



**UNIVERSITÀ DEGLI STUDI DI PARMA**

Dottorato di Ricerca in Ecologia - XXVII° Ciclo

**Infectious diseases in Emilia-Romagna (Italy) dairy farms: ecological modelling and network analysis**

**Coordinatore:**

*Chiar.mo Prof. Paolo Menozzi*

**Tutor:**

*Chiar.mo Prof. Giulio A. De Leo*

**Co-Tutors:**

*Dott. Luca Bolzoni, Dott. Stefano Pongolini, Dott. Simone Vincenzi*

**Dottorando**

*Dott. Gianluigi Rossi*

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## **Abstract**

Studying the spread of infectious diseases at the humans-domestic animals-wild animals interface is fundamental in order to preserve all three named compartments health. In particular, infection in livestock produced several impacts during the last two decades, threatening the sustainability of farming in different areas all over the world. The focus of this thesis is to disentangle the potential pathogens spread through farming systems, using ecological modelling methods. I worked on the dairy cattle system in the Emilia-Romagna (ER) region and in the Province of Parma (Italy). In the first chapter I reviewed the published models for bovine tuberculosis (bTB), a very dangerous disease for both cattle and humans health worldwide. In particular, I focused on the understanding of models structure and assumptions. In the second chapter, I evaluated the performance of bTB surveillance system in the ER system, a bTB-free region since 2007. The surveillance system is composed by three detection methods: on-farm routine skin-testing, exchanged cattle skin-testing, and slaughterhouse carcass inspection. In order to evaluate their performances, I built a stochastic network model, able to represent both within-farm and between-farm bTB dynamics, and calibrated on available data on ER dairy farms. The surveillance system was assessed through the comparative analysis of a number of statistics, including the time required for epidemic detection and the total number of infected farms during the epidemic. Results showed that slaughterhouse inspection and on-farm routine surveillance mainly contributed both to the surveillance system effectiveness, while exchanged cattle testing was relatively marginal. In the third chapter of my thesis, I analysed the indirect contacts existing between farms. These contacts are due to fomites mediated infections between farms, caused by farm-visitors moving between farms. In my case of study I analysed: private and government veterinarians, and milk trucks. My results showed that all three indirect contacts networks considered was very different in respect to direct one, and that they created an higher number of between-farm connections in respect to cattle movements. This suggested that the indirect contact route of transmission should not be neglected in control and surveillance effective strategies for highly contagious diseases.

## **General introduction**

Since the very beginning of humans civilization, satisfying alimentary needs has been one the most important challenges that mankind has been facing so far. One of the biggest turning point in human history, in fact, was the beginning of the agricultural era, during the Neolithic Demographic Transition. From that moment on, farming and breeding became the primary source of food for humans, replacing hunting and gathering. This phenomenon was possible because of geographical and climatic factors that permitted the domestication of various plant and animal species (Diamond, 2007). The production of high calorie foods, such as wheat, lentils, peas, maize, rice, and millet, indeed permitted an increase of the human population (Bocquet-Appel, 2011). Thanks to food and manpower surplus, some individuals could specialize in tasks other than food seeking (such as soldiers, teachers, and priests) starting the process that resulted in the current civilization (Diamond, 1997).

On the other hand, the domestication of various mammal species, and the close contacts with them, exposed humans to a great variety of pathogens typical of these species, and vice-versa. In fact, it has been shown that notable epidemics in human population history, such as the Black Death, Spanish Flu and HIV/AIDS, were caused by zoonotic diseases, defined as pathogens typical of animal species that, in particular conditions, “jumped” to humans (Jones et al., 2008; Lloyd-Smith et al., 2009; Taylor et al., 2001). Many of these diseases originated in the “Old World” (Africa, Asia and Europe), where an higher number of animal species have been domesticated (Diamond, 1997). The factor that helped many disease to definitely establish into human population was the higher density of human individuals, permitted by the switch to farming systems described above (Wolfe et al., 2007).

Until the XIX century the approaches used by human communities to face epidemic crises relied mostly on the isolation of infected individuals, such as hospitalization and the creation of lazarettos (towns' areas in which infected individuals were confined). The birth of the modern microbiology and

the germs theory, pursued by medical scientists such as Pasteur and Koch, radically changed the individual-level approach to infectious diseases. The discovery of micro-organisms responsible for diseases led to the development of drugs able to control them. In many cases, these drugs gave to infected people the opportunity to recover from diseases that just few decades earlier were considered lethal (Smith et al., 2005).

Another fortunate approach to infectious diseases at was the so-called classic epidemiology framework. In this case, scientists efforts were focused on the detection of risk factors, such as environmental, behavioural and genetic ones, acting at population-level and related to disease occurrence. Risk factor analyses are mainly based on statistical tools, and they are fundamental for sanitary management and decision making (Smith et al., 2005).

At the beginning of the 20th century by Ross (1911) and Kermack and McKendrick (1927) introduced a new approach for the study of infectious diseases. This approach is focused on the ecological and evolutionary relationships existing among parasites and host species. On the basis of this approach, the pattern of infection cases occurrence is the result of many biological processes, such as contacts among individuals, hosts immunity responses, parasites replication inside the host, pathogen transmission, etc. (Smith et al., 2005 ). The key concept of this approach is the description of pathogens spread among hosts through opportune mathematical models, in order to obtain predictions on the spatial and temporal course of an infection within host populations and communities (Keeling and Rohani, 2008). This approach was popularized by ecologists Anderson and May (1978), and from that moment on it has proved to be successful in several cases (Smith et al., 2005). The classic framework of epidemiological models subdivided the host population into different classes, or compartments, on the basis of their status in respect to the infection, namely: *susceptibles* (i.e. individuals previously unexposed to the pathogen); *infectious* (i.e. individuals that have been infected and can transmit the

disease); and *recovered* (or *removed*, i.e. individuals in which the disease is cleared or removed from the population, in both cases they can not infect nor been infected any more) (Kermak & McKendrick, 1927). Despite their simplicity and the rigidity of some of the considered assumptions, these models provided many insights in the eco-epidemiology theory (Keeling & Rohani, 2008). Nowadays the topic of infectious diseases is one of the most popular and challenging in modern ecology. In several occasions the ecological approach helped to address control strategies through the understanding of transmission dynamics and mechanisms. That was the case, among many others, of measles in human in England and Wales, approached by Bartlett (1957, 1960) using the classic Kermak and McKendrick framework (1927).

Despite the success of the earlier ecological models of infectious diseases, their assumptions represented a limitation for a possible application to more complex ecological and epidemiological contexts. In particular, the mean-field (or homogeneous mixing) assumption, postulating that all individuals of a host population have the same probability to interact among each other, is often violated in many real-life situations. The case of HIV/AIDS, disease responsible for a global healthcare crisis worldwide starting from the '80s and '90s of the XX century, is a well-known example of an infection with strongly non-homogeneous individual contacts. Seminal studies on the sexual behaviours in the homosexual community in San Francisco showed that the homogeneous mixing hypothesis was unsuitable in describing contacts between hosts of sexually transmitted diseases (Jay and Young, 1979). In the case of HIV/AIDS, developments in theoretical ecology helped to understand the transmission dynamics, in particular by recognising the variance in the number of per-capita sexual partners as a major determinant for the disease rate of spread (Anderson, 1988; Garnett & Anderson, 1993; May, 2004; Medley et al., 1987).

In other cases, the assumption of homogeneous mixing was violated because of the heterogeneous

spatial distribution of the host population. The UK 2001 Foot-and-Mouth disease (FMD) epidemic in cattle farms produced major impacts on the agricultural industry and even greater impacts on the rural tourism (Ferguson et al., 2001; Keeling et al., 2003). Despite the FMD within-farm transmission could be suitably described by a simple Susceptible-Infected-Recovered (SIR) model, the farm-to-farm infection dynamics proved to be much more tricky and challenging for theoretical ecologists. Indeed, to effectively represent the FMD spread, mathematical models need to incorporate different spatial scales of transmission (Boender et al., 2010; Keeling et al., 2005 and references there in); specifically: local transmission (by aerial plumes between neighbouring farms) and long distance transmission (resulting from the movement of live animals and farms personnel with their contaminated equipment or vehicles). The outcomes provided by these models led to the introduction of combined stringent movement restrictions and to ring culling around infected farms, that were able to eradicate the infection (Ferguson et al., 2001a,b; Keeling et al., 2001, 2003).

Recently, network theory have been extensively used to represent the potential spread of diseases through communities of hosts with a limited set of potential infectious contacts (Keeling et al., 2010). Network analysis was firstly implemented in social science, and has been used as an explanatory tool to describe the evolution of social dynamics (Leinhardt, 1977). In the epidemiology field, network analysis can provide a powerful tool for predictions of population-level dynamics based on individual-level observations. Moreover, control methods, such as contact tracing or ring vaccination, can only be accurately captured and modelled using network-based approaches (Keeling and Eames, 2005). In a network framework, each node of the network can represent host individuals, as in case of sexually transmitted disease frameworks, or an entire host sub-population, as in case of farmed animals. The edges between nodes represent the potential infectious contacts (Potterat et al., 2002; Keeling and Eames, 2005).



Diseases spread in livestock represents an ideal field for applying a network analysis approach to epidemiology (Danon et al., 2011). Livestock infections are serious threats for both farmed animal health and for the economic sustainability of farming systems. An emblematic case is represented by bovine tuberculosis (bTB) in UK (Johnston et al., 2011). The live animal exchange is considered the most effective route of bTB transmission between farms (Gilbert et al., 2005). However, indirect contacts due to contaminated fomites, transported by farms personnel or on shared equipment or trucks could represent another important route of transmission, for virulent disease such as FMD (Nielen et al., 1996).

The main goal of my PhD research is to provide new understanding to livestock diseases dynamics in farm networks and, consequently, to use these findings to inform disease surveillance and control strategies. In particular, the case of study presented here involves the analysis of the dairy farm network in the Emilia-Romagna region (Italy). I used an ecological modelling approach based on mathematical network models to answer different ecological and epidemiological questions regarding the spread of diseases in the farm network.

In the first chapter of my thesis, I reviewed the literature on mathematical models applied to bovine tuberculosis (bTB). This is among the major disease threats to farm animals worldwide (Skuce et al., 2012). In fact, bTB is a chronic disease fully adapted to a wide number mammalian of hosts and it is characterized by a long and highly variable incubation period (Morris et al., 1994; Phillips et al. 2003). Emilia-Romagna is a bTB-free region since 2007, however the risk of re-introduction still persists because of the presence of bTB in other Italian regions or European countries. In this review I focused my attention on the existing mathematical models developed for bTB in different ecological and epidemiological contexts. The final scope of the first chapter was to understand which was the best approach, and the necessary assumptions, in order to formulate a model able to represent a possible

bTB spread in Emilia-Romagna region, that is developed in the second chapter.

The second chapter concerns the study of the possible introduction of bovine tuberculosis (bTB) in the Emilia-Romagna dairy farm system, with a specific focus on the assessment of the disease surveillance system effectiveness. Firstly, I tried to understand the effect of farming practices in act in Emilia-Romagna on bTB within-farm transmission. As I highlighted in the first chapter, the vast majority of bTB models are suited to extensive farms, where cattle are bred in pasture lands. However, in areas as the Po Valley in Northern Italy (comprehending Emilia-Romagna region), the most widespread farming practice is represented by factory farms, where animals are kept in close contact to each other inside farm buildings. In fact, keeping animals inside buildings may reduce the possible bTB transmission between-farm, inhibiting air-borne transmission, and contact with wild species. As a consequence, the live cattle movement represents the only effective bTB transmission route in such areas (Marangon et al., 1998).

In the third and last chapter of my thesis I analysed data on potential indirect contacts (fomites) between farms. In particular, I focused on veterinarian visits and milk trucks movement among farms. Indeed, the disease transmission among cattle herds through fomites, negligible for diseases such as bTB or Jhone's disease, can be key routes of transmission for several highly pathogenic livestock pathogens, such as brucellosis and FMD (Skuce et al., 2012). In order to understand the possible spread of acute diseases in farm systems, I analysed the characteristics of indirect between-farm contact networks resulting from on-farm visits of veterinarians and milk trucks. Then, I compared the direct (i.e. cattle movements) and indirect (i.e. farm visitors) contact networks features to understand the relative importance of the two route of transmission in effectively spread diseases in the Province of Parma (an highly farm-populated province of Emilia Romagna region) dairy farm system. We used network analysis and metrics to characterize the different contact networks, and to evaluate the role of single farms in a potential epidemic spread.

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# Chapter I: Review of dynamics ecological models for the spread of bovine tuberculosis in cattle

## 1. Introduction

### 1.1. Overview

One of the most dangerous diseases at the human-livestock-wildlife interface is bovine tuberculosis (bTB; De Garine-Wichatitsky et al., 2013; Maas et al. 2013; Palmer et al., 2012). bTB aetiological agent is *Mycobacterium bovis*, member of the *Mycobacterium tuberculosis* complex. This pathogen successfully adapted to an extremely wide number of hosts, including humans, domestic species as cattle, and wild species as buffalo, badgers, wild boars and possums, among others (Fitzgerald and Kaneene, 2012; Phillips et al. 2003). bTB represents a threat for human health, the economic sustainability of agriculture and the conservation of wildlife (Donnelly et al. 2003).

bTB main host, among domesticated animal, is represented by cattle. However, growing evidence is emerging of cases in which other domesticated species could be infected, such as swine, goats, sheep, or pets (Cassidy, 2013).

In humans, tuberculosis is more frequently caused by *Mycobacterium tuberculosis* but infection with bTB is also possible: *M. bovis* and *M. tuberculosis* have a similar clinical progress in infected individuals and the specific aetiological agent can be identified only through DNA analysis (Roberts and Buikstra, 2003; Ortner, 1999). In fact, evidence of bTB infection in humans exists since the iron-age (Murphy et al., 2009, Taylor et al., 2007), and bTB infections in humans are still common in the developing countries as a consequence of un-pasteurize milk consumption. Husbandry practices and poor hygienic conditions are also risk factors for bTB infections in humans, along with compromised immunological status following HIV infection, malnutrition, and other immune-suppressing diseases (De Garine-Wichatitsky et al., 2013).

Indeed, many developed countries reduced the human cases to less than 1% of the total tuberculosis cases by implementing surveillance systems in livestock. Usually, these cases are still associated with people in close proximity to herds, such as farmers, veterinary surgeons, and abattoir workers (Gutierrez et al., 1997; Robinson et al., 1988).

bTB is endemic in wild reservoir hosts in many areas of the world. It has been hypothesized that domestic species were the initial source of infection, but then wild hosts were able to maintain the infection, allowing the disease to spill-back to domestic species (Fitzgerald and Kaneene, 2012; Renwick et al., 2007). The two most important wildlife species recognized as bTB reservoir hosts are badgers (*Meles meles*) in UK and Ireland, and possums (*Trichosura vulpecula*) in New Zealand (Smith et al., 2001). Anyhow, other significant hosts for bTB are represented by white tailed deer (*Odocoileus virginianus*) in Michigan and Minnesota states (USA) and European wild boar (*Sus scrofa*) in Iberian peninsula (Palmer et al., 2012). The awareness on bTB is also increasing in developing countries, particularly in Africa, where the main wildlife hosts are the African buffalo (*Syncerus caffer*) and the lechwe antelope (*Kobus leche*) (Fitzgerald and Kaneene, 2012). In many regions, bTB is still a problem for humans as well as for conservation and tourism economy (De Garine-Wichatitsky et al., 2013; Renwick et al., 2007).

### ***1.2. Transmission of bTB***

Between hosts bTb transmission typically occurs directly through nose-to-nose contacts and infectious aerosolized respiratory droplets or, indirectly, through infectious nasal excreta, saliva or urine when animals share the same feed or feeding site (Palmer et al., 2012). In livestock, bTB transmission is mostly due to either within-farm contacts with other infected cattle, or through contacts with infected wildlife. Cattle-wildlife contacts occur in pasture lands (both for direct contacts and through infected excreta of wild animals), while indoor contacts with wildlife is less common. Another bTB source of



infection for livestock is represented by soil previously infected by wildlife faeces, urine, saliva or nasal excretions (Phillips et al. 2003). In UK an increase in the incidence (i.e. the number of new infected individuals in a given time span) in livestock was observed in the last 30 years, probably due to spill-over and spill-back processes between domestic cattle and badgers (Karolemeas et al. 2012). In fact, disease transmission processes from cattle contaminated excreta to badgers, or other wild animals have also been observed (Morris et al., 1994). These observations contributed to great economic effort for disease eradication in wildlife: in 2009 only, the British government invested £63 million for bTB surveillance and control (Johnston et al. 2011).

In the case of humans, the most common bTB infection source is through the ingestion of unpasteurized milk or due to occupational cattle contacts (Cotter et al., 1996; Evans et al. 2007). However, environmental infection and person-to-person transmission are still possible, but the latter is rare and usually occurs only with immuno-compromised subjects. Person-to-person transmission of *M. bovis* has been recently documented also in a modern urban setting in West Midlands, UK, in 2005. All six bTB infected individuals had common social links through visits to bars in two different areas, while only one of these, probably the index case, had link with environmental sources of infection (Evans et al. 2007).

### ***1.3. Mathematical models***

Host–pathogen models are essential to explore the dynamics of infectious diseases and to design strategies for disease control and possible eradication (McCallum et al., 2001). Temporal trends and spatial patterns of disease incidence and prevalence emerge as result of a combination of complex biological, physiological, immunological, behavioural and ecological processes, that can be conveniently described and investigated through mathematical and statistical models. They can provide an essential tool to explore surveillance and control strategies and project or predict the temporal and

spatial dynamics of disease infection (Smith et al., 2005). The theory of disease dynamics began its development in the first half of the 20<sup>th</sup> century, with attempts by Ross (1911) and MacDonald (1957) to model the dynamics of malaria in humans and mosquitoes, and with work by Kermack and McKendrick (1927) to explain the dynamics of the number of cases among a host population. This early work has been later developed, expanded and popularized by the seminal work of Anderson and May (1978). A typical framework (known as SIR model) categorizes the host population into different classes, or compartments, on the basis of their status in respect to the infection, namely: *susceptibles* (S; i.e. individuals previously unexposed to the pathogen); *infectious* (I; i.e. individuals that have been infected and can transmit the disease); and *recovered* (or *removed*, R; i.e. individuals in which the disease is cleared and present temporary or permanent immunity). The earliest SIR models typically assumed that hosts are homogeneous distributed within communities, and well mixed, so that the contact risk is the same among all host individuals. This simplifying assumption allows the analytical tractability of the model, but may not adequately reflect the real contacts structure existing among hosts. Despite several limitations, homogeneous-mixing compartmental models have proved to be a robust and powerful predictive tool to analyse the dynamics of infectious diseases (Anderson and May, 1992). A large number of models has been developed in the last few decades to explicitly account for important sources of heterogeneity, such as the host population structure (as for age, sex, population structure and contact rate, etc.), the genetic structure, and the transmission strategies of the pathogen and within host dynamics (susceptibility to disease, transmission rates, route of transmission and recovery rate, etc., Bansal et al., 2007). Multi-host dynamics needs also to be accounted for those infective agents, as in bTB, with a wide spectrum of potential hosts, or when transmission occur through vector (malaria, dengue, Lyme disease, etc.), through an intermediate hosts (in the case of lots of trematode parasites), or from a wildlife or domesticated reservoir, as typically occur for zoonotic diseases (Daszak, 2000).

#### ***1.4. Aim of the study***

Several mathematical models have been developed and used in the last 20 years to investigate bTB transmission in cattle and in other host species (Smith et al., 2001). The aim of this review is to analyse the published models for bTB with a special focus, in particular, on dynamical models to investigate bTB transmission in cattle, and to assess the effectiveness and efficacy of alternative strategy for surveillance and control. Most of the analysed models have been developed with reference to a single host, typically free ranging or farmed cattle, others account also for the dynamics of the disease in the wildlife compartment and its interaction with the domesticated one (Kao et al., 1997; Brooks-Pollock et al., 2014). Whereas focus has been typically on European badgers in UK and Ireland and possums in New Zealand (Smith et al., 2001), modelling papers have been recently published to described bTB dynamics between cattle and other wildlife hosts, such as between white tailed deer in US states Michigan and Minnesota (Palmer et al., 2012).

#### **2. Bovine Tuberculosis models**

As bTB is characterized by slow and typically chronic infections with a long asymptomatic latent period, a common feature of the vast majority of bTB models has been structured in three compartments representing respectively susceptibles (S) individuals (as in classic Kormak-McKendrick framework), exposed individuals (E), those individuals that have been infected but are not yet infectious, and infectious individuals (I), i.e.. individuals that infect other susceptible hosts. The exposed class E is crucial component of bTB models because of the long, and highly variable, latent period after the initial infection, before the diseases host start to shed infective physiological liquids and contaminate the environment or, directly, other susceptible hosts.

Most models uses two exposed classes: to track asymptomatic unresponsive individuals (those exposed but unresponsive to the intradermal tuberculin test), and test-responsive exposed ones. This is because

countries implementing bTB eradication programmes often perform routine cattle herds testing through an intradermal tuberculin test, commonly known as ‘skin tests’. This test has a limited sensitivity and fail to detect individuals are right after infection (De la Rúa-Domenech et al., 2006). In particular, the exposed individuals fail the test in the first phases of the infection. In the follow, we refer to asymptomatic unresponsive individuals as  $E_1$  and exposed and responsive to skin-test individuals as  $E_2$ . Moreover, most of the bTB models for cattle do not account for the recovered class  $R$ , as life expectancy of farmed animals is quite short and those resulting positive to the test are generally removed from the population to prevent further transmission.

### ***2.1. New Zealand***

One of the most important context where models for bTB in cattle have been developed was New Zealand. New Zealand experienced high bTB prevalence in cattle herds, due also to the contribution of infection in possums. However, many within-species mathematical models have been developed, to disentangle the spread of disease in the domestic animal compartment.

The first modelling paper for bTB in New Zealand has been published by Barlow et al. (1997). They developed a deterministic and stochastic versions of a density-dependent transmission  $SE_1E_2I$  model of bTB within cattle farms. In this model, they firstly estimated the within-farm transmission coefficient by using empirical data on outbreaks, then they applied the model to assess the effects of different surveillance and control policies. The model predicted that within-herd transmission was not sufficient to maintain infection in New Zealand cattle farms without external input. Simulations of the effect of a more sensitive test showed improvement for control in bTB-free areas, whereas more frequent test improved surveillance in endemic areas.

Barlow et al. (1998) developed a similar compartmental model describing bTB between-farm transmission. To reproduce spatial heterogeneity in disease spread in New Zealand, they subdivided the

landscape in two sub-areas: a first smaller one, where infection from wildlife is present; and a much larger one, characterized by a lack external sources of infection. Under these assumptions they predicted that the recruitment of newly infected individuals was compensated by the removal of infected animals identified through routine test-and-cull policy. They showed that, if the input of infective cattle from other farms is removed, the decline in the number of infected herd is negligible and almost undetectable. However, they predicted that reducing the test interval would result in a reduced bTB incidence. Barlow et al.'s (1998) work had a significant impact on bTB surveillance in New Zealand, since an annual testing regime has been adopted in the area under study.

Another study conducted in New Zealand herds was performed by Kao and co-authors (1997). They developed a within-farm density-dependent transmission  $SE_1E_2I$  model, which included external sources of infection from wildlife. They investigated a control strategy for bTB that included cattle vaccination. The external herd infection rates were estimated to match empirical data. The model predicted that a vaccine efficacy have to increase substantially in order to reduce consistently the prevalence of bTB in six years, and that a complementary strategy coupling vaccination and wildlife reservoir together would be preferable.

Another simple model of bTB transmission in cattle in New Zealand was published by Kean and co-authors (1999). By observing the decay of prevalence in infected herds after a successful program of wildlife control, they suggest that bTB within-herd transmission may be responsible of 20-32% of infections. As in Barlow and co-authors (1997), they suggested that the rate of within-herd transmission was below the threshold required to maintain cattle bTB in the absence of infectious wildlife. Thus, their analysis supported the policy of wildlife control as the most effective strategy for reducing cattle bTB.

## ***2.2. United Kingdom***

UK experienced an increased incidence of cattle bTB in the last 20 years (Krebs et al., 1997), which caused significant economic losses (Abernethy et al., 2013; Reynolds, 2006). The control of bTB in UK is complicated by the involvement of badgers, which are considered responsible to sustain disease endemicity and to transmit bTB to cattle. For this reason, between 1975 and 1997 over 20,000 badgers were culled in the intent to control bTB, generating conflicts between conservationists and farming interest groups (Donnelly et al., 2003). Several models have been developed in order to assess the impact of badger bTB transmission to cattle (Cox et al., 2005; Smith, 2001, Smith and Cheeseman, 2002). However, more recently the focus has then moved on within-farm dynamic of bTB and on the role of cattle movement in transmitting the disease and sustaining the epidemic (Gilbert et al., 2005). Brooks-Pollock and Keeling (2009) showed that various changes in farming practices in years 2002-2007, including the increase in farms mean size, probably facilitated the growing incidence of bTB. Their conclusions relied on simulations with a within-farm SIR model applied to farms with different sizes and the comparison of their results with observed incidence data.

Conlan and co-authors (2012) investigated the within-farm bTB dynamics using two different assumptions on the transmission in skin-test unresponsive individuals, namely  $SE_1E_1I$  and  $SI_1I_2$ , models. In the latter model they assumed that animals both unresponsive and responsive to skin-test can infect other individuals. They implemented in both cases a stochastic version of the models, using Markov chains in continuous time. Moreover, they used a sequential Monte Carlo implementation of Approximate Bayesian Computation (SMC-ABC) to estimate model parameters. They demonstrated that different assumptions on the duration of latent period impacts the estimate of the effectiveness of skin-test surveillance. Moreover, both models suggested that the rate of cattle-to-cattle transmission in Great Britain herds is density dependent and highly non linear. Finally, they suggested that the key strategy to control bTB burden in UK the is to reduce within-farm transmission following an increased

surveillance, rather than including wildlife reservoir management.

A series of nested epidemic within-farm  $SE_1E_2I$  models was evaluated by O'Hare and co-authors (2014). They assumed density-dependent transmission and they accounted for an external source of infection. In addition, they included heterogeneities in disease transmission, both at individual-level (considering the presence of super-spreaders) and herd-level (using different within-herd transmission rates). Finally, they inferred the model parameters with a likelihood-based bootstrapping. Results were obtained running 10,000 stochastic simulation of a within-farm epidemics, indicating that super-spreaders individuals can have an effective role in within-farm disease transmission. Their model fitted well both in high- and low-risk areas and, similarly to previous models (Conlan et al., 2012), was applied for similar evaluations on abattoir and on-farm routine surveillance. They concluded that cattle-to-cattle transmission is largely dominant in within-farm bTB dynamics, and that badgers were only partially involved.

Similar results were found by Brooks-Pollock et al. (2014). They used a simple stochastic SEI model with frequency-dependent transmission. This was the first model for bTB in which the authors considered both the within- and between-dynamics of the disease, the latter using records on cattle movements. Moreover, they took in account for the environmental source of infection, but they did not distinguished between pasture contamination and wildlife transmission. They calibrated the model using SMC-ABC (sequential Monte Carlo implementation of Approximate Bayesian Computation) on the epidemic data. They found that herd-breakdowns trigger by a combination of environmental conditions, cattle movement, and missed infections. In particular, they attributed to the environmental transmission a central role in maintaining the infection locally, while cattle movement was mostly implicate in the generation of new herd-breakdowns.

### *2.3. USA and others*

In 2013, Smith and co-authors (a) developed a stochastic  $SE_1E_2I$  model for bTB transmission in US herds. The model was parametrized according to the published literature as time series of infections or spatial distribution were not available. The model was used to test the effectiveness of the current surveillance in US, slaughterhouse surveillance, and they found that it might be not sufficient for a quick detection of the epidemic. They tested for both density- and frequency-dependent transmission, and they found that the latter fitted better data on 10 observed outbreaks in US herds. In a further published work (Smith et al., 2013b) they made an economical evaluation of the surveillance strategy. Based on the results of their model, in fact, they recommend 2-month testing intervals for herds after an outbreak of bTB, with 2 negative whole herd tests being sufficient to lift quarantine. Moreover, they found that when the cost of losing the bTB-free status is greater than \$1.4 million, then depopulation of farms could be preferred over a test and cull program.

In The Netherlands, concern on bTB raised in 1999 after a 10-farms outbreak, with a prevalence up to 80% of cattle per farm. Consequently, a first model was developed to evaluate alternative surveillance strategies (Fischer et al., 2005), followed by a second one published in the same year (Van Asseldonk et al., 2005) that included also an economic assessment of alternative surveillance and control strategies. By using a a stochastic  $SE_1E_2I$  individual-based model with frequency-dependent transmission. They found that surveillance based on slaughterhouse inspection was optimal given the current low bTB prevalence in the country, even if risk of outbreaks, such the 1999 one, still existed. Their analysis suggested that, slaughterhouse inspection with analysis of gamma-interferon (similar to skin-test but made in lab on blood samples) or to on-farm routine testing, were optimal as well, and that other surveillance techniques such as the ELISA on milk tanks should be considered for a better performance.

Dairy production is substantial in Argentina. In this context, Perez and co-authors (2002) produced a



Red-Frost stochastic model for bTB diffusion. They used it to estimate the bTB transmission rate and his variability among herds. Data on three herd-breakdowns was used to estimate this parameter, that resulted similar to the one estimates in New Zealand herds in previous studies (Barlow et al., 1997).

A deterministic theoretical within-farm model, assuming density-dependet transmission, was formulated and qualitatively analysed by Augusto and co-authors (2011). In respect to the majority of other bTB models, this is theoretical and is not calibrated with epidemic data. The model shows that in both absence or presence of infected cattle imports, the model has a unique endemic stable equilibrium.

### **3. Discussion and final remarks**

The goal of this review was to analyse the published literature on bovine tuberculosis (bTB) dynamical models, and, in particular, to evaluate their insights from an epidemiological management point of view. Another review on bTB was proposed in 2001 (Smith, 2001). They mostly focused on bTB dynamics in wild species, in particular possums and badgers, while we focused on models explicitly describing the dynamics of infection in cattle. Our choice was due to the central role that cattle has in bTB spread, both in developed and developing countries. Moreover, it is important to understand the potential spread of bTB in regions in which wildlife reservoirs have any role, in particular in order to design effective surveillance and control systems.

As we observed in the previous section, most of the modelling effort for bTB have been made in New Zealand and UK. These two regions experienced an high prevalence of bTB in cattle herds, mostly due to contacts with infected wildlife (Hutchings et al., 2012; Johnston et al., 2011). bTB surveillance and control is a complex issue and now it is clear that when bTB is endemic in wildlife, the spill-over to human or to livestock risk becomes very likely, and eradication will be a long term commitment (Fitzgerald and Kaneene, 2012). In fact, the presence of a wildlife reservoir was often indicated as one of the major reasons for bTB diffusion and newly infected farms. However, most of the models we

analysed stated that wildlife is only partially involved in the infection, mostly for maintaining the infection locally (Brooks-Pollock et al., 2014). In fact, cattle movement has been implicated mostly in newly infected farms, while cattle-to-cattle transmission has been recognized as an important route of within-farm transmission (O'Hare et al., 2014). This is fundamental from a disease control perspective, because it moves the main target of intervention from wildlife to cattle, in particular focusing on higher on-farm surveillance (Conlan et al., 2002). Vaccination is proposed only by Kao and co-authors (1997). This is due to the low effectiveness of bTB vaccines and, in particular, to the lost skin-test responsiveness of vaccinated individuals.

Most models assume a within-farm density-dependent transmission. However, Smith et al. (2013) demonstrated that a frequency-dependent transmission gave a better fitting on bTB outbreaks data in the US. Moreover, the correlation between outbreak size and herd size is still not clear. In fact O'Hare and co-authors (2014) found that these were weakly correlated, while Conlan and co-authors suggested that the transmission rate is non-linearly dependent from herd size. This lack of knowledge is partially due to the high variability of the disease parameters, that do not permit to have sound estimates of transmission phenomena.

An important modelling improvement in Brooks-Pollock and co-authors (2014) is the inclusion of both within- and between-farm dynamics of bTB. This is a very important feature, as it allows the assessment of surveillance and control strategies at a regional-level, and not only at the farm-level, thus providing a broader perspective on bTB epidemics and control strategies. In fact, most of the bTB control strategies in developed countries are focused at avoiding new infected farms, an issue that can not be addressable without a fully understanding of the between-farms dynamics.

Surveillance mostly relies on slaughterhouse inspection and on-farm testing. Many countries, in which the prevalence is low, implement only the first strategy. However, both studies conducted in The Netherlands (Fischer et al., 2005; Van Asseldonk et al., 2005) and the US (Smith et al., 2013a,b)

showed that abattoir inspection could not be enough to quickly detect bTB outbreaks and, eventually, to avoid epidemics.

The majority of the reported bTB models, are suited to contexts in which outdoor farming is a very common practice (such as British Isles, USA, and New Zealand). However, the epidemiological risks intensive vs. free range farming can be very different. On the one hand, the outdoor farming expose cattle to local infection following contact wildlife or contaminated environment. On the other hand, cattle densities in intensive farming are significantly higher in free ranging animals and this can boost disease transmission. Simulation of bTB dynamics within intensive farms has been rarely performed, highlighting a gap in the literature.

Another interesting issue is the understanding of potentially different role of dairy and beef farms in bTB dynamics. The two sectors are characterized by remarkably different management practice and schedule, so the risk of an outbreak can be totally different as well. In particular, the differences in the life cycle length (2 and 5 years, respectively for beef and dairy cattle), and the different conditions in which they are reared.

Finally, a growing concern is raising about the diffusion of bTB into cattle in developing countries, in particular in Africa. In developing countries, not only management practices, herd sizes, environmental and hygienic conditions are very different from those in industrialized countries, but the risk of disease spill over from the cattle compartment to the human population is much higher due to raw milk consumptions. Likewise for threatened wildlife species that can be affected by bTB (De Garine-Wichatitsky et al., 2013). A modelling effort still is needed in this context too.

In conclusion, the development of a deeper understanding of the bTB dynamics and transmission patterns at all levels - within a single species, inside each one of the three of the ecological compartment (wildlife, domestic and human) and at the interface of these - could help to prevent bTB to become endemic in a given area and, thus, to become a major risk for humans, livestock and

wildlife. Cattle plays a central role in bTB spread. This is an area where good epidemiological models can provide crucial insights and foster the development and testing of effective contingency plans for disease control, surveillance and possible eradication.

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## **Chapter II: Epidemiological modelling for the assessment of bovine tuberculosis surveillance in the dairy farm network in Emilia-Romagna (Italy)**

### **1. Introduction**

Bovine tuberculosis (bTB) caused by *Mycobacterium bovis* is among the major disease threats to farm animals worldwide (Skuce et al., 2012). bTB is a chronic disease characterized by a variable and generally long incubation period, and its aetiological agent has an exceptionally wide range of hosts, including humans, domestic and wild animals, as well as high persistence in the environment (Morris et al., 1994). Besides its direct impact to the cattle industry, bTB is a zoonotic disease of great concern. For these reasons, regulatory restrictions are in place to prevent the trade of infected animals and their products within and between countries and to reduce the risk of spillover from the animal to the human compartment. Most countries and transnational areas (such as the European Union) with a thriving cattle industry have developed surveillance systems to prevent bTB outbreaks (Cousins, 2001). Despite the control efforts, bTB is still endemic in many countries, both developed and developing (World Organization for Animal Health, 2009) and its eradication has proved to be very challenging (Fitzgerald and Kaneene, 2013; Independent Scientific Group, 2007). Therefore, it is essential to develop surveillance strategies that allow for a rapid detection of infected animals both in endemic and bTB-free areas.

The organization and the components needed for an effective surveillance system in a given territory depend on bTB epidemiology, which is largely determined by the characteristics of the farming system in place. How these factors may interplay to determine the risk of outbreaks in the specific case of bTB has been well documented in the UK and Ireland, where the rising incidence of the infection in the last 20 years has caused significant economic losses (Abernethy et al., 2013; Reynolds, 2006). The direct costs of bTB for UK taxpayers in 2009 were estimated in around £63M (about 95M USD) and over

25,000 cattle were culled (Johnston et al., 2011). Outdoor farming, a common breeding system in the UK and Ireland, favours contacts between animals of different herds, one of the pathways for bTB transmission. In addition, this farming system exposes cattle to the possibly infected wildlife, such as the European badger (*Meles meles*) whose population is endemically infected by *Mycobacterium bovis* in large areas of the British isles (Cheeseman et al., 1989; Griffin et al., 2005; Morris et al., 1994). Another factor associated with recurrent bTB outbreaks in the UK is between-farm movement of live animal (Johnston et al., 2011; Reilly and Courtenay, 2007). Therefore, preventing contacts between cattle of different herds and between cattle and wildlife are considered effective measures to control bTB risk in the UK and Ireland (Johnston et al., 2011; Phillips et al., 2003; Reilly and Courtenay, 2007).

In countries where indoor farming is the most common breeding practice, such as Italy, bTB transmission rarely occurs through contacts with infected wildlife. A study on bTB risk factors in Northern Italy showed that the main risk factor for bTB breakdown was cattle movement between farms (Marangon et al., 1998). bTB is still endemic, though at very low prevalence, in some Italian regions while it has been eradicated in others.

Among the latter, Emilia-Romagna (ER), located on the right side of the river Po valley (Northern Italy), has been declared officially free from bovine tuberculosis (UE Decision n° 2007/174/CE). This Region has an important food-farming industry characterized by an intensive dairy production, and is the region of origin of Parmigiano-Reggiano cheese. According to the latest official statistics (Italian National Statistics Institute, 2010), about 550,000 cattle are reared in 7,343 farms in ER. As an epidemic of bTB in this Region would have severe economic consequences, the regional health authorities have implemented, in compliance with EU regulations (UE Decision 2002/677/CE), an integrated surveillance system to prevent bTB re-emergence in dairy cattle (Regional decision GPG/2010/1049). The system is based on three detection methods, namely: (i) periodic routine

skin-test on all animals aged over 24 months (RS); (ii) systematic skin-test on all exchanged cattle (ECT) at the destination farm; and (iii) inspection for bTB lesions of all slaughtered animals (SI).

While ER Region has managed to remain bTB-free so far, the effectiveness of the integrated surveillance system (and its individual components) in the case of re-introduction of the infection has never been assessed.

The goal of this work is to quantitatively assess the performance of the current surveillance system and its individual components in terms of (i) time needed to detect a prospective newly introduced bTB epidemic in the dairy farms of ER, and (ii) the magnitude of the epidemic, in terms of number of farms infected before the detection of the epidemic.

Since ER has been free from bTB for many years, no empirical data on the epidemiological dynamics of the infection is available. Therefore, in order to test the performance of the three surveillance methods currently in place in the Region, we developed an individual-based epidemic model capable of simulating the spread of bTB in the regional network of dairy farms following the random introduction of infected animals in the system.

Considering that the rearing system in place in ER and that the components of bTB surveillance described above are largely widespread in several developed countries, our findings can be applicable to many farming and surveillance systems worldwide.

## **2. Materials & Methods**

To assess the performance of the current surveillance system and each of its components, we built a data-driven, stochastic network model that simulates both within-farm and between-farm bTB dynamics. We assumed that the contribution of transmission routes other than cattle exchanges – such as airborne transmission or transmission mediated by wildlife or fomites – was negligible within the regional system of industrial indoor farms. Thus, we considered cattle exchanges as the only

transmission route for bTB among dairy farms.

Since bTB is a chronic disease characterized by slow transmission dynamics (see Augusto et al., 2011; Huang et al., 2013; Brooks-Pollock et al. 2014) the assumption of endemic equilibrium within a given farm is unrealistic. Then, we explicitly accounted for within-farm disease dynamics describing how epidemics evolve inside farms following the introduction of infected animals.

We represented the system of cattle exchanges of ER as a contact network where nodes are farms and directional edges represent between-farm animal movements. We reproduced the network of 4353 dairy farms and 20 intermediary trader farms (ITF) by using information from the cattle movement dataset as described hereafter.

All statistical analyses and model simulations were performed using the software R with “MASS”, “triangle” and “poilog” packages (<http://www.r-project.com>).

### ***2.1. Cattle Trade Movement Data***

Cattle movement data was provided by the Italian National Bovine database. At the end of 2010, 4353 dairy farms and 20 intermediary trader farms (ITF) were in operation in ER. For all of them we collected the farm size, i.e. number of animals per farm (Figure 1). For all the ITF and a subset of 837 dairy farms we also collected individual records of every cattle movement, incoming and outgoing, over a 100-week time span (from the beginning of February 2009 to the end of December 2010). The farms were sampled stratified by province and the obtained sample is representative of the farms size distribution observed in the region (Figure 1 and Appendix S1.1 for details). Each individual record had a unique identifier code for the animal, the birth date, sex and race of the animal, identifier codes for the farms of origin and destination, codes for farms production sector (beef, dairy or mixed), and the

movement date. We considered the movements between dairy farms only, and we excluded the movements of young calves or end-of-life cattle sent to beef farms or directly to the slaughterhouse. The final dataset was composed of 15,501 individual records reporting animal movement (Table 1).

ITF activity consists in trading cattle, not in rearing them. As a consequence, animals stay in ITF for just a few days (median 2 days, lower [upper] quartile 1 [8] days) before being moved to a destination dairy farm, a much shorter time compared to the time that individuals spend in dairy farm (1815 days, on average). In addition, the number of animals simultaneously held in ITFs is usually very low (median 0, lower [upper] quartile 0 [5] animals). Given the limited number of animals and the short time that individuals spend inside them, we assumed that the bTB transmission inside ITFs was negligible compared to dairy farms (a sensitivity analysis on the effect of this assumption on bTB dynamics and the effectiveness of surveillance is shown in the Appendix, section S3.4).

We defined the in-degree and the out-degree for farm  $i$  as the number of farms from which farm  $i$  receives and the number of farms to which farm  $i$  sends cattle, respectively. Altogether, the joint sets of in- and out-degrees are referred to as the in- and out-degree distributions of the network of dairy farms. Since movement data were available for all ITFs in operation in ER, we directly derived the in- and out-degree of each ITF in the network as well as their distributions.

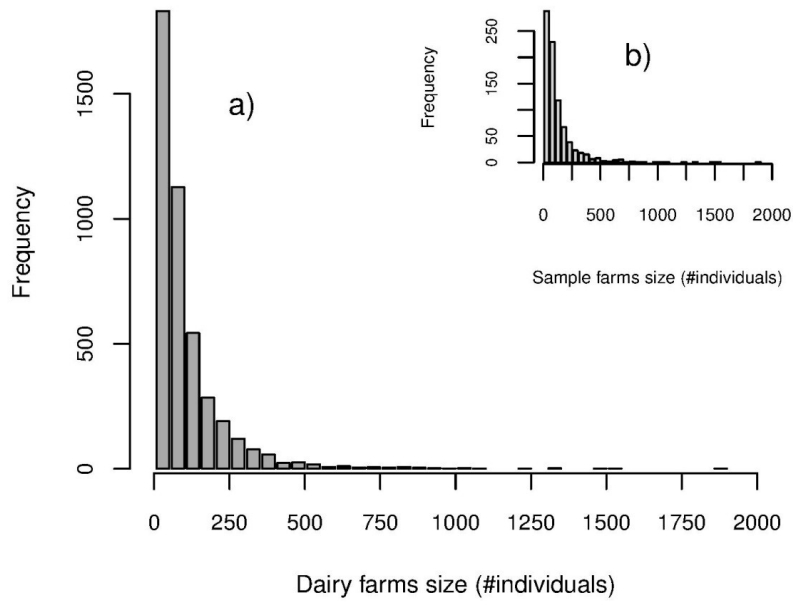


Figure 1: Distribution of Emilia-Romagna dairy farms size. Every bar correspond to a size interval of 50 individuals (0-50, 51-100, etc.). a) All dairy farms size distribution (Min = 1, Median = 63, Mean = 101.9, Max = 1889); b) sampled dairy farms size distribution (Min = 1, Median = 74, Mean = 126.6, Max = 1889).

<i>Cattle Movement Dairy Farms Data (feb-2009/dec-2010)</i>			<i>Cattle Movement ITF Data (feb-2009/dec-2010)</i>		
<b>Total Dairy Farms in Emilia Romagna</b>			<b>Total ITF in Emilia Romagna</b>		
4353			20		
<b>Samples Dairy Farms</b>			<b>Sample ITF</b>		
837			20		
<b>Sample Individual Records</b>			<b>Sample Individual Records</b>		
106499			3571		
<b>Incoming Individuals</b>	<i>Total</i>	9173	<b>Incoming Individuals</b>	<i>Total</i>	3571
	<i>from ER dairy farms</i>	7036		<i>from ER dairy farms</i>	1774
	<i>from other regions/countries</i>	2137		<i>from other regions/countries</i>	1797
<b>Outgoing individuals</b>	<i>Total</i>	28954	<b>Outgoing individuals</b>	<i>Total</i>	3476
	<i>Total to farms</i>	6328		<i>Total to farms</i>	3211
	<i>to other ER dairy farms</i>	5597		<i>to other ER dairy farms</i>	2136
	<i>to other regions/countries</i>	731		<i>to other regions/countries</i>	1075
	<b>Total slaughterhouse</b>	<b>21851</b>		<b>Total slaughterhouse</b>	<b>230</b>
	<i>to ER slaughterhouse</i>	10195		<i>to ER slaughterhouse</i>	147
	<i>to other region slaughterhouse</i>	11656		<i>to other region slaughterhouse</i>	83
<i>death/other destinations</i>	775	<i>death/other destinations</i>	35		
<b>Sample Farms Mean In-Degree</b>			<b>ITF Mean In-Degree</b>		
1.91 (SE ±0.096)			24.50 (SE ±7.185)		
<b>Sample Farms Mean Out-Degree</b>			<b>ITF Mean Out-Degree</b>		
1.67 (SE ±0.075)			19.25 (SE ±5.537)		

Table 1: Detailed data on sampled dairy farms and Intermediary Trader Farms (ITF).

Regarding farms other than ITFs, movement data were available only for a subset of 837 farms out of the total 4353 in the ER network. Thus, we derived the in- and out-degree distributions of this subset. Then, we estimated the parameters of a set of candidate probability distributions (namely: power-law, log-normal, Poisson and Yule) on the available data and selected the distribution that best fitted the data by using log-likelihood ratio (LLR) test as shown in Vuong (1989) and Clauset et al. (2009). Finally, we used the selected probability distribution to generate a synthetic network of 4353 farms having the same topological properties (i.e., the in- and out-degree distributions) observed in the subset of 837 dairy farms. The procedure for network generation is described in the following section.

## ***2.2. Network Model***

To generate a network of 4353 farms with the same topological properties observed in the subset of 837 dairy farms, we first assigned in- and out-degrees independent of each other to the 4353 nodes in the network (as in- and out-degrees were weakly correlated, see Appendix S1.3). Moreover, as both in-degrees and out-degrees were very weakly correlated with farm size in the observed dataset, they were assigned to nodes irrespective of farm size (see Appendix S1.3). According to the available movement data, we assumed that neither the in-degree nor the out-degree could be larger than the node size, i.e. the number of animals in the farm.

Once we assigned to each node an in-degree and an out-degree, we used a heuristic algorithm to exactly match the number of outgoing connections from farms in the network to that of ingoing connections to farms in the same network (Appendix S2). Then, we completed the network by adding 20 additional nodes representing the 20 ITF and assigned to each of them the observed in- and out-degrees.



### 2.3. Within-Farm Model

We described the within-farm bTB dynamics through a compartmental stochastic Susceptible-Exposed-Infected (SEI) model with homogeneous frequency dependent transmission (Agusto et al., 2011; Brooks-Pollock et al., 2014). The compartment of the susceptible individuals ( $S$ ) represents cattle that have not been infected yet. Following successful infection, each individual is classified as exposed ( $E$ ) but not infectious yet. The incubation period ends when the infected individual becomes also infectious ( $I$ ). Then, the infection dynamics for farm  $i$  can be represented by the following system of ordinary differential equations:

$$\begin{cases} \dot{E}_i = \frac{\beta_i S_i I_i}{N_i} - (\sigma + \mu) E_i - \sum_{j \neq i} \gamma_{ij} E_i + \sum_{j \neq i} \gamma_{ji} E_j \\ \dot{I}_i = \sigma E_i - \mu I_i - \sum_{j \neq i} \gamma_{ij} I_i + \sum_{j \neq i} \gamma_{ji} I_j \end{cases} \quad (1)$$

where farm size  $N_i$ , i.e. the number of cattle in farm  $i$ , was assumed constant in time, and the number of susceptible animals in farm  $i$  was computed as  $S_i = N_i - E_i - I_i$ ;  $\beta_i$  represents the within-farm transmission rate;  $\sigma$  the rate at which exposed individuals become infectious;  $\gamma_{ij}$  the movement rate of individuals from farm  $i$  to farm  $j$ , which is proportional to farm  $i$  out-degree; and  $\mu$  the rate at which individuals leave the farm system, either because they are sent to slaughterhouse, to farms outside the ER system, or because they die.

To explicitly account for the stochastic nature of the contact and transmission processes in a large network of farms, we ran stochastic simulations through an event-driven approach in which time steps between two consecutive events were described through a Poisson process with exponentially distributed means (Gillespie, 1977; Rohani et al., 2002). All the events simulated by the stochastic infection model are reported in Table 2.

## 2.4. Parameter Estimation

The basic reproduction number represents the mean number of secondary infections caused by a single infected individual introduced into a completely susceptible population (Diekmann et al., 1990). As ER is an officially bTB-free region since 7 years, there are no available outbreaks data to estimate a within-farm basic reproduction number,  $R_0$ . Thus, we set  $R_0$  value in our simulations using estimates for bTB found in the literature (Brook-Pollock and Keeling, 2009; Agosto et al. 2011; Conlan et al., 2012; O'Hare et al., 2014). In particular, we described the uncertainty in  $R_0$  by assuming a triangular distribution with mode 2.40 (which corresponds to the mean value of literature estimates), lower limit 0.83, and upper limit 4.9 (which correspond to the extreme values found), see Appendix S3.1 for details. In further simulations we tested the effect of  $R_0$  variability, comparing the obtained results against those of simulations ran using a fixed  $R_0$  value (see Appendix S3.2).

Moreover, in order to test the robustness of our results against possible variations in the within-farm bTB basic reproduction number, we run further simulations using as fixed value the highest ( $R_{0,i} \equiv 4.9$ ) and the lowest ( $R_{0,i} \equiv 0.83$ ) of the literature estimates (see Appendix S3.3).

The  $i$ -th farm transmission rate,  $\beta_i$ , was indirectly derived from the expression for the within-farm basic reproduction number of model (1), i.e. assuming infection in  $i$ -th farm only, by using Next Generation Matrix techniques (Diekmann et al., 1990):

$$\beta_i = R_0 \frac{(\sum_{j \neq i} \gamma_{ij} + \mu)(\sum_{j \neq i} \gamma_{ij} + \mu + \sigma)}{\sigma} \quad (2)$$

Following Barlow et al. (1997), we set the mean time  $\tau (= 1/\sigma)$  spent in the exposed class  $E$  to 202 days. Likewise, parameters  $\mu$  and  $\text{mean}[\gamma_{ij}]$  were estimated from movement data as the inverse of the mean time that animals spent inside the farm before being moved out of the system and toward other ER

dairy farms, respectively.

ITFs are characterized by substantial different behaviours with respect to dairy farms. As we assumed that there is no bTB transmission inside these farms, we set  $\beta_{ITF} = 0$  and we estimated ITF specific movement ( $\gamma_{ITF}$ ) and exit ( $\mu_{ITF}$ ) rates from available data.

### ***2.5. Assessment of the Surveillance System***

We used the stochastic model described in Table 2 to assess the effectiveness of the three surveillance components of the current surveillance system implemented in ER Region. Specifically: (i) a systematic control on every farm based on a tuberculin skin-tests (RS). This is performed on every individual in the farm older than 24 months, which represents about 60% of the total farm population. Following current legislation for bTB-free areas in ER all cattle herds are tested with a turnaround period of three years; (ii) a skin-test performed at the destination farm on moved cattle within 45 days after the arrival (ECT); (iii) a visual inspection for bTB characteristic lesions on cattle slaughtered (SI).

To simulate the RS we assumed that, within a turnaround period, farms are tested in a random order (with a frequency of about 4 farms a day). To simulate the other surveillance components, we assumed to test all individuals moved between dairy farms and to all individuals sent to slaughterhouses. When an animal tests positive to the tuberculin skin-test, both for RS and ECT cases, the procedures to verify whether the animal is truly infected by *M. bovis* last usually 2 months. These procedures consist in a carcass inspection for bTB typical lesions and in a bacteriological examination. During this confirmation period, cattle from the same farm can not be transferred to other farms. This protocol was simulated in the model by assuming that, when a positive individual is detected by the skin-test, the farm can not have any contact with other farms, but the simulation of disease dynamics lasts for other 2

months.

Event in farm $i$	Transition	Rate at which event occurs
<i>Infection</i>	$E_i \rightarrow E_i + I$	$\beta S_i I_i / N_i$
<i>Exposed individual become Infectious</i>	$E_i \rightarrow E_i - 1, I_i \rightarrow I_i + 1$	$\sigma E_i$
<i>Exposed individual is sent to farm <math>j</math></i>	$E_i \rightarrow E_i - 1, E_j \rightarrow E_j + 1$	$\gamma_i E_i$
<i>Exposed individual leaves the system</i>	$E_i \rightarrow E_i - 1$	$\mu E_i$
<i>Infectious individual sent to farm <math>j</math></i>	$I_i \rightarrow I_i - 1, I_j \rightarrow I_j + 1$	$\gamma_i I_i$
<i>Infectious individual leaves the system</i>	$I_i \rightarrow I_i - 1$	$\mu I_i$

Table 2: The list and rates of the possible events for the stochastic model.

The skin-test currently in use in Italy is the cervical Single Intra-dermal Test (SIT). In their review, de la Rua-Domenech and co-authors (2006) showed that the SIT sensitivity estimates fall between 0.7 and 1. However, on-farm test sensitivity can be substantially smaller than in controlled laboratory experiment trials as other factors than the imperfect nature of the test may affect test outcome, such as the training level, care and experience of the veterinarian and the actual protocol used. Therefore, following Welby et al. (2012), we simulated the uncertainty in test sensitivities by randomly drawing sensitivity values from a beta distribution with mean 0.60 and coefficient of variation 0.10.

Analogously to Barlow et al. (1997), we assumed a reduction of test sensitivity in infective but not infectious animals (mean value 0.54). Sensitivity of *post-mortem* inspection at the slaughterhouses can also be very variable (Asseged et al., 2004; Fischer et al., 2005; Smith et al., 2013; van Asseldonk et al., 2005; Welby et al., 2012). Therefore, similarly to Welby et al. (2012), we simulated the uncertainty in *post-mortem* inspection as a beta distribution with mean 0.60 and coefficient of variation 0.10 (with a reduction of sensitivity for exposed animals as in tuberculin skin-test).

In addition, with the intent to simulate different levels of accuracy and awareness in the testing

procedures, we ran a sensitivity analysis to assess the effect of *post-mortem* inspection and tuberculin skin-test sensitivity values on surveillance system performance (Hadorn and Stärk, 2008; Humblet et al., 2011). The results are shown in Appendix S4.

Disease dynamics following the introduction of an infected individual in the network of dairy farms of ER were assessed under five scenarios. Firstly, we simulated a baseline scenario of bTB epidemics spreading without surveillance so as to understand the potential impacts of uncontrolled bTB epidemics on the system. Secondly, we ran simulations on the integrated surveillance system currently in place, which includes all the three surveillance components (Scenario 1). Then, in order to assess the performances of each component of the surveillance system, we analysed three alternative scenarios in which we removed one of the three components at a time and simulated epidemic dynamics with the other two systems in place. In Scenario 2a, Scenario 2b and Scenario 2c we removed RS, ECT and SI components, respectively (Figure 2).

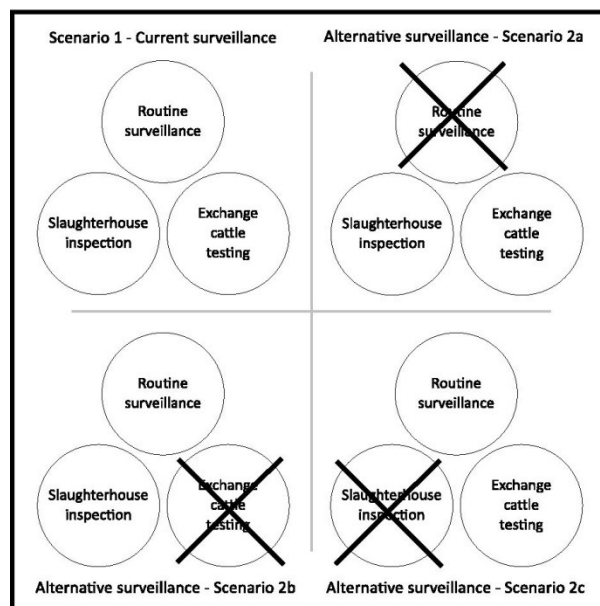


Figure 2: Different surveillance system scenarios simulated.

For each scenario we ran 10 thousand stochastic Monte Carlo simulations of disease dynamics until

infection detection or up to 10 years at most. For each simulation we generated a network of 4535 dairy farms as described above and assumed that an infected but not infective individual was randomly introduced in the network. To simulate variable test sensitivity, the values of skin test and *post-mortem* inspection sensitivity were drawn from the respective beta distributions each time a test was performed. Then, the 10 thousand replicates were used to derive a number of statistics on disease dynamics and on the performance of the specific surveillance system under assessment. In particular, for each scenario we derived: (i) the mean and 95<sup>th</sup> percentile of the time for an epidemic to be detected (when surveillance is in place); (ii) the mean and 95<sup>th</sup> percentile of the number of infected farms; (iii) the number of epidemics that involved more than one farm (i.e. multi-farms epidemics); (iv) the number of undetected epidemics after 10 years; and (v) the number of epidemics that went naturally extinct in less than 10 years.

### 3. Results

#### 3.1. Farm size, In- and Out-Degree Distributions, and parameters estimations

The size of the 4353 ER cattle farms was quite variable and ranged from just few individuals to almost two thousands: farm size distribution was highly skewed with mean equal to 102 and median 63 (Figure 1).

The average number of outgoing [ingoing] links in the 837 sampled farms was 1.67 [1.91], the median 1 [1] and the maximum number 23 [29]. The in-degree and out-degree distributions were not significantly different (Kolmogorov-Smirnov test,  $D = 0.044$ ,  $p = 0.387$ ) and were best fitted by a discrete log-normal distribution (Figure 3 and Table 3). In-degree and out-degree were weakly correlated with farm size (Kendall's  $\tau = -0.028$  and  $0.20$ , respectively). The correlation between in- and

out-degrees was also very weak (Kendall's  $\tau = -0.064$ ). Therefore, for each of the 10 thousand runs we generated a network of 4353 farms by independently drawing farms' in-degree and out-degree from their corresponding distributions, assuming no correlation between them. The resulting networks were weakly connected, with average density of  $5.2 \times 10^{-4}$  (SD  $\pm 8.7 \times 10^{-6}$ ). The value of parameters  $\mu$  and  $\text{mean}[\gamma_{ij}]$  estimated from movement data was  $0.240 \text{ y}^{-1}$  (S.E.  $\pm 0.044$ ) and  $0.043 \text{ y}^{-1}$  (S.E.  $\pm 0.008$ ), respectively, for dairy farms and,  $19.981 \text{ y}^{-1}$  (S.E.  $\pm 1.499$ ) and  $15.901 \text{ y}^{-1}$  (S.E.  $\pm 1.192$ ) for ITFs.

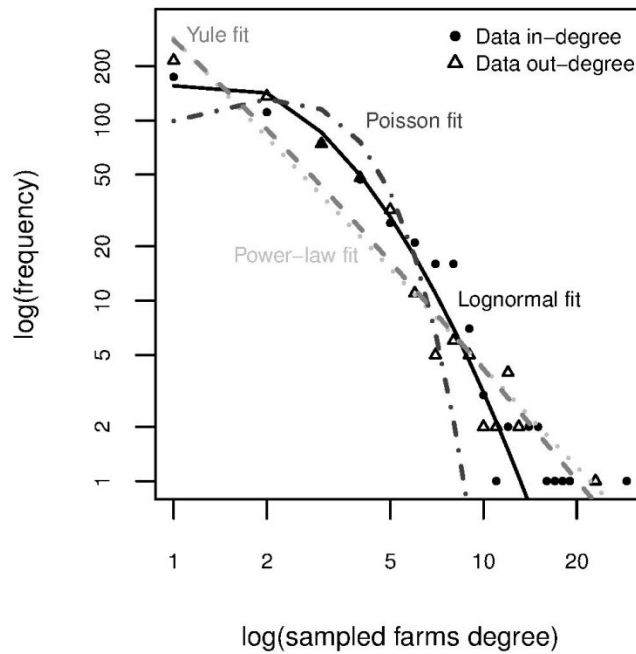


Figure 3: Points represents the observed in- (circles) and out-degree (triangles) distributions of the sampled dairy farms. Lines represent the fitting for different distributions to the empirical data: discrete log-normal (solid line), Yule (dashed line), discrete power-law (dotted line) and Poisson (dashed-dotted line).

<i>Discrete Log-Normal Distribution</i>	<i>loglike.ratio</i>	<i>mean.LLR</i>	<i>sd.LLR</i>	<i>Vuong</i>	<i>p-value</i>
<i>vs. Power Law</i>	158,903	0,151	0,647	7,57	3.6e-14
<i>vs. Poisson</i>	314,182	0,299	1,735	5,58	2.4e-08
<i>vs. Yule</i>	107,584	0,102	0,583	5,69	1.3e-08

Table 3: The result of the Vuong test on the degree data for discrete log-normal distribution vs. discrete power-law, Poisson and Yule distributions.

### 3.2. The baseline scenario: no surveillance

In the absence of any surveillance system, about half of the epidemics lasted for 10 years or more (56.05%), while the others (43.95%) naturally faded out in a median time of 14.92 months (lower [upper] quartile 5.95 [32.89] months). Epidemic size was highly skewed (Figure 4): the mean number of infected farms was 2.91 (S.E.  $\pm 0.05$ ), but 62.66% of the epidemics involved only a single farm. In the worst 5% of the cases more than 11 farms got infected, with a maximum of 89.

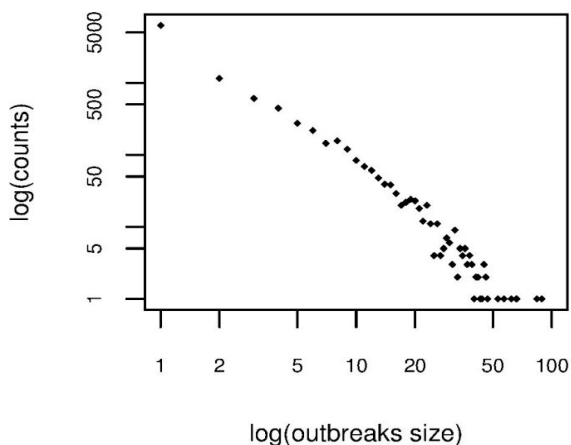


Figure 4: The distribution (in log-log scale) of maximum number of infected farms (outbreak size) in each simulated outbreak.



### 3.3. Performance of the current surveillance system

Figure 5 shows the cumulated distribution of the time of epidemic detection computed as the fraction of epidemics monthly detected by the current surveillance system. The mean detection time was 27.04 months (S.E.  $\pm 0.19$ ). Epidemics were identified more frequently by routine surveillance, RS, and by *post-mortem* inspection at the slaughterhouse, SI. In 10 years, RS identified 41.66% of the epidemics and SI the 38.59%. Only 5.10% of the epidemics were first detected by ECT, i.e. intra-dermal testing of exchanged cattle; 14.55% of the epidemics faded out unreported while only 0.1% of the epidemics lasted 10 years or more. The average number of infected farms during the epidemics was 1.12 (S.E.  $\pm 0.003$ ); the worst 5% of the epidemics lasted more than 59.20 months affecting from 2 to 5 farms at most. Multi-farm epidemics occurred in 10.15% of the replicates. The performances of the current surveillance and the alternative scenarios described hereafter are summarised in Tab. 4.

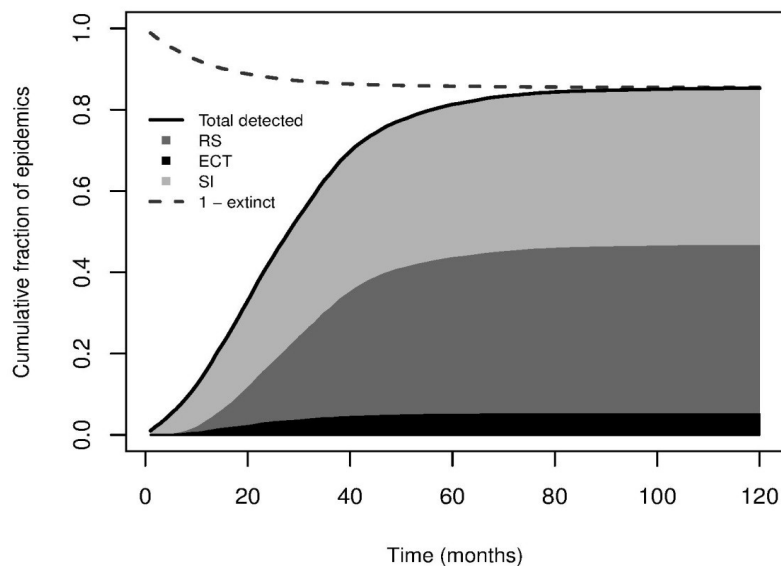


Figure 5: The cumulated fraction of simulated epidemics monthly detected by the current surveillance system. Different colours correspond to the fraction of epidemics detected by each surveillance strategy: in light grey by slaughterhouse

*inspection (SI), in dark grey by routine surveillance (RS) and in black by exchanged cattle testing (ECT). The solid line represents the fraction of total of detected epidemics while the dashed line represents the fraction of epidemics not naturally faded out, or extinct, at any given time.*

### **3.4. Performance of alternative surveillance systems**

Figure 6 shows the cumulative fraction of monthly detected epidemics registered for a surveillance system implementing only two of the three surveillance methods currently in place. Our analysis showed that removing RS testing from the integrated surveillance system (Scenario 2a) led to an almost 7-month delay in the detection time driven by a significant reduction in the number of epidemics detected starting from the second year (Figure 6). Compared with the current integrated surveillance system, this scenario was characterized by a small increase in the mean number of infected farms, in the total number of unreported epidemics lasting 10 years or more (from 0.10% to 0.70%), and in the number of unreported epidemics naturally fading out before the end of the simulation time (from 14.55% to 16.23%). In the worst 5% of the cases, the epidemics remained undetected for about 6 and a half years (76.91 months) and involved 2 to 5 farms.

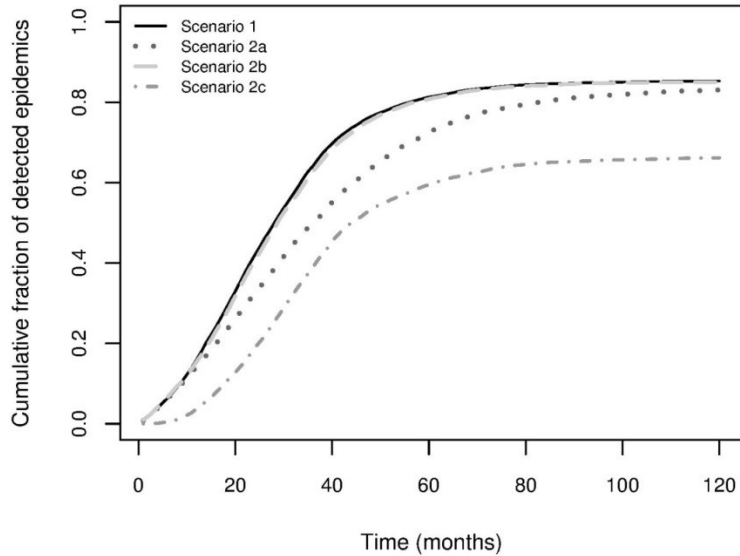


Figure 6: The cumulated fraction of simulated epidemics monthly detected by the surveillance system in Scenario 1 (solid line); Scenario 2a (only ECT and SI components, dotted line); Scenario 2b (only RS and SI components, dashed-dotted line); and Scenario 2c (only RS and ECT components, dashed line).

Removing the ECT (Scenario 2b) did not cause significant changes in any of the performance indicators. In the worst 5% of the cases the time to detection was about 60 months and the number of infected farms between 2 and 7.

The removal of SI (Scenario 2c) led to a more than 8 months increase in the mean detection time mostly driven by a significant reduction in the detection rate since the first months. The number of undetected epidemics still ongoing after 10 years did not significantly change, while the fraction of multi-farm epidemics increased from 10.15% in Scenario 1 to 12.01%. The largest change in scenario 2c was represented by the number of unreported epidemics that naturally faded out, increased from 14.55% in the Scenario 1 to the 33.57% in Scenario 2c, that explains the lower number of totally

detected epidemics (Figure 6). In the worst 5% of the cases, the epidemics remained undetected for 71 months and involved 2 to 7 farms.

#### **4. Discussion**

The goal of this study was to assess the effectiveness of the current bTB surveillance system in Emilia-Romagna (ER) and the specific role played by its three components: on-farm routine testing, exchanged animal testing and slaughterhouse inspection. To assess the performance of the bTB surveillance system we computed two main indicators: the time needed to detect a prospective newly introduced bTB epidemic in the dairy farms of ER; and the magnitude of the epidemic, in terms of number of farms infected before the detection of the epidemic. The time to epidemic detection is a very important measure of the surveillance performance (Hadorn and Stärk, 2008); firstly, because an early detection of the epidemic limits the number of both animals and farms infected; secondly, because according to Italian and European legislations at least 6 months without bTB cases are required for farms to regain their disease-free status. Indeed, the trade restrictions following an epidemic represent one of the worst consequences for the cattle industry (Gordon, 2008). The magnitude of the epidemic allows us to assess the damages caused by a bTB incursion quantifying the number of infected farms and, consequently, the total economic burden (i.e., loss of earnings, costs for animal culling and re-stocking).

Our analysis showed that the integrated surveillance system currently in place in ER can effectively reduce the number of farms involved in a potential epidemic of bTB with respect to a baseline scenario with no surveillance (see Table 4). The model predicts that about the 15% of the epidemics can not be detected by the system. However, the undetected epidemics usually faded out during the first nine months after the introduction of the infection and generally involved one or few individuals in a single farm.

<i>Scenario</i>	<i>Mean (and 95p) Time to Detection (months)</i>	<i>Mean (and 95p) number of in- fected farms</i>	<i>% of Multi-farm Epidemics</i>	<i>% of Epidemic over 10 years</i>	<i>% of Epidemic Extinct</i>
<i>No surveillance</i>	-	2.91 (11)	37,34%	56,05%	43,95%
<i>Scenario 1</i>	27.04 (59.20)	1.12 (2)	10,15%	0,10%	14,55%
<i>Scenario 2a</i>	33.77 (76.91)	1.20 (2)	16,80%	0,70%	16,23%
<i>Scenario 2b</i>	27.52 (60.09)	1.11 (2)	9,61%	0,14%	14,80%
<i>Scenario 2c</i>	35.45 (71.34)	1.15 (2)	12,01%	0,18%	33,57%

*Table 4: The main indicator for the surveillance system performances. Results for 5 different surveillance scenarios: no surveillance, 1: current surveillance, 2a: RS removed, 2b: ECT, 2c: SI removed.*

Numerical simulations also showed that *post-mortem* inspection at the slaughterhouse (SI) and on-farm routine testing (RS) were the most effective surveillance methods. These methods were significantly more effective in detecting bTB in the ER network of dairy farms than testing cattle upon moving to a new farm (ECT). Considering that the cattle exchanges were the only hypothesised route of between-farm transmission in the model, this result seems, at first sight, unexpected. However, it can be explained by the low bTB transmission rate and some specific proprieties of the ER cattle system (such as the low network density and the low number of cattle traded).

Slaughterhouse inspection turned out to be particularly effective as an early detection tool of the epidemics. This seemed to be due to the fairly regular flow of cattle sent out to slaughterhouses at the end of their productive cycle, on average at 5 years of age. Therefore, when an outbreak occurred in a farm, SI was likely the earliest diagnostic intervention able to detect it compared to ECT and RS. However, the large number of epidemics that naturally faded out when SI was absent (Scenario 2c) points out that most of the outbreaks that remained undetected at the end of the first year were likely to get extinct afterwards without developing into significant epidemics.

On-farm routine surveillance was the most effective in preventing major, or multi-farm, outbreaks. This was probably due to the low value of within-farm bTB basic reproduction number ( $R_0$ ). Indeed, a low within-farm  $R_0$  is followed by a low number of infected individuals inside farms, that means a low number of infected individuals exchanged or sent to slaughterhouses. In these epidemiological conditions, RS was the most effective method to detect outbreaks before bTB spreads to other farms. Moreover, this method was also the most effective in detecting the small portion of minor epidemics that can persist unnoticed for many years.

The performances of SI and RS, both with regard to time to bTB detection and to fraction of multi-farm epidemics, were comparable and which one performed better depended on the value of the basic reproduction number ( $R_0$ ), see Appendix S3.3, and on the values of skin-test and SI sensitivity, see Appendix S4.2.

As the  $R_0$  value increased, the number of detected epidemics by the SI tended to increase compared to the number of epidemics detected by RS (see Appendix S3.3). This result can be explained considering that a larger within-farm disease prevalence, as a consequence of a larger within-farm  $R_0$ , implies a higher probability of sending infected individuals to the slaughterhouse in the early stages of epidemics, speeding up bTB detection, while the timing of RS is not affected by farm prevalence.

The values of skin-test and carcass inspection sensitivity played an important role as well. This was particularly significant in the case of slaughterhouse inspection, since a very large range of values of inspection sensitivity has been estimated in different contexts, from 0.1 to 0.65 (Asseged et al., 2004; Fischer et al., 2005; Smith et al., 2013; van Asseldonk et al., 2005; Welby et al., 2012).

The RS turnaround period, i.e. the time frame in which all farms are tested once for bTB, was set to 3 years, as currently is in ER region. The regional administration decided to move this period from 2 to 3 years in 2010. Our results suggested that this change had a negligible impact on the overall surveillance system performance and, moreover, that moving it to 4 years would have marginal impact as well

(Appendix S5).

The limited performance improvement provided by ECT raised questions about its usefulness and whether it could be safely dropped out from ER integrated surveillance system without significantly weakening it. Of course, this kind of decision should also be based on cost-benefit analysis. On the other hand, the role of ECT on animals entering a bTB-free region is not negligible, especially for cattle originating from regions where bTB is endemic and if pre-movement testing - in contrast to post-movement testing - is adopted to prevent the risk of disease introduction (Clegg et al. 2008; Schiller et al., 2011).

In agreement with our results, Shittu et al. (2013) showed that SI plays an important role in bTB detection in the UK, both in low incidence and endemic areas, but is highly influenced by inspection sensitivity. Another study conducted in Belgium by Welby et al. (2012) showed that SI is one of the most effective surveillance strategy for bTB. However, further analyses performed with our model showed that the use of the SI component only determined a significant reduction in the surveillance system performance compared to a surveillance based on SI coupled with routine skin-testing (see Appendix S6). This result is in agreement with findings by Fischer et al. (2005) and van Asseldonk et al. (2005), who showed that SI surveillance alone is ineffective in detecting bTB in The Netherlands. This was confirmed by Schöning et al. (2013) on US cattle herds, who showed how SI needed to go along with the skin-test component in order to provide an effective surveillance system.

In this study, cattle movement data were available for a subset of the network of dairy farms in ER. These data were used to infer the fundamental topological properties of the whole system in terms of connectivity, so as to generate synthetic networks of the same size of the ER one. While we are confident that our approach provides a solid preliminary understanding of bTB dynamics on the network of dairy farms in ER, we did not have the possibility to derive more sophisticated metrics, such as the clustering coefficient and the Disease Flow Centrality, that would help to have more

detailed prediction on how diseases spread through the network, as shown by Bajardi et al. (2011) and Natale et al. (2011). We simulated disease dynamics on a static network, ignoring seasonality in cattle movement and the dynamical nature of cattle trade. Dynamical networks can effectively represent the time-varying structure of the network – which is crucial to simulate the dynamics of acute and highly contagious diseases, such as Food-and-Mouth Disease (Bajardi et al., 2011, 2012; Vernon and Keeling, 2009). However, since bTB is characterized by slow infection dynamics and it is able to remain unnoticed for a long period of time, we believe that it is not essential to track the small structural changes occurring on a short time scale for suitably describe bTB dynamics.

Other studies were previously conducted on cattle movements in Italy by Natale et al. (2009, 2011). In particular, they found that the degree distribution of cattle movement was best approximated by a *fat-tailed* power-law probability distribution. Our degree distributions best fitting was obtained through a discrete log-normal distribution, characterized by lower right-tails than the power-law (Figure 3). This could be a consequence of isolating the dairy system, i.e. by not considering the movements towards fattening farms and slaughterhouses.

This study was specifically performed on the dairy cattle system, ignoring the beef sector. The main reason was that the bTB surveillance system in ER is in place on the dairy sector only. Moreover, further reasons supported this choice: (i) the higher economic value of the dairy sector in the Region; (ii) the fact that generally beef farms do not exchange animals with other farms, they only receive male calves within 100 days of age from dairy farms and send animals to slaughterhouses at the end of the fattening process; (iii) the two systems are almost fully separated, so there is no possibility of spill-over from the beef to the dairy system; and (iv) dairy cattle live substantially longer than beef cattle, i.e. up to 5 years vs. 2 respectively, thus epidemics have more possibility to develop in the dairy system because of bTB long incubation period.

Our analyses showed that the farms in-degree and the out-degree were not coupled. In other words,



farms having a higher in-degree, i.e. that import relatively more cattle from other farms in the Region, do not necessarily have a high out-degree, and vice-versa. A similar result is outlined in Volkova et al. (2010) on Scottish farms, suggesting that this could be a recurrent pattern for cattle herds in different management contexts.

In the UK and in other countries where bTB is endemic, the high cost of the surveillance and eradication programme, namely £74-99 million a year for UK (Smith and Clifton-Hadley, 2008; Torgerson and Torgerson, 2008) has been put under scrutiny. Our results indicate that a proposal for simplification or strengthening of an integrated surveillance system should be always based on a rigorous, quantitative understanding of the role played by each of its components. In fact understanding the actual effectiveness of a specific control strategy, is not always straightforward, and can be affected by factors like network topology, the trading system, rearing methods, test frequency and test sensitivity. Moreover, the increasing density of ungulates and other potential wildlife reservoir species occurring in several European countries poses new challenges for the existing surveillance systems even in areas that are currently bTB-free (Schöning et al., 2013).

*A priori* information on the network structure of cattle exchanges during non-emergency periods can help orienting control strategies to prevent epidemics in areas characterized by a high number of industrial farms (Bajardi et al., 2012; Gilbert et al., 2005). Our framework, based on a mathematical modelling approach, provided decision-makers with a powerful cost-effective tool to assess the effectiveness of the current bTB surveillance system in ER, by highlighting strengths and weaknesses its different components.

Moreover, the methodological approach proposed here will allow the health authorities to assess the effectiveness of future alternative strategies for bTB detection that are not currently implemented in ER, such as antigen detecting tests (ELISA) on milk and blood sample (van Asseldonk et al., 2005). Furthermore, by taking into consideration the cost of different testing strategies, it will be possible to

use our modelling approach to run cost-effectiveness analyses of different options of surveillance (Anderson et al., 2013; Cameron, 2012).

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## Appendix:

### S1. Data analysis

#### S1.1. Farms sample

We worked on a sample 837 of the 4353 dairy cattle farms in Emilia-Romagna (ER) region (Italy). The sample was taken from the national bovine register and was provided by the veterinary service administration of the region (*Servizio Veterinario dell'Emilia Romagna*). The farms were sampled stratified by province (see Table S1) and the sample is representative of the farms size distribution observed in the region (i.e. size as the number of individuals reared, see main text Fig. 1). We considered the multi-site farms as single epidemiological units since different compartments of the same multi-site farm are usually very close in space and the exchanges among them are very frequent.

	<i>BO</i>	<i>FC</i>	<i>FE</i>	<i>MO</i>	<i>PC</i>	<i>PR</i>	<i>RA</i>	<i>RE</i>	<i>RN</i>	<i>Total</i>
<i>Dairy farms</i>	214	26	37	882	417	1368	25	1356	28	4353
<i>Sampled farms</i>	47	23	14	168	78	248	10	236	13	837

Table S1: the number of total and sampled dairy farm in each of the nine provinces of Emilia-Romagna (Bologna, Forlì-Cesena, Ferrara, Modena, Piacenza, Parma, Ravenna, Reggio Emilia, Rimini).

#### S1.2. Cattle movements selection

The original dataset contained more than 139,000 individual records. As our study focused on the dairy farms network, we did not consider in our analyses the movements of animals from dairy farms directed towards fattening farms. The latter mostly regarded the movement of male calves aged less than 100 days. In the case of multi-production (mixed) farms – i.e., with both dairy and beef productions – we used in our analyses the exchanges with other dairy (or mixed) farms involving cow races for dairy production only.



### ***S1.3. Correlations among farms features***

For our analyses, we identified the several features characterizing the 837 sampled farms. Specifically: the farm size (i.e. the total number of animals), the number of in- and out- degrees (i.e. the number of exchanged animals from and to other dairy farms), the number of on- and off- individuals, and the number of individuals sent to slaughterhouses during the sample period (February 2009 - December 2010). Then, we looked for possible correlations between these quantities. In figure S1, S2 and S3 we showed the Kendall rank correlation coefficient  $\tau$  between the above mentioned sampled farms features. We found a large correlation value between farm sizes and number of individuals sent to slaughterhouses during the sample period (S1:  $\tau = 0.71$ , p-value  $< 0.001$ ). This result was incorporated in our model by assuming a constant death rate ( $\mu$ ) for all farms (which implies a direct proportionality with farm size). Large correlation values were also found between the number of incoming individuals and the in-degree (S3:  $\tau = 0.78$ , p-value  $< 0.001$ ), and between the number of outgoing individuals and the out-degree (S3:  $\tau = 0.73$ , p-value  $< 0.001$ ). To include this finding in our model we assumed that the number of outgoing individuals in the  $i$ -th farm ( $\sum_j \gamma_{ij} N_i$ ) is proportional to the farm degree. The in- and out-degrees were very weakly correlated (S2:  $\tau = -0.064$ , p-value  $< 0.05$ ). It follows that, we could assign an in- and an out-degree independently to the synthetic network farms.

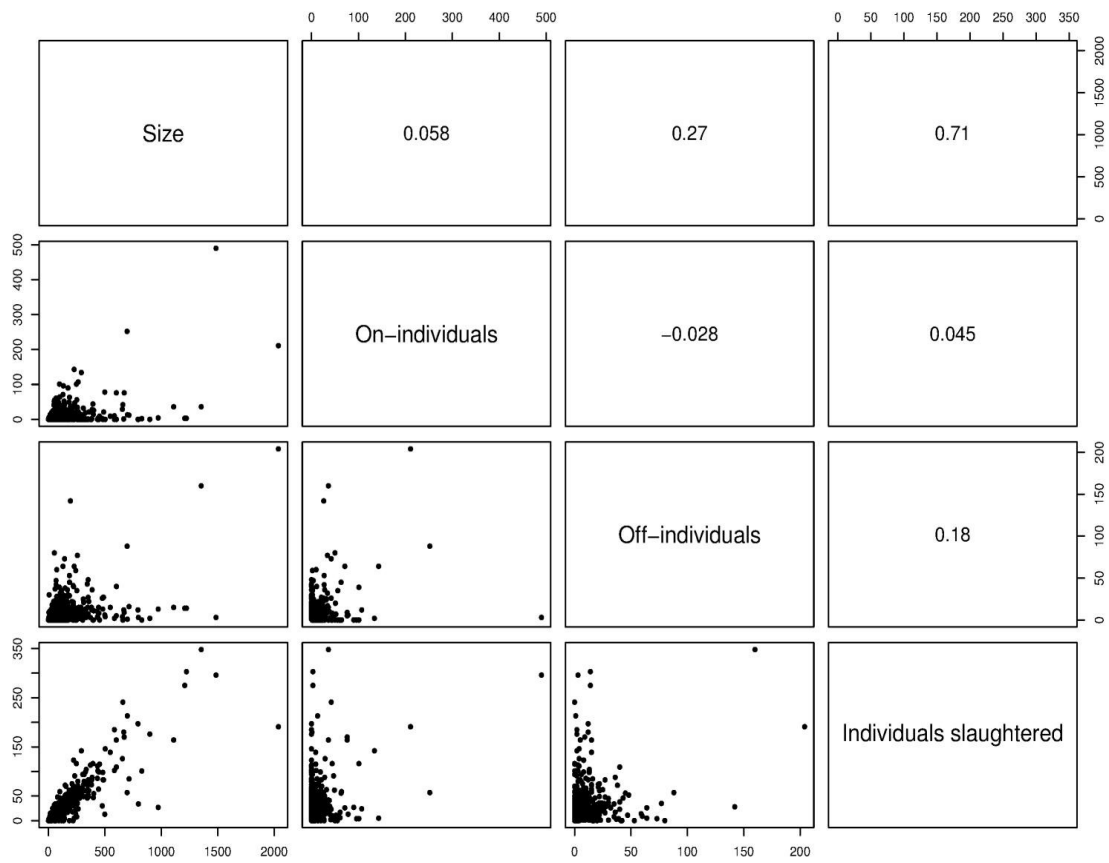


Figure S1: Kendall rank correlation coefficient  $\tau$  between observed features of the sample dairy farms. Size: number of animals farmed, On-individuals: number of animals coming from other dairy premises, Off-individuals: number of animals sent toward other dairy farms, Individuals slaughtered: individuals sent toward slaughterhouse.

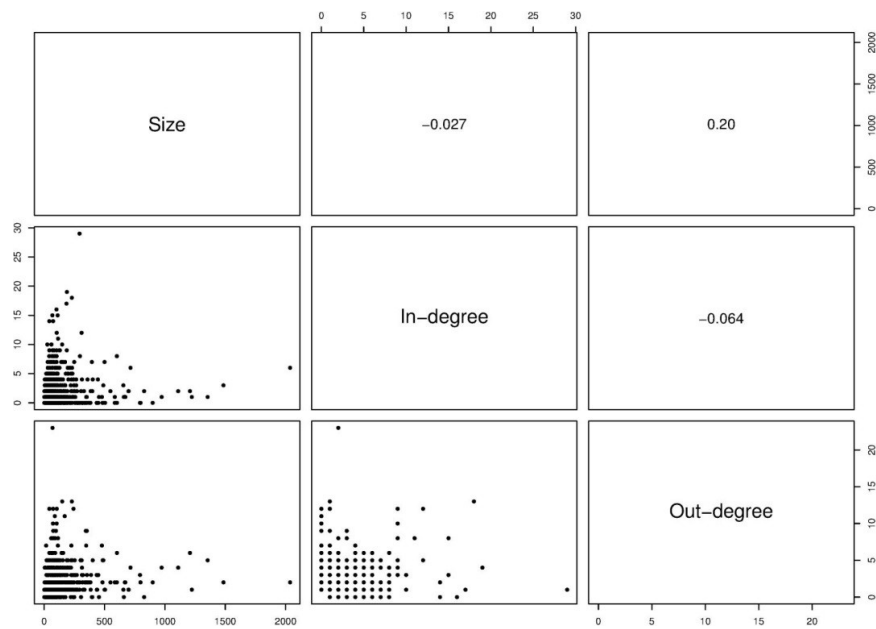


Figure S2: Kendall rank correlation coefficient  $\tau$  between observed features of the sample dairy farms. Size: number of animals farmed, In-degree: number of on-contacts from other dairy farms, Out-degree: number of off-contacts to other dairy farms.

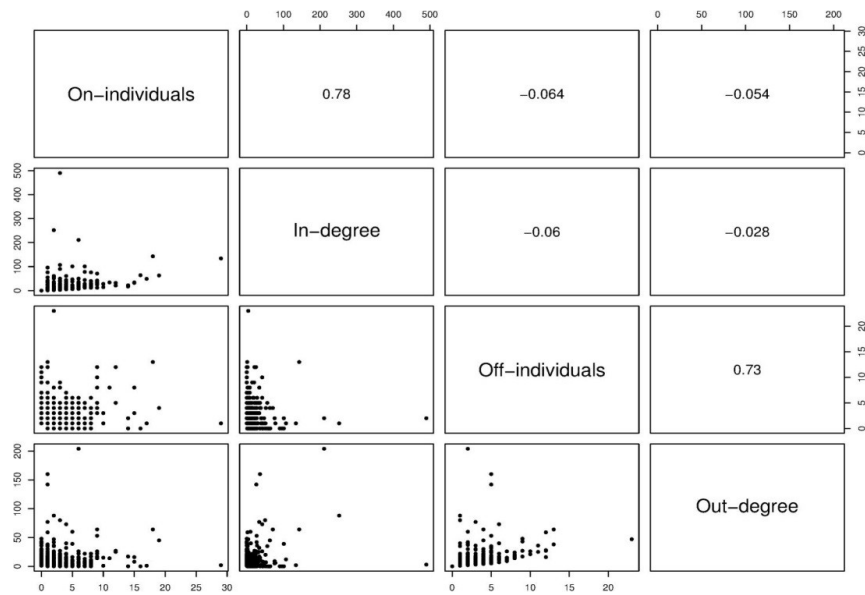


Figure S3: Kendall rank correlation coefficient  $\tau$  between observed features of the sample dairy farms. On-animals: number of animals coming from other dairy premises, In-degree: number of on-contacts from other dairy farms, Off-animals: number of animals sent toward other dairy farms, Out-degree: number of off-contacts to other dairy farms.

## **S2. Network generation algorithm**

To generate a synthetic network reproducing the main characteristics of the observed sample network, we independently assign to each of the 4353 farms: the size, as an extraction from the observed values of the Emilia Romagna dairy farms size (see Main text, Figure 1), and the in- and out-degree, as extractions from the estimated degree distributions (see Main text). In order to match the connections among nodes, firstly we ordered the nodes from the highest to the lowest value of in-degree (the choice to proceed using the in-degree or out-degree was not influential in the outcomes). Then, we randomly assigned to each in-stub of each node an out-stub of another node (excluding multiple connections for the same pair of nodes) until we ran out of in- or out-stubs. Then, we coupled the remaining in (or out) stubs with randomly selected nodes. Since the in-degree and out-degree values were drawn from the same distribution, the total number of in- and out-stubs (number of truncated connection per node) extracted in each simulation are usually similar, then the last step in the procedure generally involved a very limited number of stubs.

## **S3. Within-farm basic reproduction number estimate and sensitivity analysis**

### ***S3.1. $R_0$ literature estimates***

As shown in Section 2.4 in the main text, we used the mean of six estimated values derived from four different literature references to set a value of within-farm basic reproduction number,  $R_0$  (Brook-Pollock & Keeling, 2009; Augusto et al. 2011; Conlan et al., 2012; O'Hare et al., 2014; see Table S2 for details).

### S3.2. Constant vs. normally distributed $R_0$

To test the effect of among-farm  $R_0$  variability on simulation results, we compared the outcomes of simulations in the main text (in which the  $R_0$  value is drawn from a triangle distribution with mode 2.4 and bounded by the highest and the lowest literature values, see Table S2) and simulations in which  $R_0$  is kept constant in every farm (see main text). As showed in Table S3 (and Figure S4), the two scenarios display very similar output. In the simulations with constant  $R_0$  the average detection time decreased of only the 0.59%, while the number of multi-farm outbreaks increased of the 0.46%.

<i>Reference</i>	<i>Estimated <math>R_0</math> (or median)</i>	<i>Range</i>	<i>Notes</i>
<i>Brooks-Pollock &amp; Keeling 2009</i>	3.50	-	-
<i>Agusto et al. 2011</i>	2.08	-	-
<i>Conlan et al. 2012</i>	1.50	0.26 – 4.9	<i>Small farms (<math>\approx 30</math> individuals)</i>
<i>Conlan et al. 2012</i>	<b>4.90</b>	0.99 – 14.00	<i>Big farms (<math>\approx 400</math> individuals)</i>
<i>O'Hare et al. 2014</i>	<b>0.83</b>	0.6 – 1.4	<i>Low risk Areas</i>
<i>O'Hare et al. 2014</i>	1.62	1.3 – 1.9	<i>High Risk Areas</i>
<b><i>Average</i></b>	<b>2.40</b>	-	-

Table S2: Estimate of bTB within-farm  $R_0$  from different studies.

<i><math>R_0</math> Mean</i>	<i><math>R_0</math> Boundaries</i>	<i>Mean Time to Detection (months)</i>	<i>Mean number of infected farms</i>	<i>% of Multi-farm Epidemics</i>	<i>% of Epidemic over 10 years</i>
2.40	0.83 – 4.90	27.04	1.12	10.15%	0.10%
2.40	2.40 – 2.40	27.24	1.11	9.81%	0.12%

Table S3: The main indicators for the surveillance system performance. Results for 2 scenarios with different values of  $R_0$  standard error.

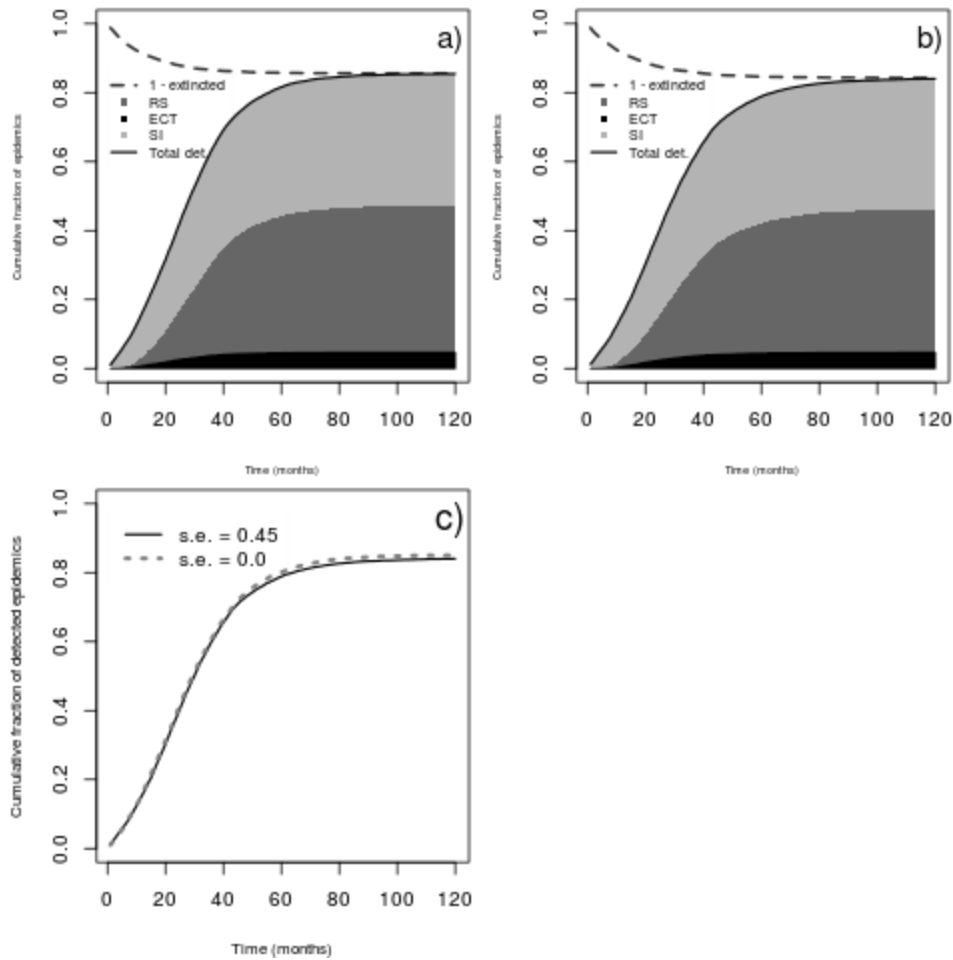


Figure S4: The cumulated fraction of simulated epidemics detected monthly by the three surveillance system components: slaughterhouse inspection (SI), routine surveillance (RS) and exchanged cattle testing (ECT) and the total detected epidemics (solid line). a)  $R_0$  mode = 2.40 and bounds 0.83 – 4.90; b)  $R_0 = 2.40$  and c) the comparison of the total detected epidemics in the two cases. Other parameters as in Figure 5 (main text).

### S3.3. Effect of different $R_0$ values on surveillance

Here, we tested the  $R_0$  estimation sensitivity of the on our results. We performed the analyses provided in Figure 5 of the main text by using different values of  $R_0$ . In particular, we used the higher ( $R_0 = 4.9$ ) and lower ( $R_0 = 0.83$ ) literature values, estimated respectively by Conlan et al. (2012) and O'Hare et al.

(2014). As showed in Table S4 and Figure S5, as  $R_0$  was higher, the time to detection of bTB epidemics was shorter. This was due to the increased number of infected individuals inside infected farms and, consequently, the increased chance of infected individuals to be sent to slaughterhouses or being exchanged.

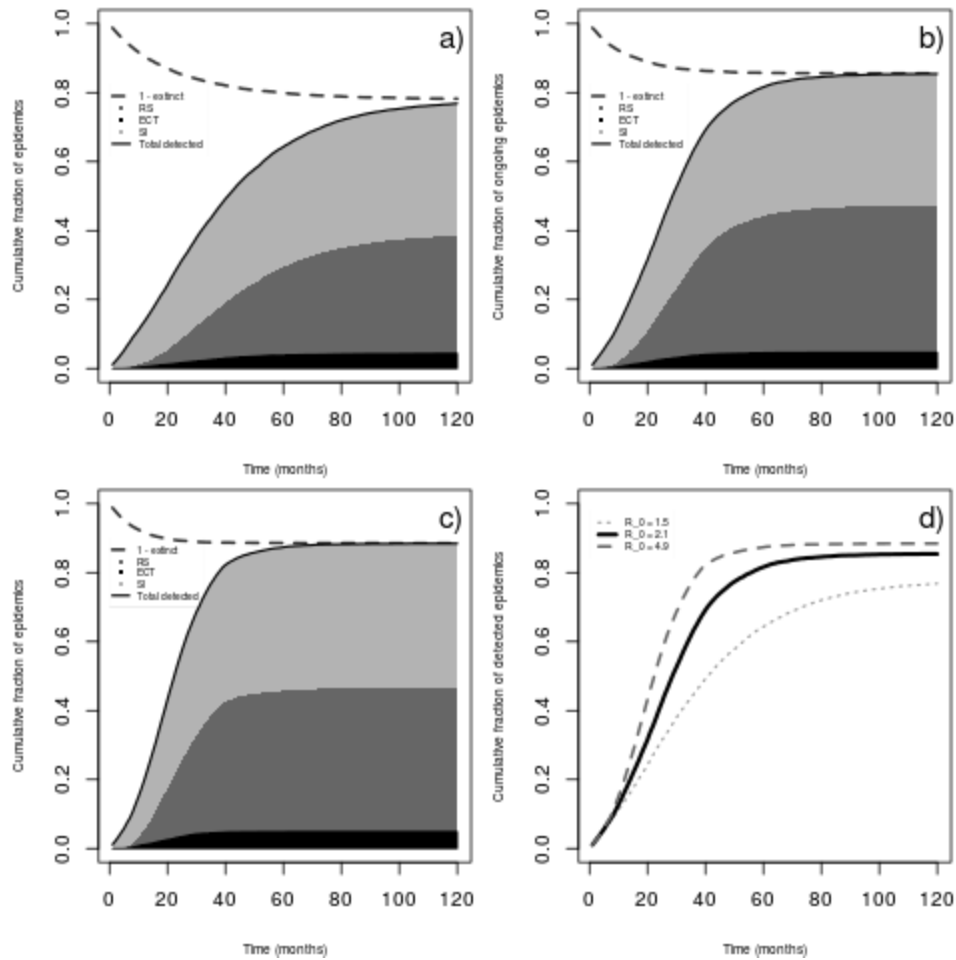


Figure S5: The cumulated fraction of simulated epidemics detected monthly by the three surveillance system components: slaughterhouse inspection (SI), routine surveillance (RS) and exchanged cattle testing (ECT) and the total detected epidemics (solid line). a)  $R_0 = 0.83$  (O'Hare et al. 2014); b)  $R_0 = 2.40$  (data), c)  $R_0 = 4.9$  (Conlan et al. 2012); and d) the comparison of the total detected epidemics in the three cases. Other parameters as in Figure 5 (main text).

$R_0$ Mean	Mean Time to Detection (months)	Mean number of infected farms	% of Multi-farm Epidemics	% of Epidemic over 10 years
0.83	35.91	1.11	9.70%	1.00%
2.40	27.04	1.12	10.15%	0.10%
4.90	21.80	1.12	10.05%	0.09%

Table S4: The main indicators for the surveillance system performance. Results for 3 scenarios with different values of  $R_0$  mean.

### S3.4. Transmission in Intermediary Trader Farms

Given the low number of animals inside the intermediary trader farms (ITF), and the short time that they spend there, we assumed in the main text that bTB transmission inside ITF was negligible.

However, in order to test the robustness of this assumption, we run 1000 simulations in which infected contacts inside the ITF were allowed. We assigned to each ITF variable-in-time size value, uniformly distributed between 0 and 5 (the 25<sup>th</sup> and 75<sup>th</sup> quantile of the observed value in data). As shown in Figure S6 and Table S5 the differences on bTB surveillance outcomes between the scenarios were negligible: in case assuming infections inside the ITFs the average detection time and the number of multi-farm outbreaks respectively increased of 1.34% and 1.05%.

$R_0$ Mean	Infection in ITFs	Mean Time to Detection (months)	Mean number of infected farms	% of Multi-farm Epidemics	% of Epidemic over 10 years
2.40	NO	27.04	1.12	10.15%	0.10%
2.40	YES	27.40	1.13	11.07%	0.04%

Table S5: The main indicators for the surveillance system performance. Results for 2 scenarios assuming the absence or the presence of bTB transmission inside intermediary trader farms.



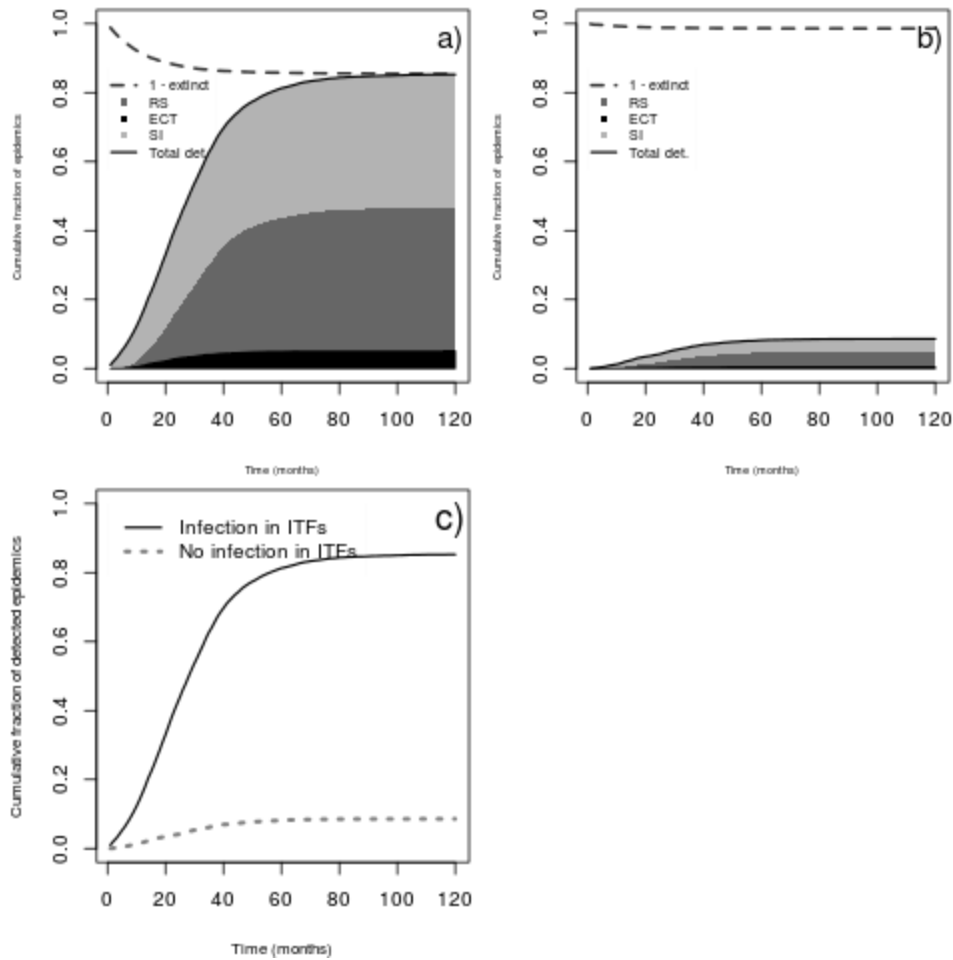


Figure S6: The cumulated fraction of simulated epidemics detected monthly by the three surveillance system components: slaughterhouse inspection (SI), routine surveillance (RS) and exchanged cattle testing (ECT) and the total detected epidemics (solid line). a) No infection in Intermediary Trader Farms (ITF); b) Infection inside ITF; and c) the comparison of the total detected epidemics in the two cases. Other parameters as in Figure 5 (main text).

#### S4. Alternative Skin-Test and Carcass Inspection Sensitivities

To test the robustness of our results to the control parameters, we ran additional simulations of bTB epidemics spread in the Emilia-Romagna Region for different values of the tuberculin Single Intradermal Tuberculin Test (SIT) and the carcass inspection sensitivities (which are characterized by

large uncertainty and variability, Asseged et al., 2004; Barlow et al., 1997; Brooks-Pollock and Keeling, 2009; Karolemeas et al., 2012; Smith et al., 2013; De La Rúa-Domenech et al., 2006; Fischer et al. 2005; Van Asseldonk et al., 2005; Welby et al., 2012,). For the same scenarios analysed in the main text, we assessed the effectiveness of the surveillance system under different hypotheses on test sensitivity by drawing the values from beta distributions having given means (0.8, 0.6 and 0.4) and coefficient of variations ( $CV = 0.25, 0.10$  and 0).

#### ***S4.1. Alternative Coefficient of Variation in sensitivities***

Firstly, we simulated different CV of the beta distributions from which the sensitivity values of skin-tests and slaughterhouse inspections are drawn. The rationale relies in taking into account for the operator effect (due to inter-individual variation among different veterinaries who apply the skin-test on the live cattle or on the operators that inspect the carcasses at the slaughterhouse looking for bTB typical lesions). Then, for a fixed mean value of the distribution ( $= 0.60$ ), we simulated 3 different values of CV ( $= 0.25, 0.10, 0$ ). In Table S6 we showed the results of simulations for the current surveillance scenario. The performance indicators show that there were not significant differences among the performances of the surveillance system in all three cases (Figure S7, d), thus, highlighting that the main results obtained with the model were not affected by the assumptions of the sensitivity distributions.

<i>Sensitivity Coefficient Variation</i>	<i>Sensitivity Mean</i>	<i>Mean Time to Detection (months)</i>	<i>Mean number of infected farms</i>	<i>% of Multi-farm Epidemics</i>	<i>% of Epidemic over 10 years</i>
0.00	0.60	26.86	1.11	9.92%	0.09%
0.10	0.60	27.04	1.12	10.15%	0.10%
0.25	0.60	27.09	1.11	9.87%	0.11%

Table S6: The main indicators for the surveillance system performance. Results for 3 cases varying the beta distribution coefficient of variation for the test/inspection sensitivity drawing.

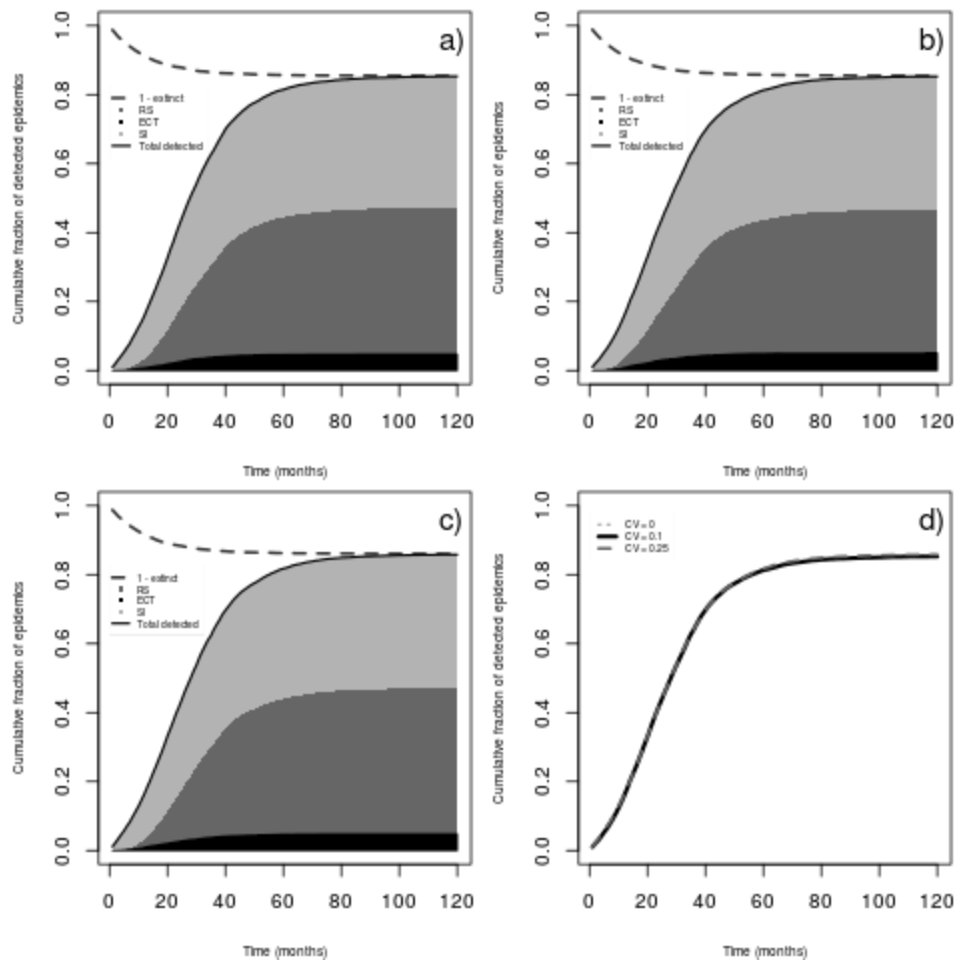


Figure S7: The cumulative fraction of simulated epidemics detected monthly by the three surveillance system components: slaughterhouse inspection (SI), routine surveillance (RS) and exchanged cattle testing (ECT) and the total detected epidemics (solid line). Three different coefficient of variation for the beta distribution (mean 0.60) from which we draw the

*test and inspection sensitivity: a) 0.00, b) 0.10, c) 0.25 and d) the comparison of the total detected epidemics in the three cases. Other parameters as in Figure 5 (main text).*

#### ***S4.2. Alternative Mean values of Sensitivity***

Similarly than in the case of CV, we simulated bTB spread by using different values for the mean sensitivity of the surveillance methods. The rationale relies on the uncertainty on the estimation of both carcass inspection and tuberculin skin test sensitivities (see the main text for details). Specifically, we simulated 3 different mean values of sensitivity (= 0.8, 0.6, 0.4). We performed the analyses in 4 scenarios: the current surveillance system (Scenario 1); and the scenarios obtained by removing one of the surveillance components at the time: removing routine surveillance (RS) component, Scenario 2a; removing exchanged cattle testing (ECT) component, Scenario 2b; and removing slaughterhouse inspection (SI) component, Scenario 2c (see the Main text for details).

As pointed out in Table S7, we did not observe significant differences for two of the indicators (i.e. the fraction of undetected epidemics and mean infected farms). On the other hands, the number of multi-farms epidemics increased in all the 4 scenarios by diminishing the mean value of sensitivity. In particular, in the worst case scenario (Scenario 2a with mean = 0.4), we observed that in almost one over five cases, the epidemic infected more than one farm. Comparing the detection time of the epidemic for different mean values of sensitivity, we observed in the 4 scenarios an increase of 9-11%, on average (from sensitivity = 0.80 to 0.60) and an increase of 14-16% (from sensitivity = 0.60 to 0.40). In all the four scenarios we observed an earlier plateau in the cumulative fraction of epidemics detected monthly (Figures S8, S9, S10 and S11) in the higher sensitivity case.

Scenario	Sensitivity Mean	Sensitivity Coefficient Variation	Mean Time to Detection (months)	Mean number of infected farms	% of Multi-farm Epidemics	% of Epidemic over 10 years
Complete surveillance (Scenario 1)	0.40	0.10	31.49	1.15	12.58%	0.28%
	0.60	0.10	27.04	1.12	10.15%	0.10%
	0.80	0.10	24.34	1.09	8.51%	0.07%
No routine surveillance (Scenario 2a)	0.40	0.10	40.11	1.27	20.42%	1.88%
	0.60	0.10	33.77	1.20	16.80%	0.70%
	0.80	0.10	29.31	1.17	15.05%	0.44%
No exchanged cattle test (Scenario 2b)	0.40	0.10	32.08	1.16	12.67%	0.26%
	0.60	0.10	27.52	1.11	9.61%	0.14%
	0.80	0.10	24.45	1.10	8.60%	0.14%
No slaughterhouse inspection (Scenario 2c)	0.40	0.10	40.96	1.19	14.98%	0.65%
	0.60	0.10	35.45	1.15	12.01%	0.18%
	0.80	0.10	32.20	1.11	10.12%	0.10%

Table S7: The main indicator for the surveillance system performance. Results for three different values of the beta distribution mean for the test/inspection sensitivity drawing, in four different surveillance system scenarios.

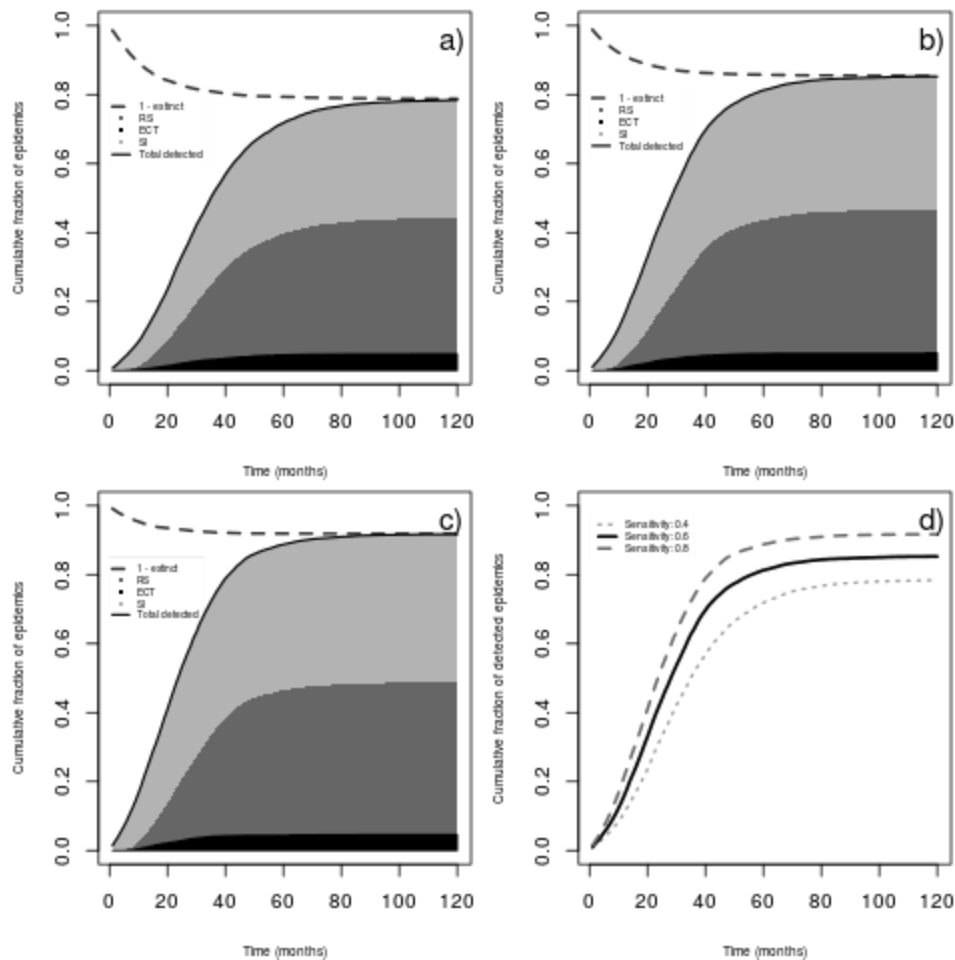


Figure S8: The cumulative fraction of simulated epidemics detected monthly by the three surveillance system components:

slaughterhouse inspection (SI), routine surveillance (RS) and exchanged cattle testing (ECT) and the total detected epidemics (solid line). We used three different mean for the beta distribution (coefficient of variation 0.10) from which we draw the skin-test and inspection sensitivity: a) 0.40, b) 0.60, c) 0.80 and d) the comparison of the total detected epidemics in the three cases. Other parameters as in Figure 5 (main text).

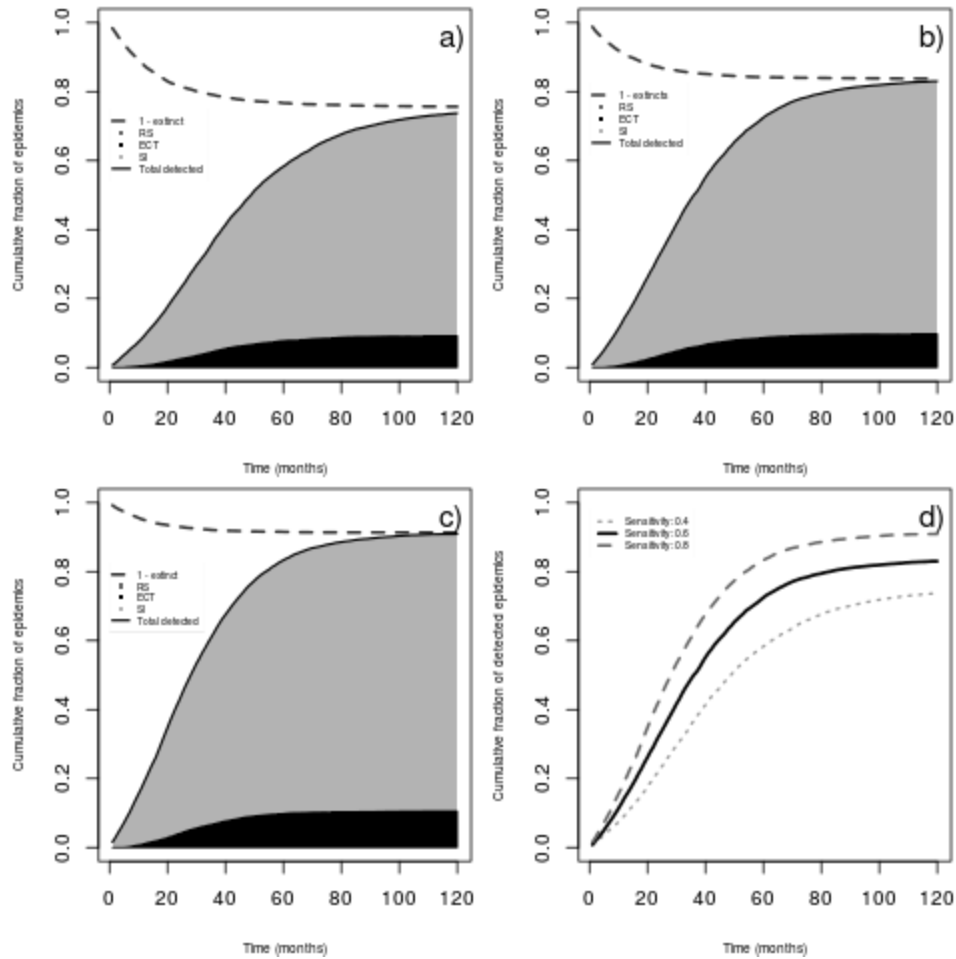


Figure S9: The cumulative fraction of simulated epidemics monthly detected by the slaughterhouse inspection (SI) combined with the exchanged cattle testing (ECT) and the total detected epidemics (solid line). We used three different mean for the beta distribution (coefficient of variation 0.10) from which we draw the skin-test and inspection sensitivity: a) 0.40, b) 0.60, c) 0.80 and d) the comparison of the total detected epidemics in the three cases. Other parameters as in Figure 5 (main text).

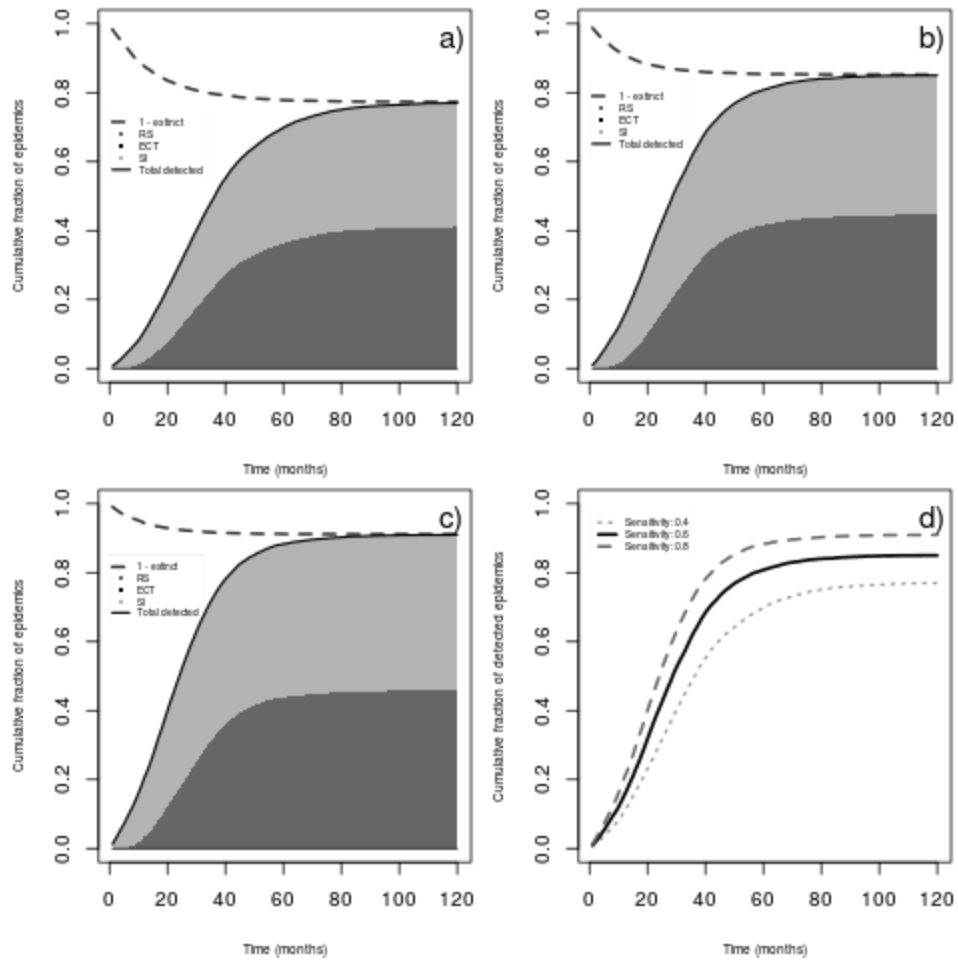


Figure S10: The cumulative fraction of simulated epidemics monthly detected by the slaughterhouse inspection (SI) combined with the routine surveillance (RS) and the total detected epidemics (solid line). We used three different mean for the beta distribution (coefficient of variation 0.10) from which we draw the skin-test and inspection sensitivity: a) 0.40, b) 0.60, c) 0.80 and d) the comparison of the total detected epidemics in the three cases. Other parameters as in Figure 5 (main text).

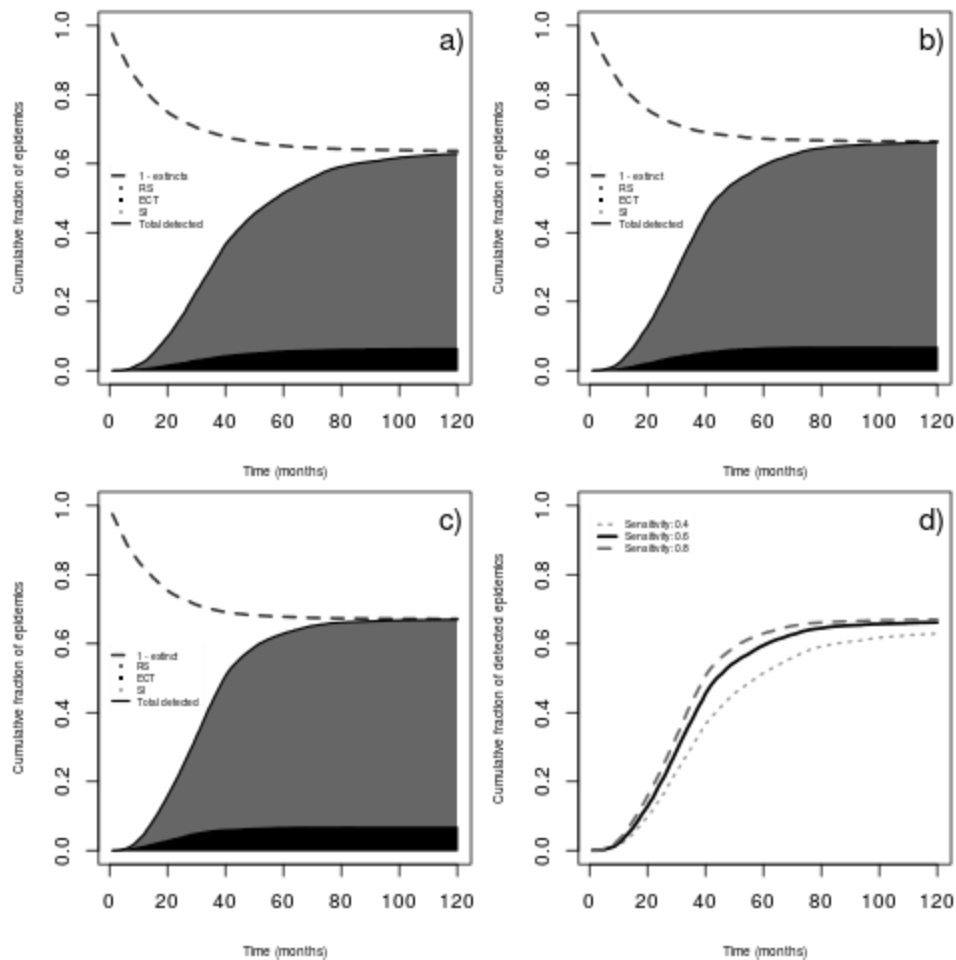


Figure S11: The cumulative fraction of simulated epidemics monthly detected by the exchanged cattle testing (ECT) combined with the routine surveillance (RS) and the total detected epidemics (solid line). We used three different mean for the beta distribution (coefficient of variation 0.10) from which we draw the skin-test and inspection sensitivity: a) 0.40, b) 0.60, c) 0.80 and d) the comparison of the total detected epidemics in the three cases. Other parameters as in Figure 5 (main text).

The increase in the mean time to detection is not negligible, and the consequences of a late detection could make the disease very difficult to eradicate and regular commercial activities (such as trading) very slow to recover, with a big impact on the local economy. This result highlights the importance of the awareness of the operators performing tests, in order to apply the controls in the best possible



conditions.

### ***S4.3. Alternative Mean Sensitivity in the Single Component Cases***

In this section we showed the effect of the mean sensitivity on alternative surveillance systems based on one component only (among RS, ECT and SI). In Table S8 we reported the results for all indicators taken into account. As expected we observed a general increase in the performance of all the three components by increasing the mean skin test or inspection sensitivity. The improvement in the performance is particularly significant for the mean time to bTB detection. More importantly, Table S8 showed that the ranking in the performance of SI and RS strongly depends on the assumptions on the mean values of sensitivity, while ECT always performed worse in the analysed scenarios.

<i>Scenario</i>	<i>Sensitivity Mean</i>	<i>Sensitivity Coefficient Variation</i>	<i>Mean Time to Detection (months)</i>	<i>Mean number of infected farms</i>	<i>% of Multi-farm Epidemics</i>	<i>% of Epidemic over 10 years</i>
Routine surveillance only	0.40	0.10	43.18	1.2303	15.45%	0.62%
	0.60	0.10	37.23	1.1595	11.94%	0.29%
	0.80	0.10	33.67	1.1362	10.60%	0.06%
Exchanged Cattle Test only	0.40	0.10	64.62	1.6869	37.82%	29.26%
	0.60	0.10	59.70	1.5552	37.07%	25.70%
	0.80	0.10	56.04	1.5074	38.61%	22.82%
Slaughterhouse Inspection only	0.40	0.10	41.81	1.3088	20.14%	1.68%
	0.60	0.10	35.18	1.2328	17.18%	0.90%
	0.80	0.10	31.14	1.2053	15.56%	0.40%

*Table S8: The main indicators for the surveillance system performance. Results for three different surveillance system components alone, varying the skin-test and carcass inspection sensitivity.*

### ***S4.4. Different mean sensitivity for skin-test and carcass inspection***

In this section we explored the effect on surveillance performances of assigning different mean sensitivities to the SIT skin-tests (RS and ECT) and to carcass inspection (SI). Specifically, we run simulations assigning to the carcass inspection a lower sensitivity value (= 0.4) than the skin-tests (=

0.6). The assumption relies on the uncertainty around the carcass inspection sensitivity (see main text and references therein). The results of the simulations are summarized in Table S9 and Figure S12. Table S9 shows that the main indicators of the surveillance performance do not substantially change in the case of lower SI sensitivity. However, Figure S12 shows a decrease [increase] in the fraction of epidemics detected by carcass inspection [routine skin-test] in the case of lower SI sensitivity. These results suggest that RS was able to eventually compensate for the poor sensitivity in SI, while ECT still performed poorly compared to SI also in the case of lower carcass inspection sensitivity.

<i>Mean Skin-Test Sensitivity</i>	<i>Mean Carcass Inspection Sensitivity</i>	<i>Mean Time to Detection (months)</i>	<i>Mean number of infected farms</i>	<i>% of Multi-farm Epidemics</i>	<i>% of Epidemic over 10 years</i>
0.60	0.60	27.04	1.12	10.15%	0.10%
0.60	0.40	28.88	1.12	10.41%	0.16%

*Table S9: The main indicators for the surveillance system performance. Results in two scenarios with different carcass inspection sensitivities.*

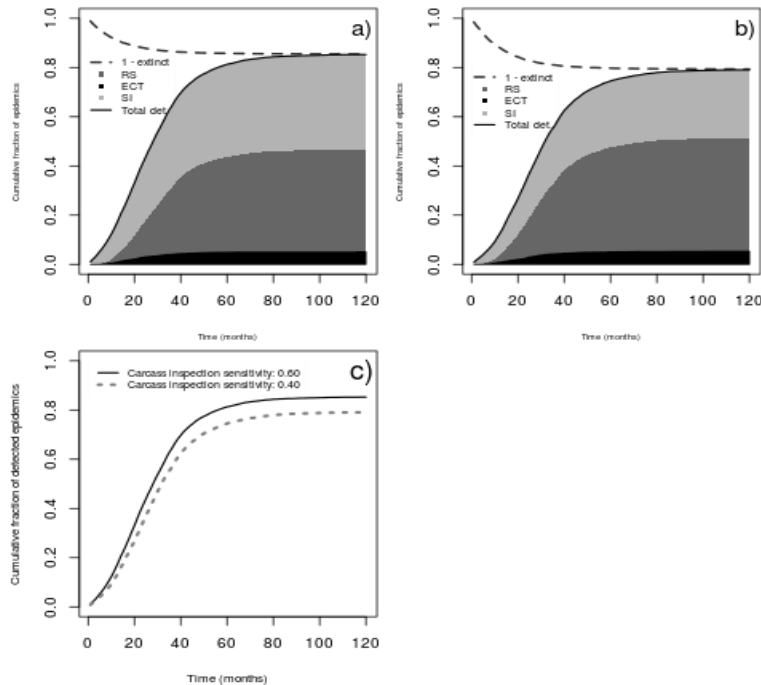


Figure S12: The cumulative fraction of simulated epidemics monthly detected by the three surveillance system components: slaughterhouse inspection (SI), routine surveillance (RS) and exchanged cattle testing (ECT) and the total detected epidemics (solid line). Two different cases of carcass inspection sensitivity: a) 0.6, and b) 0.4. Panel (c) shows the cumulative fraction of detected epidemics in the two scenarios. Other parameters as in Figure 5 (main text).

## S5. Alternative Routine Test Turnaround Period

In our model we used the routine surveillance (RS) turnaround time (i.e. the time needed for the health authorities to monitor the entire regional farm system) fixed at 3 years, as currently set in Emilia-Romagna region. The Regional administration increased the RS turnaround period from 2 to 3 years in 2010. In order to have a quantitative evaluation of the possible consequences of this choice we run our simulations setting the turnaround period at 2 years, and comparing the results with the 3-years turnaround time. Moreover, we run simulations setting RS turnaround period at 4 years. The indicators did not report big differences in the three cases (see Table S10). The detection time increases of the

2.1% if we set the RS turnaround time to 4 years, and decrease of the 0.2% if we set it at 2 years. By looking at the cumulative fraction of epidemic monthly detected (Figure S13, d) we can not detect any substantial difference among the three cases, suggesting that the surveillance system performance would not be affected by the recent increase of the turnaround period from 2 to 3 years.

<i>Routine Surveillance Recurrence (years)</i>	<i>Health Authorities Test Effort (farm/year)</i>	<i>Sensitivity Mean</i>	<i>Sensitivity Coefficient Variation</i>	<i>Mean Time to Detection (months)</i>	<i>Mean number of infected farms</i>	<i>% of Multi-farm Epidemics</i>	<i>% of Epidemic over 10 years</i>
2	2187	0.60	0.10	27.08	1.11	9.76%	0.11%
3	1458	0.60	0.10	27.04	1.12	10.15%	0.10%
4	1093	0.60	0.10	26.48	1.12	10.39%	0.11%

Table S10: The main indicators for the surveillance system performance. Results in three cases using different on-farm routine skin-testing (RS) turnaround period.

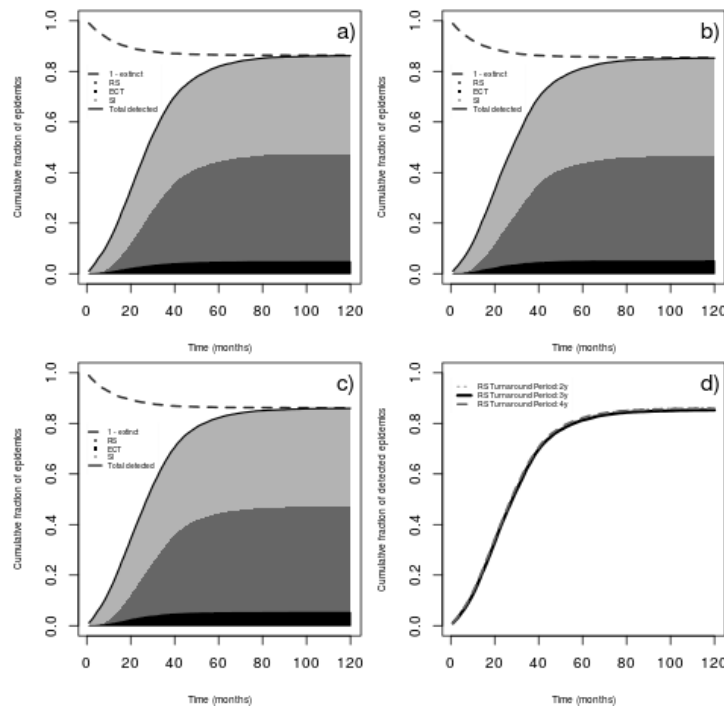


Figure S13: The cumulative fraction of simulated epidemics monthly detected by the three surveillance system components: slaughterhouse inspection (SI), routine surveillance (RS) and exchanged cattle testing (ECT) and the total detected epidemics (solid line). Three different cases of turnaround period for the RS: a) 2 years, b) 3 years, c) 4 years and d) the comparison of the total detected epidemics in the three cases. Other parameters as in Figure 5 (main text).

## S6. Single methods analysis

In this section we analysed the performance of a surveillance system based on on-farm routine surveillance (RS) and on slaughterhouse inspection (SI) only. In particular, we compared the output obtained with the current surveillance system (Scenario 1 in the main text) – composed by SI+RS+ECT – with the Scenario 2b in the main text – composed by SI+RS – and a surveillance system based on one component only (alternatively RS or SI).

As pointed out in Table S11 and in Figure S14 there were not significant differences in the surveillance system performance by eliminating the ECT component (see main text for details). However, by using a surveillance strategy based on one component only, the performances substantially decreased. In particular, by eliminating the SI component the time to detection substantially increased, while by eliminating the RS is the number of multi-farm outbreaks that increased mostly. This suggested that these two components compensate for each other and, thus, that they should be used together to have an effective surveillance system for dairy farms in ER.

<i>Surveillance</i>	<i>Sensitivity Mean</i>	<i>Sensitivity Coefficient Variation</i>	<i>Mean Time to Detection (months)</i>	<i>Mean number of infected farms</i>	<i>% of Multi-farm Epidemics</i>	<i>% of Epidemic over 10 years</i>
Complete (SI+RS+ECT)	0.60	0.10	27.04	1.12	10.15%	0.10%
SI + RS	0.60	0.10	27.52	1.11	9.61%	0.14%
RS	0.60	0.10	37.23	1.16	11.94%	0.29%
SI	0.60	0.10	35.18	1.23	17.18%	0.90%

*Table S11: The main indicators for the surveillance system performance. Results for three surveillance system scenarios, hypothesizing the integrated surveillance system composed by: three components (slaughterhouse inspection, on-farm routine skin-testing, and exchanged cattle skin-testing (SI+RS+ECT), by two components (SI+RS), and by one component (RS or SI).*

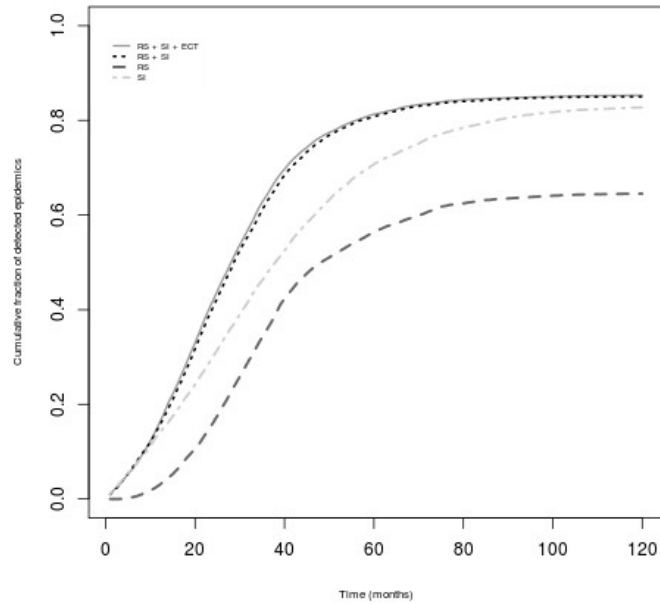


Figure S14: The comparison of the cumulative fraction of simulated epidemics monthly detected by the surveillance system in three cases: assuming a system composed by all three components (slaughterhouse inspection, routine on-farm skin-testing and exchanged cattle skin-testing SI+RS+ECT; solid line), by two components (SI+RS; dotted line), and by one component (RS only, dashed line; SI only, dashed-dotted line). Other parameters as in Figure 5 (main text).

## **Chapter III: Network analysis of direct and indirect contacts between dairy farms in the Province of Parma (Emilia-Romagna, IT)**

### **1. Introduction**

The problem of infectious diseases spread in livestock is still an open challenge for epidemiologists, disease ecologists, veterinarians and health administrations. Infections in livestock cause severe implications for animal health and for economic sustainability of farming systems. Further implications also exist for human health (in particular food safety) and for wildlife species (Daszak et al., 2000).

Because the contact structure existing between host individuals is a key determinant to comprehend the ability of infectious diseases to spread in a given population (Woolhouse et al., 2005), the study of infections transmission through network analysis techniques has recently become a very prolific research field (Keeling et al., 2010; Watts and Strogatz, 1998). In particular, networks can successfully represent farming systems, characterized by many nodes, i.e. farms, and links between them, i.e. cattle movements, indirect contacts, etc. (Dubè et al., 2008; Eames et al., 2014). Live animals exchange is considered the most effective route of disease transmission among farms (Fevre et al., 2006; Gilbert et al., 2005; Sanson et al., 1993). For this reason several countries have developed extensive databases where farmed animal movements are tracked. Cattle movement data have provided the knowledge background for several studies investigating the main features of contact networks structure and their implications for the potential disease spread (Bajardi et al., 2011; Noremark et al, 2011; Volkova et al, 2010).

However, diseases such as the FMD, classical swine fever, bovine viral diarrhoea and Aujeszky's disease, can also spread through wind-borne spores and fomites. The contacts mediated by external operators (or fomites) are usually defined as indirect contacts (Noremark et al, 2013). In fact, an operator may be contaminated during a visits in an infected farm, then following visits may result in

transmission of infectious agent to other farms. This includes sharing of equipment, movement of people and vehicles (Brennan et al., 2008). Conversely than livestock movement, the understanding of the role of fomites on livestock diseases spread has received less attention so far. This gap of knowledge is mainly due to the multiplicity and the complex nature of the potentially infectious indirect contacts. For these reasons data retrieval of indirect contacts on a temporal and spatial scale which can be significant from an epidemiological point of view proved to be a challenging task. Until now the understanding acquired on this topic has mainly relied on voluntary questionnaires proposed to farmers regarding farm visits in a limited time span, few weeks, and/or at a small spatial scale, tens to hundreds farms involved (Bates et al., 2001; Brennan et al., 2008; Nielen et al. 1996; Sanson et al., 1993). The questionnaires usually focus on the visits received within farms in a given time period. Because of their voluntary nature, the return rate of the questionnaires is usually low. As a consequence, these studies are more suitable in evaluating the frequency of different type of visit inside farms than investigating the structure of the farm network in a given area. Despite the described limitations, some studies applied network analysis techniques on questionnaire-based data (Brennan et al., 2008; Dommergues et al., 2012).

In recent years, especially after the 2001 FMD outbreak in the UK, many studies have been focusing on including indirect contacts in epidemiological models for disease spread in livestock (see Keeling, 2005, and references there in). The introduction of disease transmission through fomites in epidemic models for FMD was a consequence of the observed disease spread in UK farms despite the banning of cattle movements (Gibbens et al., 2001). Usually, indirect routes of transmission in epidemic models have been represented using risk kernel functions (Ferguson et al., 2001; Keeling et al., 2002). Local risk kernels are functions that assign the probability  $p_{ij}$  of disease spread from farm  $i$  to farm  $j$  as a function of the inter-farm distance. However, the approach based on kernel functions was unable to suitably describe the differences among indirect routes of transmission and to take into account for



other spatial heterogeneities.

The goal of this work is to assess the implications of between-farms indirect contacts in a potential epidemic transmission. In particular, we focused on the interplay of direct and indirect contacts in disease spread. We pursued this scope by: (i) assessing the structure of the indirect contact farm networks; (ii) comparing it with the direct contact network, evaluating the eventual differences or similarities.

The case of study was the Province of Parma (Emilia Romagna, Italy) dairy farms system. The study area accounts for more than 1300 dairy farms and over 180 dairy factories operating.

We investigated the structure of both direct (i.e. cattle movements) and indirect contacts between farms. In particular, in the category of indirect contacts, we considered: (i) government veterinarians (or inspectors), (ii) private veterinarians, and (iii) milk trucks. The first category, government inspectors, are veterinarians officially sent by the Province health administration for various purposes, such as animal health inspection and disease surveillance. We considered separately contacts due to government and private veterinarians since they perform different activities inside farms and because of the different type of data we could access: the actual on-farm visits list for government inspectors, and the list of drug prescribed to each farm by private veterinarians, as a proxy measure for private veterinarians visits.

We firstly described all contact network, , whether direct or indirect, using the opportune statistics and networks analysis metrics. Then, we focused on the single farms role in potential disease spread. We recognized which farm could act as a hub (or super-spreader) in the direct and indirect contact cases, and we then described the differences among these two cases. Finally, we measured how the farms ranking changed, in respect to their centrality values, assuming different levels of infection probability for indirect contacts.

## **2. Materials and Methods**

### ***2.1. Data description and network building***

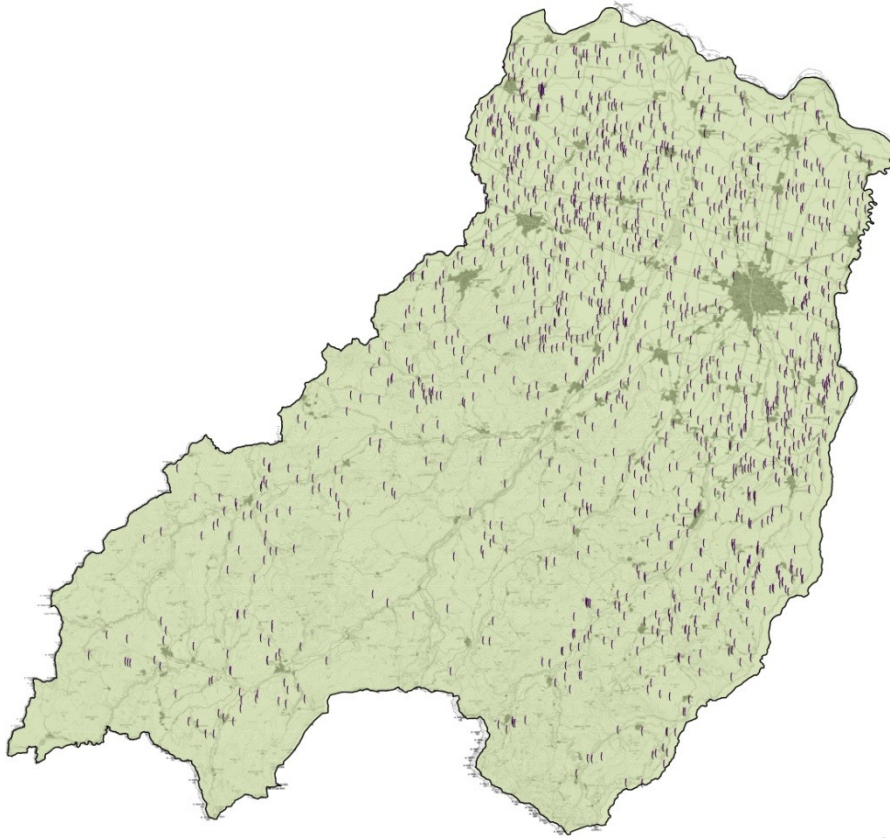
The case of study is represented by the dairy farm system of the Parma Province (Emilia Romagna, Italy) for the year 2013. In the Province of Parma, the dairy sector is more developed in respect to the beef one (1349 dairy farms vs. 423 beef farms actives in 2013). Moreover, dairy farms have several characteristic that make them more interesting from an epidemiological point of view (especially for disease transmission through fomites). Firstly, dairy cattle live substantially longer than beef cattle, i.e. up to 5 vs. 2 years respectively, thus veterinary healthcare management is more focused on the first ones. Secondly, by living longer, dairy cattle have more chances to develop chronic infectious diseases, and such as bovine tuberculosis and Johne's disease. Thirdly, milk trucks (which can represent a high source of contamination) visit only dairy farms.

All statistical analyses, network analyses and drawings were performed using the software R with “igraph”, “spatstat”, “tnet”, “ggplot2”, “reshape2” and “plyr” packages (<http://www.r-project.com>).

#### ***2.1.1. Dairy farms data***

The dairy farms were selected from the Italian National Bovine database (BDN), among all cattle farms of the Province of Parma, on the basis of their production sector. The resulting number of dairy farms in 2013 was 1349.

The available data for each dairy farm are: a unique identifier code, the coordinates in space (Figure 1) and the size (i.e. the number of animals farmed). Specifically, the size records referred to the number of animals farmed on the 31th December 2012 and on the 31th December 2013. We defined the farm size as the mean of the two values.



*Figure 1: Dairy farms in the Province of Parma (Emilia-Romagna region, Italy)*

### **2.1.2. Cattle movement**

Cattle movements data were obtained from the BDN. For each dairy farm we collected data on all cattle movements from other dairy farms of the Parma Province in 2013. We also took into account for the trader intermediated movements among dairy farms.

The total number of recorded individual movements was 16,647. Each individual record consisted in a unique identifier code for the animal, the birth date, sex and race of the animal, identifier codes of the farms of origin and destination, codes for farms production sector (beef, dairy or mixed), and the movement date.

Each contact between farms due to cattle movements corresponds to an edge in the network describing the direct contacts and, then, to a non-zero value in the corresponding adjacency matrix. According to

the literature, we weighted the edges using three different criteria: (i) unweighted (presence or absence of the contact, corresponding to 1 or 0 in the adjacency matrix); (ii) weighted on the number of cattle shipments, or batches; and (iii) weighted on the number of animals exchanged (Volkova et al., 2010).

### **2.1.3. Government inspectors**

Data on government veterinarians, or inspectors, visits were provided by the Parma Province health administration (AUSL: Azienda Unità Sanitaria Locale). The database collected all the inspections that the public health administration performed on dairy farms during the year 2013. The dataset comprehended data on 7950 on-farm visits performed by 62 veterinarians. For each visit the record contains the unique identifier farm code, the veterinarian name and the visit date.

In order to build the inspectors contact network, we assigned a directed edge connecting a given farm with those visited at later stages by the same veterinarian in a given time span. This time span represents the maximum period in which the veterinarian or its equipment can remain contaminated by the pathogenic agent (contamination period,  $h$ ). The contamination period strongly depends on the pathogen survival period. There is a lot of uncertainty and variability on the survival of viruses and bacteria in environmental conditions (Bartley et al., 2002). To assess the effect of the contamination period on the shape of the government inspectors network, we computed the basic network features for different  $h$  values: 0 (i.e. within the same day), 3, 7, 15, 30 and 60 days. We used the network built with  $h = 15$  days as the benchmark case.

Since the order of the veterinarians visits in the same day was not specified, we simulated the itinerary of inspectors within the same day. Specifically, we built 1,000 simulated government inspectors networks with random itineraries of same-day visits. We used the simulated networks to test the effect of the uncertainty on the government inspectors network measures.

Analogously than in the cattle movement network, we developed two version of the government

inspector network: (i) unweighted, and (ii) weighted on the frequency of contacts, i.e. the number of time that a connection between two farms is realized within the year 2013.

#### ***2.1.4 Proxy indirect contacts data***

Data on private veterinarians and milk trucks visits in dairy farms were not directly observed. However, we used proxy information to derive the contact structure among farms due to these potential routes of transmission.

Data on private veterinarians were obtained from the list of cattle drug prescriptions by veterinarians collected by the Parma Province AUSL for year 2013. Each record contains the veterinarian name, the farm unique identifier code, and the date in which the prescription, for one or many drugs, was made. The dataset totally accounted for 11,611 prescriptions performed by 181 veterinarians. Since veterinarians do not prescribe drugs in each on-farm visit, the prescription database is under-representative in respect to the real number of private veterinarian on-farm visits. However, is a common practice for most dairy farms to rely on a limited number of veterinarians, that periodically visit the farm, about 1 to 4 time per month (Bates et al., 2001; Mc Reynolds et al., 2014; Matteucci and Massirio, 1999; Noremark et al., 2013; Richert et al, 2013). We assumed that farms was periodically visited by each veterinarian observed among their prescription records and that all farms served by the same veterinarian are fully connected among them.

Dairy-factories records were available from livestock production service website (<http://agrinet.crpa.it>). Each record contains the name of the dairy factory, the farm unique identifier code and the date of the last milk deliver from such a farm to the dairy factory. We used this database to identify the farms delivering milk to dairy factories in 2013. Then, we built a network where farms delivering milk to the same dairy factory are connected by its trucks. We recorded 1189 delivering farms and 182 dairy factories operating in the Province of Parma.

For both private veterinaries and milk trucks, we built the contact network assuming each farm belonging to the same pool is completely connected with other farms of the same pool.

## ***2.2. Structural properties of single layer networks and contacts distance***

We computed some important network features, including (i) the links density; (ii) the in- and out-degree; (iii) the giant strongly connected component (GSCC); (iv) the global clustering coefficient; and (v) the in- and out-closeness centrality.

These measure are defined as follows:

- (i) Links density in a network of  $n$  nodes is the fraction of observed over the possible number of links (Wasserman and Faust, 1994). This measure reflects whether nodes are well connected among each other, or not. In low density (or sparse) networks spreading processes are more difficult because of the lack of connections among nodes;
- (ii) The GSCC is defined as the biggest portion of the network in which each node is reachable from any other node. This measure has been proposed has the lower bound of a maximum epidemic size, and the distribution of the strongly connected components of a network generally represents the distribution of the epidemic size (Kao et al., 2006). In case of undirected networks GSCC is simply defined as giant connected component (GCC);
- (iii) The clustering coefficient is a measure of how clustered the network is, in other words it measure the probability that two farms, that are both connected to a third one, are connected to each other. The measure ranges from 0, not clustered, to 1, completely clustered (Watts and Strogatz, 1998);
- (iv) The  $i$ -th farm degrees are defined as the number of farms from which farm  $i$  receives (in-degree) and the number of farms to which farm  $i$  sends cattle (out-degree). This is an important and intuitive measure of farm centrality, i.e. the importance of a node within the network;
- (v) The closeness centrality, measures the mean distance of a given node to all the other nodes in the

network. As the movement and the government inspector networks are directed, we considered two closeness centrality measures. The in-closeness centrality of farms  $i$  as:

$$C_i^I = \sum_{j \neq i} \frac{1}{g_{ji}}; \quad (1)$$

in which  $g_{ji}$  is the shortest path length from node  $j$  to node  $i$ ; and the out-closeness centrality as

$$C_i^O = \sum_{j \neq i} \frac{1}{g_{ij}}. \quad (2)$$

By using this definition we could calculate the closeness centrality also for those farms that did not reach the entire network (Newmann, 2010). A generally high closeness centrality means that farms are in closer contacts with the rest of the network, so that they could be reached by [in-] or spread [out-] the infection through few steps (Buttner et al., 2013).

As the coordinates of each farm was provided, we could calculate the Euclidean distance between each pair of farms. We computed the Euclidean above other distances since Savill et al. (2006) showed that straight distance was a better predictor of the risk of transmission in respect to the shortest or quickest road length between farms. For each of the four different type contact networks we calculated the median between farms Euclidean distance. Moreover, we compared the distances of edges grouped in respect to the number of different networks (from 0 to 4) in which they are observed.

We calculated also the number of totally isolated farms for each one of the four networks and, moreover, in both the aggregated indirect contact network (i.e. the government inspectors, private veterinarians, and dairy truck networks joined), and the overall aggregated network (i.e. joining the indirect contact aggregated network and the direct contact network).

### ***2.3. Hubs detection in direct and indirect contact networks***

Centrality measures are very important for surveillance and contingency planning. They are often used to identify specific farms which are highly connected and, thus, represent an higher epidemic risk for the entire system (Christley et al., 2005). Graph theory jargon defines highly connected nodes as hubs, while in epidemiology they are known as super-spreaders (Looyd-Smith et al., 2005). We considered as hubs the 5% of farms with the highest centrality values (i.e. over the 95<sup>th</sup> percentile) for both in and out measures. To compare the different transmission routes, we used the weighted version of the respective networks which take into account for the frequency of the contacts. We considered the direct contacts network (adjacency matrix **M**) edges weighted on the number of animals exchanged between two farms, while the weights of indirect contacts correspond to the yearly frequency at which a given contact between two farms was realized during the year 2013. As explained in section 2.1.3, we weighted government inspector network (**G**) for the times an inspector visited the same two farms within the contagion period  $h$  (15 days, in the case of this analysis). In case of private veterinarians network (**V**) we collected indirect information of on-farm visits frequency. Thus, we estimated the frequency of the veterinarian visits in dairy farms from the literature, corresponding to 27 (range 13 - 44) visits a year (Bates et al., 2001; Mc Reynolds et al., 2014; Matteucci and Massirio, 1999; Noremark et al., 2013; Richert et al., 2013). We assumed the same visit frequency for each farm, thus the turnaround period for veterinarian visits was about 13.5 days (365/27). We previously assumed that the benchmark contamination period (see section 2.1.3) is equal to 15 days. As this period was higher than the veterinarian visits turnaround period, we assumed that each veterinarians visits all his client farms in a 15 days period. Thus, we assigned the same weight to each observed link. Finally, milk trucks collects milk on a daily basis, so within 24 hours they visits all their pool of farms. Given that, we assigned to the milk trucks adjacency matrix (**T**) a weight of 365 visits per year. Finally, we generated the indirect contacts weighted network (**I**) by summing the three adjacency matrix:



$$\mathbf{I} = \mathbf{G} + \mathbf{V} + \mathbf{T} . \quad (3)$$

As these networks are weighted, we calculated the closeness centrality (in and out) using the definition of weighted shortest path given by Opsal and co-authors (2010), that consider highly weighted edges preferred in respect to lower weighted edges.

#### ***2.4. Relative indirect contacts infection probability***

Usually, different routes of transmission are associated to different risks for a potential infectious contact to result in an actual infection. In particular, it is well known that in the case of farming systems the most effective route of between farms transmission is live animals exchange (Anderson, 2002; Gilbert et al., 2005). However, as we wrote in the *Introduction* section, in many cases indirect contacts proved to be crucial in enhancing infections spread. To assess the effect of indirect contacts on the overall risk of infections spread, we considered direct and indirect contacts networks as different layers of the aggregated multi-layer network ( $\mathbf{O}$ ), applying to the indirect contacts frequency-weighted matrix ( $\mathbf{I}$ ) a coefficient of relative infection risk ( $r$ ). This coefficient represents the contagion effectiveness of indirect in respect to direct contacts. Then, the aggregated multiplex matrix is calculated as follows:

$$\mathbf{O} = \mathbf{M} + r\mathbf{I} . \quad (4)$$

Unfortunately, evaluating the real infection risk due to fomites contacts is a difficult task in environmental conditions (Bartley et al., 2002). Thus, we assumed that  $r$  can vary between the two limit cases of 0 (i.e. when indirect contacts are not effective at all) and 1 (i.e. when indirect contacts are as effective as cattle movement). In one of the few existing studies on farm visitors contamination risks, Nielen and co-authors (1996) categorized in case of FMD infection on-farm veterinarians and trucks visits in the same infectious risk category. Then, following Nielen et al. (1996), we applied the same  $r$  coefficient to all indirect contacts.

We performed a sensitivity analysis on the coefficient  $r$ , in particular we assessed the variability of

farm ranking, in respect to their value of closeness centralities, for different values of  $r$  (from 0 to 1).

We measured the ranking shift using of the Kendall's  $\tau$  correlation coefficient calculated between **O** and **M** networks.

### **3. Results**

#### ***3.1. Dairy farms data***

The mean [median] observed dairy farms size for year 2013 was 113.17 [67] individuals, and the values ranged from small house farms (45 farms with 5 or less animals) to big herds (maximum farm size = 1897). In the dataset there were 39 missing size data.

#### ***3.2. Single Networks Analyses***

##### ***3.2.1. Cattle movement network***

As described in the Materials & Methods section, the available dataset on cattle movement included all animals exchanged between dairy farms of the Province of Parma during year 2013. In the overall system, the total number of individuals moved between dairy farms was 16647, the mean [median] number of cattle moved per day was 45.61 [39], with a maximum of 277. The total number of batches moved were 3150, the mean [median] number of batches moved per day was equal to 8.63 [8], with a maximum of 26 shipments during the same day. The mean farm in- and out-degree was 1.06, the mean number of incoming or outgoing batches per farm was 2.34, and the mean number of imported or exported individuals was 12.34. The median of both in- and out-degree is equal to zero, and this is a consequence of the large number of farms with no incoming (688) or outgoing (719) contacts with other dairy farms during year 2013. The correlation coefficient (Kendall's  $\tau$ ) between the mean farm size and both the in-degree and the out-degree were low, respectively -0.0051 and 0.04 (p-values < 0.05), while in- and out-degree were also poorly correlated (Kendall's  $\tau$  = 0.18, p-value < 0.01).

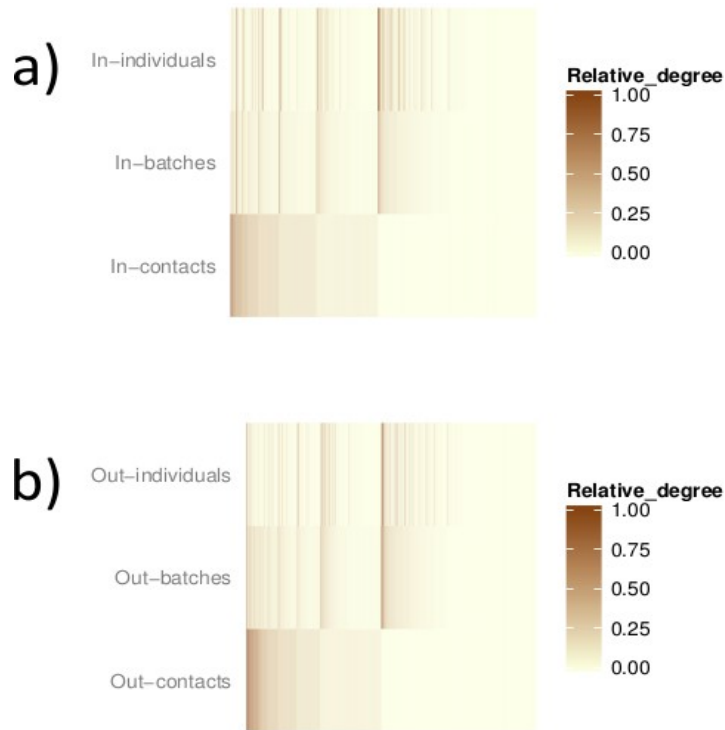


Figure 2: Farms ranking comparison in respect to different degree measures: a) number of in-contacts, number of in-batches and number of in-individuals; b) number of out-contacts, number of out-batches and number of out-individuals.

All values are rescaled for their maximum.

In Figure 2 we reported a colour grid representing, in panel a[b], the farms ranking in respect to in[out] number contacts, in[out] number of batches exchanged, and in[out] number of individuals exchanged. For each measure, darker brown slices represent farms with higher measure (normalized to the maximum). As Figure 2 shows, farms ranks in a very different way in respect to the three measures. Despite this, the in-degree is highly correlated with the in-batches and in-individuals (Kendall's  $\tau = 0.86$  and  $0.79$ , respectively, and in both cases  $p\text{-value} < 0.01$ ), and the out-degree is highly correlated with the out-batches and the out-individuals (Kendall's  $\tau = 0.87$  and  $0.81$ , respectively,  $p\text{-value} < 0.01$ ). The network measures (i-vi) are reported in Table 1. The mean [standard deviation] frequency for links in the cattle movement weighted network is  $11.62$  [ $33.21$ ].

	<i>Movement network</i>	<i>Inspectors network*</i>	<i>Veterinarians network</i>	<i>Dairy-trucks network</i>
<i>Mean degree</i>	1,06	56.26 (0.04)	23,71	9,90
<i>Median in-degree</i>	0	51.09 (0.30)	19	7
<i>Median out-degree</i>	0	51.52 (0.50)	19	7
<i>95° in-degree percentile</i>	4	138.67 (0.32)	64,6	29
<i>95° out-degree percentile</i>	5	134.03 (0.63)	64,6	29
<i>Links density (x 10<sup>-2</sup>)</i>	0,08	4.17 (0.3 x 10 <sup>-2</sup> )	1,76	0,73
<i>GSCC (or GCC)</i>	18	1260.67 (1.14)	955	52
<i>Clustering coefficient</i>	0,058	0.646 (0.08)	0,691	0,977
<i>In-Closeness centrality (mean and SD)</i>	4.4 (9.4)	459.4 (132.6)	254.5 (168.1)	11.8 (11.4)
<i>Out-Closeness centrality (mean and SD)</i>	4.4 (9.1)	459.4 (133.3)	254.5 (168.1)	11.8 (11.4)

\*  $h = 15$  days, mean (standard deviation) out of the 1000 simulations

*Table 1: Network measures for different type of contact among dairy farms. We showed: the mean degree, the median in- and out-degree, the 95<sup>th</sup> percentile of in- and out-degree, the links density, the giant strongly connected component (GSCC), the global clustering coefficient, , and the in- and out-closeness centrality. All measures are calculated for the unweighted single-layer networks.*

### **3.2.2. Government inspectors network**

The 62 government veterinarians, or inspectors, performed on average 128.2 visits in 2013 (median 132), ranging from 1 to 417 visits per operator. The mean [median] number of visits per day was 21.78 [20] (range 0-118). Dairy farms were visited a mean [median] of 5.89 [5] times per year (ranging from 0 to 55). The number of yearly visits for farm was weakly correlated with the farms size (Kendall's  $\tau = 0.3$  and p-value  $< 0.01$ ).

In Figure 3 we reported the links density (panel a), the median and 95th percentile of in-degree (panel b) and out-degree (panel c), and the GSCC (panel d) of the government inspector network. In each of the four panels, the corresponding measure was calculated for 6 case of  $h$  (0, 3, 7, 15, 30 and 60 days), and for 1000 simulations of the inspectors within-day itineraries for different contagious period  $h$  (from

0 to 60 days).

By considering the benchmark case, where  $h$  was set to 15 days, we observed the mean in- and out-degrees partially correlated among them (Kendall's  $\tau = 0.63$  and  $p\text{-value} < 0.01$ ), while they were poorly correlated with the mean farms size (Kendalls'  $\tau = 0.30$  and  $0.31$ , respectively; both  $p\text{-values} < 0.01$ ). We reported the network measures (i-vi) in Table 1. The mean [standard deviation] frequency for links in the government inspector network is  $1.38 [0.76]$ .

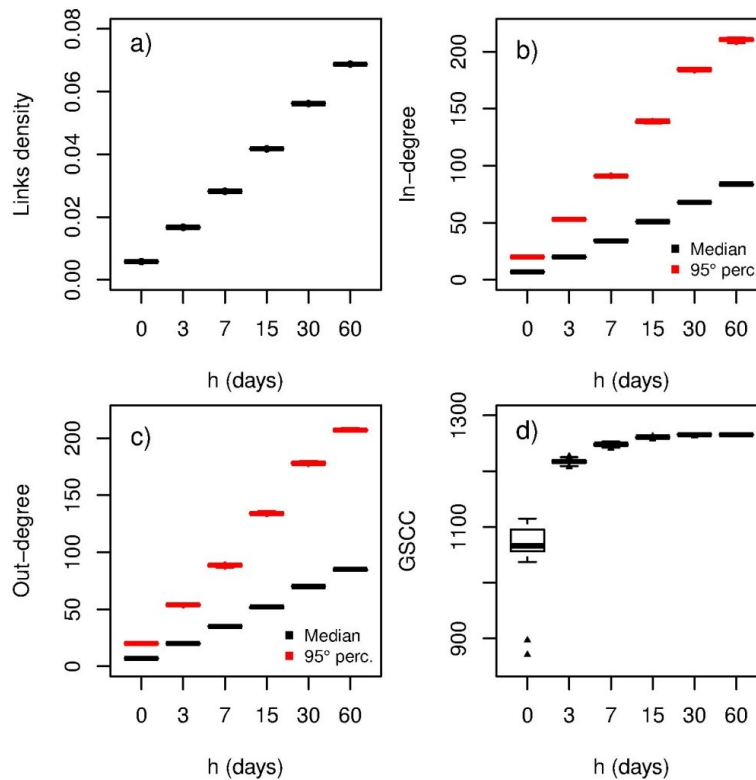


Figure 3: Government inspector network measures for different  $h$  values (0, 3, 7, 15, 30, and 60 days) and, for each case, a 1000 simulations: a) links density; b) median and 95th percentile (red) of in-degree; c) median (black) and 95th percentile (red) of out-degree; d) giant strongly connected component (GSCC).

### 3.2.3. Proxy indirect contacts measures

The private veterinarians network mean [median] degree is equal to  $23.71 [19]$ , with 95th percentile equal to  $64.6$  (see Table 1), the links density is  $1.76 \times 10^{-2}$ , the GCC is equal to  $955$ , and the clustering coefficient value is  $0.69$ . The degree value was poorly correlated with mean size (Kendalls'  $\tau 0.12$ ,

p-values < 0.01).

The number of dairy factories operating in the Province of Parma in 2013 is 181. Most farms, 1032, are linked with one dairy factory, while 32 farms are linked with two and only one farm with 3 factories.

The remaining 282 farms did not delivered milk to dairy factories in 2013. The dairy trucks network mean [median] degree is 9.90 [7], with 95th percentile equal to 29, links density is  $0.73 \times 10^{-2}$ , and clustering coefficient value is 0.98 (see Table 1). The GCC is 52 and there exist 112 clusters of average [median] size of 9.4 [6] farms.

#### ***3.2.4. Contact distance analysis and isolated farms***

The mean [median] distance between the 1349 farms in the Province of Parma was 27.78 [26.12] km, while the maximum inter-farm distance was 86.62 km.

The median [95 percentile] distance between farms connected through direct contacts, i.e. cattle movement (M) is 11.77 [44.75] km (Figure 4,a). For indirect contacts, the median [95th percentile] distances between farms connected through government inspectors (G), private veterinarians (V), and milk trucks (T) are 9.24 [23.80] km, 9.51 [29.98], and 6.42 [20.71], respectively.

As showed in Figure 4,b, we calculated the median distance for all links observed in only 1 out of 3 indirect contact networks, in 2 out of 3, in all 3, and in all 3 plus on the cattle movement network. We observed that the median [95th percentile] distance of links observed in only one indirect contact network was 11.93 [26.97] km, for links observed in at least two was 7.00 [17.01] km, for links observed in all three networks was 4.64 [11.33] km, and for links appeared in all three indirect contact networks plus in the direct contact network was 2.60 [5.32] km. The median [95th percentile] distance of not-observed links (not connected farms) was 27.22 [55.78] km.

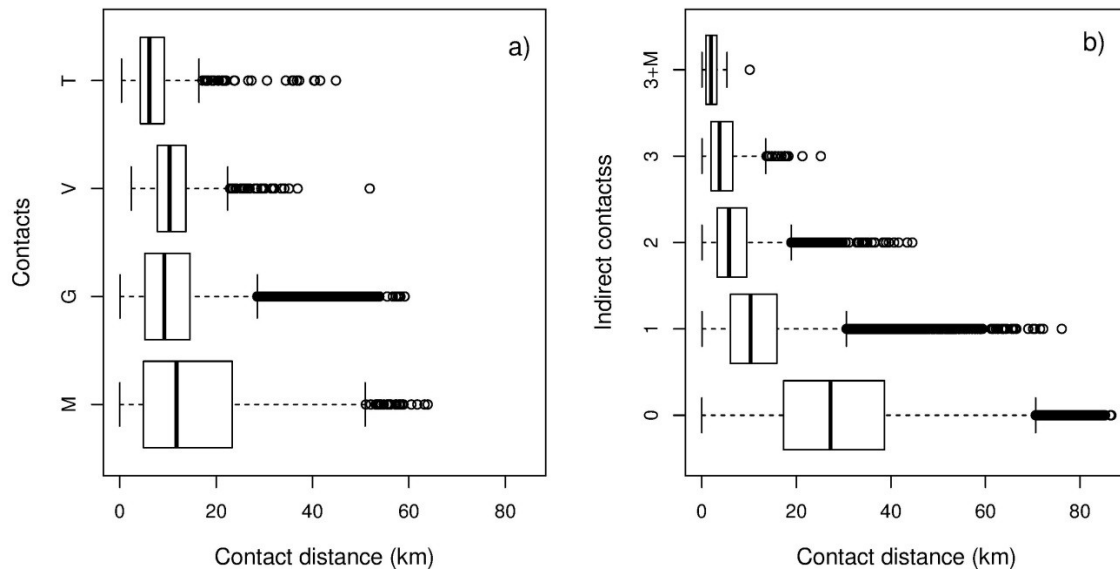


Figure 4: a) Between farm contact distance values in case of four contact networks (M: cattle movement, G: government inspectors, V: private veterinarians, T: dairy trucks). b) Between farm contact distance values for not observed links, for links observed in at least one, two and three indirect networks, and for links observed in all four contact networks.

The number of isolated farms in each network were: 488 for cattle movements, 82 for inspector network, 383 for private veterinarians, and 296 for milk trucks. However, by considering all indirect contacts together, the isolated farms drop to 49; while, by considering both direct and indirect contacts, there were only 6 isolated farms recorded in the 2013 dairy farms network.

### 3.3. Hubs detection and relative indirect contacts infection probability

In Figure 5 we showed the in- and out-closeness centrality scores (panels a and b, respectively), for the direct vs. indirect contacts. Red stars indicate farms representing hubs in both layers.

In Figure 6 we showed the  $\tau$  correlation coefficients, calculated between farms closeness centrality values in **M** and **O** for different values of  $r$ . The curves correspond to the correlation coefficient calculated between values of in-closeness,  $C^I$  (in black), and out-closeness,  $C^O$  (in red).

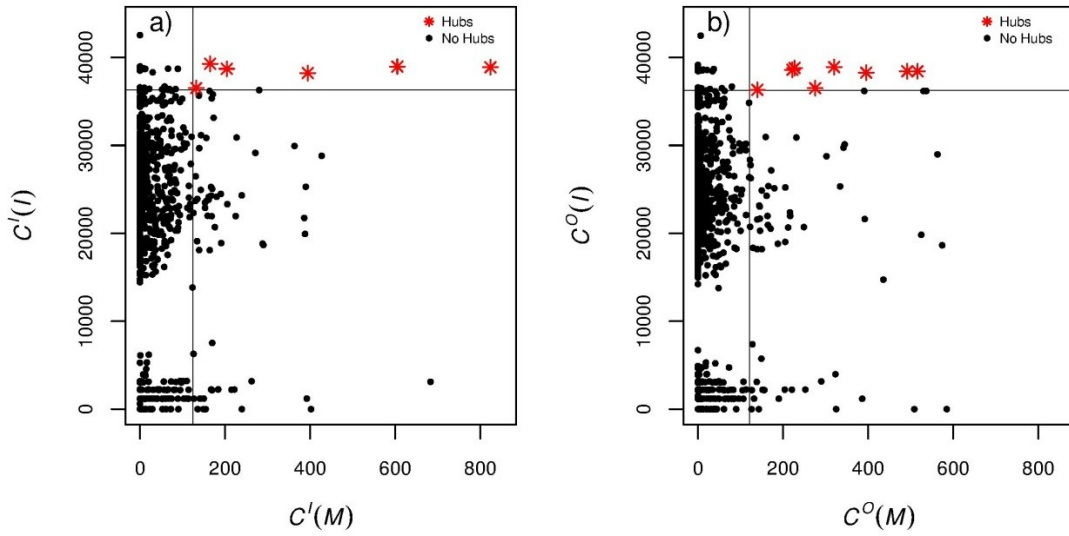


Figure 5: Z scores of direct contact layer  $M$  vs. aggregated indirect contact layer  $I$  (both weighted on frequency). a)  $Z_I$  scores (for in-degree) and b)  $Z_O$  scores (for-out-degree). In both panels, red-star represents hubs for both layers, dark-grey crosses hubs in one out of two layers, and light-grey dots for none.

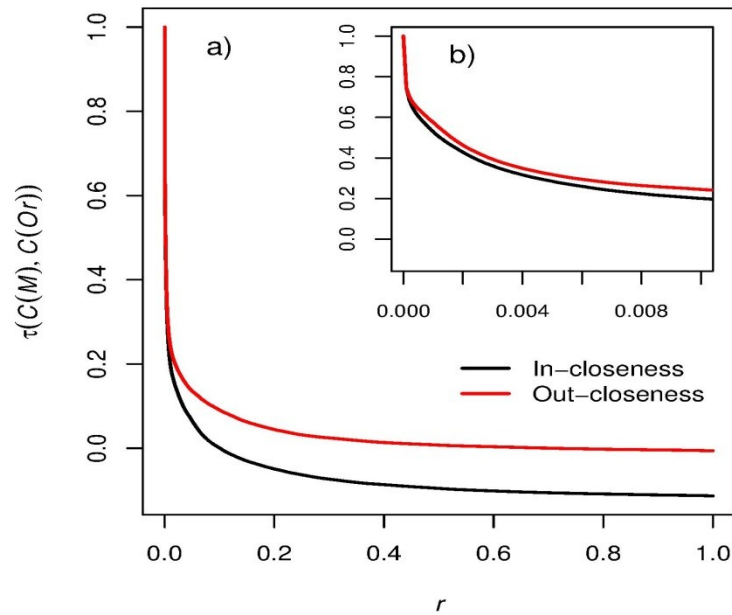


Figure 6: Kendall's  $\tau$  correlation coefficient between farms' in (black line) and out (red line) closeness centrality of direct contact network ( $C(M)$ ) of aggregated multi-layer network ( $C(O)$ ), in respect to different values of relative infection probability  $r$ .

#### 4. Discussion

The goal of this study was to investigate the features of different indirect contact networks among dairy  
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farms, and to understand the role of indirect contacts in the potential spread of infectious diseases in farm systems by comparing them with the characteristics of the direct contact network. The comparison with direct contacts was significant, since of the majority of the studies on farms network structure only relied on animals movement data, i.e. direct contacts, neglecting other routes of transmission (Bajardi et al., 2012; Noremark et al., 2011; Volkova et al., 2010).

To compare the risk represented by different type of contacts (cattle movement, government inspectors, private veterinarians, and milk trucks), we calculated a number of network metrics that can be associated to infection spread among farms. These metrics, can be divided between: measures characterizing the structure of the whole networks, and measures characterizing the proprieties of the single nodes (i.e. farms).

We found that the government veterinarian network is characterised by the higher link density, followed by private veterinarians, milk trucks, and finally animal movement network. As inspectors visits are scheduled by the health administration on the whole province, there are no effect of commercial partnership or fidelity (as typical for cattle exchanges, milk delivery, and private veterinarians) and this may explain the higher observed density. Valdano and co-authors (in press), define “loyalty” as a local measure of the tendency to maintain contacts with the same nodes over time. Moreover, the analysed databases recorded 61 inspectors operating in the province in 2013, about one third in respect to both dairy-factories (180) and private veterinarians (181). This means an higher probability for different farms to be visited by the same inspector in respect to the same private veterinarian or milk truck.

On the other hand, this effect of “loyalty” is evident in milk trucks network, and this is reflected by the high clustering coefficient. Movement network is sparse in respect to indirect contacts, and this is reflected in both values of density and clustering coefficient. In other study by Natale and co-authors (2009) on general cattle movements in Italy, they found a similar clustering coefficient.

The measure of GSCC (giant strongly connected component), or GCC, reflected the structures observed for links density. In the case of cattle movement the obtained value of GSCC (18, corresponding to 1.3% of the total network) was lower in respect to what found in other contexts, such as in Scotland herds (Volkova et al., 2010). This is probably due to the fact that we considered only the dairy sector and excluded the beef one: in the second one a higher number of cattle are usually exchanged, in particular young males from the dairy to the fattening farms. Among the indirect contacts, the GCC value of milk trucks network, 52 farms (3.8% of the total network), was significantly lower than in other indirect contact networks (GSCC correspond to 1260.7 farms on average, or 93%, for inspector, and to 955 farms, or 71%, for veterinarian networks). This depends on the milk trucks network structure, in which farms are highly connected in relatively small clusters (up to 52 farms each). This result, couple it with the high frequency of milk trucks contacts, is particularly significant from an epidemiological point of view. In fact, while other contacts, such as inspectors and veterinarians, could potentially spread a disease in a bigger portion of the network (as a consequence of high values of GCC), the frequency of these contacts is much lower in respect to milk trucks (1.38 and 27 contacts/year, respectively). As a consequence, the milk trucks contact network, characterised by high contact frequency and high clustering, could be effective in maintaining a constant spread inside a limited cluster of farms; while, different milk truck clusters can be connected between each other by lower frequency contacts, such as inspectors or veterinarian contacts.

In a more general way, the introduction of different indirect contacts in the analyses provided a huge impact on networks connectivity. The number of isolated farms was between 80 and 400 in the case of single networks, but decreased to only 6 farms in the case of the aggregated network. This suggests that, considering different routes of transmission highlight that almost no farms are free from the epidemic risk.

Volkova et al. (2010) showed that contacts due to cattle movements can be weighted in different ways

(unweighted, weighted for batches and individuals exchanged). Here, Figure 3 shows how the choice of a different weighting systems could change the ranking of farm centrality in the direct contact network. We highlighted that the three alternatives could be representative of different transmission ways. In particular, contacts in case of frequency-dependent transmitted diseases can be better represented by the number of batches exchanged, since batches represent the number of commercial contacts between farms in a given time period. On the other hand, contacts in case of density-dependent transmitted diseases can be better represented by the number of individuals exchanged, since larger numbers of individuals represent higher probabilities of infection under this hypothesis.

The analysis of the contacts distance, showed in Figure 4.b, suggests that farms closer in space are more likely to have a potential infectious contacts. This is in agreement with epidemic models that used a spatial kernel to represent indirect contacts route of transmission (Boender et al., 2007, 2010; Ferguson et al., 2001; Keeling et al., 2003, Savill et al., 2006). However, the median distance that we found for indirect contacts is always higher than the local contacts distance estimated by epidemics models, that is about 4 km for foot-and-mouth disease spread (Ferguson et al., 2001; Keeling et al., 2001). This is probably due to different reasons: first, the median distance outlined by risk kernel models comprehend also the local infectious contact (due to shared fences and small animals as vectors). Moreover, the distribution of the distances in traced contacts of the 2001 FMD epidemic in UK may well be biased toward small distances (as suggested by Ferguson et al., 2001), since only about the 5% of the infections were traced.

To characterize the farms role in the different networks, we computed for each farm its values of closeness centrality in each network. This measure shows how close are the farms in the network, highlighting whether a given farm is in close contacts with the rest of the network or not. Moreover, as observed by Buttner and co-authors (2013), this measure behave similarly to infectious chains measures, a recently introduced metric that measures the number of farms reachable by a given farm,

also accounting for the temporal sequence of the observed links (Dubè et al., 2008; Noremark et al., 2011). However, conversely than Buttner and co-authors, we found a wide range of both in- and out-closeness centrality in all networks (as showed Table 1), and that these measure were consistent with other centrality measures such as in- and out-degree (data not showed).

We used in- and out-closeness centrality to detect the hub farms in cattle movement network (**M**) and in the aggregated indirect contacts network (**I**). As showed in Figure 5, only 6 and 8 farms (in case of in- and out-closeness centrality, respectively) are considered hubs in both networks, corresponding to the 9% and 12% of the total hubs. This indicate how these two sub-systems behave totally differently, and that we could not assimilate direct with indirect contacts, or vice-versa. Moreover, this result indicated that farm role in respect to different routes of transmission could be very different.

However, it is well known that for the majority of farmed animals diseases, the most effective route of transmission is represented by animal movement (Gilbert et al., 2005). To introduce the differential risk of infection associated to different layers, we applied a coefficient of relative infection probability ( $0 < r < 1$ ) of indirect respect to direct contacts. Since the role of indirect contacts may be different in different infections, we performed a sensitivity analysis on  $r$ . As showed in Figure 6, as the coefficient  $r$  grows, the correlation between the closeness centralities of the direct contact layer and the overall aggregated multiplex rapidly decreases. From Figure 6 we remark that, assuming a probability of infection due to cattle movement hundreds time higher than that due to indirect contacts (i.e.  $r = 0.01$ ), the correlation among closeness centrality scores is about 0.2. Last finding means that, also when the infection transmission through animal movements is 100 times higher than through indirect contacts, neglecting the indirect contacts in the transmission structure implies missing the correct ranking for more than the 80% of the farms. This could lead to a loss of effectiveness for eventual control measures targeting the highest central farms.

In order to build the government inspector network, we assumed a contamination period length in

which to consider an inspector still infective between two visits, namely  $h$ . This period can represent the pathogen survivor in fomites. We considered the foot-and-mouth disease as typical example of fomites transmitted infection and we set  $h$  in accordance to FMD literature. Despite the numerous literature on that topic, the FMD virus survival in the environment is still not clear (Bartley et al., 2002). Thus, we performed a sensitivity analysis on  $h$ , to understand the effect of the assumption on our results. As showed in Figure 5, the links density substantially increases for  $h$  going from 3 to 60 days, while GSCCs tend to a plateau. We noticed that, as  $h$  increases, median and 95<sup>th</sup> percentile of the degrees diverge, with the 95<sup>th</sup> percentile growing much faster than the median. This means that for long living pathogens in fomites, we would expect a longer tail in the degree distribution, with a higher number super-spreaders.

We assumed that farms visited by the same private veterinarian or milk truck are fully connected to each other. However, this assumption could over-estimate the number of links, in particular for milk trucks network. This over-estimate can be due to dairy factories collecting milk by using different trucks, which means that trucks connect only a fraction of the farms delivering milk to the same factory.

In conclusion, all measures on the four contact networks showed non-trivial differences among the considered routes of transmission, in particular in respect to the number of connections and to the contact frequency. In addition, we observed that the joint structure of the network could effectively enhance spread, this is due to the contrast between high frequencies contacts, that can facilitate the infection spread within relatively small clusters of farms, and low frequency contacts, that can spread the disease between clusters. This kind of structure could be observed also in the human movement network (Balcan et al., 2009; Poletto et al., 2013).

In the analysed farming system, we showed that the contribution to connectivity by the indirect contacts was crucial in shaping the overall system network structure. This happened despite indirect

contacts low rate of infection in respect to cattle movement.

Then, our results imply that only by having a broad view on all potential farm connections, we could obtain the necessary understanding of infection processes through the system; which is essential to develop contingency plans able to effectively face epidemic crisis.

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## General Conclusions

My PhD thesis was focused on the dynamics of infectious diseases spread in farm networks. The case of study was represented by the dairy farm system of the Emilia Romagna region (Italy). In Emilia Romagna, the dairy industry represents a strategic activity for the local food-farming economy, that is mostly based on high-quality food products. Intensive (or factory) farming is the most common practice in the region.

In the first chapter I reviewed the existing literature on mathematical models for bovine tuberculosis (bTB) dynamics. From the late 90's on, I found several models published on this topics. However, most of these models are suited for extensive farming contexts (in particular UK and New Zealand), with focus cattle-wildlife contacts as a potential route of transmission in addition to between-farm animal exchange. Moreover, most of these models did not include the between-farm dynamics of the disease, focusing on the within-farm dynamics.

In the second chapter I evaluated the bTB surveillance system of the Emilia-Romagna region, characterised by three main detection methods; namely: on-farm routine testing, exchanged cattle testing, and slaughterhouse, or abattoir, inspection. In order to evaluate the surveillance effectiveness, I built a data-driven stochastic model able to represent the potential spread of bTB in the Emilia Romagna dairy farms. The model developed here differed substantially from the majority of the reviewed models already present in the literature. Firstly, I accounted for both within- and between-farm dynamics of the disease; secondly, I assumed animal exchange as the only between-farm route of transmission. In respect to other context, in fact, the wildlife source of infection was negligible because of the limited contacts with cattle reared indoor. I used the stochastic model to perform a number of simulations of a potential bTB introduction in the region, testing the effectiveness of the surveillance system in detecting the infection. The model results indicated that the on-farm routine testing and slaughterhouse surveillance are both effective in bTB detection, while the effectiveness of

the exchanged animal testing is only marginal. At first place the ineffectiveness of animal exchange testing could seem counter-intuitive, because it represents the only effective route of transmission for bTB spread. However, this result becomes straightforward given the low connectedness of the Emilia Romagna dairy system. From the modelling point of view, the inclusion in bTB dynamics of both within- and between-farm transmission represents a significant improvement respect to classical bTB models. This inclusion helps to improve the representation of within-farm disease dynamics, which is highly dependent on the time of farm infections, because of the slow dynamics peculiar of bTB. This peculiarity reduces the number of infected individuals moving between-farm in the short period in respect to faster diseases, but, from a longer perspective, it makes the disease more difficult to eradicate. From an epidemiological point of view, the differences between indoor intensive and outdoor extensive farming can not be neglected, and intensive farming systems can not be properly analysed using models developed for extensive farming systems. On one hand, higher densities of individuals inside farm-buildings might enhance the spread of diseases, obviously only in case of density-dependent transmission infection. On the other hand, indoor farming prevents the spread among different herds thorough shared fences and decrease the number of contacts chance between cattle and potentially infected wildlife reservoirs. As a consequence, in Emilia-Romagna farm system the wildlife-to-cattle transmission can be neglected, while it does not represent a suitable assumption in many other contexts (such as UK, Ireland and New Zealand).

Despite it is well known that live animals exchange represents the most effective between-farm disease transmission pathway (Gilbert et al., 2005), between-farm transmission through fomites carried by farms visitors and their contaminated material or vehicles can not be neglected for several acute diseases, as Foot-and-mouth disease (FMD) or Aujesky's disease (Nielen et al., 1996; Noremark et al., 2013). In the third chapter, I analysed indirect contacts routes of transmission using data on three kind of professional farm-visitor, for the Province of Parma. These are: government veterinarian inspectors,

private veterinarians, and milk trucks. We compared the structure of the direct contact (i.e. live animal exchange) network with the indirect contact network built by using the on-farm visits data of the three listed categories. My analysis showed that the contact structures obtained from the three professional visitors were very different among them, in particular by looking at the number of connections and frequency of contacts. Moreover, I showed that, even for low probabilities of pathogen transmission through indirect contacts in respect to direct contacts, the indirect routes of transmission can be determinant in shaping the disease transmission network structure. Finally, I showed that the farms role in respect to a potential spread could be very different whether considering both direct and indirect contacts or direct contacts only. These results are particularly significant since the indirect routes of transmission are often neglected, as a consequence of the lack of data on this topic, in modelling representation of disease spread.

The results obtained in my PhD work are epidemiologically relevant for the understanding of pathogen transmission in farms network. My work highlighted the necessity to separate within- and between-farm dynamics, in particular for slow-growing diseases such as bovine tuberculosis, and they showed how determinant could be a minor route of transmission, such as indirect contacts in farms system, that is often neglected. In my work, ecological modelling tools were fundamental to obtain these results.

Moreover, the results of my work open the door for a number of new issues about pathogen diffusion in farmed animals populations. First, the assessment of surveillance strategies could be followed by a cost-benefit analysis on different alternatives. In fact, an important aspect, that was beyond the scope of the work showed here, was the surveillance system economic efficiency analysis. Moreover, another possible follow-up could be the analysis of the current and alternative control strategies. In the case of bTB this is particularly relevant, because the most of bTB detection tests produce a number of false-positive individuals, that need to be managed in order to avoid over-reactions.

In order to extend these analyses to other diseases, in particular to Foot-and-mouth disease, we can not neglect the indirect contacts route of transmission. The showed results demonstrate that direct and indirect contact networks structure are substantially different and, for a proper stochastic model simulating a FMD epidemic, they need both to be accounted. Only through the use of all routes of transmission it would be possible to perform analysis on surveillance and control systems, as we did for bTB.