Universitat Autònoma de Barcelona

# Epidemiology of Bovine Tuberculosis: evaluation of surveillance and sociological factors in Spain

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Tesi doctoral presentada per ***Giovanna Ciaravino*** per accedir al grau de Doctora en Veterinària dins del programa de Doctorat en Medicina i Sanitat Animals de la Facultat de Veterinària de la Universitat Autònoma de Barcelona, sota la dirección del Dr. **Alberto Allepuz Palau** i el Dr. **Sebastian Napp Avelli**.

Bellaterra (Barcelona), 2018.

FACULTAT DE VETERINÀRIA

El Dr. **Alberto Allepuz Palau**, profesor del Departament de Sanitat i d'Anatomia Animals de la Facultat de Veterinària de la Universitat Autònoma de Barcelona i investigador del Centre de Recerca en Sanitat Animal (CReSA-IRTA), i **Sebastian Napp Avelli**, investigador del Centre de Recerca en Sanitat Animal (CReSA-IRTA).

Certifica:

Que la tesi doctoral titulada "**Epidemiology of bovine tuberculosis: evaluation of surveillance and sociological factors in Spain**" presentada per **Giovanna Ciaravino** per l'obtenció del grau de Doctora en Veterinària, s'ha realitzat sota la seva direcció a la Universitat Autònoma de Barcelona i al CReSA-IRTA.

I per tal que consti als efectes oportuns, signem la declaraciò a Bellaterra (Barcelona), a 5 de setembre de 2018.

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# LIST OF ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| AUC | Area Under the Curve |
| BCG | Bacillus Calmette and Guérin |
| bTB | Bovine Tuberculosis |
| CMI | Cell-Mediated Immunity |
| DTH | Delayed-Type Hypersensitivity |
| ECDC | European Centre for Disease Prevention and Control |
| EFSA | European Food Safety Authority |
| ELISA | Enzyme-linked immunosorbent assay |
| EU | European Union |
| FAO | Agriculture Organization of the United Nations |
| HCPC | Hierarchical Clustering on Principal Components |
| HPA | High Prevalence Areas |
| IFN-γ | interferon-gamma assay |
| LPA | Low Prevalence Areas |
| LPS | Lipopolysaccharide |
| MCA | Multiple Correspondence Analysis |
| MS | Member State |
| MTC | *Mycobacterium tuberculosis*-complex |
| NTM | Non-Tuberculous Mycobacteria |
| OIE | World Organisation for Animal Health/Office International des Epizooties |
| OR | Odds Ratio |
| OTF | Officially Free of Bovine Tuberculosis |
| PCR | Polymerase Chain Reaction |
| PPDs | Purified Protein Derivatives |
| SICTT | Single Intradermal Comparative Tuberculin Test |
| SITT | Single Intradermal Tuberculin Test |
| WHO | World Health Organization |

# ABSTRACT

The present PhD thesis aimed to increase knowledge on bTB epidemiology and control and to investigate sociological factors that might hinder the success of the bTB eradication programme.

In the first study, we developed a stochastic dynamic model that allowed estimating the cattle-to-cattle bTB transmission parameters within Spanish herds, using field data from the eradication campaign. Then, we used those parameters to simulate the average number of secondary cases caused by a single infected animal introduced into a herd ($R_h$), considering different control frequencies. The median transmission coefficient ($\beta$) was 5.2 newly infected animals per infectious animal per year; however, results evidenced a great variability in the estimates among the 22 study-herds, with median estimates ranging between 1.8 and 8.3. The overall median duration of the latent period ($\alpha$) was 3.2 months, with an interquartile range varying from 2.4 to 5.4 months. Considering a 6-month interval between tests, the mean $R_h$ was 0.23, increasing to 0.82 for annual intervals, and to 2.01 and 3.47 with testing intervals of 2 and 4 years, respectively.

The second study was directed at evaluating the efficiency of the components of bTB surveillance system: routine skin testing, slaughterhouse surveillance and pre-movement testing; and, to assess their variability among Spanish provinces, by using a modified version of the model previously developed. Under the average Spanish conditions, the overall sensitivity (i.e. probability of detection per year) of the national bTB surveillance system was 79.7% and the mean time until detection 221.6 days. Routine testing was the most efficient component, while the efficiency of slaughterhouse surveillance and pre-movement testing was much lower; although these components also contributed to detection of some infected herds. Looking at the province level, the efficiency of the different components of the bTB surveillance varied significantly, but no obvious spatial pattern was identified. Our results evidence that in many Spanish provinces, the intensity of surveillance efforts was not correlated to the herd prevalence in the area.

In the third study, we used qualitative approaches to investigate opinions and attitudes of farmers and veterinarians toward the Spanish bTB eradication programme. Face-to-face exploratory interviews were used to identify main themes, followed by in-depth interviews. Main results suggested that the bTB programme is perceived as a law enforcement duty

without an adequate motivation of some stakeholders. The complex bTB epidemiology combined with gaps in knowledge and weak communication among stakeholders contributed to generate disbeliefs towards control measures and, in turn, different kinds of guesses on the disease. Low reliability in the skin test was expressed and some pressures faced by private veterinarians during field activities also emerged. People perceived very few benefits of being bTB-free and comparative grievances to wildlife, other domestic reservoirs and bullfighting farms arose.

In the fourth study, the sociological factors previously identified were investigated through a structured questionnaire, telephonically interviewing a sample of 706 farmers and 180 veterinarians. Multiple Correspondence Analysis, followed by Hierarchical Clustering on Principal Components were used to identify opinion profiles; and, a logistic regression model was developed to quantify the main differences between groups. Different attitudes toward the bTB eradication programme were characterised by opinions on the bTB diagnostic tests, the perception on the impact of bTB and the importance of other domestic and wildlife species. There were people with positive and with negative attitudes toward the programme and a third group with a clear tendency to not respond. Opposite profiles were observed among farmers. Differently, veterinarians were more homogeneous and the vast majority of them expressed a positive attitude; however, some veterinarians showed a negative attitude toward the bTB eradication programme, which deserve a special attention.

# RESUMEN

El objetivo de esta tesis ha sido aumentar el conocimiento sobre la epidemiología y el control de la Tuberculosis Bovina (TBb) e investigar factores sociológicos que podrían obstaculizar su erradicación en España.

En el primer estudio, desarrollamos un modelo que permitió estimar los parámetros de transmisión de la TBb dentro de los rebaños utilizando datos de la campaña de erradicación. Dichos parámetros se emplearon para simular el número medio de casos secundarios causados por un solo animal infectado introducido en un rebaño ($R_h$), considerando diferentes frecuencias de control. El coeficiente medio de transmisión fue de 5,2 animales infectados por animal infeccioso y año; sin embargo, los resultados evidenciaron una gran variabilidad entre los 22 rebaños estudiados, con valores que oscilaron entre 1,8 y 8,3. La duración media del período de latencia fue 3,2 meses, con un rango intercuartil de 2,4 a 5,4 meses. Considerando un intervalo de 6 meses entre las pruebas, el valor medio de $R_h$ fue 0,23, aumentando a 0,82 para intervalos anuales, y a 2 y 3,5 para intervalos de pruebas de 2 y 4 años, respectivamente.

En el segundo estudio se evaluó la eficiencia de los componentes del sistema de vigilancia: vigilancia de rutina, vigilancia en mataderos y pruebas pre-movimientos; y su variabilidad entre las provincias españolas con una versión modificada del modelo desarrollado. Bajo las condiciones españolas, la sensibilidad (probabilidad de detección por año) del sistema de vigilancia fue 79,7% y el tiempo medio hasta la detección de 221,6 días. La vigilancia de rutina fue el componente más eficiente, mientras que la eficacia de la vigilancia en matadero y las pruebas pre-movimientos fue menor; aunque estos componentes también contribuyeron a la detección de algunos rebaños infectados. La eficiencia de los diferentes componentes de vigilancia entre las provincias fue muy variable, pero sin un patrón espacial evidente. Nuestros resultados muestran que, en muchas provincias, la intensidad de la vigilancia no se correlaciona con la prevalencia.

En el tercer estudio, utilizamos enfoques cualitativos para investigar opiniones y actitudes de ganaderos y veterinarios hacia el programa de erradicación. Para ello se emplearon entrevistas exploratorias y entrevistas en profundidad. Los principales resultados sugirieron que el programa se percibe como una obligación impuesta por la ley sin una adecuada motivación. La compleja epidemiología de la TBb combinada con una falta de conocimiento

y carencias en la comunicación contribuyen a generar desconfianza hacia las medidas de control y, a su vez, a la generación de diferentes tipos de conjeturas sobre la enfermedad. También se mencionó una baja fiabilidad en la prueba cutánea, así como presiones a los veterinarios durante el saneamiento. En general, no se perciben beneficios de ser libres y los ganaderos perciben agravios comparativos respecto a la fauna silvestre, otros reservorios domésticos y granjas de lidia.

En el cuarto estudio, los factores sociológicos previamente identificados se investigaron mediante un cuestionario estructurado, entrevistando telefónicamente a 706 ganaderos y 180 veterinarios. Un análisis de correspondencia múltiple, seguido de un análisis de conglomerados, nos permitió identificar tres perfiles de opinión; además, se desarrolló un modelo de regresión logística para cuantificar las principales diferencias entre los grupos. Dichos perfiles se diferenciaron principalmente por su opinión sobre las pruebas de diagnóstico, su percepción sobre el impacto de la TBb y la importancia de otras especies domésticas y silvestres. Algunas personas mostraron actitudes positivas y otras negativas hacia el programa, y un tercer grupo una clara tendencia a no responder. Entre los ganaderos se observaron perfiles opuestos mientras que los veterinarios fueron más homogéneos y la mayoría de ellos expresaron una actitud positiva; sin embargo, algunos veterinarios mostraron una actitud negativa que merece particular atención.

# PUBLICATIONS

The studies presented in this thesis have been published in international scientific peer-reviewed journals:

**Study I: Assessing the variability in transmission of bovine tuberculosis within Spanish cattle herds impact**

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**Study III: Farmer and veterinarian attitudes towards the bovine tuberculosis eradication programme in Spain: what is going on in the field?**

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# Introduction

## Chapter I

# 1.1. Definition of Bovine Tuberculosis

## 1.1.1. Aetiology

Bovine tuberculosis (bTB) is a chronic infection of cattle (including all *Bos* species, and *Bubalus bubalus*) and bison (*Bison bison*) caused by any mycobacterial species within the *Mycobacterium tuberculosis*-complex (MTC) (EFSA AHAW Panel, 2017; SANCO WD, 2013). By far, the most important etiologic agents of bTB in cattle are *M. bovis*[1] and, to a lesser extent, *M. caprae*, recognised as an independent mycobacterial specie since 2003 (Aranaz et al., 2003; OIE, 2015; Rodriguez-Campos et al., 2014; Schiller et al., 2010).

Figure 1: *Mycobacterium* bacteria. Scanning electron microscopic (SEM) digitally colorized in red. Photo produced by the National Institute of Allergy and Infectious Diseases in 2010. *Source: CDC - Public Health Image Library (PHIL) – ID 18139. This image is in the public domain and thus free of any copyright restrictions.*

The MTC represents one of the three groups into the genus *Mycobacterium*[2] (Fig. 1) together with *M. leprae* and the group of non-tuberculous mycobacteria (NTM) (i.e., mycobacteria other than the MTC and *M. leprae*). It comprises a range of mycobacterial species causing tuberculosis in humans and animals that are highly related among them (i.e., 99.9% homogeneity in the nucleotides sequence and virtually identical 16S rRNA sequences) (Böddinghaus et al., 1990; Rodriguez-Campos et al., 2014; Sreevatsan et al., 1997; Thoen et al., 2010). Despite their great genetic relatedness, MTC species differ in terms of pathogenicity, geographical distribution and preferred host. Moreover, they also differ in some biochemical characteristics, cultural requirements and for several molecular markers (Rodriguez-Campos et al., 2014).

Mycobacteria are considered to have existed for more than 150 million years (Hayman, 1984). It is likely that all members of the MTC might have evolved as host-adapted ecotypes from a common African ancestor (possibly *M. canettii*) about

[1] *M. bovis* was officially recognised as a mycobacterial species in 1970 by Lessel and Karlson (Lessel and Karlson, 1970), although, already in 1898, Theobald Smith differentiated the tubercle bacilli isolated from humans from those isolated from cattle

[2] The first scientific taxonomy of mycobacteria began in 1896, when the genus *Mycobacterium* was erected by Lehmann and Neumann

15,000–20,000 years ago, via successive DNA deletions/insertions (Brosch et al., 2002; Good et al., 2018; Good & Duignan, 2011; Patané et al., 2017). The evidence that human tuberculosis is coincident with animal domestication in the Near East at the beginning of the Neolithic, gave rise to the speculation that *M. tuberculosis* and the other human-infecting mycobacteria evolved from an ancient *M. bovis* strain through a zoonotic transmission from cattle (Brosch et al., 2002; Galagan, 2014; Rodriguez-Campos et al., 2014).

Traditionally, the division into different species is based on the host preference (i.e., phenotypic variations related to host adaptability and virulence), defining human-associated species and animal-adapted lineages with zoonotic potential for humans, and it is supported by molecular phylogenetics (Brosch et al., 2002; Galagan, 2014; Rodriguez-Campos et al., 2014). In addition to *M. bovis* and *M. caprae*, the other most important MTC members are *M. tuberculosis* and *M. africanum* that are typical human-associated species; *M. canettii,* the most divergent within the MTC also isolated in humans, *M. microti* (originally described in wild rodents)*, M. pinnipedii* (originally described in seals and sea lions) and the dassie bacillus (isolated in rock hyraxes) (Aranaz et al., 1999; Brosch et al., 2002; Cousins et al., 2003; Michel et al., 2010). In recent years, also *M. mungi* (banded mongooses) and *M. suricattae* (meerkats), closely related to the dassie bacillus, and *M. orygis* (*Bovidae* family, i.e., oryxes, gazelles, deer, antelope and waterbucks) have been elevated to independent mycobacterial species (Alexander et al., 2010; EFSA AHAW Panel, 2017; Parsons et al., 2013; van Ingen et al., 2012). This group also includes the vaccine strain *M. bovis* Bacillus Calmette and Guérin (BCG), the only licensed and most widely used human vaccine, available since 1921 that provides protective immunity to challenge with *M. tuberculosis* (Rodriguez-Campos et al., 2014).

It is worth to mention that mycobacterial species have undergone different taxonomic and nomenclatural changes. As matter of fact, the structure of the MTC is in constant evolution due to the improvement of molecular diagnostic techniques, allowing a better understanding of the evolutionary processes and relationship among mycobacterial species (Patané et al., 2017; Riojas et al., 2018; Rodriguez-Campos et al., 2014).

The controversial structure of the MTC (i.e., its members have been considered species, subspecies or ecotypes) may have possible diagnostics and legal implications.

That was the case, for example, of *M. caprae* and its role in animal tuberculosis, which led in many countries to important changes in the legislation in order to address infections due to *M. caprae* in a similar manner to *M. bovis* (Rodriguez-Campos et al., 2014). A rapid and reliable identification of the members of the *M. tuberculosis* complex is critical in guiding public health and primary care decision-making (Olea-Popelka et al., 2017). The MTC members are acid-fast gram-positive bacteria, strictly intracellular and characterized by a very complex cell wall envelope, which impacts the cell permeability and allows for the differential staining procedure (Ziehl Neelsen, i.e., acid-alcohol resistance) (Forrellad et al., 2013). The mycobacterial cell wall has been described as having three layers, an outer layer of lipopolysaccharide (LPS), an intermediate layer of LPS-lipid-protein complex, and an inner layer of LPS muco-peptide (Imaeda et al., 1968). Moreover, the capsular structure contributes to the intracellular survival of the bacteria and its immune modulating abilities (Rastogi et al., 2001).

### 1.1.2. Pathology, Pathogenesis and Lesions

Despite MTC species lack toxins, they have several virulence genes, which mostly encode for enzymes of lipid pathways, cell surface proteins, regulators, or proteins of the signal transduction system. Moreover, other genes are involved in mycobacterial survival inside the host macrophages, encoding for proteins inhibiting the antimicrobial effect of macrophages, including phagosome arrest and inhibition of apoptosis (Forrellad et al., 2013). Therefore, the tuberculous infection is characterised by the activation of an exacerbated inflammatory process (i.e., caseous-necrotising), as host response to virulence factors and antigen stimulation. This process leads to the formation of the typical lesions of the MTC, the *granulomas*, which represent the intent of the organism to limit tissue damage and restrict microbial dissemination (Domingo et al., 2014; Pollock & Neill, 2002; Saunders et al., 1999; Waters et al., 2014).

In cattle, the *granulomas* are characterized by a central core of caseous, often mineralized material, surrounded by infiltrates of epithelioid macrophages, Langhan's type multinucleated giant cells and lymphocytes (Zachary & McGavin, 2012). This structure is often enclosed by a fibrous capsule which level of fibrous encapsulation depends on the chronicity of infection.

Within the granuloma, the mycobacteria may remain dormant for decades without any clinical disease (i.e., latent tuberculosis) (Domingo et al., 2014; Pollock & Neill, 2002; Saunders et al., 1999). Subsequent immune suppression could allow activation of the dormant bacteria, followed by replication and spread; consequently, a proportion of infected cases may not develop any active tuberculosis (Saunders et al., 1999; Smith, 2003). The mechanisms responsible for latency in tuberculosis are not well understood; potential latent infections are suspected in cattle, thought their occurrence remains unclear (Domingo et al., 2014; Pollock & Neill, 2002; Waters et al., 2014).

The progression of bTB in the body's host is characterized by two stages: the initial infection (primary complex) and a chronic post-primary dissemination.

The entrance of mycobacteria is followed by a lesion at the point of entry. If the infection is not controlled, mycobacteria spread to the respective draining lymph node, producing a new lesion. The developing necrotic focus is soon surrounded by granulation tissue, monocytes, and plasma cells, and the establishment of the pathognomonic "tubercle"; calcification may also occur.

In some instances, the lesion at the point of entry may heal and disappear or not be visible. Depending on the presence of the lesion at the site of entry the primary complex is classified as complete, when both lesions, at the point of entry and in the lymph node, are present, or incomplete, when only the lesion in the lymph node remains (Domingo et al., 2014). However, the spectrum and location of the lesions observed in bTB is also determined by the route of transmission: a lesion at the point of entry is common when infection is by inhalation, whereas, if the infection occurs via the alimentary tract, a lesion at the site of entry is unusual and, commonly, the only observable lesion is in the pharyngeal or mesenteric lymph nodes (Domingo et al., 2014; Menzies & Neill, 2000).

According to the efficiency of the immunological response, from the primary complex, dissemination may occur via both lymphatic and haematogenous spread or via pre-existing anatomical channels in the organs. Therefore, the post-primary phase may take the so-called form of "chronic organ tuberculosis" or may generalise, which is called late generalisation. When the host response is largely ineffective, generalisation may also occur during the initial stage (i.e., early generalisation). In cattle, generalization is commonly characterized by numerous and small nodular lesions in various organs, called miliary tuberculosis (Constable et al., 2017; Domingo et al., 2014; Radostits et al. 2007; Waters et al., 2014).

### 1.1.3. Clinical Sign and Immune Response in cattle

Depending on the sites of localization of infection, clinical signs may vary. Usually, clinical signs of bTB in cattle take months to develop due to the chronic character of bTB, and initially they are unspecific (i.e., weakness, debility, fluctuating fever, intermittent hacking cough, loss of appetite and progressive emaciation) (Constable et al., 2017; Radostits et al. 2007). Therefore, bTB can be difficult to diagnose based only on the clinical signs; moreover, it is worth to mention, that some cattle with extensive miliary tubercular lesions may appear clinically normal (Constable et al., 2017).

Localized lesions are frequently found in lungs and the pulmonary tract, which may result in a chronic cough. In the advanced stages, animals may become extremely emaciated and develop active respiratory distress; dyspnoea and depth of respiration becomes apparent and tuberculous pleuritis may occur (Constable et al., 2017; Radostits et al. 2007).

When the gastrointestinal tract is affected, visible lesions on the intestinal wall are generally absent and, rarely, the presence of tuberculous ulcers of the small intestine causes diarrhoea. Uterine tuberculosis causing reproductive disorders is uncommon; whereas, the tuberculous mastitis is difficult to differentiate from other forms of mastitis, and is of major importance due to the risk of spread of the disease through the milk (Constable et al., 2017; Radostits et al. 2007).

An essential component of the immunological response to bTB in cattle is the cell-mediated immunity (CMI) and it is responsible for both the defence from the infection and the development of lesions (de la Rua-Domenech et al., 2006; Domingo et al., 2014; Waters et al., 2014). In particular, a key role is played by T lymphocytes (‘T cells’) (i.e., T-helper1 - CD4 T cells) that are responsible for the production of interferon (IFN)-$\gamma$ and, when sensitized by contact with antigen, drive the so called delayed-type hypersensitivity (DTH) response, a localized inflammatory reaction, also mediated by macrophages, which typically occurs at least 48 hours after exposure to an antigen.

On the other hand, humoral immune responses are considered supportive rather than essential, and the specific role of the B cells remains controversial (Waters et al., 2014). Experimental infection of cattle with virulent strains of *M. bovis* showed that a robust cellular immune responses (e.g., IFN-γ and DTH responses) begins as early as 2–3 weeks after challenge (Pollock et al., 2001; Waters et al., 2003; Waters et al., 2012); whereas, the humoral immune responses (both IgM and IgG) appear in the more advanced stages of the infection, starting 2–4 weeks later (Waters et al., 2006) (Fig. 2).

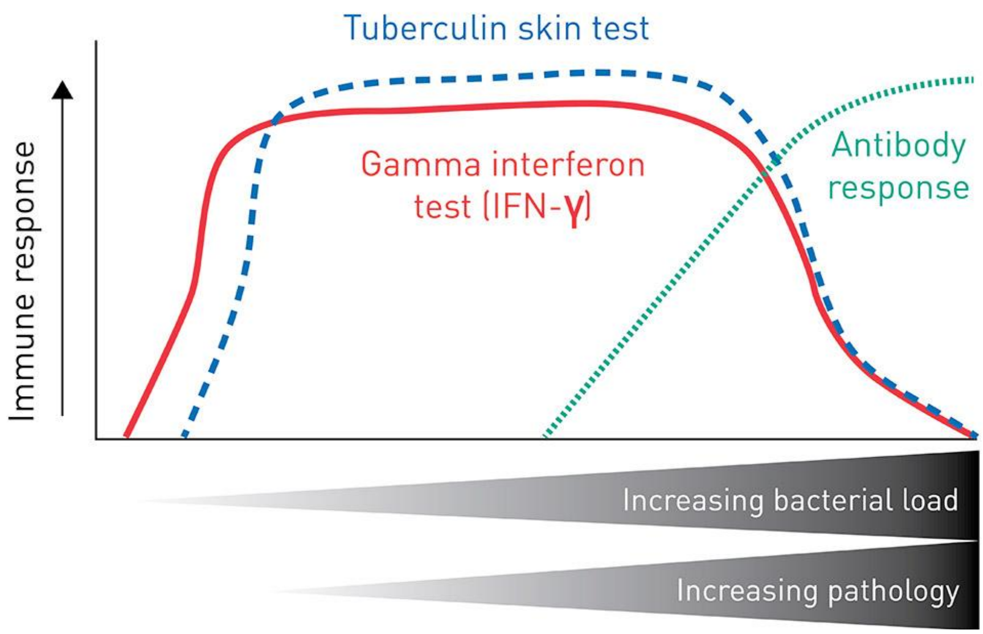



Figure 2: Response of the immune system to bTB infection in cattle with respect to different *ante-mortem* diagnostic methods as the disease progresses. The red line and the blue dotted line indicate the cell-mediated immune response and the detectability by IFN-γ and Tuberculin skin test (respectively); the green dotted line shows the antibody response. Source: adapted from Vordermeier et al. (2004).

## 1.2. Epidemiology of Bovine Tuberculosis

### 1.2.1. Susceptible Hosts and Reservoirs

*Mycobacterium bovis* has been the commonest isolated mycobacteria from tuberculous cattle over centuries (Pollock & Neill, 2002); domestic cattle and species of the *Bovidae* family (i.e., buffalo and bison) are the most susceptible and represent the main animal reservoirs ((Brosch et al., 2002); albeit, possible differences in susceptibility between different cattle subspecies have been hypothesized (i.e., *Bos taurus* and *Bos indicus*) (Rodriguez-Campos et al., 2014).

However, the host range of the *M. bovis* is exceptionally wide (Brosch et al., 2002; Cousins, 2001; O'Reilly & Daborn, 1995; Rodriguez-Campos et al., 2014), it has been isolated from domestic ruminants other than cattle, camelids and many other domesticated animals, such as pigs, cats, dogs, equines, and parrots (Good & Duignan, 2011; Michel et al., 2010; O'Reilly & Daborn, 1995; Pesciaroli et al., 2014); in addition, *M. bovis* has been reported in several non-domesticated animals (Humblet et al., 2009) and wildlife species (Good & Duignan, 2011; OIE, 2015; Rodriguez-Campos et al., 2014; Waters et al., 2014).

The exceptionally broad host range of *M. bovis* is also reflected in a widespread reservoir in diverse species (Gortázar et al., 2015). Depending on the characteristics of the local host community, domestic species other than bovid may also act as bTB reservoirs (EFSA AHAW Panel, 2017; Pesciaroli et al., 2014).

In particular, goats seem to be very susceptible to bTB infections (Pérez de Val et al., 2011) and it has been suggested their role as possible reservoirs (Napp et al., 2013; Zanardi et al., 2013); sheep have been traditionally considered less susceptible than cattle and goats (Caswell & Williams, 2016), however, increasing evidence highlighted that, in certain epidemiological situations, this specie could also have a role in the maintenance of the disease (Broughan et al., 2013; Muñoz Mendoza et al., 2012; Muñoz-Mendoza et al., 2016; Pesciaroli et al., 2014). Moreover, the potential role of pigs as reservoir has also been reported (Amato et al., 2018; Bailey et al., 2013; Di Marco et al., 2012) and it was demonstrated that the same *M. bovis* stains circulate in pigs, wild boar and cattle (Bailey et al., 2013; Parra et al., 2003; Santos et al., 2009). Finally, among domesticated animals also alpaca and llama are considered domestic reservoir (García-Bocanegra et al., 2010; Twomey et al., 2007); whereas, horses, cats and dogs are considered spillover hosts.

Beside, in different countries, specific wildlife species, playing a role of major reservoirs of *M. bovis*, have been reported, as for example, white-tailed deer (USA), fallow dear (Spain) , red-deer (Spain and Canada), Eurasian wild boar (Spain), brushtail possums (New Zealand), African buffalo (South Africa), Eurasian badgers (United Kingdom and Ireland) (Fitzgerald & Kaneene, 2013; Gortázar et al., 2011; Hardstaff et al., 2014; Naranjo et al., 2008; Palmer et al., 2013; Parra et al., 2005;

Waters et al., 2014). In these areas, the presence of these species may hinder attempts to control and eradicate bTB in livestock (Hardstaff et al., 2014).

Although *Mycobacterium tuberculosis* remains the main causative agent of human TB, humans can also be infected by *M. bovis* (i.e., zoonotic tuberculosis) which causes a clinically undistinguishable disease from that of human origin, making bTB an important zoonotic disease of public health concern (Evans et al., 2007; Michel et al., 2010; Olea-Popelka et al., 2017; O'Reilly & Daborn, 1995; Palacios et al., 2016). Animal-to-human transmission is the main origin of zoonotic tuberculosis, however, less commonly, human-to-human transmission of *M. bovis* has been demonstrated among both immune deficient (Evans et al., 2007) and immune-competent patients (Palacios et al., 2016; Sunder et al., 2009).

It is worth to mention that, in several areas, bTB is maintained in a multi-host-pathogen system with *M. bovis*, and where present *M. caprae*, circulating between domesticated and wildlife reservoirs. In these contexts, the maintenance of the disease is ensured by multiple species epidemiologically linked among them, which may include multiple reservoir hosts and several routes of transmission (Cowie et al., 2016; Good et al., 2018; Gortázar et al., 2015; Palmer, 2013).

### 1.2.2. Modes of Transmission

*M. bovis* is extremely resistant in the environment and can survive under extreme conditions (Courtenay et al., 2006; Fine et al., 2011). Depending on weather conditions, it can be isolated from carcasses for up to 6 weeks and from faecal matter during summer (at temperatures of 24-43ºC under the sunlight) for up to 4 weeks, but its survival can be longer than 5 months under diffuse sunlight conditions. Its persistence in the environment can reach one year during winter (at temperatures of 12 - 14ºC) and more than 2 years if covered in dung. Moreover, *M. bovis* is able to survive up to 58 days in contaminated water, and it can also be spread by rains to contaminate grazing areas. However, the amount required for indirect transmission is clearly higher than that needed for direct or aerosol transmission. Even though indirect transmission due to environmental contamination can occur, direct transmission from infected animals is considered the main source of new infections (Cosivi et al., 1995; Fine et al., 2011; Sweeney et al., 2007). Once bTB is established in a herd, it spreads via aerosols, suckling, direct contact between animals and sharing of water and feed, persisting in cattle through horizontal transmission (Biet et al., 2005). Obviously, animals with gross lesions that communicate with airways or intestinal lumen are the most efficient disseminators of infection (Radostits et al., 2007).

The aerogenous (i.e., respiratory secretions) is the most frequent excretion route. It occurs intermittently and mycobacteria are mainly excreted through the exhaled air (aerosol droplets); however, cattle may also excrete viable mycobacteria in nasal discharge and tracheal mucus during the early stages of the disease, before the occurrence of any visible lesion (Mcllroy et al., 1986). Thus, in the animal-to-animal transmission (i.e., direct contact), the primary route of transmission for *M. bovis* is the respiratory through the inhalation of aerosols containing mycobacteria and the vast majority of infections among cattle occur this way (Domingo et al., 2014; Morris et al., 1994; Neill et al., 2005).

Infected cattle might excrete mycobacteria also through, faeces, urine and milk. It has been reported that about a 10% of heavily infected cattle can excrete *M. bovis* in faeces, however, other authors increase this percentage up to 80% (Reuss, 1955 in Phillips et al., 2003). In fact, it has been recently suggested that the oropharyngeal route could have a more significant role in the transmission and maintenance of bTB than previously reported in literature (Domingo et al., 2014; Serrano et al., 2018). In addition, the oral transmission has a major role in the bTB transmission to calves during suckling from infected cows excreting mycobacterium in milk (Goodchild & Clifton-Hadley, 2001).

The oral transmission is an important pathway also in the transmission to other domestic species and humans. Dogs and cats are more frequently infected by this route due to their habits (i.e., drinking infected milk, feeding on infected carcasses or coming into contact with infected pus secreted through open lesions), even though they can also get the infection through direct contact with infected cattle (Gilsdorf et al., 2006; Fitzgerald et al., 2016). In humans, *M. bovis* is largely transmitted through consumption of unpasteurized infected milk, but there is also the possibility of inhalation of aerosols due to contact with cattle, especially for some professions (i.e., livestock keepers, abattoir workers, or veterinarians) or in areas where people live in strict and direct contact with animals (El Idrissi & Parker, 2012; Michel et al., 2010; Thoen et al., 2006; Thoen et al., 2009; Thoen et al., 2010; Vayr et al., 2018).

Excretion of *M. bovis* through semen, vaginal and uterine discharges and discharges from open peripheral lymph nodes are also possible but highly infrequent (Constable et al., 2017; Radostits et al. 2007. Others route as the congenital, or entry through open wounds are uncommon (Good & Duignan, 2011).

### 1.2.3. States of Infection

Bovine TB in cattle is characterized by a chronic progression. The dynamics of *M. bovis* transmission are not completely understood, and the conditions under which infected cattle become effectively infectious are not fully defined. Knowledge on bTB infection dynamics mostly derived from experimental and field studies (Goodchild & Clifton-Hadley, 2001; Menzies & Neill, 2000; Pollock & Neill, 2002), and, more recently, from the application of conceptual mathematical models (Álvarez et al., 2014a). Investigations mainly focused on the infectiousness of infected cattle, and the relationship between different immunological statuses with respect to diagnostic test. Those investigations highlighted the existence of different stages of infection and tried to estimate their duration.

After the infection, cattle undergo two periods (Fig. 3): the pre-infectious and the infectious stages; their duration is highly variable and it depends on several factors related to the host, the route and dose of infection (Francis, 1947; Goodchild & Clifton-Hadley, 2001; Menzies & Neill, 2000; Pollok & Neill, 2002). The time from infection to excretion (i.e., pre-infectious period) is reported to range between 3 and 35 months (21 months on average) (Barlow et al., 1997; Kao et al., 1997; Fischer at al., 2005; Smith et al., 2013 (reviewed in Álvarez et al., 2014a)). On the other hand, the time cattle needed to develop a (cell-mediated) immune response detectable by diagnostic tests, known as pre-allergic or occult period, may range between 14 and 119 days (41 days on average) (Barlow et al., 1997; Kao et al., 1997; Fischer at al., 2005; Conlan et al., 2012; Smith et al., 2013 (reviewed in Álvarez et al., 2014a)). Animals in the more advanced stages of the disease might enter into a state of anergy, with a depressed cell-mediated immune response, which makes them unresponsive to the traditional, cell-mediated, diagnostic tests (i.e., tuberculin and gamma-interferon); however, these anergic animals might be detected by serological assays (mainly ELISA-type) which measure the antibodies against *M. bovis* (de La Rua-Domenech et al., 2006).

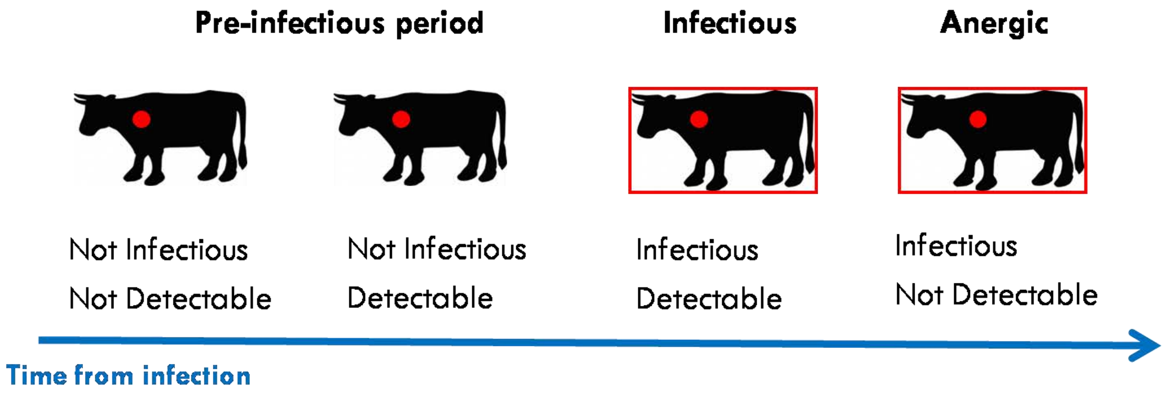



Figure 3: bTB infection dynamics in cattle: different stages of infection and immunological statuses with respect to the response to in vivo diagnostic test as the disease progresses. Source: adapted from Barlow et al., 1997.

### 1.2.4. Geographical distribution

Bovine TB is a notifiable disease listed by World Organisation for Animal Health (OIE) (box 1), and therefore its occurrence must be reported to the OIE and designated national institutions (Awada et al., 2018; EFSA AHAW Panel, 2017).

Over the last 30 years (from 1986 to 2016), data reported to the OIE evidences a regular and significant improvement in the global control of bTB worldwide. In all geographical regions, the proportion of reporting countries notifying bTB decreased significantly, with an overall reduction by more than 30%, though, the rate of decrease has not been homogeneous all over the world (Awada et al., 2018).

The largest decrease in regional bTB trends was observed in Oceania and Europe (i.e., by more than 45%), followed by Asia (i.e., 38% decrease); whereas, the decrease in bTB notification was slower in Africa and the Americas, with a reduction of 25% and 18%, respectively, over the 30-year period (Awada et al., 2018).

In 2017, bTB was present in the 43% of the OIE reporting countries and was present in every region of the world, being bTB widespread in Africa, Central and South America, parts of Asia and some Middle Eastern countries (Fig. 4). In the United States (U.S.), the disease has been eliminated in most but not all territories, where bTB remains prevalent in domestic and wildlife reservoirs (i.e., low prevalence level in cattle but high prevalence in the wildlife); for example, in last years, outbreaks in cattle herds have been reported in Michigan, Texas, New Mexico and California (Kaneene & Pfeiffer, 2006).

## BOX 1: The OIE: diseases notification and international standards

The OIE is the intergovernmental organization responsible for improving animal health worldwide and, in 2017, had a total of 181 member countries.

It was created in 1924 with the aim of exchanging information on animal diseases between countries, thus, ensuring transparency of the animal health; and it is recognized as the reference organization by the World Trade Organization.

The first assigned name was the "Office International des Epizooties (OIE)"; later, in 2003, it became the "World Organisation for Animal Health", but kept its historic acronym 'OIE' (Awada et al., 2018).

Initially all notifiable animal diseases were included in two former lists (i.e., list A and B). Between 2004 and 2005, the two lists were replaced by one unique list that entered into force in 2006. The criteria to identify diseases to be included in this OIE single list were also established, and referred to the risks of spread of the infectious microorganism internationally, together with the consequences for humans, domestic livestock and wildlife; and, the availability of reliable methods for diagnosis and detection (Awada et al., 2018). This list and it is reviewed on a regular annual basis and, for year 2018, it includes 117 animal diseases (available at: http://www.oie.int/en/animal-health-in-the-world/oie-listed-diseases-2018/).

Two specialist OIE commissions are in charge for the development and the update of international standards and recommendations that are formally adopted by the World Assembly of Delegates of the OIE.

These international standard settings are published by the OIE in the Animal Health Code and the Manual of Diagnostic Tests and Vaccines (i.e., both for terrestrial and aquatic animals) (Awada et al., 2018). The first *Terrestrial Animal Health Code* was published in 1968. It sets out standards for the improvement of animal health and welfare and veterinary public health, including standards for safe international trade in animals and their products. The *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, covering infectious and parasitic diseases, was first published in 1989 with the aim to provide internationally agreed diagnostic laboratory methods and requirements for the production and control of vaccines and other biological products.

Similarly, in New Zealand, despite considerable progress has been made, the elimination of the disease is still ongoing due to the presence of wildlife infected possum acting as reservoir (i.e., brush-tailed possums) (Awada et al., 2018; Ryan et al., 2006). In Australia, bTB has been successfully eradicated from animals and humans' populations, being the elimination of wild water buffalo and feral cattle from endemic areas one of major components of the eradication campaign (Awada et al., 2018; Good & Duignan, 2011; Radunz, 2006).

Between 2015-2016, bTB was also notified in a total of 19 wild species (Fig. 4), with the highest number of cases at global level reported in wild boar (*Sus scrofa*), European badger (*Meles meles*) and African buffalo (*Syncerus caffer*), which appear to be the main reservoir species (Fitzgerald & Kaneene, 2013).

It is worth to mention that for many OIE member countries there is not enough data available to assess the real presence and burden of bTB, in particular in wildlife, with important gaps of information in some developing countries, mainly in Africa, Asia and South America (Awada et al., 2018).

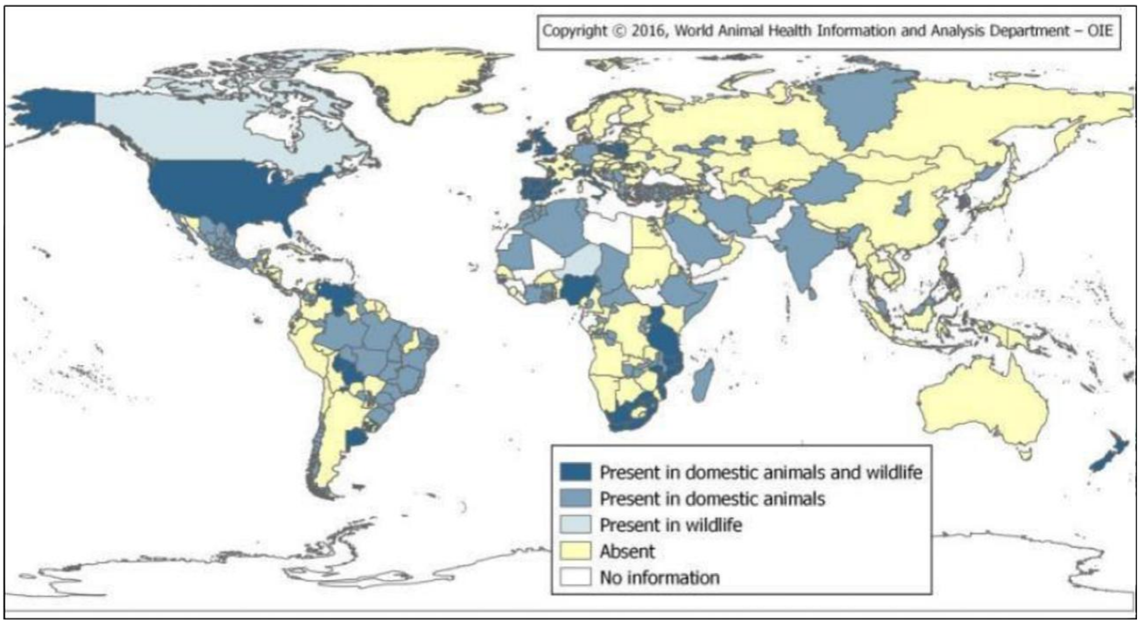



Figure 4: WAHIS Reports of *M. bovis* in wildlife and domestic animals, 2015 - 2016 (*Source*: OIE in "activities conducted under One Health Concept / One Health: Neglected Zoonoses"; available at http://www.rr-asia.oie.int/activities/regional-activities/one-health/neglected-zoonoses/2017-brucellosis-ws-bangkok/presentation/)

In Europe, the overall proportion of bTB infected/positive cattle herds is very low (0.7% in 2016). However, the distribution of the disease across countries is highly heterogeneous (Fig. 5), with prevalences ranging from zero to higher than 15% in some areas (EFSA &ECDC, 2017).

According to the data published by the European Food Safety Authority (EFSA) and the European Centre for Disease Control (ECDC) for 2016, 21 countries confirmed their status as Officially Free of Bovine Tuberculosis[3] (OTF) (Fig. 6), whereas 10 Member States had not achieved the OTF status at country-level yet (non-OTF MS); rather, within non-OTF MS, the overall bTB herd prevalence increased from 1.1% in 2010 to 1.6% in 2016 (EFSA & ECDC, 2017).

The observed epidemiological situation seems to be greatly affected by the existence of different breeding systems and a variety of environmental conditions (i.e., badgers as reservoir in Ireland and the United Kingdom or semi-free ranging systems in Spain and Portugal) (Parra et al., 2003; Reviriego & Vermeersch, 2006).

It is worth to mention that, compared to *M. bovis*, the geographical spread of *M. caprae* is quite limited. It has been mainly recognised in Spain and Central and Western European countries, such as Austria, France, Germany, Hungary, Italy, Slovenia, and the Czech Republic (Good & Duignan, 2011). Interestingly, many countries, reporting the isolations of *M. caprae*, are virtually free of bTB caused by *M. bovis* (Rodriguez-Campos et al., 2014).

[3] In accordance with EU regulation, the Officially Tuberculosis Free (OTF) status, granted to a country or part of a country (i.e., namely 'region'), implies the reporting during 6 consecutive years of an apparent area prevalence below 0.1 % and at least 99.9% of the herds within that country or region bTB-free. The minimal size of a 'region' is defined by the regulation (i.e., 2.000 $km^2$) and 'regions' are based on administrative units in each country. The official status of an area with regard to bTB will in turn influence the surveillance programme implemented in that area, resulting in different regimes of sampling and testing.


Figure 5. Proportion of cattle herds infected / positive for bTB, EU/EEA, 2016. Source: the European Union Summary Report on Zoonoses and Food-borne Outbreaks 2016 (EFSA & ECDC, 2017).

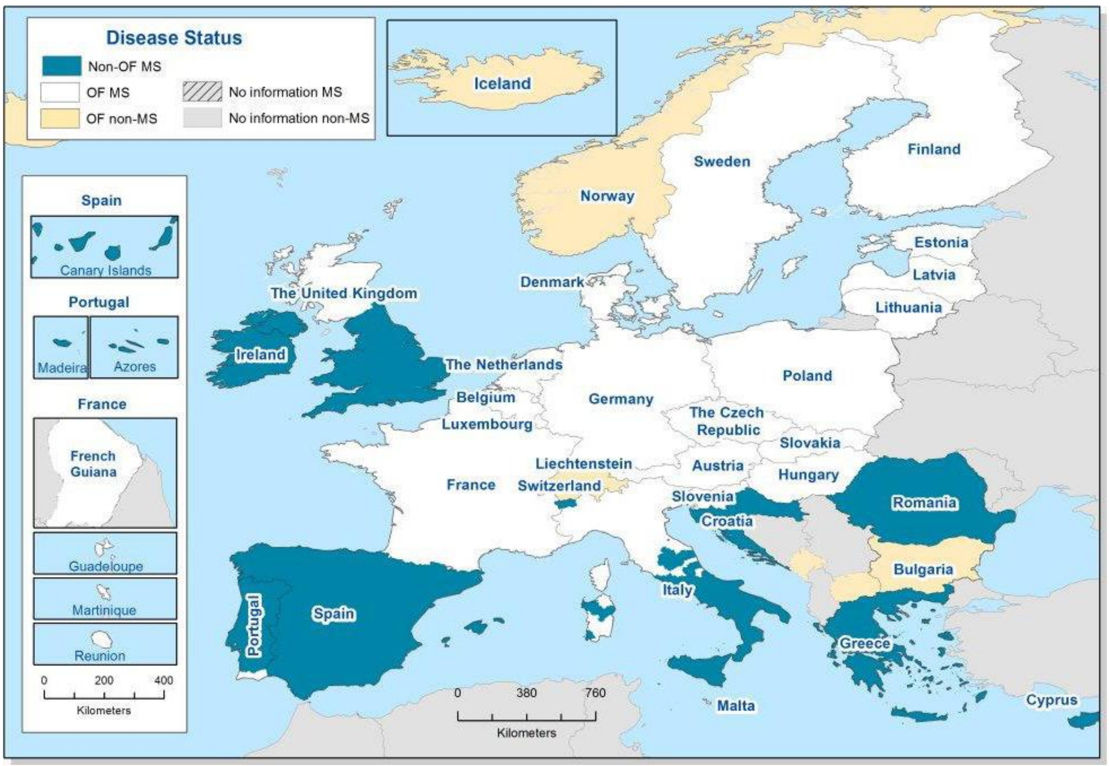



Figure 6: Official status of the EU/EEA Countries on bTB, 2016. Source: the European Union Summary Report on Zoonoses and Food-borne Outbreaks 2016 (EFSA & ECDC, 2017).

In Spain, the bTB herd prevalence strongly decreased from the end of the 80s to the beginning of 2000. Since then, no further decline in cattle herds' prevalence has been reported (Anon., 2018). Actually, after an impasse in the reduction which lasted for about a decade (from 1.8% in 2004 to 1.7% in 2014), a significant increasing trend was observed. In 2016, the overall bTB herd prevalence reported in Spain was 2.9%, with an increase of about 70% compared to 2014 (EFSA & ECDC, 2017; Anon., 2018).

Besides, the epidemiological situation across the country is extremely heterogeneous (Allepuz et al., 2011; García-Saenz et al., 2014) (Fig. 7): The Balearics and The Canaries islands are free of bTB; others regions, mainly located in the north of Spain, show very low prevalences (i.e., 0.05% in Galicia or 0.3% in Catalonia); while, the central and southern-west areas of the country, report very high herd prevalences (e.g. 17.1% in Andalusia in 2016) (Anon., 2018).

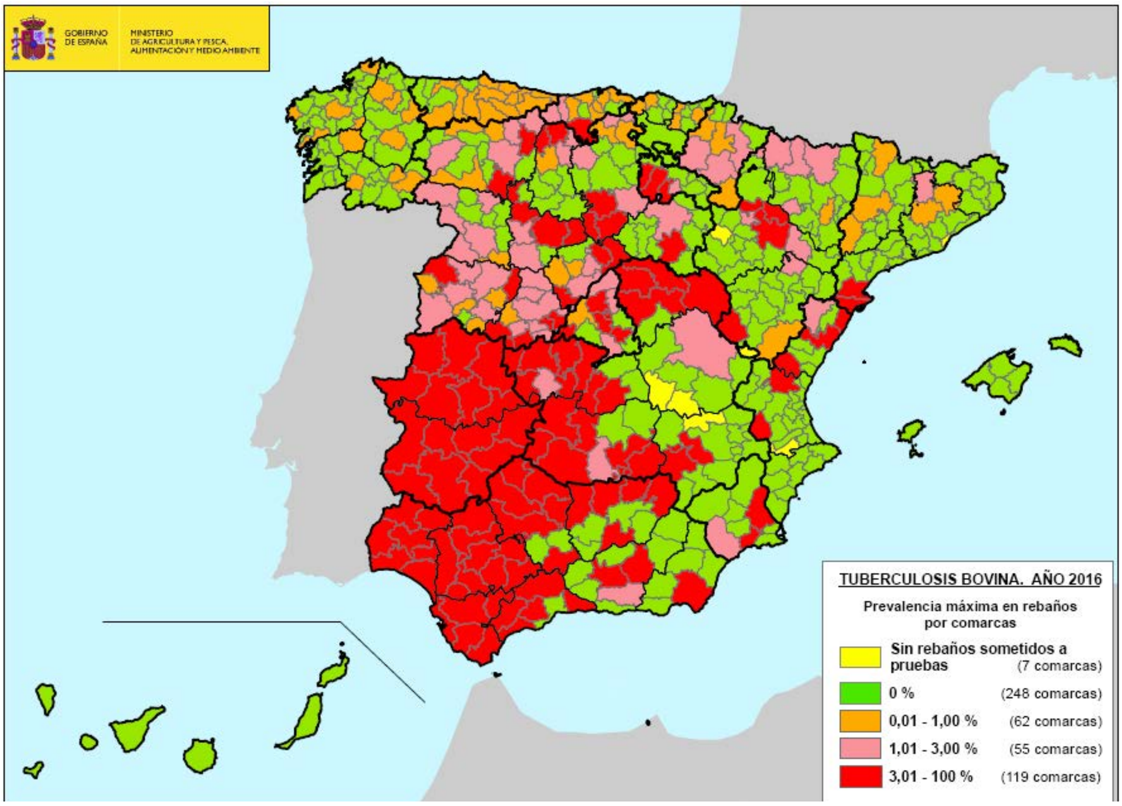



Figure 7: Cattle herd prevalence of bTB in Spain, 2016. Source: "Programa Nacional de Erradicación de Tuberculosis Bovina, España 2018" (Anon., 2018).

## 1.3. Control policy for Bovine Tuberculosis and diagnostic tools

Historically, bTB was one of the main diseases of domestic animals throughout the world, most likely distributed by the movement of domesticated cattle and exacerbated by the gradual intensification of cattle production (Francis, 1947; Rodriguez-Campos et. al, 2014).

The disease has been described in slaughtered cattle since the early 1800s and, in 1895, the zoonotic risks of *M. bovis* due to consumption of infected milk was already known (Good & Duignan, 2011; Good et al., 2018), although it was not until the beginning of 1900, that bTB finally received political attention and some governments decided to implement measures against it (Francis, 1947; Good & Duignan, 2011; Good et al., 2018). The first actions adopted to control bTB, including milk pasteurization, were strongly disapproved by the farming community and the milk industries, evidencing the low awareness of the risk posed by bTB to the health of people (Francis, 1947; Good & Duignan, 2011; Good et al., 2018).

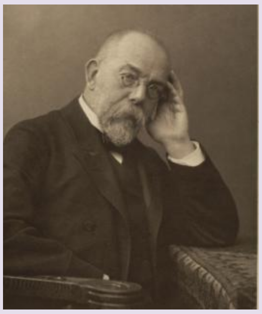
As matter of fact, applications of control plan in cattle herds were firstly introduced (between 1917 and 1919) in the United States (U.S.) and Canada, in order to prevent both entries of infected animals from Europe and disease spread within the States (Gilsdorf et al., 2006; Good & Duignan, 2011). Other pioneer countries in the implementation of national bTB control programmes were Finland, Denmark and The Netherlands that introduced national bTB eradication plans between 1893 and 1922 (Good & Duignan, 2011; Good et al., 2018). The Danish programme in cattle was the first in using the tuberculin test as official diagnostic tool on a national scale (i.e., the "Bang method", box 2). However, it was not until the end of the 20th century that most of European Countries introduced national control programmes for bTB and, since then, huge efforts have been made to eradicate the disease (Conlan & Wood, 2018; Good & Duignan, 2011; Good et al., 2018).

## Box 2: The origin of the Tuberculin Skin Test

Robert Koch discovered the "Tubercle bacillus" in 1882 and, in 1890, developed the tuberculin (i.e., an extract of the TB bacilli) trying to demonstrate its therapeutic qualities for the treatment of human tuberculosis. As soon as the possibility of using the tuberculin to detect infected animals was recognized, the first tests were quickly developed (Good et al., 2018; Good & Duignan, 2011).

The diagnostic potential of the tuberculin was highlighted by Bernhard Bang; who, during the early 1890s, introduced the tuberculin test, using Koch's Old Tuberculin (KOT), as official diagnostic tool in the Danish bTB eradication programme. The so called "Bang method" consisted in the repetitive use of KOT, at regular testing interval of six month, in order to identify reactor animals (Good et al., 2018). The testing of cattle using KOT, up to day, remains the basis of all bTB control programs (Good et al., 2018; Francis, 1947).

In the 1930s, Florence Seibert developed a process for isolating and purifying the active protein of the tuberculin with antigenic proprieties (Seibert & Glenn, 1941). In producing the first purified protein derivative tuberculin (PPD), Seibert enabled the first reliable tuberculin test (i.e., the skin test), since prior to this, the tuberculin used was not consistent or standardized. Seibert did not patent the technology that was adopted as the standard by the United States in 1941 and by the World Health Organization in 1952 (Harding 2017). This skin test which uses PPD and avails of a cell-mediated response, became the world standard diagnostic tool; it is listed on the World Health Organization's essential medicines list and is still in use today for the diagnosis of tuberculosis in man and animals.



**Koch, Robert (1843-1910)** *Source*: The National Library of Denmark and Copenhagen University Library

**Bang, Bernhard (1848-1932).** *Source*: The National Library of Denmark and Copenhagen University Library

**Seibert, Florence (1897-1991).** *Source*: Acc. 90-105 - Science Service, Smithsonian Institution Archivess

The international acknowledgement of the significance of the threat from zoonotic tuberculosis resulted in the inclusion of bTB among the OIE-listed diseases[4], in 1964, in the gradual implementation of stringent meat inspections and in the introduction of mandatory sanitation of milk for human consumption (i.e., pasteurization[5] or boiling) (Awada et al., 2018). In 1983, the OIE adopted of a resolution calling for the eradication of *M. bovis*, for both public health and economic reasons (Good et al., 2018; Kleeberg, 1984).

Nowadays, national bTB eradication programmes have been introduced in many countries throughout the world, either as voluntary or compulsory. Control programs for bTB have been primarily focused on control of *M. bovis* infections in cattle (Skuce et al., 2018) and consisted of three main components: prevention, surveillance and eradication; whereas, in most of countries, the treatment of bTB in cattle is not permitted (Anon., 1994; El Idrissi & Parker, 2012).

In particular, the prevention, mainly based on herd hygiene and biosecurity practices, is aimed to reduce the exposition to the pathogen; the routine surveillance, usually, includes *ante-mortem* testing of livestock and slaughter surveillance. The eradication of *M. bovis* from domestic herds can be achieved through the whole-herd depopulation strategy (i.e. stamping-out) followed by the restocking of the farm after a waiting period. However, the emergence of financial and animal welfare constraints, as well as the emotional impact on individual farmers and the opinion of public community (i.e., consumers) made this strategy be indicated only on rare occasions and under specific circumstances (Good & Duignan, 2011; Kaneene & Pfeiffer, 2006; Schiller et al., 2010).

As an alternative strategy, "test-and-slaughter" policies, based on the testing of the whole herd and the culling of positive cattle (i.e., reactors), were implemented. An essential aspect of this strategy is that once at least one animal from a herd tests

[4] Bovine tuberculosis was included in the OIE list of notifiable diseases (i.e., initially the List B) following a revision by the International Committee of the OIE that led to the establishment of a new list of diseases. This revision took into changes in the national zoosanitary legislation of member countries during the previous 40 years, and the specific request by some international organizations, such as the Food and Agriculture Organization of the United Nations (FAO), and the European Economic Community (EEC). (Awada et al., 2018)

[5] Between 1930 and 1960 several experiments were performed on the "High Temperature Short Time (HTST)" procedure for milk pasteurization in order to assess the efficacy to inactivate pathogens. Among the target organism were *M. bovis* and *M. tuberculosis*. As a result, the HTST-pasteurization is prescribed by the *Codex Alimentarius* as a standard treatment to reduce pathogens in milk. (Hammer, 2004, FAO & WHO, 2011).

positive, no movements of animals other than to slaughter are allowed until the herd tests negative again (Good & Duignan, 2011; Kaneene & Pfeiffer, 2006; Pfeiffer, 2013; Schiller et al., 2011). Mandatory eradication programs based on test-and-slaughter policies successfully eliminated bTB from livestock in most of high-income industrialized countries. However, the maintenance of *M. bovis* infection in wildlife reservoirs has compromised eradication efforts in some countries such as in the United Kingdom, Ireland, New Zealand and parts of the United States of America (Hardstaff et al., 2014; Thoen et al., 2009).

In Europe, bTB has been an important issue since the beginning of the European Economic Community (EEC); and, still today, its eradication remains a major common objective and a financial target within EU countries (Reviriego & Vermeersch, 2006). The current EU policies on the bTB eradication are based on the principle that the Member States are primarily responsible for the bTB eradication and may receive community financial support from the EU on the condition that those programs have been approved by the European Commission (Reviriego & Vermeersch, 2006).

Measures adopted in Europe[6] are mostly based on "test-and-slaughter" strategies (i.e., routine application of tuberculin testing and culling of reactor cattle). The official diagnostic test for bovine TB in live animals is the intradermal tuberculin test (Council Directive 64/432/CEE) and, since 2002, the interferon-γ assay (IFN-γ) is accepted and may be authorized for its use as ancillary test to the tuberculin test to maximize detection of infected cattle (Commission Regulation (EC) No. 1226/2002), in line with last updates of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, produced by the OIE[7] (Reviriego & Vermeersch, 2006; SANCO WD, 2013).

Despite the fact that many animal species can be infected with *M. bovis* and *M. caprae*, the current European eradication programmes target primarily cattle (Reviriego & Vermeersch, 2006). Other domestic species (e.g., goats and sheep and pigs) are not routinely included in the eradication programmes or they are included

[6] The most relevant legislation regarding the eradication of bTB in Europe was recently summarized in the "Working Document on Eradication of Bovine TB in the EU" and its Annexes (SANCO/10067/2013). Available online at: https://www.visavet.es/bovinetuberculosis/documents.php

[7] The interferon-γ assay (IFN-γ) is recommended by the OIE since 1996 (OIE Terrestrial Manual) as ancillary laboratory-based test to the tuberculin intradermal test.

only in specific circumstances (i.e., co-existence in the same farm of goats and cattle), or in pilot programmes (e.g., irregular programmes with spotty coverage).

In Spain, it was not until 1993 when most dairy and beef herds were included within the national bTB eradication program (Allepuz et al., 2011), which, currently, is based on following pillars:

Regular periodic screening of cattle herds, testing all animals older than six months, performed by authorized private veterinarians (i.e., *ante-mortem* testing). The frequency of the routine screening ranges from six months to two years interval, depending on the prevalence of the area (i.e., prevalence<1%; prevalence >1 or prevalence>3%). Cattle that test positive are culled (with compensation of farmers) and subjected to *post-mortem* examination at the slaughterhouses. According to the Spanish legislation (RD 2611/1996), the Single Intradermal Tuberculin Test (SITT) is the official test for bTB detection in the routine screening, and positivity is confirmed by culture of the mycobacteria. Depending on the epidemiological situation, also the Single Intradermal Comparative Tuberculin Test (SICTT) and the IFN-γ can be authorized.
Compulsory pre-movement tests on purchased cattle (introduced in 2006 for safe trading) (Anon., 2010).
Systematic *post-mortem* examinations at the abattoir (i.e. slaughterhouse surveillance), with reporting of all suspicious lesions to the Laboratory for analysis and confirmation.

Furthermore, other measures and interventions integrated in the Spanish eradication plan include: a) epidemiological investigations through a standard questionnaire (BRUTUB) of all new tested positive farms; b) Ongoing training of Official Veterinary Services (OVS); c) mandatory training for private veterinarians performing the routine testing (i.e., required for official accreditation); d) auditing of the testing practices of private veterinarians (audited by OVS); e) surveillance plan for wildlife reservoirs (introduced in 2009); f) testing of goat herds (i.e., by SITT or SICCT and IFN-γ) that live together with cattle, or that share pasture with cattle, or that are epidemiologically linked to positive cattle herds (Anon., 2018).

*Ante-mortem* tests are a critical component of any bTB control programme. According to the international legislation, the tuberculin test (i.e., delayed hypersensitivity test), the most widely used as the screening technique, is the official prescribed method for the *in vivo* diagnosis of bTB for international trade and national control, while the gamma-interferon assay (blood-based laboratory test) is listed as the alternative *in vitro* test for international trade (Bezos et al., 2014; de la Rua-Domenech et al., 2006; OIE, 2015; Reviriego & Vermeersch, 2006). In the case of bTB, both *in vivo* and *in vitro* assays are performed using purified protein derivatives (PPDs) of *M. bovis* (bovine PPD) and *M. avium* (avian PPD) (de La Rua-Domenech et al., 2006) and rely on the detection of early cell-mediated immune responses (CMI) to the tuberculin protein. In fact, infected animals turn allergic to the PPD and, when exposed to those proteins, develop characteristic delayed-type hypersensitivity reactions (de La Rua-Domenech et al., 2006; Kaneene & Pfeiffe, 2006; Radostits et al. 2007).

The main variants of the tuberculin test in use today are known as the Single Intradermal Tuberculin Test (SITT), which is performed using only bovine tuberculin PPD, and the Single Intradermal Comparative Tuberculin Test (SICTT), which is performed using both bovine and avian tuberculin PPDs in combination (Bezos et al., 2014; Good & Duignan, 2011; Karolemeas et al., 2012; Monaghan et al., 1994).

Generally, the SITT has a higher sensitivity, while the SICTT has a higher specificity (de la Rua-Domenech et al., 2006; Karolemeas et al., 2012; Schiller et al., 2010). In the majority of cases, the SICCT is applied to differentiate between responses from exposure to *M. bovis* or to other mycobacteria. In fact, non-specific sensitisation to the bovine tuberculin PPD (i.e., cross-reactions) may be caused also by other pathogenic mycobacteria, such as *Mycobacterium paratuberculosis* subsp. *avium*, and non-pathogenic environmental mycobacteria (Good & Duignan, 2011; Karolemeas et al., 2012).

However, the sensitivity of the SITT is influenced by several different factors, such as the immunological status of hosts (i.e., early infection, anergy, age, immunosuppression, co-infection or pre-exposure to other mycobacteria), the characteristics of the PPDs (i.e., expired products, storage conditions, manufacturing, potency), or the methodology used to perform the test (i.e., doses, site of injection, experience of veterinarians) (Álvarez et al., 2012a; de la Rua-Domenech et al., 2006; Good & Duignan, 2011; Good et al., 2018; Humblet et al., 2011).

The site of injection has been one of the most studied factors influencing the accuracy of the tuberculin tests (Good et al., 2018; Good & Duignan, 2011); the tuberculin test can be performed on the neck region (i.e. cervical SITT), with the middle third of the neck described as the optimal injection site (Casal et al., 2015), or it can be performed in the caudal fold of the tail (CFT). The cervical SITT is more sensitive than the CFT (Good et al., 2018; Good & Duignan, 2011; OIE, 2015) and, in order to compensate this difference, higher tuberculin doses are allowed in the CFT (OIE, 2015). The CFT has been widely used in the U.S. and New Zealand; and, it was also used in Australia during their bovine TB eradication campaign. The SITT is adopted by most European Union Member States; whereas, the SICTT has been mainly used in Great Britain, Ireland and Portugal (Good & Duignan, 2011; Karolemeas et al., 2012).

The tuberculin test has demonstrated to be an effective tool when applied at herd level, although a lack of sensitivity at the individual animal level is recognised to be its limitation (EFSA AHAW, 2012). To overcome this problem, the cut-off point of the tuberculin test can be changed (i.e., standard interpretation versus severe interpretation). Moreover, the IFN-γ is also authorized as ancillary laboratory-based test, and it may be used as parallel test to the tuberculin test in order to maximise sensitivity (EFSA AHAW, 2012; Good & Duignan, 2011; Good et al., 2018). Besides, following the EU-approved use, many countries have adopted protocols for the use of the IFN-γ assay as a serial test to the tuberculin test in order to increase the specificity.

It has been demonstrated that the IFN-γ test has a higher sensitivity compared to the diagnostic performance of the tuberculin test, but its specificity is lower than that of the SITT and the SCITT. The higher sensitivity of the IFN-γ test compared to the tuberculin test is likely due to the fact that the IFN-γ test detects bTB infected animals as early as 14 days following infection, whereas reactivity to the SITT usually develops between 3 and 6 weeks post-infection (de la Rua-Domenech et al., 2006; Serrano et al., 2018). Thus, using the IFN-γ test, the pre-allergic phase, or occult period (i.e., time to develop an immune response detectable by diagnostic test), is shorter than using the SITT. As consequence there is a reduction of false negative reactions in recently infected animals and, therefore, an increase of sensitivity. The interpretation criteria of the IFN-γ assay can be adapted based on the epidemiological situation, disease prevalence and the stage of the bTB control program; an overview of different criteria applied in European Countries was published by the EFSA (2012) (EFSA AHAW, 2012).

As of 2016, other diagnostic techniques for bTB include the lymphocyte proliferation assay and the Enzyme-Linked Immunosorbent Assay (ELISA) described as alternative blood-based laboratory tests. The microscopic examination, culture and nucleic acid recognition methods are recommended for agent identification (Awada et al., 2018; OIE, 2015).

Until today, antibody-based assays have shown a poor sensitivity due to the characteristic of the humoral immune responses to the mycobacterial infection, which is quite delayed in the case of bTB (Pollock et al., 2001; Waters et al., 2006). Therefore, its use as diagnostic test for the early detection of tuberculous cattle has been quite limited (Pollock et al., 2001; Schiller et al., 2010). However, serologic assays, such as ELISA, may be particularly useful as complementary tools to detect infected animals missed by cell-mediated response-based tests, as for example in the case of chronically-infected/ anergic cattle that may be acting as bTB reservoirs (de la Rua-Domenech et al., 2006; Radostits et al. 2007; Schiller et al., 2010; Waters et al., 2006).

Mycobacterial culture is regarded as a gold standard for confirmatory post-mortem diagnosis of bTB and it offers the advantage of species identification (Patané et al., 2017). Typing methods allow identifying the mycobacterial species on a molecular basis, differentiating *M. bovis* strains from the other strains of the MTC (Brosch et al., 2002). However, culture presents certain limitations (i.e., the difficulty of obtaining samples, the need for pre-treatment, slow growth of the agents and additional time for identification) (Patané et al., 2017; Schiller et al., 2010).

Recently, huge progresses have been achieved in the fields of culture technologies (e.g. liquid culture systems) and the molecular typing of mycobacterial strains is becoming an important tool for studying the epidemiology of bTB (Brosch et al., 2002). For example, the increased use of rapid typing techniques, based on PCR amplification (i.e., spoligotyping), and more recently, the ‘whole-genome’ sequencing (Kao et al., 2016), to characterize mycobacterial isolates from domestic livestock and wildlife has provided important insights into the sources of infection, the spread and maintenance of bTB, allowing the establishment of epidemiological links, necessary for the development of successful control and eradication strategies (Schiller et al., 2010).

## 1.4. Impact of Bovine Tuberculosis

Despite control efforts in the past 100 years, bTB still represents a global threat that generates a wide range of socio-economic impacts and public and animal health concerns. Impacts of bTB may be further classified as direct and indirect and being associated with the overt disease (i.e., control) or with the disease risk (i.e., prevention) (Perry et al., 2002).

Nowadays, the occurrence of bTB is geographically heterogeneous; mostly reflecting the political and economic situation of different countries (El Idrissi & Parker, 2012; Zinsstag et al., 2006). In fact, although still present in some high-income industrialized countries, the major impact of bTB falls on low- and middle-income countries that lack of the adequate institutional network and economic resources, including human (i.e., labour and management) and non-human resources (i.e., capital goods, financial resources and technology), to regularly apply expensive control strategies (Ayele et al., 2004; Azami & Zinsstag, 2018; Cosivi et al., 1998; El Idrissi & Parker, 2012).

### 1.4.1. Economic impact

At the beginning of 1900 bTB was one the most prevalent infectious disease of cattle, causing vast agricultural losses, to the extent that, in 1901, during his Nobel lecture, Von Behring stated that "*As you know, tuberculosis in cattle is one of the most damaging infectious diseases to affect agriculture. It causes premature death in affected animals, damages nutrition and milk production and is the cause of inferior meat*"[8].

Currently, the global economic impact of bovine TB on livestock production is extremely difficult to determine accurately, since available information is scarce and refers only to some specific countries. However, data suggest that economic costs associated to bTB are significant, causing worldwide annual agricultural losses of several billion dollars, with devastating consequences for the cattle industry (Garnier et al., 2003; Perry et al., 2002; Zinsstag et al., 2006);

In absence of control measures or effective surveillance plans, bTB has prejudicial implications for the livestock industry, the public health sector and the national

[8] The Von Behring Nobel lecture is available at: https://www.nobelprize.org/nobel_prizes/medicine/laureates/1901/behring-lecture.html

economy (Zinsstag et al., 2006). At national level, the most noticeable losses from bTB in cattle is the reduced benefit for farmers (i.e., direct and indirect "on-farm" costs), which include losses from decreased milk and meat production, calf losses, herd restrictions (i.e., movements and reduced herd size), the increased reproduction efforts and replacement costs for infected cattle. Studies published between 1969 and 1997 in several countries, such us Germany, Canada, Spain and the U.S., estimated decreases in milk productivity of about 10% and reduction in meat production of about 5%, rising to 10% in calves born from infected cows (Berga, 1987; Gilsdorf et al. 2006; Zinsstag et al., 2006). The fertility and demographic composition of the herd were also affected by bTB, positive animals showed a 5% decrease in annual calving rates and replacement losses for about 15% (Zinsstag et al., 2006); moreover, a reduction in calf weight around 20% was also reported (Gilsdorf et al. 2006). A more recent study carried out by Boland et al. (2010) in Irish dairy herds between 2004 and 2005 (i.e., high bTB prevalence despite the implementation of a national eradication programme) confirmed a decrease in milk yields for bTB positive cows. They evidenced that bTB reactors produced significantly less milk than non-reactor cows, with differences ranging from 120 kg to 573 kg that correspond to about the 10% of the average annual production (Boland et al., 2010). Further losses for farmers are due to carcass or organ condemnation at the abattoir when animals show gross visible lesions suggestive of bTB (Michel et al., 2010). It was estimated that, the culling loss due to the disease (i.e., the difference between the estimated economic value of beef or dairy breeding cattle and the purchased value at slaughter) may reach 30-50% (Zinsstag et al., 2006). Studies published in Spain at the end of *80s*, have reported losses of 1.4%, 7.7% and 20.1% of condemned carcasses, livers and lungs, respectively (Berga, 1987).

Apart from the reduced farmers' benefits, bTB has economic consequences also on trade and national economy. On an international scale, losses caused by bTB are mostly due to the limited access to foreign markets for livestock and animal products (i.e., import/export bans for enzootic countries). This has also major implications for all the economic sectors linked to livestock production (Zinsstag et al., 2006).

Finally, bTB economically affects also the public health sector, in terms of drug expenses and health-care costs, besides the payments for income loss (opportunity costs) and the burden on human health (i.e., Disability Adjusted Life Year[9]).

The economic impact of bTB is further exacerbated in low-income countries, mostly threatening the livelihoods of poor and marginalised communities due to the absence of adequate infrastructures, scarce means and the lack of financial resources for bTB control, which lead to a vicious cycle in which increased poverty affects the resources for control and vice versa (El Idrissi & Parker, 2012; Zinsstag et al., 2006). Moreover, in those countries, the public and political awareness on bTB is usually low, the institutional framework is weak (i.e., veterinary and public health services) and, compared to industrialized countries, the already limited access to international markets and trade make them particularly vulnerable to the protectionist application of sanitary international measures (Zinsstag et al., 2006).

In countries with bTB eradication plans operating on a regular and mandatory basis and effective surveillance programmes, clinical evidences in cattle are seldom encountered because infected cattle are detected and eliminated at very early stages of infection, before signs appear. Therefore, losses due to the decline in animal productivity and livestock deaths are extremely reduced (Cousins, 2001; Domingo et al., 2014; Good et al., 2018; Olea-Popelka et al., 2017; Zinsstag et al., 2006).

However, bTB still has an economic impact for both farmers and countries, mainly due to the cost of eradication programmes (i.e., surveillance and regular testing; movement control on infected herds, removal of infected cattle with compensation for farmers and other administrative costs), reduced value or condemnations of carcases, restrictions for markets access and international trade of animals and their products. Moreover, in some high-income countries, bTB eradication is more difficult and costly due to the presence of wildlife reservoirs, with implications for food security and for areas of private interest, such as tourism or hunting game farms (Zinsstag et al., 2006).

[9] The Disability Adjusted Life Year (DALY) is recommended by the World Health Organisation (WHO) as outcome measure of human population health in order to determine the relative burden of disease in different settings, thus it is widely used at international level. DALYs are composed of years of life lost and years of healthy life lost because of disability (Murray et al., 1994). It is particularly useful in cost-effectiveness analysis for economic evaluations.

### 1.4.2. Public health impact: zoonotic tuberculosis and the one-health approach

Bovine TB can be transmitted from animal to humans and vice versa, and, to a lesser extent, from humans to humans (EFSA AHAW Panel, 2017; Evans et al., 2007; Sunder et al., 2009; Waters et al., 2014), reducing the quality of life of human populations (i.e., disability and premature death) and leading to serious consequences for public health (Ayele et al., 2004; Cosivi et al., 1998; Good et al., 2018; Good & Duignan, 2011; C. Thoen et al., 2006; Zinsstag et al., 2006). Zoonotic TB is clinically, radiologically, and histopathologically indistinguishable from infections caused by *M. tuberculosis* (de la Rua-Domenech et al., 2006; Michel et al., 2010a; O'Reilly & Daborn, 1995); therefore, the only way to discriminate these pathogens is to identify isolates to species level.

Since the primary location of lesions differ according to the route of infection, in humans, lesions due to *M. bovis* are usually extra-pulmonary (i.e., non-pulmonary forms), as a result of drinking or handling contaminated unpasteurized milk or consuming dairy raw products (Good et al., 2018; O'Reilly & Daborn, 1995). However, following the exposure to infected domestic or wildlife animals, carcasses or contaminated environment, zoonotic bTB transmission may also occur by inhaling contaminated droplets or aerosols, leading to the development of typical pulmonary forms (Cosivi et al., 1998; Michel et al., 2010; Pérez-Lago et al., 2014).

Infants, children and women are described as the most vulnerable groups to bTB, thought the frequency of infection may vary because of the cultural habits of people (Good et al., 2018; Pérez-Lago et al., 2014). Some professional categories are especially exposed to the risk of contracting bTB (i.e., occupational exposure), for example hunters, abattoir workers, veterinarians, farm workers (i.e., herd owners, milkmaids, animals' keepers and attendants) and their families (Ayele et al., 2004; El Idrissi & Parker, 2012; Michel et al., 2010; Olea-Popelka et al., 2017; Pérez-Lago et al., 2014; Vayr et al., 2018). Transmission of *M. bovis* to humans due to recreational exposure to wildlife has also been reported (Good et al., 2018). Moreover, people with problems related to suppression of the immune system (i.e., HIV-infected persons) are highly susceptible to *M. bovis* (Good et al., 2018); for example, dual HIV and *M. bovis* infections have been reported in high and low-income countries (Cosivi et al., 1998; Grange et al., 1994; LoBue, 2006).

The transmission of *M. bovis* from cattle to humans was once very common (Francis, 1947; Good et al., 2018; Waters et al., 2014). Since the first half of the 20th Century, especially in high-income countries, huge control efforts have been made through the launch of bTB eradication programmes, the introduction of enhanced food safety measures (i.e., mandatory pasteurization, hygiene practices and meat inspections) and the modernization of farming management systems, leading to a decrease of bTB in cattle herds and in humans (Azami & Zinsstag, 2018; Good et al., 2018; Good & Duignan, 2011; Michel et al., 2010; Zinsstag et al., 2006). By the 1990s it was estimated that 3.1% of human TB cases worldwide were due to *M. bovis* with the vast majority occurring in low-income countries (Cosivi et al., 1998; LoBue, 2006; Michel et al., 2010; O'Reilly & Daborn, 1995), that showed the same conditions observed in Europe during 1930s and 1940s (Cosivi et al., 1995). In 2013, a systematic review, mandated by the World Health Organization (WHO) (Müller et al., 2013) stated that zoonotic TB accounted for about 1.4% and 2.8% of all TB cases outside of and within Africa, respectively. Despite the significant progresses made towards the elimination of bTB, human cases of bTB continue to be reported worldwide and zoonotic TB is still a concern, even in the developed world (Good et al., 2018; Olea-Popelka et al., 2017; Pérez-Lago et al., 2014).

In 2016, the World Health Organization (WHO) estimated[10] 147.000 (IC95% 71.800-249.000) new cases of zoonotic TB in humans, and 12.500 deaths reported from all WHO regions, with the African and the South-East Asian regions carrying the heaviest burden (Fig. 8a, 8b) (Anon., 2017a).

According to the last European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) report (EFSA & ECDC, 2017), across European Member States (EU-MS) zoonotic tuberculosis is rare; the notification rates have been stable in 2012–2016 (0.04 cases per 100,000 populations), and the number of cases reported in the last five years were: 132, 144, 167, 181 and 170 between 2012 and 2016, respectively.

[10] Estimates of TB cases in human population published by the WHO are based on case notifications in countries that have high-performance surveillance systems, mainly high-income countries. Otherwise, estimates were obtained through prevalence surveys, results from inventory studies and capture–recapture analysis and case notification combined with experts opinion. Estimates are updated on annual basis and are available online at: http://www.who.int/tb/publications/global_report/en/

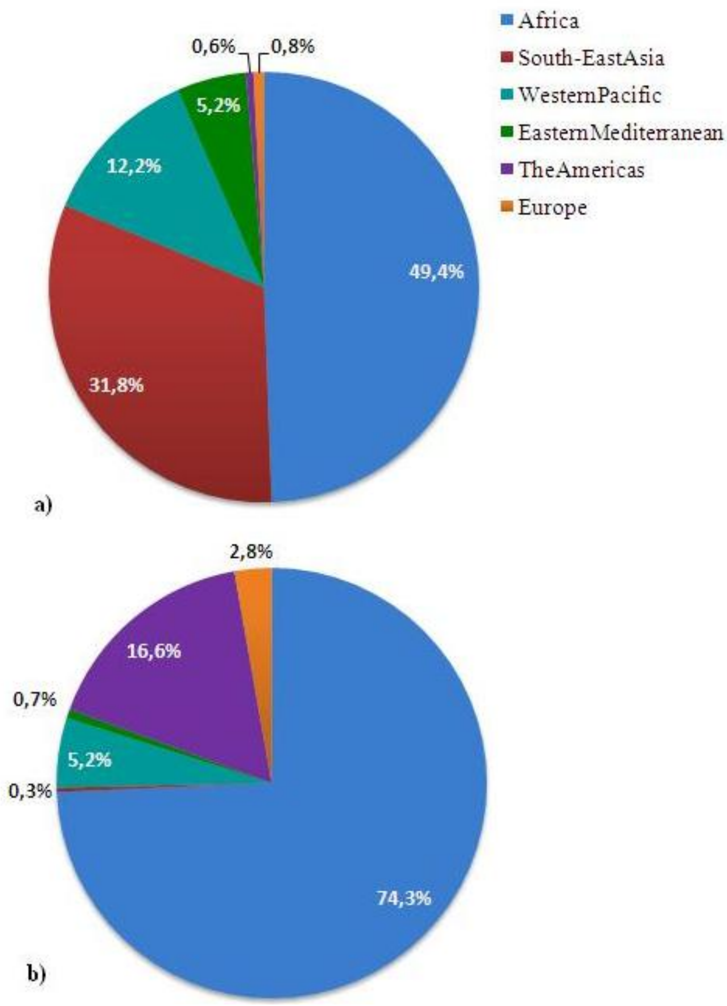



Figure 8: Estimated incidence **(a)** and mortality **(b)** due to *M. bovis* in human population by WHO Regions in 2016. Proportions are calculated on a total of 147.000 estimated incident bTB cases and 12.500 estimated bTB deaths. *Source: Anon., 2017a.*

In 2016, human cases were reported by 12 EU-MS, with the highest rate observed in Belgium (0.12 cases per 100,000 populations). Zoonotic TB was reported by Austria (1 case, OTF declared), Belgium (14, OTF), Czech Republic (1, OTF), Denmark (1, OTF), Germany (52, OTF), Ireland (3), Italy[11] (13), the Netherlands (12, OTF), Romania (2), Spain (26), Sweden (5, OTF), Norway (5, OTF), Switzerland and Liechtenstein (5, OTF) and the United Kingdom (39).

The majority of reported cases (105/170) were of EU origin (native cases and/or cases originating from other MS), and most of them (67.5%) were born in non-OTF countries (Fig. 9) (EFSA & ECDC, 2017).

[11] In Italy, 7 Regions and 14 Provinces are OTF

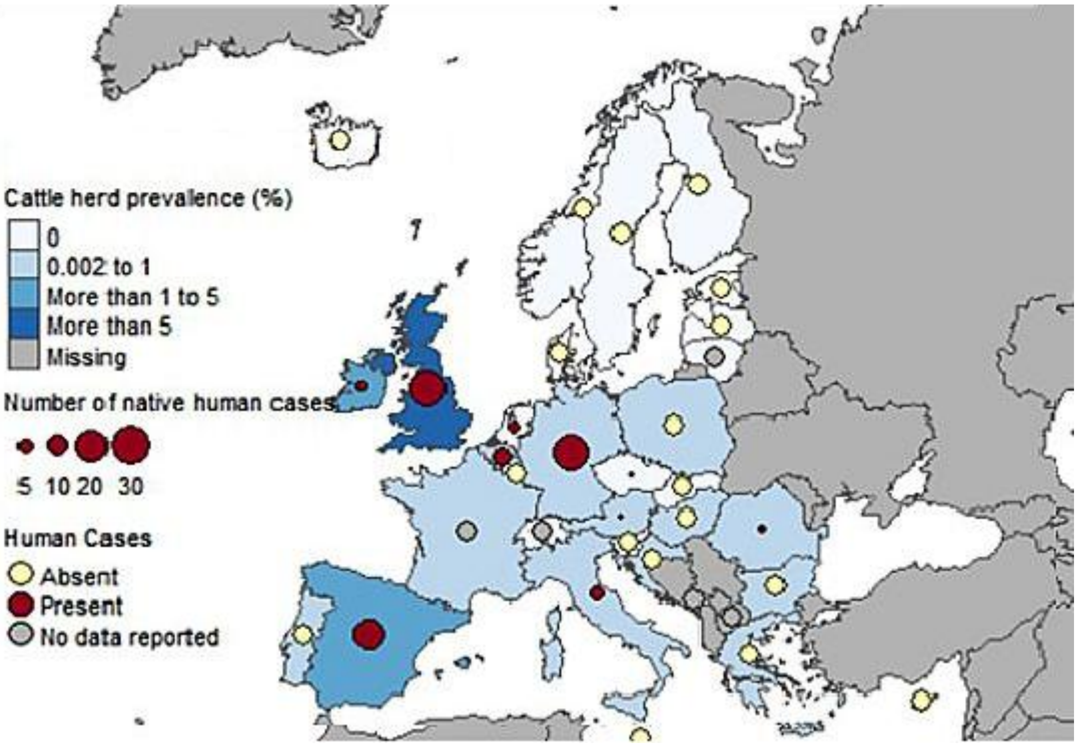



Figure 9: Number of confirmed human tuberculosis cases of EU origin due to *M. bovis* and country-level aggregated prevalence of bTB-positive cattle herds (due to *M. bovis* and/or *M. caprae*), EU - MS, 2016. Source: EFSA & ECDC, 2017

Although, the association between a country's OTF status and its notification rate in humans was not clear (EFSA & ECDC, 2017), recent studies evidenced the risk of transmission due to the exposure to infected cattle (i.e., high proportion of match between *M. bovis* strains isolated from patients with those isolated in cattle herds (Lombardi et al., 2017; Palacios et al., 2016).

The real burden of zoonotic TB is difficult to be determined and the true incidence of bTB in humans remains uncertain (Anon., 2017a; Anon. 2017b; EFSA & ECDC, 2017; Olea-Popelka et al., 2017). As recently highlighted, the report of zoonotic TB as a proportion of all tuberculosis cases may lead to misunderstand the real size of the problem since data used are not representative of national populations (i.e., derived from specific studies instead of national surveillance systems) (Müller et al., 2013; Olea-Popelka et al., 2017). Moreover, information might be biased by some technical constraints; such as the difficulties in differentiating *M. tuberculosis* from *M. bovis* or *M. caprae*, which requires, first, the isolation of the mycobacteria on selective culture media, and its subsequent identification by the use of biochemical tests or molecular diagnostic methods (e.g., spoligotyping or other genotyping techniques), not always available or routinely used (Müller et al., 2013). Furthermore, biochemical methods may be relatively unreliable for the identification of *M. bovis* or *M. caprae* strains, and routine culture methods for *M. tuberculosis* are suboptimal to detect strains of *M. bovis* (Ayele et al., 2004; Cosivi et al., 1998). Therefore, TB cases caused by *M. bovis* may be systematically under-diagnosed and/or under-reported (Müller et al., 2013; Olea-Popelka et al., 2017; Pérez-Lago et al. 2014; Waters et al., 2014), leading to the misconception that only a small proportion TB cases is due to *M bovis*, which, in turn, may result in a general low awareness among consumers, health-care providers and public health officials (Olea-Popelka et al., 2017; Thoen et al., 2006).

These constrains affect both low and high-income countries, although in a different way and measure. In the majority of low income countries, the occurrence of *M. bovis* infections in humans is likely to be underestimated since there is no systematic national surveillance neither in humans and in animals (Anon., 2017a; Olea-Popelka et al., 2017; Pérez-Lago et al., 2014; Thoen et al., 2010). Moreover, laboratory capacity is very limited in many of those countries, and therefore mycobacterial culture is not routinely performed (Collins and Grange, 1983; Michel et al., 2010; Muller et al., 2013; Thoen et al., 2010). In fact, most often, the diagnosis of tuberculosis relies exclusively on microscopy; thus, many cases are only assumed to be caused by *M. tuberculosis* (Ayele et al., 2004; Cosivi et al., 1998; Olea-Popelka et al., 2017; Zinsstag et al., 2006). In high-income countries, zoonotic TB zoonotic TB might also be underestimated because the most commonly used procedures for identification and laboratory routine methods do not differentiate MTC species (Good et al., 2018; Lombardi et al., 2017; Palacios et al., 2016; Pérez-Lago et al., 2014). Moreover, the lack of information exchanges and common strategies between human and animal health authorities/sectors, together with an insufficient typing of animals' isolates may further contribute to underestimate the burden of zoonotic TB in low-prevalence settings (Palacios et al., 2016).

The problem concerning the spread of zoonotic TB in humans and the heterogeneous distribution across countries is not only associated with its prevalence in cattle. Other factors include the presence of wildlife reservoirs, food hygiene practices and population habits, political situations and socio-economic conditions and the quality of veterinary and public health services. Moreover, different pathways of transmission require specific public health strategies to be prevented (El Idrissi & Parker, 2012; Zinsstag et al., 2006).

Therefore, the prevention and control of zoonotic TB clearly needs to be approached through a cross-sectorial and multidisciplinary approach, linking animal, human, and environmental health. Besides controlling bTB in cattle and promote high food hygiene standards, that have been historical milestones in the control of zoonotic TB, the elimination of tuberculosis from human society will necessitate understanding and controlling this organism in all its reservoirs. At the same time, continued professional training for specific groups and improved health education for people are also needed for effective control interventions (Good et al., 2018; Zinsstag et al., 2006).

The importance of a multidisciplinary "One Health" approach was also remarked in the zoonotic TB roadmap launched, in 2016–2017, as joined tripartite effort between WHO, OIE and FAO[12] (Anon., 2017b). The multidisciplinary roadmap, emphasizing the interdependence of humans and animals health, defined a common strategy in 10 priority actions which were grouped under three core themes (Anon., 2017b; Good et al., 2018; Zinsstag et al., 2015): i) Improve the scientific evidence base (i.e., increase awareness and knowledge on bTB and improve diagnostic capacities); ii) Reduce transmission at the animal–human interface (i.e., advocate for the control of the disease at the animal source, including all domestic and wildlife reservoirs); iii) Strengthen inter-sectoral and collaborative approaches (i.e., enhance the exchange of data and discussions among veterinary and public health authorities and develop locally adapted control strategies through participatory approaches).

## 1.5. Toward the eradication: Biological & Non-biological constrains

Eradication of bTB is a challenge (Cousins, 2001; Humblet et al., 2009; Good et al., 2018; Olea-Popelka et al., 2017; Pfeiffer et al., 2013). In some countries, the application of systematic testing and culling of reactor animals (i.e., "test-and-slaughter" policy) has been highly effective in eradicating the bTB from cattle populations. However, this strategy has not been universally successful; and, despite all the efforts and the huge amount of economic resources invested, in some other countries, among which is Spain, bTB eradication has not been achieved yet, persisting in cattle, wildlife reservoirs and humans.

[12] The Food and Agricultural Organization of the United Nations (FAO)

The complexity posed by the eradication of bTB can be explained with the existence of a range of epidemiological factors, which may influence the effectiveness of both surveillance and control activities (EFSA AHAW Panel, 2014), as for example:

The involvement of other domestic reservoirs, mainly goats, but also sheep and pigs (Muñoz-Mendoza et al., 2016; Napp et al., 2013, García-Bocanegra et al., 2012)
The existence of different wildlife reservoirs, such as badgers, red deer and wild boar (Fitzgerald & Kaneene, 2013; Naranjo et al., 2008);
Limitations associated to the available *ante-mortem* diagnostic tools (de la Rua-Domenech et al., 2006);
Local differences in management systems, productive type and farming practices (Álvarez et al., 2012b; Humblet et al., 2009; Reviriego & Vermeersch, 2006);
The implication of socio-economic aspects (i.e., non-biological context) (Enticott, 2014; Pfeiffer et al., 2013).

The complex interaction pathogen-hosts-local environments in bTB infection dynamics, implies that the effect of specific interventions and measures may differ from the expected outcome, when applied to different epidemiological contexts (EFSA AHAW Panel, 2014; Olea-Popelka et al.,2017; Schiller et al., 2010).

In this frame, the EFSA AHAW Panel published in 2014 a conceptual framework on bTB aimed to support the understanding of the bTB epidemiology and to guide the identification of principal biological and non-biological factors influencing bTB infection, detection and control of bTB (EFSA AHAW, 2014). The EFSA statement considered three different levels as "units of interest": the animal, the herd and the area levels (Fig. 10), as already suggested by Humblet and collaborators (2009) in their classification of bTB risk factors. Moreover, non-biological aspects were also considered as factors that might influence the outcome of interventions at herd and area levels (Fig. 11).

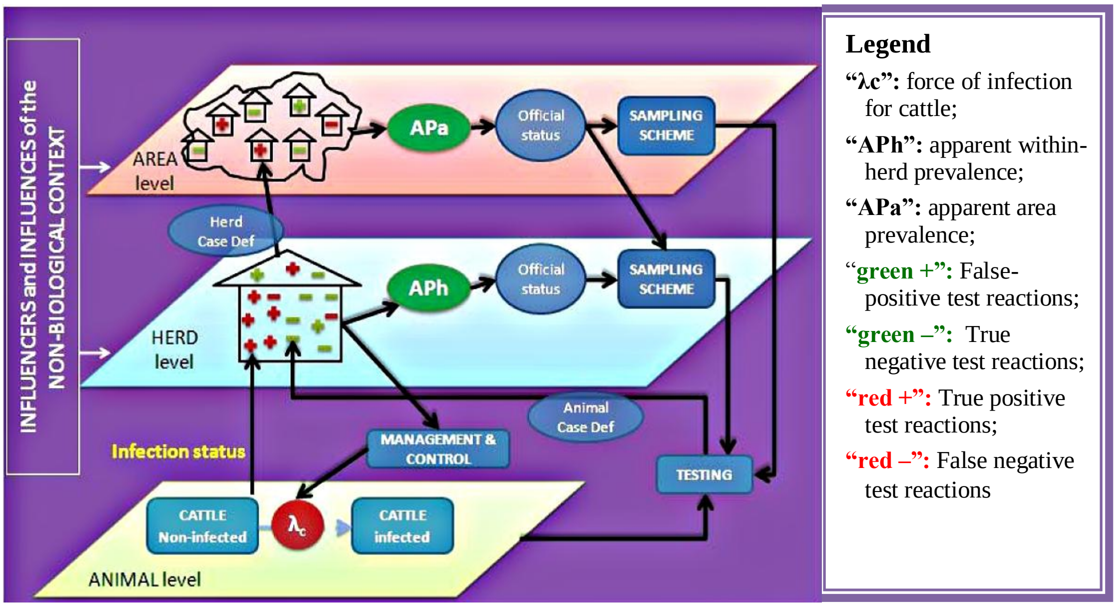



Figure 10: Anchor model representing the conceptual framework on bTB developed by the EFSA - AHAW Panel. The model describes interactions between main biological parameters involved in bTB infection, detection and control (biological context) according to the animal, the herd and the area levels. All these biological factors are influenced by non-biological aspects. Source: EFSA AHAW, 2014. Available online: www.efsa.europa.eu/efsajournal

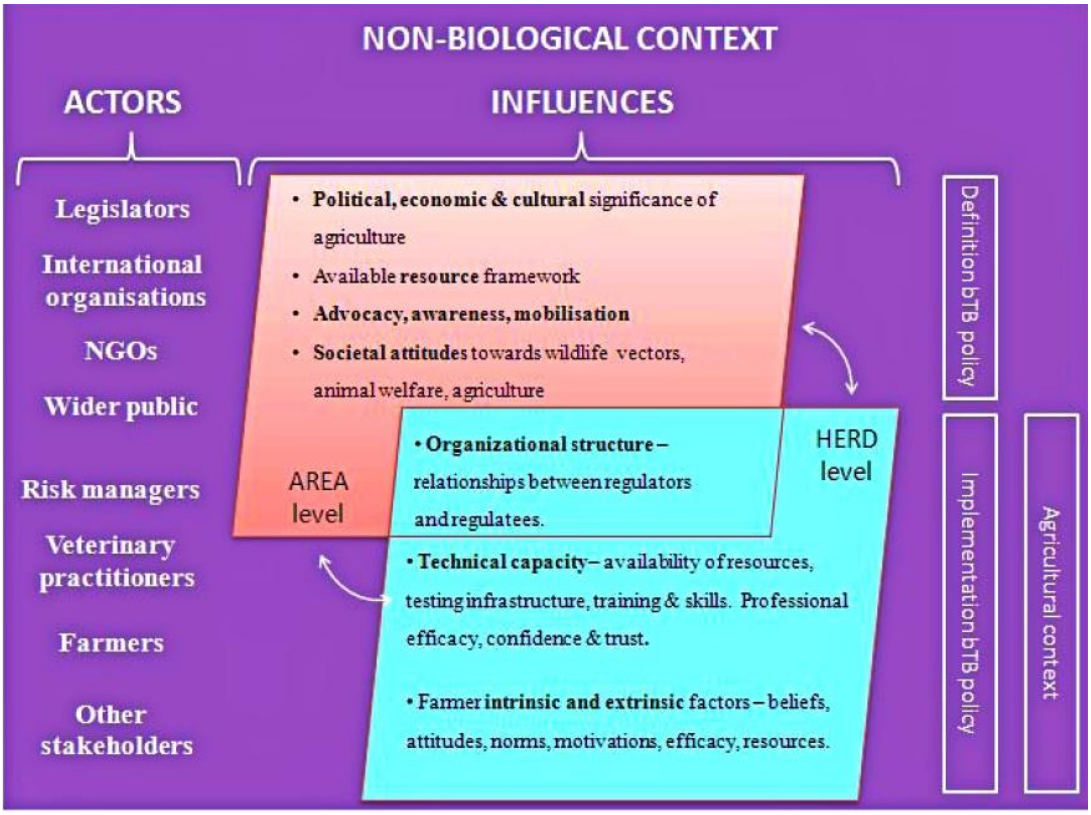



Figure 11: Main actors and influences of the non-biological context on bTB at herd and area levels. Source: EFSA AHAW, 2014. Legend: NGOs, non-governmental organisations; bTB, bovine tuberculosis. Available online: www.efsa.europa.eu/efsajournal

The animal level comprises specific characteristics of hosts (e.g. age, breed, immune status, etc.) and the pathogen characteristics (EFSA AHAW, 2014). At this level, susceptible cattle will become infected according to the overall 'force of infection' (λ) that is the cumulative dynamic rate of all the forces of infection from different sources. The force of infection, λ, describes therefore the rate at which susceptible individuals contract the infection at each point in time (t); thus, considering the within-herd cattle–to–cattle transmission, it reflects the transmission coefficient ($\beta$) and the number of infectious and susceptible cattle present in the herd; and, as such, it can change over time: $\lambda = \beta I_{(t)}/N_{(t)}$

where, $I/N_{(t)}$ is the fraction of population that is infectious and $\beta$, the transmission coefficient, is the average number of individuals that are newly infected from an infectious individual per unit of time (De Jong, 1995).

All events taking place 'within a herd' are considered part of the 'herd level', whereas, the 'area level' is represented by all activities and events taking place 'between herds'(EFSA AHAW, 2014).

On the other hand, farming management and control activities (i.e., removal of infected cattle, movement restrictions, hygiene practices, separation of animals, indoor housing and external biosecurity measures) that are mainly implemented within a herd (part of the 'herd level'), have a primary effect at the "animal level" by reducing the exposure of susceptible animals or the contact rate between infected and susceptible animals (i.e., act on the force of infection) (EFSA AHAW, 2014).

Although several control measures are established at the area level, the herd remains the key level at which interventions are implemented (e.g. culling, hygiene and biosecurity measures). Therefore, policy-implementation is influenced by several day-to-day dynamics, including non-biological factors (i.e., individual perceptions and external factors), such us availability of economic resources, testing infrastructure, past experiences, motivations and attitudes of veterinarians, farmers and other stakeholders as well as the training level, professional experience and personal relationships among people involved in the implementation of eradication programmes (EFSA AHAW Panel, 2014; Enticott, 2014; Meskill et al., 2013).

In order to overcome these constrains and achieve the bTB eradication, it is important to identify and subsequently quantify these risk factors. Although some risk factors are well known (Humblet et al., 2009; García-Saenz et al., 2014; Guta et al., 2014a; Guta et al., 2014b), others are not well understood yet (i.e., bTB transmission dynamics) or they have been recently recognised (i.e., the influence of non-biological factors). Therefore, multi-disciplinary research efforts to enhance knowledge and understanding on the epidemiology of bTB are crucial for the development of sustainable and effective surveillance and control strategies, thus, toward the achievement of bTB eradication.

In Spain, bTB studies have mainly focused on the role of wildlife reservoirs (Barasona et al., 2014; Barasona et al., 2017; De Mendoza et al., 2006; García-Jiménez et al., 2013; Gortázar et al., 2011; Muñoz-Mendoza et al., 2013; Pérez de Val et al., 2017; Risco et al., 2013; Vicente et al., 2007) or on the evaluation of diagnostic methods and test results (Bezos et al., 2012; Álvarez et al., 2012a; Álvarez et al., 2014b). Investigations on the epidemiology of bTB in domestic livestock have also been carried out, in particular on: the spatial and spatio-temporal dynamics of the disease (Allepuz et al., 2011; de la Cruz et al., 2014; García-Saenz et al., 2014); on risk factors associated with bTB persistence and occurrence of bTB in cattle herds (Guta et al., 2014a; Guta et al., 2014b; Martínez-López et al., 2014); and, on the role of other domestic reservoirs (Muñoz-Mendoza et al., 2012; Muñoz-Mendoza et al., 2016; Napp et al., 2013; Rodríguez-Prieto et al., 2012).

However, very few studies have investigated the within-herd bTB transmission dynamics (Álvarez et al., 2012b) and the effect that sociological factors may have at herd levels on the implementation of bTB control measures have never been central in such investigations. Moreover, due to local differences or particular epidemiological situations, some risk factors and infection dynamics may be relevant in certain contexts but not in others (Humblet et al., 2009), which makes it difficult to extrapolate results from studies carried out in other countries (Álvarez et al., 2014a).

It is, therefore, necessary to increase knowledge and improve the understanding of those aspects that have not been fully addressed (Anon., 2012) in order to identify the limitations of the national eradication program and allow the design of new and more appropriate strategies (Good & Duignan, 2011).

## 1.6. References

Anon. (1994). Zoonotic tuberculosis (*Mycobacterium bovis*): memorandum from a WHO meeting (with the participation of FAO). Bulletin of the World Health Organization, 72(6), 851–857

Anon., 2010. Programa Nacional de Erradicación de Tuberculosis Bovina presentado por España para el año 2010. Available online: https://www.mapama.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/pnetb_2010_tcm30-111302.pdf

Anon. (2012) Task Force ES Anónimo (2012). Report of the Task Force Meeting of the Bovine Tuberculosis Sub-Group, Santiago de Compostela, Spain, October 4-5. http://ec.europa.eu/food/animal/diseases/docs/tb_subgroup_spain_oct2012_en.pdf

Anon. (2017a). World Health Organization (WHO). Global Tuberculosis Report 2017. Available online: http://www.who.int/tb/publications/global_report/en/ .

Anon. (2017b). World Animal Health Organization (OIE), Food and Agricultural Organization of the United Nations (FAO) & World Health Organization (WHO). Roadmap for zoonotic tuberculosis. Available online: http://www.who.int/tb/publications/2017/zoonotic_TB/en/

Anon. (2018). Programa Nacional de Erradicación de Tuberculosis Bovina presentado por España para el año 2018. Available online: https://www.mapama.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/pnetb_2018_tcm30-436761.pdf

Alexander, K. A., Laver, P. N., Michel, A. L., Williams, M., van Helden, P. D., Warren, R. M., & Gey van Pittius, N. C. (2010). Novel *Mycobacterium tuberculosis* complex pathogen *M. mungi*. Emerging Infectious Diseases, 16(8), 1296–1299. https://doi.org/10.3201/eid1608.100314.

Allepuz, A., Casal, J., Napp, S., Saez, M., Alba, A., Vilar, M., ... & Álvarez, J. (2011). Analysis of the spatial variation of Bovine tuberculosis disease risk in Spain (2006–2009). Preventive veterinary medicine, 100(1), 44-52.

Álvarez, J., Perez, A., Bezos, J., Marqués, S., Grau, A., Saez, J. L., … Domínguez, L. (2012a). Evaluation of the sensitivity and specificity of bovine tuberculosis diagnostic tests in naturally infected cattle herds using a Bayesian approach. Veterinary Microbiology, 155(1), 38–43. https://doi.org/10.1016/j.vetmic.2011.07.034.

Álvarez, J., Perez, A. M., Bezos, J., Casal, C., Romero, B., Rodriguez-Campos, S., ... & Domínguez, L. (2012b). Eradication of bovine tuberculosis at a herd-level in Madrid, Spain: study of within-herd transmission dynamics over a 12-year period. BMC veterinary research, 8(1), 100.

Álvarez, J., Bezos, J., de la Cruz, M. L., Casal, C., Romero, B., Domínguez, L., … Pérez, A. (2014a). Bovine tuberculosis: within-herd transmission models to support and direct the decision-making process. Research in Veterinary Science, 97 Suppl, S61-68. https://doi.org/10.1016/j.rvsc.2014.04.009.

Álvarez, J., Perez, A., Marqués, S., Bezos, J., Grau, A., de la Cruz, M. L., ... & Mínguez, O. (2014b). Risk factors associated with negative in-vivo diagnostic results in bovine tuberculosis-infected cattle in Spain. BMC veterinary research, 10(1), 14.

Amato, B., Di Marco Lo Presti, V., Gerace, E., Capucchio, M. T., Vitale, M., Zanghì, P., ... & Boniotti, M. B. (2018). Molecular epidemiology of Mycobacterium tuberculosis complex strains isolated from livestock and wild animals in Italy suggests the need for a different eradication strategy for bovine tuberculosis. Transboundary and emerging diseases, 65(2), https://doi.org/10.1111/tbed.12776.

Aranaz, A., Liébana, E., Gómez-Mampaso, E., Galán, J. C., Cousins, D., Ortega, A., … Domínguez, L. (1999). Mycobacterium tuberculosis subsp. caprae subsp. nov.: a taxonomic study of a new member of the Mycobacterium tuberculosis complex isolated from goats in Spain. International Journal of Systematic Bacteriology, 49 Pt 3, 1263–1273. https://doi.org/10.1099/00207713-49-3-1263.

Aranaz, Alicia, Cousins, D., Mateos, A., & Domínguez, L. (2003). Elevation of Mycobacterium tuberculosis subsp. caprae Aranaz et al. 1999 to species rank as Mycobacterium caprae comb. nov., sp. nov. International Journal of Systematic and Evolutionary Microbiology, 53(Pt 6), 1785–1789. https://doi.org/10.1099/ijs.0.02532-0.

Awada, L., Tizzani, P., Erlacher-Vindel, E., Forcella, S., & Caceres, P. (2018). Bovine Tuberculosis: Worldwide Picture. In “Bovine Tuberculosis”, pp 1-15. M. Chambers, S. Gordon, F. Olea-Popelka, P. Barrow (eds). CAB International.

Ayele, W. Y., Neill, S. D., Zinsstag, J., Weiss, M. G., & Pavlik, I. (2004). Bovine tuberculosis: an old disease but a new threat to Africa. The International Journal of Tuberculosis and Lung Disease: The Official Journal of the International Union Against Tuberculosis and Lung Disease, 8(8), 924–937.

Azami, H. Y., & Zinsstag, J. (2018). Economics of Bovine Tuberculosis: A One Health Issue. In “Bovine Tuberculosis”, pp 31-42. M. Chambers, S. Gordon, F. Olea-Popelka, P. Barrow (eds). CAB International.

Bailey, S. S., Crawshaw, T. R., Smith, N. H., & Palgrave, C. J. (2013). Mycobacterium bovis infection in domestic pigs in Great Britain. Veterinary Journal (London, England: 1997), 198(2), 391–397. https://doi.org/10.1016/j.tvjl.2013.08.035.

Barasona, J. A., Vicente, J., Díez-Delgado, I., Aznar, J., Gortázar, C., & Torres, M. J. (2017). Environmental Presence of Mycobacterium tuberculosis Complex in Aggregation Points at the Wildlife/Livestock Interface. Transboundary and Emerging Diseases, 64(4), 1148–1158. https://doi.org/10.1111/tbed.12480.

Barasona, Jose A., Latham, M. C., Acevedo, P., Armenteros, J. A., Latham, A. D. M., Gortázar, C., … Vicente, J. (2014). Spatiotemporal interactions between wild boar and cattle: implications for cross-species disease transmission. Veterinary Research, 45, 122. https://doi.org/10.1186/s13567-014-0122-7.

Barlow, N.D., Kean, J.M., Hickling, G., Livingstone, P.G., Robson, A.B., 1997. A simulationmodel for the spread of bovine tuberculosis within New Zealand cattle herds. Preventive Veterinary Medicine 32, 57–75.

Berga A.M. (1987). Incidencia econdmica de la sanidad animal. Secretarfa General Tecnica M.A.P.A., Madrid, pp. 243.

Bezos, J., Álvarez, J., Romero, B., Aranaz, A., & de Juan, L. (2012). Tuberculosis in goats: assessment of current in vivo cell-mediated and antibody-based diagnostic assays. The Veterinary Journal, 191(2), 161-165.

Bezos, J., Casal, C., Romero, B., Schroeder, B., Hardegger, R., Raeber, A. J., ... & Domínguez, L. (2014). Current ante-mortem techniques for diagnosis of bovine tuberculosis. Research in veterinary science, 97, S44-S52.

Biet, F., Boschiroli, M. L., Thorel, M. F., & Guilloteau, L. A. (2005). Zoonotic aspects of Mycobacterium bovis and Mycobacterium avium-intracellulare complex (MAC). Veterinary Research, 36(3), 411–436. https://doi.org/10.1051/vetres:2005001.

Böddinghaus, B., Rogall, T., Flohr, T., Blöcker, H., & Böttger, E. C. (1990). Detection and identification of mycobacteria by amplification of rRNA. Journal of Clinical Microbiology, 28(8), 1751–1759.

Boland, F., Kelly, G. E., Good, M., & More, S. J. (2010). Bovine tuberculosis and milk production in infected dairy herds in Ireland. Preventive Veterinary Medicine, 93(2–3), 153–161. https://doi.org/10.1016/j.prevetmed.2009.09.021.

Brosch, R., Gordon, S. V., Marmiesse, M., Brodin, P., Buchrieser, C., Eiglmeier, K., … Cole, S. T. (2002). A new evolutionary scenario for the Mycobacterium tuberculosis complex. Proceedings of the National Academy of Sciences of the United States of America, 99(6), 3684–3689. https://doi.org/10.1073/pnas.052548299.

Broughan, J. M., Crawshaw, T. R., Downs, S. H., Brewer, J., & Clifton-Hadley, R. S. (2013). Mycobacterium bovis infections in domesticated non-bovine mammalian species. Part 2: A review of diagnostic methods. Veterinary Journal (London, England: 1997), 198(2), 346–351. https://doi.org/10.1016/j.tvjl.2013.09.007.

Casal, C., Alvarez, J., Bezos, J., Quick, H., Díez-Guerrier, A., Romero, B., … de Juan, L. (2015). Effect of the inoculation site of bovine purified protein derivative (PPD) on the skin fold thickness increase in cattle from officially tuberculosis free and tuberculosis-infected herds. Preventive Veterinary Medicine, 121(1–2), 86–92. https://doi.org/10.1016/j.prevetmed.2015.07.001.

Caswell, J. L., & Williams, K. J. (2016). Respiratory System. In Jubb, Kennedy & Palmer's Pathology of Domestic Animals: Volume 2 (pp. 465-591.e4). Elsevier. https://doi.org/10.1016/B978-0-7020-5318-4.00011-5.

Conlan, A.J.K., McKinley, T.J., Karolemeas, K., Pollock, E.B., Goodchild, A. V., Mitchell, A.P., Birch, C.P.D., Clifton-Hadley, R.S., Wood, J.L.N., 2012. Estimating the Hidden Burden of Bovine Tuberculosis in Great Britain. PLoS Comput. Biol. 8. doi:10.1371/journal.pcbi.1002730.

Conlan, A. J., & Wood, J. L. (2018). The Epidemiology of Mycobacterium bovis Infection in Cattle. In "Bovine Tuberculosis", pp 43-57. M. Chambers, S. Gordon, F. Olea-Popelka, P. Barrow (eds.). CAB International.

Constable, P. D., Hinchcliff, K. W., Done, S. H., & Grünberg, W. (2017). Systemic and Multi-Organ Diseases. In Veterinary Medicine (pp. 2002–2214), Eleventh Edition, Elsevier Health Sciences W.B. Saunders. https://doi.org/10.1016/B978-0-7020-5246-0.00021-8.

Cosivi, O., Meslin, F. X., Daborn, C. J., & Grange, J. M. (1995). Epidemiology of Mycobacterium bovis infection in animals and humans, with particular reference to Africa. Revue Scientifique et Technique-Office International des Epizooties, 14(3), 733-750.

Cosivi, O., Grange, J. M., Daborn, C. J., Raviglione, M. C., Fujikura, T., Cousins, D., … Meslin, F. X. (1998). Zoonotic tuberculosis due to Mycobacterium bovis in developing countries. Emerging Infectious Diseases, 4(1), 59–70. https://doi.org/10.3201/eid0401.980108.

Courtenay, O., Reilly, L. A., Sweeney, F. P., Hibberd, V., Bryan, S., Ul-Hassan, A., ... & Wellington, E. M. H. (2006). Is Mycobacterium bovis in the environment important for the persistence of bovine tuberculosis?. Biology letters, 2(3), 460-462.

Cousins, D. V. (2001). *Mycobacterium bovis* infection and control in domestic livestock. Revue Scientifique et Technique-Office International des Epizooties, 20(1), 71-85.

Cousins, D. V., Bastida, R., Cataldi, A., Quse, V., Redrobe, S., Dow, S., … Bernardelli, A. (2003). Tuberculosis in seals caused by a novel member of the *Mycobacterium tuberculosis* complex: *Mycobacterium pinnipedii* sp. nov. International Journal of Systematic and Evolutionary Microbiology, 53(Pt 5), 1305–1314. https://doi.org/10.1099/ijs.0.02401-0.

Cowie, C. E., Hutchings, M. R., Barasona, J. A., Gortázar, C., Vicente, J., & White, P. C. L. (2016). Interactions between four species in a complex wildlife: livestock disease community: implications for *Mycobacterium bovis* maintenance and transmission. European Journal of Wildlife Research, 62(1), 51–64. https://doi.org/10.1007/s10344-015-0973-x.

de la Cruz, M. L., Perez, A., Bezos, J., Pages, E., Casal, C., Carpintero, J., … Álvarez, J. (2014). Spatial dynamics of bovine tuberculosis in the Autonomous Community of Madrid, Spain (2010-2012). PloS One, 9(12), e115632. https://doi.org/10.1371/journal.pone.0115632.

de la Rua-Domenech, R., Goodchild, A. T., Vordermeier, H. M., Hewinson, R. G., Christiansen, K. H., & Clifton-Hadley, R. S. (2006). Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. Research in Veterinary Science, 81(2), 190–210. https://doi.org/10.1016/j.rvsc.2005.11.005.

De Mendoza, J. H., Parra, A., Tato, A., Alonso, J. M., Rey, J. M., Pena, J., ... & Cerrato, R. (2006). Bovine tuberculosis in wild boar (*Sus scrofa*), red deer (*Cervus elaphus*) and cattle (*Bos taurus*) in a Mediterranean ecosystem (1992–2004). Preventive Veterinary Medicine, 74(2-3), 239-247.

De Jong, M.C.M., 1995. Mathematical modelling in veterinary epidemiology: why model building is important. Prev. Vet. Med. 25, 183–193. doi:10.1016/0167-5877(95)00538-2.

Di Marco V, Mazzone P, Capucchio MT, Boniotti MB, Aronica V, Russo M, Fiasconaro M, Cifani N, Corneli S, Biasibetti E, Biagetti M, Pacciarini ML, Cagiola M, Pasquali P, Marianelli C (2012). Epidemiological significance of the domestic black pig (Sus scrofa) in maintenance of bovine tuberculosis in Sicily. J Clin Microbiol 50:1209–1218.

Domingo, M., Vidal, E., & Marco, A. (2014). Pathology of bovine tuberculosis. Research in Veterinary Science, 97 Suppl, S20-29. https://doi.org/10.1016/j.rvsc.2014.03.017.

El Idrissi, A., & Parker, E. (2012). Bovine tuberculosis at the animal-human-ecosystem interface. EMPRES Transboundary Animal Diseases Bulletin, 40, 2-11.

Enticott, G. (2014). Relational distance, neoliberalism and the regulation of animal health. Geoforum, 52, 42–50. https://doi.org/10.1016/j.geoforum.2013.12.004.

European Food Safety Authority - Panel on Animal Health and Welfare (EFSA AHAW). (2012). Scientific Opinion on the use of a gamma interferon test for the diagnosis of bovine tuberculosis. EFSA Journal 10(12). https://doi.org/10.2903/j.efsa.2012.2975.

European Food Safety Authority - Panel on Animal Health and Welfare (EFSA AHAW). (2014). Statement on a conceptual framework for bovine tuberculosis. EFSA Journal, 12(5). https://doi.org/10.2903/j.efsa.2014.3711.

European Food Safety Authority - Panel on Animal Health and Welfare (EFSA AHAW). More, S., Bøtner, A., Butterworth, A., Calistri, P., Depner, K., … Bicout, D. (2017). Assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): bovine tuberculosis. EFSA Journal, 15(8). https://doi.org/10.2903/j.efsa.2017.4959.

European Food Safety Authority (EFSA) & European Centre for Disease Prevention and Control (ECDC). (2017). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA Journal, 15(12). https://doi.org/10.2903/j.efsa.2017.5077.

Evans, J. T., Smith, E. G., Banerjee, A., Smith, R. M., Dale, J., Innes, J. A., … Sonnenberg, P. (2007). Cluster of human tuberculosis caused by Mycobacterium bovis: evidence for person-to-person transmission in the UK. Lancet (London, England), 369(9569), 1270–1276. https://doi.org/10.1016/S0140-6736(07)60598-4.

FAO, & WHO. (2011). Alimentarius Codex. “Milk and milk products.” Rome: World Health Organization and Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/docrep/015/i2085e/i2085e00.pdf

Fine, A. E., Bolin, C. A., Gardiner, J. C., & Kaneene, J. B. (2011). A study of the persistence of Mycobacterium bovis in the environment under natural weather conditions in Michigan, USA. Veterinary medicine international, 2011.

Fischer, E.A.J., Van Roermund, H.J.W., Hemerik, L., Van Asseldonk, M.A.P.M., De Jong, M.C.M.. (2005). Evaluation of surveillance strategies for bovine tuberculosis (Mycobacterium bovis) using an individual based epidemiological model. Prev. Vet. Med. 67, 283–301. doi:10.1016/j.prevetmed.2004.12.002.

Fitzgerald, S. D., & Kaneene, J. B. (2013). Wildlife reservoirs of bovine tuberculosis worldwide: hosts, pathology, surveillance, and control. Veterinary Pathology, 50(3), 488–499. https://doi.org/10.1177/0300985812467472.

Fitzgerald, Scott D., Hollinger, C., Mullaney, T. P., Bruning-Fann, C. S., Tilden, J., Smith, R., … Kaneene, J. B. (2016). Herd outbreak of bovine tuberculosis illustrates that route of infection correlates with anatomic distribution of lesions in cattle and cats. Journal of

Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc, 28(2), 129–132. https://doi.org/10.1177/1040638715626484.

Forrellad, M. A., Klepp, L. I., Gioffré, A., Sabio y García, J., Morbidoni, H. R., de la Paz Santangelo, M., … Bigi, F. (2013). Virulence factors of the Mycobacterium tuberculosis complex. Virulence, 4(1), 3–66. https://doi.org/10.4161/viru.22329.

Francis, J. (1947). Bovine Tuberculosis Including A Contras With Human Tuberculisis. London W: Staples Press Limited Cavendish Place.

Galagan, J. E. (2014). Genomic insights into tuberculosis. Nature Reviews. Genetics, 15(5), 307–320. https://doi.org/10.1038/nrg3664.

García-Bocanegra, I., de Val, B. P., Arenas-Montes, A., Paniagua, J., Boadella, M., Gortázar, C., & Arenas, A. (2012). Seroprevalence and risk factors associated to Mycobacterium bovis in wild artiodactyl species from southern Spain, 2006–2010. PLoS One, 7(4) https://doi.org/10.1371/journal.pone.0034908.

García-Jiménez, W. L., Benítez-Medina, J. M., Fernández-Llario, P., Abecia, J. A., García-Sánchez, A., Martínez, R., ... & Gómez, L. (2013). Comparative pathology of the natural infections by Mycobacterium bovis and by Mycobacterium caprae in wild boar (Sus scrofa). Transboundary and emerging diseases, 60(2), 102-109.

Garcia-Saenz, A., Saez, M., Napp, S., Casal, J., Saez, J. L., Acevedo, P., ... & Allepuz, A. (2014). Spatio-temporal variability of bovine tuberculosis eradication in Spain (2006–2011). Spatial and spatio-temporal epidemiology, 10, 1-10.

Garnier, T., Eiglmeier, K., Camus, J.-C., Medina, N., Mansoor, H., Pryor, M., … Hewinson, R. G. (2003). The complete genome sequence of Mycobacterium bovis. Proceedings of the National Academy of Sciences, 100(13), 7877–7882. https://doi.org/10.1073/pnas.1130426100.

Gilsdorf, M. J., Ebel, E. D., & Disney, T. W. (2006). Benefit and Cost Assessment of the U.S. Bovine Tuberculosis Eradication Program. In "Mycobacterium bovis Infection in Animals and Humans", pp. 89–99. C. O. Thoen, J. H. Steele, & M. J. Gilsdorf (Eds.), 2nd Edition. Oxford, UK: Blackwell Publishing Ltd. https://doi.org/10.1002/9780470344538.ch11.

Good, M., Bakker, D., Duignan, A., & Collins, D. M. (2018). The History of In Vivo Tuberculin Testing in Bovines: Tuberculosis, a “One Health” Issue. Frontiers in Veterinary Science, 5. https://doi.org/10.3389/fvets.2018.00059.

Good, M., & Duignan, A. (2011). Perspectives on the History of Bovine TB and the Role of Tuberculin in Bovine TB Eradication. Veterinary Medicine International, 2011, 1–11. https://doi.org/10.4061/2011/410470.

Goodchild, A. V., & Clifton-Hadley, R. S. (2001). Cattle-to-cattle transmission of Mycobacterium bovis. Tuberculosis (Edinburgh, Scotland), 81(1–2), 23–41. https://doi.org/10.1054/tube.2000.0256.

Gortázar, C., Vicente, J., Boadella, M., Ballesteros, C., Galindo, R. C., Garrido, J., … de la Fuente, J. (2011). Progress in the control of bovine tuberculosis in Spanish wildlife. Veterinary Microbiology, 151(1–2), 170–178. https://doi.org/10.1016/j.vetmic.2011.02.04

Gortázar, C., Che Amat, A., & O'Brien, D. J. (2015). Open questions and recent advances in the control of a multi-host infectious disease: animal tuberculosis: Recent advances in animal tuberculosis control. Mammal Review, 45(3), 160–175. https://doi.org/10.1111/mam.12042.

Grange, J. M., Daborn, C., & Cosivi, O. (1994). HIV-related tuberculosis due to Mycobacterium bovis. The European Respiratory Journal, 7(9), 1564–1566.

Guta, S., Casal, J., Garcia-Saenz, A., Saez, J. L., Pacios, A., Garcia, P., … Allepuz, A. (2014). Risk factors for bovine tuberculosis persistence in beef herds of Southern and Central Spain. Preventive Veterinary Medicine, 115(3–4), 173–180. https://doi.org/10.1016/j.prevetmed.2014.04.007.

Guta, Sintayehu, Casal, J., Napp, S., Saez, J. L., Garcia-Saenz, A., Perez de Val, B., … Allepuz, A. (2014a). Epidemiological investigation of bovine tuberculosis herd breakdowns in Spain 2009/2011. PloS One, 9(8), e104383. https://doi.org/10.1371/journal.pone.0104383.

Hammer, P. (2004). Heat inactivation of classical mycobacteria in milk: A historical review. Bulletin-International Dairy Federation, (392), 49-52.

Hardstaff, J. L., Marion, G., Hutchings, M. R., & White, P. C. L. (2014). Evaluating the tuberculosis hazard posed to cattle from wildlife across Europe. Research in Veterinary Science, 97 Suppl, S86-93. https://doi.org/10.1016/j.rvsc.2013.12.002.

Harding, E. (2017). Woman's Mysteries: Ancient & Modern. Shambhala Publications.

Hayman, J. (1984). Mycobacterium ulcerans: an infection from Jurassic time? Lancet (London, England), 2(8410), 1015–1016.

Humblet, M.-F., Boschiroli, M. L., & Saegerman, C. (2009). Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. Veterinary Research, 40(5), 50. https://doi.org/10.1051/vetres/2009033.

Humblet, M.-F., Walravens, K., Salandre, O., Boschiroli, M. L., Gilbert, M., Berkvens, D., … Saegerman, C. (2011). Monitoring of the intra-dermal tuberculosis skin test performed by Belgian field practitioners. Research in Veterinary Science, 91(2), 199–207. https://doi.org/10.1016/j.rvsc.2010.12.004.

Imaeda, T., Kanetsuna, F., & Galindo, B. (1968). Ultrastructure of cell walls of genus Mycobacterium. Journal of Ultrastructure Research, 25(1–2), 46–63. https://doi.org/10.1016/S0022-5320(68)80059-0.

Kaneene, J. B., & Pfeiffer, D. (2006). Epidemiology of Mycobacterium bovis. In " Mycobacterium bovis Infection in Animals and Humans", pp 34-48. Thoen, C. O., Steele, J. H., & Gilsdorf, M. J. (Eds.), 2nd Edition. Oxford, UK: Blackwell Publishing Ltd. https://onlinelibrary.wiley.com/doi/abs/10.1002/9780470344538.ch5.

Kao, R.R., Roberts, M.G., Ryan, T.J.. (1997). A model of bovine tuberculosis control in domesticated cattle herds. Proc. Biol. Sci. 264, 1069–76. doi:10.1098/rspb.1997.0148.

Kao, R. R., Price-Carter, M., & Robbe-Austerman, S. (2016). Use of genomics to track bovine tuberculosis transmission. Revue scientifique et technique, 35(1), 241-58.

Karolemeas, K., De la Rua-Domenech, R., Cooper, R., Goodchild, A. V., Clifton-Hadley, R.S., Conlan, A.J.K., Mitchell, A.P., Hewinson, R.G., Donnelly, C.A., Wood, J.L.N., McKinley, T.J., 2012. Estimation of the relative sensitivity of the comparative tuberculin skin test in tuberculous cattle herds subjected to depopulation. PLoS One 7. doi:10.1371/journal.pone.0043217.

Kleeberg, H. H. (1984). Tuberculose humaine d’origine bovine et santé publique: -EN- -FR- -ES-. Revue Scientifique et Technique de l’OIE, 3(1), 11–76. https://doi.org/10.20506/rst.3.1.156.

LoBue, P. (2006). Public Health Significance of M. bovis. In "Mycobacterium bovis Infection in Animals and Humans", pp 6-12. Thoen, C. O., Steele, J. H., & Gilsdorf, M. J. (Eds.) 2nd Edition. Oxford, UK: Blackwell Publishing Ltd. https://onlinelibrary.wiley.com/doi/abs/10.1002/9780470344538.ch2.

Lombardi, G., Botti, I., Pacciarini, M. L., Boniotti, M. B., Roncarati, G., & Dal Monte, P. (2017). Five-year surveillance of human tuberculosis caused by Mycobacterium bovis in Bologna, Italy: an underestimated problem. Epidemiology and Infection, 145(14), 3035–3039. https://doi.org/10.1017/S0950268817001996.

Martínez-López, B., Barasona, J. A., Gortázar, C., Rodríguez-Prieto, V., Sánchez-Vizcaíno, J. M., & Vicente, J. (2014). Farm-level risk factors for the occurrence, new infection or persistence of tuberculosis in cattle herds from South-Central Spain. Preventive Veterinary Medicine, 116(3), 268–278. https://doi.org/10.1016/j.prevetmed.2013.11.002.

McIlroy, S. G., Neill, S. D., & McCracken, R. M. (1986). Pulmonary lesions and Mycobacterium bovis excretion from the respiratory tract of tuberculin reacting cattle. The Veterinary Record, 118(26), 718-721.

Menzies, F. D., & Neill, S. D. (2000). Cattle-to-Cattle Transmission of Bovine Tuberculosis. The Veterinary Journal, 160(2), 92–106. https://doi.org/10.1053/tvjl.2000.0482.

Meskell, P., Devitt, C., & More, S. J. (2013). Challenges to quality testing for bovine tuberculosis in Ireland; perspectives from major stakeholders. The Veterinary Record, 173(4), 94. https://doi.org/10.1136/vr.101676.

Michel, A. L., Müller, B., & van Helden, P. D. (2010). Mycobacterium bovis at the animal–human interface: A problem, or not? Veterinary Microbiology, 140(3–4), 371–381. https://doi.org/10.1016/j.vetmic.2009.08.029.

Monaghan, M. L., Doherty, M. L., Collins, J. D., Kazda, J. F., & Quinn, P. J. (1994). The tuberculin test. Veterinary Microbiology, 40(1–2), 111–124.

Morris, R. S., Pfeiffer, D. U., & Jackson, R. (1994). The epidemiology of Mycobacterium bovis infections. Veterinary Microbiology, 40(1–2), 153–177.

Müller, B., Dürr, S., Alonso, S., Hattendorf, J., Laisse, C. J. M., Parsons, S. D. C., … Zinsstag, J. (2013). Zoonotic Mycobacterium bovis-induced tuberculosis in humans. Emerging Infectious Diseases, 19(6), 899–908. https://doi.org/10.3201/eid1906.120543.

Muñoz Mendoza, M., Juan, L. de, Menéndez, S., Ocampo, A., Mourelo, J., Sáez, J. L., … Balseiro, A. (2012). Tuberculosis due to Mycobacterium bovis and Mycobacterium caprae in sheep. Veterinary Journal (London, England: 1997), 191(2), 267–269. https://doi.org/10.1016/j.tvjl.2011.05.006.

Muñoz-Mendoza, M., Romero, B., Del Cerro, A., Gortázar, C., García-Marín, J. F., Menéndez, S., … Balseiro, A. (2016). Sheep as a Potential Source of Bovine TB: Epidemiology, Pathology and Evaluation of Diagnostic Techniques. Transboundary and Emerging Diseases, 63(6), 635–646. https://doi.org/10.1111/tbed.12325.

Muñoz-Mendoza, Marta, Marreros, N., Boadella, M., Gortázar, C., Menéndez, S., de Juan, L., … Balseiro, A. (2013). Wild boar tuberculosis in Iberian Atlantic Spain: a different picture from Mediterranean habitats. BMC Veterinary Research, 9, 176. https://doi.org/10.1186/1746-6148-9-176.

Napp, S., Allepuz, A., Mercader, I., Nofrarías, M., López-Soria, S., Domingo, M., … Pérez de Val, B. (2013). Evidence of goats acting as domestic reservoirs of bovine tuberculosis. The Veterinary Record, 172(25), 663. https://doi.org/10.1136/vr.101347.

Naranjo, V., Gortázar, C., Vicente, J., & De La Fuente, J. (2008). Evidence of the role of European wild boar as a reservoir of Mycobacterium tuberculosis complex. Veterinary microbiology,127(1-2), 1-9.

Neill, S. D., Skuce, R. A., & Pollock, J. M. (2005). Tuberculosis--new light from an old window. Journal of Applied Microbiology, 98(6), 1261–1269. https://doi.org/10.1111/j.1365-2672.2005.02599.x.

OIE, W. O. for A. H. (2015). Chapter 2.4.7: Bovine tuberculosis adopted. In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2009. Retrieved from http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.06_BOVINE_TB.pdf

Olea-Popelka, F., Muwonge, A., Perera, A., Dean, A. S., Mumford, E., Erlacher-Vindel, E., … Fujiwara, P. I. (2017). Zoonotic tuberculosis in human beings caused by Mycobacterium bovis-a call for action. The Lancet. Infectious Diseases, 17(1), e21–e25. https://doi.org/10.1016/S1473-3099(16)30139-6.

O'Reilly, L. M., & Daborn, C. J. (1995). The epidemiology of Mycobacterium bovis infections in animals and man: a review. Tubercle and Lung Disease: The Official Journal of the International Union Against Tuberculosis and Lung Disease, 76 Suppl 1, 1–46.

Palacios, J. J., Navarro, Y., Romero, B., Penedo, A., González, Á. M., Hernández, M. D. P., ... & Domínguez, L. (2016). Molecular and epidemiological population-based integrative analysis of human and animal Mycobacterium bovis infections in a low-prevalence setting. Veterinary microbiology, 195, 30-36.

Palmer, M. V. (2013). Mycobacterium bovis: characteristics of wildlife reservoir hosts. Transboundary and Emerging Diseases, 60 Suppl 1, 1–13. https://doi.org/10.1111/tbed.12115.

Parra, A., Fernandez-Llario, P., Tato, A., Larrasa, J., Garcıa, A., Alonso, J. M., ... & De Mendoza, J. H. (2003). Epidemiology of Mycobacterium bovis infections of pigs and wild boars using a molecular approach. Veterinary Microbiology, 97(1-2), 123-133.

Parra, A., Larrasa, J., Garcia, A., Alonso, J. M., & De Mendoza, J. H. (2005). Molecular epidemiology of bovine tuberculosis in wild animals in Spain: a first approach to risk factor analysis.Veterinary Microbiology, 110(3-4), 293-300.

Parsons, S. D. C., Drewe, J. A., Gey van Pittius, N. C., Warren, R. M., & van Helden, P. D. (2013). Novel cause of tuberculosis in meerkats, South Africa. Emerging Infectious Diseases, 19(12), 2004–2007. https://doi.org/10.3201/eid1912.130268.

Patané, J. S. L., Martins, J., Castelão, A. B., Nishibe, C., Montera, L., Bigi, F., … Setubal, J. C. (2017). Patterns and Processes of Mycobacterium bovis Evolution Revealed by Phylogenomic Analyses. Genome Biology and Evolution, 9(3), 521–535. https://doi.org/10.1093/gbe/evx022.

Pérez de Val, B., Napp, S., Velarde, R., Lavín, S., Cervera, Z., Singh, M., ... & Mentaberre, G. (2017). Serological Follow-up of Tuberculosis in a Wild Boar Population in Contact with Infected Cattle. Transboundary and emerging diseases, 64(1), 275-283.

Pérez de Val, B., López-Soria, S., Nofrarías, M., Martín, M., Vordermeier, H. M., Villarreal-Ramos, B., … Domingo, M. (2011). Experimental model of tuberculosis in the domestic goat after endobronchial infection with Mycobacterium caprae. Clinical and Vaccine Immunology: CVI, 18(11), 1872–1881. https://doi.org/10.1128/CVI.05323-11.

Pérez-Lago, L., Navarro, Y., & García-de-Viedma, D. (2014). Current knowledge and pending challenges in zoonosis caused by Mycobacterium bovis: a review. Research in Veterinary Science, 97 Suppl, S94–S100. https://doi.org/10.1016/j.rvsc.2013.11.008.

Perry, D., T.F. Randolph, J.J. McDermott, K.R. Sones, and P.K. Thornton. 2002. Investing in Animal Health Research to Alleviate Poverty. International Livestock Research Institute. Available: www.ilri.cgiar.org/InfoServ/Webpub/fulldocs/investinginanimal/index.htm.

Pesciaroli, M., Alvarez, J., Boniotti, M. B., Cagiola, M., Di Marco, V., Marianelli, C., … Pasquali, P. (2014). Tuberculosis in domestic animal species. Research in Veterinary Science, 97, S78–S85. https://doi.org/10.1016/j.rvsc.2014.05.015.

Pfeiffer, D. U. (2013). Epidemiology caught in the causal web of bovine tuberculosis. Transboundary and Emerging Diseases, 60 Suppl 1, 104–110. https://doi.org/10.1111/tbed.12105.

Phillips, C. J. C., Foster, C. R. W., Morris, P. A., & Teverson, R. (2003). The transmission of Mycobacterium bovis infection to cattle. Research in veterinary science, 74(1), 1-15.

Pollock, J. M., Buddle, B. M., & Andersen, P. (2001). Towards more accurate diagnosis of bovine tuberculosis using defined antigens. Tuberculosis (Edinburgh, Scotland), 81(1–2), 65–69. https://doi.org/10.1054/tube.2000.0273.

Pollock, J. M., & Neill, S. D. (2002). Mycobacterium bovis infection and tuberculosis in cattle. Veterinary Journal (London, England: 1997), 163(2), 115–127.

Radostits, O. M., Gay, C. C., Hinchelift, K. W. and Constabel, P. D. (2007) Veterinary Medicine. A text book of the disease of cattle, sheep, pig, goat and horses. 10th ed., Elsevier, London. Pp. 1007-1040.

Radunz, B. (2006). Surveillance and risk management during the latter stages of eradication: experiences from Australia. Veterinary Microbiology, 112(2–4), 283–290. https://doi.org/10.1016/j.vetmic.2005.11.017.

Rastogi, N., Legrand, E., & Sola, C. (2001). The mycobacteria: an introduction to nomenclature and pathogenesis. Revue Scientifique Et Technique (International Office of Epizootics), 20(1), 21–54.

Reviriego Gordejo, F. J., & Vermeersch, J. P. (2006). Towards eradication of bovine tuberculosis in the European Union. Veterinary Microbiology, 112(2–4), 101–109. https://doi.org/10.1016/j.vetmic.2005.11.034.

Riojas, M. A., McGough, K. J., Rider-Riojas, C. J., Rastogi, N., & Hazbón, M. H. (2018). Phylogenomic analysis of the species of the Mycobacterium tuberculosis complex demonstrates that Mycobacterium africanum, Mycobacterium bovis, Mycobacterium caprae, Mycobacterium microti and Mycobacterium pinnipedii are later heterotypic synonyms of Mycobacterium tuberculosis. International Journal of Systematic and Evolutionary Microbiology, 68(1), 324–332. https://doi.org/10.1099/ijsem.0.002507.

Risco, D., Fernández-Llario, P., García-Jiménez, W. L., Gonçalves, P., Cuesta, J. M., Martínez, R., … de Mendoza, J. H. (2013). Influence of Porcine Circovirus Type 2 Infections on Bovine Tuberculosis in Wild Boar Populations. Transboundary and Emerging Diseases, 60, 121–127. https://doi.org/10.1111/tbed.12112.

Rodriguez-Campos, S., Smith, N. H., Boniotti, M. B., & Aranaz, A. (2014). Overview and phylogeny of Mycobacterium tuberculosis complex organisms: implications for diagnostics and legislation of bovine tuberculosis. Research in Veterinary Science, 97 Suppl, S5–S19. https://doi.org/10.1016/j.rvsc.2014.02.009.

Rodríguez-Prieto, V., Martínez-López, B., Barasona, J. A., Acevedo, P., Romero, B., Rodriguez-Campos, S., … Vicente, J. (2012). A Bayesian approach to study the risk variables for tuberculosis occurrence in domestic and wild ungulates in South Central Spain. BMC Veterinary Research, 8, 148. https://doi.org/10.1186/1746-6148-8-148.

Ryan, T. J., Livingstone, P. G., Ramsey, D. S. L., de Lisle, G. W., Nugent, G., Collins, D. M., & Buddle, B. M. (2006). Advances in understanding disease epidemiology and implications for control and eradication of tuberculosis in livestock: the experience from New Zealand. Veterinary Microbiology, 112(2–4), 211–219. https://doi.org/10.1016/j.vetmic.2005.11.025

SANCO WD. (2013). Working Document on Eradication of Bovine Tuberculosis in the EU. Accepted by the Bovine tuberculosis subgroup of the Task Force on Monitoring Animal Disease Eradication. Brussels SANCO/7059/2013.

Santos, N., Correia-Neves, M., Ghebremichael, S., Källenius, G., Svenson, S. B., & Almeida, V. (2009). Epidemiology of Mycobacterium bovis infection in wild boar (Sus scrofa) from Portugal. Journal of wildlife diseases, 45(4), 1048-1061.

Saunders, Frank, & Orme. (1999). Granuloma formation is required to contain bacillus growth and delay mortality in mice chronically infected with Mycobacterium tuberculosis. Immunology, 98(3), 324–328. https://doi.org/10.1046/j.1365-2567.1999.00877.x.

Schiller, I., Oesch, B., Vordermeier, H. M., Palmer, M. V., Harris, B. N., Orloski, K. A., … Waters, W. R. (2010). Bovine tuberculosis: a review of current and emerging diagnostic techniques in view of their relevance for disease control and eradication. Transboundary and Emerging Diseases, 57(4), 205–220. https://doi.org/10.1111/j.1865-1682.2010.01148.x.

Schiller, I, Waters, W. R., RayWaters, W., Vordermeier, H. M., Jemmi, T., Welsh, M., … Oesch, B. (2011). Bovine tuberculosis in Europe from the perspective of an officially tuberculosis free country: trade, surveillance and diagnostics. Veterinary Microbiology, 151(1–2), 153–159. https://doi.org/10.1016/j.vetmic.2011.02.039.

Seibert, F. B., & Glenn, J. T. (1941). Tuberculin purified protein derivative. Preparation and analyses of a large quantity for standard. American Review of Tuberculosis and Pulmonary Diseases, 44(1), 9-25.

Serrano, M., Sevilla, I. A., Fuertes, M., Geijo, M., Risalde, M. Á., Ruiz-Fons, J. F., … Garrido, J. M. (2018). Different lesion distribution in calves orally or intratracheally challenged with Mycobacterium bovis: implications for diagnosis. Veterinary Research, 49(1), 74. https://doi.org/10.1186/s13567-018-0566-2.

Smith, I. (2003). Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. Clinical Microbiology Reviews, 16(3), 463–496. DOI: 10.1128/CMR.16.3.463-496.2003.

Smith, R.L., Schukken, Y.H., Lu, Z., Mitchell, R.M., Grohn, Y.T.. (2013). Development of a model to simulate infection dynamics of Mycobacterium bovis in cattle herds in the United States. J. Am. Vet. Med. Assoc. 243, 411–423. doi:10.2460/javma.243.3.411.

Sreevatsan, S., Stockbauer, K. E., Pan, X., Kreiswirth, B. N., Moghazeh, S. L., Jacobs, W. R., … Musser, J. M. (1997). Ethambutol resistance in Mycobacterium tuberculosis: critical role of embB mutations. Antimicrobial Agents and Chemotherapy, 41(8), 1677–1681.

Skuce, R. A., Byrne, A. W., Lahuerta-Marin, A., & Allen, A. (2018). Mycobacterium bovis Molecular Typing and Surveillance. In “Bovine Tuberculosis”, pp 58-79. M. Chambers, S. Gordon, F. Olea-Popelka, P. Barrow (eds). CAB International.

Sunder, S., Lanotte, P., Godreuil, S., Martin, C., Boschiroli, M. L., & Besnier, J. M. (2009). Human-to-human transmission of tuberculosis caused by Mycobacterium bovis in immunocompetent patients. Journal of Clinical Microbiology, 47(4), 1249–1251. https://doi.org/10.1128/JCM.02042-08.

Sweeney, F. P., Courtenay, O., Hibberd, V., Hewinson, R. G., Reilly, L. A., Gaze, W. H., & Wellington, E. M. H. (2007). Environmental monitoring of Mycobacterium bovis in badger feces and badger sett soil by real-time PCR, as confirmed by immunofluorescence, immunocapture, and cultivation. Applied and environmental microbiology, 73(22), 7471-7473.

Thoen, C., Lobue, P., & de Kantor, I. (2006). The importance of Mycobacterium bovis as a zoonosis. Veterinary Microbiology, 112(2–4), 339–345. https://doi.org/10.1016/j.vetmic.2005.11.047.

Thoen, C. O., Lobue, P. A., Enarson, D. A., Kaneene, J. B., & de Kantor, I. N. (2009). Tuberculosis: a re-emerging disease in animals and humans. Veterinaria Italiana, 45(1), 135–181.

Thoen, C. O., LoBue, P. A., & de Kantor, I. (2010). Why has zoonotic tuberculosis not received much attention? The International Journal of Tuberculosis and Lung Disease: The Official Journal of the International Union Against Tuberculosis and Lung Disease, 14(9), 1073–1074.

Twomey, D. F., Crawshaw, T. R., Anscombe, J. E., Farrant, L., Evans, L. J., McElligott, W. S., … de la Rua-Domenech, R. (2007). TB in llamas caused by Mycobacterium bovis. The Veterinary Record, 160(5), 170.

van Ingen, J., Rahim, Z., Mulder, A., Boeree, M. J., Simeone, R., Brosch, R., & van Soolingen, D. (2012). Characterization of Mycobacterium orygis as M. tuberculosis Complex Subspecies. Emerging Infectious Diseases, 18(4), 653–655. https://doi.org/10.3201/eid1804.110888.

Vayr, F., Martin-Blondel, G., Savall, F., Soulat, J.-M., Deffontaines, G., & Herin, F. (2018). Occupational exposure to human Mycobacterium bovis infection: A systematic review. PLOS Neglected Tropical Diseases, 12(1), e0006208. https://doi.org/10.1371/journal.pntd.0006208.

Vicente, J., Höfle, U., Garrido, J. M., Acevedo, P., Juste, R., Barral, M., & Gortazar, C. (2007). Risk factors associated with the prevalence of tuberculosis-like lesions in fenced wild boar and red deer in south central Spain. Veterinary research, 38(3), 451-464.

Vordermeier, M., Goodchild, A., Clifton-Hadley, R.S., & Rua, R.D. (2004). The interferon-gamma field trial: background, principles and progress. The Veterinary record, 155, 2: 37-8.

Waters, W. R., Palmer, M. V., Whipple, D. L., Carlson, M. P., & Nonnecke, B. J. (2003). Diagnostic Implications of Antigen-Induced Gamma Interferon, Nitric Oxide, and Tumor Necrosis Factor Alpha Production by Peripheral Blood Mononuclear Cells from Mycobacterium bovis-Infected Cattle. Clinical and Vaccine Immunology, 10(5), 960–966. https://doi.org/10.1128/CDLI.10.5.960-966.2003.

Waters, W. R., Palmer, M. V., Thacker, T. C., Bannantine, J. P., Vordermeier, H. M., Hewinson, R. G., … Lyashchenko, K. P. (2006). Early antibody responses to experimental Mycobacterium bovis infection of cattle. Clinical and Vaccine Immunology: CVI, 13(6), 648–654. https://doi.org/10.1128/CVI.00061-06.

Waters, W. R., Thacker, T. C., Nonnecke, B. J., Palmer, M. V., Schiller, I., Oesch, B., … Estes, D. M. (2012). Evaluation of Gamma Interferon (IFN-γ)-Induced Protein 10 Responses for Detection of Cattle Infected with Mycobacterium bovis: Comparisons to IFN-γ Responses. Clinical and Vaccine Immunology, 19(3), 346–351. https://doi.org/10.1128/CVI.05657-11.

Waters, W. Ray, Maggioli, M. F., McGill, J. L., Lyashchenko, K. P., & Palmer, M. V. (2014). Relevance of bovine tuberculosis research to the understanding of human disease: historical perspectives, approaches, and immunologic mechanisms. Veterinary Immunology and Immunopathology, 159(3–4), 113–132. https://doi.org/10.1016/j.vetimm.2014.02.009.

Zachary, J.F., McGavin, M.D., 2012. Pathologic Basis of Veterinary Disease, 5th ed. Elsevier.

Zanardi, G., Boniotti, M. B., Gaffuri, A., Casto, B., Zanoni, M., & Pacciarini, M. L. (2013). Tuberculosis transmission by Mycobacterium bovis in a mixed cattle and goat herd. Research in Veterinary Science, 95(2), 430–433. https://doi.org/10.1016/j.rvsc.2013.04.019.

Zinsstag, J., Schelling, E., Roth, F., & Kazwala, R. (2006). Economics of Bovine Tuberculosis. In " Mycobacterium bovis Infection in Animals and Humans", pp. 68–83. Thoen, C. O., Steele, J. H., & Gilsdorf, M. J. (Eds.), 2nd Edition. Oxford, UK: Blackwell Publishing Ltd. https://doi.org/10.1002/9780470344538.ch9.

Zinsstag, J., Schelling, E., Waltner-Toews, D., Whittaker, M., & Tanner, M. (Eds.). (2015). One Health: the theory and practice of integrated health approaches. Wallingford: CABI. https://doi.org/10.1079/9781780643410.0000.

# Objectives

## Chapter II

The general aim of this PhD thesis is to enhance knowledge on the epidemiology of bTB and its control in Spanish cattle herds and to investigate sociological aspects that may hinder the success of the bTB eradication program in Spain.

The specific objectives that have guided this PhD research are:

- To assess the spread of bTB within Spanish cattle herds;
- To evaluate the efficiency of the bTB surveillance system in Spain and the relative contribution of the system's components;
- To estimate the spatial variability of the bTB surveillance among Spanish provinces
- To gather detailed information on the main arguments circulating among farmers and veterinarians about the bTB eradication programme;
- To identify the existence of different profiles of opinions towards the bTB eradication programme;
- To quantify similarities and differences in opinions and attitudes of farmers and veterinarians in relation to the bTB eradication programme.

# Study I

## Chapter III

## Assessing the variability in transmission of bovine tuberculosis within Spanish cattle herds impact

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## 3.1. Abstract

In Spain, despite years of efforts to eradicate bovine tuberculosis (bTB), the disease is still endemic, with some areas of high prevalence. In this context, the surveillance and control plans may need to be re-evaluated, and understanding the dynamics of bTB spread within Spanish herds may help to develop new strategies for reducing the time for detection of infected herds and for the elimination of bTB from the herds already infected.

Here, we developed a compartmental stochastic model to simulate bTB within-herd transmission, fed it with epidemiological data from 22 herds (obtained from a previous work) and carried out parameter inference using Approximate Bayesian Computing methods. We also estimated the "Within-herd transmission potential Number" (Rh), i.e. the average number of secondary cases generated by a single animal infected introduced into a totally susceptible herd, considering different scenarios depending on the frequency of controls.

The median global values obtained for the transmission parameters were: for the transmission coefficient ($\beta$), 0.014 newly infected animals per infectious individual per day (i.e. 5.2 per year), for the rate at which infected individuals become infectious ($\alpha$), 0.01 per day (equivalent to a latent period of 97 days), and for the rate at which infected individuals become reactive to the skin test ($\alpha_1$), 0.08 per day (equivalent to a period of 12 days for an infected animal to become reactive). However, the results also evidenced a great variability in the estimates of those parameters (in particular $\beta$ and $\alpha$) among the 22 herds. Considering a 6-month interval between tests, the mean Rh was 0.23, increasing to 0.82 with an interval of 1 year, and to 2.01 and 3.47 with testing intervals of 2 and 4 years, respectively.

## 3.2. Introduction

Bovine Tuberculosis (bTB) is defined as a chronic infectious disease of cattle (including all *Bos* species, and *Bubalus bubalis*) and bison (*Bison bison*) caused by any of the disease-causing mycobacterial species within the *Mycobacterium tuberculosis*-complex (Anon., 2013a). Cattle are mainly affected by *Mycobacterium bovis* and *Mycobacterium caprae*, which can also affect other domestic and wild animals as well as humans (Anon., 2013b; De la Rua-Domenech et al., 2006; Aranaz et al., 2003). Due to its zoonotic nature and the high economic impact on livestock production, the objective within EU countries is the elimination of bTB through the implementation of eradication programs (Reviriego Gordejo and Vermeersch, 2006).

In Spain, it was not until 1993 that most dairy and beef herds were included within the bTB national eradication program (Anon., 2010). According to the programme, all cattle herds are routinely screened by the single intradermal tuberculin test (SITT), testing all animals above 6 weeks of age. Private veterinarians, accredited to provide government services, are in charge of performing the tests, which are usually carried out annually, although the frequency may be increased depending on the prevalence in the area. Positive cattle (reactors) are slaughtered and subjected to *post-mortem* examination at the slaughterhouses. Positivity is confirmed by culture of the mycobacteria. Other measures include passive surveillance for bTB lesions at the slaughterhouses. Thanks to the application of the national eradication program in cattle, bTB herd prevalence in Spain decreased from 5.90% in 1993 to 1.80% by the end of 2004 (Anon., 2015a). Afterwards, the bTB prevalence remained quite stable for over one decade (1.72% in 2014), despite the implementation of further measures such as the introduction of compulsory pre-movement tests in 2006 or the establishment of a surveillance plan for wildlife reservoirs in 2009. In 2015 there was a major setback, as bTB prevalence increased to 2.81%, similar to the levels Spain had in 2001 (Anon., 2015b). Within the country the situation is also quite heterogeneous with some regions free of bTB (e.g. the Canary Islands) or with very low prevalence (mainly the north of Spain), and others with very high prevalence, mainly central and southern Spain (e.g. herd prevalence in Andalusia in 2015 was 17.2%) (Allepuz et al., 2011; García-Saenz et al., 2014; Anon., 2015b).

Those results demonstrate the need to re-evaluate the measures currently in place if eradication is to be achieved. Understanding the dynamics of bTB spread within Spanish herds would be helpful for the design of new surveillance and control strategies that would reduce the time needed for both the detection of infected herds and the elimination of the disease from the infected herds.

Dynamic modelling of bTB has been widely applied because studying bTB spread in infected herds is hindered by the long incubation periods; and, therefore, models offer the opportunity to assess bTB transmission in a more cost-effective way (Brooks-Pollock et al., 2014; Conlan et al., 2012; Pérez et al., 2002). Different mathematical models have been used to describe the dynamics of bTB infection in the herd, with the purpose of estimating bTB within-herd transmission rates and evaluating the effectiveness of surveillance and control strategies (Barlow et al., 1997; Pérez et al., 2002; Álvarez et al., 2012a; Bekara et al., 2014; Brooks-Pollock et al., 2014; O'Hare et al., 2014). As a result, the bTB transmission parameters estimated are quite variable, which may be partially explained by the intrinsic variability in the transmission process, but also on factors such as the modelling approach used, the assumptions made, or the type and quality of the data used to feed models. Transmission dynamics is also influenced by the herd production type or the management practices, and therefore it is essential that parameters are obtained using data from herds that are representative of the bTB context in Spain.

In the present work, we first estimated the variability in the parameters related to bTB transmission in Spanish herds. Then, we used those parameters to simulate the average number of secondary cases caused by a single infected animal introduced into a herd, calling this "quantity" the "Within-herd transmission potential Number" ($R_h$).

## 3.3. Materials and Methods

### 3.3.1. Selection of herds for parameter inference

In Spain, when a newly infected herd is confirmed by bacteriological culture, a veterinary officer carries out an epidemiological questionnaire, and the data is recorded in a database called BRUTUB, which is maintained by the Spanish Ministry of Agriculture, Fisheries, Food and Environment (Anon., 2010). In a previous work, Guta et al., (2014) developed a methodology to determine the most likely source of infection of bTB affected herds. Briefly: seven possible origins of infection were considered: i) residual infection; ii) purchase of cattle; iii) sharing of pastures; iv) neighbours; v) contact with domestic goats; vi) interaction with wildlife reservoirs and vii) contact with humans. Decision trees were developed for each of the different sources of infection, and a group of bTB experts assigned the probabilities for the possible events on those decision trees. By feeding the data from a given farm (contained in the BRUTUB questionnaire) to the decision trees, the probabilities of the farm being infected by each of the seven possible sources were quantified.

For the inference of bTB transmission parameters, we selected only infected herds in which we had some certainty that the introduction of bTB into the herd had occurred through purchase of animals, by adapting the methodology developed by Guta et al., (2014). More specifically, from the herds recorded in the BRUTUB database between 2010 and 2013:

a) First, we selected herds that met the criteria in relation to introduction through purchase of animals, that is: i) cattle had been purchased between the last negative control and the detection of infection in the herd of destination; ii) at least one of the purchased animals reacted positive to the SITT at the time of detection; iii) the herd of origin of cattle was subsequently confirmed as bTB infected; iv) and the same spoligotype was isolated in both herds or the same spoligotype was isolated during the previous year in the municipality of the herd of origin of introduced cattle.

b) Then, from the herds selected, we excluded those that did not meet the criteria of exclusivity in relation to the introduction of bTB only through purchase of animals. It means we further excluded all herds in which the introduction of the disease through any of the other sources was possible. In order to do that, we defined some other "key events" as exclusion criteria. For example, herds with evidence of the presence of some reactor 3 years prior to the last negative control were excluded because of potential residual infection; and herds that reported some sort of contact with wildlife reservoir species were excluded because of potential infection from wildlife.

Besides, any herd with missing data that did not allow ruling out any of the possible origins was also excluded for parameter inference.

### 3.3.2. Herd data for parameter inference

On those selected herds, data available included:

a) Date of purchase of animals from the herd subsequently found to be infected, i.e. the likely date of introduction of bTB into the herd.

b) Date of bTB detection in the herd.

We assumed that the difference between both dates represented the time available for the spread of bTB.

c) Number of animals in the herd on the date of bTB detection.

We assumed a constant population size between infection of the herd and detection.

d) Number of positives on the date of bTB detection.

e) Number of positives among the purchased animals. As it is estimated at the time of detection, not at the time of purchase, it actually represents the maximum number of infected animals introduced into the herd (i.e. the number of occult animals introduced is modelled as a *Uniform* distribution between 1 and the number of positives among the purchased animals).

The difference between the number of infected among the purchased animals and the total infected animals in the herd on the date of bTB detection represented the spread of the infection within the herd since the introduction of bTB.

### 3.3.3. Development of the bTB spread model

Bovine tuberculosis within-herd transmission was simulated using a compartmental stochastic SOEI (Susceptible, Occult, Exposed and Infectious) model (Conlan et al., 2012; Barlow et al., 1997) (Figure 1). In this model, occult animals (O) represented animals that were infected, but were not yet detectable by SITT and were not infectious either. Exposed animals (E) represented animals that were infected and were detectable by SITT, but were not infectious yet. Finally, infectious animals (I) represented animals that were infected, were detectable by SITT and were also infectious.

A homogeneous-mixing model with frequency-dependent (i.e. true mass-action) transmission was assumed as described in previous studies (Bekara et al., 2014; Smith et al., 2013; Álvarez et al., 2012a; Fischer et al., 2005; Pérez et al., 2002). Although herd size is known to be correlated with the persistence of the bTB (Brooks-Pollock et al., 2014) and several authors opted for density-dependent models (O'Hare et al., 2014; Barlow et al., 1997; Kao et al., 1997), recent comparison of models have demonstrated a higher predictive ability for the frequency-dependent models (Álvarez et al., 2014; Smith et al., 2013). In contrast to wildlife or human populations, in cattle holdings there is an upper limit to the number of contacts that animals may have, so it is unlike that an increase in the size of the herd would lead to an increase in animal interactions (Sánchez and Hudgens, 2015; Vynnycky and White, 2010).

Although the simplest compartmental models implicitly assume that the sojourn time in any of the states is exponentially distributed, from a biological point of view, in some situations, the use of more flexible non-exponential residence-time distributions for latent and infectious periods may represent a reasonable alternative (Streftaris and

Gibson, 2004, Wearing et al., 2005, Feng et al., 2007; Huppert et al., 2013). In our study, we assumed that the occult and exposed sojourn states followed the Erlang distribution, a subset of the gamma probability density function, with integer-valued shape parameter (Ibe, 2009). The Erlang distribution, due to its proprieties, offers a computationally tractable way to incorporate gamma-like distributed sojourn times into a compartmental model (Lloyd, 2001; Bame et al., 2008; Yan and Feng, 2010). While this modification does not affect the development of the epidemic as such, it leads to a more flexible and reasonable representation of the occult and exposed sojourn times (Barlow et al., 1997; Lloyd, 2001; Streftaris and Gibson, 2004; Wearing et al., 2005; Feng et al., 2007; Huppert et al., 2013). The Erlang distributed occult and exposed periods were introduced into the compartmental model by using a "box-car" approach, to take advantage of the so-called "linear chain trick" (Wearing et al., 2005; Feng et al., 2007; Lloyd, 2009). The O and E compartments were subdivided into *m* and *n* sequential sub-compartments, respectively. We assumed 3 sub-compartments for each state (*m*=*n*=3), dubbing the model as $SO^mE^nI$ (Figure 1).

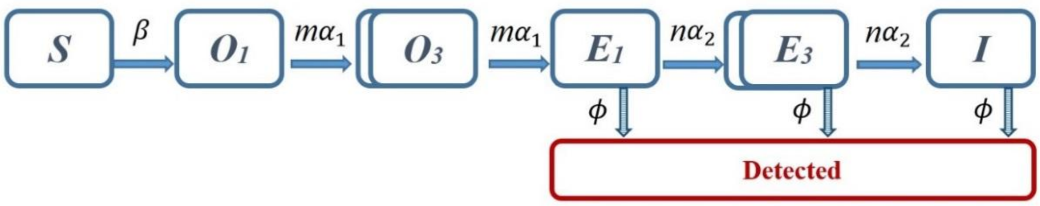


Figure 1: Flow diagram of the compartmental stochastic $SO^mE^nI$ (Susceptible, Occult, Exposed and Infectious) model with Erlang-distributed occult and exposed sojourn times (where *m=n=3*), representing the dynamics of the bTB spread within the herd. Animals susceptible to *M. bovis* (S) become occult (O), infected but nor detectable by SITT neither infectious, through the contact with shedding cattle at a rate $\beta$, the transmission coefficient. Occult cattle become exposed (E), not infectious yet but detectable by SITT, at a rate $\alpha_1$. Exposed animals become infectious and detectable by SITT (I) at a rate $\alpha_2$. Exposed (E) and Infectious (I) cattle can be detected as bTB positive based on the SITT sensitivity ($\phi$).

To ensure that the overall average times spent in the occult and exposed classes were still $1/\alpha_1$ and $1/\alpha_2$, respectively, we constructed the original single compartments as the sum of the respective sub-compartments and the transition rates between successive occult and exposed sub-compartments were defined as $m*\alpha_1$ and $n*\alpha_2$, respectively (Figure 1).

Infection dynamics were modelled in continuous time (with days as units), using the

Gillespie's direct algorithm (Vynnycky and White, 2010; Keeling and Rohani, 2008). At each time step transitions between compartments of the $SO^mE^nI$ model occurred according to the following differential equations:

$$\frac{dS}{dt} = -\frac{\beta S(t)I(t)}{N(t)}$$

$$N(t) = S(t) + O(t) + E(t) + I(t)$$

$$\frac{dO_1}{dt} = \frac{\beta S(t)I(t)}{N(t)} - m\alpha_1 O_1(t)$$

.

.

.

$$\frac{dO_m}{dt} = m\alpha_1 O_{m-1}(t) - m\alpha_1 O_m(t)$$

$$\frac{dE_1}{dt} = m\alpha_1 O_m(t) - n\alpha_2 E_1(t)$$

.

.

.

$$\frac{dE_n}{dt} = n\alpha_2 E_{n-1}(t) - n\alpha_2 E_n(t)$$

$$\frac{dI}{dt} = n\alpha_2 E_n(t)$$

where *m* and *n* represented the different sub-compartments within the occult and exposed stages, respectively. The transmission coefficient ($\beta$) is defined as the average number of individuals that are newly infected from an infectious individual per unit of time (De Jong, 1995). The parameter $\alpha_1$ is defined as the rate at which infected non-detectable and non-shedding cattle (O) become reactive to the SITT (E). Thus $1/\alpha_1$, known as *occult period*, is the average time between the infection of the animal and the moment in which that animal is able to develop a (cell-mediated) immune response detectable by SITT. The parameter $\alpha_2$ is defined as the rate at which infected detectable but non-shedding cattle (E) become infectious (I). The value of $\alpha_2$ is obtained as:

$$\alpha_2 = \frac{1}{\left(\frac{1}{\alpha} - \frac{1}{\alpha_1}\right)}$$

where $\alpha$ is the rate at which infected individuals become infectious, and $1/\alpha$ is the *latent period*, i.e. the average time between infection of a cow and the moment when that animal becomes infectious.

The only way of measuring the progress of the infection within the farm is through the detection of infected animals by means of the *in-vivo* diagnostic tests (mainly SITT). As tests are not perfect, some infected animals may be missed. In fact, in the case of the SITT, there is a great deal of uncertainty about the true sensitivity of this test applied in the field (Álvarez et al., 2012b). In this study, we defined a short *occult period*, in which animals were not reactive to the cervical SITT, and then the same sensitivity ($\phi$) was assumed for both exposed and infectious individuals. Consequently, the number of animals detected in the herd at any point in time can be estimated as:

$$Detected_t \sim binomial\ ((E_t + I_t), \phi)$$

We assumed a test sensitivity ($\phi$) of 94%, the median value for the SIT (cervical) reported in the comprehensive review carried out by the EFSA (EFSA-AHAW Panel, 2012).

As purchased animals were assumed to have been subjected to pre-movement tests, the infected animals introduced into the herd were assumed to be in the occult state (O). The within-herd transmission model was built in R version 3.2.1 (R Core Team., 2015).

### 3.3.4. Parameter inference

While it is often straightforward to build models that may describe our observations, or even feed some parameters to a model to simulate an artificial data set, it is usually more difficult to estimate the parameter values that could have given rise to a given data set, i.e. carry out parameter inference (Beaumont, 2010). Because of that, some deterministic methods, mainly based on maximum-likelihood estimation, were developed for parameter estimation, but they were constrained by the stochasticity, which is an inherent part of many biological systems (Hartig et al., 2011; Toni et al., 2009). To overcome those limitations further inference methods were developed; among them, the Approximate Bayesian Computing (ABC) (Beaumont, 2010; Tavaré et al., 1997). ABC methods are based on the calculation of summary statistics for a given configuration of the parameters obtained from the stochastic simulation model. Acceptance of that configuration is based on the comparison between observed and simulated data, and that comparison enables us to obtain an approximated posterior distribution of the model parameters (Hartig et al., 2011). The simplest ABC algorithm is the ABC rejection sampler, but it has the disadvantage that the rate of acceptance may be quite low when non-informative prior distributions are used (Toni et al., 2009). Therefore, we used a random walk ABC Markov chain Monte Carlo (MCMC) algorithm (see Toni et al., 2009 for a detailed description; Marjoram et al., 2003) to generate the posterior distributions of the bTB transmission parameters ($\beta, \alpha, \alpha_1$ and $\alpha_2$) within Spanish cattle herds. To build the posterior chains, the algorithm drew candidate samples from a proposal distribution that was normally distributed, centred at the previous state of the chain, and with standard deviations set at 0.003 for $\beta$, 0.002 for $\alpha$, and 0.007 for $\alpha_1$.

The study-herds were analyzed individually by running MCMC chains with 1,000,000 steps, with the posterior distributions thinned to return 10,000 samples. Therefore, we obtained 22 posterior distributions for each of the parameters estimated. ABC-MCMC simulations were assessed using the "coda" package (Plummer et al., 2006). The estimated posterior distributions of the bTB transmission parameters ($\beta, \alpha, \alpha_1$ and $\alpha_2$) within Spanish cattle herds are summarized with their mean and quantiles, and also displayed graphically as box-and-whiskers plots. For each of the transmission parameters we also calculated a global median value (i.e., aggregated value), obtained by binding together the posteriors distributions inferred from the 22 selected Spanish cattle herds, after determining that each of the individual posterior distributions were satisfactory. Algorithms were implemented within the R environment version 3.2.1 (R Core Team., 2015).

Definition of prior distributions: The uncertainty of $\beta, \alpha, \alpha_1$ and $\alpha_2$ parameters was accounted for by the use of prior distributions. Prior distributions for the different parameters, and the sources from which they were derived, are described in table 1.

| Parameter | Description | Distribution | Inputs of the distribution | Source |
|---|---|---|---|---|
| $\beta$ | Transmission coefficient | *uniform(min, max)* | Minimum= 0.0003 days$^{-1}$ | Bekara et al., 2014 |
| | | | Maximum=0.0276 days$^{-1}$ | Bekara et al., 2014 |
| $\alpha$ | Rate at which infected individuals become infectious. | *uniform(min, max)* | Minimum=0.0009 days$^{-1}$ | Bekara et al., 2014 |
| | | | Maximum=0.0164 days$^{-1}$ | Bekara et al., 2014 |
| $\alpha_1$ | Rate at which infected individuals become reactive to SITT. | *pert*(min, most likely, max)* | Minimum=1/63 days | De la Rua-Domenech et al., 2006 |
| | | | Most likely= 1/uniform(21,42) days | De la Rua-Domenech et al., 2006 & OIE Terrestrial Manual |
| | | | Maximum=1/7 days | De la Rua-Domenech et al., 2006 |

Table 1: Prior distributions for the bTB within-herd transmission model parameters, their values and the sources from which those values were derived. **Pert distribution*: a special version of the beta distribution defined by the minimum, most likely and maximum values (Vose, 2008).

## Optimization of the sampling algorithm

A potential disadvantage of the ABC-MCMC algorithm is that when there is a high degree of uncertainty in relation to the prior distributions, the candidate parameters sampled from those priors may be potentially very far from the posterior distribution, and the ABC-MCMC may result in low acceptance rates (Toni et al., 2009). In order to avoid that problem and optimize the sampling, we developed an algorithm that, before the initiation of the Markov chains, drew samples from the prior distributions, simulated the spread within a given herd, calculated the summary measure for that simulation and compared it with summary measure observed for that herd. Samples were drawn until the difference of those summary measures was within the tolerance limit (set at 0.1), in which case, the values sampled from the priors were accepted, and used as the values that initiated the Markov chains. That enabled us to avoid samples from priors that are too distant from posterior values.

## Choice of the summary measure (SM)

The most obvious approach for comparing the bTB within-herd spread observed in the herds with the values simulated using the within-herd spread model, would be to use the difference in the absolute number of infected animals. However, while a difference of a few infected animals may be considered as acceptable in a large herd, the same difference may not be acceptable in a small herd. On the other hand, if we used prevalence to account for the size of the herd, while a relatively small difference in prevalence may be considered as acceptable in a small herd, the same difference may not be acceptable in a large herd (as it would represent a huge difference in the number of infected animals). Because of that, we chose a combination of absolute number of infected animals and prevalence (i.e. number of infected animals times prevalence) as the summary measure. The tolerance limit of SM was set at 0.1, which corresponds to a difference (between observed and simulated values) of 0 infected animals for herds with less than 10 animals; 1 infected animal for herds between 11 and 39 animals; 2 infected animals for herds between 40 and 90 animals; 3 infected animals for herds between 91 and 159 animals, and so on.

### 3.3.5. Estimation of the average number of secondary cases (within-herd transmission potential number, $R_h$)

The basic reproduction ratio ($R_0$) is the most extensively used parameter in epidemic theory and it is an essential tool for understanding the behaviour of infectious diseases. It is defined as the average number of secondary cases produced when a single infected individual is introduced into a fully susceptible population (Anderson and May, 1991). If $R_0 > 1$ then the disease tends to persist within that population, while if $R_0 < 1$ the disease tends to die out, and this threshold behaviour makes $R_0$ the most useful measure of the transmission potential of a pathogen within a population (Heffernan et al., 2005). It also allows evaluating which control measures would be most effective in reducing $R_0$ below one and therefore eliminating the disease from that population (Heffernan et al., 2005; Diekmann et al., 2010).

In our study, we used an intuitive epidemiological approach to quantify the number of secondary cases produced by the introduction of an infected animal into a totally susceptible herd, and we called this quantity the “Within-herd transmission potential Number” ($R_h$). In order to do that, we used the compartmental transmission model described in section 3 to simulate bTB spread after the introduction into the herds of a single infected animal. Given that in Spain cattle are subjected to pre-movement tests, the introduced infected animal was assumed to be in the occult stage (O). By tracking down the number of new infections generated, we obtained an estimate of $R_h$. As once infectious, animals are considered to remain in that state for life, the number of secondary infections generated will depend on the time available for disease spread. We assumed that bTB spread within the herd until the disease was discovered by routine

SITT testing. Therefore, bTB spread, and ultimately $R_h$, depend on the frequency of those controls.

We simulated bTB transmission within the herds considering different times for the disease to spread freely within the herd, which is equivalent to the assumption that the disease was indeed detected after those periods. The periods chosen for the simulations were related to the frequency of testing considered within the Spanish eradication program. In Spain the spatial distribution of bTB is highly heterogeneous (Allepuz et al., 2011; Garcia-Saenz et al., 2014), and therefore, the frequency of routine testing was adapted to account for that. In general, herds are subject to one whole herd test per year. However, within regions where the herd prevalence is below 1% (low prevalence regions), the provinces where the herd prevalence has remained below 1% for two consecutive years may reduce the frequency to one testing every two years. In contrast, within regions where the herd prevalence is above 1% (high prevalence regions), the counties where the herd prevalence is above 3% need to increase the frequency of controls to two per year. Therefore, the spread of the disease was then simulated in absence of control interventions, for fixed time periods of 90, 180, 365 and 730 days. Where for example a time period of 90 days represents the average time bTB would have to spread when routine testing are carried out twice a year.

For each of the 22 selected herds, we simulated the number of secondary infections generated by the introduction of a single occult animal using the compartmental transmission model from section 3 with the values of the posterior distributions of bTB transmission parameters ($\beta$, $\alpha$, $\alpha_1$ and $\alpha_2$) inferred for that herd. For each herd and each time-spread period, the model was run for 1,000 iterations. For each time-spread period, the global values of $R_h$ were obtained by combining the estimates from the 22 study-herds. We also estimated the proportion of simulations in which $R_h$ was zero (i.e. no bTB transmission) and the proportion of simulations in which $R_h$ was equal or higher than one (i.e. bTB transmission) for the different time-spread periods. To gain a deeper knowledge of the mechanisms of transmission, within simulations in which $R_h$ was zero, we quantified the cases in which the infected animal, a) remained as occult, b) became exposed, or c) reached the infectious state. And within simulations in which $R_h$ was equal or higher than one, we calculated the proportion of cases in which a) the transmission occurred but secondarily-infected cattle did not have enough time to become infectious; and b) the transmission occurred and at least one of the secondarily-infected cattle became infectious.

## 3.4. Results

### 3.4.1. Herds selected for parameter inference

Of the 1,869 bTB-infected herds recorded in the BRUTUB system between 2010 and 2013, only 22 met the inclusion and exclusion criteria (i.e. infection likely to have been caused by the purchase of an infected animal and not by other causes). The majority of holdings were located in South-Central Spain, including 13 herds in Andalusia, six in Extremadura and two in Castile La Mancha, while there was only one herd from the North of Spain, Navarre region. All the selected herds were extensive beef herds, with sizes ranging between 26 and 213 cattle heads, although the majority were small to medium size beef herds (only 27% had more than 100 cows).

### 3.4.2. bTB spread model and parameter inference

The median global value for the transmission coefficient ($\beta$) was 0.014 newly infected animals per infectious individual per day (percentiles 5 and 95 of 0.002 and 0.026, respectively) (figure 2, table 2), equivalent to a median of 5.2 newly infected animals per infectious individual per year (percentiles 5 and 95 of 0.69 and 9.49, respectively). The individual median $\beta$ values inferred from the 22 herds (figure 2) ranged between 0.005 and 0.023 (corresponding to a range of 1.8 and 8.3 newly infected animals per infectious individual per year, respectively). Further details on the estimated $\beta$ posterior distributions obtained for the 22 study-herds are given in the Supplementary material (Tab. S1).

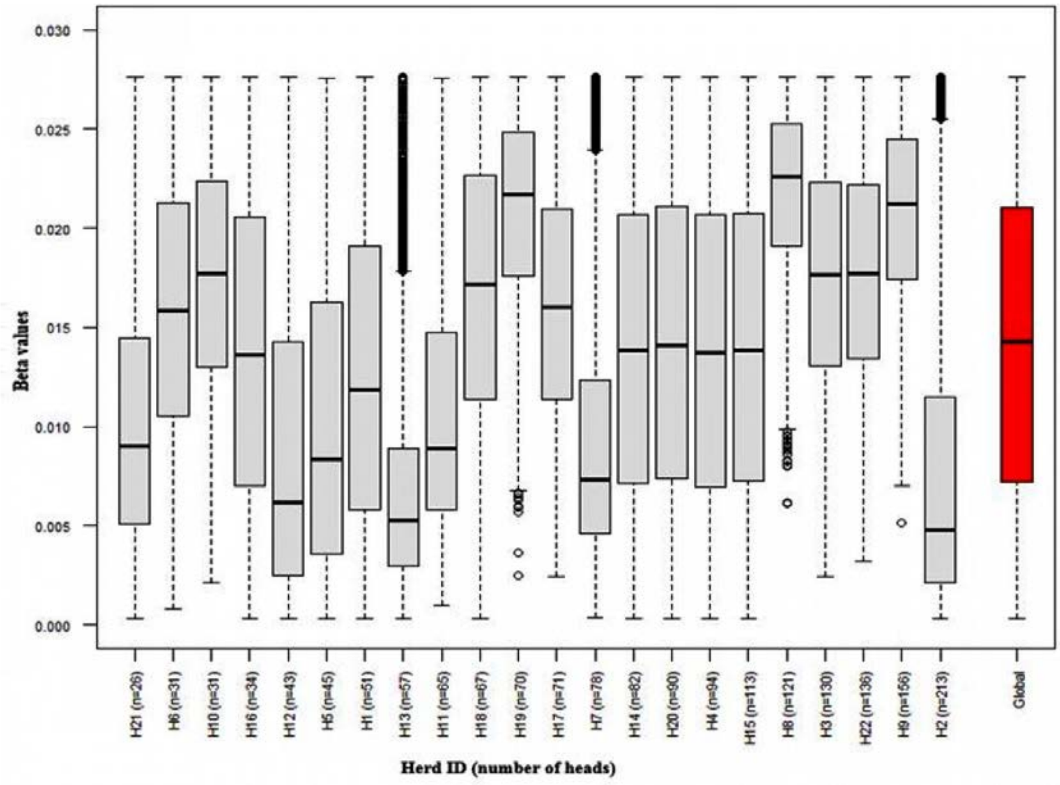


Figure 2: Box and whisker plots summarizing the posterior distribution of the $\beta$ parameter. The horizontal line inside the box represents the median value (Q50%), and the limits of the box are the lower (Q25%), and upper quantiles (Q75%). The upper and lower whiskers (the two lines extending vertically from the box) represent respectively the highest datum still within the 1.5 interquartile range (IQR) of the upper quartile and the lowest datum still within the 1.5 IQR of the lower quartile. Values higher than the upper whisker and lower than the lower whiskers are considered "outliers" and plotted as individual points. In grey: the 22 posterior distributions of the bTB transmission coefficient ($\beta$) obtained for the individual herds. The x-axis indicates the herd's ID number, and for each herd, the corresponding herds' size (cattle heads) is indicated in brackets; herds are ordered by its size. In red: the global $\beta$ value, calculated binding together the posteriors distributions inferred from the 22 selected Spanish cattle herds.

| **bTB Transmission parameter** | **Mean** | **Quantiles** | | | | |
|---|---|---|---|---|---|---|
| | | **5%** | **25%** | **50%** | **75%** | **95%** |
| $\beta$ | 0.014 | 0.002 | 0.007 | 0.014 | 0.021 | 0.026 |
| $\alpha$ | 0.010 | 0.002 | 0.006 | 0.010 | 0.014 | 0.016 |
| $\alpha_1$ | 0.080 | 0.022 | 0.049 | 0.081 | 0.112 | 0.137 |
| $\alpha_2$ | 0.014 | 0.002 | 0.007 | 0.012 | 0.017 | 0.026 |

Table 2: Mean and quantiles obtained for the global value of the bTB transmission parameters ($\beta$, $\alpha$, $\alpha_1$ and $\alpha_2$).

The median global value for $\alpha_1$ (i.e. the rate at which infected non-detectable and non-shedding cattle (O) become reactive to the SITT (E)) was 0.081 per day (percentiles 5 and 95 of 0.022 and 0.137, respectively) (table 2). Thus, the median estimate of the occult stage (i.e. the time between the infection of an animal and when it becomes detectable by SITT), $\frac{1}{\alpha_1}$, was 12 days (percentiles 5 and 95 of 7.3 and 45.5 days, respectively). Median estimates of the individual occult stage obtained from the 22 selected herds ranged between 11 and 13 days (see Supplementary material (Tab. S2) for the summary of the posterior $\alpha_1$ distributions for each of the 22 study-herds).

The median global value for $\alpha_2$ (i.e. the rate at which infected cattle reactive to the SITT but not infectious (E) yet, become infectious (I)) was 0.012 per day (percentiles 5 and 95 of 0.002 and 0.026, respectively) (table 2). Therefore, the median estimate of the exposed stage (i.e. the time between when an infected animal becomes detectable by SITT and when that animal becomes infectious), $\frac{1}{\alpha_2}$, was 82 days (percentiles 5 and 95 of 39 and 500, respectively). Median estimates of the exposed stage obtained for each of the 22 selected herds ranged between 59 and 263 days. Further details on the posterior $\alpha_2$ distributions obtained for the 22 herds are given in the Supplementary material (Tab. S3).

The median global value for $\alpha$ (i.e. the rate at which infected non-detectable and non-shedding cattle (O) become infectious (I)) was 0.010 per day (percentiles 5 and 95 of 0.002 and 0.016, respectively) (figure 3, table 2). Therefore, the median estimate for the latent period (i.e. the time between the infection of an animal and when it becomes infectious), $\frac{1}{\alpha}$, was 97 days (with percentiles 5 and 95 of 62 and 500, respectively). The median value for $\alpha$ inferred from the individual herds ranged between 0.004 and 0.014 (corresponding to 72 and 250 days, respectively) (see figure 3 and Supplementary material (Tab. S4)).

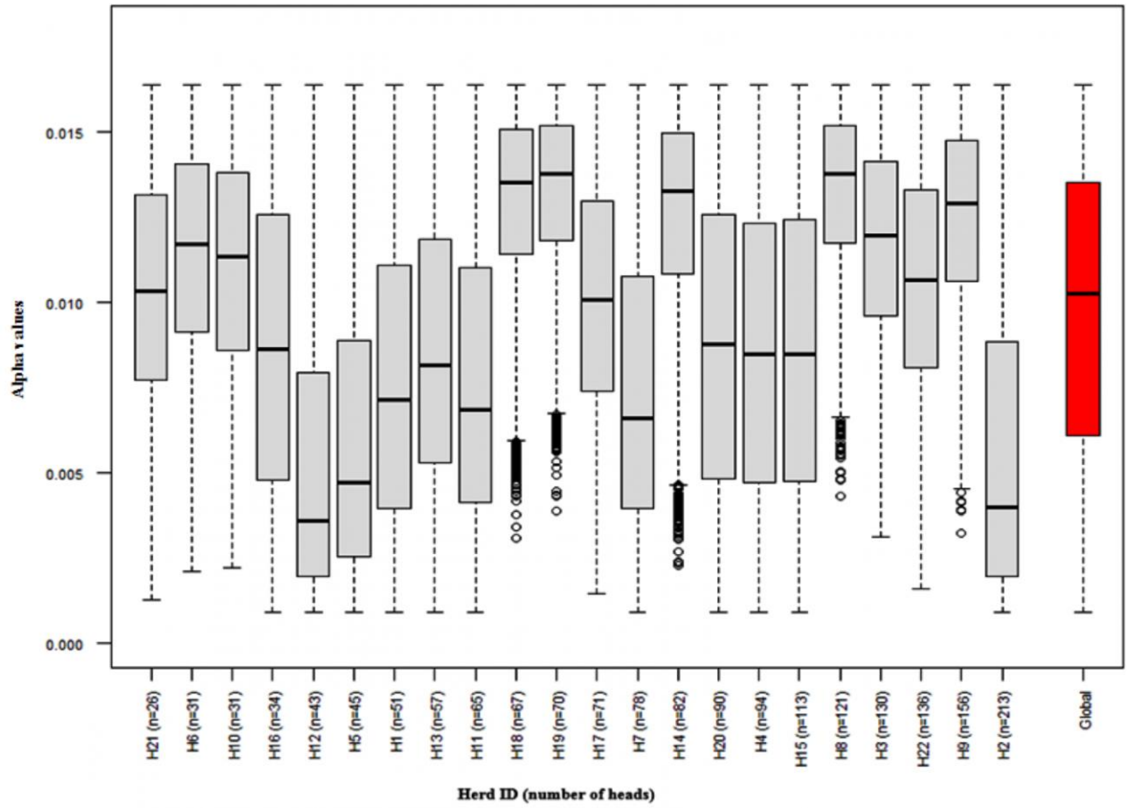


Figure 3: Box and whisker plots summarizing the posterior distribution of the $\alpha$ parameter. The horizontal line inside the box represents the median value (Q50%), and the limits of the box are the lower (Q25%), and upper quantiles (Q75%), The upper and lower whiskers (the two lines outside the box) represent respectively the highest datum still within the 1.5 interquartile range (IQR) of the upper quartile and the lowest datum still within the 1.5 IQR of the lower quartile. Values higher than the upper whisker and lower than the lower whiskers are considered "outliers" and plotted as individual points. In grey: the 22 posterior distributions of the parameter $\alpha$ obtained for the 22 study-herds. The x-axis indicates the herd's ID number, and for each herd, the corresponding herds' size (cattle heads) is indicated in brackets; herds are ordered by its size. In red: the global $\alpha$ value, calculated binding together the posteriors distributions inferred from the 22 selected Spanish cattle herds.

### 3.4.3. Within-herd transmission potential number for Spanish herds

Summary statistics of the distributions obtained for the global within-herd transmission potential number ($R_h$) at times of 90, 180, 365 and 730 days are shown in figure 4. Our results indicate that when bTB was allowed spread for 90 days, the global mean value of $R_h$ was 0.23 (percentiles 2.5 and 97.5 of 0 and 2, respectively), which increased to 0.82 (percentiles 2.5 and 97.5 of 0 and 3, respectively) when the time for spread was 180 days. The mean $R_h$ value rose to 2.01 (percentiles 2.5 and 97.5 of 0 and 6, respectively) when the spread period was 365 days and to 3.47 (percentiles 5 and 95 of 0 and 8, respectively) when the period was 730 days. Further details on the $R_h$ estimates obtained for each of the 22 study-herds are given in the Supplementary material (Tab. S5).

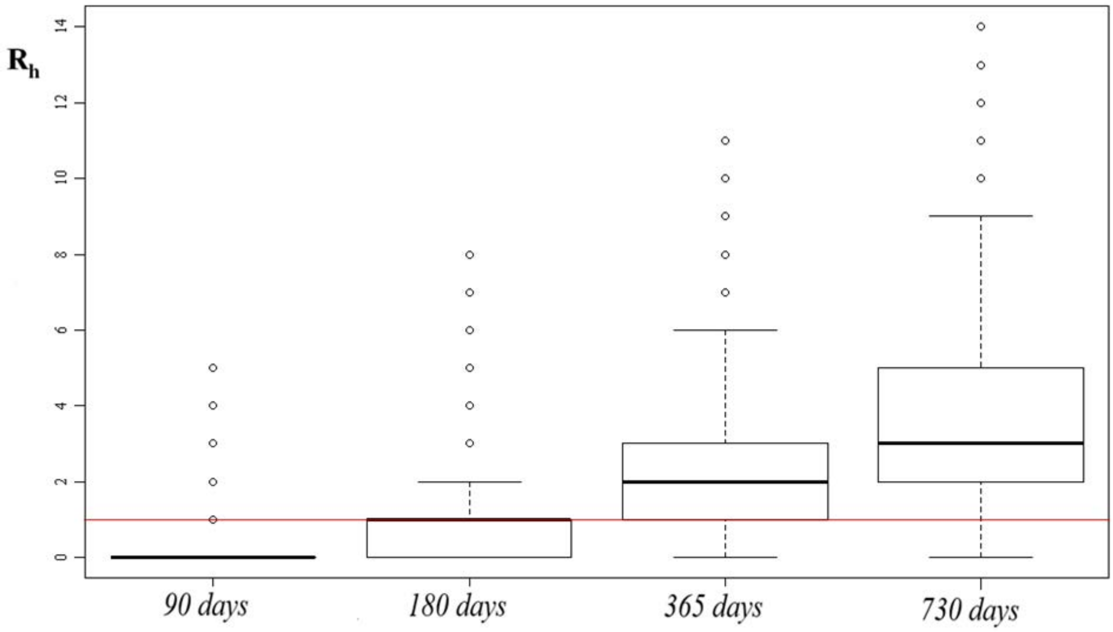


Figure 4: Box and whisker plots summarizing the $R_h$ estimates at times 90, 180, 365,730 days (x-axis). For each time, the horizontal line inside the box represents the global median value (Q50%) including all the 22 herds, and the limits of the box are the lower (Q25%) and upper quartiles (Q75%).The upper and lower whiskers (the two lines extending vertically from the box) represent respectively the highest datum still within the 1.5 IQR (interquartile range) of the upper quartile and the lowest datum still within the 1.5 IQR of the lower quartile. Values higher than the upper whisker and lower than the lower whiskers are considered “outliers” and plotted as individual points. The horizontal continuous line (in red), set at the Rh point value of one, indicates that transmission occurred.

We also estimated the proportion of simulations in which $R_h$ was equal to zero, equal to one, between two and four, between five and nine and equal or higher than 10 (figure 5), using the same times for disease spread as previously described. For disease-spread periods of 90 days, there was an 81.5% probability that $R_h$ was equal to 0, while the probability of $R_h$ being equal to one was 14.8%, and only in 3.7% of simulations $R_h$ was higher than 1. For disease-spread periods of 180 days, the probability of $R_h$ being equal to zero decreased to 49.4%, while the probability of $R_h$ being equal to 1 was 28.5%, and in 22.1% of simulations $R_h$ was higher than 1. When bTB was allowed spread for 365 days there was a 21.8% probability that $R_h$ was equal to zero, a 22.1% probability that $R_h$ was equal to one, there was a 47.5% probability for $R_h$ being between 2 and 4, and in 8.6% of simulation $R_h$ was higher than 4. Finally, for disease-spread periods of 730 days, the probability that $R_h$ was equal to zero dropped to 8.1%, the probability of $R_h$

being equal to 1 was 11.4% and there was a 50.1% probability for $R_h$ being between 2 and 4. In 29.9% of the simulations $R_h$ was between five and nine, and in 0.41% equal or higher than 10.

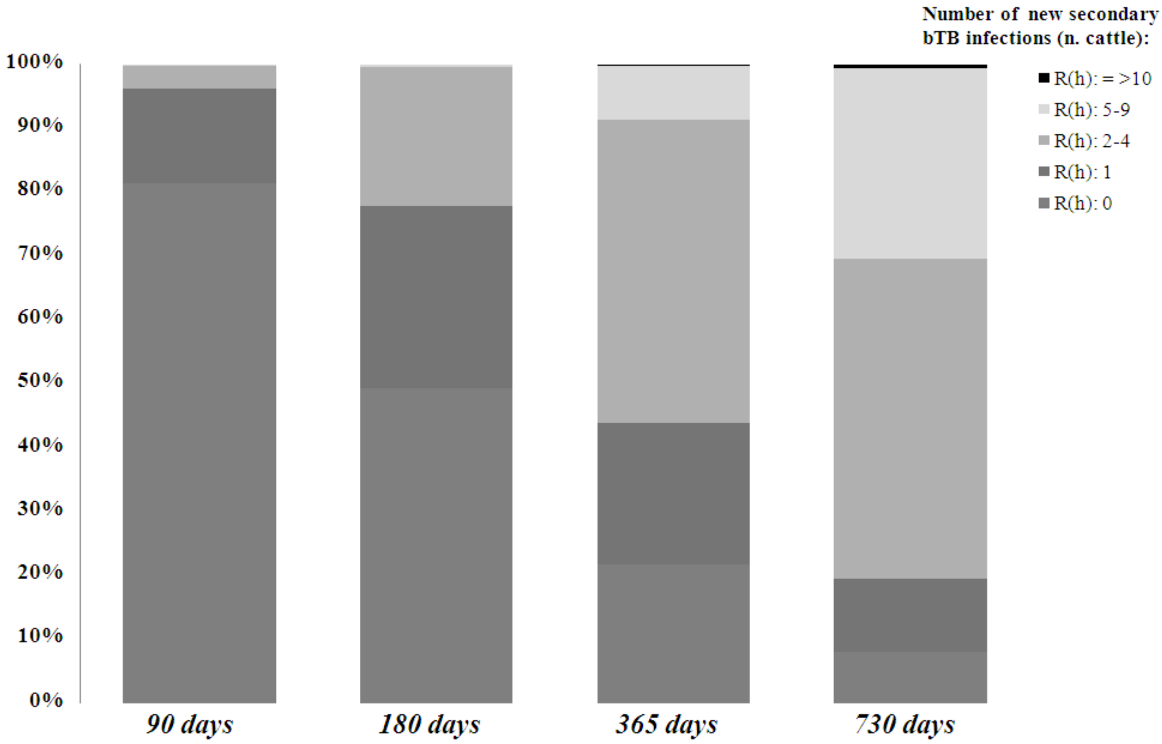


Figure 5: Range of $R_h$ values considering 90, 180, 365 and 730 days for disease spread (bar graphs from left to right). The average number of secondary cases generated after introducing an occult animal into a totally susceptible herd was categorized in 5 groups: $R_h$ equal to zero, $R_h$ equal to one, $R_h$ ranging between two and four, between five and nine and $R_h$ higher or equal to 10. Categories are indicated with the different gradients of grey (see legend in the figure).

Considering 90 days for disease-spread, in 49.7% of simulations the infected animal introduced did not have enough time to become infectious, while in 15.8% of cases bTB transmission occurred, but the secondary cases did not have enough time to become infectious (table 3). For disease-spread periods of 180 days, in 27.6% of cases the animal introduced was able to become infectious but failed to transmit the disease; and in 25% of simulations the transmission occurred but the secondarily-infected cattle had not enough time to become infectious (table 3). For disease-spread periods of 365 and 730 days, the probabilities that at least one of the secondarily new infected cattle became infectious were 64.6% and 86.0%, respectively (table 3).

| | 90 days (n. 22,000) | | 180 days (n. 22,000) | | 365 days (n. 22,000) | | 730 days (n. 22,000) | |
|---|---|---|---|---|---|---|---|---|
| | (N) | (%) | (N) | (%) | (N) | (%) | (N) | (%) |
| No transmission, one O animal | 77 | *0.4%* | 1 | *0.0%* | 0 | *0.0%* | 0 | *0.0%* |
| No transmission, one E animal | 10866 | *49.4%* | 4808 | *21.9%* | 1869 | *8.5%* | 547 | *2.5%* |
| No transmission, one I animal | 6983 | *31.7%* | 6062 | *27.6%* | 2936 | *13.3%* | 1226 | *5.6%* |
| **NO bTB transmission, Total** | **17,926** | ***81.5%*** | **10,871** | ***49.4%*** | **4,805** | ***21.8%*** | **1,773** | ***8.1%*** |
| Transmission, one I animal only | 3472 | *15.8%* | 5551 | *25.2%* | 2990 | *13.6%* | 1318 | *6.0%* |
| Transmission, more I animals | 602 | *2.7%* | 5578 | *25.4%* | 14205 | *64.6%* | 18909 | *86.0%* |
| **bTB transmission, Total** | **4,074** | ***18.5%*** | **11,129** | ***50.6%*** | **17,195** | ***78.2%*** | **20,227** | ***91.9%*** |

Table 3: Possible events in the case of a) No bTB transmission (infected animal remains as occult, becomes exposed, or reaches the infectious state); and b) bTB transmission (one infectious animal or more than one infectious animal)

## 3.5. Discussion

In Spain, even though the bTB eradication program has been implemented at the national level for almost 25 years, the Officially Tuberculosis-Free (OTF) Status is far from being achieved. Given the situation, new strategies for improving the detection of infected herds and then to help to eliminate bTB from those herds, are needed, and for that, knowledge of the dynamics of bTB spread within Spanish herds is essential. However, the long time-scales associated with the disease, the lack of clinical symptoms in infected animals, the ambiguity of the mechanisms of transmission or the effect of varying control policies complicate the study of bTB dynamics (Brooks-Pollock et al., 2014). Because of that, mathematical models have been extensively used for improving our knowledge on bTB transmission and developing evidences that can help decision-making (Álvarez et al., 2014). However, there are factors such as the type of model used and the assumptions made, or the type and quality of the data used to feed models, that have a critical impact on the values of the transmission parameters estimated, and therefore the extrapolation of the results from other studies is not recommended (Álvarez et al., 2014; Bekara et al., 2014). Within-herd transmission dynamics is also influenced by the herd production type or the management practices, and that is why it is essential that parameters are obtained using data from herds that are representative of the bTB context in Spain.

The availability and the quality of data is one of the main limitations when trying to estimate bTB transmission parameters. In fact, data obtained under experimental conditions (Neill et al., 1988, 1989; Costello et al., 1998; Dean et al., 2005) may not be representative of the infection dynamics under natural field conditions. Some authors have based their parameter estimations on data obtained from field studies, but with a low number of observations (Fischer et al., 2005; Pérez et al., 2002; Barlow et al., 1997), which may not reflect the whole complexity and variability of bTB spread among different farms. On the other hand, when local (Bekara et al., 2014; Álvarez et al., 2012a) or national-based data sets are used (O'Hare et al., 2014; Conlan et al., 2012; Kao et al., 1997), they are unlikely to contain the level of detail needed for the accurate estimation of transmission parameters. To overcome those difficulties, we took advantage of the information recorded between 2010 and 2013 in the national BRUTUB database by the Spanish Ministry of Agriculture and Fisheries, Food and Environment, that contained very detailed data of the epidemiological investigations carried out by the veterinary officers. Based on the methodology developed by Guta et al. (2014), we applied a very restrictive selection criteria for a) the inclusion of herds where we had clear evidence that bTB had been introduced through the purchase of infected animals, and b) the exclusion of herds that may have been infected by any other origin. By doing so, we ended up with 22 herds for which we had all the data we needed for the inference of the bTB transmission parameters. They were small to medium size extensive beef herds, located mainly in South-Western Spain. Those are indeed the type of herds that represent the majority of bTB-infected herds in Spain, and the location also coincides with the areas of Spain with the highest risk of infection (Allepuz et al., 2011; García-Saenz et al., 2014). Therefore, they may be considered as representative of the herds affected by bTB in Spain.

In relation to the types of models, different approaches have been used to evaluate within-herd transmission, including deterministic models (Barlow et al., 1997), though in small populations stochastic models are preferred (Vynnycky & White, 2010; Keeling and Rohani, 2008). Transmission parameters for bTB have been also calculated using modifications of the Reed-Frost model (Pérez et al., 2002; Álvarez et al., 2012a), but they imply strong assumptions, for example in relation to the duration of the latent and infectious periods. We developed a stochastic continuous-time compartmental model with gamma distributed occult and exposed period ($SO^nE^mI$), assuming a frequency-dependent transmission, as used in the majority of bTB models, and as recommended by different authors (Álvarez et al., 2014; Smith et al., 2013).

In relation to parameter estimation, to avoid the limitations of deterministic methods, we used an ABC Markov chain Monte Carlo (MCMC) algorithm. As the ABC-MCMC algorithm may result in low acceptance rates when non-informative prior distributions are used, we developed an algorithm that, ensured that the values drawn from the prior distributions for the initiation of the Markov chains were not too distant from posterior values, and that enabled us to improve the computational efficiency.

For the estimation of bTB within-herd transmission parameters, we considered that spread was only the result of the transmission from one or more infected animals introduced into the herd. Although not implicitly stated, that transmission may include not only direct, but also some sort of indirect transmission. We did not consider any external sources of infection such as wildlife reservoirs or spread from neighbouring herds, which have been included in other models (Kao et al., 1997; Brooks-Pollock et al., 2014; O'Hare et al., 2014). However, in the process of selecting the herds to be included in the study, we did exclude the possibility of infection by other sources such as wildlife reservoirs or infected neighbours.

Considering only cattle-to-cattle transmission, our median estimate of $\beta$ for extensively reared beef herds in Spain, was 0.014 newly infected animals per infectious individual per day, equivalent to 5.2 per year. The median transmission coefficient ($\beta$) calculated by Álvarez et al. (2012a) for Spanish beef herds was 2.3, lower than our estimate, however when the improvements introduced in the eradication program in 2006 were taken into account, they observed an increase in the values of $\beta$ for beef to 5.7, much similar to our estimate. Barlow et al. (1997), estimated a $\beta$ value of 2.6 new infections per infectious animal per year, but the value was for a typical dairy herd in New Zealand (200 cattle heads in a pasture-based system). Similarly, Pérez et al. (2002) obtained a $\beta$ value of 2.2 for dairy herds managed in pasture in Argentina. Bekara et al. (2014) reported a median $\beta$ value of 5.16 per year during the stabling period, but only of 0.96 per year during the grazing period. Variations in the transmission coefficient ($\beta$) estimated for the different countries may be explained by differences in the model design and assumptions made, but also by differences in management practices.

Moreover, we observed a wide variation in the median estimates of $\beta$ among the 22 herds included in the study, ranging between 1.8 and 8.3 newly infected animals per infectious cow per year. Although certain variability in the estimations of $\beta$ is described in the literature, such extreme differences are rarely reported.

Variations in $\beta$ estimates among herds do not seem to be related to the size of the herd, but may be the result of other factors such as the implementation of different herd management practices (that may help or prevent the transmission of bTB). Discrepancies in $\beta$ may also be the result of factors related to individual animals. Differences in the infectiousness of the infected animals have been reported: while most individuals seem not to be very infectious, the presence of "super-spreaders" has also been described (Goodchild & Clifton-Hadley, 2001; O'Hare et al., 2014). The level of infectiousness of individual animals may reflect differences in terms of the infective dose of *M. bovis* received or in terms of the immune status of the individuals (Neill et al., 1988; Morrison et al., 2000; Menzies and Neill, 2000; Goodchild and Clifton-Hadley, 2001; Pollock and Neill, 2002). Variations in $\beta$ estimates may also reflect differences in behavior and/or social ranking of infected cattle (some animals, usually those on the top of the social hierarchy, are more curious and dominant than others, increasing the probability of infection by increasing both number and intensity of contacts) (Menzies & Neill, 2000; Goodchild & Clifton-Hadley, 2001). The $\beta$ parameter was by far the most influential parameter in bTB transmission within herds, and therefore the study of the factors, either related to the herd management or related to the individual animals, which influence $\beta$, deserves further attention.

Previous studies evidence a high degree of uncertainty in relation to the duration of the latent period (i.e. from the infection of an animal until it becomes infectious) (Barlow et al., 1997; Goodchild & Clifton-Hadley, 2001; Conlan et al., 2012). Even though we used weakly informative priors for the duration of the latent period (uniform: 2-36 months), we obtained a median latent period of 97 days with a narrow interquartile range (i.e. $25^{th}$ and $75^{th}$ percentiles (IQR), 74 and 164 days, respectively). This result is consistent with those of other models (Barlow et al., 1997; Bekara et al., 2014; O'Hare et al., 2014) and some experimental studies (Neill et al., 1991; Menzies & Neill, 2000), which described the total duration of the latent period ranging between 2 and 9 months. In contrast to other studies reporting latent periods longer than 20 months (Kao et al., 1997; Pérez et al., 2002; Smith et al., 2013), we did not obtain median values above 9 months in any of the herds evaluated. Observed variation in latency may be influenced by the intermittency of shedding, or reflect differences in factors such as the infective dose, the individual host susceptibility or environmental factors (for example housing condition or nutritional status, which may affect the level of stress of animals, which, may in turn, influences immune competence) (Menzies & Neill, 2000; Goodchild & Clifton-Hadley, 2001; Pollock & Neill, 2002).

The *in-vivo* diagnostic tests for bTB are mainly based on the detection of the cellular mediated immune (CMI) response, since it is the predominant mechanism of defence in infected cattle, and antibodies against *M. bovis* are generated only in the more advanced stages of infection (De la Rua-Domenech et al., 2006). However, there is a period between the infection of an animal and the development of a detectable cellular immune response, known as *occult* or *unreactive* period, during which infected animals test negative to the SITT (Vordermeier et al., 2004; De la Rua-Domenech et al., 2006). Even though some models did not consider this occult stage (Pérez et al., 2002; Bekara et al., 2014), we included it, because it influences our capacity to detect bTB-infected animals, and there is a lot of uncertainty about its duration. We estimated a median duration of the occult stage of 12 days (IQR: 9 – 21 days), with very low variability among the 22 herds studied (median values ranging between 11 and 13 days). Although slightly lower, our median estimate of the duration of the unreactive period remains in line with observations reported from experimental studies, which report a period of 3 weeks (Thom et al., 2006), and with the values estimated by Conlan et al. (2012), which calculated a mean duration of 28 days. Differences observed to values reported by Conlan et al. (2012) may be due to the assumed sensitivity of the test and the choices made on priors distribution of the model parameter.

There are numerous factors that may affect the detection of bTB infection by the tuberculin test (reviewed by De la Rua-Domenech et al., 2006), including factors related to the animal (e.g. concurrent infections, immunosuppression post-partum or nutrition deficiencies) and factors related to the test (e.g. failures of the tuberculin or errors in administration or interpretation).

In the advanced stages of bTB infection (generalisation phase), some animals may spontaneously revert to an anergic state in which they would not react to the diagnostic tests measuring the CMI response (i.e. tuberculin test and $\gamma$-Interferon test), although they would potentially be detected by tests that measure the humoral immune response (Domingo et al., 2014; Pollock and Neill, 2002). However, we did not include such a stage in our model because the mechanism of bTB-associated anergy is not well understood and the frequency of this phenomenon is unknown (Pollock and Neill, 2002). Besides, in countries such as Spain, where eradication programs (with regular test and slaughter) have been applied for many years, anergy tends to be less frequent (García-Saenz et al., 2015).

The great variation in the values of the parameters inferred (mainly $\beta$ and the parameters related to the latent period) are partially related to the variability that is to be expected in nature, but also to the uncertainty associated to them. The available information on bTB transmission parameters ($\beta, \alpha, \alpha_1, \alpha_2$) is scarce and compromised by the difficulties in their estimation, as well as the heterogeneity of the methods by which they were obtained. Therefore, further research would be essential for increasing the precision of those estimates, and ultimately, help in the decision-making process. In any case, while for some herds $\beta$ and $\alpha$ estimates were not very informative (evidenced by wide interquartile ranges), for others (i.e., Herds 'ID 19, 8 and 9) their posterior distributions were narrower than the priors, which indicates that data provided additional information and the model allowed us to obtain more accurate estimates of those parameters.

Considering a period between two consecutive tests of 6 months (as in highly prevalent counties), which results in average period for disease spread of around 90 days, the results of our model (given the assumptions) indicate that bTB transmission would not be efficient (mean $R_h$ value of 0.23). In fact, in more than 80% of cases transmission would not occur, and in almost half of the cases, the infected (occult) animal introduced would not even reach the infectious stage. Considering a period between two consecutive tests of 1 year (as for the majority of herds in Spain), which results in average period for disease spread of around 180 days for the spread of bTB, the results of our model indicate that while mean $R_h$ value remains below 1 (0.82), and bTB transmission would occur in approximately half of the cases. Increasing the period between testing to 2 years (as in low-prevalence provinces), which represents an average period for disease spread of around 365 days, would result in mean values $R_h$ clearly above 1 (2.01). In fact, in almost half of the cases $R_h$ would reach values between 2 and 4, and in almost ten percent of cases higher than 4. Even longer periods (testing every 4 years) would result in mean $R_h$ values of 3.47, and bTB transmission would occur in more than 90% of the cases.

Our results indicate that in Spain frequencies of routine SITT testing above once a year would not be effective to control bTB. Even annual testing would result in bTB being transmitted in half of the cases, which would increase the probability of at least one of the infected animals not being detected and preventing the elimination of bTB from the herd. Clearance of bTB from the herds is often a lengthy process that results in serious economic burden for both the farmers and the Public Administration.

Although our estimates of $R_h$ are not directly comparable with the $R_0$ estimates reported by other authors due to the differences in the modelling approach and/or the assumptions made, our findings that when the time between controls is short, the mean value of $R_h/R_0$ remains below 1 coincide with those of other authors. For example, for a period between tests of 6 months, we obtained a mean $R_h$ value of 0.23, while Smith et al. (2013), under the assumption of a test-based culling strategy implemented at 3-month intervals calculated a mean $R_0$ estimate of 0.02. However, they also estimated that, $R_0$ would remain lower than 1 if testing was performed more frequently than every 4 years; and estimated a $R_0$ of only 4.13 without test-based culling 10 years-after the disease introduction (Smith et al., 2013). In contrast, our mean estimate of $R_h$ was 3.5 already with testing every 4 years. On the other hand, Conlan et al. (2012) calculated median $R_0$ estimates of 1.5 in a herd of 30 cattle and 4.9 in a herd of 400 cattle, considering testing every 5 years; and O'Hare et al. (2014), estimated that the within-herd $R_0$ in Great Britain ranged between 1.3 and 1.9 for high-risk areas tested annually and between 0.6 and 1.4 for low-risk areas under quadrennial testing. The observed differences may reflect the impact of the testing frequency, herd management practices and pattern of movements according to the size of the herd and the prevalence of the area.

Even though the sensitivity of the SITT is not 100%, and therefore a small proportion of the infected animals introduced into the herd may be actually exposed or infectious, accounting for that would result in the introduction of much more uncertainty in the parameters estimated. Since only bTB-free herds are allowed to move animals, that all herds are subjected to regular controls for detection of infection, and that all purchased animals are subjected to pre-movement tests, which have very high sensitivity for exposed and infectious individuals, the assumption that that only occult animals were introduced into the herds seems sensible.

Finding the right balance between the capturing the complexity of the biological processes and the computational feasibility of the model is challenging. In general, model complexity involves a trade-off between simplicity and accuracy of the model: adding complexity improves the realism of a model, but, at the same time, it can pose computational problems and instability, and make the model difficult to understand and analyse (Vynnycky and White, 2010). Here, we developed a method to estimate the variability of the transmission parameters for bTB within-herd spread using field data from the Spanish eradication campaign. The results obtained can be used to improve the strategies for both the detection of bTB in infected herds and the elimination of bTB from affected herds. This methodology could be applied for the estimation of the within-herd transmission parameters of other infectious diseases given that a limited number of inputs are available.

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## 3.8. References

Allepuz, A., Casal, J., Napp, S., Saez, M., Alba, A., Vilar, M., Domingo, M., González, M.A., Duran-Ferrer, M., Vicente, J., Álvarez, J., Muñoz, M., Saez, J.L., 2011. Analysis of the spatial variation of Bovine tuberculosis disease risk in Spain (2006–2009). Prev. Vet. Med. 100, 44–52. doi:10.1016/j.prevetmed.2011.02.012.

Álvarez, J., Bezos, J., de la Cruz, M.L., Casal, C., Romero, B., Domínguez, L., de Juan, L., Pérez, A., 2014. Bovine tuberculosis: Within-herd transmission models to support and direct the decision-making process. Res. Vet. Sci. 97, S61–S68. doi:10.1016/j.rvsc.2014.04.009.

Álvarez, J., Pérez, A.M., Bezos, J., Casal, C., Romero, B., Rodriguez-Campos, S., Saez-Llorente, J.L., Diaz, R., Carpintero, J., de Juan, L., Domínguez, L., 2012a. Eradication of bovine tuberculosis at a herd-level in Madrid, Spain: study of within-herd transmission dynamics over a 12-year period. BMC Vet. Res. 8, 100. doi:10.1186/1746-6148-8-100.

Álvarez, J., Pérez, A., Bezos, J., Marqués, S., Grau, A., Saez, J.L., Mínguez, O., De Juan, L., Domínguez, L., 2012b. Evaluation of the sensitivity and specificity of bovine tuberculosis diagnostic tests in naturally infected cattle herds using a Bayesian approach. Vet. Microbiol. 155, 38–43. doi:10.1016/j.vetmic.2011.07.034.

Anderson, R.M. & May, R.M., 1991. Infectious Diseases of Humans: Dynamics and Control. Oxford University Press, New York.

Anon., 2010. Programa Nacional de Erradicación de Tuberculosis Bovina presentado por España para el año 2010 (http://www.mapama.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/pnetb_2010_tcm7-428877.pdf)

Anon., 2013a. SANCO: Working Document on Eradication of Bovine Tuberculosis in the EU accepted by the Bovine tuberculosis subgroup of the Task Force on Monitoring Animal Disease Eradication. Brussels, SANCO/10067/2013.

Anon., 2013b. SANCO: Working Document on Causal Agents of Bovine Tuberculosis.Brussels, SANCO/7059/2013.

Anon., 2015a. Programa Nacional de Erradicación de Tuberculosis Bovina presentado por España para el año 2015-2016 (http://www.mapama.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/pnetb_2015_tcm7-428870.pdf).

Anon., 2015b. Informe final técnico-financiero, programa nacionalde la tuberculosis bovina, año 2015 (http://www.mapama.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/informe_tb_2015_tcm7-428843.pdf).

Aranaz, A., Cousins, D., Mateos, A., Domínguez, L., 2003. Elevation of *Mycobacterium tuberculosis* subsp. *caprae* Aranaz *et al.* 1999 to species rank as *Mycobacterium caprae* comb. nov., sp. nov. Int. J. Syst. Evol. Microb. 53, 1785–1789. doi:10.1099/ijs.0.02532-0.

Bame, N., Bowong, S., Mbang, J., Sallet, G., and Tewa, J., 2008. Global stability analysis for SEIS models with n latent classes. Math Biosci Eng. 5(1): 20-33.

Barlow, N.D., Kean, J.M., Hickling, G., Livingstone, P.G., Robson, a B., 1997. A simulation model for the spread of bovine tuberculosis within New Zealand cattle herds. Prev Vet Med 32, 57–75. doi:10.1016/S0167-5877(97)00002-0.

Beaumont, M.A., 2010. Approximate Bayesian Computation in Evolution and Ecology. Annu. Rev. Ecol. Evol. Syst. 41, 379–406. doi:10.1146/annurev-ecolsys-102209-144621.

Bekara, M.E. l A., Courcoul, A., Bénet, J.J., Durand, B., 2014. Modeling tuberculosis dynamics, detection and control in cattle herds. PLoS One 9, e108584. doi:10.1371/journal.pone.0108584.

Brooks-Pollock, E., Roberts, G.O., Keeling, M.J., 2014. A dynamic model of bovine tuberculosis spread and control in Great Britain. Nature 511, 228–231. doi:10.1038/nature13529.

Conlan, A.J.K., McKinley, T.J., Karolemeas, K., Pollock, E.B., Goodchild, A. V., Mitchell, A.P., Birch, C.P.D., Clifton-Hadley, R.S., Wood, J.L.N., 2012. Estimating the Hidden Burden of Bovine Tuberculosis in Great Britain. PLoS Comput. Biol. 8. doi:10.1371/journal.pcbi.1002730.

Costello, E., Doherty, M.L., Monaghan, M.L., Quigley, F.C., O'Reilly, P.F., 1998. A study of cattle-to-cattle transmission of *Mycobacterium bovis* infection. Vet J 155, 245–250.

De Jong, M.C.M., 1995. Mathematical modelling in veterinary epidemiology: why model building is important. Prev. Vet. Med. 25, 183–193. doi:10.1016/0167-5877(95)00538-2.

De la Rua-Domenech, R., Goodchild, A.T., Vordermeier, H.M., Hewinson, R.G., Christiansen, K.H., Clifton-Hadley, R.S., 2006. Ante mortem diagnosis of tuberculosis in cattle: A review of the tuberculin tests, γ-interferon assay and other ancillary diagnostic techniques. Res. Vet. Sci. 81, 190–210. doi:10.1016/j.rvsc.2005.11.005.

Dean, G.S., Rhodes, S.G., Coad, M., Whelan, a O., Cockle, P.J., Clifford, D.J., Hewinson, R.G., Vordermeier, H.M., 2005. Minimum effective dose of *Mycobacterium bovis* in cattle. Infect. Immun. 73, 6467–6471. doi:10.1128/IAI.73.10.6467.

Diekmann, O., Heesterbeek, J.A., Roberts, M.G., 2010. The construction of next-generation matrices for compartmental epidemic models. J R Soc Interface. Jun 6;7(47):873-85. doi: 10.1098/rsif.2009.0386. Epub 2009 Nov 5.

Domingo, M., Vidal, E., Marco, A., 2014. Pathology of bovine tuberculosis. Res Vet Sci. Oct;97 Suppl:S20-9.

EFSA-AHAW (EFSA Panel on Animal Health and Welfare), 2012. Scientific Opinion on the use of a gamma interferon test for the diagnosis of bovine tuberculosis.EFSA Journal 2012;10 (12):2975 [63 pp.] doi:10.2903/j.efsa.2012.2975.

Fischer, E.A.J., Van Roermund, H.J.W., Hemerik, L., Van Asseldonk, M.A.P.M., De Jong, M.C.M., 2005. Evaluation of surveillance strategies for bovine tuberculosis (*Mycobacterium bovis*) using an individual based epidemiological model. Prev. Vet. Med. 67, 283–301. doi:10.1016/j.prevetmed.2004.12.002.

Feng, Z., Xu, D. and Zhao, H., 2007. Epidemiological models with non-exponentially distributed disease stages and applications to disease control. Bulletin of Mathematical Biology, 69: 1511-1536.

García-Saenz, A., Saez, M., Napp, S., Casal, J., Saez, J.L., Acevedo, P., Guta, S., Allepuz, A., 2014. Spatio-temporal variability of bovine tuberculosis eradication in Spain (2006-2011). Spat Spatiotemporal Epidemiol. Jul;10:1-10.

García-Saenz, A., Napp, S., López, S., Casal, J., Allepuz, A., 2015. Estimation of the individual slaughterhouse surveillance sensitivity for bovine tuberculosis in Catalonia (North-Eastern Spain). Prev. Vet. Med. 121, 332-337. doi: 10.1016/j.prevetmed.2015.08.008.

Goodchild, A.V., Clifton-Hadley, R.S., 2001. Cattle-to-cattle transmission of *Mycobacterium bovis*. Tuberculosis 81, 23–41. doi:10.1054/tube.2000.0256.

Guta, S., Casal, J., Napp, S., Saez, J.L., García-Saenz, A, et al. (2014) Epidemiological Investigation of Bovine Tuberculosis Herd Breakdowns in Spain 2009/2011. PLoS ONE 9(8): e104383. doi:10.1371/journal.pone.0104383.

Hartig, F., Calabrese, J.M., Reineking, B., Wiegand, T., Huth, A., 2011. Statistical inference for stochastic simulation models - theory and application. Ecol. Lett. 14, 816–827. doi:10.1111/j.1461-0248.2011.01640.x.

Heffernan, J.M., Smith, R.J., Wahl, L.M., 2005. Perspectives on the basic reproductive ratio. J R Soc Interface. Sep 22;2(4):281-93.

Huppert, A., and Katriel, G., 2013. Mathematical modelling and prediction in infectious disease epidemiology. Clinical Microbiology and Infection 19 (11): 999-1005.

Ibe, O.C., 2009. Markov Processes for Stochastic Modeling. Elsevier Academic Press.

Kao, R.R., Roberts, M.G., Ryan, T.J., 1997. A model of bovine tuberculosis control in domesticated cattle herds. Proc. Biol. Sci. 264, 1069–76. doi:10.1098/rspb.1997.0148

Keeling, M. J., & Rohani, P., 2008. Modeling infectious diseases in humans and animals. Princeton University Press, New Jersey.

Lloyd, A.L., 2001. Realistic distributions of infectious periods in epidemic models: changing patterns of persistence and dynamics. Theor Popul Biol. 60(1):59-71.

Lloyd, A.L., 2009. Sensitivity of Model-Based Epidemiological Parameter Estimation to Model Assumptions. In: Chowell G., Hyman J.M., Bettencourt L.M.A., Castillo-Chavez C. (eds) Mathematical and Statistical Estimation Approaches in Epidemiology pp 123-141. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-2313-1_6.

Marjoram, P., Molitor, J., Plagnol, V., Tavare, S., 2003. Markov chain Monte Carlo without likelihoods. Proc. Natl Acad. Sci. USA. 100, 15 324–15 328.

doi:10.1073/pnas.0306899100

Manual O.I.E., 2012. Bovine Tuberculosis, in: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 7th Edition, chapther 2.4.6. ISBN 978-92-9044-878-5 Ref: A 203. Available online: http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/.

Menzies, F.D., Neill, S.D., 2000. Cattle-to-Cattle Transmission of Bovine Tuberculosis. Vet. J. 160, 92–106. doi:10.1053/tvjl.2000.0482.

Morrison W.I., Bourne F.J., Cox D.R., Donnelly C.A., Gettinby G., McInerney J.P. and Woodroffe R., 2000. Pathogenesis and diagnosis of infections with Mycobacterium bovis in cattle. Independent Scientific Group on Cattle TB, . Vet. Rec. 146, 236 - 242.

Neill, S. D., Hanna, J., O'Brien, J.J., McCracken, R. M., 1988. Excretion of *Mycobacterium bovis* by experimentally infected cattle. Vet. Rec. 123:340–343.

Neill, S. D., Hanna, J., O'Brien, J.J., McCracken, R. M., 1989. Transmission of tuberculosis from experimentally infected cattle to in-contact calves. Vet. Rec. 124:269-271.

Neill, S. D., O'Brien, J.J., Hanna, J., 1991. A mathematical model for *Mycobacterium bovis* excretion from tuberculous cattle. Vet. Microbiol. 28:103-109.

O'Hare, A., Orton, R.J., Bessell, P.R., Kao, R.R., 2014. Estimating epidemiological parameters for bovine tuberculosis in British cattle using a Bayesian partial-likelihood approach. Proc. R. Soc. B 281.

Pérez, A.M., Ward, M.P., Charmandarián, A., Ritacco, V., 2002. Simulation model of within-herd transmission of bovine tuberculosis in Argentine dairy herds. Prev. Vet. Med. 54, 361–372. doi:10.1016/S0167-5877(02)00043-0.

Pollock, J.M., Neill, S.D., 2002. *Mycobacterium bovis* Infection and Tuberculosis in Cattle. Vet. J. 163, 115–127. doi:10.1053/tvjl.2001.0655

R Core Team, 2015. R: A Language and Environment for Statistical Computing (version 3.2.1). R Foundation for Statistical Computing, Vienna, Austria (2015). http://www.R-project.org/

Plummer, M., Best, N., Cowles, K., & Vines, K., 2006. CODA: Convergence diagnosis and output analysis for MCMC. R news, 6(1), 7-11.

Reviriego Gordejo, F.J., Vermeersch, J.P., 2006. Towards eradication of bovine tuberculosis in the European Union. Vet. Microbiol. 112, 101–109. doi:10.1016/j.vetmic.2005.11.034

Sánchez, J.N., Hudgens, B.R., 2015. Interactions between density, home range behaviors, and contact rates in the Channel Island fox (*Urocyon littoralis*). Ecol. Evol. 5, 2466–2477. doi:10.1002/ece3.1533.

Smith, R.L., Schukken, Y.H., Lu, Z., Mitchell, R.M., Grohn, Y.T., 2013. Development of a model to simulate infection dynamics of *Mycobacterium bovis* in cattle herds in the United States. J. Am. Vet. Med. Assoc. 243, 411–423. doi:10.2460/javma.243.3.411.

Streftaris, G., and Gibson, G. J., 2004. Bayesian inference for stochastic epidemics in closed populations. Statistical Modelling 4(1), 63-75.

Tavaré, S., Balding, D. J., Griffiths, R. C., & Donnelly, P., 1997. Inferring coalescence times from DNA sequence data. Genetics, 145(2), 505-518.

Thom, M.L., Hope, J.C., McAulay, M., Villarreal-Ramos, B., Coffey, T.J., Stephens, S., Vordermeier, H.M., Howard, C.J., 2006. The effect of tuberculin testing on the development of cell-mediated immune responses during *Mycobacterium bovis* infection. Vet. Immunol. Immunopathol. 114, 25–36. doi:10.1016/j.vetimm.2006.07.001.

Toni, T., Welch, D., Strelkowa, N., Ipsen, A., Stumpf, M.P., 2009. Approximate Bayesian computation scheme for parameter inference and model selection in dynamical systems. Interface 6, 187–202.

Vynnycky, E., & White, R., 2010. An introduction to infectious disease modelling. Oxford University Press, New York.

Vordermeier, M., Goodchild, A., Clifton-Hadley, R., De la Rua-Domenech, R., 2004. The interferon-gamma field trial: background, principles and progress. Vet Rec. Jul 10;155(2):37-8.

Vose, D. Risk analysis: a quantitative guide. John Wiley & Sons, 2008.

Wearing, H.J., Rohani, P., Keeling, M.J., 2005. Appropriate models for the management of infectious diseases. PLoS Med. Jul;2(7):e174. Epub 2005 Jul 26. Erratum in: PLoS Med. 2005 Aug;2(8):e320.

Yan, P., and Feng, Z., 2010. Variability order of the latent and the infectious periods in a deterministic SEIR epidemic model and evaluation of control effectiveness. Mathematical biosciences, 224(1), 43-52. https://doi.org/10.1016/j.mbs.2009.12.007.

# Study II

# Chapter IV

## Evaluation of the efficiency of the surveillance for bovine tuberculosis in Spain

In preparation for submission

# 4.

## 4.1. Introduction

Bovine Tuberculosis (bTB) is a chronic infectious disease of cattle caused by any of the mycobacterial species within the *Mycobacterium tuberculosis*-complex (MTC), mainly *Mycobacterium bovis*, but also *Mycobacterium caprae* (Anon., 2013). Besides cattle, bTB may affect other domestic species, mainly goats, and also wildlife, of which wild boars and red deer are the main reservoirs in Spain (De Mendoza et al., 2006). Humans are also susceptible to bTB (i.e. zoonotic TB), which makes the disease a public health concern (Cosivi et al., 1998; Thoen et al., 2010). Traditionally, the major impact of zoonotic TB was considered to fall on low-income countries; while, in high-income countries, with mandatory eradication programmes in place, bTB cases in humans were rare events (Müller et al., 2013; Olea-Popelka et al., 2017). However, recent publications have highlighted that the real burden of zoonotic TB might be underestimated in both developing and developed countries, mainly due to technical constrains in the isolation and differentiation of MTC members (Lombardi et al., 2017; Olea-Popelka et al., 2017; Palacios et al., 2016c).

Given bTB's zoonotic potential and its high economic impact, the objective within the EU countries is the eradication (Reviriego Gordejo & Vermeersch, 2006). In Spain, eradication programs with "test-and-slaughter" strategies have been implemented for decades but, despite the progressive improvements, bTB has not been eradicated yet. To the contrary, bTB herd prevalence has increased in recent years, from 1.3% in 2012 to 2.9% in 2016 (Anon., 2018). Besides, the distribution of the disease is highly heterogeneous, with herd prevalences close to zero in the north of Spain, but as high as 17.1% in the south of the country (Andalusia region).

In this context, the measures being implemented within the eradication program may need to be re-evaluated. Understanding the dynamics of bTB spread within Spanish herds is essential for the design of new surveillance and control strategies that allowed reducing the time needed for the detection of infected herds (Ciaravino et al., 2018). However, the study of bTB dynamics is complicated because of factors such as the long incubation periods, the lack of clinical symptoms in infected animals, or the uncertainty in relation to the mechanisms of transmission, and that is why mathematical modelling offers an alternative option (Brooks-Pollock et al., 2014).

Currently, in Spain, the detection of infected cattle herds relies mainly on the periodic screening of all cattle herds with the Single Intradermal Tuberculin Test (SITT), followed by the culling of positive cattle (i.e., reactors). Herd-testing interval varies between once every two years and twice a year depending on the prevalence in the area where the herd is located.

Regular testing of bTB is complemented by the slaughterhouse surveillance (i.e. meat inspection), as all cattle intended for human consumption undergo routine *post-mortem* examination at the slaughterhouses (Anon., 2018). If lesions compatible with bTB are detected, samples are collected and sent for laboratory confirmation. Moreover, since 2006, the Spanish eradication program has established the need of testing of animals transported to other herds with the aim of preventing the infection of bTB-free herds through cattle trade. Thus, with a few exceptions, all cattle are subject to mandatory SITT testing prior to the movement, which also contributes to the detection of infection in some herds. Therefore, three major components are considered within the bTB surveillance system in Spain: a) routine skin testing, b) slaughterhouse surveillance, and c) pre-movement controls.

While it is clear that those three components contribute to the detection of bTB, their relative contribution has never been evaluated. Therefore, the first objective of the current work was to assess the efficiency of those three components in bTB detection, as well as the overall efficiency of the bTB surveillance system in Spain. To measure the efficiency, we evaluated both the sensitivity of detection (i.e. the probability of detection per year), and the time until detection.

The Spanish eradication program is not applied homogeneously throughout the territory. It allows some variations in the control measures applied (e.g. in the frequency of testing) depending on the bTB prevalence in the area. Therefore, the second objective was to assess how the efficiency of bTB surveillance varied spatially (at province) level, and to estimate whether that intensity of surveillance efforts actually correlated with what was required according to the actual prevalence in the province. Finally, the third objective was to evaluate how the variation of different factors (e.g. in the SITT test sensitivity or frequency of SITT testing), influenced the efficiency of bTB detection.

To carry out those objectives, we modified the model previously developed by Ciaravino and collaborators (2018) for bTB within-herd transmission in Spanish herds, to allow the assessment of the three components of the Spanish bTB surveillance programme.

## 4.2. Materials and Methods

### 4.2.1. bTB within-herd model

Bovine tuberculosis within-herd transmission was simulated using a compartmental stochastic SOEI (Susceptible, Occult, Exposed and Infectious) model (Ciaravino et al 2018). Where, occult animals (O) represented animals that were infected but were not yet detectable by SITT and were not infectious either. Exposed animals (E) represented animals that were infected and were detectable by SITT but were not infectious yet. Finally, infectious animals (I) represented animals that were infected, were detectable by SITT and were also infectious. A homogeneous-mixing model with frequency-dependent (i.e. true mass-action) transmission was assumed.

We assumed that the occult and exposed sojourn states followed the Erlang distribution; thus, the respective O and E compartments were divided into 3 sequential sub-compartments each (m=n=3) (see Ciaravino et al., 2018 for further details), dubbing the model as $SO^mE^nI$ (Fig. 1). Animals susceptible to *M. bovis* (S) become occult (O), through the contact with infectious cattle at a rate $\beta$, the transmission coefficient. Occult cattle become exposed (E), at a rate $\alpha_1$. Exposed animals become infectious and detectable by SITT (I) at a rate $\alpha_2$. Animals are born as susceptible at a rate $\mu$ (Fig. 1).

Infection dynamics were modelled in continuous time (with days as units), using the Gillespie's direct algorithm (Vynnycky & White, 2010; Keeling & Rohani, 2008). At each time step transitions between compartments of the $SO^mE^nI$ model occurred according to the corresponding differential equations (see Ciaravino et al., 2018 for further details). The values of the transmission parameters ($\alpha_1$, $\alpha_2$ and $\beta$) used in the simulations were randomly drawn from the probability distributions for those parameters estimated by Ciaravino and collaborators for Spanish cattle herds (Ciaravino et al., 2018). The within-herd transmission models were built in R version 3.4.3 (R Core Team., 2013). Regardless of the mechanism of introduction of bTB within the herd, infection was assumed to start in a single infected animal (time 0), and the model was run for up to 5 years.

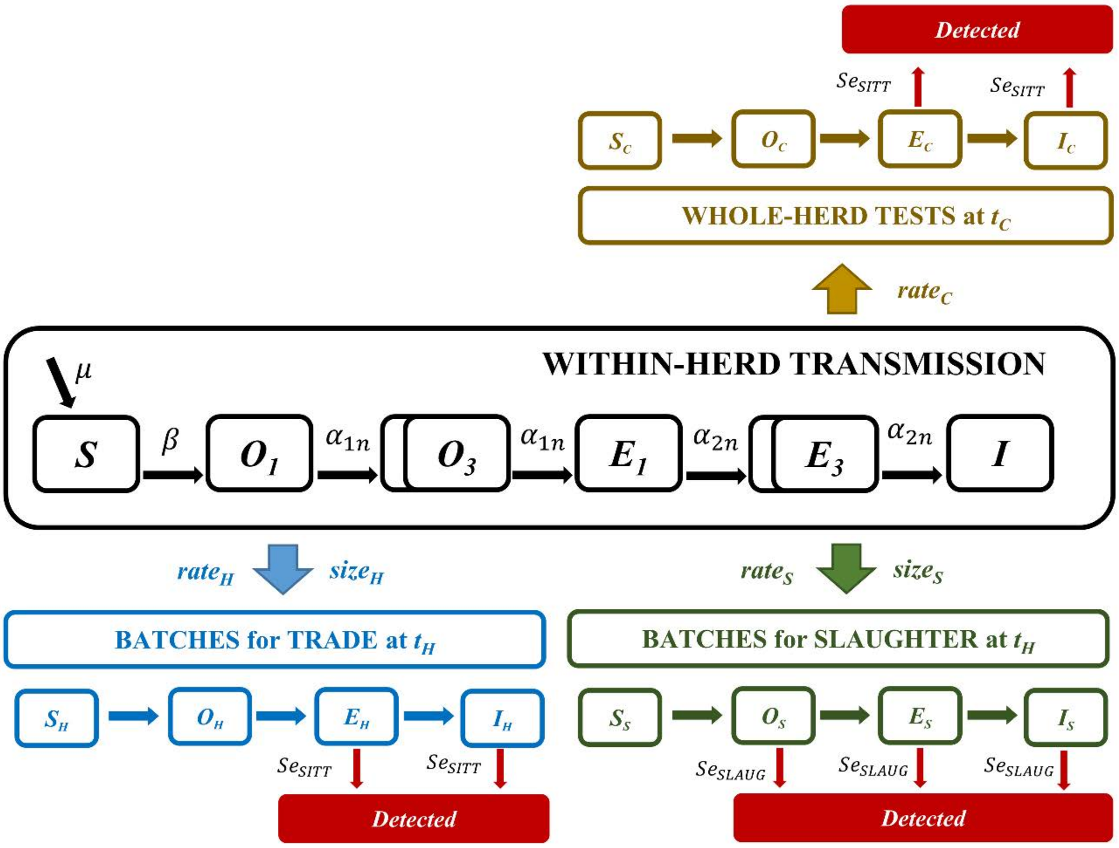


Figure 1: Modification of within-herd transmission model from Ciaravino and collaborators (2018) to include births (with birth rate $\mu$), plus models for the different components of the bTB surveillance system: a) whole-herd tests (i.e. routine testing), b) cattle trade (i.e. pre-movement testing), and c) slaughterhouse surveillance.

### 4.2.2. Modifications to include the different components of the surveillance for bTB in Spain

The within-herd transmission developed by Ciaravino and collaborators (2018) had to be modified to include the different components of the surveillance of bTB in Spain, that is: a) bTB detection at slaughterhouses, b) pre-movement testing, and c) routine testing by SITT (Fig. 1).

Modelling of bTB detection at slaughterhouses

Data on cattle movements to slaughterhouses was provided by the Spanish Ministry of Agriculture, Fisheries, Food and Environment (MAPAMA). Data fed to the model included average number of batches sent to the slaughterhouse per year, as well as average size (number of animals) of those batches for each geographical unit (the whole of Spain for objective 1, and the different provinces for objective 2).

Therefore, given the frequency of movement of animals to the slaughterhouse ($rate_S$, measured in batches transported per year), the time of the first movement of animals to the slaughterhouse ($t_{S_1}$) was simulated. At time $t_{S_1}$, the spread of the disease within the herd determines the number of animals in the different compartments ($S$, $O$, $E$ and $I$). Therefore, the composition of the batch ($S_{S_1}$, $O_{S_1}$, $E_{S_1}$and $I_{S_1}$) will be dependent on the average number of animals per batch ($size_S$) and on the composition of the herd at time $t_{S_1}$.

The probability of detection (at the slaughterhouse) for that batch at time $t_{S_1}$ ($Se_{S_1}$) will be given by the probability that at least one of the infected animals is detected at the post-mortem inspection:

$$Se_{S_1} \sim binomial\,((O_{S_1} + E_{S_1} + I_{S_1}), Se_{slaug})$$

where $E_{S_1}$ and $I_{S_1}$are the number of exposed and infected individuals on the batch sent to the slaughterhouse at time $t_{S_1}$ and $Se_{slaug}$ is the (individual) sensitivity of post-mortem detection at the slaughterhouse.

The value of $Se_{slaug}$ used in the model was 31.4%, which was derived from the study carried out by García-Saenz and collaborators (2015) in North-Eastern Spain (Catalonia). They estimated the individual slaughterhouse surveillance sensitivity for bTB in northern Spain as the consequence of three consecutive steps. First, the probability that a bTB-infected animal arrived at the slaughterhouse presenting bTB-Macroscopically Detectable Lesions (MDL). Second, the probability that MDL, from cattle belonging to bTB negative farms, were detected by the routinely meat inspection procedure carried out in the slaughterhouse. And finally, the probability that the veterinary officer suspected of bTB and sent the sample to the laboratory for confirmation, or notified directly to the authorities.

In the herd, after the movement of a batch of animals to the slaughterhouse, the composition is re-adjusted by subtracting the number of animals in the different compartments in the batch ($S_{S_1}$, $O_{S_1}$, $E_{S_1}$and $I_{S_1}$), from the number of animals in the different compartments ($S$, $O$, $E$ and $I$) in the herd. And if any infected animal ($O$, $E$ or $I$) remains in the herd, within-herd spread is resumed. If further movements to slaughterhouses fell within period considered for the simulation of within-herd spread, the whole process was repeated.

Modelling of bTB detection by pre-movement testing

The Spanish eradication program considers the need to test (by SITT) the cattle before they are transported to other herds, with the aim of preventing the infection of bTB-free herds through cattle movement, although there are a few exceptions to that rule. Data on cattle movements to other herds was also provided by the MAPAMA. Data included the average number of batches transported per year, as well as average size (number of animals) of those batches for each province.

Therefore, given the frequency of those movements ($rate_H$, measured in batches per year), the time of the first movement ($t_{H_1}$) was simulated. At time $t_{H_1}$, the spread of the disease within the herd determines the number of animals in the different compartments ($S$, $O$, $E$ and $I$). Therefore, the composition of the batch ($S_{H_1}$, $O_{H_1}$, $E_{H_1}$and $I_{H_1}$) will be dependent on the average number of animals per batch ($size_H$) and on the composition of the herd at time $t_{H_1}$.

The probability of detection for that batch at time $t_{H_1}$ ($Se_{H_1}$) will be given by:

$$Se_{H_1} \sim binomial\,((E_{H_1} + I_{H_1}), Se_{SITT})$$

where $E_{H_1}$ and $I_{H_1}$are the number of exposed and infected individuals on the batch sent to other herds (and subject to pre-movement tests) at time $t_{H_1}$. And where $Se_{SITT}$ is the (individual) sensitivity of the SITT. The value of $Se_{SITT}$ used in the model was 94%, as in Ciaravino and collaborators (2018).

In the herd, after the movement, the composition is re-adjusted by subtracting the number of animals in the different compartments in the batch ($S_{H_1}$, $O_{H_1}$, $E_{H_1}$and $I_{H_1}$), from the number of animals in the different compartments ($S$, $O$, $E$ and $I$) in the herd. And if any infected animal ($O$, $E$ or $I$) remains in the herd, within-herd spread is resumed. If further movements to other herds fell within period considered for the simulation of within-herd spread, the whole process was repeated.

Routine testing by SITT

In Spain, the majority of infected cattle herds are detected through the periodic screening of all cattle herds with the single intradermal tuberculin test (SITT). The frequency of routine controls varies between once every two years and twice a year depending on the prevalence in the area where the herd is located.

Therefore, given the frequency of routine controls per year ($rate_C$), the time of the first routine control ($t_{C_1}$) was simulated as:

$$t_{C_1} \sim uniform\ (0, 365/rate_C)$$

At time $t_{C_1}$, the spread of the disease within the herd determines the number of animals in the different compartments ($S_{C_1}$, $O_{C_1}$, $E_{C_1}$ and $I_{C_1}$).

Therefore, the probability of detection for that first control ($Se_{C_1}$), which occurs at time $t_{C_1}$ will be given by:

$$Se_{C_1} \sim binomial\ ((E_{C_1} + I_{C_1}), Se_{SITT})$$

where $E_{C_1}$ and $I_{C_1}$ are the number of exposed and infected individuals at the time of the first routine control ($t_{C_1}$). And where $Se_{SITT}$ is the (individual) sensitivity of the SITT. The value of $Se_{SITT}$ used in the model was 94%, as in Ciaravino and collaborators (2018).

The times for the subsequent routine controls followed a regular pattern, where:

$$t_{C_2} = t_{C_1} + (365/rate_C)$$

$$t_{C_3} = t_{C_2} + (365/rate_C)$$

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If further routine controls fell within period considered for the simulation of within-herd spread, the process was repeated.

### 4.2.3. Further modifications of the bTB within-herd model

The inclusion of animal movements to both slaughterhouses and to other herds implies the progressive decrease of the number of animals in the herd. To avoid that, a crude birth rate ($\mu$) term (Keeling & Rohani, 2008) was included in the equation that determines the variation in the number of susceptible animals, which implies that all animals are born as susceptible. Therefore, differential equation for susceptibles is modified to:

$$\frac{dS}{dt} = \mu - \frac{\beta S(t)I(t)}{N(t)}$$

To allow the maintenance of a more or less constant number of animals, $\mu$ was estimated as a function of the number and size of the batches sent to both slaughterhouses and to other herds:

$$\mu = (rate_S \times size_S) + (rate_H \times size_H)$$

### 4.2.4. Evaluation of the efficacy of bTB surveillance

At each iteration, the spread of bTB within herds, as well as movements to other herds or to slaughterhouses, and routine SITT controls, were simulated (Fig. 2), and results of whether detection by any of the components of bTB surveillance occurred, were recorded. Efficacy of surveillance, either of the whole system or the different components individually, was evaluated on the basis of a) probability of detection within a year (i.e. sensitivity of surveillance), and b) time to detection.

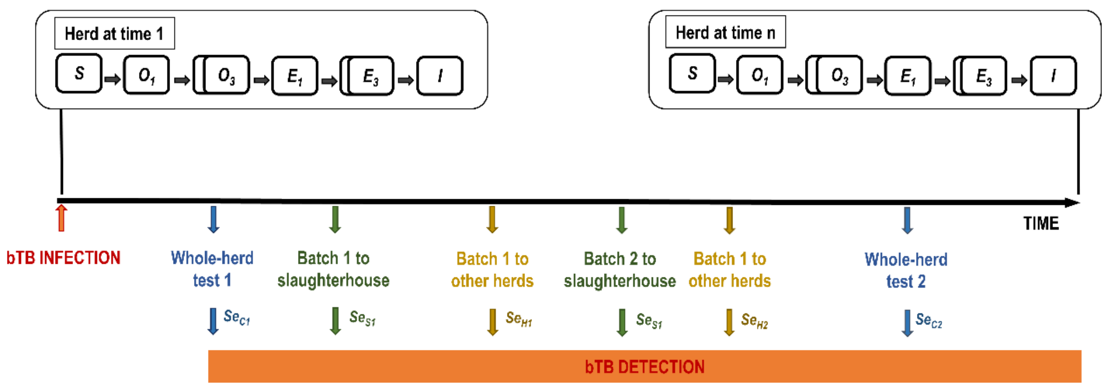


Figure 2: Since the introduction of bTB, infection progresses within the herd. That progress determines the composition of the herd at each point in time, which will influence on the probability of detection by the different components of the bTB surveillance system.

We fed the model with the input data (e.g. frequency and size of movements to slaughterhouses and other herds or frequency of routine SITT controls). When those input values represented the average values for Spain, the efficiency of the whole bTB surveillance system in Spain, as well as the relative contribution of each of the components of the bTB surveillance, could be assessed (objective 1). When those input values represented the average values from different provinces in Spain, the spatial variation in sensitivity of bTB detection among those areas may also be assessed (objective 2). Then, we also evaluated whether the the overall sensitivities of bTB surveillance in the different provinces correlated with the bTB prevalences in those provinces. To allow that comparison, we standardized the overall sensitivities of bTB surveillance and bTB prevalences in the different provinces, by their highest values (i.e. we set overall sensitivities and prevalences in a scale from 0 to 100):

$$Prev.ST_i = \frac{Prev_i}{max(Prev_i)} \times 100$$

$$Se.ST_i = \frac{Se_{ALLi}}{max(Se_{ALLi})} \times 100$$

Then, we estimated, for each province, a parameter that we called discrepancy as:

$$Discrepancy_i = Se.ST_i - Prev.ST_i$$

Finally, by allowing different model parameters to vary within a range of values, we evaluated the influence of those factors on the efficiency of bTB detection (objective 3). The parameters evaluated included:

Sensitivity of the routine test: A value of 94% was assumed for the SITT based on the average value given in the comprehensive review carried out by the European Food Safety Authority (EFSA) (EFSA AHAW Panel, 2012). However, in field conditions, factors such as the difficulties of carrying out the skin tests in extensively-reared cattle (Álvarez et al, 2012a), or the pressure faced by the veterinarians when performing the tests in the presence of farmers (Ciaravino et al., 2017), may result in much lower sensitivities. Therefore, besides the value of 94%, we included another two scenarios in which the sensitivity of the SITT was reduced to 70% and 50%.

In some countries such as the UK, the the single intradermal comparative cervical test (SICCT) is the primary screening test for TB in cattle (De la Rua-Domenech et al., 2006). The SICCT requires the simultaneous injection of bovine and avian tuberculins, and its interpretation is based on the observation that *M. bovis*-infected cattle tend to show a greater response to bovine tuberculin than to avian tuberculin (De la Rua-Domenech et al., 2006). While the use of the SICCT allows to increase the specificity (i.e. reduces the risk of a false positive) as compared with the SITT, it comes at the price of a reduced sensitivity (i.e. increases the risk of a false negative). In fact, an average sensitivity of 61% is attributed to the SICCT considering a severe interpretation, and 49% considering a standard interpretation (EFSA AHAW Panel, 2012).

Frequency of routine controls per year ($rate_C$). In Spain all cattle herds are subject to bTB testing (by SITT) with a periodicity which varies depending on the prevalence in the area where the herd is located. Therefore, we included three scenarios with frequencies of once every two years, once a year and twice a year (i.e. the range of frequencies found in Spain).

For the frequency of cattle movements to slaughterhouses ($rate_S$), the frequency of cattle movements to other herds ($rate_H$), the average size batches to slaughterhouses ($size_S$) and the the average size batches to other herds ($size_H$), three scenarios corresponding to low, medium and high frequency, were considered. They correspond to the 5$^{th}$, 50$^{th}$ and 95$^{th}$ percentiles of the values provided by the MAPAMA for the different provinces (Table 1).

| | **Herd size** | **Routine SITT controls (per year)** | **Cattle movements to slaughterhouses (per year)** | **Average size batches to slaughterhouses** | **Cattle movements to other herds (per year)** | **Average size batches to other herds** |
|---|---|---|---|---|---|---|
| **Average values** (Spain) | 49 | 0.77 | 2.53 | 2 | 0.67 | 4 |
| **Minimum** (province) | 6 | 0.09 | 0,1 | 1 | 0.01 | 1 |
| **Maximum** (province) | 207 | 1 | 61,5 | 4 | 14.2 | 8 |

Table 1: Data inputs for models used for objective 1 (average values for Spain) and objective 3 (average values for Spain plus values for the provinces with the minimum and maximum values). For objective 2, the specific values for the different provinces were used (data not shown).

Therefore, simulations were carried out in the following way:

1 - Input selection: First, a herd of a given size is selected. Then, inputs related to movements to slaughterhouses (rate and size of the batches) are selected. Finally, inputs related to movements to other herds (rate and size of the batches) are selected. For objective 1 (model 1), average values from Spain were always used (see table 1). For objective 2 (model 2), a Spanish province was randomly selected, and the average values for that province were used. For objective 3 (model 3), either average value for Spain or the values for the provinces with the minimum or the maximum values, were randomly chosen (see table 1). For the transmission

parameters ($\alpha_1$, $\alpha_2$ and $\beta$), a value for each of them was randomly selected from their estimated probability distributions (Ciaravino et al., 2018).

2 - Then, the bTB spread as well as movements to slaughterhouses or to other herds are simulated.

3 - Finally, detection by routine SITT, slaughterhouse detection and pre-movement tests are also simulated.

## 4.3. Results

### 4.3.1. The efficiency of the bTB surveillance system in Spain (Objective 1)

By feeding the model with the input data which represent the average values for Spain (table 1), the relative contribution of each of the components of the bTB surveillance in Spain, was assessed (objective 1). The mean sensitivity for the slaughterhouse detection was 8.7%. That means that in Spain herds infected by bTB have an 8.7% probability of being detected by slaughterhouse within one year of infection (table 2). For pre-movement testing, the mean sensitivity in Spanish cattle herds was 11.6%. For routine SITT testing, the mean sensitivity in Spanish cattle herds was 74.2%. The overall sensitivity of the surveillance system for bTB was 79.7%.

| | **Sensitivity (%)** | **First detected by (%)** | **Mean time to detection (days)** | **Not detected within 5 years (%)** |
|---|---|---|---|---|
| **Slaughterhouse detection** | 8.7% | 4.8% | 826.8 | 5.9% |
| **Pre-movement testing** | 11.6% | 7.0% | 825.6 | 11.0% |
| **Routine testing** | 74.2% | 87.2% | 236.2 | 2.1% |
| **Overall bTB surveillance** | 79.7% | NA | 221.6 | 1.0% |

Table 2: Average values of efficiency of bTB surveillance in Spain (objective 1): results for the different components, as well as for the overall surveillance system.

In 87.2% of the times, bTB was first detected by routine testing, in 7.0% by pre-movement testing, in 4.8% by slaughterhouse detection. The mean time to detection was 236.2 days for routine testing, 825.6 days for pre-movement testing, and 826.8 for slaughterhouse detection. Considering the three components, in Spain bTB was detected, on average, 221.6 days after the infection of the herd. The probability of bTB not being detected within the 5-years period considered, was 2.1% for routine testing, 11.0% for pre-movement testing, and 5.9% for slaughterhouse detection. Only 1% of bTB infected herds were not detected by any of the components after 5 years.

### 4.3.2. The efficiency of the bTB surveillance system among Spanish provinces (Objective 2)

By feeding the model with the data which represent the average values for the different Spanish provinces, spatial differences in the efficiency of bTB surveillance in Spain, was assessed (objective 2).

| | | Sensitivity (%) | First detected by (%) | Mean time to detection (days) | Not detected within 5 years (%) |
|---|---|---|---|---|---|
| **Slaughterhouse detection** | *Minimum* | 0.7% | 0.5% | 567 | 22,4% |
| | *Maximum* | 23.3% | 33.4% | 1139 | 76.3% |
| **Pre-movement testing** | *Minimum* | 3.9% | 2.2% | 415 | 17.3% |
| | *Maximum* | 44.0% | 42.0% | 923 | 66.4% |
| **Routine testing by SITT** | *Minimum* | 9.5% | 34.8% | 167 | 0,3% |
| | *Maximum* | 96.0% | 97.3% | 1392 | 15.7% |
| **Overall bTB surveillance** | *Minimum* | 14.5% | NA | 152 | 0,0% |
| | *Maximum* | 96.8% | NA | 837 | 4.6% |

Table 3: Values of efficiency of bTB surveillance in the different Spanish provinces (objective 2): results for the different components, as well as for the overall surveillance system.

<u>Efficiency of the bTB surveillance at provincial level</u>

The values of sensitivity for the different components of the bTB surveillance system varied significantly among provinces. The sensitivity of slaughterhouse detection varied between 0.7 and 23.3% (Table 3; Fig. 3A). Provinces with the highest sensitivities of slaughterhouse detection were located mainly in Central and Northern Spain (e.g. Salamanca, Caceres and Ciudad Real) (Fig. 3A). The sensitivity of pre-movement detection varied between 3.9 and 44.0% (Table 3; Fig. 3B). The provinces with the highest sensitivities were Salamanca, Caceres and Huelva, although in this case there was no clear geogrphical pattern.

The sensitivity of routine testing varied significantly among provinces (Table 3; Fig. 3C), from only 9.5% in Tenerife (a province free of bTB) to more than 96% in Tarragona, and the value was associated to the number of tests per year. In general, overall bTB sensitivity was dependent mainly on the sensitivity of routine testing, although in some provinces (e.g. Salamanca), slaughterhause detection and/or pre-movement testing contributed significantly to the global sensitivity (Table 3; Fig. 3D).

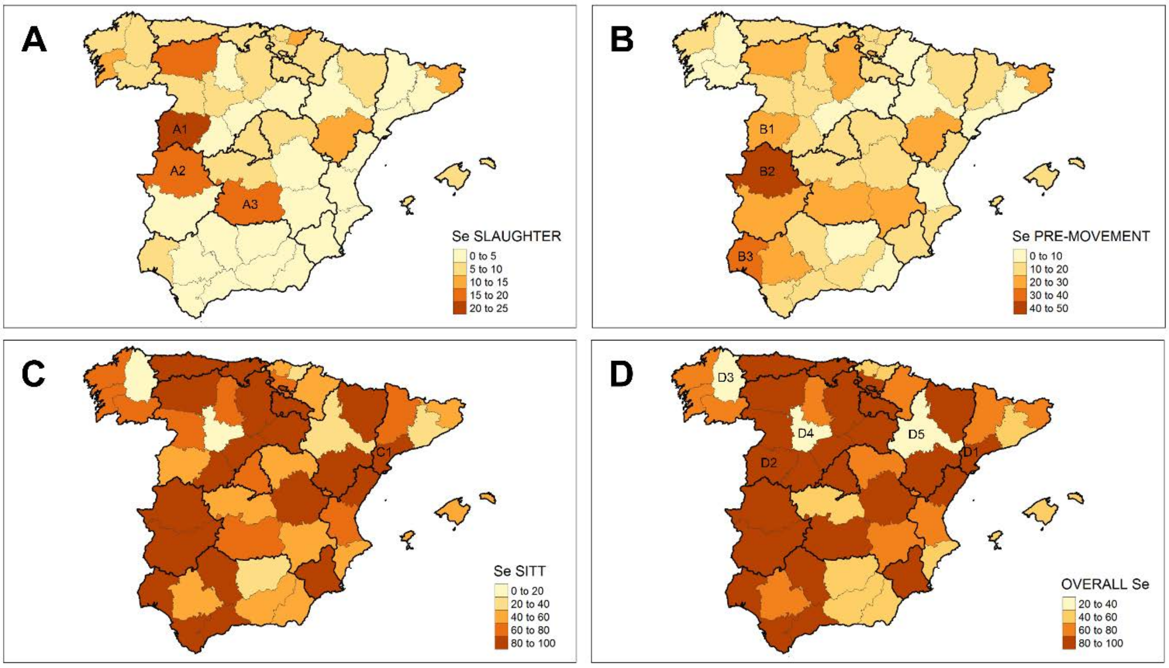


Figure 3: Maps of sensitivities of the whole bTB surveillance system (overall Se) and each of the different components by Spanish provinces. A: Slaughterhouse detection. A1 corresponds to Salamanca, A2 to Caceres and A3 to Ciudad Real. B: Pre-movement detection. B1 corresponds to Salamanca B2 to Caceres and B3 to Huelva. C: Routine SITT test detection. C1 corresponds to Tarragona. D: Whole bTB surveillance system (overall sensitivity). D1 correspond to Tarragona, D2 to Salamanca, D3 to Lugo, D4 to Valladolid and D5 to Zaragoza. ** Be aware that the scales for the maps are different **.

The times to detection of the bTB surveillance also varied significantly among provinces, for both the whole system and for each of the different components (Fig. 4) and, as expected, the times to detection were inversely correlated to the sensitivities of surveillance systems. The average times to detection by slaughterhouse surveillance were above 500 days for all Spanish provinces, although for the majority of them times were above 800 days (Fig. 4A). The average times to detection by pre-movement tests were in the range between 500 and 700 days for the majority of Spanish provinces (Fig. 4B). In relation to routine testing, times to detection were much lower with 32 of the 50 provinces with times below 300 days (Fig. 4C). The average time needed to detection for the overall surveillance system ranged from 152 to 837 days (Fig. 4D). In the majority of provinces, the time remained below 200 days (Fig.4D), although, in one province, Lugo (Fig. 4D), it took up to 700 days, and in two provinces, Zaragoza and Valladolid (Fig. 4D), the time until detection was above 500 days.

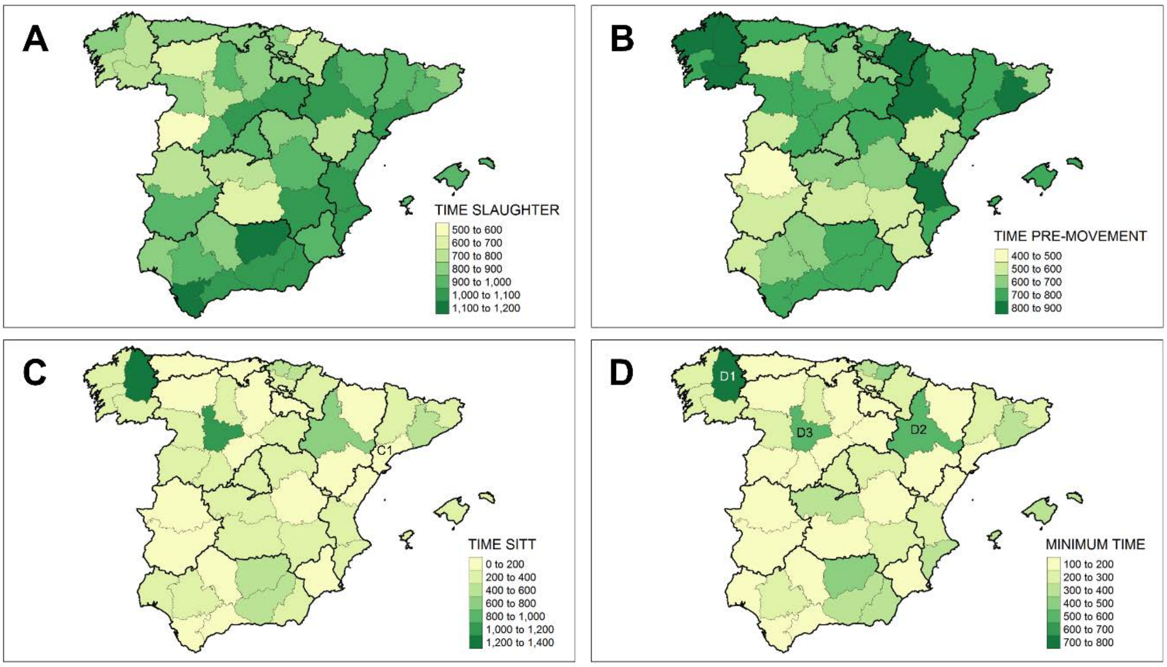


Figure 4: Maps of times to detection for the different components of the bTB surveillance system. A: Slaughterhouse detection. B: Pre-movement detection. C: Routine SITT test detection. D: Whole bTB surveillance system. D1 corresponds to Lugo, D2 to Zaragoza and D3 to Valladolid *Be aware that the scales for the maps are different.

The probability of bTB being first detected by the different components of the bTB surveillance system also varied significantly among provinces. For slaughterhouse inspection it ranged from 0.5 to 33.4% (Fig. 5A), for pre-movement testing it ranged 2.2 and 42.0% (Fig. 5B), and for routine testing it ranged 34.8 and 97.3% (Fig. 5C). The provinces of Lugo (A1, B1 & C1 in Fig. 5) and Valladolid (A2, B2 & C2 in Fig. 5) were characterized by frequent detection by both slaughterhouse and pre-movement tests and infrequent detection by SITT. Variations among provinces in the probability of no detection within 5 years for the different components of the bTB surveillance system are shown in Table 3.

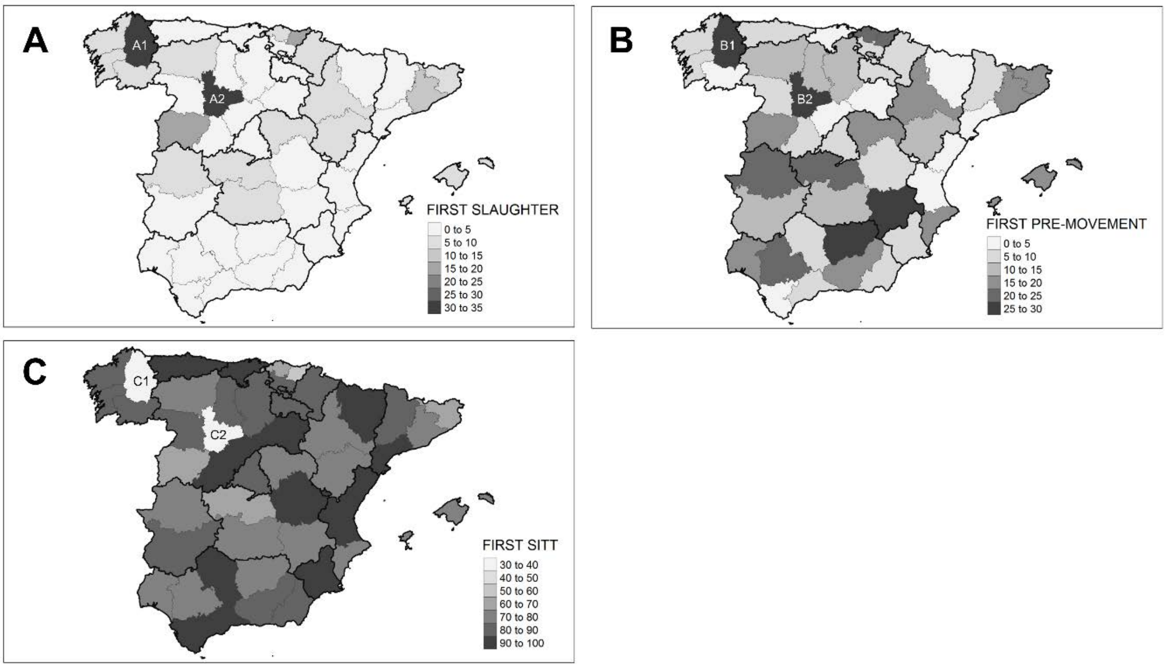


Figure 5: Maps of the proportion of times bTB is first detected by the different components of the bTB surveillance system. A: Slaughterhouse detection. B: Pre-movement detection. C: Routine SITT test detection. D: Whole bTB surveillance system.

Discrepancy between sensitivity of detection and bTB herd prevalence in Spain

We also evaluated whether the intensity of surveillance efforts in each province was adequate to its level of bTB herd prevalence, by calculating the parameter discrepancy. We identified three provinces with values of discrepancy below zero, Guadalajara, Almería and Jaén (Fig. 6). They were the result of the combination of very high prevalences (between 20.5 and 28.6%) with relatively low overall sensitivities (between 41.2 and 68.4%), mainly because of low number of routine SITT controls. There were also provinces with very high values of discrepancy. The clearest example is Tarragona with a discrepancy of 100, as aresult of a very high overall sensitivity (96.8%) although the prevalence for that year was zero.

### 4.3.3. Factors influencing the efficiency of bTB surveillance in Spain (Objective 3)

By changing the values of some of the parameters used in models, we assessed the influence of those factors on the efficiency of the bTB surveillance.

The higher the sensitivity of the diagnostic test is, the higher the sensitivities of both the herd testing and pre-movement detection components are. That resulted in an increase of the overall sensitivity of bTB surveillance and a reduction of the time to detection, from a sensitivity of 74.6% and a time for detection of 227 days for a test-sensitivity of 50%, to a sensitivity of 88.1% and a time for detection of 153 days for a test-sensitivity of 94% (Table 4).

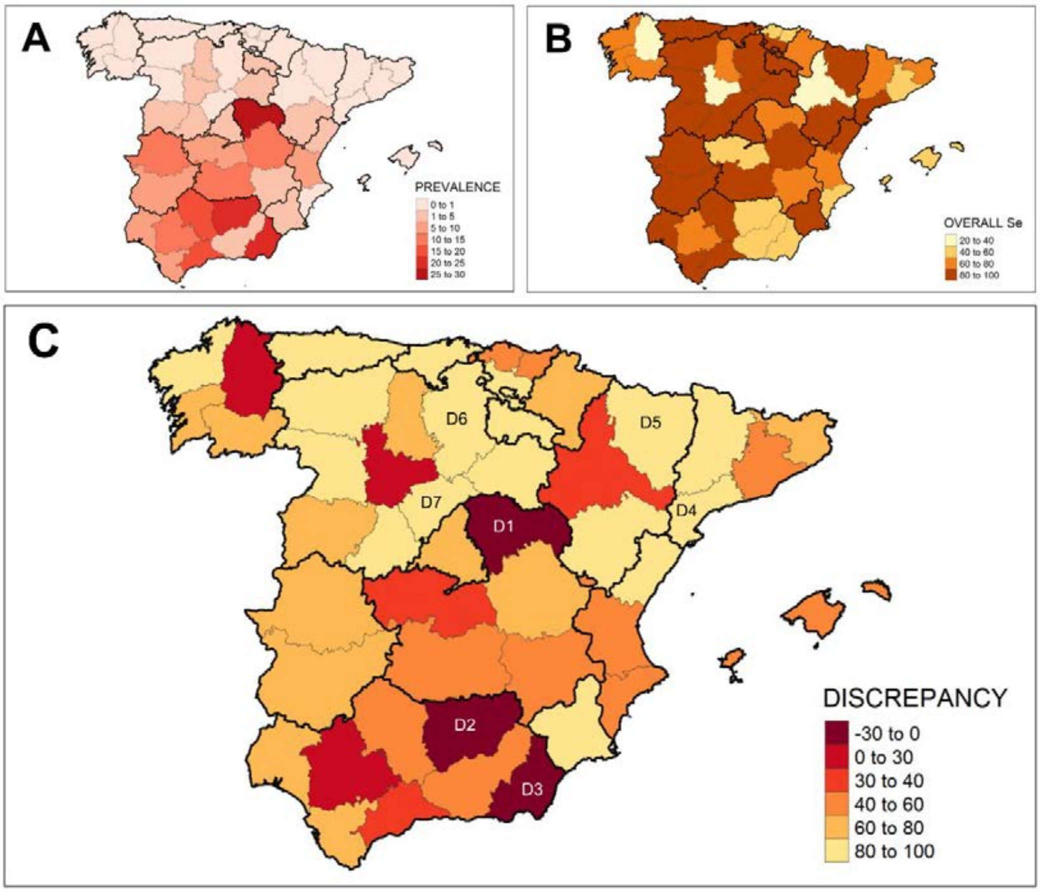


Figure 6: **A:** Map of prevalence. **B:** Map of the overall sensitivity for bTB. **C:** Map of discrepancy. D1, D2 and D3 correspond to the provinces of Guadalajara, Jaen and Almería, respectively. D4 corresponds to Tarragona, D5 to Huesca, D6 to Burgos and D7 to Segovia.

The higher the frequency of routine controls is, the higher the overall sensitivity of bTB surveillance is; so that for one control every 2 years, the overall sensitivity of bTB surveillance was 64.3%, while for one control every half year, the overall sensitivity of bTB surveillance was 93.7% (Table 4). The increase in the frequency of routine testing allows reducing the time to detection, from 280 days with one control every 2 years, to only 115 days with two controls per year.

While an increase in the frequency of cattle movements to slaughterhouses had a large effect on the sensitivity of slaughterhouse detection, the effect on the overall sensitivity of bTB detection was much more limited, resulting in a slight increase (Table 4). On the other hand, an increase in the average size batches to slaughterhouses resulted in a slight increase of the sensitivity of slaughterhouse detection, while it did not seem to have a significant effect on the overall sensitivity of bTB detection. The increase of the frequency of cattle movements to other herds had a very large effect on the sensitivity of pre-movement testing, but also a significant effect on the overall sensitivity of bTB detection (Table 4). The increase in the average size of the batches sent to other herds also resulted in a large effect on the sensitivity of pre-movement testing and a significant effect on the overall sensitivity of bTB detection.

| | Categories | Value | Overall Se ($Se_{ALL}$) | Se specific component |
|---|---|---|---|---|
| **Sensitivity of the routine test** | Minimum | 50% | 74.6% | $Se_{TEST}$=56.0% & $Se_{HERD}$=22.9% |
| | Other (SICCT) | 61% | 79.0% | $Se_{TEST}$=61.6% & $Se_{HERD}$=25.8% |
| | Medium | 70% | 82.0% | $Se_{TEST}$=65.3% & $Se_{HERD}$=28.0% |
| | Maximum | 94% | 88.1% | $Se_{TEST}$=73.9% & $Se_{HERD}$=32.7% |
| **Frequency of the routine controls (per year)** | Minimum | 0.5 | 64.3% | $Se_{TEST}$=35.4% |
| | Medium | 1 | 84.8% | $Se_{TEST}$=70.5% |
| | Maximum | 2 | 93.7% | $Se_{TEST}$=86.8% |
| **Frequency of cattle movements to slaughterhouses (per year)** | Minimum | 0.1 | 80.3% | $Se_{SLAUG}$=0.4% |
| | Medium | 2.5 | 80.1% | $Se_{SLAUG}$=2.4% |
| | Maximum | 61.5 | 82.4% | $Se_{SLAUG}$=58.4% |
| **Average size batches to slaughterhouses** | Minimum | 1 | 81.4% | $Se_{SLAUG}$=16.3% |
| | Medium | 2 | 80.9% | $Se_{SLAUG}$=21.0% |
| | Maximum | 4 | 80.4% | $Se_{SLAUG}$=23.6% |
| **Frequency of cattle movements to other herds (per year)** | Minimum | 0.01 | 75.9% | $Se_{HERD}$=0.2% |
| | Medium | 0.7 | 80.0% | $Se_{HERD}$=23.3% |
| | Maximum | 14.2 | 86.9% | $Se_{HERD}$=59.1% |
| **Average size batches to other herds** | Minimum | 1 | 78.3% | $Se_{HERD}$=13.8% |
| | Medium | 4 | 81.6% | $Se_{HERD}$=30.0% |
| | Maximum | 8 | 82.9% | $Se_{HERD}$=38.3% |

Table 4: Results of the evaluation of the influence of different factors on the efficiency of the bTB surveillance (objective 3). Where SeTEST is the sensitivity of routine testing, SeHERD is the sensitivity of pre-movement testing and SeSLAUG is the sensitivity of slaughterhouse detection.

## 4.4. Discussion and Conclusion

In recent years, the progress towards the eradication of bTB in Spain has had some setbacks, particularly, in some specific regions where the prevalence of bTB has increased significantly. Given such difficulties, there is a need to re-evaluate the strategies currently implemented. Eradication of bTB relies on the timely detection of infected herds. Therefore, in the present work, we evaluated the efficiency of the different components of the Spanish bTB surveillance system, we assessed its spatial variations (at province level), and finally, we evaluated what were the most influential factors in that efficiency.

The evaluation of the relative contribution of the three components of the bTB surveillance system evidenced that in Spain the detection of bTB-infected herds is highly dependent on routine SITT testing. In fact, under the average conditions, the estimated sensitivity of the routine testing was 74.2%, while the overall bTB sensitivity was 79.7%. According to our estimates, almost 90% of the infected herds would be decected by routine testing, and that would take on average 236 days (i.e. 7 months) since their infection. The importance of the routine testing is consistent with the fact that bTB eradication has been achieved in most EU Member States mainly through the regular skin testing of cattle and the elimination of infected animals. In contrast, while slaughterhouse surveillance and pre-movement testing also contributed to detection of bTB infection, their efficiency was much lower, with sensitivity values of 8.7 and 11.6%, respectively, and average times to detection in both cases of more than 27 months.

Contrary to our findings, studies carried out in Belgium (Welby et al., 2012), Denmark (Calvo-Artavia et al., 2013) or Canada (El Allaki et al. 2016), estimated that the slaughterhouse surveillance was highly effective. Several factors might explain these differences. First, the fact that those countries were Officially Tuberculosis Free (OTF), and, therefore, their surveillance systems do not necessary includes the same components. In fact, some studies (El Allaki et al. 2016; Calvo-Artavia et al., 2013) did not consider periodic tuberculin screenings as surveillance components, but only the testing for traded animals; and others (Welby et al., 2012) considered reduced herds screenings. Furthermore, the assumed individual sensitivity of detection at the slaughterhouse ($Se_{SLAUG}$) (i.e., the probability that a randomly selected animal is detected by slaughterhouse surveillance when it is truly infected) differed considerably. Welby and collaborators (2012) assumed for Belgium an individual *post-mortem* sensitivity ranging between 50% and 99%, with a most probable value of 70%, which was much higher than the 31.4% used in our study. This estimate was obtained by

Garcia-Saenz and collaborators (2015) in a study carried out in North-Eastern Spain; the main reason for such a low value was that they estimated that only 44.8% of infected animals arrived to the slaughterhouse with macroscopic lesions detectable by routine meat inspection. That is related to the chronic nature of the disease, and to the fact that in Spain cattle herds are subject to regular controls, and therefore chronic lesions are expected to be less common than in OTF countries.

In agreement with our results, other studies (Fischer et al. 2005; van Asseldonk et al. 2005; Schöning et al. 2013; Rossi et al., 2015) have highlighted the limitations of the slaughterhouses surveillance for bTB detection. However, the comparison with studies carried out in other countries is difficult due to the variability in methodologies applied, and interpretations should take with caution.

Even though we estimated that slaughterhouse detection contributted to the detection of only 4.8% of bTB infected herds, the results of the audit carried out by the European Comission on the progress of the Spanish bTB eradication program indicate that the contribution of the slaughterhouse component may be even lower (Anon., 2016). In 2015, 102 potential cases of animals with compatible lesions found during *post-mortem* inspection in animals from OTF herds, were investigated in Spain, but in only 6 cases of bTB could be confirmed (although some further cases were still pending at the time of the audit) (Anon., 2016). Reasons that may contribute to a low rate of detection include the lack of competence/awareness of meat inspectors and veterinarians or inadequate facilities/conditions (e.g. lighting or line speed) (Garcia-Saenz et al., 2015; Anon., 2013; Hadorn et al., 2008). Moreover, in Spain, a lack of coordination between the authorities responsible for animal health (bTB eradication programme) and the authorities responsible for food safety (slaughterhouse inspection) was pointed out (Anon., 2016), which might also represent a limitation. However, despite its apparent limited contribution to the overall sensitivy of the bTB surveillance system, the slaughterhouse surveillance may play an import role in the detection of “anergic” animals (Domingo et al., 2014). These are cronically infected animals in which cell-mediated immune response may be depressed, and therefore may not be detectable by skin test or gamma-interferon, but which are likely to have developed MDL and therefore be detected by *post-mortem* inspection at the slaughterhouses (De la Rua-Domenech et al., 2006).

With regard to the pre-movement testing, our results suggest that in Spain this component is slightly more sensitive than slaughter surveillance (11.6% vs. 8.7%), although the mean times until detection by either component were quite similar (around 825 days). The proportion of bTB-positive herds first detected by pre-movement testing was higher than those first detected by slaughterhouse surveillance (i.e., 7% and 4.8%, respectively). There are significant differences between these two components in the probability of detection of individual batches, as, according to our estimates 98.2% of infected batches sent to other herds were detected as compared to only 38.6% of infected batches sent to slaughterhouses. That is mainly the result of the huge differences in the probability of detection of infected animals by SITT (sensitivity of 94%) and by slaughterhouse inspection (sensitivity of 31.4%). The difference in the probability of detection of individual batches is partly compensated by the fact that the number of movements (i.e. transported batches) to slaughterhouses is larger as compared to movements to other herds (249,279 and 65,868, respectively, in 2017). Our estimate that pre-movement detection contributted to 7% of the detections of infected herds is slightly higher than the values reported in the DG SANTE document: over 5% in 2012 (53 out of 1010 herds), and nearly 4% in 2013 (39 out of 994) and 2014 (43 out of 1148) (Anon., 2016). One reason for that difference may be that our estimate did not take into account the exceptions in relation to pre-movement testing (i.e. when animals are moved from a herd that have had an OTF status for at least three years and the movement takes place within a geographical unit with annual herd prevalence lower than 1%). Even though some doubts in relation to the cost-effectiveness of this component have been raised, its contribution to prevent bTB transmission, mainly from areas of high bTB prevalence has also been highlighted (Anon., 2016).

Looking at a smaller geographical scale (i.e. provinces), very significant diferences in the efficiency of the different components of the bTB surveillance, as well as of the overall surveillance, were revealed. There was a wide range of variation in sensitivity of slaughterhouse detection (between 0.7 and 23.3%), with higher values in general in Central and Northern Spain (Fig. 3A). Higher values were associated in some cases with large number of movements (e.g. Caceres province), but also to larger sizes of the batches transported to slaughterhouses (e.g. Ciudad Real province). Also, because of the assumption of frequency-dependent transmission, smaller herd sizes (e.g. Salamanca province) resulted in higher probabilities that the batch included an infected animal, and therefore higher sensitivities. In the case of pre-movement detection, the range of variation in sensitivities was even larger (between 3.9 and 44.0%), although in this case no clear spatial pattern was observed. Higher values of sensitivity of pre-movement testing were sometimes associated with large number of movements (e.g. Huelva province). In other cases, high sensitivities were associated with larger sizes of the batches (e.g. Caceres province) or with smaller sizes of the herds (e.g. Salamanca province). In all provinces the routine testing was the most sensitive surveillance component. In general, routine testing sensitivities were relatively high with 42% of provinces with values above 80%, although in some provinces the estimate was lower than 20% (Fig. 3). High sensitivity values were clearly associated with the frequency of testing, but no obvious spatial pattern was identified. The overall sensitivities of the bTB surveillance system in Spanish provinces ranged between 14.5% (Tenerife province) and 96.8% (Cuenca province), and those values were highly associated with those of routine testing, therefore, the spatial pattern in not clear either.

The times to detection by either either slaughterhouse surveillance or pre-movement testing were generally quite large (only below 500 days for pre-movement testing in Caceres province). That evidences that those components alone would imply a long delay between infection of the herd and detection of bTB, during which disease may spread to other herds. The time until detection was generally below 300 days in the majority of provinces. Mainly as a result of the efficiency of routine testing, the times to detection by the whole bTB surveillance system were below 200 days in 44% of provinces, but for example in Tenerife (an OTF region) the value was 837 days.

In some provinces, either detection at the slaughterhouses or by pre-movement test contributted to a significant proportion of the cases detected (Fig. 5). However, that was not necessarily associated to the fact that the efficiency of either component in those provinces was particularly high, but to the fact that the sensitivities of the routine testing component were low. The fact that the distribution of bTB in Spain is highly heterogeneous is widely known (Allepuz et al., 2011; García-Saenz et al., 2014); in fact, the Spanish eradication program is designed to try to account for that heterogeneity (Anon., 2018). However, our results evidence that in many Spanish provinces, the intensity of surveillance efforts in the province were not well correlated to the level of surveillance required according to its prevalence (as measured by the discrepancy parameter). In some provinces with very low prevalences of bTB (e.g. Tarragona, Huesca, Burgos and Segovia) (Fig. 5A), the sensitivity of the bTB surveillance system was very high (Fig. 5B), resulting in very high discrepancy values (Fig. 5C). And more importantly, in some provinces with very high prevalences of bTB (e.g. Guadalajara, Jaen and Almería) (Fig. 5A), the sensitivity of the bTB surveillance system was low or very low (Fig. 5B), resulting in discrepancy values even below 0 (Fig. 5C). Low discrepancy values were always associated to low frequencies of routine controls. For example, in Jaen and Almería the number of SITT controls per year, and therefore the sensitivity of detection is much lower than in the provinces located in Western Andalusia (Fig. 5B), which are the provinces where the prevalence of bTB was traditionally higher (Allepuz et al., 2011). There is no clear spatial pattern in the values of discrepancy, with provinces with low estimates scattered through the different Spanish regions. Even though many of the measures within the Spanish eradication program are implemented at a higher geographical level (i.e. Autonomous Communities), significant differences in the efficiency of bTB surveillance are observed between provinces of the same Autonomous Community. The causes of such differences deserve further investigation. Increasing the flexibility in the allocation of resources for the surveillance of bTB would allow to save resorces where they are less needed and use them where they are more needed, allowing to improve the cost-efficiency of the program and contributing to the eradication of bTB in the long term.

The sensitivity of the SITT has been generally considered as high, in fact in the meta-analysis carried out by the EFSA, the average value was 94% (EFSA AHAW Panel, 2012). However, much lower values have been reported when the SITT is applied in field conditions (Álvarez et al., 2012b; Humblet et al., 2011). Among the reasons for the reduction of the sensitivity is the on-farm testing conditions, in particular for specific productive types, such as in the case of extensively managed bulls from beef herds and fighting bulls (Humblet et al., 2009, Humblet et al., 2011; Álvarez et al., 2014; Meskell et al., 2013; García-Saenz et al., 2014). Also, the pressure linked with the “patronage relationship” between farmers and private veterinarians, because the latter carry out other duties besides bTB testing and are paid by farmers (Ciaravino et al., 2017). Besides, factors related to the professional skills and awareness of veterinarians have also been pointed out (Ciaravino et al., 2017; Meskell et al., 2013; Humblet et al., 2011). There are also differences in sensitivity depending on the type of test selected for routine testing (De la Rua-Domenech et al., 2006). In some countries such as the UK and Ireland, the single intradermal comparative cervical test (SICCT) is the primary screening test for bTB in cattle (De la Rua-Domenech et al., 2006; Frankena et al., 2007). While the use of the SICCT allows to increase the specificity (i.e. reduces the risk of a false positive) as compared with the single intradermal tuberculin test (SITT), it comes at the price of a reduced sensitivity (i.e. increases the risk of a false negative). The sensitivity of the SICCT estimated in the meta-analysis carried out by the EFSA was 61% with the severe interpretation, and 50% with the standard interpretation (EFSA AHAW Panel, 2012).

Our estimates indicate that a decrease in the individual sensitivity of the routine test results in a decrease of the sensitivities of both the pre-movement and routine testing components and, consequently, in a further reduction of the overall sensitivity of the bTB surveillance system. The fact that the overall sensitivity of bTB surveillance decreased from 88.1% to 74.6%, despite a decrease in test sensitivuty from 94% to 50% is because the pre-movement and routine testing are interpreted at herd level. However, given the difficulties in bTB eradication, especially in the later stages, any decrease in sensitivity may compromise the achieviement of the eradication. In fact, in our scenarios, the average time to bTB detection was estimated to be 153 days with with the SITT test (assuming a 94% sensitivity), while in the case of SICCT tests the average time to bTB detection was 204 days (with the severe interpretation) and 227 (with the standard interpretation).

Our results evidence that the frequency of routine controls has a huge effect on the sensitivity of routine testing and, consequently, on the sensitivity of the whole bTB surveillance system. Changing from 2 controls per year to one control every two years would result in a reduction of the overall sensitivity of bTB surveillance from 93.7% to 64.3%, and an increase of the time to detection from 115 to 280 days. That is in agreement with previously reported results (Fischer et al. 2005; Rossi et al. 2015), who observed that variations in the sensitivity of bTB surveillance were primarily due to the frequency of testing.

Routine testing represents a substantial part of the total cost of the Spanish eradication program, 23.7 out of 38.8 million euros (Programa, 2018). However, reducing the frequency of testing would come at the price of a significant reduction of the probability of detecting infected herds and an increase of the time until detection.

Our simulations show that a significant increase on the number of movements to the slaughterhouse (from the lowest to the highest values in the Spanish provinces) would result in an increase of the sensitivity of slaughterhouse detection of 58%, but an increase of the sensitivity of the whole bTB surveillance system of only 2.1%. The assessment of an increase in the size of the batches (from 1 to 4) showed an increase of the sensitivity of slaughterhouse detection of 7%, but did not increase the sensitivity of the whole bTB surveillance system (in fact, a decrease of sensitivity, result of an artifact was observed).

The parameters related to movement to other herds seemed to have a more significant effect. An increase on the number of movements to the other herds (from the lowest to the highest values in the Spanish provinces) would result in an increase of the sensitivity of pre-movement detection of 58.9%, but also an increase of the sensitivity of the whole bTB surveillance system of 11%. And an increase in the size of the batches (from 1 to 8) showed an increase of the sensitivity of of pre-movement detection of 14.7%, and an increase of the sensitivity of the whole bTB surveillance system of 4.6%.

## 4.5. References

Allepuz, A., Casal, J., Napp, S., Saez, M., Alba, A., Vilar, M., ... & Alvarez, J. (2011). Analysis of the spatial variation of Bovine tuberculosis disease risk in Spain (2006–2009). Preventive veterinary medicine, 100(1), 44-52.

Álvarez, J., Perez, A., Bezos, J., Marqués, S., Grau, A., Saez, J. L., … Domínguez, L. (2012a). Evaluation of the sensitivity and specificity of bovine tuberculosis diagnostic tests in naturally infected cattle herds using a Bayesian approach. Veterinary Microbiology, 155(1), 38–43. https://doi.org/10.1016/j.vetmic.2011.07.034

Álvarez, J., Perez, A. M., Bezos, J., Casal, C., Romero, B., Rodriguez-Campos, S., ... & Domínguez, L. (2012b). Eradication of bovine tuberculosis at a herd-level in Madrid, Spain: study of within-herd transmission dynamics over a 12-year period. BMC veterinary research, 8(1), 100.

Álvarez, J., Bezos, J., de la Cruz, M. L., Casal, C., Romero, B., Domínguez, L., … Pérez, A. (2014). Bovine tuberculosis: within-herd transmission models to support and direct the decision-making process. Research in Veterinary Science, 97 Suppl, S61-68. https://doi.org/10.1016/j.rvsc.2014.04.009

Anon., 2013. SANCO WD: Working Document on Eradication of Bovine Tuberculosis in the EU. Accepted by the Bovine tuberculosis subgroup of the Task Force on Monitoring Animal Disease Eradication. Brussels, SANCO/7059/2013.

Anon., 2016. DG(SANTE): Informe final de una auditoría efectuada en España entre el 1 de febrero de 2016 y el 12 de febrero de 2016 con objeto de evaluar la eficacia del programa cofinanciado por la unión europea para erradicar la tuberculosis bovina y los progresos realizados por dicho programa. DG(SANTE) 2016-8771 — MR.

Anon., 2018. Programa Nacional de Erradicación de Tuberculosis Bovina presentado por España para el año 2018. Available online: https://www.mapama.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/sanidad-animal/enfermedades/tuberculosis/Tuberculosis_bovina.aspx

Brooks-Pollock, E., Roberts, G.O., Keeling, M.J., 2014. A dynamic model of bovine tuberculosis spread and control in Great Britain. Nature 511, 228–231. doi:10.1038/nature13529.

Ciaravino, G., García-Saenz, A., Cabras, S., Allepuz, A., Casal, J., García-Bocanegra, I., ... & Napp, S. (2018). Assessing the variability in transmission of bovine tuberculosis within Spanish cattle herds. Epidemics, 23, 110-120. doi: 10.1016/j.epidem.2018.01.003.

Ciaravino, G., Ibarra, P., Casal, E., Lopez, S., Espluga, J., Casal, J., ... & Allepuz, A. (2017). Farmer and Veterinarian attitudes towards the Bovine Tuberculosis eradication Programme in spain: What is going on in the Field?. Frontiers in Veterinary Science, 4, 202.

Cosivi, O., Grange, J. M., Daborn, C. J., Raviglione, M. C., Fujikura, T., Cousins, D., … Meslin, F. X. (1998). Zoonotic tuberculosis due to Mycobacterium bovis in developing countries. Emerging Infectious Diseases, 4(1), 59–70. https://doi.org/10.3201/eid0401.980108.

De la Rua-Domenech, R., Goodchild, A. T., Vordermeier, H. M., Hewinson, R. G., Christiansen, K. H., & Clifton-Hadley, R. S. (2006). Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. Research in Veterinary Science, 81(2), 190–210. https://doi.org/10.1016/j.rvsc.2005.11.005.

De Mendoza, J. H., Parra, A., Tato, A., Alonso, J. M., Rey, J. M., Pena, J., ... & Cerrato, R. (2006). Bovine tuberculosis in wild boar (Sus scrofa), red deer (Cervus elaphus) and cattle (Bos taurus) in a Mediterranean ecosystem (1992–2004). Preventive Veterinary Medicine, 74(2-3), 239-247.

Domingo, M., Vidal, E., & Marco, A. (2014). Pathology of bovine tuberculosis. Research in Veterinary Science, 97 Suppl, S20-29. https://doi.org/10.1016/j.rvsc.2014.03.017.

El Allaki, F., Harrington, N., & Howden, K. (2016). Assessing the sensitivity of bovine tuberculosis surveillance in Canada’s cattle population, 2009–2013. Preventive veterinary medicine, 134, 145-152.

Garcia-Saenz, A., Saez, M., Napp, S., Casal, J., Saez, J. L., Acevedo, P., ... & Allepuz, A. (2014). Spatio-temporal variability of bovine tuberculosis eradication in Spain (2006–

2011). Spatial and spatio-temporal epidemiology, 10, 1-10.

García-Saenz, A., Napp, S., López, S., Casal, J., Allepuz, A., (2015). Estimation of the individual slaughterhouse surveillance sensitivity for bovine tuberculosis in Catalonia (North-Eastern Spain). Prev. Vet. Med. 121, 332-337. doi: 10.1016/j.prevetmed.2015.08.008.

European Food Safety Authority - Panel on Animal Health and Welfare (EFSA AHAW). (2012). Scientific Opinion on the use of a gamma interferon test for the diagnosis of bovine tuberculosis. EFSA Journal 2012;10 (12):2975 [63 pp.] doi:10.2903/j.efsa.2012.2975.

Fischer, E.A.J., Van Roermund, H.J.W., Hemerik, L., Van Asseldonk, M.A.P.M., De Jong, M.C.M., (2005). Evaluation of surveillance strategies for bovine tuberculosis (Mycobacterium bovis) using an individual based epidemiological model. Prev. Vet. Med. 67, 283–301. doi:10.1016/j.prevetmed.2004.12.002

Frankena, K., White, P.W., O'Keeffe, O., Costello, E., Martin, S.W., Grevenhof, E.M., van More, S.J., (2007). Quantification of the relative efficiency of factory surveillance in the disclosure of tuberculosis lesions in attested Irish cattle. Vet. Rec. 161, 679–684.

Hadorn, D. C., & STäRk, K. D. (2008). Evaluation and optimization of surveillance systems for rare and emerging infectious diseases. Veterinary research, 39(6), 1.

Humblet, M.-F., Walravens, K., Salandre, O., Boschiroli, M. L., Gilbert, M., Berkvens, D., … Saegerman, C. (2011). Monitoring of the intra-dermal tuberculosis skin test performed by Belgian field practitioners. Research in Veterinary Science, 91(2), 199–207. https://doi.org/10.1016/j.rvsc.2010.12.004

Keeling, M. J., & Rohani, P., 2008. Modeling infectious diseases in humans and animals. Princeton University Press, New Jersey.

Lombardi, G., Botti, I., Pacciarini, M. L., Boniotti, M. B., Roncarati, G., & Dal Monte, P. (2017). Five-year surveillance of human tuberculosis caused by Mycobacterium bovis in Bologna, Italy: an underestimated problem. Epidemiology and Infection, 145(14), 3035–3039. https://doi.org/10.1017/S0950268817001996

Meskell, P., Devitt, C., & More, S. J. (2013). Challenges to quality testing for bovine tuberculosis in Ireland; perspectives from major stakeholders. Veterinary Record, 173(4), 94-94.

Müller, B., Dürr, S., Alonso, S., Hattendorf, J., Laisse, C. J. M., Parsons, S. D. C., … Zinsstag, J. (2013). Zoonotic Mycobacterium bovis-induced tuberculosis in humans. Emerging Infectious Diseases, 19(6), 899–908. https://doi.org/10.3201/eid1906.120543

Olea-Popelka, F., Muwonge, A., Perera, A., Dean, A. S., Mumford, E., Erlacher-Vindel, E., … Fujiwara, P. I. (2017). Zoonotic tuberculosis in human beings caused by Mycobacterium bovis-a call for action. The Lancet. Infectious Diseases, 17(1), e21–e25. https://doi.org/10.1016/S1473-3099(16)30139-6

Palacios, J. J., Navarro, Y., Romero, B., Penedo, A., González, Á. M., Hernández, M. D. P., ... & Domínguez, L. (2016). Molecular and epidemiological population-based integrative analysis of human and animal Mycobacterium bovis infections in a low-prevalence setting. Veterinary microbiology, 195, 30-36.

Perez-Lago, L. , Navarro, Y. , Garcia-de-Viedma, D. (2014). Current knowledge and pending challenges in zoonosis caused by Mycobacterium bovis: a review. Res Vet Sci, 97, 94-100.

Reviriego Gordejo, F. J., & Vermeersch, J. P. (2006). Towards eradication of bovine tuberculosis in the European Union. Veterinary Microbiology, 112(2–4), 101–109. https://doi.org/10.1016/j.vetmic.2005.11.034

Rossi, G. A. M., de Simoni, H. A. S., Lopes, W. D. Z., de Souza Almeida, H. M., Soares, V. E., Vidal, A. M. C., ... & Mathias, L. A. (2016). Prevalence and geospatial distribution of bovine cysticercosis in the state of Mato Grosso, Brazil. Preventive Veterinary Medicine, 130, 94-98.

Schöning, J.M., Cerny, N., Prohaska, S., Wittenbrink, M.M., Smith, N.H., Bloemberg, G.,Pewsner, M., Schiller, I., Origgi, F.C., Ryser-Degiorgis, M.-P., (2013). Surveillanceof bovine tuberculosis and risk estimation of a future reservoir formation inwildlife in Switzerland and Liechtenstein. PLoS ONE 8(1), e54253.

Thoen, C. O., LoBue, P. A., & de Kantor, I. (2010). Why has zoonotic tuberculosis not received much attention? The International Journal of Tuberculosis and Lung Disease: The Official Journal of the International Union against Tuberculosis and Lung Disease, 14(9), 1073–1074.

van Asseldonk, M.A.P.M., van Roermund, H.J.W., Fischer, E.A.J., de Jong, M.C.M.,Huirne, R.B.M., (2005). Stochastic efficiency analysis of bovine tuberculosis-surveillance programs in the Netherlands. Prev. Vet. Med. 69, 39–52.

Vidal, E., Tolosa, E., Espinar, S., de Val, B. P., Nofrarias, M., Alba, A., ... & Abarca, M. L. (2016). Six-Year Follow-up of Slaughterhouse Surveillance (2008–2013) The Catalan Slaughterhouse Support Network (SESC). Veterinary pathology, 53(3), 532-544.

Vynnycky, E., & White, R., 2010. An introduction to infectious disease modelling. Oxford University Press, New York.

Welby, S., Govaerts, M., Vanholme, L., Hooyberghs, J., Mennens, K., Maes, L., & Van Der Stede, Y. (2012). Bovine tuberculosis surveillance alternatives in Belgium. Preventive veterinary medicine, 106(2), 152-161.

# Study III

## Chapter V

# Farmer and veterinarian attitudes towards the bovine tuberculosis eradication programme in Spain: what is going on in the field?

Front. Vet. Sci., (2017), 4:202

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# 5.

## 5.1. Abstract

The effectiveness of health interventions against bovine tuberculosis (bTB) is influenced by several "*non-biological*" factors that may hamper bTB detection and control. Although the engagement of stakeholders is a key factor for the eradication programme's success, social factors have been often ignored in the control programmes of animal diseases, especially in developed countries. In this study, we used a qualitative approach to investigate perceptions, opinions, attitudes and beliefs of farmers and veterinarians that may influence the effectiveness of the Spanish bTB eradication programme. The study was carried out in two phases. Firstly, 13 key representatives of different groups involved in the programme were interviewed through exploratory interviews to identify most relevant themes circulating in the population. Interviews focused on strong and weak points of the programme; reasons for failure to achieve eradication; benefits of being disease free; future perspectives and proposed changes to the programme. Based on these results, a thematic guide was developed, and detailed information was gained through face-to-face in-depth interviews conducted on a purposive sample of 39 farmers and veterinarians. Data was analysed following an ethnographic methodology. Main results suggested that the bTB programme is perceived as a law enforcement duty without an adequate motivation of some stakeholders and a general feeling of distrust arose. The complexity of bTB epidemiology combined with gaps in knowledge and weak communication throughout stakeholders contributed to causing disbeliefs, which in turn generated different kinds of guesses and interpretations. Low reliability in the routine skin test for bTB screening was expressed and the level of confidence on test results interpretation was linked with skills and experience of public and private veterinarians in the field. Lack of training for farmers and pressure faced by veterinarians during field activities also emerged. Few benefits of being bTB free were perceived and comparative grievances referred to wildlife and other domestic reservoirs, sector-specific legislation for bullfighting farms and the absence of specific health legislation for game hunting farms were reported. Understanding reasons for demotivation and scepticism may help institutions to ensure stakeholders' collaboration and increase the acceptability of control measures leading to an earlier achievement of eradication.

## 5.2. Introduction

The influence of social factors on public health interventions is well known in human medicine and several studies taking these aspects into account have been done (1–3), however these aspects have been often ignored in the implementation of animal health programmes. Recently, the situation has changed and the interest on the influence of social factors in the control programmes of animal diseases has greatly increased. As a matter of fact, several studies have highlighted the importance of understanding the attitudes and behaviours of the different stakeholders involved, as their actions have a great influence on the effectiveness and sustainability of such programmes (4–9).

The use of participatory approaches to investigate attitudes and behaviours is a valuable tool to conduct such studies (6). The fundamental principle of participatory research is that emphasizes "*knowledge for action*" and a "*bottom up approach*" in contrast to conventional research, which is more "*top-down*" (10). The use of such approaches provides a voice to the different stakeholders increasing, in that way, the understanding of health problems and the options for their prevention, control and surveillance (11).

In the last years, different qualitative methods, such as semi-structured interviews, focus group discussions, ranking and scoring methods or visualisation and diagramming, have been used in the field of Veterinary Medicine (6). The increased interest in these approaches has been reflected in an increase in participatory epidemiology (PE) activities in animal health, especially from 2012; however, most of them have been implemented in Asia and Africa but not so much in Europe (12).

The engagement of stakeholders and the level of acceptability of the interventions are key factors for the success of control programmes and surveillance systems (13). The application of qualitative methods can ensure the access to specific type of information and local knowledge otherwise impossible to collect; it can contribute to identifying information gaps, understanding local cultures and beliefs, and setting priorities (11,14). Moreover, it allows investigating risk perception amongst stakeholders and the impact it may have on their response and commitment towards health policies. Finally, since the application of qualitative methods results in a high level of community participation throughout the decision process of designing health interventions, it ensures a more accurate implementation and helps in developing good relationships with communities and in reducing later conflicts.

Bovine Tuberculosis (bTB) in Europe represents a significant obstacle to the sustainability of the livestock sector and since 1964 many efforts have been made to eradicate it (15). Even though, substantial improvement in the prevalence reduction has been achieved, the eradication of bTB remains a challenge. While in some countries such as Germany, The Netherlands and Belgium the eradication campaigns have been successful, in other countries, such as the United Kingdom, Ireland, Italy and Spain, the disease is still endemic. Furthermore, recently the re-emergency of the disease in Officially bTB free (OTF) countries has been reported (16).

In Spain, several aspects of bTB epidemiology have been investigated. In particular, research has been conducted on: spatial and spatio-temporal dynamics of the disease (17–19); risk factors associated with bTB persistence and new infections in cattle herds (20–22); the role of wildlife reservoirs (23–30) and the role of other domestic reservoirs (31–33)In spite of all these studies, no major decrease in the bTB herd prevalence has been observed in Spain over the last decade (1.8% in 2004 and 1.7% in 2014) and, in 2015, the bTB prevalence has increased to 2.8% (34). This context makes it necessary to study other factors that might influence the success of the national bTB eradication programme, such as sociological and anthropological factors that have never been central in such investigations.

In this study, we aim to investigate farmers and veterinarians' perceptions, opinions, attitudes and beliefs about the Spanish bTB eradication programme by using a qualitative approach in order to assess the influence that these aspects may have on the effectiveness of the programme.

## 5.3. Materials and Methods

### 5.3.1 Study areas

The study was carried out in two Autonomous Communities of Spain, Andalusia and Catalonia, as representatives of high and low prevalence areas, respectively (Fig. 1).

In Spain, Regional Veterinary Services (RVS) has been set up in each Autonomous Community under the coordination of the Spanish Ministry of Agriculture and Fisheries, Food and Environment. Moreover, each administrative county has a Local

Veterinary Service (LVS) attached to the RVS. Besides, there are accredited veterinarians working in the field (private sector) that collaborate in carrying out disease prevention programmes. Often, they are also responsible for hygiene, productivity and treatment programmes of the same farms.

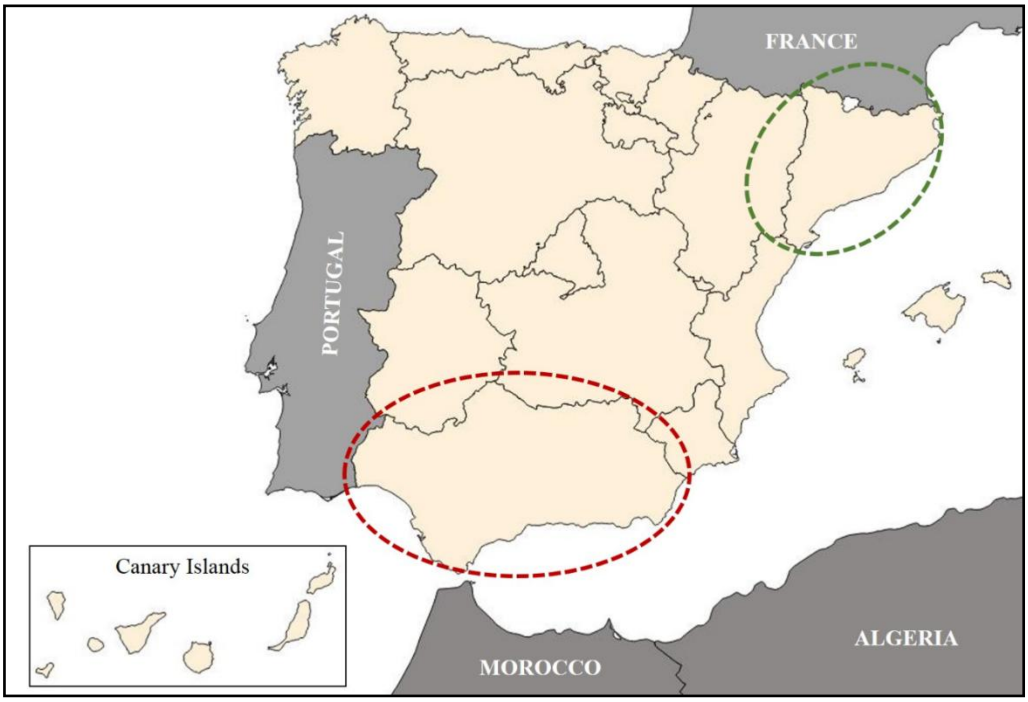


Figure 1: Map of Spain by Autonomous Communities is shown in the figure. Dotted ellipses indicate the two study areas. In red dotted ellipses: Andalusia, high prevalence area. In green dotted ellipses: Catalonia, low prevalence area. The Canary Islands, an Autonomous Community of Spain located in the Atlantic Ocean (west of Morocco), are illustrated in box at the bottom-left corner of the map.

**a) Catalonia**

Catalonia is located on the north-eastern extremity of the Iberian Peninsula; it consists of four provinces and 42 counties. The Autonomous Community can count on 47 official veterinarians working on bTB at the LVS and 113 specialized private veterinarians supporting the routine screening tests for bTB in about 1.900 beef herds, 700 dairy herds and a few bullfighting herds. Since 2008, the bTB herd prevalence at regional level remained lower than 1%, decreasing to 0.04% in 2013, but in 2015 bTB herd prevalence slightly increased to 0.32% (34).

**b) Andalusia**

Andalusia is located in southern Spain and it is divided into eight provinces and 62 counties. There are 63 official veterinarians, operating at the LVS, directly engaged with the bTB eradication programme. These are assisted by about 270 specialized private veterinarians for the implementation of routine screening in about 5.300 beef farms, 800 dairy farms and 400 bullfighting farms. Over last 10 years, herd prevalence for bTB in this region has persisted above 4% and in the last two years has dramatically increased to11% in 2014 and 17% in 2015 (34).

## 5.3.2 Study design

The present study was carried out in two phases, firstly exploratory interviews followed by qualitative in-depth interviews, and it was conducted by a team of veterinarians, sociologists and anthropologists. In both phases, people to be interviewed were selected through a purposive sampling.

In accordance with the national and institutional guidelines, ethical approval was not required for this study as it did not include samples or experiments on people but only their expression of opinions in relation to a specific topic.

With regard to the informed consent of participants: as the interviews were anonymous, the data were analysed anonymously and the decision to participate in the study was solely up to each contacted person, we did not consider it necessary to obtain a written consent. We orally informed all participants of the elements of consent and permission was obtained verbally before starting the interview.

At the beginning of each interview: Interviewers introduced themselves and the contacted person was informed on the study design and its objectives.

It was explained that the participation was voluntary and completely anonymous (data collection and analysis) and that they could stop the interview at any time. It was explained that there were no expected risks and no expected personal benefits associated with participation in the study. We also asked their approval for using information collected through the interview and for using direct quotes from them and these would only be cited as from a “farmer” or “veterinarian”, keeping the anonymity.

### a) Exploratory interviews

The aim of these interviews was to identify major themes to be considered and further investigated in the qualitative in-depth interviews. For that purpose, we used a stakeholder sampling strategy (35) by which we selected a particular segment of the population having concrete experience with the issue at stake (bTB) or being strongly affected by it. The concrete population segments were “farmers” and “veterinarians” of the study areas.

Overall, 13 key representatives were interviewed. In the high prevalence area (Andalusia), three veterinarians of the public sector (one from the RVS, one from the LVS and one from the diagnostic laboratory), two private veterinarians (operating in two different counties) and three farmers, covering the main livestock production types: beef, dairy and bullfighting farms were interviewed. In the low prevalence area (Catalonia), two veterinarians of the public sector (RVS and LVS), one private veterinarian and two farmers (beef and dairy farmers) were also interviewed.

The interviews were conducted face-to-face following a general script in order to allow, as much as possible, open and informal conversations in which key aspects on the bTB management could emerge.

Each interview lasted between 50 and 120 minutes and focused on the following six points: i) Strong points of the bTB eradication program; ii) Weak points of the bTB eradication program; iii) Reasons for the failure of bTB eradication; iv) Future perspectives; v) Proposed changes to the bTB eradication program; vi) Benefits of being bTB free.

Two of the researchers, taking handwritten notes, were present at each interview. After the interview, notes from both researchers were compared in order to transcribe the main arguments expressed. The review of the transcription of the different exploratory interviews was done in different steps. In a first step, the transcription of the exploratory interviews was sent to all the team members (paper’s authors) and then we organized a group meeting where all team members discussed together the results from those interviews. After that, the team of sociologist prepared a first draft of the interview guide for the qualitative in-depth interviews and they send it to all the authors of this paper for the final discussion and agreement.

Interviews in Andalusia were conducted at the beginning of December 2014 (from 1/12 to 11/12), whereas in Catalonia they were performed in two rounds: middle July 2015 (from 17/07 to 22/07) and middle September 2015 (from 15/09 to 21/09).

**b) Qualitative in-depth interviews**

This study phase was aimed at gaining detailed information on the themes that emerged from the exploratory interviews in order to understand perceptions of farmers and veterinarians and their interpretation of problems related to the eradication of the disease in Spain. A “thematic guide” was developed based on previous results and it provided an orienting framework of the different stakeholder groups.

Overall, 14 veterinarians and 25 farmers were interviewed (Table 1), applying a maximum variation sampling strategy in order to identify as many different “speeches” as possible (36). By this way, we aim to sample for heterogeneity in order to understand how bTB was perceived by people holding different social positions in the field. With this strategy in mind, we selected a small number of samples maximizing the diversity relevant to the research question. Diversity was achieved by segmenting the sample (both of farmers and veterinarians) through two key criteria guaranteeing very different daily experiences: territorial criteria (high / low prevalence areas) and type of farming (beef, dairy and bullfighting farmers). By doing so, we obtained a wide spectrum of daily experiences and points of view, enough to 'saturate the discursive space' related to the subject, which is what was intended by our qualitative sampling procedure.

| | **Low prevalence area (Catalonia)** | **High prevalence area (Andalusia)** | **N** |
|---|---|---|---|
| **Farmers (N=25)** | Six beef farmers | Eight beef farmers | 14 |
| | Four dairy farmers | Three dairy farmers | 7 |
| | One bullfighting farmer | Three bullfighting farmers | 4 |
| **Veterinarians (N=14)** | Three veterinarians of the public sector (official vets) | Four veterinarians of the public sector (official vets) | 7 |
| | Three private veterinarians | Four private veterinarians | 7 |
| **Total** | **17** | **22** | **39** |

Table 1: Structure of the sample for the qualitative in-depth interviews

Semi-structured face-to-face interviews, lasting between 90 and 150 minutes, were used for this study phase in order to provide in-depth understanding of the participant’s perspective and, at the same time, to allow all opinions and viewpoints to be brought up during interviews. Only one interviewer was present for each interview (an anthropologist in Andalusia and two different sociologists in Catalonia). Interviews were tape-recorded and transcribed by the team of sociologist and anthropologists.

Prior to the interview, a formal letter (headed by the university logo and signed by the research team) was hand delivered to each interviewee and permission was secured at all levels. Participants were informed about: a) the purpose of the study; b) the research team members and their university department (with the address, telephone and email of the main researcher); c) the freedom to accept or not to do the interview and to withdraw from it at any time; d) the explicit guarantee of anonymity and confidentiality of their personal opinions. Interviews only took place after they were read, and verbal consent was obtained from each participant.

In order to make respondents as comfortable as possible during the interview and encourage them to talk extensively and "freely ramble on", all in-depth interviews started with a few general questions, which respondents could answer easily. These questions were related to their professional career, type of livestock farm, daily working activities (i.e., activities performed in current job position, in the field, in the farms, etc.) and variation in their workday across the year. As the interview progressed, the interviewer gradually introduced new elements in the conversation directing it to more specific and targeted topics.

Interviews in Andalusia were conducted and transcribed between March and October 2015 whereas in Catalonia they were conducted and transcribed between January and June 2016.

To ensure the protection of sensitive data, recordings and transcripts were stored by the research team, and access to them is reserved exclusively for members linked to this research, who have undertaken to maintain the confidentiality and anonymity specified in the mentioned letter. All the real names of individuals and companies, entities or institutions were eliminated in order to ensure anonymity. Instead, an alphanumeric code that identifies each sample was assigned to each interviewed person. Each interviewee was warned that if any of the phrases pronounced during the interview were used to illustrate results in some public document, and that in no case would the person's name be mentioned, but replaced by the mentioned code or attributed to the sample as a whole.

An ethnographic methodology was used in this study. Interview transcriptions were analysed through a method inspired on the grounded theory approach, based on the constant comparisons between data of the whole dataset (of all transcripts) and on the use of a repeated coding, which provided a scheme of the main perceptions, opinions and beliefs circulating in the discourses of the study population (37). The records of the interviews were examined thematically by noting and coding each piece of information in the transcriptions. The coding allowed highlighting all central emerging themes. In relation to the internal reliability, the interviews' transcriptions were compared and discussed between three different members of the research team. Each researcher did it separately, and they met to agree on the relevance of the emerging themes and its interpretation. A single meeting was enough to agree on a common interpretation because there were no major discrepancies.

For each theme that emerged, the most representative sentences were transcribed in their original language (i.e., Spanish or Catalan) and included in the supplementary material. From here onwards in the text, we will refer to each sentence as {Sn}, where "S" means "sentence" and the "n" is an integer number whose value represents the unique identifier of the sentence.

## 5.4. Results

### 5.4.1 Exploratory interviews

Following the general script previously described, the exploratory interviews allowed us to identify the following themes to be further investigated in the second study-phase.

***i) Strong points of the bTB eradication program;***

In general, the programme was perceived as technically correct. The increased implication of veterinary services, the systematic use of the interferon-γ assay (IFN-γ) and the implementation of mandatory training courses for veterinarians (public and private) organized by the Spanish Ministry of Agriculture and Fisheries, Food and Environment were perceived as major improvements of the programme in the last years (Figure 2).

***ii) Weak points of the bTB eradication program;***

Main weak points were related to the communication flow, organizational issues and the suitability of the human and economic resources currently assigned to the programme (Figure 3). Concerns were expressed in relation to the coordination with the labs, the experience of official veterinarians who supervise private veterinarians in performing the single intradermal test (SIT), the lack of homogeneity in the implementation criteria of the bTB eradication programme and the lack of human resources. Interviewees also mentioned that some of the implemented control measures were too restrictive or infeasible.

Some stakeholders reported the comparative grievance that is generated due to the special legislation that is in place for bullfighting herds, as in herds with cattle that is older than 24 months bTB testing is not performed. Moreover, the presence of wildlife and other domestic bTB reservoirs not included in the eradication programme was perceived as a comparative grievance by farmers and contributed to generate uncertainty on the achievement of bTB eradication.

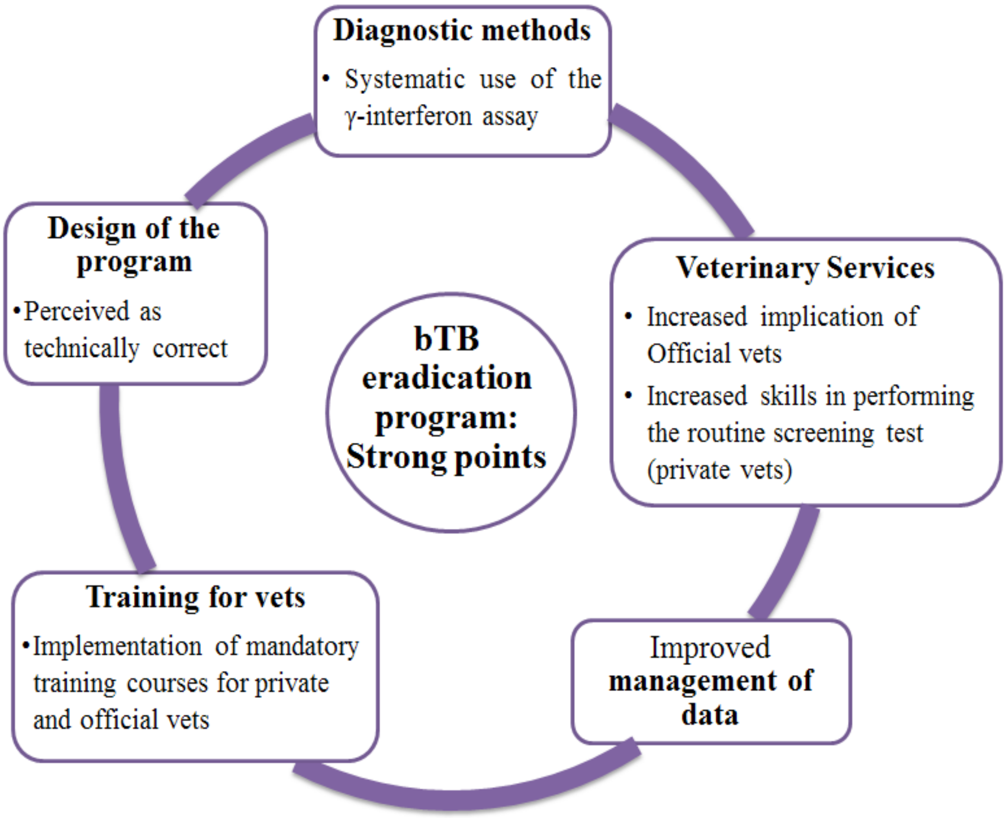


Figure 2: Schematic representations of the main themes emerged from exploratory interviews as "Strong points of the bTB eradication program"; results for Andalusia and Catalonia are presented together.

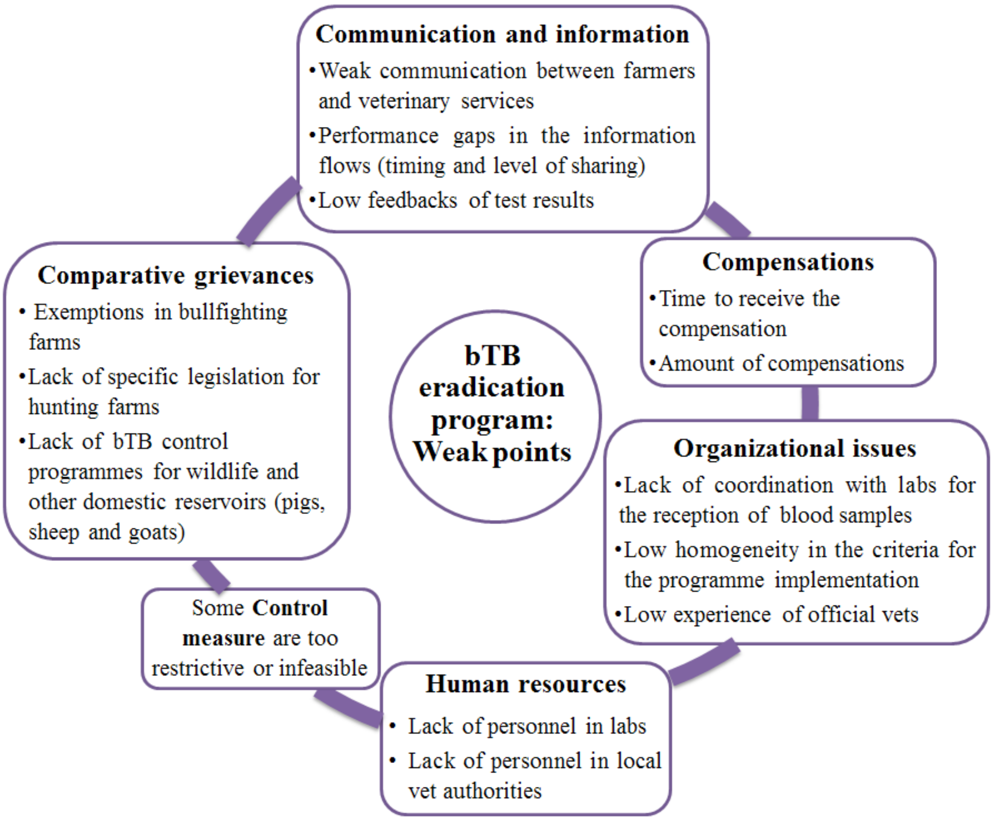


Figure 3: Schematic representations of the main themes emerged from exploratory interviews as "Weak points of the bTB eradication program"; results for Andalusia and Catalonia are presented together.

### *iii) Reasons for the failure of bTB eradication*

Arguments that emerged in this section were related to the lack of confidence in the results of the diagnostic tests, the heterogeneity in the bTB detection capacity among the different slaughterhouses, the relationships among stakeholders and pressures faced by private veterinarians when interpreting the skin test (Figure 4).

The importance of the level of implication of the different actors in the bTB eradication programme (i.e., farmers, private and official veterinarians) and the lack of trust between farmers and official veterinarians were also mentioned.

Moreover, the reason for certain sanitary measures was somewhat unclear or not well understood and the presence of infected wildlife animals was perceived as a major obstacle for the bTB eradication, especially in the south of Spain.

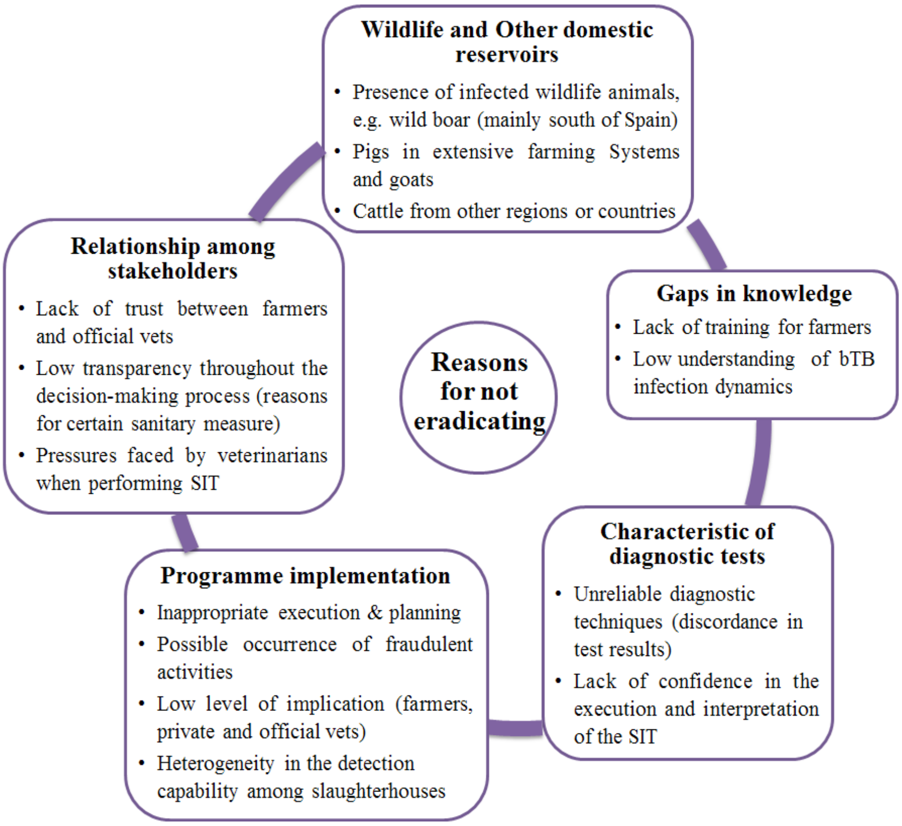


Figure 4: Schematic representations of the main themes emerged from exploratory interviews as "Reasons for the failure of bTB eradication"; results for Andalusia and Catalonia are presented together.

### *iv) Future perspectives*

In this section, very different views were expressed (Figure 5): some people considered that it was at all possible to eradicate the disease and others considered that it will only be possible to maintain a low prevalence.

### *v) Proposed changes to the bTB eradication program;*

The different stakeholders considered that improvements to the bTB programme should focus on training (especially for farmers) and communication. It was also mentioned that measures related to movement restrictions should be relaxed (Figure 5).

### *vi) Benefits of being free of bTB*

Except for some awareness on the potential zoonotic risk of bTB reported from some people, few benefits of being bTB free were perceived (Figure 5). The perceived economic impact of the disease was mainly related to the consequences of animal movement restrictions and, therefore, benefits of being bTB free were mainly related to the reduction of control activities at herd level (i.e., frequencies of routine screening) and the removal of restrictive measures on animal trade.

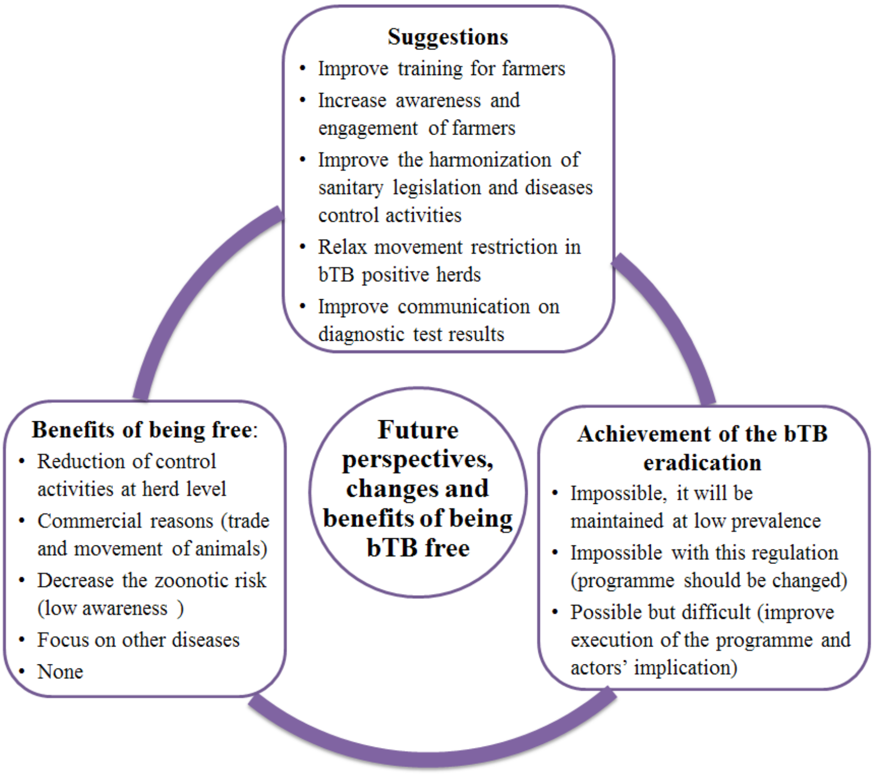


Figure 5: Schematic representations of the main themes emerged from exploratory interviews as "Future perspectives", "Proposed changes to the bTB eradication program" and "Benefits of being bTB free"; results for Andalusia and Catalonia are presented together.

Based on these results, we developed a thematic guide to be used in the qualitative in-depth interviews (Table 2) which outlined the most relevant themes identified and itemized as follows:

i) bTB detection and control (reliability of diagnostic techniques, organization and human resources, measures provided for by the programme).

ii) Training, information and communication (training for farmers and veterinarians, level of implication of different actors and communication and information flows within and between levels and categories).

iii) Role of wildlife and other domestic reservoirs (wildlife reservoir and other domestic species, game hunting areas and farms, specific legislation for bullfighting farms).

iv) Perception of social aspects (i.e., reciprocal relationships among stakeholders).

v) Risk perception on bTB and benefits of eradication (risk perception of economic aspects, such as costs of implementing the programme or direct and indirect losses due to the disease).

vi) Future perspective on the progress of bTB and proposed changes to the programme.

| |
|---|
| **Establishing the first contact: Short opening questions** |
| What is your professional career path? |
| What are your main daily work activities? (i.e., activities performed in current job position, in the field, in the farms, etc.) |
| What is your typical workday like? How does it change throughout the year? |
| What is your experience with the eradication programme? (if not already mentioned) |
| **Topic 1: Evaluation of the bTB eradication program and control measures (adequate, insufficient, excessive or illogical)** |
| Are frequencies of routine bTB screening adequate? |
| Ask about diagnostic test: Reliability of single intradermal test (SIT) and the interferon-γ assay (IFN-γ), differential diagnosis and diagnostic interference with PTB |
| Coordination with labs and availability of diagnostic kits for the interferon-γ assay (IFN-γ) |
| SIT execution: are good practice applied? (i.e., cutimeter use, measure fold, etc.) |
| What do you think about the official controls on the execution of the SIT? (adequate, insufficient, excessive...). Should they be addressed appropriately? How? |
| What do you think about the sector specific legislation for bullfighting herds? (bTB screening exemption for cattle older than 24 months, legal argument that justifies this measure). |
| Do you think that the applied control measures are adequate? Too strict? Are they feasible and applicable? (existence of fraudulent activities, reasons for fraudulent activities to occur, effects of administrative pressures on fraud and motivation) |
| **Topic 2: Other reservoirs** |
| What do you think about the role played by wildlife species in the maintenance of the disease? Is it a real problem or just an excuse? Is the administration doing enough to control and solve this matter? |
| What do you think about hunting areas and activities, hunting farms and the mixed hunting-farming subsistence strategy? |
| What do you think about the role of other domestic species? (sheep, goats and pigs in extensive systems, others…) |
| **Topic 3: Perception on social aspects, management and organizational dynamics** |
| Relationship with other social factors and institutions (dependence, confidence, mistrust and mutual perception): |
| Official and private vets |
| Private veterinarian group (ADGS). |
| Slaughterhouses (evaluation of activities) |
| Farmers and farmers' association |
| Veterinary medicine companies |
| Administration (evaluation of communication and administration operations) |
| What you think about the organization and the mode of operation of the ADGS? |
| Inter and intra-institutional coordination (between different Ministries or between central and local level of the same institution) |
| Implication and transparency of administration (particularly in respect to the diagnostic test results) |
| Information flow and training for farmers and veterinarians (level of dissemination, evaluation of courses and events on bTB, etc. |
| What kind of information, format and method would be the most effective and appropriate to train the different groups about the risk of bTB and its control? |
| **Topic 4: Risk perception on bTB and its economic impact** |
| Do you think bTB can produce direct and indirect losses on production? |
| Do you think bTB can represent a risk for human health? |
| Are the human resources destined to the implementation of the bTB eradication programme adequate? (impact on testing frequencies and test execution) |
| What do you think about the administrative sanctions and their application? Are they adequate? |
| What do you think about the farm subsidies? Are they adequate? May they influence farmers' decision process regarding management of animals and farm's infrastructures? How? |
| What do you think about financial compensation paid to farmers for the slaughter of bTB test-positive cattle? (adequacy of compensation, agility of procedures, etc.) |
| Influence of the farming type and farms' characteristics to the correct implementation of the programme (i.e., difficulties due to the extensive farming system, adequate state of, reluctance among bullfighting farmers to test animals for difficulties in management) |
| **Topic 5: Proposed changes to the programme** |
| What would you change of the bTB eradication programme? |
| Would you improve some control measures already in place? |
| **Topic 6: Future perspective on the progress of bTB** |
| What are main benefits to be bTB free? |
| What do you think on the failure of bTB eradication campaign? |
| Is the failure of bTB eradication mainly due to the persistence or to a continuous spread of the disease? |
| Can the eradication be achieved? How? When? |

Table 2: Thematic guide used in the qualitative in-depth interviews aimed at gaining detailed information on perceptions and opinions of farmers and veterinarians about the bTB eradication programme in Spain.

### 5.4.2 In-depth qualitative interviews

Main results obtained from the ethnographic reports of both areas are described below. Since we did not observe major differences in attitude and opinions between the two study-areas, results are presented together and we report differences when these were identified.

***i) bTB detection and control***

A generalized lack of confidence in the bTB diagnostic tests clearly emerged during the in-depth interviews. Both farmers and veterinarians expressed strong uncertainties on the reliability of test results, although this perception was widespread especially among farmers; so much that some people used the term "lottery" when explaining their perception about test results {S1}. Actually, farmers expressed that they do not want to have any bTB-infected animal in their herd, but that they want to be sure that the test-positive animal is truly infected {S2}.

Uncertainties were mostly associated to the SIT and mainly attributed to the lack of confirmation of positive results and they asked for the application of complementary tests for the verification of the final results {S3}. Reasons provided were the absence of visible lesions in slaughtered animals {S4}, discordance of results between the SIT and the IFN-$\gamma$ {S5} and the use, as screening test, of the SIT instead of the single intradermal comparative cervical test (SICCT), as it could give cross-reactions with paratuberculosis or other environmental mycobacteria {S6}.

Concerns with the existence of false negative results were also mentioned but mainly by the official veterinarians and related with bad practices in the field and erroneous execution of the SIT. This group, more than others, disagreed on the systematic use of the SICCT and defended the use of SIT as the screening test. Even though, they admitted a certain degree of subjectivity in the interpretation of the SIT results and a great influence of the level of experience of the veterinarian in question {S7} emphasising and warning about the importance of the professional training of veterinarians {S8}.

Private veterinarians also highlighted that a correct application of the SIT is not always easy as some cattle are difficult to manage and farms do not always have the necessary infrastructure. The importance of having good infrastructure was highlighted by several interviewed, not only to correctly perform the SIT, but also to prevent veterinarians from risk of injuries and lesions. The lack of support from the official veterinary services to ensure the existence of adequate infrastructures for bTB testing {S8b} was also mentioned.

On the other hand, the IFN-γ was generally perceived as a better diagnostic test than the SIT; thus, its introduction and systematic use was perceived as positive by most of the participants in the study {S9}. Especially, veterinarians highlighted that the IFN-γ is a valid and helpful tool to dispel doubts on diagnostic results {S10} and that it reduces pressure on veterinarians during field activities as it is performed in labs {S11}. However, some concerns were expressed on the IFN-γ regarding the possible existence of false positive animals {S12} and the high cost of this diagnostic test that makes its systematic use not always feasible {S13, S14}. Furthermore, the difficulties of sending blood samples to the laboratory on time from remote areas and the lack of support from the labs {S15} were also reported. Finally, another issue mainly expressed by private veterinarians and farmers was the over-saturation of some laboratories and the consecutive delay in receiving the results due to the lack of coordination {S16}; on their side, official veterinarians acknowledged that organizational problems have happened in some occasions due to the lack of enough personnel in the lab. Lack of enough human resources for bTB activities was also related to a deficient post-mortem inspection in the slaughterhouses or field activities supervision {S17, S18}.

Another important issue that emerged in relation to the perception of the diagnostic techniques as unreliable was the lack of understanding of test results (e.g., doubtful results in animals around one year of age). Both farmers and private veterinarians mentioned experiences with doubtful results that nobody has been able to explain and clarify {S19, S20} and they asked for further investigation and more efficient dissemination of information {S21}.

In the last years, official veterinarians were in charge of supervising the performance of the skin test done by private veterinarians. This has generated some conflicts as some private veterinarians consider that the official veterinarians who have to supervise them do not always have sufficient experience {S22}. Furthermore, the eradication programme in areas of high prevalence (as is the case of the south of Spain) has established a stricter lecture of the SIT in infected farms by which doubtful results are considered as positive. This measure has not been well accepted by the interviewed farmers and private veterinarians who would wish to verify positive results {S23}, whereas official veterinarians do think that it is a good change that will benefit the eradication program.

The screening intervals set by the bTB eradication programme for routine testing were considered functional and adequate by official veterinarians and most of private veterinarians and farmers, albeit they asked for more coordination among different sanitary controls to avoid generating stress in animals and workers {S24}.

Only in certain rural areas of Andalusia, the implementation of two screening round per year was perceived as excessive, especially by farmers, due to the difficult management of beef cattle in extensive farming systems. In addition, farmers expressed the management difficulties that they face during the bTB testing, especially in those farms with extensive managements or in bullfighting farms {S25}. Direct loses due to abortions, work hours, injured animals and decrease in milk production were mentioned as a major issue related with bTB testing, especially in those infected herds subjected to a high frequency of tests.

Some criticisms were reported in Andalusia with regard to the sector-specific legislation for bullfighting cattle farms (bTB screening exemption for cattle older than 24 months), although different points of views were expressed {S26-S28}. Some interviewees considered that no exceptions should be allowed with bullfighting animals, while others justified this measure and evaluated it as reasonable on the basis of their difficult management, the risk of injuries in animals of high value or changes in their behaviour making them unfit for bullfighting {S29}. However, even within the group of farmers that agree with the exemption of bTB testing, not everyone agreed with the argument of difficult management as still these animals are subjected to other health measures (such as vaccination or deworming). The high genealogical value of bullfighting animals and the economic difficulties that the sector is going through were considered as more relevant for these persons.

In relation to the control measures provided by the program, the huge economic consequences derived from movement restrictions was mentioned, especially for those farms without infrastructures for fattening animals. This measure was perceived as too restrictive and as the origin of fraudulent activities. Nevertheless, in the last years, farmers have been allowed to send these animals to specific fattening units; a measure that has been positively received, despite that calves are sold at a lower price {S30}.

***ii) Training, information and communication***

An improvement in the application of the bTB programme in the last years was highlighted and mainly attributed to the organization of mandatory training courses. Both official and private veterinarians acknowledged that some bad practices in the field were largely caused by a lack of knowledge and training among veterinarians {S31}.

Official and private veterinarians also expressed the importance of organizing such activities also for farmers, ensuring that they could have access to all the available information by increasing awareness and knowledge on the diseases as well as on its impact to the farm {S32, S33}. Some of the interviewees also emphasised the importance of training for farmers in order to improve the understanding of sanitary measures provided for the bTB eradication programme and increase its acceptability {S34}.

Among farmers, the lack of understanding of test results and control measures gave rise to some disbelief and to different guesses, as for example that a high mutability rate of the Mycobacterium invalidates the diagnostic tests and that bTB is just an excuse to reduce the cattle population in Southern Countries {S35}.

It was not clear which should be a more efficient way to deliver such training as some people expressed concerns due to the high number of courses that are already organized for farmers {S36} and a lack of motivation in relation to animal health by some of them {S37}. Among the different stakeholders, private veterinarians were identified as one of the more adequate professionals to inform farmers and raise their awareness on the disease, as they are the ones that usually inform farmers on other matters {S38}.

In relation to the effectiveness of communication between stakeholders, different opinions were reported. On the one hand, some farmers expressed the lack of meeting places to exchange information and to express doubts and concerns on the disease and its control. As a matter of fact, most times they have learnt about the bTB eradication programme and changes in the regulation by talking to other farmers in the bars {S39, S40}.

On the other hand, some other farmers expressed that the communication through their private veterinarian group (ADGS) was good enough and they were informed of any changes through them {S41}. Most of the farmers also reported that they would prefer attending informative days about specific issues rather than formal courses and that it would be preferred to organize these meetings during animals' markets.

Regarding the communication of bTB test results, differences emerged between the two study areas. In Catalonia it was described by farmers and veterinarians as adequate {S42, S43}; while in Andalusia a general perception of low feedbacks on test results was reported and both farmers and private veterinarians demanded easier and more flexible procedures to get all needed information on lab results {S44, S45}, results of the post-mortem inspections and the cultures {S46}.

***iii) Role of wildlife and other domestic reservoirs***

The existence of bTB wildlife reservoirs was mentioned as a major obstacle for bTB eradication in Andalusia and Catalonia, but was especially highlighted in those areas with high prevalence and extensive herd management in Andalusia. Different opinions on the role of wildlife reservoirs arose; some people attributed a secondary role in the maintenance of the disease to these species while others were of the opinion that wildlife reservoirs could represent a primary source of infection for cattle {S47-S48}.

In general, controlling bTB in these animals was perceived as a very difficult task and several people expressed the hope of having a vaccine in the future to control the disease in these animals. The development of biosecurity plans to reduce the risk of transmission from wildlife to cattle was also mentioned. However, different views were expressed and some people considered it possible whereas others considered it impossible to prevent cattle and wildlife interaction {S49}.

Other factors that in the opinion of some people increased the risk of bTB transmission was related to hunting activities and the lack of biosecurity, as different groups of dogs, vehicles, people, etc., interacted with infected wild animals and could spread the disease to other places {S50}. In this regards farmers and veterinarians agreed on asking for more controls in wildlife, especially in hunting farms as they are managed as livestock farms {S51-S52}.

Several interviewees negatively perceived the supplementary feeding for hunting purposes, as it was linked to an increase of wildlife population and as a consequence an increased risk of infection for cattle herds. Moreover, the economic benefits provided by hunting activities was suggested to lead to the establishment of several mixed farms (wildlife and cattle) therefore increasing the risk of bTB transmission. In this sense the importance of the coordination between the different governmental statements responsible to manage animal health and the environment was highlighted {S53}.

In relation to other bTB domestic reservoirs not subjected to any control programme, the potential role of goats, sheep and extensively reared pigs (the latter particularly in Andalusia) was mentioned. The interviewees reported that sharing pasture by cattle and these other domestic reservoirs poses another risk of infection for cattle and complained about the lack of specific legislation for this matter.

***iv) Perception on social aspects***

Although the relationship between farmers was considered good, bTB was described as a sensitive issue that is normally avoided in their talks. In some occasions conflicts between neighbouring farmers were generated to the perception that the adjacent farm was responsible for the bTB infection of the herd as the neighbouring farmer has not complied with the eradication programme and has been the source of the outbreak {S54, S55}.

The relationship between farmers and private veterinarians was described as good as in general, it is an enduring relationship and farmers tend to have a very high confidence on them {S56}. However, the existence of a "patronage relationship" between some farmers and private veterinarians was also mentioned, because private veterinarians conduct in the farm other duties than only the bTB testing that are paid by farmers. This fact could generate pressure on private veterinarians, which might not always act with professionalism as could be strongly influenced by the consequences for farmers due to the bTB control measures and for the fear of losing "customers" {S57}. In this regard, some of interviewees also mentioned that sometimes the pressure faced by veterinarians generated conflicts, as the most rigorous veterinarians were not well accepted by all farmers {S58, S59}. In this sense, to have a greater support from the official veterinary services was perceived as a way to reduce pressure to private veterinarians {S60}.

There were different opinions about the relationship between official veterinarians from Local Veterinary Services and private veterinarians and farmers. Some people reported to have a close and effective relationships and a good coordination with them, despite official veterinarians have the role to control and inspect them {S61, S62}. Others described the relationship as tense and of mutual mistrust. Main reason for this difficult relation was due to the perception of fraudulent activities with bTB testing.

The existence of fraudulent practices was acknowledged by some farmers, however, they also argued that, even though not all farmers act the same, they are all treated the same way, and they perceive that the official veterinary services are treating all of them as “delinquents” {S63, S64}.

Concerning the fraudulent practices, the missed communication of animals with doubtful test results and the non-rigorous reading of the SIT were the most reported by both farmers and veterinarians {S65, S66}. These behaviours contributed to generate demotivation especially among farmers but also among veterinarians {S67, S68}

***v) Risk perception on bTB and benefits of eradication;***

Some differences arose between groups on the perceived burden of the bTB. Official and private veterinarians acknowledged both the health and the economic impact of the disease. They emphasized that animal health is the base of the development of the livestock sector and it is fundamental to an efficient animal production and, therefore, to food security and human health {S69}. The group of veterinarians expressed the need to eradicate bTB also because it represents a public health problem, not only because of the obvious trade benefits and because of the positive repercussions on animal health {S70}.

On farmer’s point of view, bTB is not seen as an important animal health problem. Most of the farmers perceived that benefits of eradication were mainly commercial, as bTB was not considered having an impact on public health neither a disease causing production losses. The fact that the meat from infected animals can be passed as ‘fit for human consumption’ after the removal of the affected tissue (unless the carcass is generally emaciated and the lesions are generalized) generated doubts about the public health implications of bTB {S71 - S73}. Moreover, they strongly disagreed that veterinary services focus so much on bTB instead of controlling other diseases that they consider more severe for human health {S74}.

Generally, farmers did not perceive any production losses due directly to bTB and some of them referred that bTB does not affect animal at all. Only few farmers perceived a direct relationship in the long term between the productivity of animals and the presence of the disease {S75,-S76}. In this sense, veterinarians admitted that due to the early detection of the disease, most infected animals do not develop lesions and, in this context, it is difficult to make farmers aware on the impact of the disease {S77}. Thus, farmers mainly perceived the control of bTB as an imposition rather than a necessary activity to protect their animals {S78, S79}. They also mentioned that few studies have been done so far to quantify production losses due to bTB in the current epidemiological context and asked for updated scientific evidence on it. Nevertheless, the economic impact of the disease was strongly underlined by all interviewed groups and the commercial consequences of being bTB positive were perceived as worrisome {S80}. It was reported that some farmer abandoned the sector due to economic cost faced for the control of bTB. This is because, despite the fact that the Central Veterinary Service provides the diagnostic tests and current law provides for indemnity for slaughtered cattle, farmers assume the rest of the costs, mainly due to restrictions on trade and animal movements and field activities for the routine screening (i.e, veterinarian for screening, extra-personnel for animal management, derived damages on animals) {S81}.

With regard to the amount of the indemnification, veterinarians generally opined that it is adequate and that increasing indemnity payments would mean rewarding the maintenance of the disease; they also reported that no significant complaints have been received from farmers {S82, S83}.

***vi) Future perspective and proposed changes to the programme.***

Most of the interviewees were sceptics on the possibility of eradication mainly due to the presence of wildlife and other domestic reservoirs. The possibility of maintaining the disease at low levels was seen as the more realistic option but it was conditioned to the existence of a stable regulation {S84}.

Some farmers also doubted about the need of so restrictive measures (slaughter of positive animals, movement restrictions, etc.) taking into account the possibility of developing a vaccine for cattle {S85}. Others would prefer to live together with the disease rather than applying such restrictive measures that, on their opinion, will end up penalizing the cattle industry in the country {S86}.

Suggestions and changes proposed to the programme were related to the main problems highlighted, as for example, more investigation on diagnostic test, to improve the control on fraudulent activities, to increase the personnel of the Local Veterinary Services and the implementation of controls plan also on other reservoirs and wildlife.

## 5.5. Discussion

The continuous evaluation of the bTB programme, in order to identify limitations and modifications needed, requires taking into account the “non-biological” context, as it might influence the effectiveness of the eradication plan (16). However, despite the acknowledged importance of these “non-biological” factors, few studies have attempted to evaluate them (38–41) and they have mainly used structured questionnaires.

In this study, we used a qualitative approach in order to identify social aspects that may influence the effectiveness of the Spanish bTB eradication programme. The use of qualitative methods, such as the semi-structured interviews that we used in this study, might have some advantages in relation to the use of structured questionnaires for these types of studies. The main advantage is the fact that they allowed to develop long conversations through which people could describe their personal experiences and opinions in their own words. This generates a discourse that is neither fragmented nor pre-coded, as it happens with structured questionnaires (42). However, it is worth taking into account that qualitative interviews (as well as surveys) can inform on what people say they do, but not what they actually do. These means that the objectively knowledge about their daily practices and perceptions would require the use of other techniques, such as participant observation or systematic observation methods (43). In order to reduce this bias, in-depth interviews were conducted always in private and started with general “warm-up” questions. In this way, we intended to generate an atmosphere of conversation rather than of interview, maximizing therefore the possibility of achieving honest answers.

A disadvantage of qualitative interviews is that they do not allow making a direct inference of results to the whole population as the number of samples is normally low and the type of sampling is not random. However, this was not the objective of this study as we intended to know the main arguments that are circulating in the study population. In this context, the use of purposive sampling can ensure representativeness and diversity in the obtained results since it allows incorporating people of all possible typologies relevant to the research. This kind of sampling is the most effective technique when one needs to study a certain cultural domain or to explore all existing opinions circulating in the study-populations (44).

Considering both study phases, the main stakeholders involved in the Spanish bTB eradication program were included in our study. We interviewed cattle farmers (beef, dairy and bullfighting); Researchers with experience on bTB; Veterinarians working in the diagnostic labs: with responsibilities in the performance of the tests (gamma interferon, culture, etc) that are performed in the bTB eradication program; Private veterinarians that conduct bTB testing; and Official veterinarians working at different levels: i) Autonomous community level (Regional Veterinary Authority) with responsibilities in the coordination of the program in their Autonomous Community. These veterinarians, together with official veterinarians of other Autonomous Communities, also participate in the technical meetings organized at national level to review and discuss the bTB program; ii) County level: with responsibilities in the coordination of the program in their area.

Although it is true that some stakeholder profiles are missing, as for example we did not included veterinarians working in the slaughterhouses, trading partners or consumers, however, we have included representatives from the groups most involved in the implementation of the National bTB eradication programme. Therefore, we believe that the results of this study may have a wide applicability as we have gained information on the main discourses.

Overall, 52 people were interviewed (13 people for exploratory and 39 for in-depth interviews), among those there were 22 veterinarians and 30 farmers. The selected number of participants relied on previous studies based on grounded theory, and wanted to maintain a balanced emphasis between the homogeneity (requiring smaller size) and the heterogeneity (requiring larger size) of the sampling target (45,46). In the case of farmers' selection, the size of herds, the production type and bTB prevalence at county level were taken into account; while, in the case of veterinarians, the years of experience working with the bTB programme, their roles and responsibilities at the workplace and the disease prevalence at county level were considered. Doing this, we wanted to avoid failures in capturing insights, experiences, and activities and therefore achieve the theoretical saturation of data (45).

In recent years, the application of ethnographic methods has been extended to the description and analysis of social relations within any group of people: social, professional or conceptual (47), making this strategy of analysis particularly suitable for our study. Moreover, this methodology is optimal if people to interview tend to disguise their way of acting and / or thinking, as could be the case in the bTB eradication program.

One of the main results of this study was an apparent lack of motivation of some stakeholders and a general feeling of distrust in control measures and disbelief in test results. The complexity of the disease combined with gaps in knowledge and the lack of an efficient communication about the interpretation of diagnostic test results and control interventions seems to be important causes of disbeliefs, which in turn might generate different kinds of guesses and interpretations. Good communication and coordination between the different stakeholders have been previously described as having paramount importance in any health program, since it might be a critical factor for the success of bTB control interventions (40,41). The implementation of official communication plans on bTB and the selection of the most appropriate strategy would be an interesting research topic to tackle. Moreover, our results also points out the importance of informal places for discussion and solving doubts and the primary role of private veterinarians influencing farmers' opinions.

Similar to our findings, Calba et al. (2016), in a study conducted in Belgium, reported the key role that private veterinarians have in the surveillance and communication with farmer; they found that private veterinarians are under pressure of their client (farmer), making necessary a greater support by the official veterinary services, and highlighted the importance to address such issues in order to improve the acceptability level of the bTB surveillance system. In agreement with Calba et al. (2016), we found that the lack of support by the official veterinary services has mostly likely contributed to the feeling of distrust towards official vets, to the absence of adequate infrastructures to perform the SIT and to the pressure faced by private vets.

Perceived inaccuracies in bTB detection increased mistrust and demotivation, especially among farmers. Discordant results between diagnostic tests, the lack of guides and standards for interpretation of diagnostic results and the absence of lesions at the post–mortem inspection have been already described as possible barriers toward bTB eradication in previous studies, as they might reduce the engagement of farmers in preventive health interventions (4,8,40). Our results further highlight that the level of confidence on the interpretation of SIT results was often linked with skills and experience of official and private veterinarians involved in the field activities of the testing campaign.

Along these lines, since expert estimations of the risk of bovine tuberculosis contain many and high levels of uncertainty,  it is perfectly rational for farmers not to limit themselves merely to these estimations when evaluating the magnitudes of risks, as stated by some scholars (48,49). It is therefore logical to also ask about such issues as how much trust the institutions involved in risk management deserve: "I have argued that public perceptions of and responses to risks are rationally based on judgements of the behaviour and trustworthiness of expert institutions, namely those that are supposed to control the risky processes involved" (Wynne, 1996). The results of our research seem to fit well with this hypothesis, as far as public and private institutions in charge of tuberculosis control are implementing actions perceived as ambiguous or not always coherent by the farmers.

The lack of the application of sanitary measures to wildlife, goats and pigs in extensive farming systems were pointed out and it was perceived as a comparative grievance to what is done in cattle, as measures on cattle were perceived as much more strict. In this regard, all groups asked for improvement in coordination between institutions and implementation of specific measures and better management of wildlife, especially for hunting farms. In this regard, is worthy to mention that recently it has been launched a reinforced surveillance programme for bTB in wildlife named PATUBES (34) which was not known by the interviewers as it was not publically available at that time. Thus, it would be worthy to update opinions and beliefs in the future in the light of the results of this reinforced program.

In relation to other domestic reservoirs, the Spanish bTB eradication programme only includes the testing in goats that are epidemiologically related to infected cattle herds and sheep and extensive pigs are not included in the program. With the exception of goats (32) the role of sheep and pigs in bTB epidemiology is still controversial, but some stakeholders had the perception that they are important reservoirs. In this sense, more research might be needed in order to communicate effectively their role to the different stakeholders.

Some other factors also mentioned in this study such as some non-specific SIT reactions in young animals might also need further research in order to fill gaps and enhance communication.

Moreover, farmers perceive very few benefits of being bTB-free and that the economic impact of the disease is due to its control rather that to its presence. Additionally, a low awareness on the zoonotic risk of bTB also emerged; these aspects might discourage farmers in implementing preventive measures against bTB since the cost for such implementation would outweigh perceived benefits. This perception might be another major factor influencing the effectiveness of the programme as preventive measures might be undertaken by farmers if they clearly perceive that the benefits outweigh the costs (8).

The lack of enough human resources for bTB activities, as reported by the group of official veterinary services, might also deserve further attention. The support of official veterinary services to private veterinarians beyond official control inspections could help to enhance relationships and communications between groups.

## 5.6. Conclusion

The use of a qualitative approach allowed us to catch specific information related to the local context and highlight aspects that could be missed by applying quantitative epidemiological methods. Our findings represent a good part of the probable sphere of perceptions, opinions, behaviour, attitudes and knowledge of the study population and several key critical points that may hinder the success of the bTB eradication program in Spain were identified.

Major issues were related to the perception of the bTB programme as a law enforcement duty and to the lack of an adequate motivation, as a general feeling of distrust towards official veterinary services was expressed. The improvement of communication strategies should be considered as a priority, as it seems to be a major factor influencing the trust between stakeholders and the effectiveness of the eradication plan. Lack of understanding of test results and control measures, lack of perceived benefits of being bTB free, gaps on knowledge together with the complex epidemiology of bTB deserves further efforts on communication. Private veterinarians had a major role in influencing farmers' opinions but their feeling of inadequate support from veterinary services should be taken into account.

These results can be extremely useful to develop some context-dependent recommendations and interventions in order to increase the acceptability of the bTB eradication programme and ensure its proper implementation.

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## 5.8. Conflict of Interest Statement

Authors have no conflict of interest to declare

## 5.9. Author Contributions

Conceived and designed the study: GC, AA, JE
Performed and transcribed qualitative exploratory interviews: GC, AA, SN, JC
Performed and transcribed qualitative in-depth interviews: PI, EC, SL
Analysed collected data: GC, AA, PI, EC, SL
Wrote the paper: GC, AA
Revised the paper: AA, JE

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## 5.11. References

1. Bach M, Jordan S, Hartung S, Santos-Hövener C, Wright MT. Participatory epidemiology: the contribution of participatory research to epidemiology. Emerg Themes Epidemiol (2017) 14:2. doi:10.1186/s12982-017-0056-4
2. Berkman L, Kawachi I. A historical framework for social epidemiology. In: Berkman LF, Kawachi I, editors. *Social Epidemiology*. New York: Oxford University Press (2000). p. 3–12.

3. Honjo K. Social Epidemiology: definition, history, and research examples. *Environ Health Prev Med* (2004) 9(5):193–9. doi:10.1007/BF02898100.

4. Brennan ML, Wright N, Wapenaar W, Jarratt S, Hobson-west P, Richens IF, et al. Implementing Cattle Disease Prevention and Control Great Britain. *Animals* (2016) 6(10):61. doi:10.3390/ani6100061.

5. Catley A, Alders RG, Wood JLN. Participatory epidemiology: approaches, methods, experiences. *Vet J* (2012) 191:151–60. doi:10.1016/j.tvjl.2011.03.010.

6. Enticott G, Maye D, Carmody P, Naylor R, Ward K, Hinchliffe S, et al. Farming on the edge: farmer attitudes to bovine tuberculosis in newly endemic areas. *Vet Rec* (2015) 177:439–439. doi:10.1136/vr.103187.

7. McAloon CG, Macken-Walsh Á, Moran L, Whyte P, More SJ, O'Grady L, et al. Johne's disease in the eyes of Irish cattle farmers: a qualitative narrative research approach to understanding implications for disease management. *Prev Vet Med* (2017) 141:7–13. doi:10.1016/j.prevetmed.2017.04.001.

8. Moda G. Non-technical constraints to eradication: the Italian experience. *Vet Microbiol* (2006) 112(2–4):253–8. doi:10.1016/j.vetmic.2005.11.021

9. Pfeiffer DU. Epidemiology caught in the causal web of bovine tuberculosis. *Transbound Emerg Dis* (2013) 60:104–10. doi:10.1111/tbed.12105

10. Reason P, Heron J. Research with people: the paradigm of cooperative experiential inquiry. *Person Cent Rev* (1986) 1(4):456–76.

11. Jost CC, Mariner JC, Roeder PL, Sawitri E, Macgregor-Skinner GJ. Participatory epidemiology in disease surveillance and research. *Rev Sci Tech* (2007) 26(3):537–49. doi:10.20506/rst.26.3.1765.

12. Allepuz A, de Balogh K, Aguanno R, Heilmann M, Beltran-Alcrudo D. Review of participatory epidemiology practices in animal health (1980–2015) and future practice directions. *PLoS One* (2017) 12:e0169198. doi:10.1371/journal.pone.0169198

13. Pfeiffer DU. Communicating risk and uncertainty in relation to development and implementation of disease control policies. *Vet Microbiol* (2006) 112(2–4):259–64. doi:10.1016/j.vetmic.2005.11.020

14. Mariner JC, Paskin R. Manual on Participatory Epidemiology: Methods for the Collection of Action-Oriented Epidemiological Intelligence. Food and Agricultural Organization of the United Nations (FAO), Animal Production and Health Div., FAO Animal Health Manual. (Vol. 10). **FAO**, Rome, Italy (2000). 81 p.

15. Reviriego Gordejo FJ, Vermeersch JP. Towards eradication of bovine tuberculosis in the European Union. *Vet Microbiol* (2006) **112**:101–109. doi:10.1016/j.vetmic.2005.11.034.

16. EFSA AHAW Panel. Statement on a conceptual framework for bovine tuberculosis□: Scientific Opinion. *Eur Food Saf Auth EFSA J* (2014) **12**:3711. doi:10.2903/j.efsa.2014.3711.

17. Allepuz A, Casal J, Napp S, Saez M, Alba A, Vilar M, Domingo M, González MA, Duran-Ferrer M, Vicente J, et al. Analysis of the spatial variation of Bovine tuberculosis disease risk in Spain (2006–2009). *Prev Vet Med* (2011) **100**:44–52. doi:10.1016/j.prevetmed.2011.02.012.

18. de la Cruz ML, Perez A, Bezos J, Pages E, Casal C, Carpintero J, Romero B, Dominguez L, Barker CM, Diaz R, et al. Spatial Dynamics of Bovine Tuberculosis in the Autonomous Community of Madrid, Spain (2010–2012). *PLoS One* (2014) **9**:e115632. Available at: https://doi.org/10.1371/journal.pone.0115632.

19. Garcia-Saenz A, Saez M, Napp S, Casal J, Saez JL, Acevedo P, Guta S, Allepuz A. Spatio-temporal variability of bovine tuberculosis eradication in Spain (2006-2011). *Spat Spatiotemporal Epidemiol* (2014) **10**:1–10. doi:10.1016/j.sste.2014.06.002.

20. Guta S, Casal J, Napp S, Saez JL, Garcia-Saenz A, Perez de Val B, Romero B, Alvarez J, Allepuz A. Epidemiological Investigation of Bovine Tuberculosis Herd Breakdowns in Spain 2009/2011. *PLoS One* (2014) **9**:e104383. Available at: https://doi.org/10.1371/journal.pone.0104383.

21. Guta S, Casal J, Garcia-saenz A, Saez JL, Pacios A, Garcia P. Risk factors for bovine tuberculosis persistence in beef herds of Southern and Central Spain. *Prev Vet Med* (2014) **115**:173–180. doi:10.1016/j.prevetmed.2014.04.007.

22. Martínez-López B, Barasona JA, Gortázar C, Rodríguez-Prieto V, Sánchez-Vizcaíno JM, Vicente J. Farm-level risk factors for the occurrence, new infection or persistence of tuberculosis in cattle herds from South-Central Spain. *Prev Vet Med* (2014) **116**:268–278. doi:10.1016/j.prevetmed.2013.11.002.

23. Barasona JA, Vicente J, Díez-Delgado I, Aznar J, Gortázar C, Torres MJ. Environmental presence of *Mycobacterium tuberculosis* complex in aggregation points at the wildlife/livestock interface. *Transbound Emerg Dis* (2016) 64(4):1148–58. doi:10.1111/tbed.12480

24. Barasona JA, Latham MC, Acevedo P, Armenteros JA, Latham ADM, Gortazar C, et al. Spatiotemporal interactions between wild boar and cattle: implications for cross-species disease transmission. *Vet Res* (2014) 45:122. doi:10.1186/s13567-014-0122-7

25. García-Jiménez WL, Benítez-Medina JM, Martínez R, Carranza J, Cerrato R, García-Sánchez A, et al. Non-tuberculous mycobacteria in wild boar (*Sus scrofa*) from Southern-Spain: epidemiological, clinical and diagnostic concerns. *Transbound Emerg Dis* (2013)

62(1):72–80. doi:10.1111/tbed.12083

26. Hermoso de Mendoza J, Parra A, Tato A, Alonso JM, Rey JM, Peña J, et al. Bovine tuberculosis in wild boar (*Sus scrofa*), red deer (*Cervus elaphus*) and cattle (*Bos taurus*) in a Mediterranean ecosystem (1992–2004). *Prev Vet Med* (2006) 74(2–3):239–47. doi:10.1016/j.prevetmed.2005.10.005
27. Muñoz-Mendoza M, Marreros N, Boadella M, Gortázar C, Menéndez S, de Juan L, et al. Wild boar tuberculosis in Iberian Atlantic Spain: a different picture from Mediterranean habitats. *BMC Vet Res* (2013) 9:176. doi:10.1186/1746-6148-9-176
28. Pérez de Val B, Napp S, Velarde R, Lavín S, Cervera Z, Singh M, et al. Serological follow-up of tuberculosis in a wild boar population in contact with infected cattle. *Transbound Emerg Dis* (2017) 64:275–83. doi:10.1111/tbed.12368
29. Risco D, Fernández-Llario P, García-Jiménez WL, Gonçalves P, Cuesta JM, Martínez R, et al. Influence of porcine circovirus type 2 infections on bovine tuberculosis in wild boar populations. *Transbound Emerg Dis* (2013) 60: 121–7. doi:10.1111/tbed.12112
30. Rodríguez-Prieto V, Martínez-López B, Barasona J, Acevedo P, Romero B, Rodriguez-Campos S, et al. A Bayesian approach to study the risk variables for tuberculosis occurrence in domestic and wild ungulates in South Central Spain. *BMC Vet Res* (2012) 8:148. doi:10.1186/1746-6148-8-148
31. Vicente J, Höfle U, Garrido JM, Fernández-de-mera IG, Acevedo P, Juste R, et al. Risk factors associated with the prevalence of tuberculosis-like lesions in fenced wild boar and red deer in south central Spain. *Vet Res* (2007) 38:451–64. doi:10.1051/vetres:2007002
32. Muñoz-Mendoza M, Romero B, del Cerro A, Gortázar C, García-Marín JF, Menéndez S, et al. Sheep as a potential source of bovine TB: epidemiology, pathology and evaluation of diagnostic techniques. *Transbound Emerg Dis* (2016) 63:635–46. doi:10.1111/tbed.12325
33. Napp S, Allepuz A, Mercader I, Nofrarías M, López-Soria S, Domingo M, et al. Evidence of goats acting as domestic reservoirs of bovine tuberculosis. *Vet Rec* (2013) 172(25):663. doi:10.1136/vr.101347
34. Anonymous. Spanish Eradication Programme for Bovine Tuberculosis. Approved by Commission Decision SANTE/2017/ES/SI2.749855. Minist. Agric. Aliment. y medio Ambient, **Madrid, Spain** (2017). Available at: http://www.mapama.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/pnetb_2017_3_tcm7-443753.pdf.
35. Patton MQ. Two Decades of Developments in Qualitative Inquiry. *Qual Soc Work* (2002) **1**:261–283. doi:10.1177/1473325002001003636.

36. Flick U. *The SAGE Handbook of Qualitative Data Analysis*. (2014). doi:10.4135/9781446282243.
37. Strauss AL. *Qualitative analysis for social scientists*. (1987). doi:10.1017/CBO9780511557842.
38. Broughan JM, Maye D, Carmody P, Brunton LA, Ashton A, Wint W, et al. Farm characteristics and farmer perceptions associated with bovine tuberculosis incidents in areas of emerging endemic spread. *Prev Vet Med* (2016) 129:88–98. doi:10.1016/j.prevetmed.2016.05.007
39. Calba C, Goutard FL, Vanholme L, Antoine-Moussiaux N, Hendrikx P, Saegerman C. The added-value of using participatory approaches to assess the acceptability of surveillance systems: the case of Bovine tuberculosis in Belgium. *PLoS One* (2016) 11(7):e0159041. doi:10.1371/journal.pone. 0159041
40. Cowie CE, Gortázar C, White PCL, Hutchings MR, Vicente J. Stakeholder opinions on the practicality of management interventions to control bovine tuberculosis. *Vet J* (2015) 204:179–85. doi:10.1016/j.tvjl.2015.02.022
41. O'Hagan MJH, Matthews DI, Laird C, Mcdowell SWJ. Farmers' beliefs about bovine tuberculosis control in Northern Ireland. *Vet J* (2016) 212:22–6. doi:10.1016/j.tvjl.2015.10.038
42. Alonso LE. La mirada cualitativa en sociología: una aproximación interpretativa. Colección Ciencia. (Vol. 218). **Editorial Fundamentos**, Madrid, Spain (1998).
43. Gravlee CC. "Research Design and Methods in Medical Anthropology," in *A Companion to Medical Anthropology*, 69–91. doi:10.1002/9781444395303.ch4.
44. Palys T. Purposive Sampling 1. *SAGE Encycl Qual Res methods* (2008) **2**:698–699. doi:10.1109/IPDPS.2011.210.
45. Guest G, Arwen B, Johnson L. How many interviews are enough? An experiment with data saturation and variability. Field methods (2006) 18(1):59–82. doi:10.1177/1525822X05279903.
46. Palinkas LA, Horwitz SM, Green CA, Wisdom JP, Duan N, Hoagwood K. Purposeful Sampling for Qualitative Data Collection and Analysis in Mixed Method Implementation Research. *Adm Policy Ment Health* (2015) **42**:533–44. doi:10.1007/s10488-013-0528-y.
47. Hagberg S, Widmark C. *Ethnographic Practice and Public Aid: Methods and Meaning in Development Cooperation*. (2009).
48. Irwin A, Simmons P, Walker G. Faulty Environments and Risk Reasoning: The Local Understanding of Industrial Hazards. *Environ Plan A* (1999) **31**:1311–1326. doi:10.1068/a311311.

49. Wynne B. "May the sheep safely graze? A reflexive view of the expert-lay knowledge divide," in *Risk, Environment and Modernity: Towards a New Ecology*, 44–83. doi:10.4135/9781446221983.

# Study IV

## Chapter VI

**Farmer and Veterinarian Attitudes toward the Bovine Tuberculosis Eradication Programme in Spain: similarities and differences**

In preparation for submission

# 6.

## 6.1. Abstract

In Spain, despite the amount of efforts made, the eradication of the bovine tuberculosis (bTB) has been not achieved yet. The prolonged failure of control measures could have caused mistrust and non-compliance among people involved in the programme. In particular, the commitment of farmers and veterinarians have a significant influence on the effectiveness of bTB control efforts and the understanding of their opinions and attitudes may lead to more efficient bTB policies.

This study is part of a multidisciplinary investigation; by using a quantitative approach, we investigated the most relevant opinions of farmers and veterinarians towards the bTB eradication programme, previously identified through qualitative methodologies. Data were collected by a structured questionnaire using a telephone survey. At first, we made a comparison of answers' frequencies between farmers and veterinarians; Multiple Correspondence Analysis, followed by the Hierarchical Clustering on Principal Components were used to identify opinion profiles; and, a logistic regression model was developed to evaluate the main differences between the two groups.

A sample of 706 farmers and 180 veterinarians was interviewed. We identified the existence of three different opinion profiles mainly characterised by the attitude toward bTB diagnostic tests, the perception on the seriousness and the zoonotic impact of bTB and the perceived importance of other domestic reservoirs. There were people with positive and people with negative attitudes towards the programme and a third group with the tendency to not respond. The presence of opposite profiles was observed among farmers. Differently, veterinarians were more homogeneous and the vast majority of them expressed a positive attitude toward the programme; however, we also found that some veterinarians showed the same negative attitude as farmers, deserving a special attention. Most people did not believe in the achievement of the bTB eradication and the presence of the disease was often perceived as determined by factors out of their control. Farmers from high prevalence areas were those who more distrust in the skin test. Most of veterinarians reported that skin test is reliable, but uncertainness arose about the possibility of false positive cattle at the skin test and about results' confirmation in independent laboratories.

Our results highlight that, in Spain, both education and communication are of paramount importance and should be improved to generate motivation and positive attitudes toward the disease and the eradication programme, especially among farmers. Particular attention should also be paid to farmers with a positive attitude since they may represent a key group for an earlier achievement of the bTB eradication.

## 6.2. Introduction

Bovine tuberculosis (bTB) is a chronic infectious disease caused by any mycobacterial species included in the *Mycobacterium tuberculosis complex* (MTC) (OIE, 2015; SANCO WD, 2013). In livestock, M. bovis is the most common etiologic agent of bovine tuberculosis followed by M. caprae. Both species can cause disease in cattle and other domestic and wildlife animals (Aranaz et al., 2003; OIE, 2015; Pesciaroli et al., 2014; Rodríguez et al. 2011) as well as in humans (namely, zoonotic tuberculosis) (Cvetnic et al. 2007, OIE, 2015).

Since the end of the 20th century, huge efforts have been made to eradicate bTB in cattle through the introduction of control measures mainly based on a "test-and-slaughter" strategy (i.e., routine application of tuberculin testing and culling of reactor cattle). The early detection and elimination of infected cattle led to a reduction of economic losses caused by livestock deaths and decline in animal productivity; besides, in countries with bTB eradication programmes in place, the zoonotic tuberculosis became a rare event (EFSA & ECDC, 2016.). However, bTB remains a public health concern as recent publications highlighted that the real burden of zoonotic bTB may be underestimated (Good et al., 2018; Lombardi et al., 2017; Olea-Popelka et al., 2017; Palacios et al., 2016), and it still has an economic impact for farmers and countries, mainly due to the cost of eradication programmes (i.e., surveillance and regular testing and removal of infected cattle), restrictions for trade of animals and their products both at the local and international level.

Although the application of "test-and-slaughter" policy has been highly effective to eradicate the bTB in some countries; it has been less successful in others, where the eradication has not been achieved yet, indicating that targeted interventions need to be pursued (Good & Duignan, 2011; Good et al., 2018; Olea-Popelka et al., 2017; Schiller et al., 2010).

Several aspects, operating in local contexts, may influence the success of bTB eradication campaigns and many of those require collaborative efforts between all people involved to be overcame (Good et al., 2018). Especially in the final stages of the campaign, as the bTB prevalence decreases, the rise of the percentage of tuberculin reactors with no visible lesions at the post-mortem examination may lead to significant public relations difficulties influencing perceptions and concerns of stakeholders about the disease (Constable et al., 2017). Eradication efforts may also be impeded by transmission between wildlife reservoirs and cattle or between other domestic species sharing the environment with cattle; thus, to successfully achieve the eradication, bTB transmission has to be tackle in all species through appropriate control strategies. Moreover, methods of husbandry (e.g., extensive farming conditions and pasturing of cattle), community attitudes toward wildlife animals (e.g., badger protection policy in UK), the level of compliance of stakeholders with the programme and the attitude of individual farmers toward the adoption of appropriate preventive measure are also recognized as important factors that might hinder an effective control of the disease (Allen et al., 2018; Enticott, 2015; EFSA AHAW Panel, 2017; Humblet et al., 2009). Therefore, the complexity of bTB epidemiology deserves the need of implementing cross-sectorial approaches and multidisciplinary collaborations in order to address the context-specific constraints and achieve the eradication of this disease.

In Spain, the bTB eradication programme is based on the regular testing of all cattle herds by authorized private veterinarians. The testing frequency is set in accordance with the prevalence of the area and the health status of the herd. Cattle tested positive are slaughtered (with compensation to farmers) and subjected to post-mortem examination at the slaughterhouses, followed by culture confirmation. In bullfighting farms, breeders and fighting bulls are differentiated and the TB-testing is carried out only in breeding animals, whereas fighting bulls destined for the bullring are not included in the programme (Anon., 2018). Private veterinarians in charge of the routine testing are subjected to official auditing of their testing practices, which is carried out by the Official Veterinary Services (OVS). Moreover, compulsory pre-movement tests on purchased cattle and post-mortem surveillance at the abattoir are also carried out (Anon., 2018). The Simple Intradermal Tuberculin Test (SITT) (i.e., injection of bovine tuberculin only) is used for the routine screening; however, according to the epidemiological situation, the Single Intradermal Comparative Tuberculin Test (SICTT) (i.e., injection of both bovine and avian tuberculin at separate sites) and the interferon-gamma assay (IFN-γ) may be also authorized as ancillary tests. Despite major efforts to achieve the bTB eradication, an increase trend in the herd prevalence has been reported during the last two years and the epidemiological situation across the country is extremely heterogeneous, with regional prevalence ranging from 0.2% to 17.1% (Anon., 2018). In this context, to achieve the bTB eradication, it is essential to ensure the highest level of commitment of all people involved in the implementation of the eradication programme, independent of their respective roles and responsibilities.

In a previously published study, Ciaravino et al. (2017) investigated opinions and beliefs of farmers and veterinarians about the Spanish bTB eradication programme by using a qualitative approach. Their main results suggested that people involved in the programme were sceptics on the possibility of eradicating the disease and perceived very few benefits of being bTB-free, with the exception of the commercial benefit, which in turn generated a lack of an adequate motivation towards the programme implementation. They also highlighted feelings of distrust towards the Official Veterinary Services (OVS) and disbelieves towards the results of diagnostic tests. Difficulties and physical risk in the execution of the routine screening test due to the lack of good infrastructure and the existence of a “patronage relationship” between some farmers and private veterinarians were also mentioned. Moreover, it was reported that bTB wildlife reservoirs were perceived as a major obstacle for bTB eradication by most people, and the existence of criticisms regarding the sector-specific legislation for bullfighting cattle farms was also observed. With reference to other bTB domestic reservoirs not subjected to any control programme, people suspected that goats, sheep and extensively reared pigs might have a potential role in the maintenance of the disease with the aggravating circumstance that they are not subjected to control programs.

Although the application of a qualitative method contributed to understand reasons for demotivation and scepticism, it did not allow inferring the results to the whole population, thus informing the decision makers. Consequently, in order to infer our results to the studied population and explore the relations between the aspects previously identified, in this study we used different quantitative methods aimed to i) quantify how many people among farmers and veterinarians share the same arguments or opinions in relation to the bTB eradication program; ii) identify individuals sharing similar opinion profiles and investigate the relationship between opinions and perceptions of the different professional groups and iii) evaluate in which aspects the opinion of veterinarians and farmers might differ.

## 6.3. Materials and Methods

### 6.3.1. Study area, target population and survey design

The study area and the target population were based on those previously described in the qualitative research published by Ciaravino et al. (2017). Therefore, we carried out our study in Andalusia and Catalonia, as representative of high (HPA) and low (LPA) bTB prevalence areas in Spain, including dairy (dF) and beef farmers (bF); official veterinarians in charge of the infectious diseases regional control programmes (oV) and private veterinarians involved in the bTb eradication campaign (pV).

A cross sectional design was used to obtain people’s opinions, perceptions and attitudes on different aspects related with the bTB eradication programme. Two questionnaires were designed: one for veterinarians and the other for farmers. Survey items were developed from the results described by Ciaravino et al. (2017), referring to the same thematic blocks: i) bTB detection and control activities; ii) role of wildlife and other domestic animals in bTB epidemiology; iii) personal relations and perception on social aspects; iv) bTB risk perception and benefits of eradication; v) future perspectives and proposed changes to the bTB eradication programme. A few socio-demographic questions (such as age or years of experience) were also included (Table 1).

In addition, the farmers’ questionnaire contained a specific thematic block on “Training, information and communication”, including questions on the perception about knowledge level among farmers, their attitude toward training courses and meetings on bTB and on the use of different types of information sources. Moreover, farmers were also asked about the employment relationship with the farm (i.e. owner or farm worker) as well as if they have had bTB cases in the farms during the last two years and, if so, how long it took to solve the outbreak. On the other hand, the veterinarians’ questionnaire included five additional specific questions on diagnostic methods and the execution of the bTB eradication programme and three more questions on Perception on Social Aspects.

| ***Variables code/name*** | ***Variables meaning*** | ***Original variables type & values*** | ***Variables transformations*** | ***Thematic block*** |
|---|---|---|---|---|
| QLUGAR | AND / CAT | Binary | | Socio-demographic questions |
| CATEG | VET / GAN (farmers) | Binary | | |
| TIPOL | Vet.san (pv)<br>Vet.of (ov)<br>LECHE (df)<br>CARNE (bf) | Categorical (4 categories) | | |
| P2_COD (Exper.) | Numbers of years | Integer | | |
| Age | Year of birth | Integer | | |
| P5_1 | The SITT is a realable diagnostic test | **5 points likert-scale:** 1)Totally Agree, 2)Agree, 3) Neutral, 4)Disagree, 5) Totally disagree | * Binary for logistic regression : 1+2+3= disagree; 4+5 = agree. * 3 categories in MCA & Cluster: "Agree", "Neutral", "Disagree" | bTB detection and control |
| P5_2 | Animals tested positive with no lesions at the slaughterhouse should be considered false positive | | | |
| P5_3 | To allow confirming SITT results in independent labs | | | |
| P6_1 | G-interferon is usefull to erradicate btb from a herd | **5 points likert-scale:** 1)Totally Agree, 2)Agree, 3) Neutral, 4)Disagree, 5) Totally disagree | * Binary for logistic regression : 1+2+3= disagree; 4+5 = agree. * 3 categories in MCA & Cluster: "Agree", "Neutral", "Disagree" | |
| P6_2 | G-interferon is too expensive, it would be better to allocate resources to other activities | | | |
| P7_1 | The frequency of routine testing in HPA is too high | **5 points likert-scale:** 1)Totally Agree, 2)Agree, 3) Neutral, 4)Disagree, 5) Totally disagree | * Binary for logistic regression: 1+2+3= disagree; 4+5 = agree. * 3 categories in MCA & Cluster: "Agree", "Neutral", "Disagree" | |
| P7_2 | Frequency of routine testing in LPA is too high | | | |
| P7_3 | The central administration is not transparent enough in the communication of diagnostic results | | | |
| P8_1 | The level of professionalism and experience of pv | 5 points likert-scale: 1)Very low; 2)Low; 3)Neutral; 4)High; 5)Very high; | * Binary for logistic regression : 1+2+3= Low; 4+5 = High. * 3 categories in MCA & Cluster: "High", "Neutral", "Low" | |
| P8_2 | The level of professionalism and experience of ov | | | |
| P9_1 | It's important to erradicate because of the zoonotic risk | **5 points likert-scale:** 1)Totally Agree, 2)Agree, 3) Neutral, 4)Disagree, 5) Totally disagree | * Binary for logistic regression : 1+2+3= disagree; 4+5 = agree. * 3 categories in MCA & Cluster: "Agree", "Neutral", "Disagree" | Risk perception on bTB and benefits of eradicate the disease |
| P9_2 | It's important to erradicate because of restictions on animals movements and exportations | | | |
| P9_3 | It's not a serious disease, it is just an excuse to reduce cattle population in the South | | | |
| P11 | Cost for btb detection and control | 3 categories: 1) shared farmers & Admin.; 2) Mainly Admin.; 3) Mainly farmers. | | |

| | | | | |
|---|---|---|---|---|
| P12 | Role of wildlife | Main or secondary role | Binary | Role of wildlife and other domestic reservoirs |
| P13 | Biosegurity measures against wildlife | 3 categories: 1)Not economically feaseble; 2) exsist and are economically feaseble; 3) effective measures do not exsist | * Binary for logistic regression : 1+2+3= Low; 4+5 = High. * 3 categories in MCA & Cluster: "High", "Neutral", "Low" | |
| P14_1 | Importance of goats | 5 points likert-scale:1)Very low; 2)Low; 3)Neutral; 4)High; 5)Very high; | * Binary for logistic regression : 1+2+3= Low; 4+5 = High. * 3 categories in MCA & Cluster: "High", "Neutral", "Low" | |
| P14_2 | Importance of pigs in extensive systems | | | |
| P14_3 | Importance of sheep | | | |
| P14_4 | Importance of cattle from other regions | | | |
| P14_5 | Importance of cattle from other countries | | | |
| P14_6 | Importance of wildlife | | | |
| P20 | "patronage" relashionship between farmers and privte vets | Binary | | Perception on Social Aspects |
| P21 | Achievement of btb erradication | 3 categories: 1) Never, only low prevalence levels; 2) Yes, appling well the programme; 3) yes, changing the programme. | * Binary for logistic regression: 1+2+3= Low; 4+5 = High. * 3 categories in MCA & Cluster: "High", "Neutral", "Low" | Future perspective and proposed changes |
| P22_1 | To incentivate culling of older animals | **5 points likert-scale:** 1)Totally Agree, 2)Agree, 3) Neutral, 4)Disagree, 5) Totally disagree | | |
| P22_2 | To ensure good condition of cattle crushes | | | |
| P22_3 | To infcrease sanctions for non-compliant farmers | | | |
| P22_4 | To increase the level of training of people who execute the btb programme | | | |
| P22_5 | To implement a control programme in goats | | | |
| P22_6 | To implement a control programme in sheep | | | |
| P22_7 | To implement a control programme in pigs extensively farmed | | | |

Table 1: Description of the questionaire items asked to farmers and veterinarians (common questions core) and relative variables included in the study

The draft questionnaires were then discussed in an expert meeting with veterinary and social experts and the final ones were subsequently piloted amongst veterinarians working at the Faculty of Veterinary Medicine, Autonomous University of Barcelona, Spain and some of the farmers involved in our earlier qualitative research (Ciaravino et al. 2017). After making some amendments, the two questionnaires (available in Spanish on request) included a total of 50 questions for veterinarians and 47 for farmers (with a common core of 37 questions). All questions but two had a closed format and used a combination of multiple-choice (i.e., the respondent could choose only one among a list of alternatives) and 5-points Likert scale (i.e., 1 = completely disagree; 2 = disagree; 3 = neutral; 4 = agree; 5 = completely agree). The only two questions with an open-format response mode were the age (year of birth) and the professional experience (number of years) of the interviewed. A "No-Response" option was always available.

***a) Questionnaire distribution and Interviews***

Individuals were selected randomly from four different sample frames (one for each of the target population categories) available at regional level. Telephone numbers of farmers and veterinarians were obtained from the autonomous government of Andalusia and Catalonia, respectively.

People were interviewed between October 2016 and January 2017 through a computer-assisted telephone interviewing system (CATI) using a digitally-recorded questionnaire that was undertaken by trained interviewers. Before starting the interview, people were informed about the study objectives; then were asked about their willingness to participate to the survey and, finally, we asked them the permission for recording the interview's audio. If the last was denied, the interview was carried out without recording the audio. Responses were collected anonymously and digital files containing both the interviews data and telephone audio tracks were encrypted.

***b) Sampling design***

The sample size was calculated considering a variation in the frequency of opinions of 50%, a confidence level of 95% and a 5% of accuracy in each study area and professional category (i.e. farmers and veterinarians from HPA and from LPA). Furtherly, the study population was stratified by typology (i.e. dF; bF; oV; and pV). Typologies were sampled proportionally to their stratum size and people to be included in the study were selected randomly within each stratum. Farms with less than 20 animals or private veterinarians that have performed less than 116 SITT were excluded from the study. More details about the sample size are given in table 2.

| | HPA (Andalusia) | | | | LPA (Catalonia) | | | |
|---|---|---|---|---|---|---|---|---|
| **Target population (N)** | **Farmers** 5,006 | | **Veterinarians** 342 | | **Farmers** 2,000 | | **Veterinarians** 159 | |
| ***Sample by professional category*** | **371** | | **185** | | **334** | | **114** | |
| *Typologies in each stratum* | *Beef Farmer* | *Dairy Farmer* | *Official Veterinarians* | *Private Veterinarians* | *Beef Farmer* | *Dairy Farmer* | *Official Veterinarians* | *Private Veterinarians* |
| *Proportion in each stratum* | 87% | 13% | 18% | 82% | 69% | 31% | 26% | 74% |
| ***Sample by typology*** | ***321*** | ***50*** | ***38*** | ***147*** | ***230*** | ***104*** | ***33*** | ***81*** |

Table 2: Estimated sample size

### 6.3.2. Statistical analyses and data coding procedures

Applied methods will be described according to the study objectives. All statistical analyses were performed in version 1.1.423 of RStudio (RStudio Team, 2016) using the statistical software R (Version 3.4.3; R Core Team, 2010).

***a) Quantification of opinions and shared arguments among farmers and veterinarians***

Collected data were summarized by using descriptive statistics. Significant differences in answers were evaluated by using the appropriate tests according to the data (Pearson's Chi-squared or Fischer' exact tests, Kruskal test or Wilcoxon test).

To investigate the existence of collinearity in the core of 37 common variables, we calculated the Spearman correlation coefficient (threshold > 0.4). When a significant correlation was present, the most complete variable was selected for further analysis. "No-Response" (NR) categories showing frequencies lower than 10% were considered missing values.

Questions developed specifically for farmers or for veterinarians were only described and results are presented in specific sections.

Statistical test and figures were completed using R packages: Hmisc (Version 4.1.1), stats (Version 3.1.2), Psych (Version 1.5.8; Revelle, 2015) corrplot (Version 0.84) and reshape (Version 0.8.7).

***b) Assessment of opinion profiles among farmers and veterinarians***

Multiple Correspondence Analysis (MCA) and a Hierarchical Clustering on Principal Components (HCPC) were carried out. In particular, we used the MCA to visualize and explore the dataset at hand before applying the HCPC, which allowed us to assess the existence and characterize specific "clusters of opinions" among the different professional groups (i.e., oV, pV, dF, bF).

Previous to the analysis all the 5-points Likert scale variables were reclassified into a different number of categories, i.e. Agree (= point 4 + 5), Neutral (= point 3), Disagree (= point 1 + 2). For those variables in which the frequency of the "Neutral" category was very low (i.e. lower than 10% of the observations) we decided to include this category in the "Disagree" category. Variables showing collinearity and those related with suggestions and bullfighting farms were excluded from this analysis.

***Multiple Correspondence Analysis (MCA)*** was performed as extension of the simple Correspondence Analysis to more than two categorical variables (Greenacre, 1993; Greenacre, 2007). This analysis illustrates data as points in a multidimensional space, reducing the dataset into a small number of dimensions with minimal loss of information (Di Franco, 2016; Torres & Van de Velden, 2007). Therefore, MCA allowed us to graphically display the distances between variables categories and interviewed people in a low-dimensional space, where the first dimension retains the maximum explained variance, the second the second largest variance and so on (Husson et al., 2010). We opted for applying the classical approach based on the indicator matrix.

The number of dimensions to retain was determined by examining the eigenvalues of each dimension and relative percentage of explained variance. A histogram of the eigenvalues (scree plot) was used to visualize the point at which the screen plot showed a bend (so called "elbow"), considered as the indicator of the optimal dimensionality. Most correlated variables to each of the MCA dimensions were identified through correlation plots. Contribution and quality of variables categories were visualized using bar plots. Two-dimensional diagrams (biplots) were used to explore visually similarities and differences among individuals and variables categories and to interpret the distances between points, where the similarity was depicted by the closeness of points, thus identifying conceptual profiles (Gabriel, 1971; Greenacre, 2007; Hair et al., 2010).

***Hierarchical Clustering on Principal Components (HCPC)*** was performed on the dimensions obtained as result from the MCA. Each dimension had associated a set of variables according to their coefficient correlation values; therefore, the information of each variable in the dimension was used to perform the cluster analysis. We could combine the three methods (i.e., principal component method, hierarchical and partitional clustering) since the same distance between individuals was used (i.e., the Euclidean one) and the application of the Ward's criterion as clustering method allowed to minimize the within cluster variance (Husson et al., 2010). The initial partitioning was performed by cutting the hierarchical tree according to a suggested level that was calculated on the inertia gains between two partitions. Besides, to identify which variables characterized more the partition of clusters a chi-square test ($\chi^2$ test) was performed in the HPCP. Clusters were interpreted and described by the variables and visualized by factor maps.

The package "FactoMineR" (Version 1.32; Husson et al., 2013) was used to compute MCA and HCPC analyses and the packages "factoextra" (Version 1.0.3; Kassambara et al., 2016) and ggplot2 (Version 2.1.0; Wickham, 2009) were employed for data visualization.

***c) Evaluation of main aspects in which the opinion of veterinarians and farmers might differ***

For this analysis we developed a logistic-binomial regression model. Before running the model, the 5-points Likert scale variables were reclassified into: Agree (= point 4 + 5) and Disagree (= point 1 + 2 + 3) categories.

Our outcome variable was the professional category (i.e., veterinarians versus farmers). A univariate analysis was run for 22 variables, after excluding those that showed collinearity during the descriptive analysis and the variables related with suggestions and bullfighting farms. Variables with a p-value lower than 0.2 for at least one of the variable's categories were considered eligible candidates for the multivariate model.

An automated method based on an information-theoretic approach (Burnham et al., 2002; Calcagno & Mazancourt, 2010) was used for model selection and to estimate the relative importance of the different predictor variables, including interactions between main effects. Only interactions with a significant impact on the model and a biological plausibility were included in the final model. Fit of the model was evaluated through the McFadden's test (pseudo $R^2$) and the Area Under the Curve (AUC).

The model was run using the R Package stats (Version 3.1.2); for model selection we used glmulti (Version 1.0.7; Calcagno, 2013); fit of the model were evaluated using the R Packages pscl (Version 1.4.9), lmtest (Version 0.9-34) and pROC (Version 1.8; Robin et al., 2011). Coefficients estimates of variables results from the regression model have been interpreted using the Package R "Emmeans" (Version 1.1.2).

## 6.4. Results

### 6.4.1. Obtained Sample

In both study areas, we obtained a 100% response rate from beef and dairy farmers, whereas, among veterinarians, it remained below 70% (i.e., 65% in HPA and 52% in LPA). The response rate was especially low in the pV group for which we got 59% and 47% response rate in HPA and LPA, respectively. Among oV, we obtained an 89% response rate in HPA and 64% in LPA.

Thus, the final sample included 180 veterinarians and 706 farmers; 493 and 393 people were interviewed in HPA and LPA, respectively. With regard to the professional groups, we included 552 beef farmers (bF) and 154 dairy farmers (dF). Among veterinarians, 55 were official (oV) and 125 private (pV).

### 6.4.2. Quantification of opinions and shared arguments among farmers and veterinarians

- ***Socio-demographic characteristics of the study population***

Farmers were slightly older than veterinarians, especially in HPA. The age of interviewed was correlated with their years of professional experience (spearman coefficient > 0.4; p-values<0.001); farmers had a higher number of years of professional experience than veterinarians (i.e., median of 30 and 17 years for farmers and veterinarians, respectively) with significant differences in both study areas.

- ***bTB detection and control activities***

Uncertainness on the reliability of the bTB diagnostic tests arose among both veterinarians and farmers. In relation to the single intradermal tuberculin test (SITT), farmers expressed a wide range of opinions on the trustworthiness of the results according to the prevalence in the area. In the LPA the proportion of farmers that trust in the SITT was significantly higher than in HPA (58% in LPA and 28% in HPA; p-value<0.0001) whereas most of the veterinarians from both areas agreed to consider the SITT as a reliable technique. A clear difference between farmers and veterinarians was observed on the perception of false positives animals to the SITT. Nearly all of the farmers (78%) agreed with the statement "*an animal positive to the SITT with no visible lesions at the slaughterhouse should always be considered a false positive*", whereas veterinarians tend to disagree with it. However, it is worthy to mention that some veterinarians also agreed with this statement, in particular in LPA (32% *versus* 17% in HPA; p-value<0.001) and that, in both areas, several of them remained neutral (i.e., score of 3: 13% in HPA and 17% in LPA). Moreover, 76% of farmers would like to be allowed to verify SITT positive results in independent laboratories; whereas, in both areas, veterinarians reported a diverse set of opinions and, surprisingly, a relevant proportion of them (47%) agreed on the confirmation of the SITT results by independent laboratories, especially among pV (63% of pV and 15% of oV; p-value<0.001). Around 60% of the interviewed people were in agreement that the gamma interferon (IFN-γ) is useful to eliminate bTB from a herd. Interestingly, farmers from LPA agreed on this more than veterinarians from both areas (p-value<0.05) and more than farmers from HPA (p-value<0.0001). On the other hand, despite perceiving the utility of the γ-INF to eradicate bTB, 50% and 32% of farmers in HPA and LPA, respectively, agreed that "*the γ-INF is very expensive and it would be better to invest the money in other activities*", differing from veterinarians that were mostly in disagreement (i.e., 63% and 59% in HPA and LPA, respectively).

Only people interviewed in the HPA were asked on the adequacy of the time interval for the routine screening (currently set at six-month for HPA); 67% of farmers opined that one annual screening would be enough, contrarily, only 12% of veterinarians agreed with this opinion (p-value<0.0001). Similarly, we asked to all interviewed about the routine screening intervals in LPA (currently set at one year), 56% of farmers considered that the time interval could be lengthen while 70% of veterinarians disagreed with this (p-value<0.0001). Moreover, in both areas, about 45% of farmers and 30% of veterinarians agreed that the Central Veterinary Services are not very transparent in the communication of the bTB test results.

When asked about the costs for bTB detection and control, 72% of people opined that farmers assume the majority of the expenses. In HPA, farmers were the most convinced

(79%) significantly differing from the veterinarians of the same area, among whom the 27% answered that the administration assumes the majority of the costs and another 24% believe that the costs are shared; whereas, in LPA, no differences were found.

People were also asked on the sector-specific legislation for bullfighting farms. Only 26% of interviewed were aware of its existence, with significant difference between HPA and LPA, where 37% and 12%, respectively, knew that bullfighting farms are under a sector-specific legislation. The oV were the "most-aware" group (85% in HPA and 57% in LPA) and the "less-aware" professional group was the dF (10% in HPA and 7% in LPA).

Among who reported to know the legislation on bullfighting farms (N= 228), very few people were in agreement with the bTB testing exception for bulls older than 24 months (29%), mostly in HPA (60 out of 65), especially among bF (n=25) and pV (n=25). People in agreement with the testing exception were asked to evaluate the importance of the main arguments behind it. Out of 65 interviewed, 72% agreed on the risk of breaking the horn of the bull and 78% on the difficulties in animal handling during the routine screening by SITT.

- ***Role of wildlife and other domestic animals in bTB epidemiology***

Wildlife animals were generally perceived as having a main role in the maintenance of the disease by most of interviewed (71%) and especially among farmers; in both areas, bF and pV were the most convinced of the primary role played by wildlife reservoirs. This question was correlated with the question on the importance of wildlife animals as bTB reservoir (p-value<0.001), that was excluded to further analyses.

Most of interviewed people did not believe in the existence of effective biosecurity measures to prevent bTB transmission between cattle and wildlife animals. In both study areas, this opinion was shared by more than 60% of farmers and the 15% did not respond. Veterinarians opined differently according to the study area; in HPA, 39% of them did not believe in the existence of effective measure, however another 40% opined that effective measures do exist but their application is not economically feasible. In contrast, in LPA, about 65% considered that such measures do not exist. It is worth to mention that 14% of oV in LPA did not respond to this question and their opinion was significantly different from the opinion of oV in HPA (p-value<0.05).

With regard to the importance of other domestic bTB reservoirs as source of bTB infection, we asked to evaluate the importance of goats, sheep and pigs farmed in extensive systems and cattle imported from other Regions or Countries. It is worth to mention that, for each of these questions, a proportion of people ranging from 15% to 22% - mainly farmers - did not respond. About 45% of farmers and 50% of veterinarians attributed an important role to goats; farmers from HPA were those who attributed less importance to this specie. A low importance was attributed to sheep as source of bTB infection, especially by veterinarians (i.e., score 1 and 2: 68% and 32% of veterinarians and farmers, respectively). This variable correlated with the one on the importance of goats as bTb reservoir (p-value<0.001), thus it was excluded to further analyses. Pigs in extensive farming systems were perceived as an important source of infection mainly in HPA and especially by veterinarians (i.e., 55% provided a score > 3); whereas, in LPA veterinarians though pigs have very low importance (i.e., 56% provided a score < 3) and another 12% did not respond. About 35% of farmers evaluated pigs as important bTB source. About 50% of veterinarians considered cattle imported from other regions an important source of bTB infections *versus* the 30% of farmers (p-value<0.01); this divergence remained significant in HPA but not in LPA. Similar attitudes were observed toward cattle imported from other countries and, since this variable resulted significantly correlated to the previous one, it was excluded to further analyses.

- ***Personal relations and perception on social aspects***

With regard to the evaluation of the competence of the veterinarians involved in the bTB eradication campaign, 91% and 82% of farmers and veterinarians, respectively, opined that pV have a high professional level. Farmers tend to have on pV a consideration significantly higher than veterinarians. No differences between farmers and veterinarians were found about the evaluation of the competence of oV: 68% of farmers and 65% of veterinarians opined that oV have a high professional level. The evaluation of the competence level of oV was generally lower than that of pV.

People were also asked about the "patronage relationship" between farmers and private veterinarians; more than 80% of farmers considered it as positive because it generate an atmosphere of trust that facilitate the execution of the routine testing by SITT; whereas veterinarians were almost equally divided between who perceived it as positive (56%) and who perceived it as negative (44%) since it could generate pressure on the pV at the moment of the interpretation of SITT results. It is worth to note that, in both areas, the majority of pV considered it as a positive relationship (i.e., 65% in HPA 82% in LPA) reversely to what was opined by the oV (i.e., negative: 84% in HPA 62% in LPA).

- ***bTB risk perception and benefits of eradication (P9)***

With regard to the need of eradicate bTB and the benefits of being free of the disease, the vast majority of interviewed (80%) were aware on the economic impact for positive herds due to the restrictions in export and animal movement but the risk for public health was less perceived, especially in the HPA. Significant differences were found about the statement *"bTB is not a serious disease and it is just an excuse to reduce the cattle population in the Southern countries"*. This "rumour" circulated mainly in HPA and almost exclusively among farmers.

- ***Future perspectives and proposed changes to the bTb eradication programme***

With regard to the future perspective on bTB, 56% of veterinarians and 40% of farmers did not believe in the achievement of the bTB eradication, but only in its control at low prevalence levels. This polarization in the attitude toward the bTb eradication was true in both farmers and veterinarians; however, it is worth to mention that the 19% of farmers did not respond. Among those who believed in the achievement of the eradication, people seem to be equally divided between those who though that the programme should be modified and those who though that eradication will be achieved complying with the programme.

Three suggestions for the bTB eradication program were the most agreed in both study areas: i) that the administration should guarantee the presence of an adequate crush in order to be able to perform bTB testing adequately (81% of people agreed); ii) the creation of incentives for the removal of old cattle from bTB positive herds (73% of people agreed); and iii) the implementation of a bTB control programme in goats (70% of people agreed). Regarding the first suggestion, in both areas, veterinarians were significantly more convinced than farmers (i.e., 93% of veterinarians versus 78% of farmers). The suggestion of creating incentives for the removal of old cattle in bTB positive herds was well accepted by farmers from both study areas (i.e., 75% and 70% in HPA and LPA, respectively) while veterinarians in HPA were more convinced of those in LPA about the usefulness of this measure (i.e., 82% versus 62% respectively; p-value<0.001). With regard to the third most accepted suggestion, 84% of veterinarians perceived the need of implementing a bTB control programme in goats *versus* the 68% of farmers (p-value<0.005); it is worth to note that in both study areas about 15% of farmers did not respond to this question.

Other proposed changes to the bTB eradication programme were the improvement of the training level of people executing the bTB programme, which was well accepted by 67% of people, and that the administration should penalize more the non-compliant farmers (agreed by 58% of interviewed). However, these suggestions were differently perceived by farmers and veterinarians within the HPA, where 76% of farmers agreed on the improvement of training compared to the 60% of veterinarians (p-value<0.001); and, on the other hand, the 77% of veterinarians agreed that the administration should penalize more the non-compliant farmers versus the 57% of farmers (p-value<0.05).

Finally, we also asked about the need of implementing bTB control programmes in sheep and pigs in extensive farming systems. It is worth to note that that a high proportion of farmers did not respond to these questions (i.e., 17% and 20%, respectively). A wide difference in opinions was found with regard to the implementation of a bTB control programme in sheep: 63% of farmers agreed contrarily to veterinarians, among which only 31% agreed (p-value<0.001) and the observed difference was significant in both areas. Among who responded about the implementation of a bTB control programme in extensively farmed pigs, in LPA farmers were significantly more in agreement than veterinarians (p-value<0.001), among which only the 34% agreed and another 22% did not respond. The opposite was observed in HPA, where 71% of veterinarians were in agreement compared to 56% of farmers (p-value<0.005).

Answers to the questions about the need of implementing bTB control programmes also in other domestic animals resulted correlated among them and with the perceived importance of these species as source of bTB infection (P14_1, P14_2, P14_3).

- ***Specific questions for farmers***

Among interviewed farmers, 85% were farm's owners and 10% farm's workers with no differences between the two study areas. As expected, only 5% of the farmers from LPA reported to have had bTB positive animals in their farm during the last two years *versus* 37% of the farmers from HPA (of which 89% were beef and bullfighting farms). In the LPA, the 50% of those that have been bTB positive in the last two years solved the outbreaks in less than six months whereas in the HPA 48% farms were still positive at the moment of the interview and another 13% spent more than one year to become bTB free again.

Despite the typology of farm, 68% of farmers have not attended any training course on bTB during the last year, especially in LPA (i.e., 80% in LPA *versus* 57% in HPA, p-value<0.0001). In both areas, about 50% of farmers perceived to not have an adequate level of Knowledge on bTB and its control, the 86% of them considered that it would be useful to organize regular meetings with vets to dispel doubts on the disease and its control and about 75% reported that would be willing to attend courses or conferences on bTB. Moreover, in both areas, farmers stated that their private veterinarian is the most common source of information for bTB (82% HPA, 90% LPA).

- ***Specific questions for veterinarians***

We asked, specifically to veterinarians, three more questions on bTB diagnostic tests (i.e., SITT and γ-INF tests). With regard to the perception on the difficulties in terms of practicality of the SITT, 54% of interviewed disagreed that the SITT is a difficult test to perform and in HPA oV perceive these difficulties significantly more than the pV (p-value < 0.05). On the use of the comparative intradermal tuberculin test (CIDT) as routinely screening test in the bTB eradication campaign, polarized opinions were reported by veterinarians, due to the fact that oV were in disagreement oppositely to pV (p-value in HPA < 0.001; p-value in LPA < 0.05). The majority of the veterinarians (80%) agreed that the use of the γ-INF reduces the pressure on the veterinarian during field activities. However, significant differences were found according to the prevalence in the area: in HPA the 82% of oV were totally in agreement, whereas, in LPA, only the 53% and another 23% reported to be neutral (p-value in LPA < 0.05). Moreover, veterinarians strongly agreed on the effectiveness of the slaughter of positive animals to eliminate bTB from the herd (81% HPA, 92% LPA). About 60% of the veterinarians opined that the oV use similar criteria when performing controls on the execution of the SITT and that the animal health is a priority issue for farmers with no significant differences between the two areas neither between the professional typology.

Different opinions arose on one proposed change to the bTB eradication programme: oV agreed that the last testing round before obtaining the qualification of “official bTB free” herd should be executed by oV, whereas pV expressed disagreement, especially in HPA (p-value<0.0001).

Finally, veterinarians were asked on the mutual trust between farmers and oV, farmers and pV and between oV and pV . Veterinarians considered that there is a low (41%) to moderate (34%) confidence between farmers and oV with no differences between areas (p-value: 0.43). Although, pV tended to evaluate the level of trust between farmers and oV lower than oV (i.e., 50% and 20%, respectively). Differences also emerged on the evaluation of the mutual trust between farmers and pV, that was considered a relation of high confidence by the 68% of oV and the 84% of pV (p-value<0.01). Whereas, no differences were found on the perception of the mutual trust between oV and pV, that was evaluated as high by the 60% and 56% respectively.

### 6.4.3. Assessment of profiles of opinions among farmers and veterinarians

Categorical variables eligible for their inclusion in the MCA analysis are shown in figure 1. In order to avoid the distortion of MCA results, two variables (i.e., P8_1 and P9_2) were finally excluded from the analysis since all categories but one accounted for less than 10% of observations. Finally, the MCA analysis was performed on 21 variables: 18 active and three categorical supplementary variables (study area, years of professional experience and professional typology). The number of included categories per variable ranged between 2 and 4, resulting in a total of 56 active categories and a mean number of 15.8 observations per category. The variability (i.e, the inertia) behind our data was described by 38 MCA dimensions.

In order to explore the relationship between opinions and perceptions of interviewed people, the first three were extracted from the MCA results (fig 2), retaining overall the 19% of the total variability. The first two MCA dimensions, the most important in explaining the variability in the data, were mainly defined by the perception on the seriousness of bTB (P9_3) and opinions on the reliability of bTB diagnostic tests (i.e, SITT and γ-interferon) (P5_2, P5_1 & P6_1) (Fig. 3).

The first two MCA dimensions separated the active variable categories in three groups, suggesting the existence of different profile of responses (i.e., groups of individuals incline to taking the same categories as response). The first dimension divided positive and negative attitudes toward the bTB eradication programme; the second opposes people who tend to express their opinions with those who tend to non-respond, indicating that people who did not answer to one question tend to do the same for the others. The third MCA dimension revealed another group of profile responses: who tend to remain neutral.

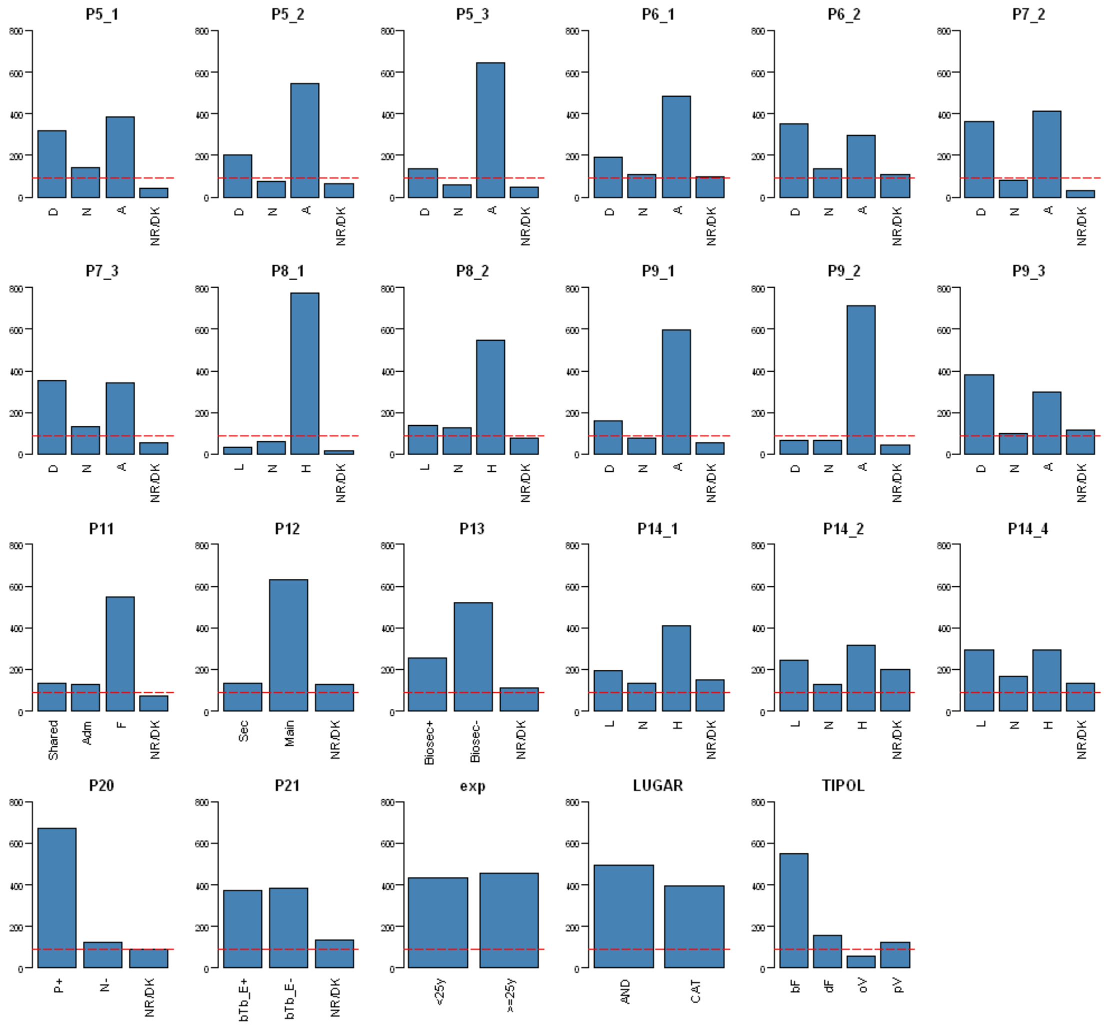



Figure 1: Barplots of variables that show the number of observations for each variable's category. The red dotted line indicates the 10% of observations

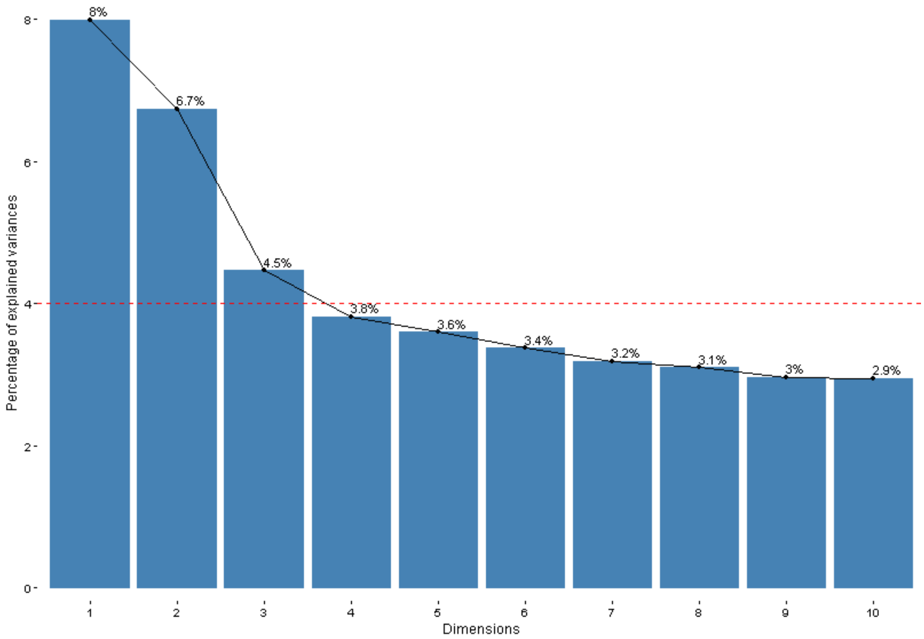



Figure 2: Scree plot showing the percentages of inertia explained by the top 10 principal MCA dimensions with a red dashed line specifying the point at which the scree plot shows a bend ( “elbow”)

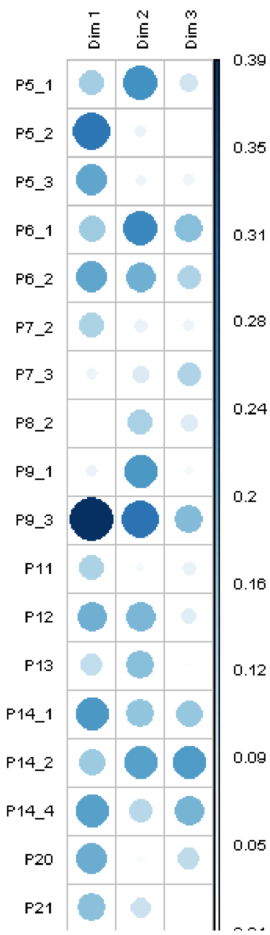
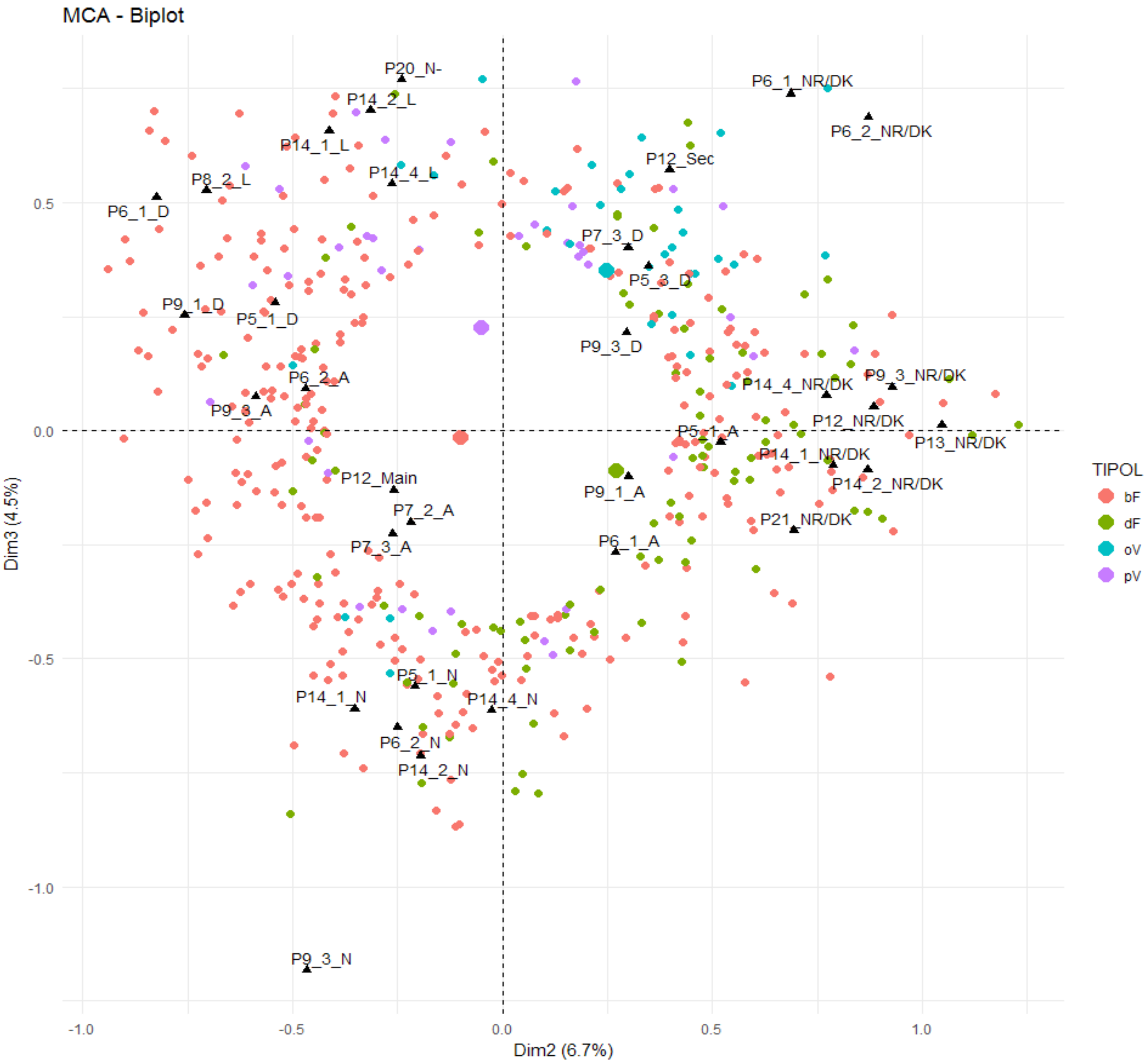



Figure 3: Corelogram plot that show the most contributing variables for each retained dimension

Biplots representing the variables categories together with individuals were used to interpret the shape of the clouds of points generated as results of the MCA analysis and the professional group was used to colour individuals (Fig. 4a, 4b, 4c). On the first two MCA dimensions, the vast majority of veterinarians was grouped in the forth quadrant of the map (i.e., people who tend to express their opinions and tend to have a positive attitude toward the bTB eradication programme) (Fig. 4a, upper left). In particular, this group was characterised by the perception of the seriousness and importance of bTB (P9_3_D, P9_1_A), by the opinion that wildlife reservoirs have a secondary role in the maintenance of the disease (P12_secondary) and by attitudes of trust toward the bTb routine tests (P5_1_A, P5_2_D, P5_3_D). The proximity of private (pV) and official veterinarians (oV) suggested a similar profile of responses, although the oV formed a close-knit group more than the pV. Differently, farmers presented a more disperse distribution and its shape (i.e., arch effect), suggested the presence of “polarized” profiles toward extreme answers; with; this polarization was not observed in the oV group. In particular, farmers occupied quadrants 1 and 2 of the map (Fig. 4a); the quadrants 1 (Fig. 4a, upper right), composed by both beef and dairy farmers, was characterized by the tendency to non-respond, especially on the role of other domestic reservoirs (P14_1_NR/DK, P14_2_NR/DK, P14_4_NR/DK), the perception on the seriousness and importance of bTB (P9_3_NR/DK) and on the achievement of the bTB eradication (P21_NR/DK). Whereas, the quadrant 2 (Fig. 4a, lower right) of the map, was characterised predominantly by beef farmers who distrust in the SITT (P5_1_D, P5_2_A) and other bTB diagnostic methods (P6_1_D, P6_2_A), perceived that oV have a low level of competence (P8_2_L) and who have a low risk perception on bTB (P9_1_D, P9_3_A). On the third dimension (Fig. 4-b and 4c, lower left) are grouped people, mainly farmers, who expressed neutrality toward the reliability of diagnostic test (P5_1_N, P6_2_N) and the role of other domestic bTb reservoirs (P14_4_N, P14_1_N, P14_2_N). Moreover, the first and third dimensions (Fig. 4c) highlighted that very few individuals, mainly oV, considered as negative the patronage relationship between farmers and pV (P20_N-) and that wildlife reservoir play a secondary role in the maintenance and transmission of bTB. In addition, it evidenced that very few people, mainly farmers, remained neutral about the idea that bTB is not a serious disease and it is just an excuse to reduce the cattle population in the Southern countries (fig P9_3_N).


MCA - Biplot
Dim1 (8%)
Dim2 (6.7%)
TIPOL
bF
dF
oV
pV
P13_NR/DK
P9_3_NR/DK
P12_NR/DK
P6_2_NR/DK
P14_2_NR/DK
P14_1_NR/DK
P14_4_NR/DK
P6_1_NR/DK
P21_NR/DK
P20_NR/DK
P5_1_A
P12_Sec
P5_3_D
P9_3_D
P5_2_D
P6_2_D
P7_3_D
P9_1_A
P6_1_A
Adm
P7_2_D
P13_Biosec+
P5_3_A
F
P5_2_A
P20_N-
P12_Main
P7_2_A
P7_3_A
P6_2_A
P5_1_D
P9_3_A
P8_2_L
P9_1_D
P6_1_D

**a)**


MCA - Biplot
Dim2 (6.7%)
Dim3 (4.5%)
TIPOL
bF
dF
oV
pV
P20_N-
P6_1_NR/DK
P6_2_NR/DK
P14_2_L
P14_1_L
P14_4_L
P8_2_L
P6_1_D
P12_Sec
P7_3_D
P5_3_D
P9_1_D
P5_1_D
P9_3_D
P6_2_A
P9_3_A
P14_4_NR/DK
P9_3_NR/DK
P12_NR/DK
P13_NR/DK
P5_1_A
P14_1_NR/DK
P14_2_NR/DK
P12_Main
P9_1_A
P7_2_A
P21_NR/DK
P7_3_A
P6_1_A
P5_1_N
P14_1_N
P14_4_N
P6_2_N
P14_2_N
P9_3_N
0.5
0.0
-0.5
-1.0
1.0

**b)**


**c)**

Figure 4: Graphical representation (biplot) of variable categories and individuals showing a global pattern within the data. Columns (variable categories) are represented by black triangles. Rows (individuals) are represented by points with different colours according to the supplementary qualitative variable “professional typology”: in red, beef farmers (bF); in green, dairy farmers (dF); in light blue, official vets (oV) and in purple, private vets. Individuals (row points) with similar profile are closed on the factor map. The same holds true for the variables categories (column points). Only the most contributing 35 variables categories and 450 individuals are shown in the biplot; the first two MCA dimensions are shown in figure 4a, the second and the third in figure 4b and the first and third dimensions in figure 4c.

***Hierarchical Clustering on Principal Components (HCPC)*** was performed on the first 30 MCA dimensions. Doing that, we included the 88% of the information on the system (i.e., percentage of cumulative variance).

The perception on the seriousness and the impact of the bTB, the attitude toward the diagnostic bTB tests and the opinions on the importance of other domestic reservoirs were the variables which characterize most the partition in three clusters and each of the clusters was characterized by a category of these variables (p-values < 0.0001).

*A*ccording to the inertia gains between partitions, three clusters were detected (fig. 5). The first cluster was composed by 346 individuals, the second by 323 and the third by 217. Individuals were graphically displayed trough a factor map according to their coordinates on each dimension, shaped according to the professional group and coloured according to the cluster they belonged to (fig. 5).

People grouped in the first cluster were aware of the zoonotic risk of bTB and disagreed on the idea that bTB is not a serious disease and that it is just an excuse to reduce the cattle population in the Southern countries (P9_3_D & P9_1_A). Moreover, they expressed positive opinions on the reliability of the diagnostic bTB tests, especially in the SITT (P5_2_D, P5_3_D, P5_1_A) and did not perceived the cost of the γ-INF as a limitation (P6_2_D). In this cluster, we found assembled the vast majority of interviewed veterinarians (i.e., 91% oV and 86% pV), though also 24% of bF and 38% of dF were grouped here. The second cluster was characterized by exactly opposite opinions to the ones expressed from people in the cluster one. The 82% (out of 323) of individuals in this cluster were bF, of which the 69% (out of 264) where from HPA, and other 12% were dF. It is worth to note that also four oV and 15 pV were grouped here. In the third cluster were together people who did not respond to the questions on wildlife and other domestic bTB reservoirs and to the questions on the γ-INF test. They were almost exclusively farmers, especially dF but also bF (i.e., 36% and 29% of interviewed, respectively) from both prevalence areas.

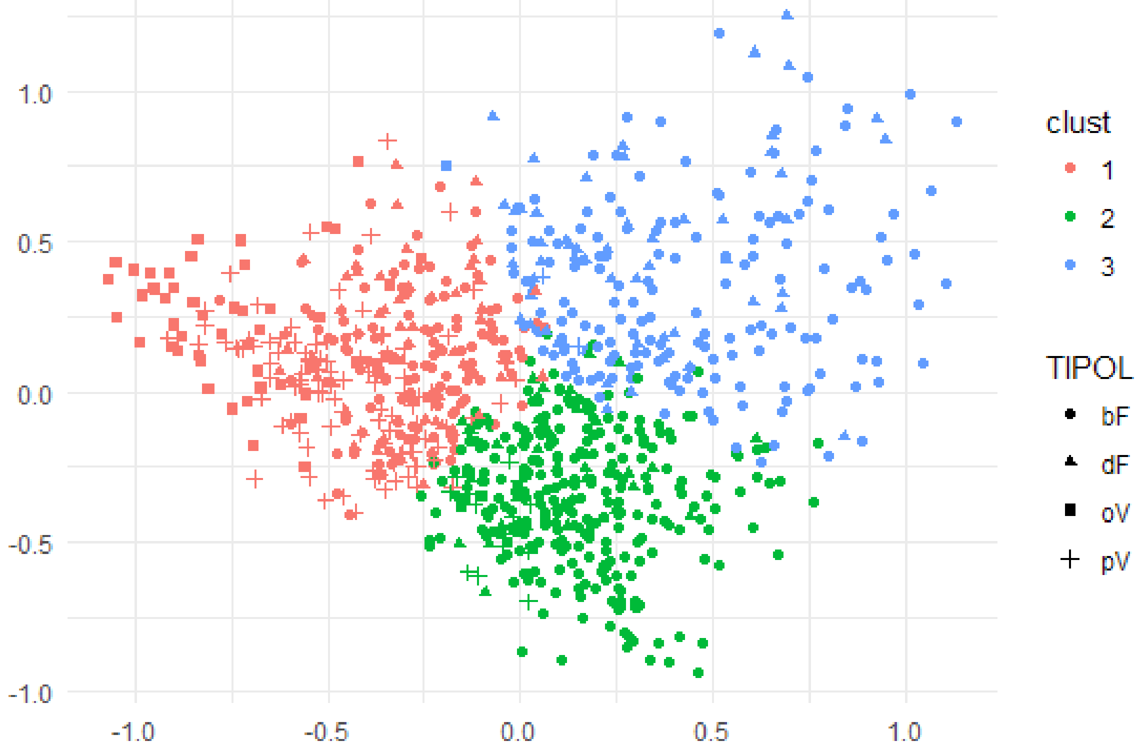



Figure 5: Factor maps of identified clusters. Each cluster is represented by a different colour. The dimentions showed in the map correspond to the first two principal components (i.e., dimensions one and two).

### 6.4.4. Evaluation of the main aspects in which the opinion of veterinarians and farmers might differ

The final selected model included 18 main effects, six interactions with prevalence area, two interactions with experience and four interactions between predictors. The total number of observations used by the model was 652 and 234 were deleted due to missingness. The McFadden's pseudo $R^2$ was 0.74, which indicates that the final model explained a high percentage of the variance in the data. The Area under the curve was 97.48% (95%CI: 96.45% - 98.51%) indicative of a model with very good ability to discriminate between veterinarians and farmers opinions. Results from the final model are shown in table 3.

Model results evidenced that farmers and veterinarians mainly differed in their attitude and opinions toward the bTB detection and control activities of the bTB eradication campaign. One of the main differences regarded the potential false positive results of the SITT (P5_2). In absence of visible lesions at the post-mortem examination (slaughterhouse), the probability of considering cattle tested positive by SITT as false-positive was 8.7 times higher (IC95%: 4.1 - 19.4) in farmers than in veterinarians.

Moreover, compared to farmers, veterinarians significantly disagreed on the possibility to verify positive bTB test results in independent laboratories (Tab. 3 - P5_3), on the increase of the interval time for routine screening in LPA (Tab. 3 - P7_2) and on the lack of transparency of the Central Veterinaries Services in the communication of bTB test results (Tab. 3 - P7_3).

In addition, with regard to the perception on the reliability of the SITT (P5_1 + interactions), we found that the attitude toward the test was linked with the study area and the opinion on the role of the wildlife animals as bTB reservoirs. In particular, in HPA, veterinarians agreed on the reliability of the SITT significantly more than farmers among those who attributed to wildlife reservoirs a secondary role (OR: 56; p-value $< 0.0001$) or among who did not respond to these questions (OR: 39; p-value $< 0.05$), while there were no differences among who believe that wildlife play a main role. Whereas, in LPA, the only significant difference was among people attributing to wildlife animals a main role; in this group, veterinarians trust in the SITT less than farmers (OR: 0.06; p-value $< 0.001$).

The attitude toward the existence of effective biosecurity measures (P13 + interactions) to prevent bTb transmission was influenced by the opinion on the competence level of pV and the prevalence in the area. Only in HPA, among those who thought that pV have a low competence level, veterinarians trusted in the existence of effective biosecurity measures more than farmers, and the divergence in opinion was most pronounced with the increase of the professional experience (exp = $<$25y: OR: 16.9, p-value $< 0.05$; exp = $>=$25y: OR: 50.6, p-value $< 0.01$).

With regard to the importance of other domestic reservoirs as source of bTB infections, we found significant differences on the perception of the role of pigs in extensive farming systems (P14_2 & interactions) only in LPA, where, compared to farmers, veterinarians had a probability 7.1 times higher (p-value 0.03) of attributing to this specie a low importance. Moreover, differences were found on the perception of cattle from other regions as source of bTB infections. Despite the prevalence in the area and the years of professional experience, veterinarians attributed significantly more importance to imported cattle than farmers (Tab. 3 - P14_4).

| *Var_names* | *Coefficients* | *Std. Error* | *OR* | *IC5% (2.5%)* | *IC95% (97.5%)* | *Significance level* |
|---|---|---|---|---|---|---|
| (Intercept) | 1.76 | 1.31 | 5.82 | 0.39 | 71.94 | |
| P5_2A | -2.16 | 0.39 | 0.12 | 0.05 | 0.24 | *** |
| P5_1A | 4.03 | 1.04 | 56.18 | 7.69 | 471.05 | *** |
| P5_3A | -1.05 | 0.42 | 0.35 | 0.15 | 0.80 | * |
| P20N- | 1.88 | 0.54 | 6.56 | 2.34 | 19.77 | *** |
| P20NR/DK | 0.18 | 0.89 | 1.20 | 0.21 | 6.92 | |
| P12Main | -0.34 | 0.64 | 0.71 | 0.20 | 2.52 | |
| P12NR/DK | -4.30 | 1.20 | 0.01 | 0.00 | 0.13 | *** |
| P9_1A | 0.11 | 0.39 | 1.12 | 0.52 | 2.42 | |
| P9_2A | -0.14 | 1.15 | 0.87 | 0.10 | 9.32 | |
| P9_3A | -0.42 | 0.53 | 0.66 | 0.23 | 1.84 | |
| P9_3NR/DK | -1.51 | 1.31 | 0.22 | 0.01 | 2.20 | |
| P7_2A | -1.52 | 0.38 | 0.22 | 0.10 | 0.46 | *** |
| P7_3A | -0.82 | 0.39 | 0.44 | 0.20 | 0.94 | * |
| P8_1H | -2.81 | 1.46 | 0.06 | 0.00 | 1.05 | . |
| P13Biosec- | -2.83 | 1.18 | 0.06 | 0.01 | 0.55 | * |
| P13NR/DK | -4.49 | 10.99 | 0.01 | 0.00 | 133.59 | |
| P14_2H | 0.81 | 0.46 | 2.25 | 0.92 | 5.61 | . |
| P14_2NR/DK | -1.74 | 1.59 | 0.18 | 0.00 | 2.55 | |
| P14_4H | 1.38 | 0.39 | 3.96 | 1.86 | 8.82 | *** |
| P14_4NR/DK | -0.43 | 1.20 | 0.65 | 0.05 | 6.05 | |
| P21bTb_E- | 2.60 | 1.08 | 13.44 | 1.80 | 133.54 | * |
| P21NR/DK | 2.12 | 2.57 | 8.30 | 0.05 | 639.03 | |
| P14_1H | -0.29 | 0.38 | 0.75 | 0.35 | 1.55 | |
| P14_1NR/DK | -0.74 | 1.04 | 0.48 | 0.06 | 3.58 | |
| LUGARCAT | 2.04 | 1.00 | 7.71 | 1.14 | 57.36 | * |
| exp>=25y | -0.06 | 1.23 | 0.94 | 0.08 | 10.56 | |
| P14_2H:LUGARCAT | -2.78 | 0.89 | 0.06 | 0.01 | 0.34 | ** |
| P14_2NR/DK:LUGARCAT | 0.82 | 1.92 | 2.27 | 0.07 | 145.21 | |
| P20N-:LUGARCAT | 4.86 | 1.32 | 129.64 | 11.04 | 1.99E+03 | *** |
| P20NR/DK:LUGARCAT | -20.42 | 2972.12 | 0.00 | 0.00 | 4.62E+41 | |
| P9_3A:LUGARCAT | -21.33 | 1488.79 | 0.00 | 0.00 | 1.58E+16 | |
| P9_3NR/DK:LUGARCAT | -18.73 | 2356.88 | 0.00 | 0.00 | 2.76E+30 | |
| P5_1A:LUGARCAT | -3.28 | 0.91 | 0.04 | 0.01 | 0.21 | *** |
| P5_1A:P12Main | -3.56 | 1.07 | 0.03 | 0.00 | 0.22 | *** |
| P5_1A:P12NR/DK | -0.37 | 1.67 | 0.69 | 0.03 | 19.04 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| P21bTb_E-:LUGARCAT | -1.84 | 0.79 | 0.16 | 0.03 | 0.71 | * |
| P21NR/DK:LUGARCAT | -13.80 | 2017.00 | 1.01E-06 | 1.31E-318 | 1.55E+27 | |
| P13Biosec-:LUGARCAT | 1.84 | 0.88 | 6.29 | 1.15 | 37.38 | * |
| P13NR/DK:LUGARCAT | 1.00 | 2.64 | 2.72 | 0.02 | 394.14 | |
| P8_1H:exp>=25y | -1.62 | 1.24 | 0.20 | 0.02 | 2.27 | |
| P13Biosec-:exp>=25y | -1.10 | 0.89 | 0.33 | 0.06 | 1.88 | |
| P13NR/DK:exp>=25y | -2.93 | 2.88 | 0.05 | 1.38E-04 | 6.81 | |
| P8_1H:P13Biosec- | 2.17 | 1.20 | 8.73 | 0.88 | 100.81 | . |
| P8_1H:P13NR/DK | 3.51 | 10.92 | 33.47 | 4.53E-03 | 9.31E+05 | |
| P9_2A:P8_1H | 3.17 | 1.31 | 23.89 | 1.94 | 334.17 | * |
| P9_2A:P21bTb_E- | -2.31 | 1.12 | 0.10 | 0.01 | 0.81 | * |
| P9_2A:P21NR/DK | -20.11 | 1687.84 | 1.85E-09 | 9.82E-273 | 4.16E+18 | |

Table 3: Results of the GLM. For each variable in the table: coefficients, ORs and relative 95% Confindence Interval are shown.
Significance level codes: '***': p-value<0.001; '**': 0.001< p-value < 0.01; '*' 0.01 < p-value < 0.05; '.' 0.05< p-value < 0.1.

Significant divergence in opinions between farmers and veterinarians were also observed about the "patronage relationship" between farmers and pV (P20): compared to farmers, veterinarians had a negative perception of it and this divergence was much greater in LPA (OR: 0.15, p-value < 0.01) than in HPA (OR: 0.001, p-value <0.0001).

Significant differences in opinions on future perspective (P21& interactions) were found when taking into account the prevalence in the area and the perception of the commercial impact of bTB. In HPA, among people that have a low perception of the commercial impact of bTB, veterinarians did not believe in achieving the bTb eradication significantly more than farmers (OR: 13.4; p-value<0.05). Whereas in LPA, a slight significant difference between farmers and veterinarians was found among people that have a high perception of the commercial impact of bTB, in this case veterinarians believed more than farmers in the achievement of bTb eradication (OR: 0.21; p-value < 0.05).

In turn, differences between farmers and veterinarians regarding the perceived benefits of eradicate bTb because of its commercial impact (P9_2 & interactions) appeared influenced by the opinion on the level of experience and competence of pV. Only among those who thought that pV have a high competence level and believed in the achievement of the bTb eradication, veterinarians agree on the commercial benefit of being bTb free more than farmers (OR: 20.7; p-value < 0.01). Whereas, among those who attributed a low competence level to pV and did not believe in the eradication, veterinarians perceived significantly less than farmers the importance of being bTb-free to avoid restrictions in export and animal movement (OR: 0.09; p-value < 0.05).

## 6.5. Discussion

The study here presented is part of a multidisciplinary investigation involving sociologist, veterinarians and epidemiologists, aimed to characterize attitudes and opinions of farmers and veterinarians towards the bTB programme. In a previous work, Ciaravino et al., (2017) identified, through qualitative methodologies, the most relevant opinions circulating among farmers and veterinarians in relation to the bTB control and infection. With the present study, by developing a structured questionnaire based on previous results, we investigated those opinions through different quantitative methodologies. The combination of qualitative and quantitative methods adds breadth to the research as both methodologies explore different aspects of the same problem (Brannen, 2005; Casebeer & Verhoef, 1997; Kelle, 2006; Sale, 2002). Therefore, a multidisciplinary approach, combining both qualitative and quantitative methods, allows the study of phenomena at different levels ensuring that study findings are ground in people' experiences (Brannen, 2005; Morgan, 1998).

The questionnaire was conducted by telephone and we obtained a lower response rate among veterinarians than among farmers, especially in LPA. This result might be due to time constraints (i.e., lack of time, time issues, time commitments), since veterinarians are already pushed for time during their average working week; or it might indicate unwillingness to answer because of some kind of pressure due to the sensitiveness and the complexity of this topic (i.e., do not want to talk about bTB and its control). Differently to what reported by other authors (Enticott et al., 2015; O'Hagan et al., 2016), we obtained a 100% response rate from farmers, highlighting the will of the farming community in Spain to talk about bTB and the eradication programme and to share their opinions.

The analysis of the results evidenced the existence of three different opinion profiles mostly differentiated by the attitude toward the SITT, the perception on the seriousness and the zoonotic impact of bTB and the perceived importance of other domestic reservoirs with regard to the maintenance of the disease. Consequently, these profiles were linked with positive and negative attitudes towards the bTB eradication programme and the level of compliance with it.

Among farmers certain variability in the responses profile was observed and the existence of opposite opinions arose, whereas the group of veterinarians, especially the official one, was more homogeneous. The existence of different attitudes toward the control of infectious diseases among farmers has been already described (Ellis-Iversen et al., 2010) and, among other factors, it was related to the perception of extrinsic barriers to the diseases control (e.g., financial constraints, lack of knowledge and lack of standardized advises).

The vast majority of veterinarians expressed a general positive attitude toward the programme while the group of people with a negative attitude towards the bTB eradication program was composed mainly by beef farmers from HPA. However, it is worth to mention that some of interviewed farmers showed a positive attitude toward the bTB eradication programme, being closer to the opinions of veterinarians; and, on the other hand, few veterinarians showed the same negative attitude than farmers, which deserves further attention due to their potential influence in the correct application of the bTB control program. These people (i.e., negative attitude) strongly distrust in the SITT, were low aware on the seriousness of bTB and its zoonotic impact and were inclined to attribute more importance to other species than cattle in the bTB transmission. Contrary to previous studies (O'Hagan et al., 2016), the control of bTB was not perceived as a priority by these farmers and the only appreciated impact of bTB was linked to the costs for its detection and control (i.e., payment of test and movement restrictions). Our results suggest that people with this negative attitude, mostly farmers, perceived the control of bTB as something out of their hands, depending from "external source" (i.e., wildlife, other domestic species not subjected to bTB testing or imported cattle). In agreement with other studies (Enticott, 2008; Enticott et al., 2015; Robinson, 2017b), this feeling together with a lack of trust in the diagnostic tests generated demotivation towards the application of control measures, fatalistic attitudes toward the disease and the perception that bTB is mostly a "political" problem (e.g., "bTb is not a serious disease but it is just an excuse to reduce cattle from southern Europe"). Previous studies (Ciaravino et al., 2017; Enticott et al., 2015; Robinson, 2017a) highlighted that farmers live as a lottery the routine screening; in line with these results, we observed that, independently of the current interval time set, farmers wanted to reduce the testing frequency; this might be due to the stress generated by the uncertain of test results, but also for the difficulty in implementing the SITT in the field (i.e., management of cattle especially in extensive farming systems) as documented by Calba et al. (2016). The third identified profile was represented by people, mainly farmers, who tended not to answer to questions on the role of wildlife and other domestic reservoirs, on the application of the γ-interferon assay and on the achievement of the bTB eradication. This might suggest they do not have a clear opinion or an adequate level of knowledge to answer on these topics; however, it might also indicate the unwillingness to respond, due to pressures related to the sensitivity of the topic and the vulnerability of the respondents (Dickson-Swift et al., 2008; Enticott et al., 2015). Moreover, farmers expressed the need for increasing their knowledge, since most of them reported to have not received any training on bTB in the last two years, and would like an easier access to information on bTB. Farmers identified their private veterinarian as the trustiest source of information, as already reported in farming communities (Ellis-Iversen et al., 2010; Enticott et al., 2015; Calba et al., 2016), and tended to have on them a significantly higher consideration than on official veterinarians, highlighting their influence in the application of field activities.

The observed distrust to the OVS, especially concerned the transparency in the communication of test results, it might also reduces the acceptability of the programme and the implementation of control measure (Broughan et al., 2016; Enticott et al., 2014). The lack of trust toward governmental institutions, mainly among farmers, has been reported elsewhere by other authors and it was linked to the level of confidence and acceptability of control interventions more than to the prevalence level in the area (Enticott, 2008; Christley et al., 2011; Broughan et al., 2016;).

In addition, previous studies have described that, even when the zoonotic risk of the disease is perceived, farmers may show resistance to the application of control measure due to the price and costs involved for the implementation of specific bTB control measures (Christley et al., 2011; O'Hagan et al., 2016) or based on other more general economic constrains (Ellis-Iversen et al., 2010). In line with these observations, we found that farmers tend to perceive the cost of the g-INF as an obstacle for its implementation (contrary to what the veterinarians believe), especially in HPA, and most people thought that farmers assume the majority of the programme's costs. The last might give rise to discontent among farmers that perceive the governmental institutions financially responsible for the control of disease (O'Hagan et al., 2016).

Interestingly, the perception on the reliability of the SITT was also linked with the perception on wildlife animals as bTB reservoir; the more importance was attributed to wildlife, the more likely respondents did not trust in the SITT. The vast majority of interviewed (i.e., mainly farmers and private veterinarians) opined that wildlife reservoirs have a main role in the transmission of the disease and felt to be under bTB threat due to wildlife more than to other cattle. Moreover, we observed this attitude independently of the study areas, although, in Spain, the presence and role of wildlife animals is not homogeneous, with more evidence of the involvement of these species in the west-southern areas (Gortázar et al., 2015, Guta et al., 2014). The same feelings toward wildlife have been described also in United Kingdom, where it has been demonstrated that badgers play a crucial role in maintenance of the bTB endemicity (Allen et al., 2018; Broughan et al., 2016; Enticott et al., 2015). Despite there are no doubt on the contribution of wildlife to the bTB maintenance, cattle and their movement remains the greatest concern for the achievement of eradication (Hardstaff et al., 2014), however, the risk represented by other cattle, imported from other regions or countries, was more perceived by veterinarians than farmers in our study.

Most but not all veterinarians perceived the SITT as a reliable test; official veterinarians were more aware than private veterinarians on the difficulties of its correct execution, on their side, private vets would prefer to use de CIDT as routing screening test. Major uncertainness arose when asking about possible false positive SITT results and about results' confirmation in independent laboratories, especially among private veterinarians in LPA. This might suggest that people (i.e., mainly farmers and some veterinarians) did not trust in the field screening tests and they perceived a test performed in a lab as more objective and reliable; as matter of fact, several interviewed reported a better opinion on the γ-INF than on the SITT. These opinions might be also linked to the predictive values of the SITT (especially in LPA); concerns about the accuracy of the diagnostic tests for bTB and the several factors that may affect their results are well described in literature (de la Rua-Domenech et al., 2006) and, although the application of the tuberculin test led to the bTB eradication in several areas, currently, the heterogeneity of the epidemiological situation highlight the need for the improvement in the diagnostic tools and the revision of testing procedures (Allen et al., 2018; Meskell et al., 2013; Schiller et al., 2011).

The results of our study highlight that, in Spain, education and communication are of paramount importance and both should be increased, especially focusing on the dynamics of bTB transmission, the interpretation and communication of test results and in the costs and benefits of the eradication. This is particularly relevant in the group with a negative attitude and in the one who tended not to answer to questions in order to increase motivation among people and take the fatalistic attitudes about bTB apart, as highlighted elsewhere (Constable et al., 2017; Ellis-Iversen et al., 2010; Calba et al., 2016; Robinson, 2017a; Enticott et al., 2015).

We believe to have provided a representative picture of the main opinions and attitudes toward bTB circulating among people involved in the eradication programme in Spain as the study population included two areas that strongly differ in terms of herd prevalence (i.e., 17.1% and 0.3% in Andalusia and Catalonia in 2016, respectively), abundance of wildlife (Gortázar et al., 2015) and type of farming systems (Garcia-Saenz, 2014). All professional typologies directly responsible for the programme implementation in the field were proportionally represented; we interviewed beef and dairy farmers, official veterinarians operating at province and district level and private accredited veterinarians authorized to perform the bTB routine testing. Despite of this, some bias associated to the method of conducting the questionnaire (i.e. telephone survey) might have been included. Collected data may underline selection bias (i.e., people without a fixed-line numbers were not interviewed and non-response errors), classification bias (i.e., questions made by different interviewers or inability of responders to answer correctly). Although bias cannot be completely controlled, the application of a computer-assisted telephone interviewing (CATI) technique is a useful and powerful tool for reducing some of these sources of bias (Sullivan, 1991). As matter of fact, the CATI system helped in the standardization of the interviews (i.e., digitally-recorded questionnaire), and in the interviewers' supervision and preparation of data sets (i.e., coding and cleaning). Moreover, it provided automated call scheduling and dialling and the non-response was followed-up (i.e., callback scheduling) (Anderson, 1991; Vasu & Garson, 1990; Tyebjee, 1979). In order to further minimize classification bias, the team of interviewers were trained on the disease and the questionnaire before starting the interviews and every question included an opt-out choice (i.e., do not know /

do not respond) that was not provided as category but available as option (e.g., in 5-points Likert scale we provided 5 response categories with "NR/DK" being the 6th option). The adoption of a probability sampling technique (i.e., stratified random sample) allowed us to numerically compare differences among farmers and veterinarians (i.e., test hypothesis related to their opinions) and make statistical inferences of results to the general population (Barnham, 2015).

Very few people answered to know the sector-specific legislation for bullfighting farms (i.e., 26% of interviewed). The low rate of answers may reflect the existence of bias due to an inappropriate question format or question context. It is likely that more people, than who responded, knew about the TB-testing exception for bulls older than 24 months, but they might not know that it was regulated by a specific legislation for bullfighting farms (i.e., apart from the national eradication programme), especially in areas where this farming type is not very common (i.e., LPA). As matter of fact, being most of bullfighting farms located in the south of the Country, it was the official veterinarians from HPA who most responded to know this legislation, whereas the less aware were beef farmers in LPA. Due to this, we opted to only describe these questions. In addition, the seven questions regarding the suggestions to improve the programme were excluded for other analysis than descriptive statistics since we considered these represented the consequence of what people opined about the different aspect of the bTB programme, already expressed through the other questions.

For data analysis, we used the MCA as an extension of the correspondence analysis, a descriptive technique on cross-tabulated data, and several descriptions on its properties are available in the literature (Greenacre, 1984; Greenacre, 1987; Torres & Greenacre, 2002; Greenacre, 2007; Hair et al., 2010). In particular, MCA allowed us to identify and interpret relations and variability among the opinions of farmers and veterinarians through the graphic representation in a low dimensional space of the distances or similarities between of both attribute ratings (variables categories) and subject profiles (interviewed profiles), allowing their representation (Torres & Van de Velden, 2007). Doing MCA, the only formal assumption is that the frequencies in the cross-table have to be positive numbers. However, variables' categories with very low frequencies and the presence of zero frequencies in cross-tabulations can distort the analysis results. On this regard, Di Franco and collaborators (2016), linked the number of observations needed to perform a robust MCA directly to the number of active variables' categories in the matrix table and suggested a threshold of 20 observations per active category. Moreover, the presence of "outliers" may also affect the interpretation of the MCA results, since they have high absolute co-ordinate values and high contributions thus they dominate the axes representations, leading the other points to be tightly clustered in the maps which become difficult to interpret (Bendixen, 2003). To overcome these problems the 5-points Likert scale variables were reclassified into fewer categories; moreover, two variables were excluded from this analysis since all categories but one accounted for frequencies lower than 10%. Doing so, we could obtain a quite robust MCA estimation (i.e., mean number of 15.8 observations per category) and reduce extreme responses.

Hierarchical Clustering on Principal Components (HCPC) was performed on 30 out of 38 dimensions obtained as results of the MCA. There is no standard rule or criteria that state how many MCA components per se have to be used afterward for the HPCP analysis, accordingly it is intended to select the components that explain as much variance as possible (Husson et al., 2010). However, we excluded the last 8 dimensions as we considered they would have incorporated only noise to the analysis (i.e. random variations), providing less stable clusters (Duda et al., 2001; Husson et al., 2010; Husson et al., 2014).

The multivariate regression model was obtained through an automated full IC-based method, available in the glmulti R package (Calcagno, 2013). We choose the AIC information criterion to compare models and, due to the higher number of possible predictors, we selected the option based on a genetic algorithm approach to explore the candidate set of models. Moreover, in some estimations more than in others, the model showed a lack of precision (i.e., large standard errors); this was due to the low number of observations of some variable categories and these results should be interpret with caution.

## 6.6. Conclusion

The combination of epidemiological and social methods allowed us to catch a significant variety of opinions and perceptions existent among farmers and veterinarians, highlighting its multi-faceted nature, similarities and differences. Our results may help decision-makers in identifying the most appropriate motivators for an enhanced bTB control.

Most people involved in the programme did not believe in the achievement of the bTB eradication and the presence of the disease was often perceived as determined by factors out of their control. The negative attitude toward the programme was mainly observed among farmers, though it was also expressed by few veterinarians. Even if not common, this attitude in official and private veterinarians deserve special awareness, since it may strongly influence the programme success, contributing in generate disbeliefs and amplify the existing demotivation among farmers.

The lack of information and low level of knowledge might contribute to generate a fatalistic attitude toward the disease and the bTB eradication programme, especially among farmers. Therefore, education and the communication should be improved and to invest in effective strategies should be considered as a priority. Particular attention should also be paid to the group of farmers that showed a positive attitude to the programme; they represent a key group for a better understanding of factors that may prevent or promote demotivation towards the bTB programme and for the implementation of effective communication and education campaigns (i.e., farmer-to-farmer programmes), leading to an earlier achievement of the bTB eradication.

## 6.7. References

Allen, A., Skuce, R., & Byrne, A. (2018). Bovine tuberculosis in Britain and Ireland–A Perfect Storm? The confluence of potential ecological and epidemiological impediments to controlling a chronic infectious disease. *Frontiers in Veterinary Science*, *5*, 109.

Anderson, R. E. (1991). Computer-assisted questioning (CAQ): Current trends and issues - A response to Shanks. In "*The information systems research challenge: Survey research methods, 3"* ed. Kenneth L. Kraemer, 237-44. Boston: Harvard Business School.

Anon. (2018). Programa Nacional de Erradicación de Tuberculosis Bovina presentado por España para el año 2018. Available online: https://www.mapama.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/pnetb_2018_tcm30-436761.pdf

Aranaz, Alicia, Cousins, D., Mateos, A., & Domínguez, L. (2003). Elevation of Mycobacterium tuberculosis subsp. caprae Aranaz et al. 1999 to species rank as Mycobacterium caprae comb. nov., sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, *53*(Pt 6), 1785–1789. https://doi.org/10.1099/ijs.0.02532-0

Bendixen, M. (1996). A practical guide to the use of correspondence analysis in marketing research. *Marketing Bulletin, 2003, 14, Technical Note 2*. Available at: http://marketing-bulletin.massey.ac.nz/V14/MB_V14_T2_Bendixen.pdf

Brannen, J. (2005) Mixing Methods: The Entry of Qualitative and Quantitative Approaches into the Research Process, *International Journal of Social Research Methodology, 8:3*, 173-184, DOI: 10.1080/13645570500154642

Broughan, J. M., Maye, D., Carmody, P., Brunton, L. A., Ashton, A., Wint, W., ... & Hinchliffe, S. (2016). Farm characteristics and farmer perceptions associated with bovine tuberculosis incidents in areas of emerging endemic spread.*Preventive veterinary medicine*, *129*, 88-98.

Burnham, K. P. & Anderson D. R.. (2002). Model selection and multimodel inference: a practical information-theoretic approach. Springer-Verlag New York, Inc - 2nd ed.

Calba, C., Goutard, F. L., Vanholme, L., Antoine-Moussiaux, N., Hendrikx, P., & Saegerman, C. (2016). The added-value of using participatory approaches to assess the acceptability of surveillance systems: the case of bovine tuberculosis in Belgium. *PLoS One*, *11*(7), e0159041.

Calcagno, V. & de Mazancourt, C. (2010) glmulti: An R Package for Easy Automated Model Selection with (Generalized) Linear Models. J. Stat. Soft. (34)12. DOI: 10.18637/jss.v034.i12

Calcagno, V. (2013). glmulti: Model selection and multimodel inference made easy. R package version 1.0.7. http://CRAN.R-project.org/package=glmulti

Casebeer, A. L., & Verhoef, M. J. (1997). Combining qualitative and quantitative research methods: Considering the possibilities for enhancing the study of chronic diseases. *Chronic diseases in Canada*, *18*(3), 130-135.

Christley, R. M., Robinson, S. E., Moore, B., Setzkorn, C., & Donald, I. (2011). Responses of farmers to introduction in England and Wales of pre-movement testing for bovine tuberculosis. *Preventive veterinary medicine*, *100*(2), 126-133.

Ciaravino, G., Ibarra, P., Casal, E., Lopez, S., Espluga, J., Casal, J., Napp, S., Allepuz, A. (2017) Farmer and Veterinarian Attitudes towards the Bovine Tuberculosis Eradication Programme in Spain: What Is Going on in the Field? Front Vet Sci. Nov 27;4:202. doi: 10.3389/fvets.2017.00202. eCollection 2017

Cvetnic, Z., Katalinic-Jankovic, V., Sostaric, B., Spicic, S., Obrovac, M., Marjanovic, S., … Vickovic, I. (2007). Mycobacterium caprae in cattle and humans in Croatia. *The International Journal of Tuberculosis and Lung Disease: The Official Journal of the International Union Against Tuberculosis and Lung Disease*, *11*(6), 652–658.

Constable, P. D., Hinchcliff, K. W., Done, S. H., & Grünberg, W. (2017). Systemic and Multi-Organ Diseases. In *Veterinary Medicine* (pp. 2002–2214), Eleventh Edition, Elsevier Health Sciences W.B. Saunders. https://doi.org/10.1016/B978-0-7020-5246-0.00021-8.

de la Rua-Domenech, R., Goodchild, A. T., Vordermeier, H. M., Hewinson, R. G., Christiansen, K. H., & Clifton-Hadley, R. S. (2006). Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. *Research in Veterinary Science*, *81*(2), 190–210. https://doi.org/10.1016/j.rvsc.2005.11.005

Dickson-Swift, V., James, E. L., & Liamputtong, P. (2008).*Undertaking sensitive research in the health and social sciences: Managing boundaries, emotions and risks*. UK: Cambridge University Press. doi:10.1017/CBO9780511545481

Di Franco, G. (2016). Multiple correspondence analysis: one only or several techniques? Quality & Quantity, 50(3), 1299–1315.

Duda, R. O., Hart, P. E., & Stork, D. G. (2001). Data description and clustering. In "*Pattern classification",* pp. 537-41, Duda, R. O., Hart, P. E., & Stork, D. G. (Rds.). New York: Wiley,.

Ellis-Iversen, J., Cook, A. J., Watson, E., Nielen, M., Larkin, L., Wooldridge, M., & Hogeveen, H. (2010). Perceptions, circumstances and motivators that influence implementation of zoonotic control programs on cattle farms. *Preventive veterinary medicine*, *93*(4), 276-285.

Enticott, G. (2008). The ecological paradox: social and natural consequences of the geographies of animal health promotion.*Transactions of the Institute of British geographers*, *33*(4), 433-446.

Enticott, G., Maye, D., Fisher, R., Ilbery, B., & Kirwan, J. (2014). Badger vaccination: dimensions of trust and confidence in the governance of animal disease. *Environment and Planning A*,*46*(12), 2881-2897.

Enticott, G. (2015). Public attitudes to badger culling to control bovine tuberculosis in rural Wales. *European Journal of Wildlife Research*, *61*(3), 387-398.

Enticott, G., Maye, D., Carmody, P., Naylor, R., Ward, K., Hinchliffe, S., ... & Upton, P. (2015). Farming on the edge: farmer attitudes to bovine tuberculosis in newly endemic areas. *Veterinary Record*, *177*(17), 439-439.

European Food Safety Authority (EFSA) & European Centre for Disease Prevention and Control (ECDC). (2017). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA Journal*, *15*(12). https://doi.org/10.2903/j.efsa.2017.5077

European Food Safety Authority - Panel on Animal Health and Welfare (EFSA AHAW). More, S., Bøtner, A., Butterworth, A., Calistri, P., Depner, K., … Bicout, D. (2017). Assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): bovine tuberculosis. *EFSA Journal*, *15*(8). https://doi.org/10.2903/j.efsa.2017.4959

Gabriel K.R. (1971). The biplot. graphical display of matrices with applications to principal component analysis. Biometrika, 58, pp. 453-467. CrossRefView Record in Scopus

Garcia-Saenz, A., Saez, M., Napp, S., Casal, J., Saez, J. L., Acevedo, P., ... & Allepuz, A. (2014). Spatio-temporal variability of bovine tuberculosis eradication in Spain (2006–2011). Spatial and spatio-temporal epidemiology, 10, 1-10.

Good, M., Bakker, D., Duignan, A., & Collins, D. M. (2018). The History of In Vivo Tuberculin Testing in Bovines: Tuberculosis, a "One Health" Issue. *Frontiers in Veterinary Science*, *5*. https://doi.org/10.3389/fvets.2018.00059

Good, M., & Duignan, A. (2011). Perspectives on the History of Bovine TB and the Role of Tuberculin in Bovine TB Eradication. *Veterinary Medicine International*, *2011*, 1–11. https://doi.org/10.4061/2011/410470

Gortázar, C., Che Amat, A., & O'Brien, D. J. (2015). Open questions and recent advances in the control of a multi-host infectious disease: animal tuberculosis: Recent advances in animal tuberculosis control. *Mammal Review*, *45*(3), 160–175. https://doi.org/10.1111/mam.12042

Greenacre, M. J. (1984). Theory and applications of correspondence analysis.

Greenacre, M., & Hastie, T. (1987). The geometric interpretation of correspondence analysis. *Journal of the American statistical association*, *82*(398), 437-447.

Greenacre M.J. (1993). Correspondence analysis in practice. Academic Press, London

Greenacre M.J. (2007). Correspondence Analysis in Practice. Chapman & Hall/CRC, Boca Raton, second edition.

Guta, S., Casal, J., Garcia-Saenz, A., Saez, J. L., Pacios, A., Garcia, P., … Allepuz, A. (2014). Risk factors for bovine tuberculosis persistence in beef herds of Southern and Central Spain. *Preventive Veterinary Medicine*, *115*(3–4), 173–180. https://doi.org/10.1016/j.prevetmed.2014.04.007

Hair, J. F., Black, W. C., Babin, B. And Anderson, R. (2010). Multivariate Data Analysis. 7th Edition. Upper Saddle River, Nj: Prentice Hall.

Hardstaff, J. L., Marion, G., Hutchings, M. R., & White, P. C. L. (2014). Evaluating the tuberculosis hazard posed to cattle from wildlife across Europe. *Research in Veterinary Science*, *97 Suppl*, S86-93. https://doi.org/10.1016/j.rvsc.2013.12.002

Humblet, M.-F., Boschiroli, M. L., & Saegerman, C. (2009). Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. *Veterinary Research*, *40*(5), 50. https://doi.org/10.1051/vetres/2009033

Husson, F., Josse, J., & Pages, J. (2010). Principal component methods-hierarchical clustering-partitional clustering: why would we need to choose for visualizing data. Applied Mathematics Department.

Husson, F., Josse, J., Le, S., & Mazet, J. (2013). FactoMineR: multivariate exploratory data analysis and data mining with R. R package version, 1, 102-123.

Husson, F., & Josse, J. (2014). Multiple correspondence analysis. In "*Visualization and Verbalization of Data"*, pp. 165-184. J. Blasius, M.J. Greenacre (Eds.), Visualization and Verbalization of Data, Chapman & Hall/CRC, London.

Husson, F., Josse, J., Le, S., & Mazet, J. (2016). FactoMineR: Multivariate Exploratory Data Analysis and Data Mining. R package version 1.32. http://CRAN.R-project.org/package=FactoMineR

Kassambara, A., & Mundt, F. (2016). factoextra: Extract and Visualize the Results of Multivariate Data Analyses. R package version 1.0.3. http://CRAN.R-project.org/package=factoextra

Kelle, U. (2006). Combining qualitative and quantitative methods in research practice: purposes and advantages. *Qualitative research in psychology*, *3*(4), 293-311.

Jackman, S. (2015). pscl: Classes and Methods for R Developed in the Political Science Computational Laboratory. Department of Political Science, Stanford University. Stanford, California. R package version 1.4.9. URL http://pscl.stanford.edu/

Lenth, R. (2018). Emmeans: Estimated marginal means, aka least-squares means. R Package Version 1.2. URL: https://github.com/rvlenth/emmeans

Lombardi, G., Botti, I., Pacciarini, M. L., Boniotti, M. B., Roncarati, G., & Dal Monte, P. (2017). Five-year surveillance of human tuberculosis caused by Mycobacterium bovis in Bologna, Italy: an underestimated problem. *Epidemiology and Infection*, *145*(14), 3035–3039. https://doi.org/10.1017/S0950268817001996

Meskell, P., Devitt, C., & More, S. J. (2013). Challenges to quality testing for bovine tuberculosis in Ireland; perspectives from major stakeholders. *Veterinary Record*, *173*(4), 94-94.

Morgan, D. L. (1998). Practical strategies for combining qualitative and quantitative methods: Applications to health research. *Qualitative health research*, *8*(3), 362-376.

O'Hagan, M. J. H., Matthews, D. I., Laird, C., & McDowell, S. W. J. (2016). Farmers' beliefs about bovine tuberculosis control in Northern Ireland. *The Veterinary Journal*, *212*, 22-26.

OIE, W. O. for A. H. (2015). Chapter 2.4.7: Bovine tuberculosis adopted. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2009*. Retrieved from http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.06_BOVINE_TB.pdf

Olea-Popelka, F., Muwonge, A., Perera, A., Dean, A. S., Mumford, E., Erlacher-Vindel, E., … Fujiwara, P. I. (2017). Zoonotic tuberculosis in human beings caused by Mycobacterium bovis-a call for action. *The Lancet. Infectious Diseases*, *17*(1), e21–e25. https://doi.org/10.1016/S1473-3099(16)30139-6

Palacios, J. J., Navarro, Y., Romero, B., Penedo, A., González, Á. M., Hernández, M. D. P., ... & Domínguez, L. (2016). Molecular and epidemiological population-based integrative analysis of human and animal Mycobacterium bovis infections in a low-prevalence setting. Veterinary microbiology, 195, 30-36.

Pesciaroli, M., Alvarez, J., Boniotti, M. B., Cagiola, M., Di Marco, V., Marianelli, C., … Pasquali, P. (2014). Tuberculosis in domestic animal species. *Research in Veterinary Science*, *97*, S78–S85. https://doi.org/10.1016/j.rvsc.2014.05.015

Rodríguez, S., Bezos, J., Romero, B., de Juan, L., Álvarez, J., Castellanos, E....Tuberculosis, M. o. (2011). Mycobacterium caprae Infection in Livestock and Wildlife, Spain. *Emerging Infectious Diseases, 17(3)*, 532-535. https://dx.doi.org/10.3201/eid1703.100618.

Sale, J. E., Lohfeld, L. H., & Brazil, K. (2002). Revisiting the quantitative-qualitative debate: Implications for mixed-methods research. *Quality and quantity*, *36*(1), 43-53.

SANCO WD. (2013). Working Document on Eradication of Bovine Tuberculosis in the EU. Accepted by the Bovine tuberculosis subgroup of the Task Force on Monitoring Animal Disease Eradication. Brussels SANCO/7059/2013.

Schiller, I., Oesch, B., Vordermeier, H. M., Palmer, M. V., Harris, B. N., Orloski, K. A., … Waters, W. R. (2010). Bovine tuberculosis: a review of current and emerging diagnostic techniques in view of their relevance for disease control and eradication. *Transboundary and Emerging Diseases*, *57*(4), 205–220. https://doi.org/10.1111/j.1865-1682.2010.01148.x

Schiller, I, Waters, W. R., RayWaters, W., Vordermeier, H. M., Jemmi, T., Welsh, M., … Oesch, B. (2011). Bovine tuberculosis in Europe from the perspective of an officially tuberculosis free country: trade, surveillance and diagnostics. *Veterinary Microbiology*, *151*(1–2), 153–159. https://doi.org/10.1016/j.vetmic.2011.02.039

Sullivan, M. (1991). Controlling non-response bias and item non-response bias using CATI techniques. *Retrieved January, 22*, 2009.

R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing; Vienna, Austria: 2010.

Revelle, W. (2015) psych: Procedures for Personality and Psychological Research, Northwestern University, Evanston, Illinois, USA, http://CRAN.R-project.org/package=psych Version = 1.5.8.

Robin, X., Turck, N., Hainard, A., Tiberti, N., Lisacek, F., Sanchez, G-C., & Müller, M. (2011). pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics, 12, p. 77. DOI: 10.1186/1471-2105-12-77

Robinson, P. A. (2017a). Farmers and bovine tuberculosis: Contextualising statutory disease control within everyday farming lives. *Journal of Rural Studies*, *55*, 168-180.

Robinson, P. A. (2017b). Framing bovine tuberculosis: a 'political ecology of health'approach to circulation of knowledge (s) about animal disease control. *The Geographical Journal*, *183*(3), 285-294.

Torres, A., & Greenacre, M. (2002). Dual scaling and correspondence analysis of preferences, paired comparisons and ratings. *International Journal of Research in Marketing*, *19*(4), 401-405.

Torres A. & van De Velden M., (2007). Perceptual mapping of multiple variable batteries by plotting supplementary variables in correspondence analysis of rating data. Food Qual Pref 18, 121-129. doi:10.1016/ j.foodqual.2005.09.005

Tyebjee, T. T. (1979). Telephone survey methods: The state of the art. *The Journal of Marketing*, 68-78.

Vasu, M. L., & Garson, G. D. (1990). Computer-assisted survey research and continuous audience response technology for the political and social sciences. *Social Science Computer Review*,*8*(4), 535-557.

Wickham, H.( 2009). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

# General discussion

## Chapter VII

The control of bTB in Spain started decades ago, but despite the fact that the strategies for its eradication have been progressively improved, the reality is that bTB not only remains endemic in Spain, but herd prevalence has increased in recent years.

The results shown in the present PhD thesis represent a step forward to understand the epidemiology of bTB in Spain and provide useful insights for the development of context-specific recommendations and enhanced control strategies that contributed to achieve the objective of bTB eradication.

Two main research lines guided the present PhD; on the one hand, we assessed the bTB transmission dynamics within Spanish cattle herds and we evaluated the bTB surveillance components in the country. On the other hand, we investigated different social factors that might have an influence on the success of the bTB eradication programme.

Mathematical models allow the simplified representation of a real-world phenomenon, and they have proven to be useful tools for understanding the complex dynamics of infectious diseases, allowing to obtain essential information on the key parameters on the infection dynamics, and to make prediction on the likely outcome of control interventions (Heesterbeek, 2002; Keeling et al., 2001; Wearing et al., 2005). Models are particularly helpful when processes under study are too complex, costly or time-consuming to be investigated through experimental or field studies.

Dynamic transmission models, rather than static, are the most suitable for evaluating pathogen's transmission and control interventions, since they take into account the rate of contact between individuals and changes over time in the risk of infection, being able to reproduce direct and indirect effects that may arise from a disease control program (Kretzschmar et al., 2009).

Dynamic models are divided into two main categories: compartmental and individual models. Compartmental dynamic models consider groups of individuals with no distinction between individuals within the same group, and they are among the most commonly used in epidemiology and health research (Homer & Hirsch, 2006; Sterman, 2006).

Individuals in the population are grouped into different “categories”, based on certain characteristics or health status (Kretzschmar et al., 2009; Koopman, 2004; Vynnycky & White, 2010).

A common type of dynamic compartmental transmission model is the so-called SEIR model, in which individuals transit between states, from susceptible (S), to exposed (E), to infected (I) and finally to removed (R) from the population (via immunity or death) (Anderson and May, 1991; Wearing et al., 2005; Vynnycky & White, 2010).

Compartmental models can be either stochastic or deterministic. Deterministic models work as fixed "clockwork" systems; it means that given the same starting conditions, the results obtained would always be the same, and that is not a realistic approximation of the dynamics of real pathogens (Keeling & Rohani, 2008).

Stochastic processes allow to reproduce the intrinsic variability of infectious diseases in the nature, thus, in principle, stochastic models are always more realistic than deterministic. Moreover, under specific circumstances stochasticity is essential, such us small populations, rare diseases or when there is an interest in studying the eradication of a disease (Keeling & Rohani, 2008; Vynnycky & White, 2010).

Even though heterogeneities are common in the real world, compartmental models usually assume homogenous mixing in the population. Accounting for some extra heterogeneity is possible, for example by including age or risk structured classes, with the advantage of increasing accuracy of estimations. However, including heterogeneities inevitably leads to an increased number of equations, since it would require many extra components, with a consequent increase of complexity and the need of precise data available (Vynnycky & White, 2010).

In contrast to compartmental models, individual-based models can easily incorporate heterogeneity, such as spatial local networks and diverse individual behaviours; thus, they may provide accurate predictions. However, there are a number of disadvantages to individual-based models over compartmental models, including slower speed, lack of analytical tractability, computational intensity and challenges in parameterization, with the need of strong assumptions (Vynnycky & White, 2010).

As a result, a tension exists in all dynamic models to make them both simple enough to be computationally stable and feasible, and complex enough not to misrepresent what is going on in the real world. Finding the appropriate balance between accuracy and simplicity is difficult. Accuracy generally improves with increasing model complexity, and the inclusion of biological detail.

Understanding the dynamics of bTB spread within cattle herds is essential for an effective management and control of the disease; though, it is hindered by factors such as the slow progress with variable rate of progression, the long incubation periods and the intermittent shedding (Brooks-Pollock et al., 2014). Therefore, dynamic modelling represents an extremely useful methodology to assess bTB transmission in a cost-effective way (Conlan et al., 2012; Brooks-Pollock et al., 2014; Álvarez et al., 2014). In addition, dynamic models may provide useful insights for the design of targeted strategies to reduce the time needed for both the detection of infected herds and the elimination of the disease from affected herds. This is reflected by the increased use of such methods for the study of the bTB spread within and between herds or to evaluate the effectiveness of control measures and the effect of varying control strategies (Álvarez et al., 2014).

Different methodological approaches have been used to evaluate within-herd transmission, among them deterministic models (Barlow et al., 1997; O'Hare et al., 2014), individual-based models (Álvarez et al., 2012; Fischer et al., 2005; Perez et al., 2002) and stochastic compartmental models. (Barlow et al., 1997; Bekara et al., 2014; Conlan et al., 2012; Smith et al., 2013). However, results are hardly comparable due to the different methodologies applied, modelling assumptions made and the heterogeneity of context-specific factors (Álvarez et al., 2014; Bekara et al., 2014).

In this PhD thesis, we developed a compartmental stochastic model (SOEI), describing conceptually the progression of bTB in cattle, in order to evaluate bTB transmission within Spanish cattle herds, by using comprehensive field data from the Spanish eradication campaign. The model allowed us to assess the variation in the cattle-to-cattle transmission rate (i.e., transmission coefficient) and the rate at which infected cattle become infectious (i.e., duration of latent period).

According to our estimations, the introduction of one infected animal into a herd would lead to a median of 5.2 newly infected animals per infectious cattle per year. We observed a high variability in the transmission coefficient, despite the fact that all farms included in the study were cattle beef farms in extensive management systems, mostly from high prevalence areas, in fact average estimates for individual herds were as low as 2.7 (IQR: 1.7 – 3.2) or as high as 7.9 (IQR: 6.4 – 8.9).

The median duration of the latent period (i.e. from the infection of an animal until it becomes infectious) was 3.2 months, with an interquartile range (IQR) varying from 2.4

and 5.4 months, which means a relatively narrow length range compared to values reported in literature.

A wide range factors, which may contribute to the observed variation variations in bTB transmission dynamics, have been described in literature (Menzies & Neill, 2000; Goodchild & Clifton-Hadley, 2001; Pollock & Neill, 2002; Álvarez et al., 2014). Many of these reflect the biological heterogeneity at individual-level which is not modifiable (e.g. age, genetic) or only partly modifiable (e.g. immune response). However, the observed variability in bTB transmission may also be the result of factors related to herd management practices, such as internal biosecurity, infrastructures and housing condition; or it might reflects differences in behaviour and "social ranking" of infected cattle (Menzies and Neill, 2000; Goodchild and Clifton-Hadley, 2001); the influence of such aspects on the within-herd bTB transmission rate deserves further investigation since its understanding may provide helpful insights for the development of more appropriate and targeted control strategies.

Our results indicate that the transmission parameters for bTB spread within Spanish herds were highly variable. Therefore, models and the conclusions derived from them for bTB control, need to take into account that variability. Otherwise, the measures applied may not be able to achieve the desired objectives. For some herds, parameter estimates showed posterior distributions narrower than the assumed priors, indicating that our results provided more accurate estimates than those reported in literature; however, for other herds, estimates were not very informative. Still, there is a lot of uncertainty associated to those parameters and further research is necessary for both the increase the precision of estimations and the identification of major sources of heterogeneity to be considered, in order to provide recommendations and useful information to support the decision-making process.

Clearance of bTB from the herds is often a lengthy process that results in serious economic burden for both the farmers and the Public Administration. The basic reproduction ratio (R0) is the most widely used parameter in epidemic theory and it is an essential tool for understanding the behaviour of infectious diseases. The developed model also allowed us to calculate the average number of secondary cases caused by a single infected animal introduced into a fully susceptible herd (i.e. a proxy of $R_0$, which we called "Within-herd transmission potential Number" ($R_h$).

Considering annual intervals testing, as for the majority of herds in Spain, the overall mean value of new infections would remain below zero (0.82). Still, there is a 51% probability of transmission between an infectious and susceptible cattle occurs, which would increase the probability of missing at least one of the infected animals, and lead to longer time for bTB elimination from the herd. The $R_h$ estimates increased proportionally to the testing frequency, and testing intervals above one per year would Rh values above one (2.01 and 3.47 for testing intervals of 2 and 4 years, respectively).

The compartmental stochastic model previously developed was modified to allow the evaluation the efficiency of bTB surveillance in Spain. The routine SITT testing of cattle herds, together with slaughterhouse surveillance (all cattle intended for human consumption) and the pre-movement testing of traded cattle represent the three major components of the bTB surveillance system implemented in the country.

In Spain, routine testing showed to be the most efficient component, with a sensitivity of bTB detection (i.e. probability of detection per year) of 74.2% and mean time until detection of about 7 months. In the current situation in Spain, this component remains essential for the achievement of the eradication of bTB. In contrast, the efficiency of slaughterhouse surveillance and pre-movement testing was much lower, with mean times to detection by any of these two components of about 27 months. The sensitivity of pre-movement testing was slightly higher than that of slaughterhouse surveillance (i.e. 7% and 4.8%, respectively). Our results highlight that the frequency of routine controls is the most influential factor on the efficiency of routine testing and, consequently, on the efficiency of the whole bTB surveillance system.

Besides the well-known heterogeneity in the distribution of bTB in Spain (Allepuz et al., 2011; García-Saenz et al., 2014), we identified a high heterogeneity in the efficiency of bTB surveillance in the country. However, the level of efficiency in bTB surveillance in a given province did not necessarily correspond with its level of bTB prevalence. It means that a lot of efforts are being used to control bTB in provinces with low prevalence, while little efforts are being used in some provinces with high bTB prevalence.

In order to improve the detection of infected herds, and eventually contribute to an early eradication of bTB, it is essential to allow more flexibility for allocating resources where they are most needed. In fact, the Spanish eradication program includes the application of reinforced measures in areas of high prevalence. Still, our analysis identified some provinces with very low efficiency of surveillance despite their high prevalence.

Control strategies against bTB need to be relentlessly pursued and continually reviewed, according to the changing epidemiological situations, in order to ensure their effectiveness. Our results provide essential information for the assessment and design of enhanced bTB control strategies. The evaluation of the surveillance components at local scale represents an essential initial step toward the development of bTB elimination strategies adapted to the local context, considering the prevalence and the specific characteristics of farms in the area (i.e. type, sizes, trade and culling rates).

The application of traditional quantitative epidemiological methods to the prevention and control of infectious diseases strongly contributes to the understanding of disease patterns and risk factors, among other biological features. However, as previously stated (EFSA AHAW Panel, 2014), the occurrence and distribution of diseases may also be influenced by several non-biological factors that can be more effectively explored by qualitative methods.

It is known that the engagement of stakeholders and the level of acceptability of health interventions are key factors for the success of control programmes and surveillance systems in animal health (Moda et al. 2006; Pfeiffer, 2006). Acceptability has been defined as the “willingness of persons and organizations to participate in the surveillance system” and it refers to the degree to which each of these people is involved in the surveillance (Calba et al., 2016; German et al., 2001). Therefore, attitudes and opinions may have a strong impact on the social acceptance at local level of animal health programmes and are a key point that policy makers should consider when designing and implementing disease management policy.

Due to the multi-factorial nature and the complex epidemiology of bTB, a full understanding of bTB dynamics inevitably requires multi-sectorial knowledge and taking into account, among other factors, the sociological context, which leads to a multi-disciplinary research approach (Zinsstag et al., 2015). A multidisciplinary approach to tackling zoonotic diseases is in line with the One Health Concept (http://www.onehealthinitiative.com) that aims to join disciplines to solve complex problems (Katinka de Balogh, personal communication). Bovine TB provides a perfect one-health model and it should be investigated through a holistic approach (Zinsstag et al.2006).

In last years, the need of integrating sociological factors in the study of the bTB epidemiology has been reflected in the increasing number of publications which highlighted the influence of such factors on control programmes (Brennan et al., 2016; Broughan et al., 2016; Catley et al., 2012; Enticott et al., 2015; McAloon et al., 2017; Moda et al. 2006; Pfeiffer, 2013). In this PhD we investigated, for the first time in Spain, sociological aspects that may have an influence on the implementation of the bTB eradication program with the collaborative support of sociologists and anthropologists. The research was carried out in three different study-steps, using at first qualitative techniques followed by the application of quantitative methods. This methodology allowed us to catch data from local field-contexts, that otherwise would have been missed; as well as, the use of semi-structured questionnaires ensured us to gather information on perception and opinions which cannot be easily captured by closed questions (Alonso & Benito, 1998; Jost et al., 2007; Mariner & Paskin, 2000; Pfeiffer, 2013); but also, the analysis of quantitative data enabled us to measure the influence of different factors and infer the results to the general population.

The logic underlying qualitative studies differs from that of quantitative research; however, regardless of their differences, both perspectives have to do with the nature of reality. These approaches do not have to be necessarily considered as diametric opposite; instead, there might be much overlap between; and, combining qualitative and quantitative techniques, referred to as ‘mixed methods’ (Creswell, 2017), offer the advantage to generate complementary knowledge for the early achievement of a common goal.

Several qualitative methods have been described in sociological investigations ranging from life-history interviews to direct observation or participant observation. Ethnography underpinned the data collection and all stages of our qualitative research; perceptions, opinions and beliefs circulating in the discourses of interviewed people were identified through a method based on the grounded theory approach (Starrin et al., 1997; Strauss, 1987).

The word ethnography has Greek origins, from the words “ethnos” (people) and “graphei” (to write) and it literally means “to write about people or cultures” (Brannen, 2005). We used this method because it is optimal in research where people interviewed may tend to disguise their way of acting and / or thinking. In addition, ethnography focuses on people in their usual environment and context, and it includes the description and analysis of social relations within groups of people: social, professional or conceptual (Eriksen, 2001). Thus, this methodology is highly suitable if the research objectives are to describe how people works and/or to explore their beliefs, behaviours and also issues faced by specific groups in their daily activities or life (Creswell, 2007).

On the other hand, quantitative data were analysed by using frequency statistics and logistic regressions. Besides, we used the Multiple Correspondence Analysis (MCA), at hand before applying a Hierarchical Clustering on Principal Components, which allowed us to assess the existence and characterize specific "clusters of opinions" or profiles among farmers and veterinarians. The MCA is part of factor analysis methods and allows summarizing a set of categorical variables through the construction of principal components (Jobson, 1992; Tenenhaus & Young, 1985). It is one of the several extensions of the correspondence analysis, used when data includes more than two categorical variables; when all variables are binary, a MCA is equivalent to a principal component analysis (Greenacre 1984). This descriptive and exploratory technique displays rows and columns in contingency table as points in a multidimensional space, allowing its visualization in a small number of orthogonal principal components (i.e., graphical displays in axes). The MCA allowed us to summarise the proximities between respondents and to determine the existent relationship among the opinions of farmers and veterinarians (i.e., the associations between variables categories). Then a hierarchical classification from these principal components allows determining clusters and this statistical approach led us to the identification of specific patterns opinions profiles.

With regard to opinions of farmers and veterinarians toward the Spanish bTB eradication program, the reliability of bTB ante-mortem diagnostic tests, especially the SITT; the perception on the seriousness of bTB and benefits of its eradication; and, the role of wildlife and other domestic reservoirs have been identified as key issues that might hinder the success of the bTB eradication program in Spain.

The above-mentioned themes also mostly contributed to characterize the three different profiles of opinions. In addition to people with positive or negative attitude towards the eradication programme, we also identified a third profile group that showed a clear tendency to not responding and which deserve further attention.

Another main fact to underline is that current controls measures against bTB are often perceived as a law enforcement duty without an adequate motivation of people involved in the bTB eradication programme. Moreover, the complexity of the bTB epidemiology combined with low levels of knowledge on the disease contribute to generate, especially among farmers, disbelief in control measures and feelings of distrust towards Public Veterinary Services and regulatory bodies.

In view of these findings, the development of effective communication strategies to increase knowledge on bTB, motivation and trustworthiness among people involved in the bTB eradication campaign appears extremely important for a proper and effective control of the disease as well as for increasing the acceptability of the bTB eradication programme in Spain.

Overcoming demotivation and dynamics of distrust towards public veterinary services and regulatory bodies is necessary and it is worth to be tried. Our results might provide useful hints for implementing new strategies aimed to increase farmers and veterinarians' awareness and engagement; as already reported in other Countries (Calba et al., 2016; Ellis-Iversen et al., 2010; Enticott et al., 2015). We found that farmers consider their private veterinarian as primary source of information, thus private veterinarians might be privilege interlocutors for increasing farmer's motivation and knowledge. On this point of view, the existence of negative attitudes among veterinarians is a concern and deserves very particular attention.

In addition, we found that the feeling of distrust towards official veterinarians, which was mainly observed among farmers and sometimes among private veterinarians, is most likely due to a lack of official veterinary service's support in the field; it is therefore essential to strengthen communication and collaboration between private and official veterinarians. Finally, we identified a group of farmers with a positive attitude towards the bTB eradication programme which deserves special consideration, since it may as flywheel for other farmers, having a strong influence on their attitude towards the programme.

However, the design of strategic communication plans deserves further investigations and it should be object of future research projects. In particular, it would be interesting to identify the most appropriate approach for both changing individuals' motivation and building trusting relationships in the Spanish context. At the same time, to explore strategies for increasing the active participation of farmers to the programme would help in the development of targeted motivational interventions and effective information programmes and it is crucial to understand how to deliver knowledge and information to the different stakeholders.

Until today in Spain, control strategies and health interventions have been mostly implemented under the conventional “top-down” approach (Reason & Heron, 1986) with public veterinary services mainly playing a sanctioning role; however, as our results pointed out, a supportive and advisory role by official veterinarians is also needed, and a combination of disciplinary and supportive actions should be provided. Moreover, an increased presence of official veterinarians in the field in supports to the activities of private veterinarians and farmers, rather than only to control them, would help to enhance relationships and communications between groups.

As matter of fact it was expressed, especially among farmers, the need for informal meetings with private and public veterinarians where people can openly discuss about the disease and its control in an atmosphere of dialogue and resolution of doubts. The implementation of participatory processes through a “bottom - up approach” (Reason & Heron, 1986) can be a beneficial and valuable tool for giving voice to the different stakeholders involved in the programme (Catley et al., 2012; Pfeiffer, 2013), identifying local needs, problems and priorities (Jost et al., 2007; Mariner & Paskin, 2000). Moreover, since participatory approaches are based on a high level of community participation on decision processes and design of health interventions, they facilitate the acceptability of control measure and ensure commitment of participating people (Jost et al., 2007; Mariner & Paskin, 2000; Pfeiffer, 2013,).

Participatory epidemiology (PE) is a branch of epidemiology applied to public health and animal health that has evolved from the principles and methods of Rapid Rural Appraisal (RRA) and Participatory Rural Appraisal (PRA) (Mariner & Paskin, 2000). PE aims to provide in depth analysis of locally identified contexts; although PE can also produce quantitative information, the flexible nature of a participatory approach fit better to qualitative analysis techniques. A wide range of methods are available and can be summarized into four main groups: i) informal semi-structured interviews; ii) focus-group discussions; iii) ranking and scoring methods; iv) and visualisation and diagramming techniques (Jost et al., 2007).

Even so, to change individuals’ motivation and dynamics of distrust are significant stumbling blocks and changes are further complicated by the characteristic of bTB. Substantial improvements in knowledge on bTB have been made, however concerns remain regarding the epidemiology and immunology of the disease. Furthermore, bTB is a chronic process and the immune response that follows infection is complex, therefore some diagnostic aspects are difficult to be explained and technical limitations exist. These aspects may hamper motivation and commitment of people and, consequently, reduce the effectiveness of participatory processes and communication campaigns. In addition, the failure of bTB eradication programs, in spite of the huge amount of resources and time dedicated to the eradication of the disease, could have also generated a firm opposition in some people, who will not change their attitudes.

Besides such limitations, the development strategies to enhance awareness and motivation would be extremely useful for the group of people that did not respond in order to increase their knowledge on the identified key themes. Moreover, these strategies may have an indirect positive effect on the general farmers' confidence toward public veterinary services, increasing, in turn, both the acceptability of control plans for other diseases and the compliance with prevention practices.

## 7.1 References

Allepuz, A., Casal, J., Napp, S., Saez, M., Alba, A., Vilar, M., ... & Alvarez, J. (2011). Analysis of the spatial variation of Bovine tuberculosis disease risk in Spain (2006–2009). Preventive veterinary medicine, 100(1), 44-52.

Alonso, L. E., & Benito, L. E. A. (1998). La mirada cualitativa en sociología: una aproximación interpretativa (Vol. 218). editorial Fundamentos.

Álvarez, J., Perez, A. M., Bezos, J., Casal, C., Romero, B., Rodriguez-Campos, S., ... & Domínguez, L. (2012). Eradication of bovine tuberculosis at a herd-level in Madrid, Spain: study of within-herd transmission dynamics over a 12-year period. BMC veterinary research, 8(1), 100.

Álvarez, J., Bezos, J., de la Cruz, M. L., Casal, C., Romero, B., Domínguez, L., … Pérez, A. (2014). Bovine tuberculosis: within-herd transmission models to support and direct the decision-making process. Research in Veterinary Science, 97 Suppl, S61-68. https://doi.org/10.1016/j.rvsc.2014.04.009

Anderson, R.M. & May, R.M., 1991. Infectious diseases of humans: dynamics and control. Oxford University Press, New York.

Barlow, N.D., Kean, J.M., Hickling, G., Livingstone, P.G., Robson, a B., 1997. A simulation model for the spread of bovine tuberculosis within New Zealand cattle herds. Prev Vet Med 32, 57–75. doi:10.1016/S0167-5877(97)00002-0

Bekara, M. E. A., Courcoul, A., Benet, J. J., & Durand, B. (2014). Modeling tuberculosis dynamics, detection and control in cattle herds. PloS one, 9(9), e108584. doi:10.1371/journal.pone.0108584

Brannen, J. (2005) Mixing Methods: The Entry of Qualitative and Quantitative Approaches into the Research Process, International Journal of Social Research Methodology, 8:3, 173-184, DOI: 10.1080/13645570500154642

Brennan, M. L., Wright, N., Wapenaar, W., Jarratt, S., Hobson-West, P., Richens, I. F., ... & O'Connor, H. M. (2016). Exploring attitudes and beliefs towards implementing cattle disease prevention and control measures: A qualitative study with dairy farmers in Great Britain. Animals, 6(10), 61. doi:10.3390/ani6100061

Brooks-Pollock, E., Roberts, G.O., Keeling, M.J., 2014. A dynamic model of bovine tuberculosis spread and control in Great Britain. Nature 511, 228–231. doi:10.1038/nature13529

Broughan, J. M., Maye, D., Carmody, P., Brunton, L. A., Ashton, A., Wint, W., ... & Hinchliffe, S. (2016). Farm characteristics and farmer perceptions associated with bovine tuberculosis incidents in areas of emerging endemic spread. Preventive veterinary medicine, 129, 88-98.

Calba, C., Goutard, F. L., Vanholme, L., Antoine-Moussiaux, N., Hendrikx, P., & Saegerman, C. (2016). The added-value of using participatory approaches to assess the acceptability of surveillance systems: the case of bovine tuberculosis in Belgium. PLoS One, 11(7), e0159041.

Catley, A., Alders, R. G., & Wood, J. L. (2012). Participatory epidemiology: approaches, methods, experiences. The Veterinary Journal, 191(2), 151-160.

Conlan, A.J.K., McKinley, T.J., Karolemeas, K., Pollock, E.B., Goodchild, A. V., Mitchell, A.P., Birch, C.P.D., Clifton-Hadley, R.S., Wood, J.L.N., 2012. Estimating the Hidden Burden of Bovine Tuberculosis in Great Britain. PLoS Comput. Biol. 8. doi:10.1371/journal.pcbi.1002730

Creswell, J. W., & Creswell, J. D. (2017). Research design: Qualitative, quantitative, and mixed methods approaches. Sage publications.

Creswell, J. W., Hanson, W. E., Clark Plano, V. L., & Morales, A. (2007). Qualitative research designs: Selection and implementation. The counseling psychologist, 35(2), 236-264.

Enticott, G., Maye, D., Carmody, P., Naylor, R., Ward, K., Hinchliffe, S., ... & Upton, P. (2015). Farming on the edge: farmer attitudes to bovine tuberculosis in newly endemic areas. Veterinary Record, 177(17), 439-439.

Ellis-Iversen, J., Cook, A. J., Watson, E., Nielen, M., Larkin, L., Wooldridge, M., & Hogeveen, H. (2010). Perceptions, circumstances and motivators that influence implementation of zoonotic control programs on cattle farms. Preventive veterinary medicine, 93(4), 276-285.

Eriksen, T. H. (2001). Small Places, Large Issues-: An Introduction to Social and Cultural Anthropolog. Pluto Press.

European Food Safety Authority - Panel on Animal Health and Welfare (EFSA AHAW). (2014). Statement on a conceptual framework for bovine tuberculosis. EFSA Journal, 12(5). https://doi.org/10.2903/j.efsa.2014.3711

Fischer, E.A.J., Van Roermund, H.J.W., Hemerik, L., Van Asseldonk, M.A.P.M., De Jong, M.C.M., 2005. Evaluation of surveillance strategies for bovine tuberculosis (Mycobacterium bovis) using an individual based epidemiological model. Prev. Vet. Med. 67, 283–301. doi:10.1016/j.prevetmed.2004.12.002

García-Saenz, A., Saez, M., Napp, S., Casal, J., Saez, J.L., Acevedo, P., Guta, S., Allepuz, A., 2014. Spatio-temporal variability of bovine tuberculosis eradication in Spain (2006-2011). Spat Spatiotemporal Epidemiol. Jul;10:1-10.

German, R. R., Lee, L. M., Horan, J., Milstein, R., Pertowski, C., & Waller, M. (2001). Updated guidelines for evaluating public health surveillance systems. Center for Disease Control and Prevention (CDC), MMWR Recomm Rep, 50(1-35).

Goodchild, A. V., & Clifton-Hadley, R. S. (2001). Cattle-to-cattle transmission of Mycobacterium bovis. Tuberculosis (Edinburgh, Scotland), 81(1–2), 23–41. https://doi.org/10.1054/tube.2000.0256

Greenacre, M. J. (1984). Theory and applications of correspondence analysis. Academic Press, London, UK. doi:10.1016/S0167-8116(02)00101-5.

Heesterbeek, J. A. P. (2002). A brief history of R 0 and a recipe for its calculation. Acta biotheoretica, 50(3), 189-204.

Homer, J. B., & Hirsch, G. B. (2006). System dynamics modeling for public health: background and opportunities. American journal of public health, 96(3), 452-458.

Jobson, J. D. (1992). Principal components, factors and correspondence analysis. In Applied multivariate data analysis(pp. 345-482). Springer, New York, NY.

Jost C, Jc M, Pl R, E S, Gj M-S. Participatory epidemiology in disease surveillance and research. Rev Sci Tech (2007) 26:537–549. Available at: http://europepmc.org/abstract/med/18293603%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/18293603

Keeling, M. J., & Rohani, P., 2008. Modeling infectious diseases in humans and animals. Princeton University Press, New Jersey.

Keeling, M. J., Woolhouse, M. E., Shaw, D. J., Matthews, L., Chase-Topping, M., Haydon, D. T., ... & Grenfell, B. T. (2001). Dynamics of the 2001 UK foot and mouth epidemic: stochastic dispersal in a heterogeneous landscape. Science, 294(5543), 813-817.

Koopman, J. (2004). Modeling infection transmission. Annu. Rev. Public Health, 25, 303-326.

Kretzschmar, M., & Wallinga, J. (2009). Mathematical models in infectious disease epidemiology. In "Modern Infectious Disease Epidemiology", pp. 209-221, Kramer A, Kretzschmar M, Krickenberg K, (eds.), Springer, New York, NY.

McAloon, C. G., Macken-Walsh, Á., Moran, L., Whyte, P., More, S. J., O'Grady, L., & Doherty, M. L. (2017). Johne's disease in the eyes of Irish cattle farmers: a qualitative narrative research approach to understanding implications for disease management. Preventive veterinary medicine, 141, 7-13.

Mariner JC, Paskin R. Manual on participatory epidemiology: methods for the collection of action-oriented epidemiological intelligence. (2000). Available at: http://www.fao.org/docrep/003/X8833E/X8833E00.HTM

Menzies, F.D., & Neill, S.D., 2000. Cattle-to-Cattle Transmission of Bovine Tuberculosis. Vet. J. 160, 92–106. doi:10.1053/tvjl.2000.0482

Moda G. Non-technical constraints to eradication□: The Italian experience. (2006) 112:253–258. doi:10.1016/j.vetmic.2005.11.021

O'Hare, A., Orton, R.J., Bessell, P.R., Kao, R.R., 2014. Estimating epidemiological parameters for bovine tuberculosis in British cattle using a Bayesian partial-likelihood approach. Proc. R. Soc. B 281.

Pérez, A.M., Ward, M.P., Charmandarián, A., Ritacco, V., 2002. Simulation model of within-herd transmission of bovine tuberculosis in Argentine dairy herds. Prev. Vet. Med. 54, 361–372. doi:10.1016/S0167-5877(02)00043-0

Pfeiffer, D. U. (2006). Communicating risk and uncertainty in relation to development and implementation of disease control policies. Veterinary microbiology, 112(2-4), 259-264.

Pfeiffer DU. Epidemiology caught in the causal web of bovine tuberculosis. Transbound Emerg Dis (2013) 60:104–110. doi:10.1111/tbed.12105

Pollock, J.M., & Neill, S.D., 2002. Mycobacterium bovis Infection and Tuberculosis in Cattle. Vet. J. 163, 115–127. doi:10.1053/tvjl.2001.0655

Reason, P., & Heron, J. (1986). Research with people: The paradigm of cooperative experiential inquiry. Person-Centered Review, Vol.1, 4. Pp. 456-476.

Smith, R.L., Schukken, Y.H., Lu, Z., Mitchell, R.M., Grohn, Y.T., 2013. Development of a model to simulate infection dynamics of Mycobacterium bovis in cattle herds in the United States. J. Am. Vet. Med. Assoc. 243, 411–423. doi:10.2460/javma.243.3.411

Starring, B., Dahlgren, L., Larsson, G., & Styrborn, S. (1997). Along the path of discovery. Quantitative methods and grounded theory. Lund, Sweden: Studentlitteratur.

Sterman, J. D. (2006). Learning from evidence in a complex world. American journal of public health, 96(3), 505-514.

Strauss, A.L., (1987). Qualitative analysis for social scientists. doi:10.1017/CBO9780511557842

Tenenhaus, M., & Young, F. W. (1985). An analysis and synthesis of multiple correspondence analysis, optimal scaling, dual scaling, homogeneity analysis and other methods for quantifying categorical multivariate data. Psychometrika, 50(1), 91-119.

Vynnycky, E., & White, R., 2010. An introduction to infectious disease modelling. Oxford University Press, New York.

Wearing, H.J., Rohani, P., Keeling, M.J., 2005. Appropriate models for the management of infectious diseases. PLoS Med. Jul;2(7):e174. Epub 2005 Jul 26. Erratum in: PLoS Med. 2005 Aug;2(8):e320.

Zinsstag, J., Schelling, E., Waltner-Toews, D., Whittaker, M., & Tanner, M. (Eds.). (2015). One Health: the theory and practice of integrated health approaches. Wallingford: CABI. https://doi.org/10.1079/9781780643410.0000.

Zinsstag, J., Schelling, E., Roth, F., & Kazwala, R. (2006). Economics of Bovine Tuberculosis. In " Mycobacterium bovis Infection in Animals and Humans", pp. 68–83. Thoen, C. O., Steele, J. H., & Gilsdorf, M. J. (Eds.), 2nd Edition. Oxford, UK: Blackwell Publishing Ltd. https://doi.org/10.1002/9780470344538.ch9

# General conclusions

## Chapter VIII

1. The median overall cattle-to-cattle transmission coefficient for Spanish cattle herds was 5.2 newly infected animals per infectious cattle per year. However, among herds, median estimates of this parameter varied significantly, from 1.8 to 8.3 new infections per infectious cattle per year.

2. Considering annual intervals for the routine skin testing, the average number of secondary cases caused by a single infected animal introduced into a fully susceptible herd remained slightly below one. This value increased to 2 and 3.5 with testing intervals of 2 and 4 years, respectively, which, therefore, would not be effective to control bTB in Spain.

3. The routine testing was found to be the most efficient surveillance component in Spain, while slaughterhouse surveillance and pre-movement testing only contributed to the detection of a small proportion of infected herds. The frequency of routine testing was the most influential parameter in the efficiency of the bTB surveillance system.

4. Among Spanish provinces, the performance of the bTB surveillance system was highly heterogeneous with no clear spatial pattern. In many provinces, the surveillance intensity was not appropriate to the prevalence level in cattle herds. The allocation of resources for the control of bTB should take into account such heterogeneities in order to improve the cost-efficiency of the bTB eradication programme.

5. With regard to the opinions of farmers and veterinarians towards the Spanish bTB eradication programme, most people do not believe in the achievement of the eradication and, especially among farmers, the presence of the disease is often regarded as an event that is out of their control.

6. Personal relationships between farmers and veterinarians have a major role in the implementation of herd-level health interventions. Private veterinarians represent key interlocutors for raising farmers' awareness and their compliance to the programme. Synergistic collaborations between private and official veterinarians are also crucial to ensure a proper implementation of bTB control measures.

7. Among veterinarians, some negative attitudes toward the bTB eradication programme exist and deserve special consideration, as it may reinforce scepticism about the bTB control and increase demotivation among farmers. Particular attention should also be paid to farmers that show a positive attitude towards the programme, since they may represent a key group for the implementation of effective farmer-to-farmer programmes, leading to an earlier achievement of the bTB eradication.

8. There is a general need to improve the level of knowledge and the flow of information among people involved in the campaign. The development of effective communication strategies should be a priority in order to increase the motivation of farmers and veterinarians, to ensure the acceptability of the bTB eradication programme, and to avoid a fatalistic attitude toward the disease and its control.

# Appendix

## Chapter IX

# Annex A – Supplemetary Information Study I

## ASSESSING THE VARIABILITY IN TRANSMISSION OF BOVINE TUBERCULOSIS WITHIN SPANISH CATTLE HERDS

Ciaravino G., García-Saenz A., Cabras S., Allepuz A., Casal J., García-Bocanegra I., De Koeijer A., Gubbins S., Sáez J.L., Cano-Terriza D., Napp S.*

Summary of the posterior MCMC distributions of the β parameter obtained for each of the 22 study-herds

| Herds' ID | Herds' size (cattle heads) | Mean | Quantiles | | | | |
|---|---|---|---|---|---|---|---|
| | | | *5%* | *25%* | *50%* | *75%* | *95%* |
| 1 | 51 | 0.0126 | 0.0014 | 0.0058 | 0.0119 | 0.0191 | 0.0258 |
| 2 | 213 | 0.0077 | 0.0006 | 0.0021 | 0.0048 | 0.0115 | 0.0239 |
| 3 | 130 | 0.0175 | 0.0081 | 0.0130 | 0.0176 | 0.0223 | 0.0265 |
| 4 | 94 | 0.0138 | 0.0016 | 0.0069 | 0.0137 | 0.0207 | 0.0263 |
| 5 | 45 | 0.0103 | 0.0009 | 0.0036 | 0.0083 | 0.0163 | 0.0251 |
| 6 | 31 | 0.0158 | 0.0051 | 0.0105 | 0.0159 | 0.0213 | 0.0262 |
| 7 | 78 | 0.0092 | 0.0024 | 0.0046 | 0.0073 | 0.0123 | 0.0224 |
| 8 | 121 | 0.0217 | 0.0135 | 0.0191 | 0.0226 | 0.0253 | 0.0272 |
| 9 | 156 | 0.0207 | 0.0124 | 0.0174 | 0.0212 | 0.0245 | 0.0270 |
| 10 | 31 | 0.0175 | 0.0073 | 0.0130 | 0.0177 | 0.0223 | 0.0265 |
| 11 | 65 | 0.0108 | 0.0034 | 0.0058 | 0.0089 | 0.0147 | 0.0239 |
| 12 | 43 | 0.0089 | 0.0007 | 0.0025 | 0.0062 | 0.0143 | 0.0248 |
| 13 | 57 | 0.0067 | 0.0012 | 0.0029 | 0.0053 | 0.0089 | 0.0178 |
| 14 | 82 | 0.0139 | 0.0016 | 0.0072 | 0.0138 | 0.0206 | 0.0262 |
| 15 | 113 | 0.0140 | 0.0017 | 0.0072 | 0.0139 | 0.0208 | 0.0261 |
| 16 | 34 | 0.0138 | 0.0016 | 0.0070 | 0.0136 | 0.0205 | 0.0262 |
| 17 | 71 | 0.0162 | 0.0065 | 0.0114 | 0.0160 | 0.0210 | 0.0261 |
| 18 | 67 | 0.0167 | 0.0049 | 0.0113 | 0.0172 | 0.0227 | 0.0266 |
| 19 | 70 | 0.0209 | 0.0119 | 0.0176 | 0.0217 | 0.0248 | 0.0271 |
| 20 | 90 | 0.0141 | 0.0017 | 0.0074 | 0.0141 | 0.0211 | 0.0263 |
| 21 | 26 | 0.0103 | 0.0018 | 0.0051 | 0.0090 | 0.0144 | 0.0233 |
| 22 | 136 | 0.0177 | 0.0088 | 0.0134 | 0.0177 | 0.0222 | 0.0264 |

Table S1: Quantiles and measures of central tendency describing the posterior distributions of the bTB transmission coefficient (β) obtained analysing each of the study-herds separately. The sizes of each herd (number of cattle heads) and its ID number are also shown in the table

Summary of the posterior MCMC distributions of the $\alpha_1$ parameter obtained for each of the 22 study-herds

| Herds' ID | Herds' size (n) | Mean | Quantiles | | | | |
|---|---|---|---|---|---|---|---|
| | | | *5%* | *25%* | *50%* | *75%* | *95%* |
| 1 | 51 | 0.078 | 0.022 | 0.047 | 0.078 | 0.110 | 0.136 |
| 2 | 213 | 0.080 | 0.022 | 0.048 | 0.081 | 0.112 | 0.137 |
| 3 | 130 | 0.082 | 0.023 | 0.050 | 0.083 | 0.114 | 0.137 |
| 4 | 94 | 0.080 | 0.022 | 0.048 | 0.079 | 0.112 | 0.137 |
| 5 | 45 | 0.079 | 0.022 | 0.047 | 0.078 | 0.111 | 0.137 |
| 6 | 31 | 0.079 | 0.021 | 0.047 | 0.079 | 0.111 | 0.136 |
| 7 | 78 | 0.078 | 0.021 | 0.045 | 0.077 | 0.110 | 0.136 |
| 8 | 121 | 0.088 | 0.028 | 0.061 | 0.091 | 0.119 | 0.138 |
| 9 | 156 | 0.084 | 0.024 | 0.054 | 0.086 | 0.116 | 0.137 |
| 10 | 31 | 0.080 | 0.023 | 0.049 | 0.081 | 0.110 | 0.136 |
| 11 | 65 | 0.081 | 0.022 | 0.049 | 0.080 | 0.113 | 0.137 |
| 12 | 43 | 0.080 | 0.022 | 0.048 | 0.081 | 0.111 | 0.136 |
| 13 | 57 | 0.078 | 0.021 | 0.046 | 0.077 | 0.111 | 0.137 |
| 14 | 82 | 0.078 | 0.020 | 0.045 | 0.078 | 0.111 | 0.136 |
| 15 | 113 | 0.080 | 0.022 | 0.048 | 0.080 | 0.111 | 0.136 |
| 16 | 34 | 0.079 | 0.022 | 0.046 | 0.078 | 0.110 | 0.136 |
| 17 | 71 | 0.083 | 0.023 | 0.051 | 0.085 | 0.115 | 0.137 |
| 18 | 67 | 0.077 | 0.021 | 0.044 | 0.077 | 0.110 | 0.136 |
| 19 | 70 | 0.087 | 0.026 | 0.059 | 0.092 | 0.117 | 0.137 |
| 20 | 90 | 0.079 | 0.022 | 0.048 | 0.079 | 0.111 | 0.137 |
| 21 | 26 | 0.078 | 0.021 | 0.045 | 0.077 | 0.110 | 0.136 |
| 22 | 136 | 0.080 | 0.023 | 0.051 | 0.081 | 0.110 | 0.136 |

Table S2: Quantiles and measures of central tendency describing the posterior distributions of the the $\alpha_1$ parameter obtained analysing each of the study-herds separately. The sizes of each herd (number of cattle heads) and its ID number are also shown in the table

Summary of the posterior MCMC distributions of the $\alpha_2$ parameter obtained for each of the 22 study-herds

| Herds' ID | Herds' size (n) | Mean | Quantiles | | | | |
|---|---|---|---|---|---|---|---|
| | | | *5%* | *25%* | *50%* | *75%* | *95%* |
| 1 | 51 | 0.010 | 0.002 | 0.004 | 0.008 | 0.014 | 0.022 |
| 2 | 213 | 0.008 | 0.001 | 0.002 | 0.004 | 0.010 | 0.019 |
| 3 | 130 | 0.017 | 0.007 | 0.011 | 0.015 | 0.018 | 0.030 |
| 4 | 94 | 0.011 | 0.002 | 0.005 | 0.010 | 0.015 | 0.023 |
| 5 | 45 | 0.008 | 0.001 | 0.003 | 0.005 | 0.010 | 0.020 |
| 6 | 31 | 0.017 | 0.007 | 0.011 | 0.014 | 0.018 | 0.030 |
| 7 | 78 | 0.010 | 0.002 | 0.004 | 0.007 | 0.013 | 0.022 |
| 8 | 121 | 0.019 | 0.009 | 0.014 | 0.017 | 0.019 | 0.029 |
| 9 | 156 | 0.018 | 0.008 | 0.012 | 0.016 | 0.019 | 0.030 |
| 10 | 31 | 0.016 | 0.006 | 0.010 | 0.014 | 0.018 | 0.028 |
| 11 | 65 | 0.011 | 0.002 | 0.004 | 0.008 | 0.013 | 0.021 |
| 12 | 43 | 0.007 | 0.001 | 0.002 | 0.004 | 0.009 | 0.019 |
| 13 | 57 | 0.012 | 0.003 | 0.006 | 0.009 | 0.015 | 0.023 |
| 14 | 82 | 0.022 | 0.008 | 0.013 | 0.017 | 0.020 | 0.038 |
| 15 | 113 | 0.013 | 0.002 | 0.005 | 0.010 | 0.016 | 0.024 |
| 16 | 34 | 0.012 | 0.002 | 0.005 | 0.010 | 0.016 | 0.024 |
| 17 | 71 | 0.016 | 0.005 | 0.008 | 0.012 | 0.016 | 0.025 |
| 18 | 67 | 0.021 | 0.009 | 0.014 | 0.017 | 0.020 | 0.038 |
| 19 | 70 | 0.019 | 0.010 | 0.014 | 0.017 | 0.019 | 0.030 |
| 20 | 90 | 0.014 | 0.002 | 0.005 | 0.010 | 0.016 | 0.024 |
| 21 | 26 | 0.015 | 0.005 | 0.009 | 0.012 | 0.017 | 0.026 |
| 22 | 136 | 0.015 | 0.006 | 0.009 | 0.013 | 0.017 | 0.025 |

Table S3: Quantiles and measures of central tendency describing the posterior distributions of the the $\alpha_2$ parameter obtained analysing each of the study-herds separately. The sizes of each herd (number of cattle heads) and its ID number are also shown in the table

Summary of the posterior MCMC distributions of the $\alpha$ parameter obtained for each of the 22 study-herds

| **Herds' ID** | **Herds' size (n)** | **Mean** | **Quantiles** | | | | |
|---|---|---|---|---|---|---|---|
| | | | ***5%*** | ***25%*** | ***50%*** | ***75%*** | ***95%*** |
| 1 | 51 | 0.008 | 0.002 | 0.004 | 0.007 | 0.011 | 0.015 |
| 2 | 213 | 0.006 | 0.001 | 0.002 | 0.004 | 0.009 | 0.015 |
| 3 | 130 | 0.012 | 0.007 | 0.010 | 0.012 | 0.014 | 0.016 |
| 4 | 94 | 0.009 | 0.002 | 0.005 | 0.008 | 0.012 | 0.016 |
| 5 | 45 | 0.006 | 0.001 | 0.003 | 0.005 | 0.009 | 0.015 |
| 6 | 31 | 0.011 | 0.006 | 0.009 | 0.012 | 0.014 | 0.016 |
| 7 | 78 | 0.008 | 0.002 | 0.004 | 0.007 | 0.011 | 0.015 |
| 8 | 121 | 0.013 | 0.008 | 0.012 | 0.014 | 0.015 | 0.016 |
| 9 | 156 | 0.012 | 0.008 | 0.011 | 0.013 | 0.015 | 0.016 |
| 10 | 31 | 0.011 | 0.005 | 0.009 | 0.011 | 0.014 | 0.016 |
| 11 | 65 | 0.008 | 0.002 | 0.004 | 0.007 | 0.011 | 0.015 |
| 12 | 43 | 0.005 | 0.001 | 0.002 | 0.004 | 0.008 | 0.015 |
| 13 | 57 | 0.009 | 0.003 | 0.005 | 0.008 | 0.012 | 0.015 |
| 14 | 82 | 0.013 | 0.007 | 0.011 | 0.013 | 0.015 | 0.016 |
| 15 | 113 | 0.009 | 0.002 | 0.005 | 0.008 | 0.012 | 0.016 |
| 16 | 34 | 0.009 | 0.002 | 0.005 | 0.009 | 0.013 | 0.016 |
| 17 | 71 | 0.010 | 0.004 | 0.007 | 0.010 | 0.013 | 0.016 |
| 18 | 67 | 0.013 | 0.008 | 0.011 | 0.014 | 0.015 | 0.016 |
| 19 | 70 | 0.013 | 0.009 | 0.012 | 0.014 | 0.015 | 0.016 |
| 20 | 90 | 0.009 | 0.002 | 0.005 | 0.009 | 0.013 | 0.016 |
| 21 | 26 | 0.010 | 0.005 | 0.008 | 0.010 | 0.013 | 0.016 |
| 22 | 136 | 0.011 | 0.005 | 0.008 | 0.011 | 0.013 | 0.016 |

Table S4: Quantiles and measures of central tendency describing the posterior distributions of the $\alpha$ parameter obtained analysing each of the study-herds separately. The sizes of each herd (number of cattle heads) and its ID number are also shown in the table

Summary of the $R_h$ estimates at times 90, 180, 365, 730 days obtained for each of the 22 study-herds

| **Herds' ID** | **Herds' size (n)** | **90 days (n. 22,000)** | | | | **180 days (n. 22,000)** | | | | **365 days (n. 22,000)** | | | | **730 days (n. 22,000)** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Mean** | **Quantiles** | | | **Mean** | **Quantiles** | | | **Mean** | **Quantiles** | | | **Mean** | **Quantiles** | | |
| | | | *2.5%* | *50%* | *97.5%* | | *2.5%* | *50%* | *97.5%* | | *2.5%* | *50%* | *97.5%* | | *2.5%* | *50%* | *97.5%* |
| 1 | 51 | 0.1 | 0 | 0 | 1 | 0.5 | 0 | 0 | 3 | 1.5 | 0 | 1 | 5 | 2.8 | 0 | 3 | 7 |
| 2 | 213 | 0.1 | 0 | 0 | 1 | 0.2 | 0 | 0 | 2 | 0.8 | 0 | 0 | 4 | 1.9 | 0 | 1 | 7 |
| 3 | 130 | 0.3 | 0 | 0 | 2 | 1.2 | 0 | 1 | 4 | 2.9 | 1 | 3 | 6 | 4.8 | 1 | 5 | 9 |
| 4 | 94 | 0.2 | 0 | 0 | 2 | 0.7 | 0 | 0 | 3 | 1.7 | 0 | 1 | 5 | 3.3 | 0 | 3 | 8 |
| 5 | 45 | 0.1 | 0 | 0 | 1 | 0.3 | 0 | 0 | 2 | 1.0 | 0 | 1 | 4 | 2.1 | 0 | 2 | 6 |
| 6 | 31 | 0.3 | 0 | 0 | 2 | 1.0 | 0 | 1 | 3 | 2.4 | 0 | 2 | 6 | 3.6 | 1 | 3 | 7 |
| 7 | 78 | 0.1 | 0 | 0 | 1 | 0.4 | 0 | 0 | 2 | 1.2 | 0 | 1 | 4 | 2.6 | 0 | 2 | 7 |
| 8 | 121 | 0.5 | 0 | 0 | 2 | 1.6 | 0 | 1 | 4 | 3.5 | 1 | 3 | 7 | 5.2 | 2 | 5 | 9 |
| 9 | 156 | 0.4 | 0 | 0 | 2 | 1.4 | 0 | 1 | 4 | 3.3 | 1 | 3 | 7 | 5.3 | 2 | 5 | 10 |
| 10 | 31 | 0.3 | 0 | 0 | 2 | 1.1 | 0 | 1 | 4 | 2.4 | 0 | 2 | 5 | 3.7 | 1 | 4 | 7 |
| 11 | 65 | 0.1 | 0 | 0 | 1 | 0.5 | 0 | 0 | 3 | 1.4 | 0 | 1 | 5 | 3.0 | 0 | 3 | 7 |
| 12 | 43 | 0.1 | 0 | 0 | 1 | 0.2 | 0 | 0 | 2 | 0.8 | 0 | 0 | 4 | 1.7 | 0 | 1 | 6 |
| 13 | 57 | 0.1 | 0 | 0 | 1 | 0.3 | 0 | 0 | 2 | 1.1 | 0 | 1 | 4 | 2.3 | 0 | 2 | 6 |
| 14 | 82 | 0.3 | 0 | 0 | 2 | 1.1 | 0 | 1 | 3 | 2.4 | 0 | 2 | 6 | 3.9 | 0 | 4 | 8 |
| 15 | 113 | 0.2 | 0 | 0 | 2 | 0.7 | 0 | 0 | 3 | 1.8 | 0 | 1 | 6 | 3.3 | 0 | 3 | 8 |
| 16 | 34 | 0.2 | 0 | 0 | 1 | 0.6 | 0 | 0 | 3 | 1.6 | 0 | 1 | 5 | 3.0 | 0 | 3 | 7 |
| 17 | 71 | 0.2 | 0 | 0 | 2 | 0.9 | 0 | 1 | 3 | 2.4 | 0 | 2 | 6 | 4.2 | 1 | 4 | 8 |
| 18 | 67 | 0.4 | 0 | 0 | 2 | 1.2 | 0 | 1 | 4 | 2.8 | 0 | 3 | 6 | 4.3 | 1 | 4 | 8 |
| 19 | 70 | 0.5 | 0 | 0 | 2 | 1.5 | 0 | 1 | 4 | 3.2 | 1 | 3 | 7 | 4.7 | 2 | 5 | 8 |
| 20 | 90 | 0.2 | 0 | 0 | 2 | 0.6 | 0 | 0 | 3 | 1.8 | 0 | 1 | 6 | 3.3 | 0 | 3 | 8 |
| 21 | 26 | 0.2 | 0 | 0 | 1 | 0.7 | 0 | 0 | 3 | 1.7 | 0 | 1 | 5 | 2.8 | 0 | 3 | 6 |
| 22 | 136 | 0.3 | 0 | 0 | 2 | 1.1 | 0 | 1 | 4 | 2.7 | 0 | 3 | 6 | 4.8 | 1 | 5 | 9 |
| | **Global value** | **0.2** | **0** | **0** | **2** | **0.8** | **0** | **1** | **3** | **2.0** | **0** | **2** | **6** | **3.5** | **0** | **3** | **8** |

Table S5: Quantiles and measures of central tendency describing the $R_h$ estimates at times 90, 180, 365, 730 days obtained analysing each of the 22 study-herds separately. The sizes of each herd (number of cattle heads) and its ID number are also shown in the table.

# Annex B – Supplemetary Information Study III

## FARMER AND VETERINARIAN ATTITUDES TOWARDS THE BOVINE TUBERCULOSIS ERADICATION PROGRAMME IN SPAIN: WHAT IS GOING ON IN THE FIELD?

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**List of Abbreviation**

*γ-IFN* = Interferon-γ assay

*SIT* = *S*ingle Intradermal Test

*bTB* = Bovine Tuberculosis

**Sentences from the qualitative in-depth interviews reported in their original language (i.e., Spanish or Catalan)**

***i) bTB detection and control***

*S1-"yo no tengo confianza en las pruebas, para mí es un poco de lotería". (farmer)*

*S2- "...porque yo animal positivo no lo quiero tener ni de coña, aunque sea la mejor vaca, como el mejor toro, o sea, seguro que no lo quiero tener, porque no es más que un problema, pero quiero tener la certeza de que es positivo" (farmer)*

*S3-"[…] en otros países se hacían las dos cosas a la vez, el γ-IFN y la SIT, y si coincidían era positiva, bueno pues yo me quedaría más tranquilo si me hicieran algo de eso, aunque me cobraran algo más. […]" (farmer)*

*S4-"tuve que matar ochenta y tantos animales, de los que no me decomisaron ni una sola pieza de nada y yo ya estaba cabreado" (farmer)*

*S5-"No es una prueba fiable, que si daba alguna en tuberculina, alguna no daba en el γ-IFN. Y alguna daba en el γ-IFN y en lo otro no, que era muy raro" (farmer)*

*S6-"A ver quién me dice a mí que muchas de mis explotaciones son de paratuberculosis y no de bTB. Nosotros no hacemos la comparada […]" (private veterinarian)*

*S7-"[…] La SIT bueno, da buenos resultados pero con muchos condicionantes que te pueden afectar […]" (official veterinarian)*

*S8-"[…] yo he ido al campo y me he encontrado en las charlas con algunos ganaderos de que faltaba rigor en la prueba. Pero no rigor por no quererlo hacer sino rigor por desconocimiento […]" (official veterinarian)*

*S8b-"[…] Te encuentras explotaciones extensivas que tienen unas mangas estupendas y tienen unas instalaciones estupendas donde los veterinarios que trabajan en el campo pues están bien, son seguras, son prácticas, son cómodas y otros sitios que están regular. Eso sí que creo que es un punto en el cual la administración de alguna manera deberíamos meternos más, tanto por la ejecución de esto como por temas de prevención de riesgos laborales. . Más de uno se ha quedado en la manga, que o se ha caído… cosas que no ha pasado nada por ahora pero podrían pasar". (official veterinarian)*

*S9- "Claro al meter el tema del γ-IFN han aparecido no más sino lo que había […]" (official veterinarian)*

*S10-"[…] y yo tengo duda con ella, el interferón me saca de dudas, ¿entiendes?" (private veterinarian)*

*S11- "[…] y el gamma, mejora la SIT en algún caso, lo mejora entre otras cosas en el tema de que el diagnóstico es en laboratorio, por lo tanto la presión en el campo desaparece." (official veterinarian)*

*S12- "el interferón también es verdad que saca vacas que no son…Hombre, saca falsos positivos, sí…No sé por qué. Eso los del laboratorio sabrán…" (private veterinarian)*

*S13- "y con esa (γ-IFN) hay gente que quiere que se la haga pero el problema es ese, no hay perras. Y el laboratorio la mitad de las veces no tiene posibilidad de hacerlas". (farmer)*

*S14- "El γ-IFN, yo personalmente le pegaba fuego. Aunque es una herramienta válida pero es cara, al ser cara depende de los presupuestos y una campaña de saneamiento continua no puede depender de que ahora tengo dinero, y mañana sí y mañana no […] Y esto no puede ser. Entonces, y esto ya lo he dicho más de una vez, mejor que el dineral tan grandísimo que cuesta los kits de γ-IFN lo invirtiésemos en más personal […]"" (official veterinarian)*

*S15- "tantos problemas porque tienes que llevarlo al laboratorio que nosotros, incluso yo le digo a tus compañeros, nos pusieron un límite de entregar la sangre, y aquí hay como poco hasta 100 km hasta laboratorio y no nos esperaban" (private veterinarian)*

*S16- "cuando todo el mundo saneamos al mismo tiempo, llega mucha sangre al laboratorio y ese laboratorio incluso…, a nosotros nos ha llegado a tardar 28 días en sacar los resultados, de publicarlos o meterlos en la base de datos". (farmer)*

*S17-"[…] Mandamos partidas a matadero que no se muestrean, porque hay varios mataderos y no tiene personal para cubrir todos los días que se matan en todos los mataderos." (official veterinarian)*

*S18"…vamos a una finca, vamos a cargar animales, y vamos a la finca y nos dicen "No, no están, están allí". Están en la finca de un vecino porque tiene mejores corrales para cargar que está calificado sanitariamente que es T3, sin embargo el que está cargando allí es positivo y está cargando en los corrales de otro que es negativo. Eso te enteras si vas a la finca, si no, no te enteras" (official veterinarian)*

*S19- "Hay otra cosa que no me acaban de aclarar y no lo acabo de entender porque yo creo que nadie lo sabe tampoco, a no ser que viniera un inmunólogo y nos lo explicara de puta madre […] deben ser reacciones esas a algún Mycobacterium, algo raro" (private veterinarian)*

*S20-"ese caso se expuso allí el día de la reunión, y tanto ni veterinarios ni como ganaderos ni técnicos, ninguno sabía darle una explicación."(farmer)*

*S21-"[…] tendrían que investigar más y cambiar el sistema para atacar la enfermedad, porque vamos yo para mí está demostrado que se han matado muchos animales y que no se ha conseguido nada, entonces habría que cambiarlo, digo yo" (farmer)*

*S22- "A nosotros nos está haciendo controles gente menos preparada que nosotros. Es que por eso se quejaban algunos compañeros, que han puesto un recurso de que no le hagan controles, que el que vaya que esté más preparado que él". (private veterinarian)*

*S23-"Hombre, si va a calificar y no da ninguna positiva, […], le estás jodiendo la calificación que le hace falta para poder vender. Entonces es absurdo, pues antes las repetíamos". (private veterinarian)*

*S24-"Yo lo que pediría de alguna manera es que se coordinara lo que es el saneamiento con los demás controles, para que una vez que tú tengas el ganado recogido y dispuesto a pasar por la manga, se hiciera todo de una vez, que no haya que estar trastornando a los animales tantas veces que algunas veces a nosotros nos hace falta aquí a la mujer, los niños, mi primo, el otro, la mujer del otro…". (farmer)*

*S25-"En cuello se hace, pero en ganado bravo, ponerte a hacerlo en cuello…, muchas veces te estás jugando el propio físico, en meter la mano hay en la mangada para pelarle el cuello, medir, pinchar… Eso es complicado. Porque el cuello es un parte de bastante movilidad animal" (farmer)*

*S26-"no puedes proteger a unos ganaderos así y a otros no. […] en este tema yo creo que están perjudicándolos más que beneficiándolos. (private veterinarian)*

*S27-"habría que aplicarles a todo el mundo lo mismo, lo veo así de claro. Todo lo demás yo pienso que es esconder el problema" (official veterinarian).*

*S28-"[…] no sé, a lo mejor es lógico que…, también si los pasas por una mangada se te puede fastidiar un cuerno, de un animal de esos que valen…" (farmer)*

*S29-"Hay quien dice que el animal cuanto menos se toque mejor, porque está más en libertad, más salvaje."(farmer)*

*S30-"Claro, sí se aprovechan, cuando van a matadero sucio se aprovechan pero bueno hubiera sido un mal menor, pero el problema gordo es tenerles que dar a los animales comida durante un montón de tiempo…" (farmer)*

***ii) Training, information and communication***

*S31 - "[…] en la bTB hemos ido saliendo de la universidad y han dicho echarse al campo y nos ha ido enseñando un compañero y como nos ha enseñado el compañero y han ido haciendo la prueba y dando un diagnóstico. […] en el curso me he dado cuenta de que eran conceptos de partida que no era ni porque ellos querían hacerlo peor sino que lo habían aprendido así y no habían visto la reacción de bTB claramente" (official veterinarian)*

*S32-"[…] Yo ahora mismo estaría por hacer lo mismo que se ha hecho con el tema de los veterinarios a nivel de ganaderos." (private veterinarian)*

*S33-"[…] la trasmisión de la información y la implicación de los ganaderos. Yo creo que eso es fundamental, y cosas que estamos ahí flojeando. Oye mira esto es así, asá y explicárselo a todo el mundo" (official veterinarian)*

*S34- "[…] Es que no tiene mucho sentido que te digan que esto es muy importante y después no te expliquen cómo funciona, ¿entonces cómo podemos combatirlo? ¿No?" (farmer)*

*S35- "[…] yo mi percepción, igual estoy equivocado, pero mi percepción es que esto se debe a cuestiones políticas, es decir, por intereses políticos europeos, interesa reducir la cabaña bovina en España y están siendo muy duros con esta enfermedad para reducir la cabaña bovina, porque si no pondrían…, si realmente fuera un problema pues investigarían más, pondrían más medios, lo harían de manera igualmente estricta con bovino y con otras especies" (farmer) […] (farmer)*

*S36- "Que no creo que sea sólo cuestión de cursos, que últimamente hemos visto que venga curso para esto, curso para lo otro, y están los pobres aburridos, que si curso de bienestar animal en el transporte, bienestar animal en la explotación, usos de biocidas en la higiene veterinaria,…" (private veterinarian)*

*S37- "Es muy difícil porque ellos tienen otras preocupaciones que no es la de la sanidad. La sanidad el ganadero no es consciente realmente de la importancia directa e indirecta que pueda tener [...]". (private veterinarian)*

*S38-"Los veterinarios de la ADSG informan a todo el mundo, ya que él quiera o no quiera, eso… Pero informar, informan" (farmer)*

*S39-"[…] A part del que es puguin explicar als bars no existeixen espais de trobada pels ramaders" (private veterinarian)*

*S40-"[…] Si hi ha canvis a les lleis o alguna cosa ningú els hi explica. Potser els explica un altre en un bar." (farmer)*

*S41-"[…] A l'ADS si que et pots informar. Hi ha l'assamblea anual on s'explica tot" (farmer)*

*S42-"[…] "La comunicació de les proves als ramaders es bona. Segons quins ramaders se'ls hi reenvia directament el correu amb els resultats. Quan arriben es truca al ramader i se'l informa" (official veterinarian)*

*S43-"[…] "La comunicación dels resultats de les proves és rápida. Les primeres són in situ i la gama interferó és bastant rápida, entre dos i quatre dies."(farmer)*

*S44-"[…] "…cuando no nos querían dar los resultados porque como éramos delincuentes. Era el único sitio donde no nos daban los resultados. Porque en teoría sólo marcábamos lo que marcaba el gamma." (private veterinarian)*

*S45-"[…] Pero si lo fastidioso es que no ves resultados. Entonces la gente está cabreada con eso". (farmer)*

*S46- "[…] a veces yo creo que tampoco se recogen las muestras como debe ser, luego no se notifica si ha dado el cultivo positivo" (private veterinarian)*

***iii) Role of wildlife and other domestic reservoirs***

*S47-"[…] nosotros hemos transmitido primero a la fauna silvestre los espoligotipos de la fauna doméstica y la fauna silvestre nos lo está devolviendo contaminándolos." (official veterinarian)*

*S48-"Yo lo veo complicadillo eso, porque mientras que no se termine con la fauna salvaje… ¿cómo vas a quitar eso? Tema de venado, tema de jabalíes […] la prueba está que en lo que es vacuno intensivo que no salen de una explotación, que te voy a decir yo, no llega al 2%..." (farmer)*

*S49-"Con respecto a la fauna salvaje, es muy complicado, porque no puedes…, los animales bueno se contagian por la hierba, por la saliva, por el agua en donde beben…, […] decían que habían inventado un bebedero para que pudieran beber las vacas y no pudieran beber los ciervos, pero al final eso dicen que tampoco ha resultado efectivo" (farmer)*

*S50-"cuando terminan, se dan la mano, empiezan a salir por la finca coches y se van extendiendo a todos lados. Y a este señor nadie le ha exigido que aquí a la salida haya un vado sanitario que desinfecte los carros, las ruedas. Estos señores que hoy están aquí a lo mejor en la comarca, mañana van a Córdoba y el viernes a Cádiz, y los perros van de aquí a aquí" (official veterinarian)*

*S51-"Que si una persona tiene dentro de una malla 600 ciervos, no digo que no los tenga, pero que los tenga con el mismo cuidado que la ganadería. Que les haga el saneamiento, porque medios para cogerlos hay". (private veterinarian)*

*S52-"[…]eso no se controla y se trata en muchos aspectos como la ganadería normal, o sea, se le da de comer como a la ganadería normal, acuden a comer como la ganadería normal, se toca el pito con el coche y se acostumbran los animales a ir a comer" (farmer)*

*S53-"[…] Explotaciones en las que el ganadero tiene parte de coto de caza… y puedes dedicarla a la actividad cinegética y además es perfectamente comprensible. Lo que pasa es que habrá que ordenar de alguna manera todo esto, ordenarlo y que empiecen a aparecer las ideas oportunas para que esto pueda minimizar las consecuencias que tiene."(official veterinarian)*

***iv) Risk perception on social aspects***

*S54-"un ganadero muy problemático que no lo hago yo, lo hace otro y está rodeado por todos los míos. Cayeron todos, y él seguía limpio, hasta que fue una vez la policía judicial y salieron positivas." (private veterinarian)*

*S55-"[…] Pero verás que yo lo puedo pensar como lo puede pensar muchísimos ganaderos, camiones, coches, personas…, que si está ahí a 20 km puede estar mañana aquí, ¿no?" (farmer)*

*S56- "Et ve a fer la prova el teu veterinary habilitat i per tant hi tens molta relació del dia a dia i colabora i ajuda en tot el possible" (farmer)*

*S57-"[…] Luego al final ¿qué pasa?, que encadenas, por no perder al cliente…, pues lo haces. Y así empezó y claro ya no era uno, ya eran varios. Y al final pues algunos nos hemos resistido y yo he perdido muchísimos clientes de bovino" (private veterinarian)*

*S58-"[…] los ganaderos siempre se han quejado. Algunos decían por aquí que yo tenía un rifle en vez de una jeringa" (private veterinarian).*

*S59- "A vegades els ramaders busquen algun culpable. Per què han sortit positius? Ells mai tenen la culpa diuen,…., quan hi ha positius la relació a vegades es tensa i es trencadirectament". (private veterinarian)*

*S60- "…para pasar a T3 esté presente la administración, a ellos les quita un montón de problemas" (official veterinarian)*

*S61- "La relació entre veterinaris de ADS i oficials és bona. Sempre hi ha persones amb qui no et portes tant bé però en general és bona" (private veterinarian)*

*S62- "Cap problema amb l'administració. Sempre que ha tingut algun problema ho ha comunicat a l'administració, al Departament i l'han atès bé, ha sortit content i si ells ho han pogut soventar ho han fet" (farmer)*

*S63- "…el problema es que sí, que por uno, dos o tres que hagan mal o unos cuantos ganaderos, estamos pagando todos" (private veterinarian)*

*S64- […] la actitud con la que nos tratan a los ganaderos, en principio nos tratan como si fuéramos delincuentes" (farmer)*

*S65- "[…] Yo sé de gente que ha saneado y ha llamado por teléfono y "Eh, ¿tienes alguna hinchada?". "No". Eso no puede ser. […]" (private veterinarian)*

*S66- "[…] Yo tengo bastante sospecha de que algunas vacas se han leído desde el coche, vamos, que lo dice mucha gente, yo se lo he oído a algún ganadero. Lo hace desde el coche y ¿cómo ves tú que aquello se ha hinchado o no? […]" (farmer)*

*S67- "[…] Ese tipo de cosas pues te descorazonan y que hay gente que no hacen las cosas y en estos años terminas enterándote de muchas cosas que han pasado y de gente que les han…, de cosas que no se hacen bien" (farmer)*

*S68-"[…] y además a todo el mundo por igual, y eso la verdad es que me duele bastante, porque en definitiva esta profesión me parece una profesión bastante digna porque lo que hacemos es producir alimentos para la sociedad y hay que producirlos con calidad, claro."(farmer)*

***v) Risk perception on bTB and benefits of eradication;***

*S69-"[…] estamos en un sector, primero muy estratégico y segundo muy miedoso, porque al final la alimentación, lo que las personas nos llevamos a la boca, en el momento en que tienen el más mínimo riesgo no quieren saber nada y simplemente la percepción aunque no sea real, genera unas pérdidas importantísimas" (official veterinarian)*

*S70- "[…] Hombre, nosotros los veterinarios lo vemos claro, ¿no? El tema de erradicar es un tema de salud pública y de sanidad animal, tienes que acabar con enfermedades más peligrosas y la TB es una de ellas, tenemos que quitarla de en medio a parte por interferencia del mercado, por tema de salud pública, por tema de la propia sanidad animal". (official veterinarian)*

*S71- "[…] ¿Qué estamos en una enfermedad que hay que erradicar por supuesto, pero que sin embargo sirve para el consumo humano?" (farmer)*

*S72- "Si et fan sacrificar 100 animals i 99 van a la cadena humana dius…els han matat perquè han volguts que els matem" (farmer)*

*S73-"[…] "Eso nunca ha afectado a la producción. Date cuenta que eso se consume por consumo humano, y eso, para mí eso es una cosa que eso cuando se consume no era malo, pero claro que los veterinarios sabrán por lo que será. […]" (farmer)*

*S74- "[…] Yo no sé exactamente cuál es lo que te puede contagiar, a las personas, porque yo creo que es nada. […] creo que hay un montón de cosas mucho más graves que eso y sin embargo no se les está dando ninguna importancia". (farmer)*

*S75-"[…] beneficios si hay porque estar libre de una enfermedad siempre es beneficioso […] las vacas no te van a parir bien si están enfermas, con los becerros exactamente igual". (farmer)*

*S76-"[…] yo he tenido animales allí que eran claramente positivos, bueno positivos no, claramente con la enfermedad, y eran animales que tú no podías explotarlos". (farmer)*

*S77-"[…] Como realizamos la prueba todo los años, actualmente no tenemos animales realmente enfermos de TBb, lo que tenemos son animales que han estado en contacto con la TBb, no han desarrollado ningún tipo de enfermedad pero como han estado en contacto con la TBb cuando le hacemos la prueba los detectamos como TBb y los sacrificamos, pero el animal desde el punto de vista reproductivo es totalmente rentable." (official veterinarian)*

*S78- "[…] "Ellos ven que las dejan circular y nada más, hombre y que está el ganado sano. Pero ellos no ven que eso sea…, es una cosa impuesta y es una cosa que hay que hacer" (private veterinarian)*

*S79- "[…] Aquí el tema sanitario se lleva un poquito por obligación no porque haya una conciencia… […]" (farmer)*

*S80- "yo creo que ahí no puede haber duda ninguna de que la erradicación tiene que ser sí o sí, eso es inevitable, porque ya sabemos lo que nos encontramos cuando nos cierran las fronteras…" (farmer)*

*S81- "[…] tú dime a mí qué hacemos si salen focos, ¿cómo vendes la leche?, ¿cómo vendes la carne? Que los más interesados de que esto no pase, son los ganaderos, aunque son los que más que pierden. […]" (farmer)*

*S82- "[…] realmente se lo pagamos como un animal enfermo. Ese hecho, pero claro aquí el problema es que si tú de alguna manera subes la indemnizaciones estás primando la enfermedad" (official veterinarian)*

*S83- "[…] La indemnización te daban, no para comprar una vaca, pero no se perdía tanto, entre la carne y la indemnización pues podías comprar una becerra, pero claro había que sanear. Yo lo veía bien y lo sigo viendo bien". (farmer)*

***vi) Future perspective and proposed changes to the programme.***

*S84- "[…] Erradicar, erradicar, va a ser muy difícil. Pero bajar la prevalencia, sí. Si se implican todos los sectores y se ponen en serio, no ahora sí, ahora no, ahora cambio la legislación porque nos convenga." (private veterinarian)*

*S85- "[…] pero ¿por qué no sacan la vacuna de la TB? ¿Sabes que la hay en humanos? ¿Lo sabías? ¿Te imaginas? Resulta que estamos metidos en un pozo cargándonos cabezas de ganaderos para una cosa que se va a solucionar dentro de 15 o 20 años, sin dar un duro, si se pusieran a poner un duro en 5 años habría un vacuna, ya la hay ¿eh?, de humanos, ¿por qué no la sacan para bóvidos? Se acabó el problema…" (farmer)*

*S86- "[…] Es que estamos viendo la enfermedad como si fuéramos a morirnos al día siguiente como si fueran manzanas envenenadas y a lo mejor hay que admitir que no vamos a superar este problema en poco tiempo, pero no tenemos que cargarnos al ganadero por en medio" (farmer)*