement. Le commerce d'animaux a été modélisé de manière déterministe à l'aide des données disponibles sur les mouvements de vaches entre troupeaux bovins laitiers en France. Le couplage du modèle intra-troupeau avec les données de mouvement des animaux a été réalisé en caractérisant chaque vache du troupeau, en fonction de son origine et de son statut infectieux, ce qui nous a permis de classer chaque vache en tant qu'animal « interne » (infecté dans le troupeau concerné) ou « externe » (infecté avant l'achat). Le couplage du modèle intra-

troupeau avec le modèle de transmission des particules infectieuses par le vent a été effectué via le compartiment environnemental. Après prise en compte des processus inter-troupeaux dans le modèle intra-troupeau, la force d'infection environnementale a pu être décomposée en deux termes liés à l'origine des animaux excréteurs ($E_{i,internal}$ et $E_{i,external}$) et un terme représentant les bactéries déposées via la transmission aérienne à partir des troupeaux sources ($\sum_j E_{j,dep}(t-1)$). Le modèle a été codé en Python, langage pertinent pour implémenter un tel modèle en métapopulation complexe et permettant de réaliser un grand nombre de simulations numériques en un temps raisonnable.

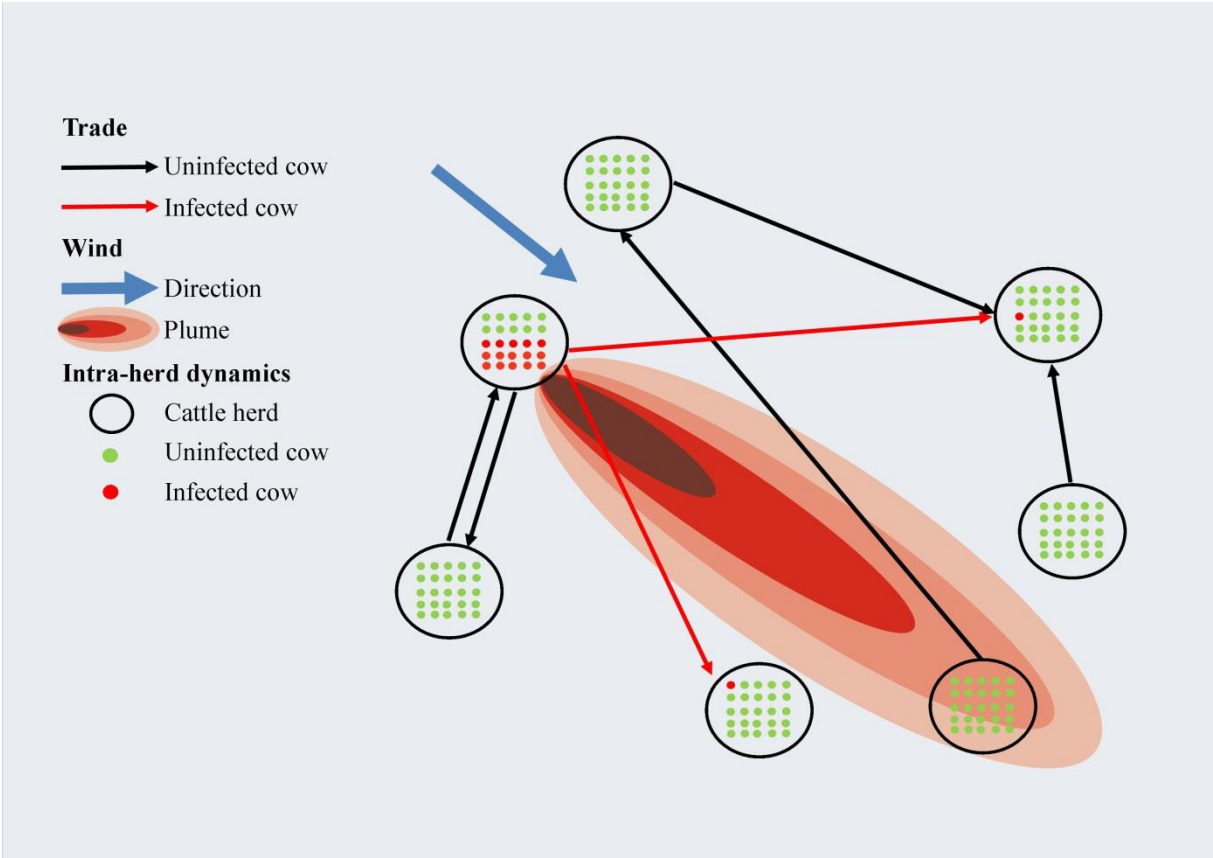


Figure 38 Figure 1 : Schéma conceptuel du modèle de métapopulation. Représentation hypothétique d'une métapopulation de troupeaux bovins avec des troupeaux indemnes (uniquement des points verts représentant des animaux sensibles) et un troupeau infecté (contenant des points rouges pour les animaux infectés). La dynamique inter-troupeaux dépend du commerce des animaux (flèches) et de la dispersion de l'agent pathogène par le vent (panache rouge).

3. Evaluation du rôle relatif du vent et du commerce des animaux dans la transmission de la fièvre Q dans les troupeaux laitiers du département du Finistère (France)

Le modèle générique, l'évaluation des prédictions qui en sont issues, et les investigations concernant les routes de transmission ont été appliqués aux données du département du Finistère, situé au Nord-Ouest de la France, et caractérisé par une forte densité de troupeaux bovins laitiers. Les données disponibles pour le département du Finistère ont été utilisées pour valider le modèle en comparant dans un premier temps les sorties du modèle avec les données observées, grâce à l'élaboration de courbes ROC (Receiver Operating Characteristic). Pour mieux comprendre le comportement du modèle, nous avons réalisé une analyse de sensibilité simple en faisant varier les valeurs des paramètres indépendamment les uns des autres. Nous avons ensuite quantifié la contribution relative de la transmission aérienne et du commerce des animaux dans la propagation régionale de *C. burnetii* dans le Finistère. De plus, nous avons estimé s'il y avait des zones de fort risque d'incidence grâce à une analyse spatiale des agrégats.

Lors de la comparaison avec des données d'observation, les sorties du modèle ont montré une sensibilité (0,71) et une spécificité (0,80) satisfaisantes pour la prédiction du statut infectieux de troupeaux situés dans un voisinage de 3km autour d'un troupeau observé comme nouvellement infecté (dit *incident*). Les prédictions issues de notre modèle appliqué au Finistère ont montré que 92% des infections dans les troupeaux indemnes étaient dues à la transmission par le vent. En dépit de cette fréquence élevée, il s'avère que la transmission aérienne engendre une propagation intra-troupeau de relativement faible ampleur et éphémère, tandis que l'introduction d'une vache infectieuse résulte en une prévalence intra-troupeau significativement plus élevée. L'analyse de sensibilité a montré que si la contribution relative des voies de transmission inter-troupeaux variait avec la valeur des paramètres, les infections liées au vent restaient prédominantes dans la plupart des scénarios. Les résultats ont indiqué également que les deux routes de transmission étaient indépendantes l'une de l'autre, sans effet de synergie, du moins sur un intervalle de temps d'une année. Ces résultats ont fait l'objet d'une publication (Pandit et al.) en cours de révision pour le journal *Veterinary Research*.

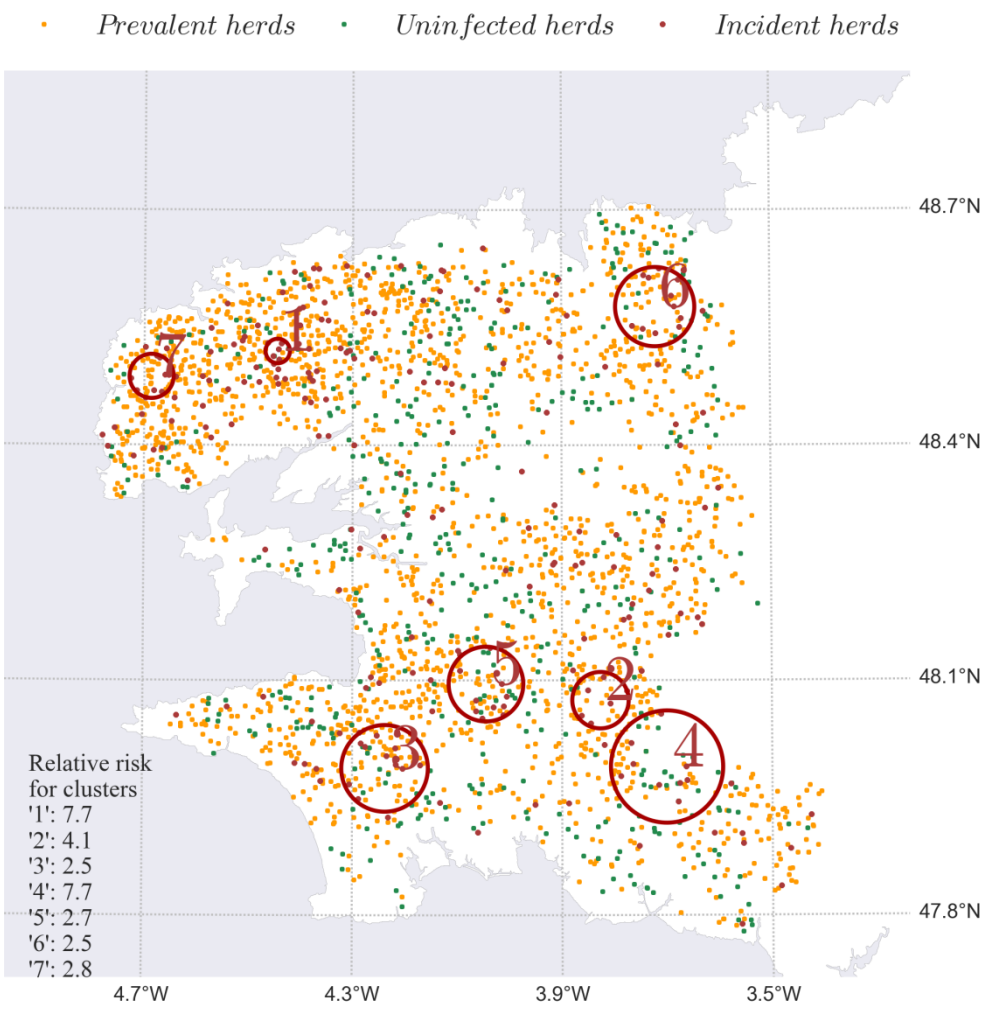


Figure 39 Agrégation spatiale de la probabilité d'infection pour le département du Finistère. Agrégats spatiaux statistiquement significatifs (cercles rouges) pour le Risque Relatif (RR) de présence of troupeaux simulés positifs (points rouges), initialement sensibles, infectés par C. burnetii un an après sa propagation entre troupeaux. Les points orange représentent les troupeaux initialement séro-prévalents (d'après les données) et les points verts les troupeaux qui restent indemnes.

4. Modélisation de l'impact de stratégies de vaccination à l'échelle régionale en conditions enzootiques

Dans le but de mener à bien notre deuxième objectif, nous avons passé en revue les stratégies de maîtrise qui pourraient être potentiellement implémentées et nous avons opté pour la vaccination comme stratégie de maîtrise prospective, et argumenté ce choix en comparaison d'autres techniques possibles. Nous avons présenté les hypothèses sous-jacentes à l'approche de modélisation de la vaccination, fondées sur des précédentes études et des données de [9].

Dans un premier temps, nous avons étudié les effets d'une durée variable de l'immunité que peut engendrer un vaccin sur la dynamique intra-troupeau de l'infection dans un troupeau bovin laitier isolé. Les objectifs de l'étude ont été par la suite d'identifier les caractéristiques principales d'un troupeau à cibler prioritairement dans le cadre d'un programme de vaccination régionale pour permettre une réduction effective de la propagation de *C. burnetii*. Nous avons estimé le taux de couverture minimal à atteindre pour réduire la prévalence dans les troupeaux bovins laitiers dans une région sous conditions d'enzootie.

D'après le modèle, la vaccination des vaches et des génisses avant leur première gestation avec un vaccin de Phase I à large échelle permet de réduire de manière significative la prévalence de *C. burnetii* dans une région. Les troupeaux ciblés pour l'implémentation de la vaccination peuvent être choisis en fonction de leur statut infectieux, de la densité animale environnante, du nombre d'animaux ou du nombre de partenaires avec lesquels ils échangent des vaches. Ces stratégies se révèlent d'une efficacité variable dans la prévention de la propagation de la maladie. Dans le cas du Finistère, la stratégie de vacciner les troupeaux déjà infectés s'avère être la plus efficace à taux de couverture donné. La stratégie de cibler les plus grands troupeaux est la meilleure pour maîtriser l'infection au niveau de l'animal, mais, pour réduire la prévalence à l'échelle du troupeau, elle n'est pas plus efficace que des stratégies ciblant les troupeaux situés dans les zones de densités les plus fortes, ou les troupeaux ayant le plus de partenaires commerciaux.

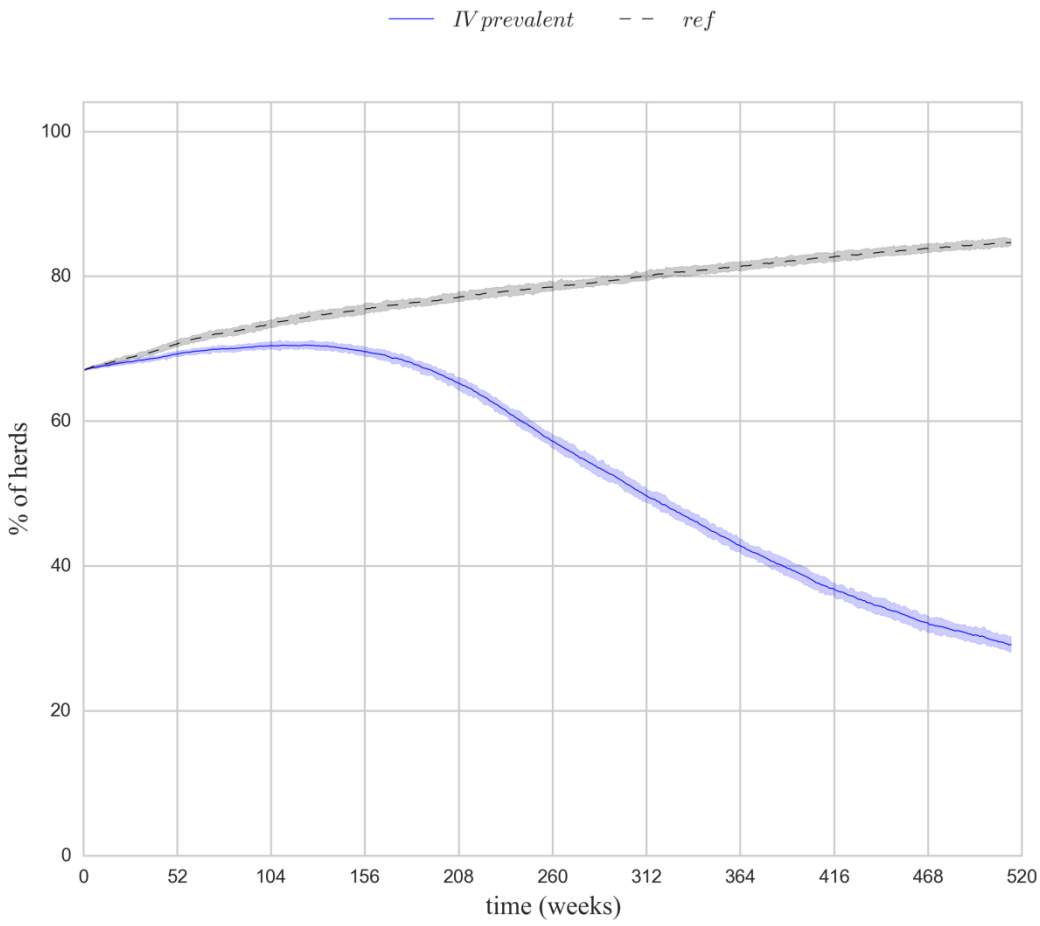


Figure 40 Réduction de la prévalence des troupeaux infectés après vaccination de tous les troupeaux détectés infectés en 2012 (bleu) en comparaison d'une stratégie sans vaccination (noir)

5. Dicussion

Dans ce chapitre final de la thèse, nous avons dans un premier temps discuté des objectifs et des résultats obtenus dans le contexte mondial de la santé publique et animale. Dans une deuxième partie, nous avons discuté des résultats les plus marquants, de leur portée en termes de biologie, et de leur importance dans des applications sur le terrain. Comme la dispersion par le vent et le commerce des animaux ont tous deux des propriétés différentes vis-à-vis de la transmission de *C. burnetii* entre les troupeaux bovins d'une région, il est essentiel de prendre en compte ces deux routes de transmission dans la mise en place d'une stratégie de maîtrise, telle que la vaccination, visant à réduire la prévalence régionale de *C. burnetii*. Nous avons également discuté des problèmes liés aux hypothèses biologiques retenues et discuté l'ensemble de la partie « Résultats » en regard de ces hypothèses. Nous avons abordé dans la discussion la validité externe des résultats et comment nous pourrions extrapoler ceux-ci à d'autres

populations (autres densités animales, autres caractéristiques météorologiques, autres réseau de contact par les mouvements d'animaux).

La partie suivante de ce chapitre a porté sur la discussion des méthodes utilisées dans la thèse. Nous avons mis en avant le caractère novateur de ces méthodes et des modèles utilisés. De plus, nous avons également discuté des inconvénients liés aux méthodes employées et proposé des suggestions d'amélioration. La partie finale a porté sur les différentes perspectives que ce travail ouvre et sur la façon dont cette étude pourrait être étendue à d'autres espèces et être utilisée pour des prédictions d'occurrence de la fièvre Q en population humaine.

Appendix II: Q fever spread between dairy cattle herds in an enzootic region: modelling contributions of windborne transmission and trade

Manuscript submitted to Journal *Veterinary Research*

Initial Submission: 13th July 2015

Editor Decision: 10th August 2015

First Author Revision: 27th August 2015

Q fever spread between dairy cattle herds in an enzootic region: modelling contributions of windborne transmission and trade

Pranav Pandit[1], Thierry Hoch[1]*, Pauline Ezanno[1*], François Beaudeau[1], Elisabeta Vergu[2]

[1]INRA, LUNAM Université, Oniris, UMR1300 BioEpAR, CS40706, F-44307 Nantes, France

[2]INRA, UR1404 Unité Mathématiques et Informatique Appliquées du Génome à l'Environnement (MaIAGE), F-78352 Jouy-en-Josas, France

Author for correspondence: Pranav Pandit; email: pranav.pandit@oniris-nantes.fr

Thierry Hoch; email: thierry.hoch@oniris-nantes.fr

Pauline Ezanno; email: pauline.ezanno@oniris-nantes.fr

François Beaudeau; email: francois.beaudeau@oniris-nantes.fr

Elisabeta Vergu; email: Elisabeta.Vergu@jouy.inra.fr

*These authors contributed equally

Abstract

Q fever, a worldwide zoonotic disease caused by *Coxiella burnetii*, is a looming concern for livestock and public health. Epidemiological features of inter-herd transmission of *C. burnetii* in cattle herds by wind and trade of cows are poorly understood. We present a novel dynamic spatial model describing the inter-herd regional spread of the *C. burnetii* in dairy herds, quantifying the ability of windborne transmission and animal trade in *C. burnetii* propagation in an enzootic region. Our model predictions indicate that the majority of infections in disease-free herds occur due to windborne transmission. Infections acquired through this pathway are shown to cause relatively small and ephemeral intra-herd outbreaks. On the other hand, disease-free herd purchasing an infectious cow will experience significantly higher intra-herd prevalence. Results also indicate that, for short duration, both transmission routes are independent from each other without any synergistic effect. The model outputs applied to the Finistère department in Western France show satisfactory sensitivity (0.71) and specificity (0.80) in predicting the infection status of herds in a neighbourhood of 3km around an expected incident herd, when compared with data. The model developed here thus provides important insights into the infection spread between dairy herds and paves the way for implementation and assessment of control strategies.

Keywords: *Coxiella burnetii*, multiscale modelling, plume model, cattle movements

Introduction

Changes in social-economical, environmental and ecological factors are driving forces for the emergence of zoonotic infections [1]. In Europe, Q fever, a re-emerging zoonosis caused by the bacterium *Coxiella burnetii*, has seen a sharp rise in the recent past, especially in the Netherlands with a large number of human cases whose sources were attributed to livestock [2]-[4]. Q fever infections are common and subclinical in cattle and generally result into reduced reproductive performance and abortions in primiparous cows [5], [6]. Infection in cattle herds is known to be widespread and enzootic [7]. Even though most of the recent human outbreaks are known to be originated from small ruminants, intensive cattle farming always becomes a looming concern for public health. Hence, investigation of infection dynamics in cattle herds at the first sign of its emergence is essential in the emergence-to-control continuum [8].

Cows acquire the infection through inhalation of bacteria shed in the environment. Infectious animals shed the bacteria with different capacities and through different routes [9]. Intra-herd infection dynamics of a dairy herd is majorly influenced by the heterogeneity in the shedding routes [10]. One of the important uncertainties concerning dynamics of infection lies in the contributions of the different routes in transmitting the pathogen between livestock herds. Although windborne transmission of *C. burnetii* is a well-documented phenomenon [11]-[13], its precise contribution to the regional spread of the infection between dairy herds is poorly understood. Besides, there is no formal testing conducted for the determination of the infection status of cows before sale or purchase. Hence, trade of animals as a direct route of transmission also can play a role in the regional spread. Recent studies have identified a risk of infection of dairy herds through dispersion of bacteria and cattle trade [14], [15], but their respective quantitative contributions to the transmission still remain unknown.

Here we present a novel individual-based mechanistic model in a stochastic framework capturing both spatial and temporal spread of *C. burnetii* between dairy herds. Infection status and trade movement

of animals between herds were individually tracked through time. The model has been applied to a case study of Finistère department located in North-Western France, characterized by a high density of dairy cattle, windy oceanic weather and relatively flat terrain. To comprehend the reliability of the model predictions, first we have assessed the model accuracy in predicting the status of individual herds and their neighbourhoods as observed during years 2012-2013 in Finistère. Then we have used the model to evaluate the contributions of transmission routes to the regional spread of the infection and their impacts on the intra-herd infection dynamics.

Materials and Methods

Data driven modelling framework

The regional spread of *C. burnetii* was conceptualised by a multiscale model (inter-herd and intra-herd scales), with spatially separated herds having their own infection and demographic dynamics, and interacting with each other via cattle trade and windborne transmission (see a schematic representation in Figure 1). The intensity and direction of cattle movements between herds were modelled as observed in available data. The health status of exchanged animals was randomly chosen according to the intra-herd prevalence in source herds, the purchase of infected cows then leading to the introduction of the bacteria into naïve herds. *C. burnetii* (as bacterial plume) also can be transmitted from infected to naïve herds by windborne transmission. This process was modelled using Gaussian dispersion model incorporating meteorological data. The individual-based stochastic intra-herd model described the infection dynamics in herds after the introduction of the pathogen via either of the two processes, subsequently becoming sources of infection to other herds. Initial conditions for simulations concerning infection status of herds were based on epidemiological data.

Data

Description of epidemiological data, animal trade data, and meteorological data used for modelling the spread of *C. burnetii* in the Finistère department is given in this subsection.

The pathogen is known to be enzootic in the cattle population of this region. In May 2012, 2,799 dairy herds (69% of all the cattle herds in Finistère), individually and spatially identified, were tested for the antibodies against *C. burnetii* in bulk tank milk (BTM) using LSI Q fever enzyme – linked immunosorbent assay (ELISA) kit® (LSI; Lissieu, France), and 1,941 were found seropositive (referred hereafter as *prevalent herds*). The results of ELISA tests were interpreted as estimates of intra-herd seroprevalence [16] and were used to set the initial conditions for simulations. Among the 858 negative herds, 826 were retested one year later in May 2013, and 306 were found positive (they represent the observed *incident herds* in the study).

For the purpose of the study, data for the individual movements of cows from one dairy herd to another only within the Finistère department were extracted for the time period of May 2012 to May 2013 from the national register (source: Groupements de Défense Sanitaire de Bretagne, France). In total, 835 out of 2,799 dairy herds participated in the 2,234 movements during the year. Among trading herds, 182 purchased at least one animal during the study period and were not infected in May 2012. On average, the number of sold animals was equal to 4.7 (n=474), the number of purchased animals was 5.3 (n=491), the number of partners for selling was 1.4 and for buying animals was 1.6.

Wind velocity data required for dispersion modelling were procured from publically available European Centre for Medium Range Weather Forecasts database [17]. Northward and eastward wind component data for Finistère department for the period of May 2012 to May 2013 was extracted. Daily data were converted to weekly averages for utilisation in the model. Details of incorporation of the data into the model are given in the Additional file 1.

Modelling description

The model is an individual-based stochastic model in discrete time with a one week simulation time step. The whole model (intra-herd dynamics and cattle trade) is restricted to cows, excluding nulliparous heifers and female calves, as they are not observed to be infected as shown in a previous longitudinal study [18]. Bulls and male calves were also excluded.

Inter-herd transmission of infection due to cattle trade

All the movements of cows between dairy herds of Finistère department were modelled according to observed data and were deterministically implemented based on source and destination herds, movement date, and age of cows. For each individual movement observed between May 2012 and May 2013, an animal of the same lactation number as the one recorded in the dataset was randomly chosen from the source (selling) herd to move to the destination herd. The probability of trading an infectious cow therefore was related to the proportion of infectious animals in the given lactation age in the source herd. Because of the comparatively low time spent by cows in markets during trading, it was assumed that there was no transmission between cows following any possible interaction between them in markets. Due to the lack of information about the prevalence outside the concerned study region, movements of cows were considered only between the herds of the study region.

Inter-herd windborne transmission of C. burnetii

In infected herds, infectious cows shed bacteria which became source of infection for animals of the herd and from which a proportion was assumed to disperse to other herds via windborne transmission. The small cell variant (SCV) of bacteria shed is very resistant to the environmental conditions and can survive well in the environment [19]. Plume transportation takes place with simultaneous deposition and settling of particles. These processes were modelled using a Gaussian Dispersion Equation, which accounts for phenomena such as transportation, settling, and gravitation [20], [21]. The concentration Ci,j,(x,y,z) [number of bacteria / m3] of bacteria reaching herd i from source herd j (where x, y are differences in respective coordinates of herds i and j, and z the height of generation plume in source herd j) was calculated using the following equation presented in Stockie et al, 2011 [21]:

$$C_{i,j,(x,y,z)} = \frac{Q_j}{2\pi U\sigma_y\sigma_z} e^{\left(\frac{-y^2}{2\sigma y^2}\right)} e^{\left(\frac{-W_{set}(z-h)}{2K_z} - \frac{W_{set}{}^2\sigma_z{}^2}{8K_z{}^2}\right)} \left[e^{\left(\frac{-(z-h)^2}{2\sigma_z{}^2}\right)} + e^{\left(\frac{-(z+h)^2}{2\sigma_z{}^2}\right)} - \frac{\sqrt{2\pi}W_o\sigma_z}{K_z} e^{\left(\frac{W_o(z+h)}{K_z} + \frac{W_o{}^2\sigma_z{}^2}{2K_z^2}\right)} \mathrm{erfc}\left(\frac{W_o\sigma_z}{\sqrt{2}K_z} + \frac{(z+h)}{\sqrt{2}\sigma_z}\right) \right]. \quad (2.1)$$

Equation (2.1) is the solution of an atmospheric advection-diffusion equation accounting for particle dispersion and deposition as developed in Ermak 1967 [20]. Quantities forming the different terms are the following: Q_j [number of bacteria / s] is the force of infection in source herd *j*; *U* [m/s] is the wind velocity; σ_y [m] and σ_z [m] are the standard deviation for dispersion coefficients, taking the form $\sigma_y(x) = a_y x^{b_y}$ and $\sigma_z(x) = a_z x^{b_z}$ with a_y, a_z, b_y, b_z corresponding to the atmospheric stability class C (3-5 m/s wind velocity, slightly unstable environment); W_0 [m/s] writes as $W_0 = W - 0.5W_{set}$, where *W* [m/s] is the deposition velocity due to gravitation and W_{set} [m/s] is the settling velocity, fixed to $\frac{2\varphi gr^2}{9\eta}\frac{2\varphi gr^2}{9\eta}$, with ϕ [kg/m^3] the particle density, *r* [m] the particle radius, η [kg/m s] the dynamic viscosity of air, and *g* [m/s^2] the gravitational acceleration; *h* [m] is the height of reception at destination herd; and K_z [m^2/s] is the coefficient of eddy diffusivity set to $K_z = 0.5a_z b_z U x^{(b_z-1)}$. In the last term of (2.1), *erfc* is the complementary error function ($erfc\,(x) = 1 - \mathrm{erf}(x)$) resulting from the approximation of the solution of the partial differential equation of advection-diffusion. Parameters were taken from the standard model presented in Stockie et al. (2011) [21]. Additional details on dispersion related parameters are given in Table 1. The relationships between $C_{i,j,(x,y,z)}$, Q_j, and the intra-herd infection dynamics at source and destination herds are presented in the next subsection.

Intra-herd dynamics of infection and coupling with cattle trade and windborne transmission of C. burnetii

The intra-herd infection dynamics, tracking the animal health statuses individually, was represented using the model introduced earlier by Courcoul *et al*. [10], whose transition parameters

between health statuses were estimated from a longitudinal observational study using Bayesian estimation methods [22], [23]. The model accounts for the heterogeneity in the routes and levels of bacterial shedding. Moreover, the population dynamics of the herd also is incorporated through probabilities for culling events and the explicit representation of cow lactation cycle.

Cows of the herd undergo transitions between health statuses after infection (Figure 2, with parameters defined in Table 2). Susceptible, non-shedder, sero-negative cows (S) become shedder sero-negative I^- cows after infection. I^- cows then either seroconvert, becoming I^+ (shedder, with antibodies) or $I^{+\,milk\,pers}$ (shedder with antibodies, and permanently shedding in milk at higher levels), or return to S status. I^+ and $I^{+\,milk\,pers}$ cows then become carriers and stop shedding C^+ (with antibodies), and subsequently C^- (without antibodies). C^+ cows can restart shedding (and then become I^+ again). Shedding cows can shed the bacteria through milk, mucus/ faeces, or through both routes (with probability distributions of shedding routes α, β, and γ depending on the infection status) at low, medium, or high levels of shedding (corresponding respectively to quantities shed equal to 1/3000, 1/30, and 1 unit of environment per week, with probability distributions of shedding levels Q1 to Q5 depending on the infection status). The proportions of cows shedding through different routes and at different levels change according to whether cows are in early lactating stage ($\leq 4\ weeks\ post\ calving$) or not. ε_1, ε_2 and ε_3 are the quantities of bacteria shed during a time step by a cow in status I^-, I^+, and $I^{milk\,pers}$, respectively, and contaminating the environment and are the sum of quantities shed by all the shedders through all the shedding routes times the fraction of bacteria ($\rho^{m/f}\ and\ \rho^m$) reaching the environment of the herd. All the parameters related to the heterogeneity of shedding are presented in Table 3.

The probability $p_i(t)$ of a susceptible cow of herd i to acquire infection at time t depends on the environmental compartment ($E_i(t-1)$) of the herd:

$$p_i(t) = 1 - e^{-(E_i(t-1))} \qquad (2.2).$$

$E_i(t)$ [number of bacteria / s] is the force of infection related to the bacterial contamination of the environment (for simplicity of writing the time step multiplying $E_i(t-1)$ was omitted in Equation (2.2)). It corresponds to the bacterial load at time *t* (expressed in infectious doses) shed by shedding animals (according to their infection statuses and the shedding routes), times the contact rate between animals and the environment, times the probability that a contact of a susceptible animal with an environment contaminated by one infectious dose leads to a successful infection event. Similar formulation of the probability of infection has been proposed previously for aerosol infection of *C. burnetii* [24].

The complete system of equations describing the infection dynamics at herd level is provided in Additional File 1.

Coupling of intra-herd model with cattle trade was done by characterizing each cow in a herd, based on its origin and health status. Cows which are borne in the same herd or when susceptible *(S)* at purchase were called *internal animals.* Cows which were infected outside the herd and that were shedders (I^-, I^+ or $I^{+\,milk\,pers}$) or carriers (C^+) at the time when they were bought were called *external animals*. The infection dynamics of the *internal animals* and *external animals* were assumed to be identical, the first contributing to the local subsection of the environmental compartment, $E_{i,internal}$, the second contributing to the external subsection, $E_{i,external}$.

Coupling of the intra-herd model with windborne transmission of infectious particles was also done through the environmental compartment. Bacteria arriving from a neighbouring herd *j* through windborne transmission accumulate in compartment $E_{i,j,dep}$. This writes as $E_{i,j,dep} = area_i\, W\, C_{i,j,(x,y,z)}$, where the area for each herd ($area_i$) was approximated using average space recommendation for a cow and the number of cows in a given herd. *W* and $C_{i,j,(x,y,z)}$ are presented in equation (2.1). Similarly, a fraction κ of the bacteria leaving the environmental compartment (due to the various mechanisms encompassed in term

μ, Table 2) was assumed to become the source for generation of the plume and was defined as $Q_i=E_i\mu_{plume\ source}$, with Q_j defined in equation (2.1) and $\mu_{plume\ source}=\kappa\mu$.

Hence, after accounting for inter-herd processes in the intra-herd model, the environmental force of infection for each herd can be decomposed into two terms related to the bacteria local shedding ($E_{internal}$) and ($E_{external}$) and one term related to deposited bacteria due to windborne transmission from all possible source herds j ($\sum_j E_{j,dep}(t-1)$). The general formulation of the environmental force of infection due to bacteria in herd i was represented as follows:

$$E_i(t) = E_i(t-1)(1-\mu) + E_{i,internal}(t-1) + E_{i,external}(t-1) + \sum_j E_{i,j,dep}(t-1)\ \mathrm{E_i(t)} =$$

$$\mathrm{E_i(t\text{-}1)\big(1\text{-}\mu\big) + Bact_{i,Local}(t\text{-}1) + Bact_{i,Foreign}(t\text{-}1) + \sum_j Bact_{Dep,i,j}(t\text{-}1)} \qquad (2.3).$$

The loss of bacteria from the environment, μ, encompassing death and plume generation, was defined as $\mu = \mu_{death} + \mu_{plume\ source}$.

Simulation settings and outputs

Initial conditions were set to mimic the spatial distribution of intra-herd seroprevalence observed in May 2012 in the Finistère department. For each prevalent herd, independent simulations of the intra-herd infection dynamics were run with one introduction of an infectious animal until they reached the observed levels of infection. Then, herds were connected and the inter-herd spread of the infection was simulated over the duration of one year, to assess if the model can predict similar spread of infection as observed in May 2013. For each scenario investigated, 100 iterations of the stochastic model were run. The spatial dynamic model was used to predict the status (in May 2013) of initially (in May 2012) susceptible herds. Introduction of infection in herds was defined as the generation of the first case among internal animals. Identifying contamination sources allowed us to allocate a cause to the primary local case and therefore to assess the relative contribution of each of the two transmission routes considered for each incident herd. Herds receiving infectious animals previous to the generation of the first local case

were designated as being infected by cattle trade, the rest of the incident herds were attributed to windborne transmission. In addition to the cause of infection, the *probability of infection* (*PI*) was also estimated for each incident herd based on the proportion of runs it experienced infection: *PI = (number of runs with at least one local case) / (total number of runs).* Herds were predicted positive by the model if their predicted *PI* was higher than a threshold, which was calibrated according to the available data, as described in the next subsection (Assessment of model predictions). Concerning the intra-herd dynamics in incident herds, four model outputs were considered: *seroprevalence, proportion of shedders* (Additional file 1, equations 7 and 8), *extinction rate* (equal to the proportion of runs with no shedding and no seropositive cow at the end of the simulation among those runs where the herd was infected) and herd incubation period (calculated as the time elapsed between exposure to the identified cause and generation of the first local case). Descriptive statistical measures (mean, median, standard deviation and percentiles) of seroprevalence and proportion of shedders in incident herds were calculated only over runs in which herds experienced an infection.

Assessment of model predictions

To assess the accuracy of the model in predicting the binary outcome (infected / non infected, as observed in May 2013) for all the initially susceptible herds, we performed a receiver operating characteristics (ROC) analysis, based on *Sensitivity* (Se) (or true positive rate of detection) and *Specificity* (Sp) (more precisely $1 - Sp$, representing the false positive rate). A ROC analysis consists in evaluating the performance of a classifier in detecting binary behaviour for different discrimination thresholds. More specifically, for each initially susceptible herd, the predicted infection status of the herd was compared against the observed one (the reference) at the end of the study period (this is what we called the herd level analysis). Each point of the ROC curve corresponds here to a different threshold to which the PI for each initially susceptible herd is compared, in order to be classified as infected or not. To assess possible improvements in prediction, we relaxed the spatial precision in the ROC analysis and compared the output for a neighbourhood around an expected incident herd (that we called the neighbourhood level analysis).

The comparison was done for neighbourhood distances of multiple radii (1, 2, 3 or 4 km). Sp for the neighbourhood level analysis was considered as equal to that of herd level analysis. AUC (Area Under the Curve) was used for assessment of model performance.

The optimum *cut-off* (threshold) values for *PI* to classify herds as positive or negative were selected based on three criteria: equality of Se and Sp, *Se =Sp*; *maximum accuracy (*Acc_{max}*)*, where *Acc = (true positive herds + true negative herds) / (total population)* or, equivalently, *Acc = Se* x *prevalence + Sp* x *(1-prevalence);*$\text{Acc} = (\text{truepositiveherds} + \text{truenegativeherds})/(\text{totalpopulation}), \text{or, equivalently, Acc} = \text{Se} \times \text{Prevalence} + \text{Sp} \times (1\text{-Prevalence})$ and *maximum Youden index* (J_{max}), where $J = Se + Sp - 1$ [25].

To identify regions with high risk of incidence, a spatial cluster analysis for predicted positive herds was done using a Poisson model (SatScan®) with a null hypothesis of expected number of cases in each area proportional to its population size, hence adjusting the model for cow density. Definition of a positive herd was based on the optimum *PI* cut-off suggested by the ROC analysis.

A preliminary sensitivity analysis was done to assess the robustness of the model predictions with respect to parameter variations. In a detailed sensitivity analysis conducted on the intra-herd infection dynamics model by Courcoul *et al*. [10], three significantly sensitive parameters were found: *Q1*, ρ, and μ. Along with these three parameters, three additional parameters from the dispersion model (κ, *r*, *W*) were tested in the analysis. The values chosen to be tested in the sensitivity analysis (details in Table 4) were those used in [10] for *Q1*, ρ, and μ. For κ, *r*, and *W*, the standard value was varied by fifty percent, in the limits of biological plausibility. Each parameter was varied independently of other parameters (univariate sensitivity analysis) and the effect of these variations was evaluated on three model outputs (relative contribution of windborne transmission to new herd infections, number of incident herds, and proportion of shedders in incident herds).

Assessment of the relative impact of transmission routes on the regional spread and intra-herd infection dynamics

To identify the contribution of cattle trade and wind dispersion as routes of transmission, we used two complementary approaches. First, we tested four scenarios to understand the role of each transmission route both independently and in association with one another: absence of inter-herd transmission (Scenario A), transmission only by movement of animals (scenario B), windborne transmission only (scenario C), both transmission routes (scenario D). The dynamics of the incidence at the herd level, the total number of incident herds, and the dynamics of shedder cows in incident herds were compared to assess the impact of presence and absence of the transmission routes on the regional spread. The second approach focused on identifying the relative roles of the two transmission pathways in introducing the infection in incident herds, by further evaluating scenario D and using the model ability to identify the cause of infection in incident herds. To investigate differences in the intra-herd dynamics within incident herds because of different causative transmission routes, comparisons of *PI*, extinction rate, and herd incubation period for herds infected by windborne transmission and infected by trade, respectively, were made using Mann-Whitney U test. A similar analysis was done on a subset of herds at risk of acquiring infection through both routes, i.e. those herds that purchased animals and were also exposed to *C. burnetii* due to windborne transmission.

Results

Incidence prediction and agreement with observed data

Out of 858 susceptible herds at the beginning of the simulation, 768 got infected at least once over the total number of runs. The *PI* predicted for incident herds showed spatial heterogeneity (Figure 3a). Most of incident herds showed low values of *PI*. Out of 768 herds, 38.8 % herds showed $PI < 0.1$, while only 1.5% herds showed $PI \geq 0.9$ (Figure 3b).

The model had moderate agreement with data at herd level. It performed better for predictions at the neighbourhood level (Figure 4a). In the radius of 2, 3, 4 km, there were on average respectively 1.7, 3.8, and 6.6 initially susceptible neighbour herds around an expected incident herd in the Finistère department. The gain in the model predictive ability in terms of AUC with the increase in the neighbourhood radius was weighed against the loss in the precision of model predictions arising because of an increase in the number of susceptible herds the calculations rely on, resulting into a subjective compromise for a neighbourhood of 3 km, retained for further analyses of model results.

Optimum cut-off values for *PI* were estimated based on three criteria: $Se = Sp$, Acc_{max}, and J_{max}. At herd level, the model was found performing better at *PI* cut-off = 0.11 for the first and third criteria ($\mathrm{se} = 0.57, \mathrm{sp} = 0.59$), $J_{max} = 0.15$), and at *PI* cut-off = 0.61 for the second one ($Se = 0.1$, $Sp = 0.95$, $Acc_{max} = 0.64$). For a neighbourhood of 3 km, the optimal cut-off was found to be 0.21 based on the first criterion($\mathrm{se} = 0.76, \mathrm{sp} = 0.75$)0.25 based on the second one ($Se = 0.71$, $Sp = 0.80$, $Acc_{max} = 0.76$), and 0.15 according to the third one ($Se = 0.86$, $Sp = 0.66$; Figure 4b and 4c). Details of the *Se*, *Sp*, *Acc*, *J*, predicted incidence, contribution of windborne transmission to the incidence, and the spatial distribution of incident herds at these cut-offs are given in Additional file 1 (Table S1 and Figure S1). The subsequent cluster analysis was performed using a cut-off value of 0.25 (i.e. herds were declared as positive if their *PI* > 0.25) as this value provided the uniformly best results with respect to the three criteria at the neighbourhood level. According to the cluster analysis, herds predicted as positive by the model at the cut-off of 0.25 showed seven non-overlapping statistical clusters, three in the north and four in the south of the Finistère department (Figure 5). A small cluster (Cluster 1, Figure 5) in northern Finistère department showed the highest relative risk of 7.7.

Model outputs were sensitive to *Q1*, ρ, μ, and κ, whereas very little perturbations were induced by variations of particle size, *r*, and deposition velocity, *W* (Figure 6). Results showed that, despite a considerable sensitivity of the model to the parameters tested (except for *r*), the relative contribution of windborne transmission in the simulated incidence remained higher than the contribution of cattle trade,

regardless the parameter values tested, except for some values of κ and μ for which this trend was reversed in the last six months of simulation duration.

Contribution of transmission pathways to the regional spread

Windborne transmission was responsible for the infection of the majority of incident herds as predicted by the model at all the optimum *PI* cut-offs derived in the ROC analysis. The contribution over these cut-offs varied from 57 % to 86 % at herd level, and from 75 % to 83 % at a neighbourhood of 3 km (see Additional file 1 Table S1). The sensitivity analyses showed that windborne transmission contributed to more than 50% and 70% of the new herd infections in 88 % and 63 % of the tested situations, respectively (Figure 6).

Figure 7 illustrates the effect of windborne transmission and cow trade on the regional spread of infection. More incident herds were seen in scenarios comprising windborne transmission (C and D, at least five times more incident herds on average than in scenario B; Figure 7a and 7b). Further analysis of scenario D carried out in the second approach provided similar results for the predicted incidence. In all the 100 iterations of the standard stochastic model, 92 % of all the new herd infections were attributed to windborne transmission, while the rest (8%) was attributed to cattle trade. The incidence dynamics over the time period attributed to these two transmission routes when acting simultaneously showed close coherence with the incidence predicted in scenarios B and C, where each transmission route was considered separately (Figure 7b). Incidence attributed to windborne transmission (scenario C and deconvolution of scenario D) showed an initial rapid increase followed by a steady growth, while the incidence attributed to cattle trade was comparatively low and constant throughout the simulation period (scenario B and deconvolution of scenario D). The analysis performed on the subset of herds at risk from getting infected through both routes, with parameter values corresponding to the standard scenario, led to results consistent to those obtained for the whole population of initially susceptible herds. On average, the majority of the new herd infections were due to windborne transmission (65%).

Impact of transmission pathways on the intra-herd dynamics

The impact of presence and absence of a transmission route on the intra-herd infection dynamics was highlighted in the four scenarios. Scenario involving only trade (B) showed higher proportion of shedders (Figure 7c) and intra-herd seroprevalence (not shown) in incident herds, than scenarios involving windborne transmission only (C) or both transmission pathways (D). When both transmission routes were accounted for, herds infected due to windborne transmission showed significantly lower levels of shedding animals than those infected after purchasing an infectious cow (Figure 7c inset). Other representative parameters of the infection dynamics also were found statistically significantly different ($p < 0.05$, Figure 8). *PI* was higher in herds infected by cattle trade, while the extinction rate was higher in windborne infected herds. These latter also took significantly longer time to generate the first local case after exposure to the respective cause than herds infected by cattle trade.

Variation in the intra-herd dynamics (proportion of shedders) followed similar trends when performed on the subset of herds exposed to both transmission routes as seen in the analysis done on all susceptible herds. Also, for all outputs considered (*PI*, *time after infection* and *extinction rate)*, statistically significant difference in herds infected by windborne transmission and herds infected by cattle trade was found.

Discussion

Our findings show that windborne transmission and movement of cows both affect the regional spread of *C. burnetii* but with different capacities. On the one hand, windborne transmission has the ability to introduce the pathogen in a large number of herds if the generation of plume occurs at high enough rates, but the generated outbreaks are generally ephemeral and small. On the other hand, animal trade results in a limited number of incident herds, but purchasing an infectious cow can instigate comparatively larger outbreaks. The differences in the impact of each transmission route on the intra-herd infection dynamics arise from the intrinsic nature of these transmission routes in spreading the infection. Regardless

the route, the first generated local case is always a cow with health status I^- as shown in Figure 2. Such a seronegative shedding cow is a transient shedder, which can become susceptible again. Therefore, in herds infected by windborne transmission, infection can easily go extinct if the transient first local case does not shed enough to generate secondary cases, which are essential for infection persistence. In herds introducing infectious cows by trade, the animal purchased can be either a transient shedder (I^-) or a permanent shedder (I^+ or $I^{milk\ pers}$). Hence, after the generation of the first local case, there are at least two shedding cows in herds purchasing infectious animals, leading to potential higher bacterial contamination and increasing the probability of intra-herd infection persistence.

Our results, based on a mechanistic dynamical model of infection spread at different scales, are consistent with a previous study from the same group [14] based on a statistical regression model, which indicated that windborne transmission and cattle trade are both risk factors for the dairy cattle herds in Finistère department. The study [14] also attributed higher proportion of cases to windborne transmission than to animal movements in areas with high cattle density. A cluster analysis performed for the 2012 seroprevalence in dairy herds showed a high-risk cluster in North-western corner of the Finistère department. Clusters for the predicted probabilities of herd infection in 2013 showed two high-risk clusters in the same area, known to have a high density in cattle.

The contribution of animal trade in transmitting livestock diseases is known to vary considerably according to the disease under study. For Q fever, cattle trade seems to explain quite a low proportion of incidence (compared to wind), at least in areas with high cattle density. It is known to play an important role in the regional spread of other infectious diseases, such as foot-and-mouth disease (FMD) and bovine viral diarrhoea virus [26], [27]. For bovine tuberculosis - as here for Q fever -, trade is correlated to a low number of infections [28] compared to other transmission routes. While these studies focus on the regional contribution of transmission pathways, here we also highlighted differences in intra-herd infection dynamics depending on these pathways. The simulated differences in the intensity of intra-herd outbreaks

experienced by herds acquiring infection by cattle trade and by windborne transmission, and the capacities of these routes to affect infection-free herds provide valuable insights for risk assessment. Even if cattle trade seems not to generate large proportion of newly infected herds in certain conditions, preventing the purchase of infected animals is still a relevant measure to limit infection spread at the intra-herd scale. From the model perspective, it is the first time, to our knowledge, that a Gaussian dispersion model for infectious particles is coupled with an intra-herd infection dynamics model to describe the spread of an enzootic livestock disease. Gaussian dispersion models previously have been employed in the description of the spread of viral diseases of livestock and poultry such as FMD and avian influenza [29], [30]. A dispersion model also has been used to detect the possible risk of Q fever occurrence in human communities from nearby sheep farms [31].

One of the main advantages of using mechanistic models is that they allow determining the causes of infection, and subsequently help assessing targeted interventions [32]. For a given scenario (characterized by a set of fixed parameter values), the mechanistic model presented here identifies the cause of infection of susceptible herds based on the dominant contributory route, at the time of generation of the first local case, and also provides very similar results with the two scenarios assuming single transmission route. Moreover, according to our investigations, the combined effect of the two processes (windborne transmission and animal trade) at a regional scale is additive and not synergistic, at least over a short period of time.

Performance measures of the model at the neighbourhood level can be interpreted as the model ability to predict an observed herd case within a given area. The increase in the AUC for the comparisons done at different neighbourhood radii also indicates the model ability to capture the spatial nature of the dispersion. Assuming that the neighbourhood range and the accuracy of the model depend on the herd density and the clustering of the infection in the study region, selection of a neighbourhood range becomes case-specific. The ROC analysis performed for different neighbourhoods is an effort to increase the sensitivity of the model without altering its specificity, with more weightage given to the capacity of the

model of identifying positive herds. The sensitivity of the model hence increases with the decreasing spatial granularity.

Irrespective of the benefits, mechanistic models are generally difficult to fit to data. Spatio-temporal outcome of FMD models, when tested against the 2001 outbreak data, have shown about 10-15% accuracy [33]. In the current Q fever model, higher accuracy of the model is probably due to the high prevalence and the enzootic nature of the infection in the study region. Models are generally used to simulate the overall spread of an infection to produce expected epidemic curve, and are often difficult to judge for their relevance, especially in the absence of detailed and accurate data. Since many models are increasingly depicting the spatial spread of infections in livestock in enzootic regions, more refined evaluation of their ability to produce spatial patterns in agreement with field observation needs to be addressed. Analysis based on ROC spatial analysis, like the one used here, can be useful in understanding the complex spatial behaviours of such models.

Although we cannot deny the possible existence of interactions between the tested parameters with potential impact on model outputs, the one-at-a-time sensitivity analysis performed supports the relative robustness of model predictions at elementary level. The main output of the model concerning the relative contributions of the transmission routes in the regional spread of *C. burnetii* showed moderate perturbations to parameter variations, especially when the plume was generated at rates high enough (allowing windborne transmission) compared to death rate of bacteria (κ related to the ratio between these two rates). To reduce the uncertainty on these parameters and hence on their effect on the infection dynamics, more data collection is essential to estimate the bacterial quantities generally found in and leaving farm buildings. The possible effects of super shedders were indirectly assessed using sensitivity analysis of the model to Q1, which is the probability distribution of the shedding levels for all the I^- and for the I^+ shedding in mucus/faeces after 4 weeks post-calving. Two of the probability distributions tested (described in Table 4) assumed proportions of high shedders of 0.25 (distribution IV) and 0.5 (distribution III), whereas the reference scenario assumed no high shedders in these classes. It seems that in scenarios

corresponding to distributions III and IV for *Q1* the contribution of trade was diminished, but this needs to be confirmed in further refined analysis.

The model is expected to underestimate the spread of the infection as we ignore beef herds in the study region, which can transmit infection to dairy herds by windborne transmission, and also as we consider cattle trade within the concerned department only. Indeed, according to the analysis of a larger database over the period 2005-2009, 22% of all the concerned transactions of cows involving dairy herds located in Finistère department corresponded to purchases from outside the department (personal communication: B. L. Dutta). However, no epidemiological information was available for these herds. Similarly the impact of small ruminant flocks also was neglected as very few small ruminant flocks are present in the region. Accuracy of the model could be further improved if epidemiological data about beef herds and other livestock flocks in and around the region were available.

The time-varying nature of the network describing cattle trade, in particular the large variability in the trade relationships between herds from one year to the next (as described in France by Dutta *et al*. [34]), suggests that the transmission route due to trade could have a larger impact on the regional dynamics over a longer duration. Indeed, new susceptible target herds could be linked to the network of herds by enlarging the time window of the study. Similarly, the capacity of windborne transmission of the bacteria is relatively unhindered and all herds get exposed in a very densely populated region without any geographical barriers such as Finistère. Hence, the regional spread and corresponding control strategies predominantly depend on the prevalence of infection, characteristics of the cattle trade network, and cattle density. On the backdrop of these, the model presented here can become a useful tool to assess the impact of relevant interventions such as vaccination of cows [35] and testing of cows for the presence of the pathogen before trading, on the control of the regional spread of infection.

Acknowledgements

This work was carried out with the financial support of the French Research Agency (ANR), Program Investments for the Future; project ANR-10-BINF-07 (MIHMES), by the European Union through the European fund for the regional development (FEDER) of Pays-de-la-Loire, and by Oniris. Data for the cattle trade was provided by the Groupements de Défense Sanitaire de Bretagne, France

Abbreviations

ROC: Receiver operating characteristic; BTM: bulk tank milk; ELISA: enzyme-linked immunosorbent assay; FMD: Foot and mouth disease

Authors' Contributions

Conceptualisation of the mathematical model, interpretation of simulation outputs, writing and critical review of the manuscript were performed by PP, TH, PE, FB and EV. Computational experiments were designed by PP, TH, PE and EV and performed by PP. Inputs in the biology and epidemiology of Q fever were provided by PP and FB.

Competing interests:

The authors declare no competing interests.

References

1. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P (2008) Global trends in emerging infectious diseases. Nature 451:990-993
2. Georgiev M, Afonso A, Neubauer H, Needham H, Thiery R, Rodolakis A, Roest H, Stark K, Stegeman J, Vellema P, *et al* (2013) Q fever in humans and farm animals in four European countries, 1982 to 2010. Euro Surveill 18
3. Schimmer B, Morroy G, Dijkstra F, Schneeberger PM, Weers-Pothoff G, Timen A, Wijkmans C, van der Hoek W (2008) Large ongoing Q fever outbreak in the south of The Netherlands, 2008. Euro Surveill 13

4. Roest HI, Tilburg JJ, van der Hoek W, Vellema P, van Zijderveld FG, Klaassen CH, Raoult D (2011) The Q fever epidemic in The Netherlands: history, onset, response and reflection. Epidemiol Infect 139:1-12
5. Hässig M, Lubsen J (1998) Relationship between abortions and seroprevalences to selected infectious agents in dairy cows. Zentralbl Veterinarmed B 45:435-441
6. Arricau-Bouvery N, Rodolakis A (2005) Is Q fever an emerging or re-emerging zoonosis? Vet Res 36:327-349
7. Guatteo R, Seegers H, Taurel AF, Joly A, Beaudeau F (2011) Prevalence of Coxiella burnetii infection in domestic ruminants: a critical review. Vet Microbiol 149:1-16
8. Murphy FA (1998) Emerging zoonoses. Emerg Infect Dis 4:429-435
9. Guatteo R, Beaudeau F, Joly A, Seegers H (2007) Coxiella burnetii shedding by dairy cows. Vet Res 38:849-860
10. Courcoul A, Monod H, Nielen M, Klinkenberg D, Hogerwerf L, Beaudeau F, Vergu E (2011) Modelling the effect of heterogeneity of shedding on the within herd Coxiella burnetii spread and identification of key parameters by sensitivity analysis. J Theor Biol 284:130-141
11. Tissot-Dupont H, Amadei MA, Nezri M, Raoult D (2004) Wind in November, Q fever in December. Emerg Infect Dis 10:1264-1269
12. Tissot-Dupont H, Torres S, Nezri M, Raoult D (1999) Hyperendemic focus of Q fever related to sheep and wind. Am J Epidemiol 150:67-74
13. Hawker JI, Ayres JG, Blair I, Evans MR, Smith DL, Smith EG, Burge PS, Carpenter MJ, Caul EO, Coupland B, *et al* (1998) A large outbreak of Q fever in the West Midlands: windborne spread into a metropolitan area? Commun Dis Public Health 1:180-187
14. Nusinovici S, Hoch T, Widgren S, Joly A, Lindberg A, Beaudeau F (2014) Relative contributions of neighbourhood and animal movements to Coxiella burnetii infection in dairy cattle herds. Geospat Health 8:471-477

15. Nusinovici S, Frössling J, Widgren S, Beaudeau F, Lindberg A (2015) Q fever infection in dairy cattle herds: increased risk with high wind speed and low precipitation. Epidemiol Infect 1-11
16. Taurel AF, Guatteo R, Joly A, Beaudeau F (2012) Relationship between the level of antibodies in bulk tank milk and the within-herd seroprevalence of *Coxiella burnetii* in cows. Epidemiol Infect 140:1710-1713
17. European Centre for Medium-Range Weather Forecasts www.ecmwf.int. Accessed Aug 2013
18. Guatteo R, Seegers H, Joly A,Beaudeau F (2008) Prevention of *Coxiella burnetii* shedding in infected dairy herds using a phase I *C. burnetii* inactivated vaccine. Vaccine 26: 4320-4328
19. McCaul TF, Williams JC (1981) Developmental cycle of Coxiella burnetii structure and morphogenesis of vegetative and sporogenic differentiations. J Bacteriol 147:1063-1076
20. Ermak DL (1967) An analytical model for air pollutant transport and deposition from a point source. Atmospheric Environment 11:231-237
21. Stockie JM (2011) The mathematics of atmospheric dispersion modeling. Siam Review 53:349-372
22. Guatteo R, Beaudeau F, Joly A, Seegers H (2007) Performances of an ELISA applied to serum and milk for the detection of antibodies to *Coxiella burnetii* in dairy cattle. Rev d méd vét 158:250-252
23. Courcoul A, Vergu E, Denis JB, Beaudeau F (2010) Spread of Q fever within dairy cattle herds: key parameters inferred using a Bayesian approach. Proc Biol Sci 277:2857-2865
24. Jones RM, Nicas M, Hubbard AE, Reingold AL (2006) The infectious dose of *Coxiella burnetii* (Q fever). Appl Biosaf, 11:32-41
25. Greiner M, Pfeiffer D, Smith RD (2000) Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev Vet Med 45:23-41
26. Green DM, Kiss IZ, Kao RR (2006) Modelling the initial spread of foot-and-mouth disease through animal movements. Proc Biol Sci 273:2729-2735

27. Courcoul A, Ezanno P (2010) Modelling the spread of Bovine Viral Diarrhoea Virus (BVDV) in a managed metapopulation of cattle herds. Vet Microbiol 142:119-128
28. Green DM, Kiss IZ, Mitchell AP, Kao RR (2008) Estimates for local and movement-based transmission of bovine tuberculosis in British cattle. Proc Biol Sci 275:1001-1005
29. Gloster J, Champion HJ, Mansley LM, Romero P, Brough T, Ramirez A (2005) The 2001 epidemic of foot-and-mouth disease in the United Kingdom: epidemiological and meteorological case studies. Vet Rec 156:793-803
30. Ssematimba A, Hagenaars TJ, de Jong MC (2012) Modelling the wind-borne spread of highly pathogenic avian influenza virus between farms. PLoS One 7:e31114
31. Wallensten A, Moore P, Webster H, Johnson C, van der Burgt G, Pritchard G, Ellis-Iversen J, Oliver I (2010) Q fever outbreak in Cheltenham, United Kingdom, in 2007 and the use of dispersion modelling to investigate the possibility of airborne spread. Euro Surveill 15
32. Brooks-Pollock E, Roberts GO, Keeling MJ (2014) A dynamic model of bovine tuberculosis spread and control in Great Britain. Nature 511:228-231
33. Tildesley MJ, Deardon R, Savill NJ, Bessell PR, Brooks SP, Woolhouse ME, Grenfell BT, Keeling MJ (2008) Accuracy of models for the 2001 foot-and-mouth epidemic. Proc Biol Sci 275:1459-1468
34. Dutta BL, Ezanno P, Vergu E (2014) Characteristics of the spatio-temporal network of cattle movements in France over a 5-year period. Prev Vet Med 117:79-94
35. Taurel AF, Guatteo R, Joly A, Beaudeau F (2012) Effectiveness of vaccination and antibiotics to control Coxiella burnetii shedding around calving in dairy cows. Vet Microbiol 159:432-437
36. World Health Organization (2007) Infection prevention and control of epidemic-and pandemic-prone acute respiratory diseases in health care: WHO interim guidelines

37. Schimmer B, Ter Schegget R, Wegdam M, Züchner L, de Bruin A, Schneeberger PM, Veenstra T, Vellema P, van der Hoek W (2010) The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. BMC Infect Dis 10:69

38. Godin M, Bryan AK, Burg TP, Babcock K, Manalis SR (2007) Measuring the mass, density, and size of particles and cells using a suspended microchannel resonator. Appl Phys Lett 91:123121

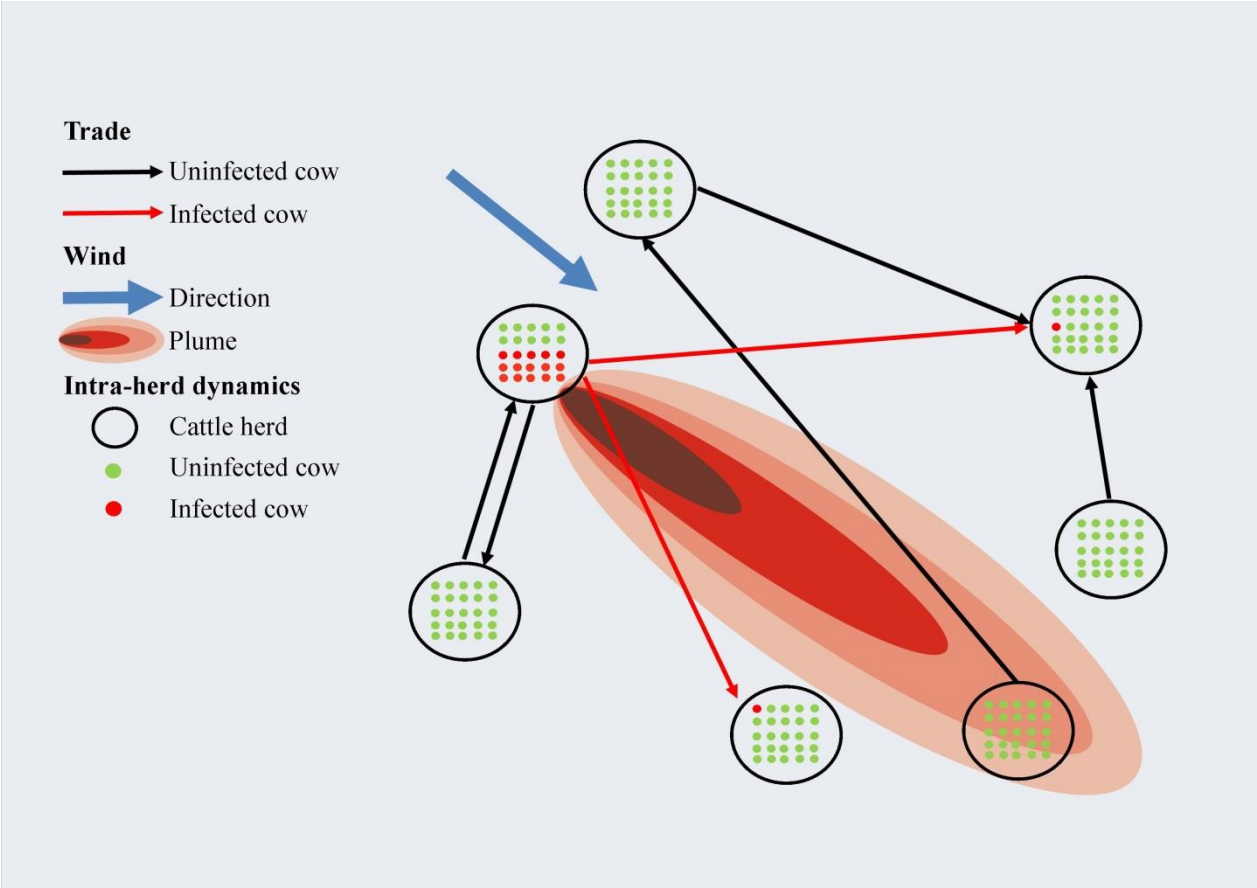

Trade
Uninfected cow
Infected cow
Wind
Direction
Plume
Intra-herd dynamics
Cattle herd
Uninfected cow
Infected cow

Figure 1

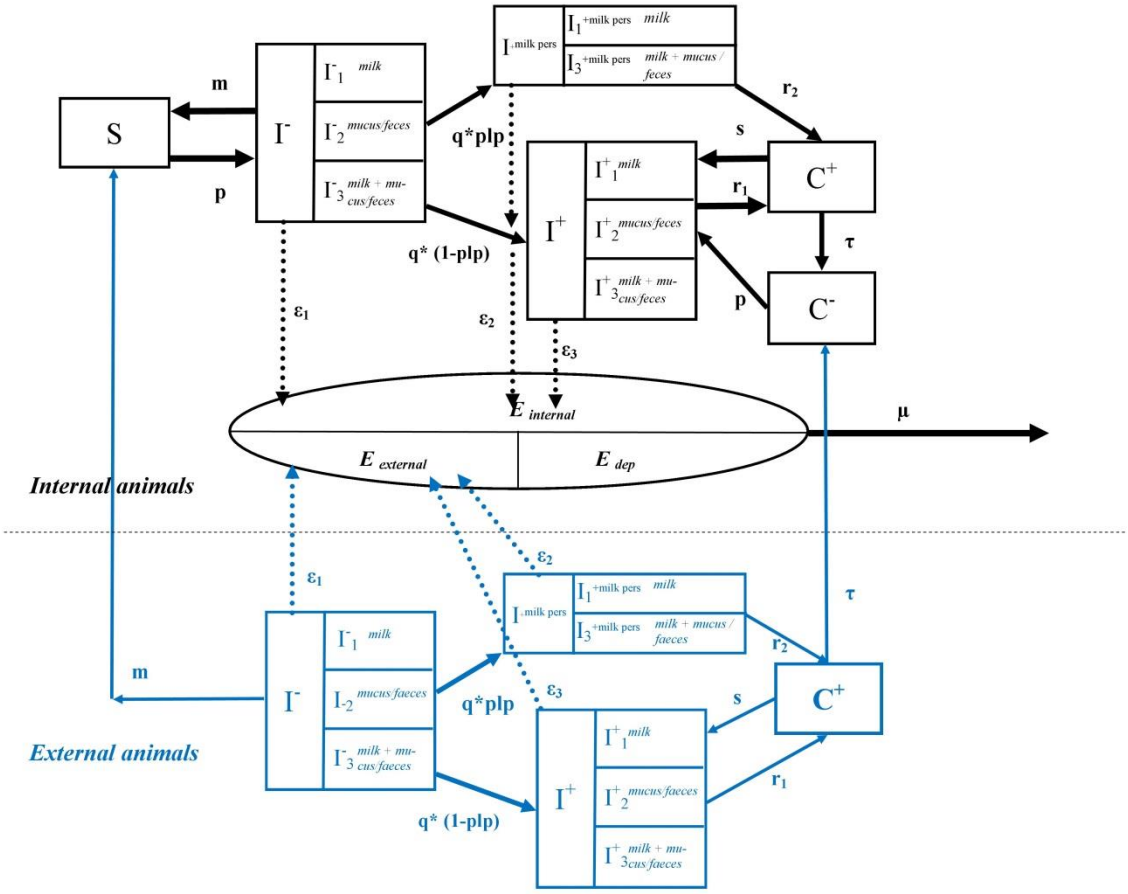

S
m
p
I-
I-1 milk
I-2 mucus/feces
I-3 milk + mucus/feces
q*plp
q* (1-plp)
I+milk pers milk
I+milk pers milk + mucus / feces
r2
s
C+
r1
τ
C-
I+
I+1 milk
I+2 mucus/feces
I+3 milk + mucus/feces
ε1
ε2
ε3
E internal
E external
E dep
μ
Internal animals
External animals

Figure 2

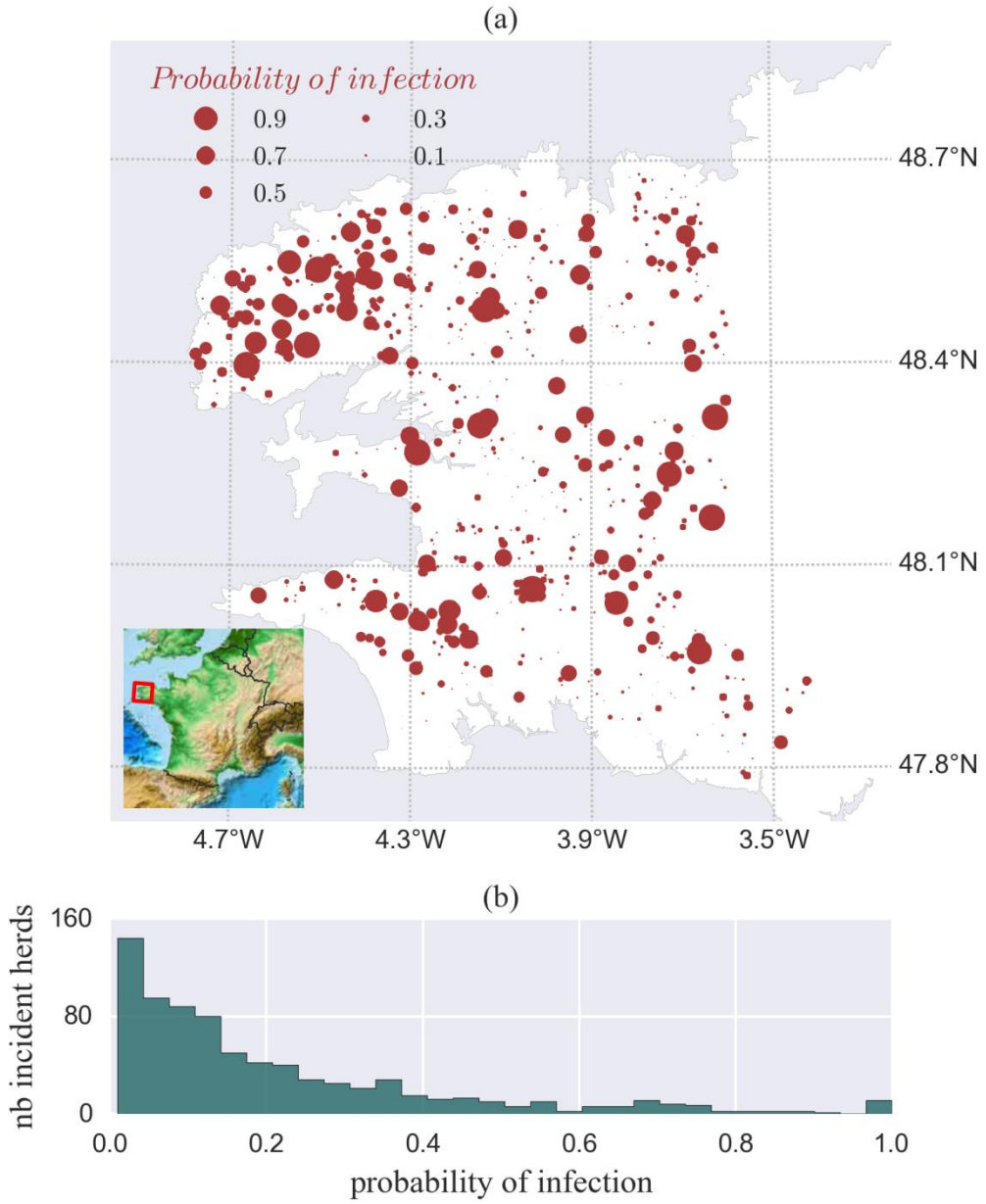

(a)
Probability of infection
0.9
0.7
0.5
0.3
0.1
48.7°N
48.4°N
48.1°N
47.8°N
4.7°W
4.3°W
3.9°W
3.5°W
(b)
nb incident herds
160
80
0
0.0
0.2
0.4
0.6
0.8
1.0
probability of infection

Figure 3

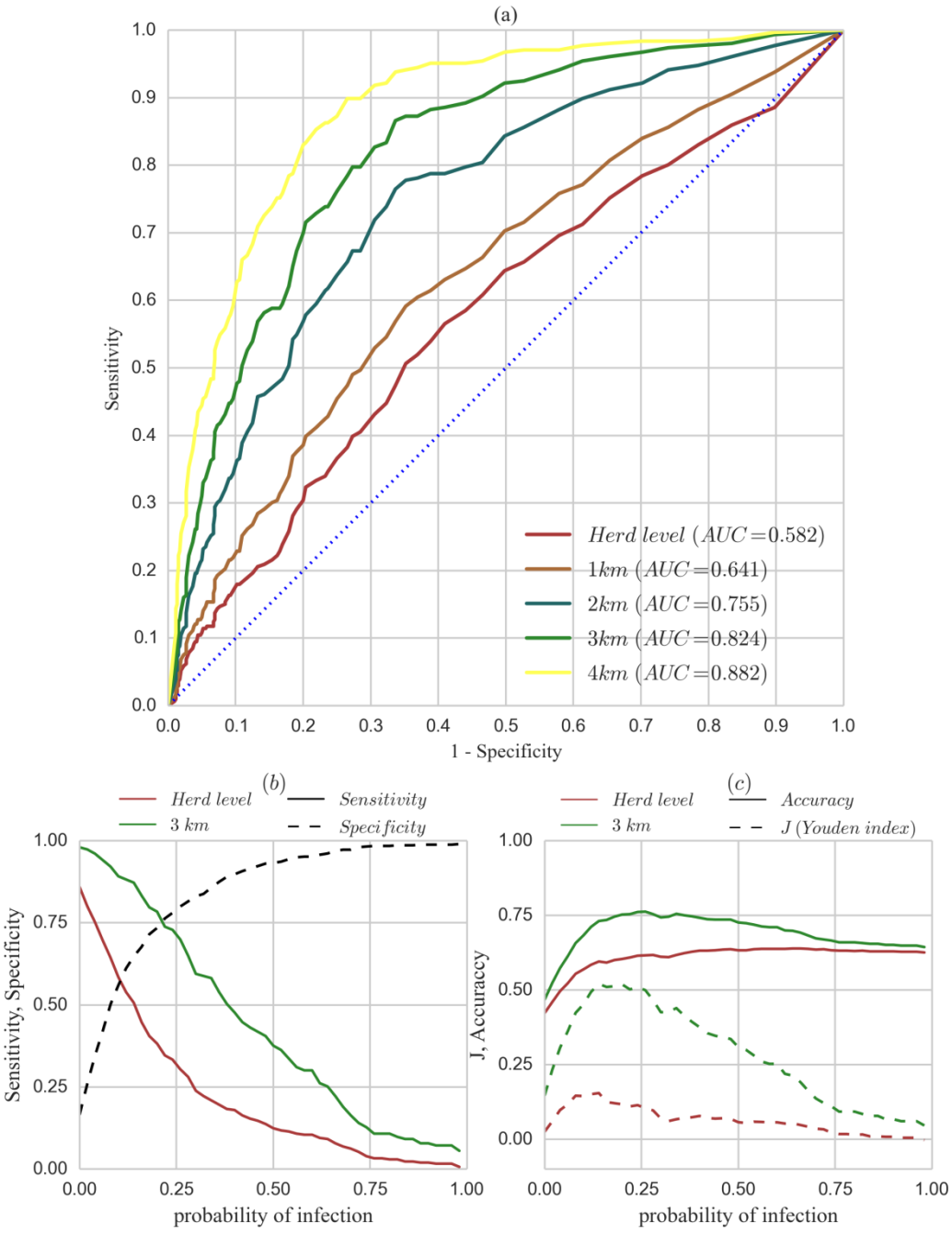

(a)
Sensitivity
1 - Specificity
Herd level (AUC=0.582)
1km (AUC=0.641)
2km (AUC=0.755)
3km (AUC=0.824)
4km (AUC=0.882)
(b)
Herd level
3 km
Sensitivity
Specificity
Sensitivity, Specificity
probability of infection
(c)
Herd level
3 km
Accuracy
J (Youden index)
J, Accuracy
probability of infection

Figure 4

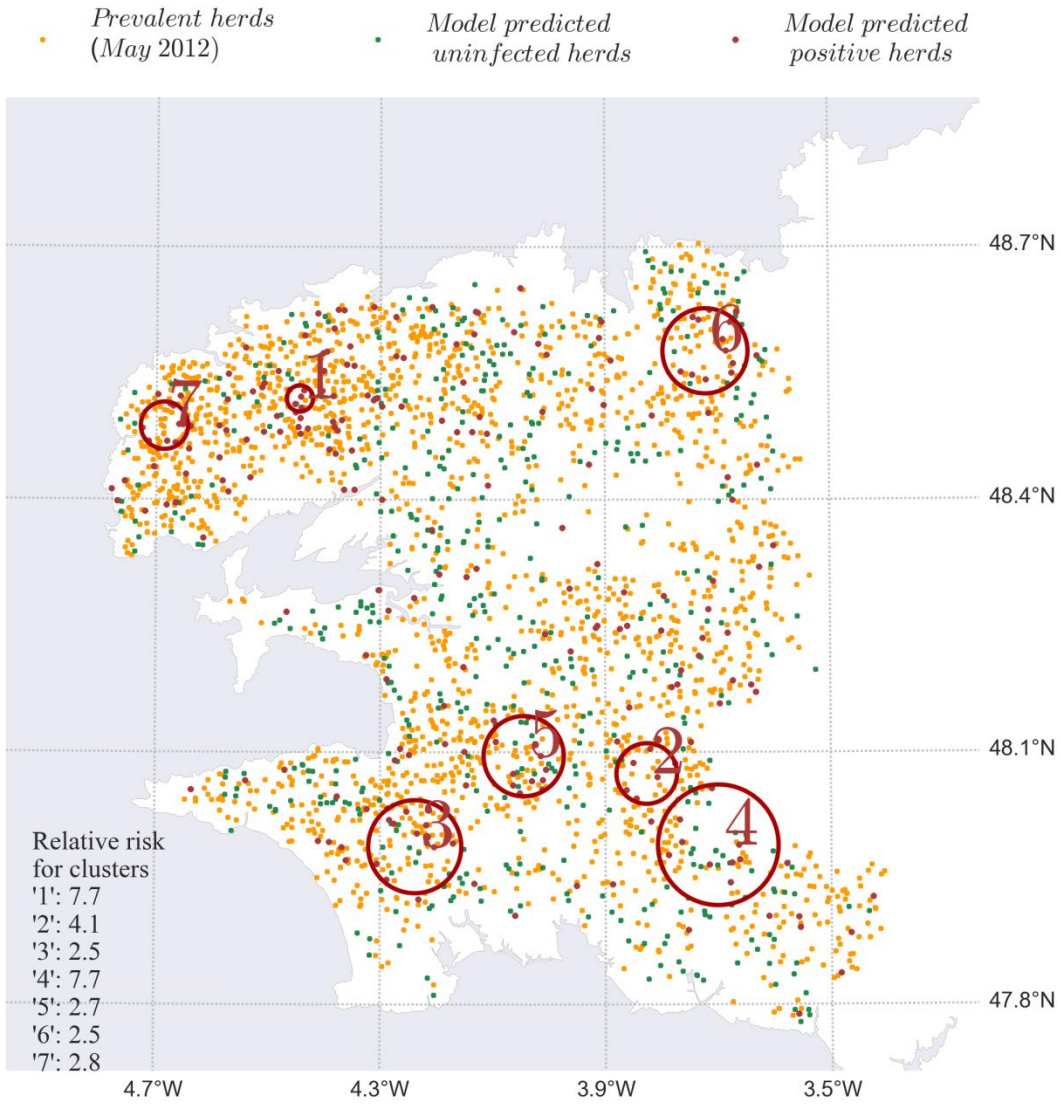

Prevalent herds
(May 2012)
Model predicted
uninfected herds
Model predicted
positive herds
48.7°N
48.4°N
48.1°N
47.8°N
4.7°W
4.3°W
3.9°W
3.5°W
Relative risk
for clusters
'1': 7.7
'2': 4.1
'3': 2.5
'4': 7.7
'5': 2.7
'6': 2.5
'7': 2.8

Figure 5

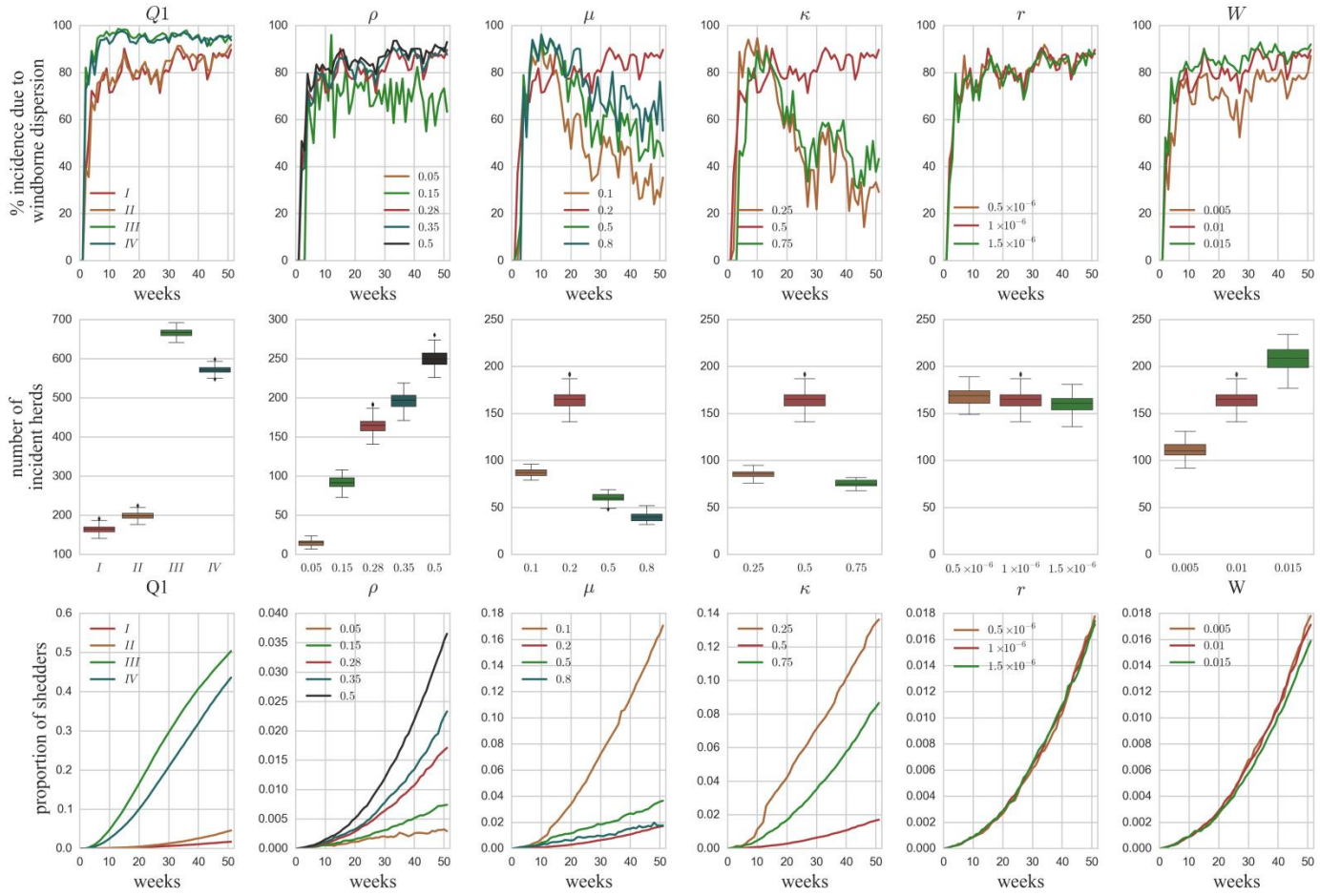

Q1
ρ
μ
κ
r
W
% incidence due to windborne dispersion
number of incident herds
proportion of shedders
weeks
I
II
III
IV
0.05
0.15
0.28
0.35
0.5
0.1
0.2
0.5
0.8
0.25
0.5
0.75
0.5 ×10⁻⁶
1 ×10⁻⁶
1.5 ×10⁻⁶
0.005
0.01
0.015

Figure 6

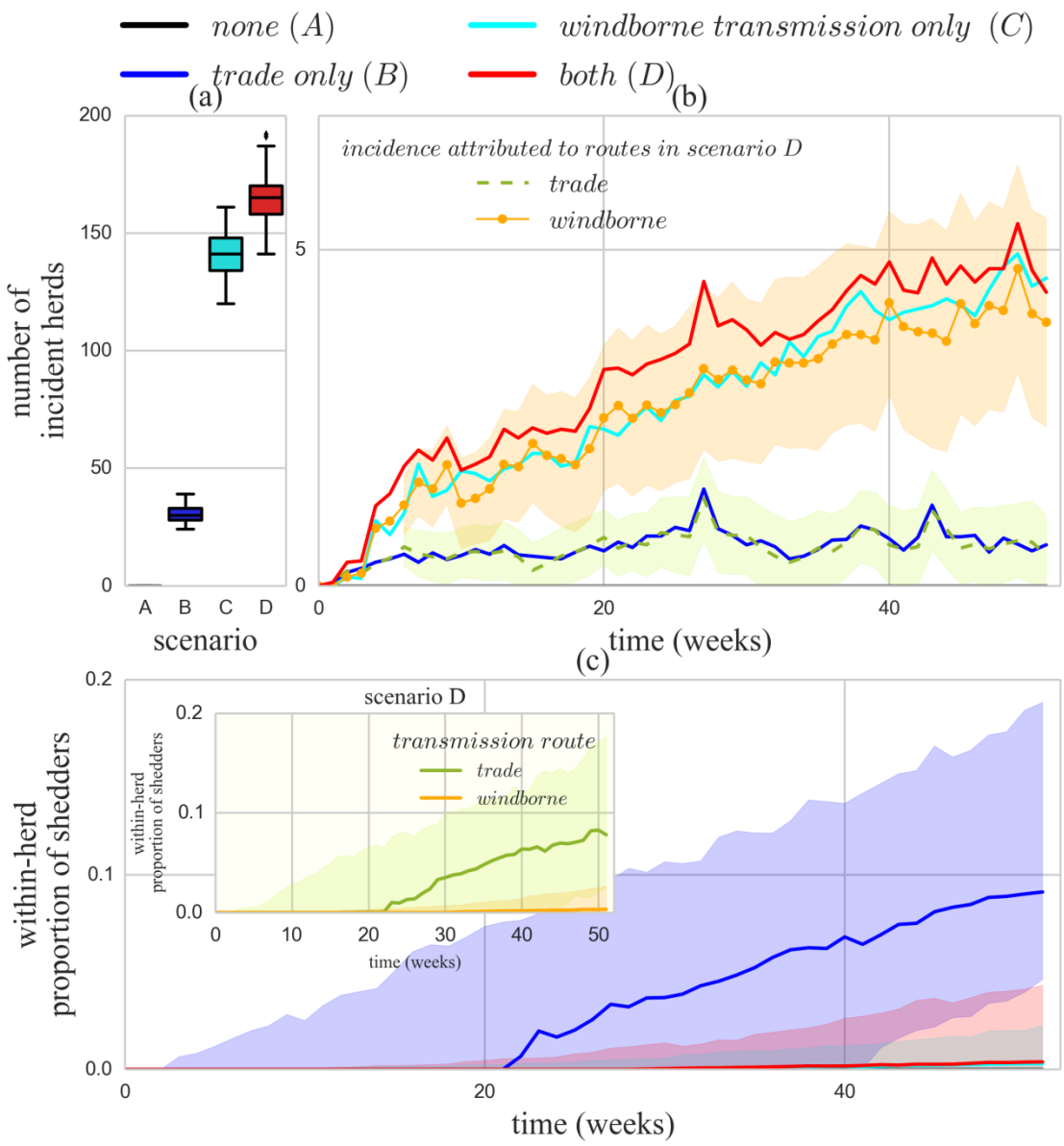

none (A)
windborne transmission only (C)
trade only (B)
both (D)
(a)
(b)
(c)
number of incident herds
scenario
A B C D
incidence attributed to routes in scenario D
trade
windborne
time (weeks)
within-herd proportion of shedders
scenario D
transmission route
trade
windborne
time (weeks)

Figure 7

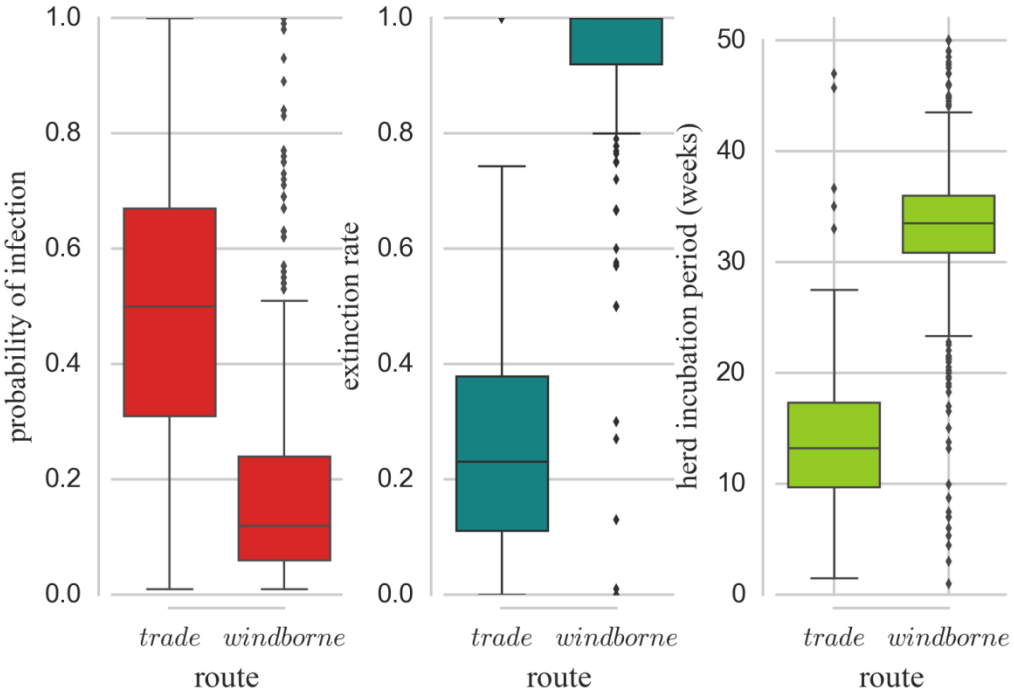

probability of infection
1.0
0.8
0.6
0.4
0.2
0.0
trade
windborne
route
extinction rate
1.0
0.8
0.6
0.4
0.2
0.0
trade
windborne
route
herd incubation period (weeks)
50
40
30
20
10
0
trade
windborne
route

Figure 8

Additional file

Additional file 1: Additional file 1 contains following sections

1. The intra-herd model: equations.

2. Windborne transmission of infectious particles in herd neighbourhood and incorporation of meteorological data

3. Estimation of the optimum cut-off values for probability of infection (*PI*) for incident herds.

Table S1: Performance of the model concerning the choice of *PI* cut-off optimal values at herd and neighbourhood levels.

Figure S2. Incidence predicted at cut-offs of 0.11 and 0.61 (optimum *PI* values for herd level analysis) and at 0.21, 0.22 and, 0.15 (optimum *PI* values for neighbourhood of 3 km).

Figure legends

Figure 1 Framework for the model of *C. burnettii* spread in a region. Hypothetical representation of a cattle herd metapopulation with infection-free herds (made of green points only – susceptible animals) and infected herds (containing some red points – infected animals). The inter-herd dynamics is governed by cattle trade (arrows) and windborne transmission of pathogen (red plume).

Figure 2 Flow diagram describing the intra-herd spread of *C. burnetii* in a dairy cattle herd. The diagram describes the health statuses of cows and transitions between these statuses, and environmental bacterial load of the herd (adapted from [10]). The blue section represents the infection dynamics of *external animals*, while the black section represents *internal animals.* $\boldsymbol{S}$: susceptible, non-shedder cows without antibodies, $\boldsymbol{I^-}$: shedder cows without antibodies, $\boldsymbol{I^+}$: shedder cows with antibodies, $\boldsymbol{I^{milk\ pers}}$: shedder cows with antibodies shedding in milk in a persistent way, $\boldsymbol{C^+}$: non-shedder cows with antibodies, and $\boldsymbol{C^-}$: non-shedder cows without antibodies which were infected and had antibodies in the past. $\boldsymbol{I^-}$ and $\boldsymbol{I^+}$ cows are in the shedding route category 1 if they shed in milk only, 2 if they shed in vaginal mucus/faeces only, and 3 if they shed in milk and vaginal mucus/faeces. $\boldsymbol{I^{milk\ pers}}$ cows are in the shedding category 1 if they shed in milk only, and 3 if they shed in milk and vaginal mucus/faeces. $\boldsymbol{E}$

represents the force of infection related to the bacterial contamination of the environment. $\boldsymbol{E_{local}}$ corresponds to the part of the force of infection due to *internal animals*, whereas $\boldsymbol{E_{external}}$ is due to *external animals* and $\boldsymbol{E_{dep}}$is due to bacteria deposited from other infectious herds by windborne transmission. ε1, ε2 and ε3 are the quantities of contributions to $\boldsymbol{E}$ during a time step by cows in statuses $\boldsymbol{I^{-}}$, $\boldsymbol{I^{+}}$, and $\boldsymbol{I^{milk\ pers}}$, respectively. These quantities are the sum of all quantities of bacterial load shed by all the shedders through all the shedding routes and reaching the environment of the herd. Details of the shedding levels and the proportions of cows shedding through different routes are given in Table 3. Description and values of the parameters used are given in Table 2.

Figure 3 Infection probabilities of initially susceptible herds. Simulated probability of infection (*PI*) by *C. burnetii* after one year of inter-herd spread, for herds observed to be infection-free in May 2012. (a) Map of Finistère department in North-Western France with the locations of incident herds (bubbles sizes are proportional to *PI*). The inset locates the Finistère department in France. (b) PI distribution amongst the 768 herds that get infected at least once in the simulations.

Figure 4 Receiver operating characteristics (ROC) analysis of model predictions. ROC analysis (data are the reference) for the simulated probability of infection (*PI*) by *C. burnetii* after one year of inter-herd spread, for herds initially susceptible. (a) ROC curves for herd level analysis and neighbourhoods of 1, 2, 3, 4 km. The AUC for each analysis is indicated in the legend. (b) and (c) Variation of the four indicators (Sensitivity (*Se*), Specificity (*Sp*), Accuracy (*Acc*), Youden Index (*J*)) used for building the three criteria (*Se*=*Sp*, max(*Acc*), max(*J*)) to optimise the cut-off of *PI* for the classification of herds as positive and negative. Calculations were performed at herd level and for a neighbourhood of 3 km. The *Sp* of the model is considered identical over the different neighbourhoods and hence is shown as a single line.

Figure 5 Spatial clustering of infection probability in Finistère department. Statistically significant spatial clusters (circled in red) with high relative risk (RR) of presence of predicted incident herds (red dots), initially susceptible and infected by *C. burnetii* after one year of inter-herd spread. The positivity of

a herd is defined based on a cut-off value of 0.25 for the probability of infection (*PI*). Herds initially seroprevalent according to the data (orange dots) and herds which remain uninfected (green dots) are also represented.

Figure 6 Univariate sensitivity analysis. Sensitivity analysis of three dynamical outputs of the model (mean proportion of herd incidence due to windborne transmission (top line), number of *incident herds* (middle line), and mean proportion of shedders in *incident herds* (bottom line) over 100 stochastic iterations of the model) with respect to the variation in six parameters (from the left to the right: $Q1$, ρ, μ, κ, r, and W) (details in Table 4).

Figure 7 Infection dynamics of *C. burnetii* spread over one year in four simulated scenarios: absence of inter-herd transmission (A, black), transmission by cattle trade only (B, blue), transmission by wind dispersion (C, cyan), and presence of both transmission routes (D, red). The subdivision of scenario D based on the identified cause of herd infection is also represented (due to animal trade – orange; by wind dispersion – green). (a) Distribution of the total number of predicted incident herds. (b) Dynamics of incidence (mean over 100 runs). Shaded regions for the subdivisions of scenario D represent 95% empirical confidence intervals. (c) Median proportion of intra-herd shedders and 80th percentile (represented by shaded area) for all the scenarios. Inset figure shows the proportion of shedders (median and 80th percentile) for subdivisions of scenario D. Median and percentiles are calculated for runs where herds experienced infection (sample sizes are 16,733 for D, 13,814 for C and 3,617 for B).

Figure 8 Distribution of *PI*, extinction rate, and herd incubation period. Distribution of simulated probability of infection (*PI*), extinction rate, and time before generation of the first case after exposure to the cause of infection in *C. burnetii* newly infected herds (one year of simulated infection dynamics) by cattle trade and windborne transmission.

Q fever spread between dairy cattle herds in an enzootic region: modelling contributions of windborne transmission and trade

Pranav Pandit[1], Thierry Hoch[1], Pauline Ezanno[1], François Beaudeau[1], Elisabeta Vergu[2]

[1]INRA, LUNAM Université, Oniris, UMR1300 BioEpAR, CS40706, F-44307 Nantes, France

[2]INRA, UR1404 Unité Mathématiques et Informatique Appliquées du Génome à l'Environnement (MaIAGE), F-78352 Jouy-en-Josas, France

Tables

Table 1 Parameters of the windborne transmission model.

Parameter	Definition	Estimation	Unit
g	Gravitational acceleration	9.8	m s^{-2}
z	Height of plume generation	4	m
h	Height of plume reception	4	m
η	Dynamic viscosity of air	$1.8x10^{-5}$	Kg $m^{-1}s^{-1}$
φ	Density of particles	1150 [37]	Kg m^{-3}
r	Radius of particle	10^{-6}*	m^{*}
W	Deposition velocity	0.01 [29]	m s^{-1}
a_y, a_z	Guifford-Pasquill stability	0.34, 0.27[21]	$m^{(1-b)}$
b_y, b_z	class 'C' stability parameters	0.82, 0.82 [21]	

* approximated from www.camfilfarr.com

Table 2 Parameters of the intra-herd infection dynamics for a dairy cattle herd (adapted from [23]).

Parameter	Definition	Value
m	Transition probability $I^{-} \Rightarrow S$	0.7
q	Transition probability $I^{-} \Rightarrow I^{+}$	0.02
pIp	Proportion of cows going from I^{-} to I^{+} and becoming $I^{milk\ pers}$	0.5
$r1$	Transition probability $I^{+} \Rightarrow C^{+}$	0.2
$r2$	Transition probability $I^{milk\ pers} \Rightarrow C^{+}$	0.02
s	Transition probability $C^{+} \Rightarrow I^{+}$	0.15
τ	Transition probability $C^{+} \Rightarrow C^{-}$	0.0096
μ	Proportion of bacteria eliminated due to death and to plume generation (can be written as $\mu_{death} + \mu_{plume\ source}$)	0.2
p	Infection probability	$1 - e^{-E}$
$\rho^{m/f}$	Proportion of bacteria shed through mucus/faeces filling the environment compartment	0.28
ρ^{m}	Proportion of bacteria shed through milk filling the environment	$0.125\rho^{m/f}$

compartment

Table 3 Description and probability distributions used for the different shedding routes and levels (from [10]).

Parameter		Definition	Value
α	α_1, milk	Probability distribution of the shedding routes for the I^- cows	0.31
	α_2, mucus/faeces		0.62
	α_3, milk+mucus/faeces		0.07
β	β_1, milk	Probability distribution of the shedding routes for the I^+ cows after 4 weeks post-calving	0.61
	β_2, mucus/faeces		0.33
	β_3, milk+mucus/faeces		0.06
β_{calv}	β_{calv1}, milk	Probability distribution of the shedding routes for the I^+ cows in the 4 first weeks post-calving	0.14
	β_{calv3}, mucus/faeces		0.5
	β_{calv3}, milk+mucus/faeces		0.36
γ	γ_1, milk	Probability distribution of the shedding routes for the $I^{milk\ pers}$ cows after 4 weeks post-calving	0.83
	γ_3, milk+mucus/faeces		0.17
γ_{calv}	γ_{calv1}, milk	Probability distribution of the shedding routes for the $I^{milk\ pers}$ cows in the 4 first weeks post-calving	0.25
	γ_{calv3}, milk+mucus/faeces		0.75
Q1	Low level	Probability distribution of the shedding levels for all the I^- and for the I^+ shedding in mucus/faeces after 4 weeks post-calving	0.85
	Mid-level		0.15
	High level		0
Q2	Low level	Probability distribution of the shedding levels for the I^+ shedding in milk after 4 weeks post-calving	0.4
	Mid-level		0.5
	High level		0.1
Q3	Low level	Probability distribution of the shedding levels for all the I^+ in the 4 first weeks post-calving	0.25
	Mid-level		0.25
	High level		0.5
Q4	Low level	Probability distribution of the shedding levels for the $I^{milk\ pers}$ shedding in mucus/faeces after 4 weeks post-calving	0.6
	Mid-level		0.4
	High level		0
Q5	Low level	Probability distribution of the shedding levels for all the $I^{milk\ pers}$ shedding in milk and for the $I^{milk\ pers}$ in the 4 first weeks post-calving	0.15
	Mid-level		0.6
	High level		0.25

Table 4: Parameters considered for the model sensitivity analysis.

Parameter	Definition	Standard value	Values tested in sensitivity analysis			
Q1	Probability distribution of the shedding	Distribution I	Distribution II	Distribution III	Distribution IV	
Low-level	levels of all the $I^-$and for the I^+ shedding	0.85	0.6	0.25	0.15	
Mid-level	in mucus/faeces after 4 weeks post calving	0.15	0.4	0.25	0.6	
High-level		0.0	0	0.5	0.25	
ρ	Proportion of bacteria shed through mucus and faeces filling the compartment	0.28	0.05	0.15	0.35	0.5
μ	Elimination rate of *C. burnetii* from the herd environment	0.2	0.1	0.5	0.8	
κ	Ratio between $\mu_{plume\ source.}$ and μ	0.5	0.25	0.75		
r	Radius of a fomite particle	1e-6	0.5e-6	1.5e-6		
W	Deposition velocity due to gravitation	0.01	0.005	0.015		

Q fever spread between dairy cattle herds in an enzootic region: modelling contributions of windborne transmission and trade

Pranav Pandit[1], Thierry Hoch[1], Pauline Ezanno[1], François Beaudeau[1], Elisabeta Vergu[2]

[1]INRA, LUNAM Université, Oniris, UMR1300 BioEpAR, CS40706, F-44307 Nantes, France

[2]INRA, UR1404 Unité Mathématiques et Informatique Appliquées du Génome à l'Environnement (MaIAGE), F-78352 Jouy-en-Josas, France

1. The intra-herd model: equations

The model for dynamics of *Coxiella burnetii* in a dairy cattle herd used in this study is a variant of the model introduced by Courcoul *el al* in 2011 [10]. Here we present the equations (1-6), which describe the updating (between time steps *(t-1)* and *t*) of variables corresponding to health states of cows in a herd *i*:

$$S_i(t) = S_i(t-1) - NI_i^-(t) + NS_i(t) + \Omega[S_i](t-1) \quad (1)$$

$$I_i^-(t) = I_i^-(t-1) + NI_i^-(t) - NS_i(t) - NI_i^+(t) - NI_i^{+milk\ pers}(t) + \Omega[I_i^-](t-1) \quad (2)$$

$$I_i^+(t) = I_i^+(t-1) + NI_i^+(t) - NI^+C_i^+(t) + NC^+I_i^+(t) + NC^-I_i^+(t) + \Omega[I_i^+](t-1) \quad (3)$$

$$I_i^{+\ milk\ pers}(t) = I_i^{+milk\ pers}(t-1) + NI_i^{+milk\ pers}(t) - NI^{+milk\ pers}C_i^+(t) + \Omega[I_i^{+milk\ pers}](t-1) \quad (4)$$

$$C_i^+(t) = C_i^+(t-1) + NI^+C_i^+(t) + NI^{+milk\ pers}C_i^+(t) - NC^+I_i^+(t) - NC^+C_i^-(t) + \Omega[C_i^+](t-1) \quad (5)$$

$$C_i^-(t) = C_i^-(t-1) + NC^+C_i^-(t) - NC^-I_i^+(t) + \Omega[C_i^-](t-1) \quad (6)$$

Based on equations (1-6), it is possible to define two main outputs of the model at the herd level as:

$$Seroprevalence_i(t) = I_i^+(t) + I_i^{+\ milk\ pers}(t) + C_i^+(t) \quad (7)$$

$$Shedders_i(t) = I_i^-(t) + I_i^+(t) + I_i^{+\ milk\ pers}(t) \quad (8)$$

Equations (9-16) here below define ingoing and outgoing flows in (1-6), with parameters defined in Table 2 (main text):

$$NI_i^-(t) \sim Bin\,(S_i(t-1), p_i(t)),\ where\ p_i(t) = 1 - e^{-(E_i(t-1))} \quad (9)$$

$$(NS_i(t), NI_i^+, NI_i^{+\,milk\ pers}) \sim Multinomial\,(I_i^-(t-1), (\frac{m}{m+q}, \frac{qpIp}{m+q}, \frac{q(1-pIP)}{m+q})) \quad (10)$$

$$NI^+C_i^+(t) \sim Bin\,(I_i^+(t-1), r_1) \quad (11)$$

$$NI^{+milk\ pers}C_i^+(t) \sim Bin\,(I_i^{+milk\ pers}(t-1), r_2) \quad (12)$$

$$(NC^+C_i^-(t), NC^+I_i^+(t)) \sim Multinomial\,(C_i^+(t-1), (\frac{\tau}{\tau+s}, \frac{s}{\tau+s})) \quad (13)$$

$$NI_i^-(t) \sim Bin\,(S_i(t-1), p_i(t)) \quad (14)$$

$$NC^-I_i^+(t) \sim Bin\,(C_i^-(t-1), p_i(t)) \quad (15)$$

$$\Omega[S,\ I^-, I^+, I^{milk\ pers}, C^+, C^-]_i(t) = \sum_{j=1}^{Nb} Multinomial\,(n_{ji}(t), P_{X,ji}(t)) - \sum_{j=1}^{Nb} Multinomial\,(n_{ij}(t), P_{X,ij}(t))$$

Where,

$P_{X,ji}(t) = \left[\frac{S_{j,l}(t)}{N_{j,l}(t)}, \frac{I_{j,l}^-(t)}{N_{j,l}(t)}, \frac{I_{j,l}^+(t)}{N_{j,l}(t)}, \frac{I_{j,l}^{milk\ pers}(t)}{N_{j,l}(t)}, \frac{C_{j,l}^+(t)}{N_{j,l}(t)}, \frac{C_{j,l}^-(t)}{N_{j,l}(t)}\right]$, was the probability of purchasing a cow with specific health state from herd *j* in the lactation year *l.*

$P_{X,ij}(t) = \left[\frac{S_{i,l}(t)}{N_{i,l}(t)}, \frac{I_{i,l}^-(t)}{N_{i,l}(t)}, \frac{I_{i,l}^+(t)}{N_{i,l}(t)}, \frac{I_{i,l}^{milk\ pers}(t)}{N_{i,l}(t)}, \frac{C_{i,l}^+(t)}{N_{i,l}(t)}, \frac{C_{i,l}^-(t)}{N_{i,l}(t)}\right]$, was the probability of selling a cow with specific health state to herd *j* in the lactation year *l.* The lactation year *l*, n_{ji}number of purchases made by herd *I* from *j*, and n_{ij} number of cows sold to herd *j* were based on to the data.

The dynamics of bacterial load in the environment is given by the following equation (17, identical to equation 2.3 in the main text):

$$E_i(t) = E_i(t-1)(1-\mu) + E_{i,internal}(t-1) + E_{i,external}(t-1) + \sum_j E_{i,j,dep}(t-1)\ \mathrm{E_i(t)} =$$

$$\mathrm{E_i(t\text{-}1)\big(1\text{-}\mu\big) + Bact_{i,Local}(t\text{-}1) + Bact_{i,Foreign}(t\text{-}1) + \sum_j Bact_{Dep,i,j}(t\text{-}1)} \quad (17)$$

The overall dynamics of the environmental bacterial load is governed by animals shedding through different routes at different stages of their reproductive cycle and at different levels of shedding. According to [10], for herd *i* this hence can be summarized in the following equation:

$$Bact_{i,x,origin}(t) = \sum_{k,l}\left(\rho^{k} Qty_{l} \sum_{i,j} n_{t,xwkl}\right)_{origin}, \quad (18)$$

where, $origin \in \{internal, external\}$ and $x \in \{I^{-}, I^{+}, I^{pm}\}$ are the different health states of cows which can shed the bacteria, $w \in \{\leq 4\ weeks\ post\ calving\ , > 4\ weeks\ post\ calving\}$ is the state of reproductive cycle of the cow, $k \in \{milk, mucus/feaces\}$, is the route by which bacteria are shed, $l \in \{low, medium, high\}$ is the level of bacterial shedding, $n_{t,xwkl} \sim Multinomial\left(N_{t,xwk}, Qc_{(x,w,k)}\right)$ with $N_{t,xwk}$ the number of animals in corresponding health state at time *t* and $Qc_{(x,w,k)}$ the probability distributions governing shedding levels. The remaining parameters are defined in Tables 2 and 3 of main text.

2. Windborne dispersion of infectious particles in herd neighbourhood and incorporation of meteorological data

The wind speed and direction data from the European Centre for Medium Range Weather Forecasts database were procured for the entire Finistère department (Western France). Data consisted of daily values of northward wind component and eastward wind component. Based on it, the wind speed was estimated as $windspeed = \sqrt{northward\ wind\ component^2 + eastward\ wind\ component^2}$, and direction of the wind flow was estimated through its angle ϕ with the original x-axis, where $\phi = \tan^{-1}\frac{northward\ wind\ component}{eastward\ wind\ component}$. Weekly averages of wind direction and speed (a unique value for the whole area considered, here the Finistère department) were used in the Gaussian dispersion model. Adjustment of the frame of the receiving *j* and source *i* herd coordinates according to the direction of wind flow was done based on the distance between the two herds ($Distance_{ij}$), direction of the wind angle between the line linking the two herds and the x-axis ($angle_{ij}$) as described in Stockie (2011) [21]: $x_{adjusted} = distance_{ij} * \cos(angle_{ij} - \phi)$ and $y_{adjusted} = distance_{ij} * \sin(angle_{ij} - \phi)$. The source for generation of the plume leaving the source herd *i* was the bacteria being lost from the environment compartment of this herd (rate of bacterial removal from the environment due to various mechanisms estimated for the intra-herd dynamics by Courcoul et al 2010 [23]). Here, we assume that a part of bacteria shed by cows through different routes form dust particles, which remain infectious and

become source for generation of plume. This plume then transported by the wind to another herd. Indeed, the smaller droplets generated by sneezing, coughing, splashing and other activities remain suspended in the air and dry fast enough to produce smaller particles called droplet nuclei, which can remain suspended in the air for long duration and can be transported along with the wind to distant places, unlike larger particles. Hence, the inherent capacity of windborne transmission of any infectious agent depends on production of appropriate range of droplet particle sizes with viable pathogens [35]. Multiple studies have suggested a higher risk of windborne transmission of Q fever within the radius of 5 km from the source in moderate environmental conditions [36], [30]. Hence, we restrict our dispersion model to a radius of 5 km from the source herd.

3. Estimation of the optimum cut-off value for probability of infection (PI) for incident herds

The cut-off PI was used to classify the herds into two categories: herds with a PI larger than the cut-off were considered positive (infected), the others were negative (uninfected). This categorisation concerns the simulated herd status at one year after the onset of pathogen spread into the metapopulation. The optimum cut-off is chosen based on comparison of simulation to data concerning herd status at time zero (of the follow-up) and at one year. The optimum cut-off value is chosen based on the epidemiological situation of the case concerned, such as prevalence in the population and consequences of false positive and false negative results. In the literature, prevalence dependent (Sensitivity (*Se*), Specificity (*Sp*), Youden index (*J*), odds ratio etc) and independent criteria (Efficiency, kappa) are both used to come up to a decision [24]. Here, three criteria were used to determine the optimal cut-off value: $Se = Sp$, maximum Youden index (J_{max}, where $J = Se + Sp - 1$) and maximum accuracy (Acc_{max}, where Acc is equal to the proportion of true negative and true positive into the population). Values of these criteria along with PI cut-off values are provided in Table S1 for both herd level and neighbourhood (3km) based on simulations (description of the simulation settings in the main text).

Predicted incident herds for different values of cut-off at one year after the onset of pathogen spread into the metapopulation, specifying the contamination route, are provided in Figure S1.

Table S1: Performance of the model concerning the choice of *PI* cut-off optimal values at herd and neighbourhood levels. (Values in bold are *PI* values at which criteria were fulfilled).

	Herd Level			**Neighbourhood (3km)**		
Criteria	Se≈Sp	Acc_{max}	J_{max}	Se≈Sp	Acc_{max}	J_{max}
PI cut-off	**0.11**	**0.61**	**0.11**	**0.21**	**0.25**	**0.15**
Sensitivity	**0.58**	0.10	0.58	**0.75**	0.71	0.86
Specificity	**0.58**	0.95	0.58	**0.75**	0.80	0.66
Accuracy	0.58	**0.64**	0.58	0.75	**0.76**	0.73
Youden index (J)	0.15	0.06	**0.15**	0.51	0.51	**0.53**
Incidence	419	58	419	259	219	346
% airborne transmission	86	57	86	78	75	83

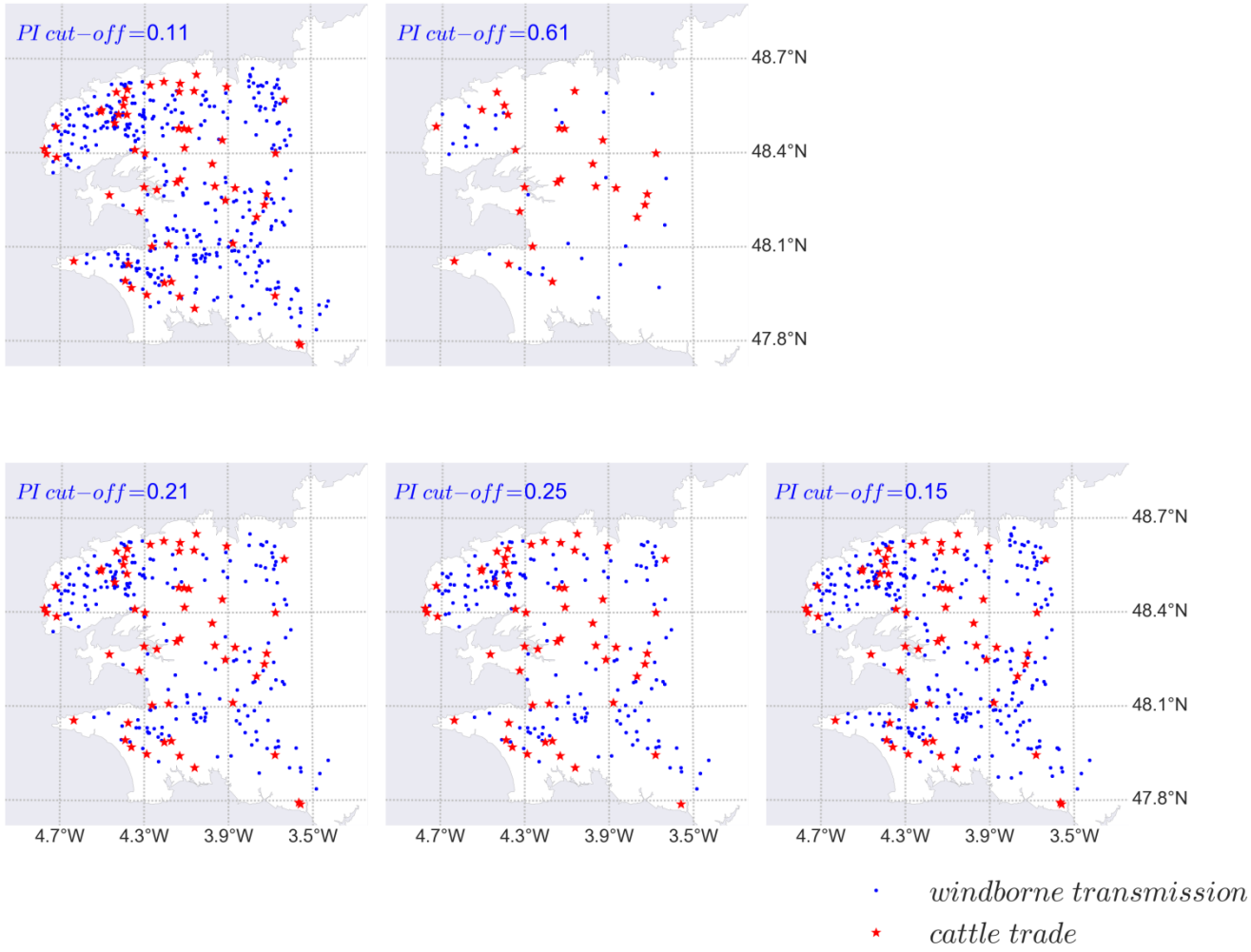


Figure S1: Incidence predicted at cut-offs of 0.11 and 0.61 (optimum PI values for herd level analysis) and at 0.21, 0.22 and, 0.15 (optimum PI values for neighbourhood of 3 km).

l'université nantes angers le mans
PÔLE DE RECHERCHE ET D'ENSEIGNEMENT SUPÉRIEUR

Oniris
École Nationale Vétérinaire, Agroalimentaire et de l'Alimentation — Nantes Atlantique
Santé et alimentation au cœur de la vie

Thèse de Doctorat

Pranav Pandit

Propagation et contrôle de la fièvre Q dans les troupeaux bovins laitiers à l'échelle régionale : une approche par modélisation multi-échelles

Regional spread and control of Q fever in dairy cattle herds: a multiscale modelling approach

Résumé

La fièvre Q, une zoonose causée par la bactérie *Coxiella burnetii*, constitue une menace croissante par la santé animale et humaine. Les caractéristiques épidémiologiques de la propagation entre troupeaux bovins de *C. burnetii* via le vent et le commerce des animaux sont peu connues. Nous avons développé un nouveau modèle dynamique spatialisé simulant la propagation entre troupeaux laitiers de *C. burnetii*, et quantifiant la contribution relative de la transmission par le vent et par le commerce des animaux dans un contexte enzootique. La propagation de *C. burnetii* entre troupeaux laitiers du Finistère (France) a été simulée et comparée avec des observations de la dynamique de l'infection. Nos prédictions indiquent que les troupeaux sensibles acquièrent majoritairement l'infection via une transmission par le vent. Cette voie de transmission engendre des infections de relativement faible ampleur et éphémères. En revanche, l'achat d'une vache infectieuse dans les troupeaux initialement sensibles pourrait résulter en une prévalence intra-troupeau plus forte. Les effets de l'implémentation d'une vaccination sur la propagation à l'échelle régionale ont été évalués en comparant différentes stratégies suivant les troupeaux soumis à vaccination. Vacciner 70% des troupeaux pendant 10 ans a résulté en une réduction importante de la prévalence des troupeaux infectés. La vaccination des troupeaux déjà infectés a été reconnue comme la stratégie la plus efficace. Ce travail apporte une meilleure compréhension de la dynamique de l'infection de *C. burnetii* à l'échelle régionale, et fournit des informations pertinentes pour maîtriser l'infection dans les populations animales.

Mots clés

***Coxiella burnetii*, modélisation multi-échelles, modèle de dispersion, mouvements d'animaux, maîtrise, vaccination**

Abstract

Q fever, a worldwide zoonotic disease caused by *Coxiella burnetii*, is a looming concern for livestock and public health. Epidemiological features of inter-herd transmission of *C. burnetii* in cattle herds by wind and trade of cows are poorly understood. We developed a novel dynamic spatial model describing the inter-herd regional spread of the *C. burnetii* in dairy herds, quantifying the ability of windborne transmission and animal trade in *C. burnetii* propagation in an enzootic region. Spread of *C. burnetii* between dairy herds of Finistère department, France was predicted and compared with observed spread of the infection. Our model predictions indicated that the majority of infections in disease-free herds occur due to windborne transmission. Infections acquired through this pathway are shown to cause relatively small and ephemeral intra-herd outbreaks. On the other hand, disease-free herd purchasing an infectious cow will experience significantly higher intra-herd prevalence. Results also indicated that, both transmission routes are independent from each other without any synergistic effect. Lastly, effects of implementation of vaccination on regional spread were assessed by comparing different strategies to select herds for vaccination. Vaccinating cows and heifers of 70% of herds using Phase I vaccine over 10 years resulted into significant reduction the prevalence of *C. burnetii* positive herds. Vaccinating already infected herds was found to be most effective strategy. Besides providing better understanding of *C. burnetii* infection dynamics at regional scale, this work also gives important insights to control the infection in animal populations.

Key Words

***Coxiella burnetii*, multiscale modelling, plume model, cattle movements, control, vaccination**

L'Université Nantes Angers Le Mans