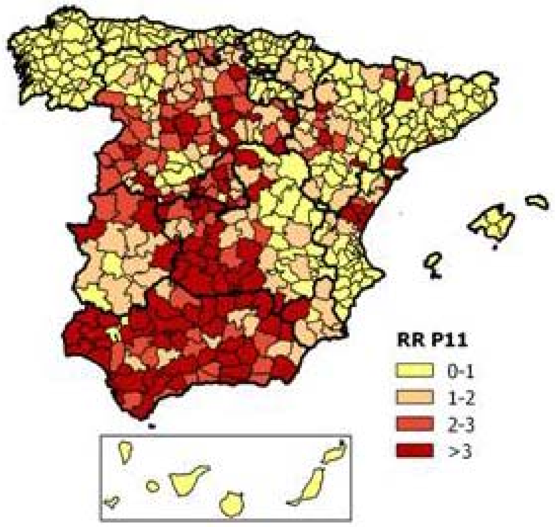
EPIDEMIOLOGICAL INVESTIGATION OF BOVINE TUBERCULOSIS CAUSES OF HERD BREAKDOWNS AND PERSISTENCE IN SPAIN



Sintayehu Guta Debela Bedhadha

PhD Thesis

2013

Universitat Autònoma de Barcelona

CReSA
Centre de Recerca en Sanitat Animal

EPIDEMIOLOGICAL INVESTIGATION OF BOVINE TUBERCULOSIS CAUSES OF HERD BREAKDOWNS AND PERSISTENCE IN SPAIN

PhD Thesis

Sintayehu Guta Debela Bedhadha

2013

Directors:

Alberto Allepuz

Jordi Casal

Alberto Allepuz y Jordi Casal, profesor lector y catedrático respectivamente del Departament de Sanitat i Anatomia Animals de la Facultat de Veterinaria de la Universitat Autònoma de Barcelona,

HACEN CONSTAR,

Que el trabajo "Epidemiological investigation of Bovine Tuberculosis causes of herd breakdowns and persistence in Spain" presentado por Sintayehu Guta, ha sido realizado en el Centre de Recerca en Sanitat Animal (CReSA) bajo su direccion.

Para que conste, firman la presente,

Alberto Allepuz Jordi Casal

Bellaterra, Junio 2013

AGRADECIMIENTOS

Mi sincero agradecimiento al Dr. Jordi Casal i Fabrega, por aceptarme y darme la oportunidad de venir desde la Universidad de Zaragoza a realizar mi tesis aquí, en la Universidad Autónoma de Barcelona, en el CReSA con el grupo de epidemiología. Por su contribución a mi formación Profesional, liderazgo consistente y disponibilidad ilimitada en todo momento.

Mi especial agradecimiento va dirigido al Dr. Alberto Allepuz, desde mí llegada me ha apoyado en la formación profesional, la integración social y en todo sentido. Por estar disponible en cualquier momento para consultarle y dar más vueltas al tema. Por su paciencia en escuchar y entender a la gente de diferente cultura e idiosincrasia. Además por las ganas de enseñar nuevas metodologías de trabajo y su alto grado de flexibilidad.

Mi agradecimiento a Dr. Sebastian Napp y Dra. Anna Alba por apoyarme en mi formación y en el desarrollo metodológico de la tesis, por sus comentarios críticos constructivos en todo momento. Rubén por el apoyo técnico. Ariadna, por su colaboración extraordinaria en la realización de la tesis. Gemma Castellà por su excelente gestión durante el proceso de admisión del doctorado. Particularmente mi agradecimiento va dirigido a la Agencia Española de Cooperación internacional para el Desarrollo por concederme la beca para mi formación. A mi mujer y mis hijos, por aguantar mi lejanía de casa y apoyarme en todo momento.

ABBREVIATIONS

AIC	Akaike information criterion
AUC	Area under curve
BRUTUB	Brucella and Tuberculosis database
bTB	Bovine tuberculosis
EC	European Commission
EEC	European Economic Commission
EFSA	European Food Safety Authority
MAGRAMA	Ministry of Agriculture Food and Environment
mycoDB	Mycobacteria data base
OIE	World Organization for Animal Health
OTF	Officially Tuberculosis free
PCR	Polymerase Chain Reaction
RD	Royale Decree
RFLP	Restricted Fragment Length Polymorphism
ROC	Receiver operating curve
SIR	Regional Information System
SICCT	Single Intra-dermal Comparative Cervical Test
SIT	Single Intra-dermal Tuberculin
SITRAN	Integrated animal traceability system
VNTR	Variable Number of Tandem Repeat

INDEX

1. INTRODUCTION

1. Introduction

1.1. History of bovine Tuberculosis (bTB) eradication scheme

The development of paleo-pathology and paleo-epidemiology in infectious diseases has allowed proving the very ancient origin of tuberculosis (Formicola et al., 1987; Buikstra, 1999). The discovery of preserved deformed bones, with apparent macroscopic TB lesions in various Neolithic sites in Italy, Denmark, Egypt and the Middle East (Smith, 2003), together with molecular studies carried out on mummies from Egypt and South America confirmed the worldwide distribution of the disease, and its presentation in different time periods (Salo et al., 1994; Nerlich et al., 1997). In Europe, the first tuberculosis (TB) report in humans corresponds to the first half of the fourth millennium BC, and was discovered on the cave of Arena Candide (Liguria) in Italy (Formicola et al., 1987). In animals, the oldest reported case of proven infection with ancestral *Mycobacterium tuberculosis* complex bacteria was found in a bison dated 17,000 years BC (Rothschild, 2001). However, no human infection older than 9,000 years has been successfully analyzed. In humans, *Mycobacterium bovis* was first evidenced in a skeleton found in a cemetery on Siberia dating from 2,000 BC (Nerlich and Lösch, 2009).

In 1882, Robert Koch (1843-1910) isolated the tubercle bacillus, and established TB as an infectious disease. From filtered cultures of human *Mycobacterium tuberculosis*, he obtained what was named as the old tuberculin (OT) of Koch. At first, it was believed to have therapeutic effects, but this hypothesis was proven wrong. However it was observed that in infected people the subcutaneous injection of OT gave a reaction, and that was the basis for the development of the tuberculin skin test by the French doctor Charles Mantoux in 1908 (Mazana, 2009; Good and Duginan, 2011).

The isolation of the tubercle bacilli in animal lesions generated a big concern about the role of animals as a source of tuberculosis for humans. At the First British Congress of Tuberculosis held in London in 1901, the zoonotic nature of bTB was widely and seriously debated. Robert Koch defended the hypothesis that milk and meat from infected cattle had no role in disease transmission, and therefore, that the control of bTB was unnecessary to prevent human disease. However, subsequent studies demonstrated that bovine tuberculosis in humans was linked to animals and that Koch thesis was not correct. The breakpoint was the International Tuberculosis Congress held in Washington in 1908. Even though Koch maintained his viewpoint, his thesis was strongly opposed by the majority of scientists, and he did not oppose to the implementation of control measures in animals (Douglas, 2008). In 1911, a report compiling all the evidence about the link between bTB and human tuberculosis was published. In that report it was concluded that the vast majority of cases of bovine tuberculosis in people could be attributed to the consumption of raw milk (Garcia, 2003).

Tuberculosis became a serious problem as industrialization crowded people together in insanitary conditions in large cities. In addition, many of the dairy herds that were kept in and around the cities to provide fresh milk were infected with bTB (Dubos and Dubos, 1952). Due to the public health and economic relevance of bTB, different countries started official control schemes. Finland was the first country, in the late 1890s, to start a successful bTB control and eradication program (Francis, 1947). As various tuberculin test methodologies were developed, other countries such as the United States in 1917, Sweden in 1927, Denmark in 1930 or United Kingdom in 1935, gradually began to implement control programs (Olmstead and Rhode, 2004; Evans and Thompson, 1981; Flückiger, 1960; Myers and Steele, 1969).

In Spain, the first bTB test was conducted on the 19th June 1950 in a dairy herd in Santander (north of Spain). Between 1956 and 1964, the first legal initiatives, based on the testing of animals and the culling of reactors, were established. However, these campaigns did not have success due to the high cost imposed to farmers. In 1986, when Spain became a member of the European Economic Community (EEC), the national programs were extended to beef cattle, but it was not until 1993, when the EU was established, that the bTB national eradication program included all dairy and beef cattle herds (Anon., 2012).

1.2. Importance of the disease

The eradication of bTB has been an important issue over years due to its public health impact and the high economic impact in livestock production.

1.2.1. Public health significance of bovine TB

Zoonotic TB is mainly caused by *Mycobacterium bovis* and *Mycobacterium caprae* (Cosivi et al., 1998; Thoen et al., 2006; Cvetnic et al., 2007; Regassa et al., 2008; Rodriguez et al., 2009; LoBue et al., 2010). It is associated mainly with the ingestion or handling of contaminated milk and dairy products. Human infection due to the inhalation of infected droplets released by animals or because of contact with infected droplets in mucous membranes and broken skin is associated to groups of people that are in close contact with animals, such as slaughterhouse workers, farmers, etc (Moda et al., 1996; de la Rua-Domenech, 2006a; Une et al., 2007).

Zoonotic TB is clinically indistinguishable from TB caused by *M. tuberculosis* and can only be differentiated by laboratory diagnostic methods. Therefore, in many countries, the proportion of cases that are caused by *M. bovis* is difficult to estimate (De la Rua-Domenech, 2006a; Cosivi, et al., 1995, Cosivi, et al., 1998; de Kantor et al., 2010). Data on zoonotic TB is believed to be well documented in the majority of industrialized countries (Collins and Grange, 1983; Cosivi et al., 1995, EFSA, 2010) and the low number of cases is associated to the implementation of bTB control and eradication programs in cattle, and the pasteurization of milk.

In many developing countries, however, animal TB is widely distributed, and control measures in animals, or pasteurization of milk are not applied in many cases. In developing countries, zoonotic TB constitute a major public health concern, and it is therefore considered as a “neglected zoonotic diseases” (Cosivi et al., 1998; Amanfu, 2006; Ayele et al., 2004; Cvetnic et al., 2007; Shitaye et al., 2007; De Kantor et al., 2010; Ehizibolo et al., 2011). In these countries, the current epidemiological situation is quite similar to that found in the 1930’s in most of the industrialized countries (Griffith, 1932). The disease is mainly contracted by the consumption of raw milk and dairy products. Besides, in some areas of Africa there is a tradition of sharing shelter with animals, and to consume raw milk, which may also contribute to disease dissemination (Moda et al., 1996; Cosivi et al., 1998; Ayele et al., 2004; Shitaye et al., 2007; Berg et al., 2009).

1.2.2. Economic importance

In most developing countries no control programs are implemented, and bTB causes severe economic losses, especially in urban and peri-urban cross breed dairy cattle due to mortality, low productivity, carcass condemnation and trade restrictions (Amanfu, 2006). In some areas, the disease may also be a serious threat to endangered wildlife species (OIE, 2006).

In industrialized countries, bTB control programs are based on meat inspection combined with skin test and slaughter of positive animals (EFSA, 2003; Reviriego-Gordejo and Vermeersch, 2006). The economic impact of these programs is high, however, they are justifiable in terms of food safety and public health, and bTB eradication is considered as an important objective to be achieved (Reviriego-Gordejo and Vermeersch, 2006; Schiller et al., 2011).

1.3. Etiology

Bovine tuberculosis is a chronic infectious disease caused by both *M. bovis* and *M. caprae* which belong to the *Mycobaterium tuberculosis* complex (MTC) group (Aranaz et al.,2003; Smith et al., 2006; Cvetnic et al., 2007; Javed et al., 2007; Duarte et al., 2008; Bezos et al., 2012). *M. bovis* and *M. caprae* can also affect other domestic and wild animals as well as humans (Aranaz et al., 2003; de la Rua -Domenech et al., 2006b; Amanfu, 2006).

Other members of the MTC group such as *M. tuberculosis, M. africanum* and *M. canetti* are predominantly human pathogens (Meyer et al., 2008; Muños Mendoza et al., 2012; Bezos et al., 2012), although infections with *M. tuberculosis* in cattle have been reported (Francis, 1947; Shitaye et al., 2007; Regassa et al., 2008; Berg et al., 2009; Romero et al., 2011). *M. pinnipedii, M. microti* and *M. mungi*, also MTC members, affects marine mammals (Kiers et al., 2008), rodents (Cavanagh et al., 2002), and banded mongoose (Mungos mungo) respectively (Alexandera et al., 2010).

MTC group members are commonly considered as subspecies and are 99.9% similar at the nucleotide level, but differ widely in terms of their host tropisms, phenotypes and pathogenicity (Brosch et al., 2002; Smith, 2003). Mycobacteria have DNA with a high proportion of guanine and cytosine which increases DNA stability. The thick and lipid-rich cell wall of mycobacteria protects DNA from attack of lytic enzymes after autolysis and necrosis of the host cell.

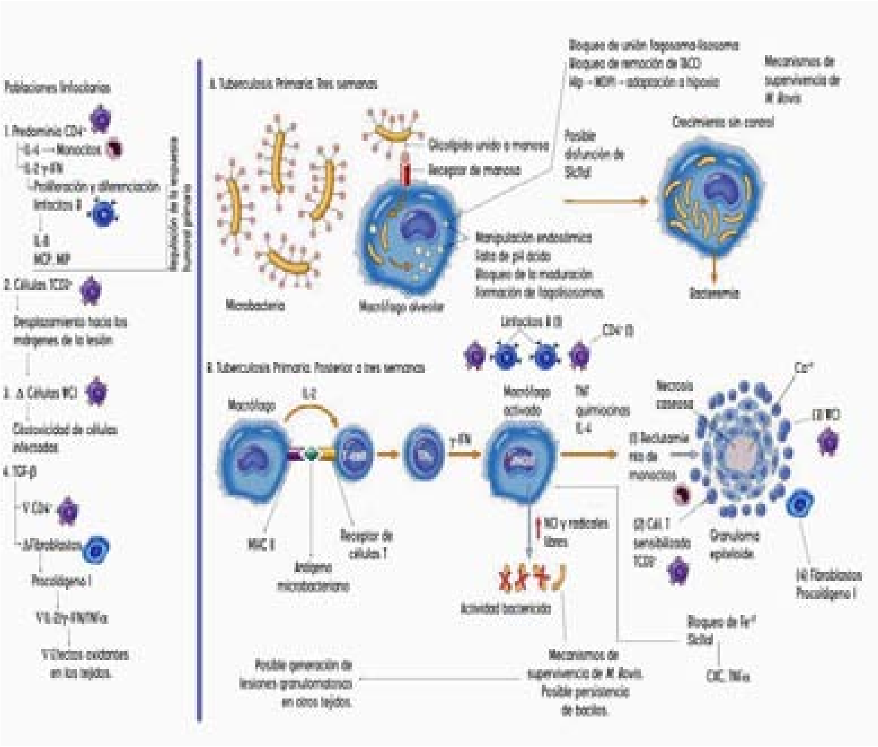
1.4. Pathogenesis

Tuberculosis spreads through the body in two stages, the primary complex and post–primary dissemination. The primary complex consists of the lesion at the point of entry and the local lymph node. Post-primary dissemination from the primary complex may take the form of acute miliary tuberculosis, discrete nodular lesions in various organs, or chronic organ tuberculosis (Francis, 1947; Radostitis et al., 2000).

The macrophages are the primary host cell for intracellular growth of *M. bovis* following an infection (Pollock et al., 2006). The gradual accumulation of macrophages in the lesion and the formation of a granulomatous response lead to the development of a tubercule (Quinn et al., 2002). The granuloma prevents the spreading of bacilli resident within macrophages. However, the latent bacilli could be later released if the immunological balance is broken, triggering disease reactivation. The characteristic lesion caused by *M. bovis* in cattle is described as having a centre of caseous necrosis, usually with some calcification, with a boundary of epithelioid cells, some of which form multinucleated giant cells and few to numerous lymphocytes and neutrophils (Neill et al., 1994).

In cattle, lesions are found most frequently in lymphatic tissues of the thoracic cavity, usually the bronchial and/or mediastinal lymph nodes (Stamp, 1948). Lymph nodes of the head region are the second most frequent site, and in many instances lesions in the retropharyngeal and sub-maxillary nodes exist in the absence of detectable lung lesions. Less frequently, lesions are found in both regions simultaneously (Neill et al., 1994; Whipple et al., 1996). Lesions occurring exclusively in the mesenteric lymph nodes are not a common finding now (Francis, 1947; Neill et al., 1988).

Figure 1: Pathogenesis of TB



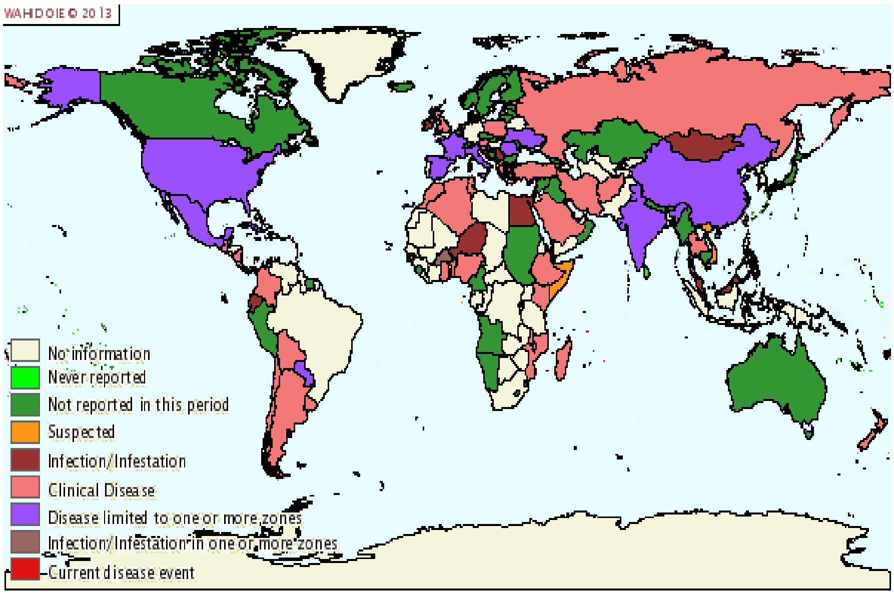
(http://medicinembbs.blogspot.com.es/2011/01/pathogenesis-of-tb.html)

1.5. Epidemiology

1.5.1. Distribution of bovine TB

Although bTB was once found worldwide, control programs have substantially reduced or nearly eradicated the disease from farm animals in many industrialized countries (OIE, 2009; Anon., 2011; EFSA, 2012a, 2012b). However, bTB is still widespread in Africa, Central and South America, parts of Asia and some Middle East countries (Figure 2) (Amanfu, 2006; OIE, 2009).

***Figure 2: Bovine Tuberculosis world distribution according to reports submitted to the World Organistaion of Animal Health (OIE) in the first semester of 2012*.**



In Europe, some countries have achieved the officially tuberculosis free (OTF) status, after reporting no more than 0.1% infected herds during 6 consecutive years (Council Directive 64/432/EC). Despite intensive eradication efforts applied over years, bTB continues to be present in some other European countries. United Kingdom and Ireland are the countries with the highest prevalences of positive herds, followed by Spain, Greece, Portugal and Italy (Figure 3) (EFSA 2012).

Figure 3: Proportion of positive Bovine Tuberculosis herds at the end of 2010 according to the European of Food Safety (EFSA).

European countries currently classified as Officially Tuberculosis Free (OTF) are represented in figure 4 and includes Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Latvia, Luxembourg, Netherlands, Poland, Slovakia, Slovenia, Sweden, Norway and Switzerland (Commission Decision 2003/467/EC). However, OTF countries are still facing continuous challenge of disease re-introduction to cattle herds from wildlife reservoirs, or due to international trade or mixing of herds from different countries on summer pasturelands (Zanella et al, 2008; Schiller et al., 2011), and therefore bTB cases continue to be detected. For instance France achieved the OTF status in 2000, but since then several cases have been detected in the departments of Côte d'Or and Dordogne (EFSA, 2010; Dommergues et al., 2011). In Austria (Tirol and Voralberg) several red deer infected with *M. caprae* were found in regions along the border of Germany and Switzerland (Schiller et al., 2011).

Figure 4: Officially Bovine Tuberculosis free countries and non-Officially Bovine Tuberculosis free countries at the end of 2010 according to the European Food Safety Authority (EFSA).

1.6. Mode of transmission

1.6.1. Cattle-to-Cattle transmission

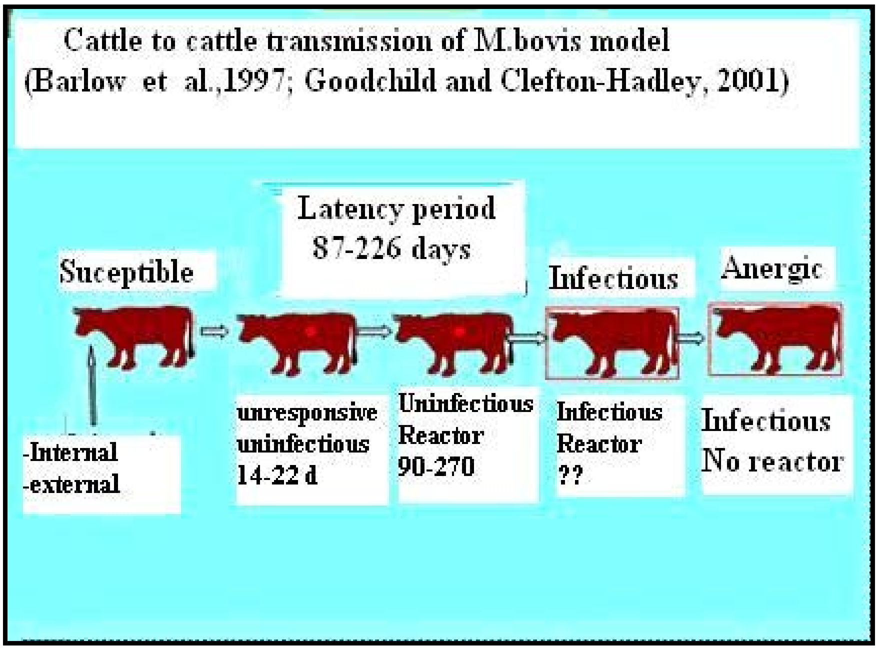
Direct horizontal transmission due to inhalation of *M. bovis* aerosol droplets is considered to be the primary mechanism by which cattle-to-cattle transmission takes place (Courtenay et al., 2006; Goodchild and Clifton-Hadley, 2001; Menzies and Neill, 2000; Phillips et al., 2003). This is supported by i) experimental studies that have shown that low numbers of bacilli are needed to experimentally infect animals via the respiratory tract, as compared to the large doses needed to infect animals via the digestive route (Neill et al., 1989; Buddle et al., 1994; Palmer et al., 2002; Menzies and Neill, 2000); and ii) the high frequency of tuberculosis lesions found in the respiratory tract and associated lymph nodes in cattle (Francis, 1972; Neill et al., 1988; Goodchild and Clifton-Hadley, 2001; Johnson et al., 2007).

Indirect transmission due to ingestion of viable *M. bovis* present in the environment is considered to be the second most common route of infection (Pritchard, 1988; Neill et al., 1994) but its role as a significant source of infection is not so clear (Courtenay et al., 2006; Young et al. 2005; Amanda et al., 2011; Okafor et al., 2011). Survival of *M. bovis* in the environment is usually considered to be short, especially if environmental conditions are dry and sunny, as they are killed by desiccation and UV light. However, experimental studies conducted in New Zealand, South Africa, Great Britain, Ireland, and the USA have demonstrated that *M. bovis* can persist in environmental substrates for varying amounts of time (Jackson et al.,1995; Tanner and Michel,1999; Gallager and Clifton-Hadley, 2000; Amanda et al.,2011).

Other routes, such as congenital transmission through the umbilical cord, genital infection during coitus or pseudovertical transmission due to udder infections have been reported, but are very uncommon in countries with intensive test and slaughter schemes (Menzies and Neill, 2000).

Infected cattle will go through different stages of the disease: i) latency stage: including an unresponsive period, in which the animal is infected but neither infectious nor reactive to the tests, and an uninfectious reactor period, in which the animal tests positive but is not yet infectious (no excretion of *M. bovis*), ii) infectious stage: the animal tests positive and it is infectious; and iii) anergic stage: infectious animals that no longer react to the tests (i.e., not a detectable cellular immunity response). The duration of each of these stages is variable. In figure 6 the duration of each of them is represented as reported in Barlow et al., (1997) and Goodchild and Clifton-Hadley (2001). In general, bTB is considered as a chronic disease with a slow within herd transmission rate (Barlow et al., 1997; Perez et al., 2002). However, there are also field reports of a higher transmission rate (Steger, 1970).

Figure 5: Bovine TB Cattle-to Cattle transmission model



1.7. The bovine tuberculosis eradication program and legal framework

The Spanish national bTB eradication program is compulsory for all bovines as defined in the Directive 64/432/EEC, incorporated into the domestic legal system by RD 2611/1996 and RD 1716/2000, which establishes the national programs for ruminant diseases eradication, and the health standards for the intra-community exchange of animals of the bovine and porcine species, respectively.

The diagnostic tests used are those laid down in RD 2611/1996 and RD1047/2003. The tuberculin skin test is the commonly used, and it is applied either as a single intra-dermal tuberculin (SIT) test using *M. bovis* as the antigen, or as the more specific single intra-dermal comparative cervical tuberculin (SICCT) test in which both *M. bovis* and M. avium are used as antigens. Both the SIT and the SICCT are tests approved for their use in the EU by EC regulation (De la Rua-Domenech et al., 2006b; OIE, 2009). EC 1226/2002 also allows the strategic use of gamma interferon test to complement the tuberculin skin test in bTB control programs. The gamma interferon test constitutes an additional ante-mortem test to identify cattle infected with *M. bovis* and it is recommended in order to maximize sensitivity (Vordermeier et al., 2006). These tests can be carried out on animals from 6 weeks of age in the case of the intra-dermal tuberculin test, and from 6 months in the case of gamma-interferon.

Conventional culture remains the gold standard for detection of *M. bovis* (OIE, 2009). However, other diagnostic tools such as molecular, pathological, histological or microbiological techniques can also be used in order to confirm the disease. Molecular diagnostic techniques such as restricted fragment length polymorphism (RFLP), PCR-based spoligotyping and Variable Number of Tandem Repeat (VNTR) analyses, have been used to trace origins of infection in herd breakdowns (Gortazar et al., 2005; Smith et al., 2006; Matos et al., 2010; Rodriguez et al., 2011).

Positive animals and herds are dealt with according to the provisions laid down in the Directive 64/432/EEC, Directive 97/12/EC and the Directive 98/46/EC. At the national level, the measures taken with positive cases are described in chapter II of the RD 2611/1996, and basically consist of compulsory slaughter of positive animals with an economic compensation, and movement restrictions for the remaining animals in the herd.

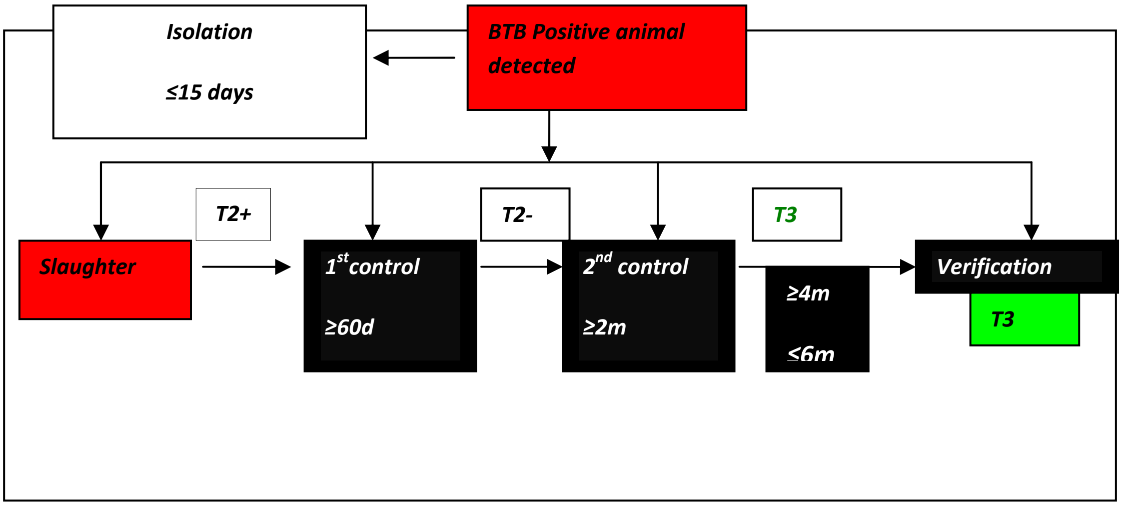
In accordance with the requirements laid down in the Community legislation, Directives 97/12/EC and 98/46/EC, and national legislation (RD 51/2004), herds are qualified according to their disease status in one of the following categories:

- T3 = officially bTB free herds (at least two consecutive negative tests).

- T2- = previous T2+ farms, but currently with negative result in the last test.
- T2+ = herds with one or more positive cattle in the last test.
- T1 = herds with unknown status.

Between 60 and 90 days after the slaughter of the bTB positive animals detected in a herd (i.e., T2+), a second bTB test must be carried out. If all animals tested are negative, the herd achieves the T2- status, that can be consolidated as officially free (i.e., T3) if all animals are also negative in the next bTB test. Figure 6 describes the procedure for achieving the different bTB qualifications.

Figure 6. Schematic procedure for achieving the different bTB herd qualifications according to national legislation



1.7.1. Additional measures:

The national bTB program has been reinforced several times since it was implemented for the first time. The main changes incorporated into the program were (Anon., 2007, 2012b):

- Increase in the frequency of controls in areas of high prevalence (i.e., herd prevalence higher than 1%).

- Pre-movement testing of animals.

- Development of standard protocols to carry out the diagnostic tests.

- Intensification of inspections of the field teams.

- Severe interpretation of the single intradermal tuberculin test (SIT) and application of gamma-interferon assays in confirmed positive herds.

- Tuberculosis control in goats in mixed farms or in farms epidemiologically related with cattle.

- Controls on communal pastures: only animals from holdings that have achieved a T3 status can access qualified pastures, and in the case that positive animals have been detected in a pasture, this should not be used for a minimum period of 60 days. Moreover, each pasture is considered a single epidemiological unit, and holds a single qualification, which affects all holdings with animals in the pasture.

- Improvement of slaughterhouse surveillance: In accordance with current legislation (Council Directive 64/433/EEC), all bovines are subjected to detailed ante- and post-mortem examination.

- Surveillance of wildlife reservoirs: performed in all autonomous communities (except in those without wildlife reservoirs, or little geographical extension, where it is optional).

-Implementation of a plan to limit contact between livestock and wildlife by peripheral fencing or reduction in the density of game species on holdings where wildlife and domestic animals are cohabiting.

- Organization of training courses for the field veterinarians involved in the program.

1.8. Tuberculosis situation in Spain

The trend of bTB in Spain in the past 25 years (1986-2011) is represented in figure 7. Herd prevalence has decreased from 11.14% in 1986 to 1.33% in 2011. During the first years, the reduction in the prevalence was important, but in the last years there has been just a moderate decline. Regarding incidence at animal level, the significant continuous decrease observed until 2005 changed in the years 2006 and 2007, most likely due to the large number of gamma-interferon tests conducted in parallel with severe interpretation of the single intradermal tuberculin (SIT) test. The descending trend was resumed in 2008 and continued in 2009, 2010 and 2011 (Anon., 2012b). At 31st of December 2011, 98.99% of the herds included in the program were negative.

In Spain bTB herd prevalence varies depending on the area and the type of production (beef, dairy or fighting bulls). Herd prevalence is higher in counties located in the central and southern Spain as compared to the rest of the country (figure 8), and in bullfighting and beef herds as compared to dairy herds (table 1).

Figure 7: Herd prevalence and animal incidence trend from 1986 to 2011 according to the Ministry of Agriculture (source: www.rasve.es)

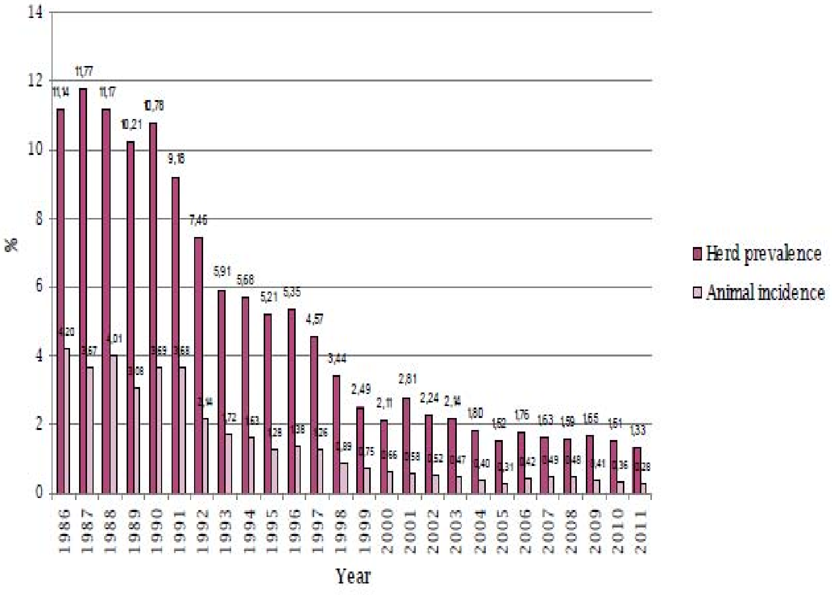


Figure8. Herd prevalence by county at the end of 2011 according to the Spanish Ministry of Agriculture

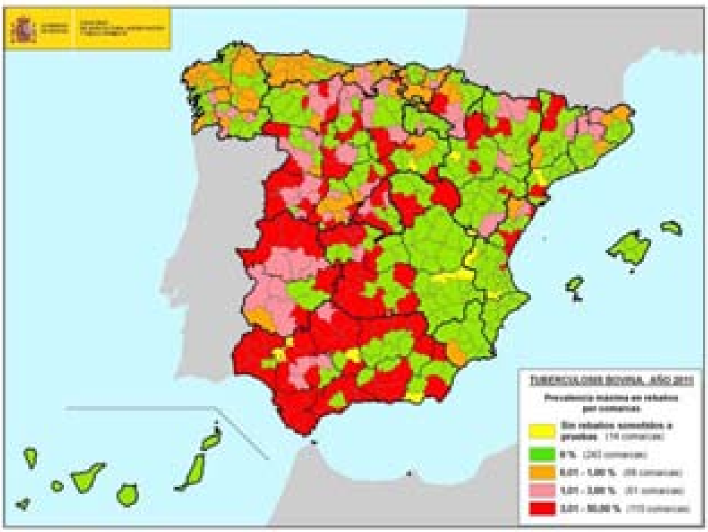


Table 1. Herd prevalence of bovine TB by farm type and year (2006-2011)

Positive herds												
Year	**Beef**			**Dairy**			**Fighting bull**			**Total**		
	Pos	**Herds**	**%**	**Pos**	**Herds**	**%**	**Pos**	**Herds**	**%**	**Pos**	**Herds**	**%**
2006	2108	105164	2.0	203	30568	0.7	98	1184	8.3	2411	136916	1.8
2007	1809	99236	1.8	199	29012	0.7	120	1815	6.6	2128	130063	1.6
2008	1703	97067	1.8	173	26454	0.7	108	1220	8.9	1984	124741	1.6
2009	1683	92684	1.8	159	25767	0.6	128	1211	10.6	1971	119662	1.6
2010	1504	90174	1.7	123	25012	0.5	128	1209	10.6	1759	116395	1.5
2011	1304	86083	1.5	97	24219	0.4	84	1153	7.3	1488	111455	1.3

The proportion of new positive herds detected also varies among the different farm types, being also higher in bullfighting herds (table 2). Approximately between 50 and 60% of the total bTB positive herds detected each year, are new positive herds (table 3), and the rest of the positives are herds that persist from the previous year.

Table 2. Cumulative herd incidence of bovine TB by farm type and year (2006-2011)

Newly detected Positive herds												
	Beef			Dairy			Fighting bull			Total		
Year	Pos	Herds	%	Pos	Herds	%	Pos	Herds	%	Pos	Herds	%
2006	987	105164	0.9	136	30568	0.4	43	1184	3.6	1166	136916	0.9
2007	1126	99236	1.1	160	29012	0.6	91	1815	5.0	1377	130063	1.1
2008	950	97067	1.0	122	26454	0.5	63	1220	5.2	1135	124741	0.9
2009	1039	92684	1.1	119	25767	0.5	73	1211	6.0	1231	119662	1.0
2010	837	90174	0.9	96	25012	0.4	57	1209	4.7	990	116395	0.9
2011	822	86083	1.0	74	24219	0.3	42	1153	3.6	938	111455	0.8

Table 3. Number of new positive herds in relation to the total number of bTB positive herds detected each year in the eradication campaign

Year	**New positive**	**Positive**	**Percentage**
2006	1166	2411	48.4
2007	1377	2128	64.7
2008	1135	1984	57.2
2009	1231	1971	62.4
2010	990	1759	56.3
2011	938	1488	63.1

1.9. Literature review of bTB herd breakdown studies:

According to Council Directive 64/432/EEC and RD 2611/1996, a herd is considered as positive (bTB breakdown) if it is at least one of the animals positive to the official diagnostic tests (both routine and supplemental).

A bTB herd breakdown can occur due to the persistence of *M. bovis* within the herd (i.e., residual infection), or because its introduction in a previously free herd. No risk factor has been found universally and consistently associated with the herd breakdown over time and across geographical regions. Nonetheless a variety of farm husbandry and management factors, wildlife and environmental factors, have been identified as potential risk factors for bTB breakdowns (DEFRA, 2007). The quantification of the relative importance of all possible sources of infection into herds is an important knowledge in order to implement the most appropriate and cost effective control measures. However, the identification of the most likely source is not an easy task, and most of the times the source of infection cannot be determined. According to the results of bTB herd breakdown studies conducted in Ireland (Griffin and Hahesy, 1992) and the UK (Wilesmith, 1983), in 35% and 40% of the breakdowns, respectively, the infection source could not be determined.

The different sources of bTB can be grouped in: recirculation due to residual infection, cross infection due to the presence of other infected domestic species (such as goats), introduction of infected cattle, neighborhood spread, interaction with a wildlife reservoir, interaction with infected domestic animals in communal pastures, environmental contamination due to the use of manure or agricultural machinery from infected herds, and interaction with infected humans.

1.9.1. Residual infection

Residual infection is the recirculation of *M. bovis* in the herd (Olea-Popelka et al. 2006; Good et al, 2010; Karolemeas et al., 2011; Conlan et al., 2012).

The three main reasons for the resurgence of bTB infection in a herd can be i) maintenance of the infection in undetected and/or anergic cattle due to the lack of test performance in terms of sensitivity and specificity (Monaghan et al., 1994;

Doherty et al., 1995; Costello et al., 1997; de la Rua-Domenech , 2006b; Vordermeier et al., 2006) or desensitization due to repeated testing (Hoyle, 1990) ii) *M. bovis* persistence in the farm environment (Courtenay et al.,2006;Amanda et al.,2011) or iii) maintenance of the infection due to the lack of good veterinary practice (Humblet et al., 2011).

In Ireland, residual infection was identified as the most likely cause of bTB breakdowns in 14% of 4000 herd breakdown investigated by Griffin and Hahesy (1992) and, in 4% of the 82 bTB herd breakdowns studied by Olea-Popelka et al., (2006).

In Great Britain (GB) around 38% of herds that have cleared bTB breakdown restrictions, experience a recurrent incident within 24 months, which is suggestive of a high probability of residual infection and disease persistence within herds. The approximate Bayesian computational model developed by Conlan et al., (2012) estimated that 24-50% of recurrent breakdowns in Great Britain could be attributed to residual infection missed by tuberculin testing.

1.9.2. Cattle movement

Movements of animals are considered to be one of the main factors in the spread of animal diseases (Keller, 1993; Fèvre et al., 2006), and it has been identified as a major risk factor in several bTB herd breakdown studies.

Gopal et al., (2006) identified the purchase of infected animals as the most likely source of the infection in 30 of 31 bTB breakdowns in north-east England. Also, in Great Britain, Wilesmith et al., (1983) linked the 42% of the breakdowns studied in the period 1972-1978 to animal movements. In Michigan, 47 out of 49 bTB infected herds had introduced cattle into the herd before the breakdown (Okafor et al., 2011). In Northern Ireland three studies (Mcllory et al. 1986, Anon., 1994 and Denny and Wilesmith, 1999) described proportions of breakdowns due to animal movements of 30%, 25% and 15-20%, respectively A lower percentage (between 10% and 15%) was found in studies conducted in different areas of United Kingdom (Wilesmith and Williams, 1986; Griffin and Hahesy, 1992; Philips et al., 2003).

Cattle movements from bTB high incidence areas have proved to be a major source of bTB introduction into cattle herds in low incidence areas (Griffin and Hahsey, 1992; Denny and Wilesmith, 1999; Phillips et al., 2003; Olea-Popelka et al., 2006; Green et al., 2008; Okafor et al., 2011; Conlan et al., 2012). Molecular analyses have evidenced an important role of these movements in the long-distance spread and establishment of novel strains in new geographic areas (Smith et al., 2003; Smith et al., 2006; Fèvre et al., 2006). Furthermore epidemiological investigations conducted in Great Britain, Oklahoma (USA) and Veneto Region (Italy) have demonstrated that cattle movement from market or directly from farms was the most likely source of bTB infection in previously free herds (Gilbert et al., 2005; Green et al., 2008; Schoembaum et al.,1992; Marangon et al., 1989).

1.9.3. Neighborhood infection / Contiguous spread

The presence of neighboring bTB positive herds could be the direct or indirect source of herd breakdowns. The transmission of *M. bovis* could be due to contiguous spread by cattle-to-cattle transmission over farm boundaries or drainage of contaminated sewage (Olea-Popelka et al., 2006; Wilesmith, 1983; McIlory et al., 1986; Griffin and Hahesy, 1992; Denny and Wilesmith, 1999; Porphyre et al., 2007; Dommergues et al., 2011).

The importance attributed to neighborhood spread as a source of new bTB breakdowns has been variable. While in studies carried out in Ireland, Northern Ireland and France a high proportion of new infections (between 25% and 47%) were attributed to the contiguous spread (McIlory et al. 1986, Griffin and Hahesy 1992, Anon. 1994 and Denny and Wilesmith 1999; Dommergues et al., 2011), in other countries such as England or New Zealand, this source represented less than 10% of the new infections (Wilesmith, 1983, Wilesmith and Williams 1986, Porphyre et al., 2007).

1.9.4. Interaction with wildlife bTB reservoirs

A number of wildlife reservoirs endemically infected by *M. bovis*, may pose a serious challenge to bTB eradication schemes in many countries. Examples of such reservoirs include the European badger (Meles meles) in Great Britain and Ireland (Cheeseman et al., 1989; Delahay et al., 2001; Delahay et al., 2002), the brushtail possum (Trichosurus vulpecula) in New Zealand (O'Neil and Pharo,1995; Porphyre et al., 2008), the African buffalo (Syncerus caffer) in South Africa (Amanfu, 2006) and the white-tailed deer (Odocoileus virginianus) in Michigan, USA (O'Brien et al., 2006; Okafor et al., 2011).

In Great Britain, badgers have been identified as the origin of a high percentage of bTB breakdowns: from 41% (Denny and Wilesmith, 1999) to 58% in the SW of England (Anon., 1991). In the last outbreak of bTB in the Czech Republic in 1995, cattle were considered to have been infected by ingestion of green fodder contaminated by wild ruminants (Pavlik et al., 2002).

In Spain, recreational hunting has been one of the most lucrative and rapidly growing industries, and in the Western and central part of the country red deer and wild boar density have consistently increased in the last years (Mackintosh et al., 2004; Fernández-de-Mera et al., 2009; Acevedo et al., 2007). The Eurasian wild boar (Sus scrofa), the red deer (Cervus elaphus) and the fallow deer (Dama dama) have been identified as bTB maintenance hosts in Spain (EFSA, 2009; Naranjo et al., 2006; Parra et al., 2006; Gortazar et al., 2007; Gortazar et al., 2011). Moreover, high bTB prevalences have been detected in these wildlife species, and therefore they could constitute an important infection source to farm animals (Parra et al., 2006; Boadella et al., 2011; Gortazar et al., 2011; Garcia-Bocanegra et al., 2012).

1.9.5. Communal grazing in local pasturelands and/or transhumance

The use of communal grazing in local pasturelands and/or transhumance, with multiple species is a common practice in the draught periods when pasture and water scarcity is marked (Reviriego-Gordejo and Vermeersch, 2006; Gortazar et al., 2011;

Alvarez et al., 2012; Anon., 2012b). In some regions of Spain and Portugal beef and fighting bull herds are kept under extensive conditions, using the traditional communal grazing in pasturelands which is a cheap source of feed for the herds (Reviriego-Gordejo and Vermeersch, 2006). However this practice predisposes contact with other herds, thereby increasing the risk of disease transmission among herds. According to national and regional regulations, pasturelands have to be epidemiologically qualified, and all herds sharing the same pastureland are expected to have the same bTB health qualification or epidemiological status. Despite this measure, there has been tangible epidemiological evidence of herd breakdown events originated in them (Anon., 2007; Anon., 2012b, Sebastian Napp, personal communication).

1.9.6. Mixed cattle farms

In many regions of Spain, mixed farms with multiple species particularly beef cattle with goats, swine, sheep, horses and other species, managed under extensive management system, are usual. Goats seem to be very susceptible to the infection by *M. bovis* and *M. caprae*, developing disseminated lesions and fast transmission within a herd (Aranaz et al., 2003; Crawshaw et al., 2008; Rodriguez et al., 2011). However, cases of tuberculosis in goats are rarely reported, as the legislation require them to be tested only in mixed farms (cattle and goats) or when they are epidemiologically related with cattle. Nevertheless, field evidence of transmission from goat to cattle herds have been reported (Napp et al., 2013) and it seems clear that the presence of infected goats within the farm could contribute to recirculation of bTB in the herd (Crawshaw et al., 2008; Alvarez et al., 2008).

1.9.7. Manure or slurry spreading on pasture

Contaminated manure could be a possible source of introduction of bTB infection into cattle herds. Nonetheless, for slurry to be a source of infection it should contain viable bacteria, and *M. bovis* has been reported to survive in the environment only for a few weeks under natural conditions (Menzies et al., 2000). Spreading manure has been identified as a risk factor when manure stored in a closed container in-door and without direct sun light exposure was used (Griffin et al., 1993; Ramirez-Villaescusa, et al., 2010; Wolfe et al., 2010; Karolemeas et al., 2011; Abernethy, et al., 2011).

1.9.8. Human to animal transmission:

Humans suffering of tuberculosis can also act as a source of infection for cattle due to contamination of environment with infected urine, feces or sputum (Krajewska et al., 2012). *M. tuberculosis* infection in cattle is associated with a non progressive disease; however, infected animals will react positive to the intradermal tuberculin test (Hardie and Watson, 1992; Grange and Yates, 1994; Erler at al., 2004). Infection of cattle by *M. tuberculosis* due to a human infection has been reported, among others, in Slovenia, Spain, Poland from Europe and in Ethiopia and Nigeria from Africa (Ocepek et al., 2005; Regassa et al.,2008; Ehizibolo et al., 2011;Romero et al., 2011; Krajewska et al., 2012). The result of case control studies conducted by Regassa et al. (2008) and Kassa et al, (2012) in Ethiopia, reported higher prevalence of bTB in cattle owned by farmers with active tuberculosis and isolation of *M. tuberculosis* from goats respectively, suggesting a potential transmission of TB from human to animals.

Table 4. Review of the most likely source of infection on different bTB herd breakdown studies

References	Movement	Neighborhood	Wildlife	Residual	Unknown	Country
Wilsmith (1983)	42%	6%	51%	-	-	Great Britain
Wilsmith and Williams(1986)	10%	1%	Major.	-	-	SW England
Mcllory et al.(1986)	30%	40%	-	-	-	N. Ireland
Anonymus (1991)	9%	-	58%	-	-	SW England
Anonyumus (1991)	50%	-	6%	-	-	The rest of GB
Griffin and Hashey(1992)	11%	25%	14%	14%	35%	Rep. Ireland
Griffin et al. (1993)	7-15%	25%	-	-	-	Rep. Ireland
Anonymus (1994)	25%	47%	3%	-	24%	N. Ireland
Denny and Wilesmith (1999)	15-20%	42%	41%	-	-	N. Ireland
Goodchild and Clifton-Hadley (2001)	50%	4%	6%	-	40%	The rest of GB
Goodchild and Clifton-Hadley (2001)	9%	2%	58%	-	31%	SW England
Phillips et al(2003)	10-15%	-	Major.	-	-	British Isles
Green et al (2008)	25%	-	-	-	-	Great Britain
Good et al(2010)	8%	37.9%	32.4%	9.2%	-	Rep. Ireland

1.10. Literature review on bTB persistence risk factor studies:

Different risk factors for bTB at animal, herd or regional level have been identified in studies conducted in both developed and developing countries (Humblet et al., 2009; Skuce et al., 2012). However, most of these studies have analyzed the risk of a bTB infection without distinguishing between transient or persistent infections. Differences in risk factors for transient and persistent bTB infections are expected to exist (Reilly et al., 2007), and the analysis on the causes of persistent infection within farms has received less attention (Brooks and Keeling, 2009). Persistent bTB infections might be determined by those factors related with the lack of detection of all the infected animals within the herd (mentioned above in the residual infection topic (point 1.9.1)), or re-introduction of infection between tests from local wildlife reservoirs or infected cattle.

In United Kingdom, Karolemeas et al., (2011) found that the confirmation status of the herd was the most important factor to explain bTB persistence. The authors attributed this result to the fact that confirmed breakdowns had a more “severe” interpretation of the tuberculin test, resulting in a higher sensitivity and lower specificity, increasing therefore the time lag needed to acquire the bTB free status. They also found that an increasing number of reactors were related with the duration of the breakdown; but, as this variable was highly correlated with the confirmation of the herd was not included in the final model.

Herd size has been another variable related with the risk of bTB, but its role in bTB persistence seems to be debatable. Reilly et al., (2007), in a case-control study carried out in the United Kingdom compared risk factors for transient and persistent infected farms, and found that an increasing herd size was related both with transient and persistently infected farms. However, Brooks and Keeling (2009) concluded that herd size was clearly positively correlated with bTB persistence, and in the study performed by Karolemeas et al., (2011) the effect of herd size was statistically significant, although its effect was low and did not contribute much to predict bTB persistence.

Management factors, such as the use of silage clamps in areas with badgers, have been also identified as important determinants for bTB persistence (Reilly et al., 2007).

In Spain, Allepuz et al., (2011) quantified the geographical variability of bTB risk in the country and showed that counties located in the central, western and southwestern part of the country had a higher risk of bTB persistence. This could be related to factors such as re-infections due to contact with infected wildlife reservoirs, management of herds or size of the herds, but as information on these factors was not available, they could not be evaluated.

In herds from the centre of Spain, Alvarez et al., (2012) identified by survival analysis that the time to recover the Official Tuberculosis Free status (OTF) was shorter in dairy herds as compared to beef or bullfighting herds. Even though in dairy herds intensive management systems and demographic-related factors contributed to a faster within herd spread of bTB, herd breakdowns were also more rapidly controlled in dairy herds than in extensively managed herd types.

The mixed farm management system characterized by handling of multiple species with cattle in the farm especially goats which are reservoirs of *M. bovis* and M. caprae could also contribute to the re-circulation of bTB in the herd (Aranaz et al.,2003; Crawshaw et al., 2008; Alvarez et al., 2008).

2. OBJECTIVES

2. OBJECTIVES

In Spain, the relatively high number of new positive farms that appear every year, together with the persistence of bTB in some herds poses an important challenge in the last steps of the eradication program. However, there is not a clear knowledge about the epidemiological circumstances that lead to the introduction or persistence of the infection.

The main objective of this PhD is to improve the understanding of bTB epidemiology in cattle in Spain. In order to achieve the general objective two specific studies were conducted:

2.1. The objectives of study I:

- To develop a methodology that enables the identification of the most likely cause of a bTB herd breakdown in cattle farms.
- To identify the most likely cause of bTB herd breakdowns in Spanish cattle farms.
- To evaluate the perception of veterinarians involved in the bTB eradication program about the most likely causes of herd breakdowns compared to the causes identified by our study.

2.2. The objectives of study II:

- To identify risk factors associated with the persistence of bTB in beef herds from central and southern Spain.

3.1 STUDY I

3.1 STUDY I: Epidemiological investigation of new bovine TB herd breakdowns in Spain

3.1.1. Introduction

Within the bTB eradication program framework, the surveillance network allows detecting new cases by annual herd testing and inspection of carcasses at slaughterhouses. Moreover, in each new detected herd, an epidemiological questionnaire is administered by official veterinarians in order to determine the possible route of infection. Therefore, bTB breakdowns can be detected mainly by three different methods: i) annual herd monitoring (routine herd test) ii) bTB compatible lesions found at slaughterhouse during meat inspection and iii) epidemiological relations with an infected herd.

3.1.2. Material and Methods

3.1.2.1. Descriptive analysis

Descriptive statistics for number of reactors and prevalence of bTB at farm level by herd type and method of detection was calculated. Analyses of variances ANOVA, Bartlett's test for inequality of population variances and Kruskal-Wallis test for two groups was carried out by Epi Info ^{TM} 7. P value of 0.05 was accepted as level of significance

3.1.2.2. Development of an approach to investigate the cause of bTB herd breakdowns:

The approach we developed to estimate the most likely source of infection on each herd was based on the following steps:

a) Data sources:

Different databases were available and additional data was provided by different organisms:

a. BRUTUB database: after the detection of a new bTB positive herd, an epidemiological questionnaire is fulfilled by official veterinary officers. Data is stored in a national database called BRUTUB (Anon., 2012). In these questionnaires data regarding the possible causes of breakdowns are collected. The questionnaire can be found on annex 1.

b. mycoDB database: in this database molecular data about the different isolates detected in Spain in domestic animals and wildlife are stored. Data are aggregated at municipality level and includes the spolygotype and the date of analysis (http://www.vigilanciasanitaria.es/mycodb/).

c. Additional molecular data from wildlife isolates at municipality level was provided by IREC and regional governments of Andalusia and Galicia.

d. Molecular data at farm level was provided by Visavet.

e. SITRAN: stores data on identification of all livestock holdings, holders, animal movements, etc. in Spain.

f. SIR: stores individual animal movement from herds located in northeastern Spain (i.e., Catalonia).

g. Data about bTB testing results in goats was also provided by different regional governments.

b) Identification of the possible causes for a bTB herd breakdown:

Based on bTB routes of transmission between herds we considered the following possibilities as a source of herd breakdown:

i) Residual infection (resurgence of an old infection).

ii) Introduction of infected animals from other farms.

iii) Sharing of pastures with other infected herds.

iv) Contiguous spread from infected neighbor herds.

v) Presence of infected goats in the farm.

vi) Interaction with wildlife reservoirs in the farm or pastures.

vii) Contact with an infected human.

Finally, we considered the “Unknown” option, if the introduction of the infection could not be explained by any of the previous sources.

c) Definition of a time period to consider an epidemiological relation as a dangerous contact:

After an animal is infected it goes through latency, infectious and anergic phases. The latency period (estimated to length 90-270 days or even up to 7 years) comprises an unresponsive and a responsive period to the in-vivo diagnostic tests. The length of the unresponsive period has been estimated to be around 30-50 days when using single intradermal test. Nevertheless, the duration of this period is variable and dependent on the route of infection or individual factors that affect the immunity status of the animal such us pregnancy, co-infections, etc (Barlow et al., 1997; Goodchild and Clifton-Hadley, 2001). For this study we decided to establish a conservative period and therefore we considered one year before the last negative test result as the time period to consider an epidemiological relation as a dangerous contact.

d) Tool development to discriminate between the different possible causes of breakdowns.

In order to discriminate among the different possible causes of bTB on a herd, we built a decision tree diagram for each of these possible causes. The decision trees developed are represented in annex 2.

Each decision tree was composed by different question nodes that were answered with all data available. These question nodes lead to different specific events within each decision tree. For example in annex 2.2, event P3 corresponds to a farm that has introduced animals during the year before the last negative test from a farm that was positive in the following test, and the same molecular type (i.e., spolygotype) was isolated from both farms.

In order to be as objective as possible, we decided to assign probabilities to each event based on expert opinion. For that purpose, the "Workshop Method", based on a modification of the Delphi method was used (Garabed et al., 2009; Ratnapradipa et al., 2010). The workshop was done in a day meeting, and followed the next steps:

1. Selection of experts: 9 national experts working on bTB in different areas were contacted to participate in the meeting. They included veterinarians from the central and local administrations, and researchers working on bTB in both domestic and wildlife.

2. Once they agreed to participate, an introduction about expert opinion methodology and the decision trees with instructions on how to assign the probabilities were send to experts by email, so that they had time to think about it before the meeting. Following recommendations by Dufour et al., (2011) probabilities on the scale of 0 to 9 (table 5) were used.

Table: 5 Ordinal scaling and adjectives used to qualify an estimated probability of occurrence

Ordinal scaling(level)	**Adjectives used (Qualification)**
0	Null
1	Nearly null
2	Minute
3	Extremley low
4	Very low
5	Low
6	Not very high
7	Quite high
8	High
9	Very high

3. The meeting was held in June 2012 in the Veterinary Faculty of the Autonomous University of Barcelona (UAB). In order to solve doubts and avoid misunderstandings, a brief introduction about expert opinion was given together with the instructions on how to assign the probabilities.

4. Time was given to the experts to, individually, assign probabilities to the different events included in each decision tree.

5. After that, break time was given to experts, and during that time all results were compiled. A graph showing the probability distribution of values of each event within each decision tree was prepared by using the R statistical software.

6. Graphs were presented and probabilities were discussed with the entire group.

7. After these discussions, experts fulfilled again the questionnaire having the chance to change their scores if they considered that they had overestimated or underestimated any of the probabilities.

8. Finally, experts' ratings given in this second questionnaire were combined to obtain the mean value for each of the probabilities.

The distribution of values obtained for each event can be found on annex 2.

e) Data management and assignment of the most likely source

Raw data included in the BRUTUB database, together with data extracted from the other databases and the additional data provided by different organisms were included in a new Excel file. For that purpose a macro was programmed using Visual Basic programming language. This macro created a new data base extracting from the different databases all the relevant data needed to answer the different question nodes included in the decision trees. The information was compiled according to the pre-defined criteria (e.g. movements one year before the first positive test on the farm, last bTB test results and so on).

A new macro was build (also using Visual Basic programming language) in order to calculate the probabilities of the seven possible sources according to the data previously extracted.

Finally, the values for the different possible causes were compared, and the one with the highest score, was assumed to be the most likely source of infection. When all the different possible causes in a herd had a score under 5 (i.e. the estimated probability was less than "*Low*") the source of infection was considered to be unknown.

3.1.2.3. Evaluation of the perception of veterinarians involved in the bTB eradication program about the most likely causes of bTB herd breakdowns and results of our study:

The epidemiological questionnaire carried out by official veterinarians on affected farms included a question about which were the most probable sources of infection according to his/her opinion (see annex 1). In order to make conclusions comparable we aggregated their responses in the categories used in our study (table 6).

The official veterinarians could choice more than one cause. If he/she had indicated more than 3 causes, we assumed that he/she had a high level of uncertainty about the origin of the breakdown, so we considered that the cause of infection for the farm remained unknown.

We made two sets of comparisons:

i) Only with those farms where the official veterinarian gave only one cause as the most likely, presumably those ones where he/she had a higher certainty about the cause of infection.

ii) All the farms: considering also two or three possible causes of infection.

The agreement between the perception of veterinarians involved in the bTB eradication program and results of our study was assessed by the Cohen's Kappa value (Cohen, 1960; Viera and Garrett, 2005) calculated with Win Episcope (2.0) software.

Table 6: Correlation between the lists of possible infection causes considered in the questionnaires and our study.

List of possible infection causes considered in the questionnaire	List of possible infection causes considered in our study
No slaughter of positives	Residual infection(recirculation)
Contaminated feed and water	
Deficient bio-security, installation and equipments	
Recirculation	
False negatives	
Deficient hygienic conditions	
Others/ incorrect testing of animals	
Others / Old animals	
Introduction of infected animals	Introduction of infected animals from Spain
Uncontrolled movements	
Sharing installation with other herds	
Transhumance/ Communal grazing	Sharing of pastures with other infected herds
Others / Neighborhood	Neighborhood
Cohabiting with other domestic species	Presence of infected goats in the farm.
Cohabiting with wildlife species	Interaction with wildlife reservoirs in the farm or pastures.
Contact with infected humans	Contact with infected human
Others / Birds	Not considered
Others / False positives	Not considered
Nearby waste disposal	Not considered

3.1.2. Results

Between 2009 and 2011, a total of 3159 herd breakdowns were detected in Spanish cattle farms. We obtained the questionnaires for 816 of these herds, most of them from years 2010 and 2011 (91.4% of the studied farms). The percentage of coverage (i.e., surveyed farms related to the total number of new positive farms) was around 26% (table 7). The beef farms represented the highest proportion of the analyzed herds (83.5%, n=679), followed by dairy (10.5%, n=85), fighting bull (5.3%, n=43) and others (0.74 %, n=6). The median herd size of these farms was 77 cows, the minimum and maximum herd size were 10 and 1028 animals, respectively. Bovine TB herd breakdowns were detected mostly by routine annual herd tests in previously negative herds (68.3%, n=557), test performed due to epidemiological relations with infected herds allowed to detect 19.3% of the cases (n=158) and the other herds (12.4%, 101) were identified by detection of MTC compatible lesions at routine meat inspection at slaughterhouses. There were no statistical significant differences in the median prevalence for slaughterhouse, epidemiological relation and annual test (4.3%, 6.4% and 5.3%, respectively). Results for the different type of herds are shown in table 8.

According to our approach, most of the new cases of infection were in fact resurgence of residual infections that had previously affected the herd (39%, n=316). New introductions of the infection were produced by contact with wildlife in 12% of cases (n=98), and by neighboring herds in 10% of cases (n=85). In 28 % (n=225) of the breakdowns, the origin of the infection remained unknown (table 9).

In 309 herds, the difference between the first and the second cause with the higher probability was small (i.e., less than 1) so, for these herds, first and second possible sources of infection could be considered (table 9). The most frequent first option was residual infection (69%, n=214) and the most frequent second options were sharing pastures with other herds (36%, n=112) and interaction with wildlife (30%, n=94).Furthermore, in 282 herds with only one cause (i.e., where the difference between the two possible causes was higher than one, the "residual" cause was also the most frequent (36%, n=102) followed by wildlife (25%, n=70), and contiguous spread (19%, n=54).

Only in 5% (n=48) of the cases the cause of infection was regarded as having a "*high*" or a "*very high*" probability, whereas the probability for the majority of the identified infection causes were qualified as *"quite high"* or "*not very high*" (60%, n=493) .The levels of probability recorded were *low* in 6% (n= 50) of the cases and below low (considered as unknown) in the remaining 27% (table 10).

The distribution of the probability values attributed to each infection route within all the studied farms is represented in figure 9 and in table 11 are represented the most likely events within each possible cause of infection. Most of the residual infections were attributed to herds that have had cases in the previous 3 years, but for which we did not have enough data to assess whether the isolates had similar molecular characteristics. This was followed by farms were the prevalence detected in the first positive control was not compatible with a recent infection (both corresponded to a *"quite high probability"*). Only 16% (n=10) of the 59 cases attributed to the introduction of infected animals were associated with *"high" or "very high"* probability while 84% (n=49) were with "*low"* or "*not very* high" likelihood. All the cases associated to goats except 1 were due to the presence of goats in the farm without data regarding their bTB status ("*not very high"* probability). More than 50% of the farms infected by neighboring spread had a positive neighbor farm with the same molecular type, whereas in 40% of them, the molecular relation between the herd breakdown and the neighboring farm could not be assessed. From the 98 cases attributed to wildlife, only in 12 the likelihood was "*high"* and corresponded to herds located near areas of hunting activity with the same molecular type isolated in the county.

Fifty-one percent of the surveyed herds were from Andalucía, 16% from Extremadura, 10% from Madrid and all the other regions contributed with less than 9 % to the studied cases. In table 12 the most likely source of infection by Autonomous Community is represented. Is remarkable the difference in the percentage of "unknown" infections between regions. While in areas of low prevalence such as Galicia or Asturias the "unknown" cause accounted for the 65% and 51% of the studied herds respectively, in other areas with higher prevalence such as Andalusia, the “unknown” cause just accounted for 17% of the cases.

There were also some differences in the cause of bTB herd breakdown according to the type of farm: in fighting bull and beef herds residual infection was the most likely cause, with 46.5% and 40.2% of the cases respectively. In dairy herds, residual infection represented just 24.7% of the cases while 60% of the herd breakdowns remained as “unknown”. Wildlife was not considered the cause of infection in any dairy herd (Table 13).

The results of the comparisons between our results and the ones of the veterinary officers are represented on table 14 and 15. We observed fair agreement within the group of herds where the veterinary officers just considered one option: most likely those herds in which they had a higher certainty about the cause of infection. Nevertheless, the agreement between the causes of infection attributed in our study and the one attributed by the veterinary officers after their epidemiological investigation was in general slight. The lowest agreement was in the case of “wildlife”. Within the group of 309 herds where the veterinary officer just considered one cause in 124 herds wildlife was considered as the most likely cause while we just found evidences to suspect of this in 33 farms.

Table 7: Number of bTB herd breakdowns occurred and surveyed by autonomous community (number of breakdowns by year obtained from www.rasve.es)

Autonomous Community	**2011**	**2010**	**2009**	**Surveys**	**Coverage (%)**
ANDALUCÍA	241	324	453	418	41
ARAGÓN	15	12	30	6	11
ASTURIAS	21	21	30	64	89
BALEARES	0	1	0	1	100
CANARIAS	0	0	0	0	no cases
CANTABRIA	43	41	56	0	0
CASTILLA LA MANCHA	24	29	84	51	37
CASTILLA Y LEÓN	250	262	195	0	0
CATALUÑA	19	18	18	27	49
EXTREMADURA	178	122	204	129	26
GALICIA	43	70	83	27	14
LA RIOJA	1	3	41	4	9
MADRID	74	44	10	79	62
MURCIA	1	5	4	0	0
NAVARRA	7	10	32	10	20
PAÍS VASCO	16	17	2	0	0
VALENCIA	5	11	6	0	0
Total	938	990	1248	816	26

Table 8: Median number of reactors and percentage of prevalence (in brackets) by detection method and herd type

Detection method	Beef				Dairy				Fighting Bull				Total			
	Obs	Median (%)	75%	Max	Obs	Median (%)	75%	Max	Obs	Median (%)	75%	Max	Obs	Median (%)	75%	Max
Slaugtherhouse	80	2.5 (5.2)	8.5(12.3)	128(81.5)	18	5.5(2.9)	20.5(15.5)	59(63.6)	3	2.5(2.2)	4(3.5)	4(3.5)	101	3.5(4.3)	9.5(12.3)	128(81.5)
Epidemiological relation	141	4.5(6.5)	7.5(15.7)	65(57.4)	6	4(5.1)	17(26.6)	37(86.1)	8	5(3.6)	16(10.3)	32(22.8)	155	4.5(6.4)	8.5(15.6)	65(86.1)
Annual testing	449	4(5.6)	8(12.7)	83(82.9)	60	2.5(3.1)	5(9.4)	110(70.9)	31	8.5(5.4)	24.5(10.8)	91(49.5)	540	4.5(5.3)	8(11.9)	110(82.9)
*Total**	670				84				42				796			

****20 farms not included (no data on method of detection)***

Table9: The most likely causes of infection of the herd breakdowns (first and second conclusions).

No	List of causes of infection	Most likely		Most likely (only one cause)*		1st most likely		2nd most likely	
		Freq.	%	Freq	%	Freq.	%	Freq.	%
1	Residual infection	316	39	102	36	214	69	37	12
2	Introduction of infected animals	59	7	33	11.7	26	8	22	7
3	Presence of infected goats	22	3	14	5	8	2.6	21	6.8
4	Neighborhood infection	85	10	54	19	31	10	23	7
5	Sharing of pastures	8	1	7	2.5	1	0.3	112	36
6	Interaction with wildlife	98	12	70	24.8	28	9	94	30
7	Contact with infected humans	3	0.4	2	0.7	1	0.3	0	0
8	Un known	225	28						
Total		816		282		309		309	

*** Only one cause refers to a difference bigger than one between the first and second cause.**

Table 10: The most likely causes by the level of probability qualifications

The most likely causes	< low	Low	Not very high	Quite high	High	Very high	Total
Unknown	225 (100%)						225
Goat			21 (95%)	1 (4%)			22
Movement		26 (44%)	23 (38%)		7 (11%)	3 (5%)	59
Wildlife		22 (22%)	64 (65%)		12 (12%)		98
Humans		2 (66%)			1 (33%)		3
Sharing of pastures			8 (100%)				8
Residual infection			7 (2%)	302 (95%)		7 (2%)	316
Neighborhood infection			32 (37%)	35 (41%)	18 (21%)		85
Total general	225 (27%)	50 (6%)	155 (18%)	338 (41%)	38 (4%)	10 (1%)	816

Figure 9. Probability distribution values for each infection pathway. The red solid line indicates the threshold to consider the cause as "unknown".

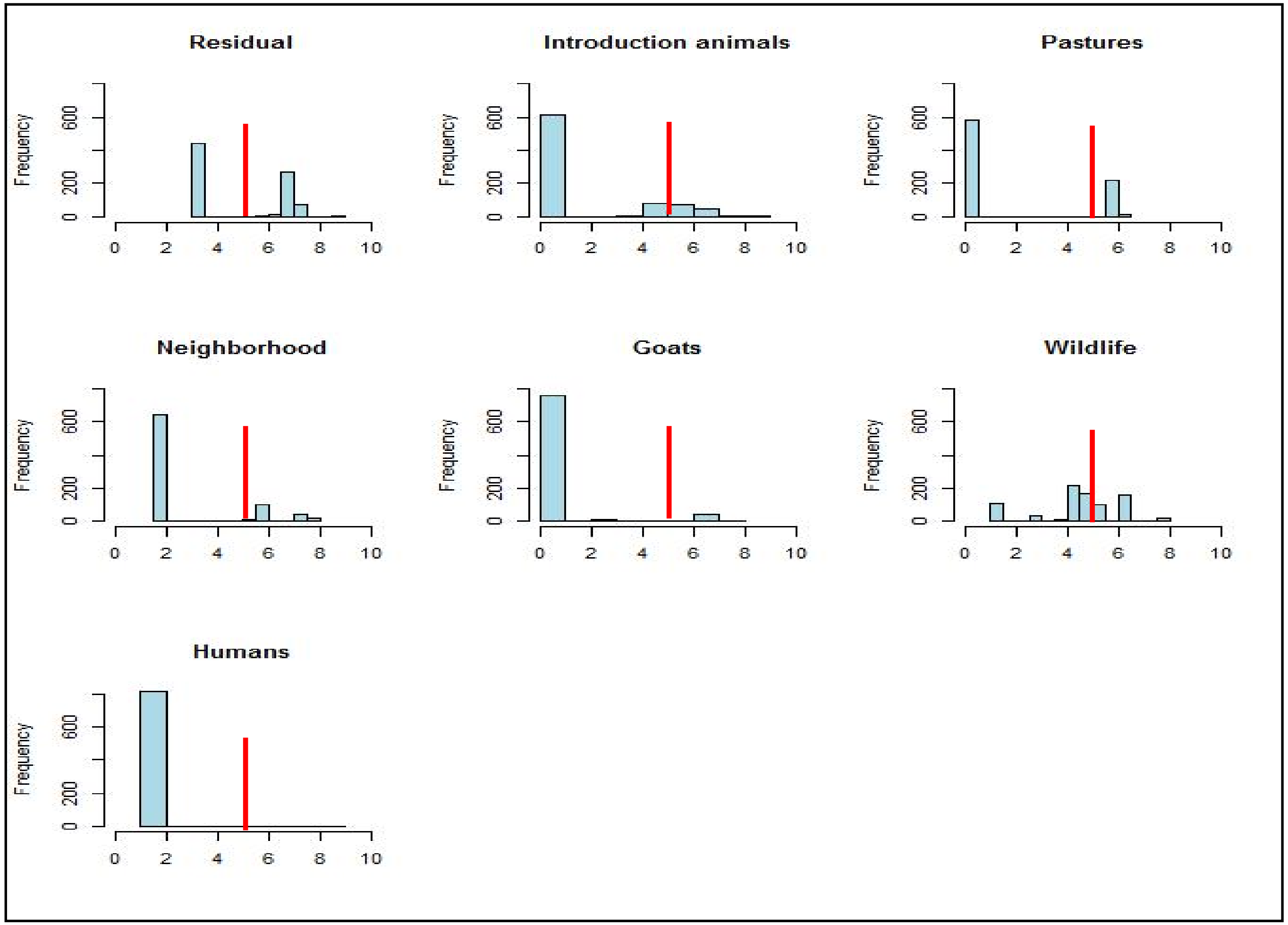


Table 11: The most likely events within each cause of infection (see annex 2 for clarifications about each event)

Residual infection		Introduction of Cattle		Goats		Neighborhood		Sharing of pastures		Wildlife		Infected Human	
Event (value)	**Herds**	**Event (value)**	**Herds**	**Event (value)**	**Herds**	**Event (value)**	**Herds**	**Event (value)**	**Herds**	**Event (value)**	**Herds**	**Event (value)**	**Herds**
P1 (6.1)	5	**P3 (8.7)**	3	**P4 (6.4)**	21	**P2 (7.9)**	18	**P4 (6.3)**	7	**P2 (5.3)**	13	**P1 (8.4)**	1
P2 (7.3)	68	**P5 (5.1)**	26	**P6 (7.3)**	1	**P4 (5.9)**	32	**P11 (6.0)**	1	**P4 (7.6)**	12	**P3 (5.1)**	2
P4 (5.6)	2	**P6 (6.4)**	6			**P5 (7.1)**	35			**P5 (5.3)**	9		
P5 (8.6)	7	**P8 (6.3)**	17							**P6 (6.2)**	41		
P6 (6.7)	234	**P9 (7.7)**	7							**P9 (6.4)**	23		
	316		59		22		85		8		98		3

Table 12: The most likely causes of infection by Autonomous Community

Autonomous Communities	**Unknown**	**Goat**	**Movement**	**Wildlife**	**Human**	**Pastures**	**Residual infection**	**Neighborhood**	**Total**	**%**
Andalucia	75 (17%)	14 (3%)	23 (5%)	55 (13%)	(0%)	(0%)	207 (49%)	44 (10%)	418	51,2
Aragon	3	0	0	2	0	0	1	0	6	0,7
Asturias	42 (65%)	2 (3%)	2 (3%)	7 (10%)	2 (3%)	(0%)	9 (14%)	(0%)	64	7,8
Baleares	1	0	0	0	0	0	0	0	1	0,1
Castilla-La mancha	18 (35%)	(0%)	1 (1%)	5 (9%)	(0%)	(0%)	22 (43%)	5 (9%)	51	6,3
Catalunya	8 (29%)	(0%)	(0%)	1 (3%)	(0%)	7 (25%)	11 (40%)	(0%)	27	3,3
Extremadura	40 (31%)	5 (3%)	22 (17%)	6 (4%)	1 (0%)	(0%)	30 (23%)	25 (19%)	129	15,8
Galicia	14 (51%)	5 (3%)	5 (18%)	1 (3%)	(0%)	(0%)	6 (22%)	1 (3%)	27	3,3
Madrid	18 (22%)	1 (1%)	5 (6%)	20 (25%)	(0%)	1 (1%)	24 (30%)	10 (12%)	79	9,7
Navarra	6	0	1	0	0	0	3	0	10	1,2
Rioja (la)	0	0	0	1	0	0	3	0	4	0,5
TOTAL	**225 (27%)**	**22 (2%)**	**59 (7%)**	**98 (12%)**	**3 (0%)**	**8 (0%)**	**316 (38%)**	**85 (10%)**	**816**	

Table 13: The most likely causes of infection identified by herd type

The most likely causes	Beef	%	Dairy	%	Fighting bull	%
Unknown	166	24.5	51	60.0	6	14.0
Goat	18	2.7	3	3.5		
Movement	44	6.5	7	8.2	6	14.0
Wildlife	93	13.7			4	9.3
Humans	1	0.2	2	2.4		
Pastures	8	1.2				
Residual infection	273	40.2	21	24.7	20	46.5
Neighborhood infection	76	11.2	1	1.2	7	16.3
Total general	679		85		43	

** 9 farms not included (other types)*

Table 14: Comparison between the most likely causes of infection determined in our study versus those causes identified by official veterinarians in those herdswhere they only concluded one option (n=309)

List of infection sources	**Our study**		**Veterinary officer**		**Agreement test**		
	Frequency	**%**	**Frequency**	**%**	**Frequency**	**Kappa**	**Agreement**
Residual infection	99	32.0	62	20.1	32	0.20	Slight
Introduction of infected animals	25	8.1	44	14.2	12	0.27	Fair
Presence of goat in the farm	9	2.9	12	3.9	2	0.16	Slight
Neighborhood infection	25	8.1	8	2.6	4	0.21	Fair
Sharing of pastures	3	1.0	7	2.3	2	0.39	Fair
Interaction with wildlife	33	10.7	124	40.1	17	0.05	Slight
Contact with infected human	2	0.6	1	0.3	1		
Unknown	113	36.6	51	16.5	39	0.32	Fair

**** NB: Interpretation of Kappa (Viera and Garrett, 2005)***

Table 15: Comparison between the veterinary officer conclusions (1, 2 or 3) and our conclusions (1 or 2)

**** If more than 3 conclusions were reported by the official veterinarian, the cause of infection was assumed to be unknown.***

List of infection sources	Conclusions of our study		Veterinary officer conclusions		Agreement test		
	Freq.	**%**	**Freq.**	**%**	**Freq.**	**Kappa**	**Interpretation**
Residual infection	353	43	427	52.3	216	0.15	Slight
Introduction of infected animals	79	10	113	13.8	28	0.20	Slight
Presence of goat in the farm	43	5	140	17.6	20	0.15	Slight
Neighborhood infection	108	13	50	6.1	22	0.21	Fair
Sharing of pastures	120	15.7	49	6.0	13	0.07	Slight
Interaction with wildlife	175	21	533	65.3	125	0.04	Slight
Contact with infected human	3	0.4	9	1.1	1	0.16	Slight
Unknown	287	35	110	13.5	62	0.13	Slight

3.1.3. Discussion

We have analyzed the most likely causes of infection of 816 herds that became infected from 2009 to 2011. This represents around 26% of the breakdowns occurred during the three years period in the whole of Spain. Moreover, if taking into account that most of the surveyed herds (all except 70) corresponded to breakdowns occurred between 2010 and 2011 (as the BRUTUB system was first implemented in 2009) this percentage increases to 39% (i.e., 746 out of 1928 breakdowns). Therefore, we consider that it is a very good representation of the most common causes of new infections in Spanish farms. However, when interpreting the results, it has to be taken into account that some regions are clearly under-represented. As it is shown in table 7, the percentage of recorded surveys in the BRUTUB system was very variable among regions. While in some areas as Asturias or Madrid the coverage was around 89% and 62% respectively, in others was very low, for example Aragon with 11%, or Castilla y Leon and Cantabria with no surveys recorded in the system. If there were real differences in the causes of infection among regions, this would not be reflected in the results of our study, and therefore care should be taken when extrapolating the results to regions not included in the study. Nevertheless, we believe that our results are quite representative as we had good coverage from autonomous communities of different areas of the country with different prevalence and which are likely to be different from an epidemiological point of view.

About 70% of the new infections were detected through the annual routine herd testing, which reflects the importance of this type of surveillance in the eradication plan for bovine TB in Spain. However, it is important to highlight that the other methods (i.e., slaughterhouse surveillance and the study of epidemiological relationships using a questionnaire) allowed an early detection of a non negligible number of new positive farms, pointing out also the paramount importance of these complementary surveillance components.

There was no difference in farm prevalence among herd breakdowns disclosed by routine herd testing, slaughterhouse surveillance and epidemiological relationships. A higher prevalence in farms not detected by routine herd testing would have been expected, as they could be expected to have been infected for a longer period of time. However, the lack of differences in herd prevalence between them may be reflecting that the slaughterhouse surveillance and epidemiological investigations are also being able to detect some early cases. It would be desirable that each region evaluated if these surveillance components could be improved, in order to achieve a better sensitivity of their regional surveillance system.

According to the results of our study, residual infection was identified as the most important cause of new bTB positive herds, being responsible for approximately the 40% of the infections detected in officially free herds. This result is in accordance with studies conducted in other bTB endemic European countries such as Ireland (Clegg et al., 2011; Good et al., 2011) and Great Britain (Goodchild and Clifton-Hadley, 2001; Carrique-Mas et al., 2008; Karolemeas et al., 2011; Conlan et al., 2012), where residual infection has been identified as a major concern of the national bTB eradication program. Griffin et al. (2005) and Olea Popelka et al. (2008) have also reported strong association between previous history of bTB in cattle herds and the risk of future breakdowns and local disease persistence in Ireland.

Reasons for the existence of residual infection includes not detecting all infected animals in previous tests within the herd because of imperfect test performance, desensitization due to repeated testing, lack of good veterinary practice (De la Rua-Domenech, 2006a; Vordermeier et al., 2006; Coad, 2010; Humblet et al., 2011) as well as persistence of *M.bovis* in the environment of the farm (Courtenay et al., 2006).

It is difficult to assess the importance of each of these factors in the resurgence of the infection in previously cleared herds. The lack of test performance could be considered as being one of the most important points. The current internationally accepted bTB screening tests is the tuberculin skin test, as a single intradermal test (SIT) or intradermal comparative test (SICCT) (Council Directive 64/432/EEC; OIE, 2009). The SIT has 80-91% sensitivity and 75.5-99.0% (median of 96.8%) specificity whereas SICCT has 88.8-100% (median of 99.5%) specificity with a reported sensitivity of 55.1-93.5%. The sensitivity of the complementary gamma interferon test ranges between 80.9 and 100% (median of 88.4%) but has a relatively lower specificity 87.7-99.2 %(median of 96.6%). Therefore none of these tests detect all bTB infected cattle in a herd and the probability of false negative results in two consecutive test rounds in farms with a single infected animal is not negligible (De la Rua-Domenech, 2006a; Vordermeier et al., 2008).Another limiting factor for detecting all the infected animals is related to management. In Spain, beef and bull fighting herds are usually kept under extensive conditions in large pastures, which might difficult the testing of all animals, and therefore increases the likelihood of not detecting one or few positive animals in two consecutive test rounds and allow the recirculation of the disease within the herd.

In some cases the prevalence found when bTB was first detected at the farm were very high (i.e., higher than 20%), which is unusual after a recent infection, and could be related to a lack of good veterinary practice. However, even though bTB is believed to have a low transmission rate within a herd, large number of infected animals in short time periods has also been observed (Steger, 1970), and therefore some of these cases could have been misclassified. On the other hand, for some farms it is not so clear whether residual infection was really the cause of infection. From the 316 herds attributed to residual infection, in 214 there was a small difference (i.e., less than one point) between the first and second cause. In these farms, the most likely second causes were sharing of pastures and interaction with wildlife. Therefore, in some of the farms where residual infection was considered as the most likely cause of infection, a reintroduction from outside the farm rather than a recirculation within the farm was also possible. If this was the case, we would have overestimated the role of residual infection.

We decided to consider a cause as the most likely cause of herd breakdown if the level of probability was at least with a “low” (i.e. with a value of 5 in the ordinal scale). On the basis of this threshold, 28% of the studied herds were classified as having an “unknown” cause of breakdown. By doing this we assumed that in the herds in which values were lower than this threshold, we did not have enough evidences to attribute to any of the possible causes of breakdown. The decision of selecting this threshold was based on biological reasons, as those events below this threshold were assumed to have a negligible probability of being the cause of breakdown.

Even though a 28% of “unknown” represents a high percentage of the studied herds, it is lower than reported in other studies. In Ireland and Great Britain, in 35% and 40% of the breakdowns, respectively, an infection source could not be established (Griffin and Hahesy, 1992; Wilesmith, 1983). The percentage of unknown cases was especially high for dairy herds (i.e., 60%). This result is more likely influenced by the quality of the data, and the fact that a high proportion of these farms are managed intensively and therefore the contact by neighborhood, pasture or wildlife is less likely. Regarding the assessment of the animal movements as possible cause of outbreaks, we had some limitations. In the epidemiological questionnaire only those movements considered to pose a risk (i.e., from herds not qualified as T3 for at least three years) were recorded. Therefore, we did not have access to all the movements and this might be influencing the result. Moreover, recorded movements were herd movements and not individual movements, which limit the assessment of the animal movement pathway. On the other hand, illegal movements, if any, are not able to be considered in this study. Also related with this limitation of the data might be the apparent relation between the prevalence of the region and the likelihood of finding consistent evidences to attribute a cause of infection. In Asturias and Galicia (areas of low prevalence (i.e., below 1%)) in more than 50% of the investigated herds the cause of infection could not be established. In contrast, in Catalonia (also an area with low prevalence) the percentage of “unknown” was lower (i.e., 30%). This difference is explained by the fact that in this region we had access to much more detailed data about animal movements, as we had data of all the positive animals detected in each breakdown. Ear tag numbers were used to extract all the individual movements from the regional database system, and thanks to this we could link some breakdowns to movement to pastures or between farms. In our opinion, access to more detailed data about animal movements would help to explain some of the origins of the breakdowns.

In Spain, the Eurasian wild boar (*Sus scrofa*), red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) have been considered to play an important role as reservoirs of infection to domestic animals (Parra et al., 2006; Boadella et al., 2011; Gortazar et al., 2011; Garcia-Bocanegra et al., 2012). This is to some extent in accordance with the results of our study where wildlife was considered to be the most likely cause of bTB herd breakdowns in 12% (n=98) from the total of 816 farms, and the second most frequent cause of bTB introduction (30%, n=94) when considering a second most likely cause in the group of 309 farms with a no clear single cause.

Different factors might bias the role of wildlife: i) interaction between domestic and wildlife was completely dependent on information given in the questionnaire survey, and could be biased by personal perceptions (wildlife has been considered most of the times the main cause of bTB infection) ii) we did not have data about the presence of bTB in wildlife (in the corresponding county) for 296 farms iii) From those that we had data, we did not have the molecular data for 27 farms iv) We only considered a county to have positive wildlife if confirmed by culture v) Even in those cases with good data about wildlife, there is a lot of uncertainty around the risk of infection for a domestic farm due to the presence of positive animals on the area and despite the probability values were assigned by an expert opinion workshop some of the probabilities might be over or underestimated.

The determination of the origin of infections, especially in chronic diseases is always a difficult task. Moreover, there is not a standard methodology to investigate the source of breakdowns. Different approaches have been applied in order to determine the possible origin of different diseases; Elbers et al. (1999) used key questions to investigate the causes of infection of classical swine fever breakdowns in The Netherlands; the European Food Safety Authority (EFSA, 2004) attributed different scores to risk factors for bovine cysticercosis by using expert opinion. By this method they assessed the risk of infection of each herd based on the characteristics of the herd. This methodology was adapted by Allepuz et al. (2009) to investigate the most likely causes of infection of bovine cysticercosis in northeastern Spain (i.e., Catalonia).

The decision trees developed in this study, and used to evaluate the cause of introduction of bTB in cattle herds, seem apparently simple in some cases. However, they were designed and adapted to get key information from each possible cause of infection, considering all possible routes for which we had information available. In our view, a key aspect of these decision trees is the assignation of a probability to each possible situation. As there is almost no information about them, we used the opinion of experts to assign those probabilities. Although, the use of the average probability values assigned by the experts for each event in the decision tree may misestimate some events; in general there was good agreement among the experts. After the discussion, experts had the chance to reconsider their first scores if they believe that had overestimated or underestimated any of the probabilities. However for some of the probabilities there was a lot of uncertainty, and it is possible that prejudgments or people defending their arguments with more determination could produce wrong perceptions and a biased value. In order to reduce the bias regarding possible prejudgments, experts with different backgrounds were included in the workshop. There was one wildlife epidemiologist, a couple of people working for the official veterinary services, two researchers from reference diagnostic laboratories (regional and national), one pathologist specialist on bTB and two epidemiologist focused on domestic animals. Moreover, experts were from different regions (north, centre and south) covering the different epidemiological scenarios. Taking into account the inherent limitations derived from obtaining estimates from expert opinion workshops, we believe the estimates we got were the best possible estimates. Nevertheless, it would be desirable to repeat this expert opinion workshop in the future in order to update these values.

Just a 5% of the cases presented a high or very high probability. This is related mainly with the limited availability of molecular data. For 53% of the herds we did not have data about the spolygotype at farm level, which limited the assessment of the different pathways, and especially the certainty attributed to a given cause. The molecular characterization of the different isolates in the breakdowns is essential to provide strong evidence about the origin of the infection. Nevertheless, it is also possible that the uncertainty regarding the origin of the breakdown would have remained high for an important proportion of farms, due to the difficulty in estimating a clear cause of infection in most of the cases. Bovine TB is a chronic disease, with low transmission rate within herds and no clear clinical signs in affected animals. All of these points make it difficult to infer with a good certainty the most likely cause of infection, as is difficult to estimate the period when the herd got infected.

The comparison carried out between our results and those fulfilled in the questionnaire by the veterinary officers showed a moderate agreement when we considered only the 309 farms where the veterinary officer just considered one possible cause (i.e., most likely those farms where he had a higher level of certainty about the cause). The level of agreement decreased to slight when we included the 816 farms and considered one or two possible causes from our study and up to three by the veterinary officer. In these cases the disagreement could be associated to the increased level of uncertainty. Both methods (decision trees and the opinion of veterinary officers) have weak and strong points and the reality might be in a medium point between the results of both methods. The decision trees are an objective procedure based on expert opinion, group discussion and literature review. However, we did not know the particularities of the management and facilities of the farm and the idiosyncrasy of the area. Besides, we had limited information on cattle movement, wildlife and other domestic species bTB status. Instead the veterinary officers had direct contact with the farm owners to get first hand information and direct on farm observation. Another likely source of discrepancy between our results and the ones of the veterinary officers is the importance attributed to the different epidemiological contacts. In our study these values were obtained from an expert opinion workshop, and the same value was applied to all the farms. In the case of the veterinary officers there might be a higher heterogeneity in the importance attributed to the different contacts due to different perceptions about the risk posed by the different epidemiological contacts. It would be desirable to harmonize the criteria used in the epidemiological investigations conducted by veterinary officers in order to get comparable results between and within the different regions of Spain.

3.2 STUDY II

3.2. STUDY 2: Bovine TB persistence associated factors (Case control study)

3.2.1. Introduction

In Spain between 50 and 60% of the total bTB positive herds detected each year, are new positive herds, and the rest of the positives are herds that persist from the previous year. During the eradication program of tuberculosis, the eradication of the infection in some herds has been very difficult and the achievement of the free status has lasted several years. The current bTB epidemiological situation poses a serious challenge to the advancement of the eradication programe toward the achievement of national OTF status. Nevertheless, there is not a clear knowledge of the epidemiological circumstances that lead to the introduction or persistence of the infection. The objective of the second study was to identify the risk factors associated with the persistence of bTB in beef herds from central and southern Spain.

3.2.2. Material and methods

We conducted a case-control study on beef farms matched by herd size and geographical location (at county level).

Case farms were defined as those farms in which bTB persisted for at least a minimum of 5 years during the last 10 years. Control farms consisted in bTB affected farms also in the last 10 years but that have achieved to eliminate the infection in a period of 1-2 years.

An epidemiological questionnaire including potential risk factors for bTB persistence was designed. In this questionnaire we tried to collect as much data as possible about factors potentially related with the probability of persistence based on existing literature. We included questions related to routine diagnostic (changes in personnel, etc), structure of the farm (pasture area, number of nuclei, neighborhood, etc), structure of the herd (number of animals by age, breed, etc), presence of other domestic species in the farm, management (origin of purchased animals, feeding practices, etc), wildlife reservoirs, health status of the herd and history of cases in people (annex 3). A pilot questionnaire was done in different herds located in northeastern Spain.

The study was designed to detect Odd Ratios differences of 2.5 with 95% level of confidence, 80% of potency and assuming exposure of 20% for the controls. A sample size of 200 (100 controls and 100 cases) beef farms was calculated. Farms were selected from Southern and Central Spain (Andalucía and Castilla La Mancha) using data on herd size, bTB status and farm location provided by the regional governments of Andalusia and Castilla La Mancha.

Personal interviews with farm owners or veterinarians responsible of the herd were carried on. Some farms could not be interviewed due to different reasons (refusal to be interviewed, farms belonging to a same epidemiological unit, etc). From the 200 farms planned for the study, questionnaire survey was carried on 150 herds (80 controls and 70 cases).

Extra data was computed in order to include it in the analysis. The abundance of red deer in Spain was obtained at UTM 10x10 km^2 grid cells from Acevedo et al. (2010). The location of cattle farms and bTB status between 2005 and 2010 were provided by the regional governments of Andalusia and Castilla La Mancha. From these data and by using Quantum GIS software Version 1.8.0 (http://www.qgis.org/) we calculated the number of cattle farms and number of positive farms in a 5 km radius around each case and control farm.

3.2.2.1. Statistical analysis

The model was built following the next steps (Dohoo et al., 2003):

a) Bivariate analysis between our outcome (i.e., case or control) and different predictor variables using a liberal p-value (we used $p< 0.30$). Categorical variables were screened using χ^2 test and continuous variables with ANOVA or Kruskal-Wallis test. Bartlett's test for inequality of variances was applied to choose between both methods. In the case of non-homogeneity of variances we used the Kruskal-Wallis test. Continuous variables were categorized before entering the model.

b) Evaluation of correlations among predictor variables: for those variables associated with the probability of persistence with a p-value lower than 0.30

we calculated the Spearman correlation coefficient, in case of correlation between them (i.e., higher than 0.5), the variable with higher biological signification was retained.

c) A manual model-building selection was conducted: for this purpose we compare all the possible models with just one variable by the AIC value. To the model with the lowest AIC value and one predictor we included all the remaining covariates and compared them based on the AIC value. This process was repeated until we got the model with lowest AIC. This was considered as the most plausible one and selected as the final model.

d) Biologically meaningful interactions were also included in the model.

e) To test the ability of the model to discriminate between case and controls we calculated a Receiver Operating Characteristic (ROC) curve and the area under the curve (AUC). An AUC value greater than 0.8 and between 0.7 and 0.8 are considered as good and moderate discriminate capacities respectively.

Epi Info 7 software was used for the bivariate analysis. The logistic multivariate analysis was conducted with SPPS software. AIC was calculated taking into account the likelihood ratio and the degrees of freedom using the next formula:

AIC= log likelihood ratio+2*(degrees of freedom+1)

3.2.3. Results

Bivariate analysis results can be found on table 7 and table 8. From the categorical predictor variables the presence of goats or pigs, the contact with pigs, doing replacement from bTB positive mothers, having contact with neighbors, contact with neighbors cattle herds or bTB positive neighbors, receiving drainage from bTB positive farms, not isolating positive cattle, not testing all cattle and the presence of deer within the farm were related with the probability of bTB persistence with p value lower than 0.30. Two quantitative predictor variables (i.e. deer density and area of pasture) had p<0.30.

For these predictor variables associated with the probability of bTB persistence in a first screening we performed a correlation matrix using the Spearman correlation coefficient. Predictor variables with correlation coefficients lower than 0.5 were retained for the multivariate analysis. In the case of correlated variables we retained those ones with higher biological signification.

Table 16: Categorical variables included in the bivariate analysis

Categorical variables	**Percentage**		**Odds- Ratio**	**95% CI**		**P value**
	Case	**Cont.**		**Lower**	**Upper**	
Routine diagnostic						
No having same enterprise	48.6	50.0	0.9	0.5	1.8	0.861
No having same veterinarian	34.3	36.3	0.9	0.5	1.8	0.801
Presence of other domestic species						
Presence of other species	62.9	65.0	0.9	0.5	1.8	0.785
Presence of sheep	25.7	20.0	1.4	0.6	3.0	0.404
Presence of goat	10.0	5.0	2.1	0.6	7.5	0.241*
Presence of pigs	17.1	32.5	0.4	0.2	0.9	0.030*
Presence of equine	31.4	30.0	1.1	0.5	2.1	0.849
Contact with sheep	15.7	11.3	1.5	0.6	3.8	0.422
Contact with goats	5.7	2.5	2.4	0.4	13.3	0.316
Contact with Pig	10.0	22.5	0.4	0.1	1.0	0.040#
Contact with Equine	30.0	26.3	1.2	0.6	2.5	0.609
Structure of the farm						

More than one holding	34.3	27.5	1.4	0.7	2.8	0.368
Having contact with neighbor	75.7	60.0	2.1	1.0	4.2	0.040#
Neighbor contact with goat	15.7	15.0	1.1	0.4	2.6	0.903
Neighbor contact with cattle	71.4	52.5	2.3	1.1	4.5	0.017#
Neighbor contact with pig	2.9	2.5	1.1	0.2	8.4	0.892
Neighbor contact with positive	55.7	35.0	2.3	1.2	4.5	0.010*
Recieve drainage from positive farm	40.0	26.3	1.9	0.9	3.7	0.073*
Not fenced farm	10.0	8.8	1.2	0.4	3.5	0.792
Forest present within farm	71.4	73.8	0.9	0.4	1.8	0.75
Management						
Being an ecological Farm	17.1	13.8	1.3	0.5	3.2	0.565
External origin of females replacement	91.4	93.8	0.7	0.2	2.4	0.586
External origin of males replacement	28.6	35.0	0.7	0.4	1.5	0.399
Replacement from positive mothers	38.6	18.8	2.7	1.3	5.7	0.006*
Doing transhumance	2.9	2.5	1.1	0.2	8.4	0.892
Pasture sharing	8.6	10.0	0.8	0.3	2.6	0.764
Give vitamine supplement	44.3	43.8	1.0	0.5	2.0	0.947
Give silage supplement	91.4	93.8	0.7	0.2	2.4	0.586
Machineary sharing	2.9	3.8	0.8	0.1	4.7	0.761
Straw from other farm	10.0	15.0	0.6	0.2	1.7	0.358
Not isolation of positive cattle	21.4	13.8	1.7	0.7	4.0	0.215*
Not all cattle tested	12.9	3.8	3.8	1.0	14.6	0.040*
Wildlife						
Game farm at the neighborhood	41.4	40.0	1.1	0.6	2.0	0.858
Hunting residues within farm	12.9	8.8	1.5	0.5	4.4	0.416
Presence of wild boar	88.6	90.0	0.9	0.3	2.4	0.777
Presence of deer	85.7	78.8	1.6	0.7	3.8	0.268*
Pressence of fallow deer	35.7	35.0	1.0	0.5	2.0	0.927
Presence of roe deer	28.6	31.3	0.9	0.4	1.8	0.72
Presence of fox	94.3	90.0	1.8	0.5	6.4	0.334
Presence of badger	45.7	46.3	1.0	0.5	1.9	0.947
Presence of chamois	41.4	45.0	0.9	0.5	1.7	0.659

* Variables entered in the multivariable analysis

Variables excluded from the multivariate analysis due to the collinearity

Table 17: Quantitative variables included in the bivariate analysis

Quantitative variables	Case/Cont	Mean(sd)	25%	Median	75%	Max	P value
Structure of the farm							
Cattle farms at 5km	Control	19.1 (20.3)	6.5	14.5	25	138	0.41
	Case	16.1 (15.3)	7	10.5	19	71	
Positive cattle farms at 5 km (2005-2010)	Control	6.5 (5.5)	2	5	9	22	0.662*
	Case	6.9 (6.3)	3.5	5	9	30	
Farm pasture area (ha)	Control	544.3 (880.7)	175	300	450	6000	0.0618
	Case	1029.4(2539.8)	200	345	700	18000	
Wildlife							
Deer Density	Control	11.3 (2.0)	10.7	12	12.5	14.8	0.061*
	Case	10.6 (2.4)	9.7	11.6	12.1	14.2	
Management							
Mean time until slaughter of positive animals (days)	Control	13.5 (6.3)	8.5	15	17	30	0.566
	Case	15.4 (9.7)	7.5	15	20	50	
Structure of the herd							
Percentage of animals from 2 to 5 years	Control	35.6 (14.7)	25.8	35.4	42.7	83.3	0.25
	Case	32.7 (13.2)	24.5	32.1	41.4	89.3	
Percentage of animals from 5 to 10 years	Control	35.1 (13.6)	26.5	34.2	43.6	73.6	0.76
	Case	34.4 (12.1)	27.9	33.7	42.8	66.6	
Percentage of animals from 10 to 15 years	Control	17.2 (10.5)	9.5	15.6	23.6	47.5	0.52
	Case	16.1 (10.3)	9.7	14.2	21.2	63.8	
Percentage of animals older than 15 years	Control	4.7 (3.3)	2.3	4.2	6.3	13.8	0.8
	Case	5.2 (5.1)	2.1	3.8	6.6	28.5	

Table 18: Variables selected for logistic regression analysis model building

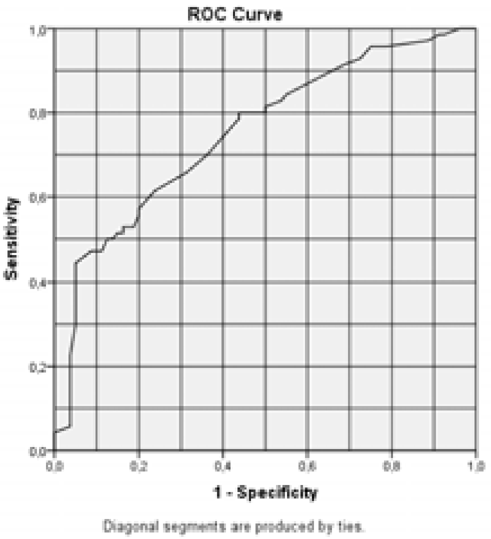
Variables	**AIC**
Not isolation of positive cattle	209.74
Pasture area	206.4
Deer density	211.50
Receive drainage from positive farms	208.07
Presence of goats	209.90
Presence of deer	210.04
Presence of pig	206.52
Not all cattle tested	206.95
Replacement from positive mothers	203.90
Neighbor contact with positive	204.76

The logistic regression model with the predictor variable "*Replacement from Positive mothers"* was the one with the lowest AIC (i.e., 203.90) of all the possible models with just one predictor variable (table 9). On this model we included a second variable and compared them based on the AIC. We repeated this procedure until getting the lowest AIC value. The model having the lowest AIC (i.e., 195.42) was selected and considered as the final model. The model includes the presence of pigs, to have contact with neighbor positive herds and the pasture area; it includes also three non significant variables: the presence of goats, maintain positive animals in the herd until slaughter and use calves born from positive dams for replacement. The odds ratio with its 95% confidence interval, standard error and p-value is represented in table19.

Table19: Results of multivariate logistic regression model. B: coefficient estimated by the model; SE: standard error; Wald: Wald statistic; df: degrees of freedom; p: p-value; OR: odds ratio (i.e., exponential of B); 95%CI: 95% confidence interval of the OR. Area, 2, 3 and 4 indicates the second, third and fourth quartiles of the farm pasture area.

Variables	**B**	**SE**	**Wald**	**Df**	**p**	**OR**	**95%CI**	
							Lower	**Upper**
Presence of goat	1.32	0.76	3.02	1	0.08	3.7	0.8	16.4
Presence of Pig	-1.02	0.45	5.04	1	0.03	0.4	0.1	0.9
Replacement from positive mothers	0.80	0.42	3.58	1	0.06	2.2	1.0	5.1
Neighbor contact with positive	0.89	0.37	5.69	1	0.02	2.4	1.2	5.1
No isolation of positive cattle	0.72	0.49	2.17	1	0.14	2.1	0.8	5.4
Area			7.49	3	0.06			
Area2	1.23	0.62	3.87	1	0.05	3.4	1.0	11.6
Area3	0.48	0.54	0.78	1	0.38	1.6	0.6	4.7
Area4	1.37	0.60	5.22	1	0.02	3.9	1.2	12.7

Figure 10: The area under the ROC curve



The area under the ROC curve (AUC) calculated for the final model was 0.75 (95% CI: 0.68-0.83), that means the model has good discrimination capacity, and in almost 75% of all possible pairs of subjects the model will assign a higher probabilities to cases with the outcome of interest (figure 10).

3.2.4. Discussion

In this study we evaluated the possible association between different factors and the success of bTB elimination, by comparing farms that achieved the officially Tb free status in less than 2 years, with farms that needed 5 or more years. We found that factors related to the structure of the farm, management and the presence of other domestic species could have an influence on the bTB eradication success.

Regarding associations related with the structure of the farm we found that the odds of bTB persistence was between 1.1 and 5.0 times higher in those herds that can contact with cattle from a neighbor positive herd. This result is in accordance with previous studies. In Ireland, Denny and Wilesmith (1999) reported that approximately 40% of breakdowns were attributed to the presence of a contiguous positive herd that had a confirmed breakdown. Local persistence of bTB infection has also been described as a key feature leading to the development of recurrent episodes in neighboring herds of United Kingdom and Ireland (Karolemeas et al., 2011; Kelly and More, 2010). This relation could be explained by the fact that the presence of bTB positive farms in the neighborhood could contribute to contiguous spread by cattle-to-cattle transmission over farm boundaries (Denny and Wilesmith, 1999; Johnston et al., 2011). Besides the transmission to previously free herds, neighborhood could also explain the difficulties in TB eradication due to periodic re-infections of affected farms.

However, it is difficult to infer the direction of the transmission. Besides the periodic re-infection coming from other farms, we must take into account the opposite direction of the infection, i.e. the surveyed herds can be the cause of the infections of farms located in the neighborhood, or, a third possibility that could explain this relation could be the presence of a wildlife reservoir, acting as a common source to the farms on the same area. In these two cases, the primary mechanism of persistence of residual infection within the farm can be due to whatever other cause not included in the study.

The exact transmission mechanisms of local bTB transmission are not clear. White et al., (2013) in a study conducted in Ireland, concluded that an infected wildlife source was the best explanation for the existence of a neighboring herd risk at distances greater than 25 meters, and just explained some of the risk at distances lower than 25 meters. These authors suggested that contiguous spread among cattle from neighboring farms had lower importance than infected wildlife reservoir as a transmission mechanism to explain neighborhood persistence. However, these results might not be directly applicable to the situation in Spain, as the wildlife reservoirs are different and therefore, the role of badgers in Ireland and wild boars or deer in Spain probably is not the same. As far as we know, in Spain, there have been no detailed studies conducted in order to elucidate the role of these different local transmission mechanisms and therefore, it is difficult to assess which would be the more plausible explanation. Further studies, attempting to gather more detailed data about this topic would be desirable.

Also related with the structure of the farm, we found an association with the size of the pasture farm land**:** Farms with larger pasture areas had an odds ratio between 1.2 and 12.7 times higher of having persistent bTB infection than farms with smaller pasture areas. In a review conducted in United Kingdom by the independent Scientific Group (2007), they also reported that an increase in farm land area was an important risk factor for bTB herd breakdowns in different regions of the country.

The size of the pasture farm land could be related to different factors potentially linked to bTB persistence. It is reasonable to speculate that farms with larger pastures have a more difficult herd management (and therefore more difficulties on testing all the animals) and an increase in the likelihood of interaction with neighboring positive herds or with infected wildlife reservoirs.

The presence of goat in the farm was not identified as a significant predictor of bTB persistence in the final result of our model (OR=3.73; 95% CI=0.846-16.417). However this could be related with an error of type II, which means that a value of $p > 0.05$ not always strictly define the absence of a significant association. This type of error is linked to the number of farms included in the study and to the small number of farms that had goats. According to the data extracted from the questionnaire survey we found that only 11 farms (7 cases and 4 controls) had goats, being this low number small to extract accurate conclusions. Nevertheless, the model selection procedure kept this variable in the final model, indicating that despite there was no significant association, it was considered as a relevant piece of information to get the more plausible model (i.e., the model that explained the higher quantity of variation with the lower number of variables). Moreover, in the last years there have been some reports suggesting a role of goats in bTB epidemiology, and the need to focus attention on them (Aranaz et al., 2003; Napp et al., 2013). Testing of goats is only compulsory on farms with cattle and goats (Anon., 2012). However, it is also remarkable that from the 11 herds reported to have goats in the farm, just four of them reported to have tested them (none with positive results).

Farms where TB has been eliminated in less than two years have more frequently pigs than farms where the infection remained for 5 or more years (OR=0.4; 95% CI=0.1-0.9). In the area of study (western and southern Spanish regions) there is an important population of Iberian breed pigs raised in a free-range system, sharing natural resources with other wild and domestic animals. In these areas, *M. bovis* infection is widespread in wild and domestic species (Gortazar et al., 2011). Moreover, according to the survey most of the pigs present in farms where in contact with domestic cattle (Spearman correlation coefficient=0.7). The Iberian pigs infected with *M. bovis* have been reported to develop severe generalized conditions with open lesions (Parra et al., 2003). Also, Di Marco et al. (2012) have reported that the Sicilian black pigs, in its ecological setting, might act as a reservoir of bTB infection on the basis of the characteristics of the lesions localization and the genetic profiles of *M. bovis* isolated during their study. In our opinion, our result could be due to an inadequate question because we are not able to distinguish between farms with one o few pigs for domestic consume and an Iberian breed farm. Another explanation is the presence of a confounding variable (such as different husbandry and management approaches conducted in these farms) that were not measured during the survey. Therefore, and despite the result of our study, the role of domestic and extensive reared pigs on bTB epidemiology might deserve further attention.

From management related factors, the replacement from positive mothers and not isolating positive animals were retained in the final model despite not having a statistical significant effect (p-value higher than 0.05). As in the case of the presence of goats within the farm, the fact that these variables reduced the AIC and were retained during the variables selection procedure is suggesting that they could have a role in the probability of bTB persistence. Transmission from cow to calf by ingestion of infected calostrum or milk has been reported to have a very limited importance in countries with regular testing programs implemented (Humblet et al., 2009). However, close contact between the calf and his infected mother could increase the likelihood of transmission via the inhalation of infected droplets released by the cow. In this sense, replacement from infected cows could be related with a higher risk of recirculation within the farm, and therefore a higher likelihood of persistence. Also, not isolating positive animals until slaughter could increase the risk of bTB persistence due to an increase in the period of time during which an infectious animal could contact with susceptible animals. Therefore, it is essential that reactors are immediately and effectively isolated on farm, and taken for slaughter as quickly as possible. On average reactors in Spain are estimated to be removed within 15 working days but, as it can be seen in table 8, there are examples of it taking significantly longer.

Not testing all cattle was a variable with a significant effect in the bivariate analysis that was not kept in the final model after the model selection procedure. Having problems to test all the animals should be related with the risk of leaving positive animals within the farm and therefore increase the likelihood of bTB persistence. The limited number of farms admitted that some animals were not tested (just 12, 9 cases and 3 controls) make our results inconclusive. It has to be taken into account that obtaining reliable information on incorrect management practices from farm owners by ordinary interview is more than a difficult task. Incorrect management factors could be related with the perception about the importance of bTB. Therefore, different and multi-disciplinary approaches, such as sociological studies, would be desirable to quantify the importance of these factors on bTB persistence.

It is also important to take into account that the study was designed as a matched case-control study by herd size and geographical location (County). By the application of this design we aimed to improve the power of our study (i.e., reduce the type II error) by comparing farms as similar as possible based on known risk factors (such as herd size), but this design does not allow to determine the possible role of these two variables on the bTB infection.

Regarding variables related with the structure of the herd, age has been identified as a risk factor in different studies due to the fact that exposure to the disease increases with age, and the possible reactivation of the *Mycobacteria* in older animals after a long period of time (Humblet et al., 2009). Due to the fact that our case-control study was matched by number of animals we analyzed this variable as a percentage, i.e., the proportion of animals representing each age category by herd. The univariate analysis did not find any association between the age structure and the risk of persistence. Nevertheless, it has to be taken into account that this data was obtained during the personal interview, and therefore is possible a lack of accuracy in some cases or even changes with time. This result should be interpreted with caution, and more accurate data would be desirable in order to assess the effect of the age structure of the herd on probability of bTB persistence. On the other hand, some of the factors initially included in the questionnaire, such as having had problems of bovine viral diarrohea (that could compromise the immunological status of the animals increasing susceptibility) or breed of the animals (potentially related with different managements) (Humblet et al., 2009) could not be assessed due to lack of reliable data (most of the owners were not sure about this) or lack of differences on the responses. Finally, other relevant data about animal individual factors such as body condition or immunosupresion by other agents was not recorded in the questionnaire and therefore could not be assessed.

4. CONCLUSIONS

4. Conclusions

1. About 69 % of the new bTB herd breakdowns were detected by annual routine herd testing, which reflects the importance of this type of surveillance in the eradication program. However, 12% and 19% of new positive farms were detected by slaughterhouse surveillance and epidemiological relations of infected farms respectively, being an important complement in the early detection of infection.

2. Residual infection seems to have an important role as a cause of bTB breakdowns. This result suggests that focusing efforts on the routine testing procedures in the positive and recently negative farms should be reflected in an improvement of the eradication program.

3. The high percentage of farms with an “unknown” cause of infection, especially relevant in areas of low prevalence and in dairy herds, reflects the lack of relevant data to infer the most likely cause of infection. Gathering more detailed epidemiological information on bTB breakdown investigations would be desirable.

4. The low agreement between the veterinary officer opinion and the results of our study reflects a lack of harmonized criteria to assess the most likely cause of infection on bTB breakdowns as well as different perceptions about the importance of the possible causes. This is especially relevant in the case of the role of wildlife reservoirs. Common procedures are recommended in order to achieve comparable results.

5. Farms with large pasture areas and positive neighbors have more difficulties in eradicating the disease and therefore, are more likely to suffer a persistent bTB outbreak.

6. Local spread seems to play a role in bTB persistence within herds. However, the transmission mechanisms involved in this local persistence are not clear. Contiguous spread among neighbor herds, wildlife reservoirs and recirculation within the herd could be involved.

5. Summary

5. Summary

In Spain, herd prevalence has decreased from 11.1% on 1986 to 1.3% on 2011. Despite this reduction on the prevalence, in the last years there has been just a moderate decline and the current bTB epidemiological situation poses a serious challenge towards the achievement of a national official tuberculosis free (OTF) status. In the country, approximately between 50 and 60% of the total bTB positive herds detected each year, are new positive herds, and the rest of the positives are herds that persist from the previous year. There is not a clear knowledge of the epidemiological circumstances that lead to the introduction or persistence of the infection. Therefore, the aim of this PhD was to improve the understanding of bTB epidemiology in Spain by assessing the most likely causes of infection and factors related with bTB persistent herds.

To estimate the most likely causes of introduction of the infection on a herd we developed a methodology based on bTB routes of transmission between herds using decision trees. We considered seven routes as a possible cause of herd breakdown. In order to discriminate among them, probabilities were assigned within each decision tree based on expert opinion. Macros were used for data management and to calculate the probabilities of the different possible causes.

According to the results of the 816 studied breakdowns (detected mainly in 2010 and 2011) most of the cases could be resurgence of residual infections that had previously affected the herd (39%, n=316). New introductions of the infection were produced by contact with wildlife in 12% of cases (n=98), and by neighboring herds in 10% of them (n=85). In 28 % (n=225) of the breakdowns, the origin of the infection remained unknown. In 309 herds, the difference between the first and the second cause with the higher probability was small (i.e., less than 1) so, for these herds, first and second possible causes of infection could be considered. Within this group the most frequent first option was residual infection (69%, n=214) and the most frequent second options were sharing pastures with other herds (36%, n=112) and interaction with wildlife (30%, n=94).

The results of the comparisons between our conclusions and the ones of the veterinary officers conducting the epidemiological investigation on the field evidenced a low agreement. The lowest agreement was in the case of “wildlife”. Within the group of herds where the veterinary officer just considered one cause (i.e., 309 herds) in 124 Wildlife was considered as the most likely cause, while we just found evidences to suspect of this in 33 farms. These discrepancies could be related to differences on access to data and perception about the importance attributed to the different causes.

In order to assess factors related to bTB persistence we conducted a case-control study and compared persistent *versus* transient bTB infected farms from southern Spain. Farms were matched by herd size and geographical location (county). A questionnaire by personal interview was carried on 150 herds (80 controls and 70 cases) from Andalusia and Castilla La Mancha regions.

According to the results of this study, farms with large pasture areas and positive neighbors had more difficulties in eradicating the disease and therefore, were more likely to suffer a persistent bTB outbreak. The odds of bTB persistence were between 1.1 and 5.0 times higher in those herds that had the possibility of contact with cattle from a neighbor positive herd. The probability of bTB persistence was also associated with the area of the pasture. Farms with larger pasture areas had odds between 1.2 and 12.7 times higher of having a persistent bTB episode than farms with smaller pasture areas. Management of positive animals such as making replacement from positive herds or not isolating positive animals and the presence of goats within the farm seem to have also an influence on bTB persistence.

Resum

A Espanya, la prevalença de ramats amb tuberculosi ha disminuït del 11,1% el 1986 a 1,3% el 2011. Malgrat aquesta reducció en la prevalença, en els últims anys s'ha moderat el descens i l'actual situació epidemiològica de bTB planteja un desafiament cara a la consecució del estatus de país oficialment indemne de tuberculosi. Entre el 50 i el 60% dels ramats positius detecta cada any, són nous ramats positius, i la resta són ramats on la infecció persisteix des de l'any anterior. No es coneixen quines circumstàncies epidemiològiques condueixen a la introducció o la persistència de la infecció. Per tant, l'objectiu d'aquesta tesi és millorar el coneixement de l'epidemiologia de la bTB a Espanya mitjançant l'avaluació de les causes més probables d'infecció i els factors relacionats amb els ramats infectats de manera persistents.

Per a l'estimació de les causes més probables d'introducció de la infecció en un ramat hem desenvolupat una metodologia basada en les diferents vies de transmissió de la bTB entre els ramats. S'han considerat set vies i per cada una s'ha realitzat un arbre de decisió sobre possibles riscos. A cada risc se l'hi ha assignat una probabilitat basada en opinió d'experts. Per a la gestió de dades i per al càlcul de les probabilitats de cada una de les diferents causes possibles s'han creat un parell de macros. D'acord amb els resultats dels 816 ramats estudiats (casos detectades principalment els anys 2010 i 2011), la majoria dels casos serien deguts al ressorgiment d'infeccions residuals que havien afectat anteriorment al ramat (39%, n = 316). La introducció de la infecció es va produir pel contacte amb animals silvestres en el 12% dels casos (n = 98), i a través de ramats veïns en el 10% (n = 85). En el 28% (n = 225) dels brots, no s'ha pogut determinar l'origen de la infecció. En 309 ramats, la diferència entre les dues causes amb la probabilitat més alta era petita (menys d'u), de manera, que per a aquestes granges tant la primera com la segona causa serien possibles. Dins d'aquest grup, la primera opció més freqüent va ser la infecció residual (69%, n = 214) i la segona de les opcions més freqüents era compartir les pastures amb altres granges (36%, n = 112) i la interacció amb la fauna silvestre (30%, n = 94).

En comparar aquestes conclusions amb les dels veterinaris oficials que havien fet la

investigació epidemiològica sobre el terreny, s'obté una concordança baixa; especialment en el cas de fauna silvestre. Dins del grup dels ramats on el veterinari només considerava una causa (és a dir, 309 ramats), en 124 la fauna silvestre va ser considerada com la causa més probable, mentre que nosaltres només ho varem concloure en 33 granges. Aquestes discordances podrien estar relacionades amb un diferent accés a les dades i també a percepcions diferents sobre la importància atribuïda a cada causa.

Per tal d'avaluar els factors relacionats amb la persistència de bTB en granges es va realitzar un estudi de casos i controls on es va comparar granges infectades que es va eliminar fàcilment la bTB amb altres on es va tardar més de 5 anys. Totes les granges eren del sud d'Espanya i es van estratificar per grandària del ramat i la ubicació geogràfica (comarca). Es va omplir un qüestionari mitjançant entrevista personal en 150 ramats (80 controls i 70 casos) de les Comunitats d'Andalusia i de Castilla-la Mancha.

D'acord amb els resultats d'aquest estudi, les granges amb més superfície de pastures i amb veïns positius, tenien més dificultats per eradicar la infecció, per tant, eren més propenses a patir un brot de bTB persistent. Les probabilitats de persistència eren entre 1,1 i 5,0 vegades més gran en aquells ramats amb possible contacte amb el bestiar d'un ramat positiu veí. La probabilitat de persistència també es va associar amb la superfície de pastures. Les finques amb àrees de pastura més grans tenien una probabilitat entre 1,2 i 12,7 vegades més alta de tenir dificultats en l'eliminació que les finques amb àrees de pastures més petits. El maneig dels animals positius, com ara utilitzar vedelles de reposició de mares positives o no aïllar els animals positius, així com la presència de cabres dins de la granja semblen tenir també una influència en la persistència de bTB.

Resumen

En España, la prevalencia de rebaños con tuberculosis ha disminuido del 11,1% en 1986 a 1,3% en 2011. A pesar de esta reducción, en los últimos años se ha moderado el descenso de la prevalencia y la situación epidemiológica actual de bTB plantea un desafío cara a la consecución del estatus de país oficialmente indemne de tuberculosis. Entre el 50 y el 60% de los rebaños positivos detectados cada año, son nuevos rebaños positivos, y el resto son rebaños donde la infección persiste desde el año anterior. No se sabe qué circunstancias epidemiológicas están implicadas en la introducción o la persistencia de la infección. Por tanto, el objetivo de esta tesis es mejorar el conocimiento de la epidemiología de la bTB en España mediante la evaluación de las causas más probables de infección y los factores relacionados con los rebaños infectados de forma persistente.

Para la estimación de las causas más probables de introducción de la infección en un rebaño hemos desarrollado una metodología basada en las diferentes vías de transmisión de la bTB entre los rebaños. Se han considerado siete vías y por cada una de ellas se ha realizado un árbol de decisión sobre posibles riesgos. A cada riesgo se le ha asignado una probabilidad basada en opinión de expertos. Para la gestión de datos y para el cálculo de las probabilidades de cada una de las diferentes causas posibles se han creado dos macros.

De acuerdo con los resultados de los 816 rebaños estudiados (casos detectados principalmente en los años 2010 y 2011), la mayoría de las nuevas infecciones en realidad son debidas al resurgimiento de infecciones que habían afectado anteriormente al rebaño (39%, n = 316). La introducción de la infección se produjo por el contacto con animales silvestres en el 12% de los casos (n = 98), y a través de rebaños vecinos en el 10% (n = 85). En el 28% (n = 225) de los brotes, no se ha podido determinar el origen de la infección. En 309 rebaños, la diferencia entre las dos causas con la probabilidad más alta era escasa (menos de uno), de manera que para estas granjas tanto la primera como la segunda causa serían posibles. Dentro de este

grupo, la primera opción más frecuente fue la infección residual (69%, n = 214) y la segunda de las opciones más frecuentes era compartir los pastos con otras granjas (36%, n = 112) y la interacción con la fauna silvestre (30%, n = 94).

Al comparar las conclusiones de nuestro estudio con las de los veterinarios oficiales que habían hecho la investigación epidemiológica sobre el terreno, se obtiene una concordancia baja, especialmente en el caso de fauna silvestre. Dentro del grupo de los rebaños donde el veterinario sólo consideraba una causa (es decir, 309 rebaños), en 124 la fauna silvestre fue la causa considerada como más probable para el veterinario, mientras que nosotros sólo lo concluimos en 33 granjas. Estas discordancias podrían estar relacionadas con diferencias en los datos a los que hemos tenido acceso así como a diferentes percepciones sobre la importancia atribuida a cada causa.

Para evaluar los factores relacionados con la persistencia de BTB en granjas se realizó un estudio de casos y controles donde se compararon granjas infectadas donde se eliminó fácilmente la bTB con otras donde se tardó más de 5 años. Todas las granjas eran del sur de España y se estratificaron por tamaño del rebaño y ubicación geográfica (comarca). Se rellenó un cuestionario mediante entrevista personal en 150 rebaños (80 controles y 70 casos) de las Comunidades de Andalucía y de Castilla-La Mancha.

De acuerdo con los resultados de este estudio, las granjas con más superficie de pastos y con vecinos positivos, tenían más dificultades para erradicar la infección, por lo tanto, eran más propensas a sufrir un brote de BTB persistente. Las probabilidades de persistencia eran entre 1,1 y 5,0 veces mayor en aquellos rebaños con posible contacto con un rebaño positivo vecino. La probabilidad de persistencia también se asoció con la superficie de pastos. Las fincas con áreas de pasto mayores tenían una probabilidad entre 1,2 y 12,7 veces más alta de tener dificultades en la eliminación que las fincas con áreas de pastos más pequeños. El manejo de los animales positivos, tales como utilizar terneras de reposición de madres positivas o no aislar los animales positivos, así como la presencia de cabras dentro de la granja parecen tener también una influencia en la persistencia de la bTB.

6. REFERENCES

6. REFERENCES

Abernethy, D. A., Walton, E., Menzies, F., Courcier, E., Robinson, P. (2011). *Mycobacterium bovis* surveillance in European badgers (*Meles meles*) killed by vehicles in Northern Ireland: an epidemiological evaluation, in Proceed. International Conference on Animal Health Surveillance, Lyon, France.

Acevedo, P., Vicente, J., Hofle, U., Cassinello, J., Ruiz-Fons, F., Gortazar, C. (2007). Estimation of European wild boar relative abundance and aggregation: a novel method in epidemiological risk assessment. Epidemiol. Infect. 135, 519–527.

Acevedo, P., Ruiz-Fons, F., Estrada, R., Márquez, A.L., Miranda, M.A., Gortázar, C., Lucientes, J. (2010).A Broad Assessment of Factors Determining *Culicoides imicola* Abundance: Modelling the Present and Forecasting Its Future in Climate Change Scenarios. *PLoS ONE* 5(12): e14236.

Allepuz, A., Napp, S., Picado, A., Alba, A., Panades, J., Domingo, M., Casal, J.(2009). Descriptive and spatial epidemiology of bovine cysticercosis in North-Eastern Spain (Catalonia).Vet. Parasitol. 159, 43-48.

Allepuz, A., Casal, J., Napp, S., Saez, M., Alba, A., Vilar, M., Domingo, M., Gonzalez, M. A., Duran-Ferrer, M., Vicente, J., Alvarez, 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.

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*. Emerg. Infect. Dis. 16, 1296–1299.

Alvarez J., de Juan L., Bezos J., Romero B., Sa´ez J.L., Reviriego Gordejo F.J., et al. (2008). Interference of paratuberculosis with the diagnosis of tuberculosis in a goat flocks with a natural mixed infection. Vet. Microbiol. 128, 72–80.

Alvarez, J., Perez, A.M., Bezos, J., Casal, C., Romero, B., Rodriguez-Campos, S., Saez-Llorente, J.L Diaz, R., Carpintero, J., de Juan, L., 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 Vet. Res. 8:100.

Amanda, E. F., Carole, A. B., Joseph, C. G., John, B. K. (2011).A Study of the Persistence of *Mycobacterium bovis* in the Environment under Natural Weather Conditions in Michigan, USA. Vet. Med. Int. doi:10.4061/2011/765430.

Amanfu, W. (2006). The situation of tuberculosis and tuberculosis control in animals of economic interest .Tuberculosis. 86, 330-335.

Anon.July 29, 1964. Off. J. Eur. Commun. 121.

Anon. (1991).Bovine Tuberculosis in Badgers: Third Report by the Ministry of Agriculture Fisheries and Food. London, HMSO.

Anon. (2007). European Commission Health and Consumer Protection Directorate General Report of the meeting of bovine tuberculosis sub-group of the task force for monitoring animal disease eradication held in Seville, Spain, 14-15 November 2007.Available al: http://ec.europa.eu/food/animal/diseases/eradication/reportsanco-10584/2007/btbsubgroupsevillarev110-1-08.pdf (Accessed 27 October, 2012).

Anon. (2007). Final report of the independent scientific Group on cattle TB (ISG).Presented to the Secretary of State for Environment, Food and Rural Affairs .The Rt Hon David Miliband MP, June 2007.

Anon. (2011).European Commission Health and Consumer Protection Directorate; 2010, annual report on notifiable diseases of bovine animals and swine. Available from: http://ec.europa.eu/food/animal/liveanimals/porcine/final_report_2010_en.pdf (accessed 28 August 2012).

Anon. (2012). Eradication program for Bovine Tuberculosis. Approved* for 2012 by Commission Decision 2011/807/EU Spain. Available from: http://ec.europa.eu/food/animal/diseases/eradication/program2012/tb_es.pdf (accessed 31 August 2012).

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. Microbiol. 53, 1785–1789.

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. Int. J. Tuberc. Lung Dis. 8, 924-937.

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.

Berg, S., Firdessa, R., Habtamu, M., Gadisa, E., Mengistu, A., et al. (2009). The Burden of Mycobacterial Disease in Ethiopian Cattle: Implications for Public Health. PLoS ONE 4(4):e5068.

Berrian, A.M., O'Keeffe, J., White, P.W., Norris, J., Litt, J., More, S. J., Olea-Popelka, F. J. (2012). Risk of bovine tuberculosis for cattle sold out from herds during 2005 in Ireland. Vet. Rec. 170, 620. .

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. Vet. J. 191, 161–165.

Bollo, E., Ferroglio, E., Dini, V., Mignone, W., Biolatti, B., Rossi, L. (2000). Detection of Mycobacterium tuberculosis complex in lymph nodes of wild boar (Sus scrofa) by a target-amplified test system. J. Vet. Med. B 47, 337–342.

Brosch, R., Gordon, S.V., Marmiesse, M., Brodin, P., Buchrieser, C., Eiglmeier, K., Garnier, T., Gutierrez, C., Hewinson, G., Kremer, K., Parsons, L.M., Pym, A.S., Samper, S.,Soolingen, D. van., Cole, S.T.(2002). A new evolutionary scenario for the Mycobacterium tuberculosis complex. Proc. Natl. Acad. Sci. USA. 99, 3684–3689.

Brooks-Pollock, E., Keeling, M. (2009). Herd size and bovine tuberculosis persistence in cattle farms in Great Britain. Prev. Vet. Med. 92, 360–365

Buddle, B.M., Aldwell, F.E., Pfeffer, A., de Lisle, G.W., Corner, L.A. (1994). Experimental *mycobacterium bovis* infection of cattle effect of dose of *M. bovis* and pregnancy on immune responses and distribution of lesions. N. Z. Vet. J. 42, 167–172.

Buikstra, J.E. (1999). Paleoepidemiology of tuberculosis in The Americas .In: Palfi, G., Dutour, O., Deak, J., Hutas, I., eds. Tuberculosis: past and present. Budapest/Szeged: Golden Book Publishers and Tuberculosis Foundation.479–94.

Carrique-Mas, J. J., Medley, G. F., Green, L. E. (2008). Risks for bovine tuberculosis in British cattle farms restocked after the foot and mouth disease epidemic of 2001. Prev. Vet. Med. 84, 85–93.

Cavanagh, R., Begon, M., Bennett, M., Ergon, T., Graham, I.M., De Haas, P.E., Hart, C.A., Koedam, M., Kremer, K., Lambin, X., Roholl, P., van Soolingen, D. (2002).*Mycobacterium microti* infection (vole tuberculosis) in wild rodent populations. J. Clin. Microbiol. 40, 3281-3285.

Cheeseman, C.L., Wilesmith, J.W., Stuart, F.A. (1989).Tuberculosis, the disease and its epidemiology in the badger, a review. Epidemiol. Infect. 103, 113–125.

Clegg, T. A., Good, M., Duignan, A., Doyle, R., Blake, M., MORE, S. J. (2011).Longer-term risk of *Mycobacterium bovis* in Irish cattle following an inconclusive diagnosis to the single intradermal comparative tuberculin test. Prev. Vet. Med. 100, 147–154

Coad, M., Clifford, D., Rhodes, S.G., Hewinson, R.G., Vordermeier, H.M., Whelan, A.O. (2010).Repeat tuberculin skin testing leads to desensitization in naturally infected tuberculous cattle which is associated with elevated interleukin-10 and decreased interleukin-1 beta responses. Vet. Res. 41: 14.

Cohen, J. 1960. A coefficient of agreement for nominal scales. Educational and Psychological Measurement .20, 37-46.

Collins, C.H., Grange, J.M. (1983).A review. The bovine tubercle bacillus. J. Appl. Bacteriol. 55, 13-29.

Conlan, A.J.K., McKinley, T.J., Karolemeas, K., Pollock, E.B., Goodchild, A.V., Mitchell, A.P., Birch, P.D.C., Clifton-Hadley, R.S., Wood, J. L. N. (2012).Estimating the Hidden Burden of Bovine Tuberculosis in Great Britain. PLoS Comput Biol 8(10): e1002730.

Cosivi, O., Meslin, F-X., Daborn, C.J, Grange, J.M. (1995).The epidemiology of Mycobacterium bovis infection in animals and humans, with particular reference to Africa. Sci. Tech. Rev. 14, 733-46.

Cosivi, O., Grange, J.M., Daborn, C.J., Raviglione, M.C., Fujikura, T., Cousins, D., Robinson, R.A., Huchzeryeyer, H.F., de Kantor, I., Meslin, F.X. (1998).Zoonotic Tuberculosis due to *Mycobacterium bovis* in developing countries. Emerg. Infect. Dis. 4, 59-70.

Costello, E., Egan, J.W., Quigley, F.C., O'Reilly, P.F. (1997).Performance of the single intradermal comparative tuberculin test in identifying cattle with tuberculous lesions in Irish herds. Vet. Rec. 141, 222–224.

Courtenay, O., Reilly, L. A., Sweeney, F. P., Hibberd, V., Bryan, S., Ul-Hassan, A., Newman, C., Macdonald, D. W., Delahay, R. J., Wilson, G. J. and Wellington, E. M. H. (2006). Is *Mycobacterium bovis* in the environment important for the persistence of bovine tuberculosis? Biol. Lett. 2, 460-462.

Crawshaw, T., Daniel, R., Clifton-Hadley, R., Clark, J., Evans, H., Rolfe, S., et al. (2008).TB in goats caused by *Mycobacterium bovis*. Vet. Rec. 163, 127.

Cvetnic, Z., Katalinic-Jankovic, V., Sostaric, B. (2007). *Mycobacterium caprae* in cattle and humans in Croatia. Int. J. Tuberc. Lung Dis. 11, 652–658.

Department for Environment, Food and Rural Affairs (DEFRA). (2007).Independent Scientific Group Final Report (ISG).Available at: http://archive.defra.gov.uk/foodfarm/Farmanimal/Diseases/atoz/tb/isg/report/final_report.pdf. (Accessed 4 February 2013).

Department for Environment, Food and Rural Affairs (DEFRA). (2011). Bovine TB Eradication Program for England. London: Department of Environment, Food and Rural Affairs. Available from: http://www.defra.gov.uk/publications/files/pb13601-bovinetb-eradication-program-110719.pdf (Accessed 12 October 2012).

De Kantor, I. N., LoBue, P.A., Thoen, C.O. (2010). Human tuberculosis caused by *Mycobacterium bovis* in the United States, Latin America and the Caribbean. Int. J. Tubercul. Lung Dis. 14, 1369-73.

Delahay, R.J., Cheeseman, C.L., Clifton-Hadley, R.S. (2001).Wildlife disease reservoirs: the epidemiology of *Mycobacterium bovis* infection in the European badger (Meles meles) and other British mammals. Tuberculosis 81, 43–49.

Delahay, R.J., De Leeuw, A.N.S., Barlow, A.M., Clifton-Hadley, R.S., Cheeseman, C.L. (2002). The status of Mycobacterium bovis infection in UK wild mammals: a review. Vet. J. 164, 90–105.

De la Rua-Domenech, R. (2006a). Human Mycobacterium bovis infection in the United Kingdom: Incidence, risks, control measures and review of the zoonotic aspects of bovine tuberculosis. Tuberculosis 86, 77-109.

De la Rua-Domenech, R., Goodchild, A.T., Vordermeier, H.M., Hewinson, R.G., Christiansen, K.H. and Clifton-Hadley, R.S. (2006b). 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.

Denny, G.O., Wilesmith, J. W. (1999). Bovine tuberculosis in Northern Ireland: a case-control study of herd risk factors. Vet. Rec. 144, 305–310.

Di Marco, V., Mazzone, P., Capucchio, M. T., Boniotti, M.B., Aronica, V., Russo, M et al., 2012. Epidemiological Significance of the Domestic Black Pig (Sus scrofa) in Maintenance of Bovine Tuberculosis in Sicily. J. Clin. Microbiol. 50, 1209-1218

Doherty, M.L., Monaghan, M.L., Bassett, H.F., Quinn, P.J. (1995). Effect of a recent injection of purified protein derivative on diagnostic tests for tuberculosis in cattle infected with Mycobacterium bovis. Res. Vet. Sci. 58, 211–217.

Dohoo, I.R., Martin, W., Stryhn, H. (2003). Veterinary Epidemiologic Research, 1st ed. Charlottetown, Prince Edward Island: AVC Inc. 36–37.

Dommergues, L., Rautureau, S., Petit, E., Dufour, B. (2011).Network of Contacts between Cattle Herds in a French Area Affected by Bovine Tuberculosis in 2010. Transbound. Emerg. Dis. 59, 292–302

Duarte, E.L., Domingos, M., Amado, A., Botelho, A. (2008). Spoligotype diversity of *Mycobacterium bovis* and *Mycobacterium caprae* animal isolates. Vet. Microbiol. 130, 415-421.

Dubos, R., Dubos, J. (1952). The white plague. Little, Brown and Co, Boston, Mass.

Douglas, Y. (2008). Tuberculosis : A Global Overview. Ethiop. J. Health. Dev.22, 97-99.

EFSA. (2003). Opinion of the Scientific Panel on Biological Hazards on a request from the Commission related on “Tuberculosis in Bovine Animals: Risks for human health and control strategies”. EFSA J. 13, 1-53.

EFSA. (2004). Risk assessment of a revised inspection of slaughter animals in areas with low prevalence of Cysticercus. EFSA J. 176, 1-24.

EFSA. (2009). Draft report on wildlife reservoirs of bovine tuberculosis in Europe.

EFSA. (2012). The community summary report on trends and sources of Zoonoses, Zoonotic agents and food-borne outbreaks in the European Union in 2010. EFSA J. 8:1496.

EFSA.(2012a). Technical meeting of the EFSA Scientific Network for Risk assessment in Animal Health and Welfare - Bovine Tuberculosis Testing Parma 21 February 2012.Available at: http://www.efsa.europa.eu/de/supporting/doc/288e.pdf (accessed December 30, 2012).

EFSA. (2012b).The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010. EFSA J. 10:2597

Ehizibolo, D.O., Ehizibolo, P.O., Ehizibolo, E.E., Sugun, M.Y., Idachaba1, S.E. (2011). The Control of Neglected Zoonotic Diseases in Nigeria through Animal Intervention. Afr. J. Biomed. Res. 81-88.

Erler, W., Martin, G., Sachse, K., Naumann, L., Kahlau, D., Beer, J., Bartos, M., Nagy, G., Cvetnic, Z., Zolnir Dovc, M., Pavlik, I. (2004). Molecular fingerprinting of Mycobacterium bovis subsp. caprae isolates from central Europe. J. Clin. Microbiol. 42, 2234–2238.

Elber, AR., Stegeman, A., Moser, H., Ekker, H.M., Smak, J.A., Pluimers, F.H. (1999). The classical swine fever epidemic 1997-1998 in The Netherlands: descriptive epidemiology. Prev. Vet. Med. 42,157-84.

Evans, H. T. J., Thompson, H. V. (1981). Bovine tuberculosis in cattle in Great Britain. I. Eradication of the disease from cattle and the role of the badger (Meles meles) as a source of *Mycobacterium bovis* for cattle. Animal Regulation Studies 3, 191-216.

Fernández-de-Mera, I. G., Vicente, J., Höfle, U., Fons, F. R., Ortiz, J. A., Gortázar, C. (2009). Factors affecting red deer skin test responsiveness to bovine and avian tuberculin and to phytohaemagglutinin. Prev. Vet. Med. 90, 119–126

Fèvre, E. M., Bronsvoort1, B. M., Hamilton, K. A., Cleaveland, S. (2006). Animal movements and the spread of infectious diseases. Trends Microbiol. 14, 125-131.

Formicola, V., Milanesi, Q., Scarsini, C. (1987). Evidence of spinal tuberculosis at the beginning of the fourth millennium BC from Arene Candide cave (Liguria, Italy). Am. J. Phys. Anthropol. 72, 1–6.

Francis, J. 1947. Bovine Tuberculosis: Including a Contrast with Human Tuberculosis, Staples Press, London, UK .220.

Francis, J. 1972. Route of infection in tuberculosis. Australian Vet. J. 48, 578.

Flückiger, G. (1960).Festschrift anlässlich der Erreichung der Tuberkulosefreiheit desSchweizerischen Viehbestandes. Eidgenössisches Veterinäramt. 1-12.

Gallager, J., Clifton-Hadley, R.S. (2000). Survival of *Mycobacterium bovis* in defined environmental conditions. Vet. Microbiol. 10, 193-197.

Garabed, R.B., Perez, A.M., Johnson, W.O., Thurmond, M.C. (2009). Use of expert opinion for animal disease decisions: An example of foot-and-mouth disease status designation. Prev. Vet Med. 92, 20–30

García, J.M. (2003).la tuberculosis bovina como zoonosis en la España contemporánea (1850-1950). Tesis doctoral, Universidad Autónoma de Barcelona, Bellaterra, Spain.

Garcıa-Bocanegra, I., Pe´rez de Val, B., Arenas-Montes, A., Paniagua, J., Boadella, M., et al. (2012). Seroprevalence and Risk Factors Associated to *Mycobacterium bovis* in Wild Artiodactyl Species from Southern Spain, 2006–2010. PLoS ONE 7(4): e34908.

Gilbert, M., Mitchell, A., Bourn, D., Mawdsley, J., Clifton-Hadley, R., Wint, W. (2005). Cattle movements and bovine tuberculosis in Great Britain. Nature 435, 491-496

Good, M., Duignan, A., Clegg, T., More, S. (2010). A review of epidemiological investigations into outbreaks of bovine TB in Ireland 1987 – 2009 Proceed. XXVI World buiatric Congress. Santiago de Chile 2010.Available at: http://www.originalprocess.it/wbc2010/AbstractCD/pdf/813.pdf (Accessed, 18 March, 2011).

Good, M., Clegg, T. A., Duignan, A., More, S. J. (2011). The impact of the national herd depopulation policy on the persistence of bovine tuberculosis in Irish herds during 2003–2005. Vet Rec. 169, 581

Good, M., Duignan, A. 2011. Perspectives on the History of Bovine TB and the Role of Tuberculin in Bovine TB Eradication. Vet. Med. Int. ID 410470. doi:10.4061/2011/410470

Goodchild, A.V., Clifton-Hadley, R.S. (2001). Cattle-to-cattle transmission of *Mycobacterium bovis.* Tuberculosis. 81, 23-41.

Gopal, R., Goodchild, A., Hewinson, G., de la Rua Domenech, R., Clifton-Hadley, R., (2006). Introduction of bovine tuberculosis to north-east England by bought-in cattle. Vet. Rec. 159, 265-71.

Gortazar, C., Vicente, J., Samper, S., Garrido, M.J., Fernandez de Mera, I.G., Gavin, P., Juste, R.A., Martin, C., Acevedo, P., de la Puente, M., Hofle, U. (2005). Molecular characterization of *Mycobacterium tuberculosis* complex isolates from wild ungulates in south-central Spain. Vet. Res. 36, 43–52.

Gortazar, C., Ferroglio, E., Ho¨fle, U., Fro¨lich, K., Vicente, J. (2007). Diseases shared between wildlife and livestock: a European perspective. Eur. J. Wildl. Res. 53, 241–256.

Gortazar, C., Vicente, J., Boadella, M., Ballesteros, C., Galindo, R. C., Garrido, J., Aranaz, A., de la Fuente, J. (2011). Progress in the Control of Bovine Tuberculosis in Spanish Wildlife. Vet. Microbiol. 151, 170-178.

Grange, J.M., Yates, M.D. (1994). Zoonotic aspects of *Mycobacterium bovis infection*. Vet. Microbiol. 40, 137–151.

Green, D.M., Kiss, I.Z., Mitchell, A.P., Kao, R.R.2008.Estimates for local and movement-based transmission of bovine tuberculosis in British cattle. Proc. Biol. Soc. 275, 1001–1005.

Griffin, J. M., Hahsey, T. (1992). Analysis of epidemiology reports on 3975 herd breakdowns in ten DVO regions during 1987–90. Irish Vet. J. 45,126.

Griffin, J.M., Hahesy, T., Lynch, K., Salman, M. D., McCarthy, J., Hurley, T. (1993). The association of cattle husbandry practices, environmental factors and farmer characteristics with the occurrence of chronic bovine tuberculosis in dairy herds in the Republic of Ireland. Prev. Vet. Med .17, 145–160.

Griffin, J.M., Williams, D.H., Kelly, G.E., Clegg, T.A., O'Boyle, I., Collins, J.D., More, S.J. (2005). The impact of badger removal on the control of tuberculosis in cattle herds in Ireland. Prev. Vet. Med. 67, 237–266.

Griffith, A.S. (1932). Observations on the bovine tubercle bacillus in human tuberculosis. Br. Med. J. 2, 501-503.

Hardie, R.M., Watson, J.M. (1992). *Mycobacterium bovis* in England and Wales, past, present and future. Epidemiol. Infect. 109, 23–33.

Hett, E.C., Rubin, E.J. (2008). Bacterial Growth and Cell Division: a Mycobacterial Perspective. Microbiol. Mol. Biol. Rev. 72, 126–156.

Hoyle, F.P. (1990). Suppression of tuberculin skin reactivity in cattle: a case report. Surveillance. 17, 28.

Humblet, M.F., Boschiroli, M.L., Saegerman, C. (2009). Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. Vet. Res. 40:50.

Humblet, M.F., Walravens, K., Salandre, O., Boschiroli, M.L., Gilbert, M., Berkvens, D., Fauville-Dufaux, M., Godfroid, J., Dufey, J., Raskin, A., Vanholme, L., Saegerman, C. (2011). Monitoring of the intra-dermal tuberculosis skin test performed by Belgian field practitioners. Res. Vet. Sci 91, 199-207.

Jackson, R., de Lisle, G. W., Morris, R. S. (1995). A study of the environmental survival of *Mycobacterium bovis* on a farm in New Zealand. N. Z .Vet J. 43, 346–352.

Javed, M.T., Aranaz, A., de Juan, L, Bezos, J., Romero, B., Alvarez, J, Lozano, C., Mateos, A., Domínguez, L., 2007. Improvement of spoligotyping with additional spacer sequences for characterization Of *Mycobacterium bovis* and *M. caprae* isolates from Spain. Tuberculosis, 87, 437–445.

Johnson, L., Dean, G., Rhodes, S., Hewinson, G., Vordermeier, M. and Wangoo, A. (2007). Low-dose *Mycobacterium bovis* infection in cattle results in pathology indistinguishable from that of high-dose infection. Tuberculosis. 87, 71-76.

Johnston, W.T., Vial, F., Gettinby, G., Bourne, F.J., Clifton-Hadley, R.S., Cox, D.R., Crea, P., Donnelly, C.A., McInerney, J.P., Mitchell, A.P., Morrison, W.I., Woodroffe, R. (2011). Herd-level risk factors of bovine tuberculosis in England and Wales after the 2001 foot-and-mouth disease epidemic. Int. J. Infect. Dis. 15, 833–840.

Karolemeas, K., McKinley, T.J., Clifton-Hadley, R.S., Goodchild, A.V., Mitchell, A., Johnston, W.T., Conlan, A.J.K., Donnelly, C.A., Wood, J.L.N. (2011). Recurrence of bovine tuberculosis breakdowns in Great Britain: Risk factors and prediction. Prev. Vet. Med. 102, 22-29.

Kassa, G.M., Abebe, F., Worku, Y., Legesse, M., Medhin, G., Bjune, G., Ameni, G. (2012). Tuberculosis in Goats and Sheep in Afar Pastoral Region of Ethiopia and Isolation of Mycobacterium tuberculosis from Goat. Vet. Med. Int. 869146. doi: 10.1155/2012/869146

Kellar, J.A. (1993). The application of risk analysis to international trade in animal products. Rev. Sci. Tech. 12, 1023–1044

Kelly, G.E., More, S.J. (2010). Spatial clustering of TB-infected cattle herds prior to and following proactive badger removal. Epidemiol. Infect. 139, 1220–1229.

Kiers, A., Klarenbeek, A., Mendelts, B., van Soolingen, D., Koëter, G. (2008).Transmission of *Mycobacterium pinnipedii* to humans in a zoo with marine mammals. Int. J. Tuberc. Lung Dis. 12, 1469 – 1473.

Krajewska, M., Kozińska, M., Zwolska, Z., Lipiec M., Augustynowicz-Kopeć, E., Szulowski, K., (2012). Human as a source of tuberculosis for cattle. First evidence of transmission in Poland. Vet. Microbiol. 159, 269-71.

LoBue, P. A., Enarson, D. A., Thoen, T. C. 2010. Tuberculosis in humans and its epidemiology, diagnosis and treatment in the United States. Int. J. Tuberc. Lung Dis 14, 1226–1232

Mackintosh, C.G., de Lisle, G.W., Collins, D.M., Griffin, J.F.T. (2004). Mycobacterial diseases of deer. N. Z. Vet. J. 52, 163–174.

Marangon, S., Martini, M., Dalla Pozza, M., Neto, F. (1998). A case-control study on bovine tuberculosis in the Veneto Region (Italy). Prev. Vet .Med. 34, 87-95.

Matos, F., Cunha, M. V., Canto, A., Albuquerque, T., Amado, A., Botelho, A. (2010). Snapshot of *Mycobacterium bovis* and *Mycobacterium caprae* Infections in Livestock in an Area with a Low Incidence of Bovine Tuberculosis. J. Clin. Microbiol. 48, 4337–4339.

Mazana, J. S. (2009). La tuberculosis y sus epónimos. Charles Mantoux (1877-1947). Rev. Esp. Sanid. Penit. 11, 27-33

Mcllory , S.G., Neill, S.D., McCracken, R.M. (1986). Pulmonary lesions and *Mycobacterium bovis* excretion from the respiratory tract of tuberculin reacting cattle. Vet. Rec. 118, 718-721.

Menzies, F.D., Neill, S.D. (2000). Cattle-to-Cattle Transmission of Bovine Tuberculosis. Vet. J. 160, 92-106.

Meyer, C.G., Scarisbrick, G., Niemann, S., Browne, E.N., Chinbuah, M.A., Gyapong, J., Osei, I., Owusu Dabo, E., Kubica, T., Rüsch Gerdes, S. (2008). Pulmonary tuberculosis: virulence of *Mycobacterium africanum* and relevance in HIV co-infection. Tuberculosis 88, 482-489.

Moda, G., Daborn, C.J., Grange, J.M. and Cosivi, O. (1996). The zoonotic importance of *Mycobacterium bovis*. Tuberc. lung Dis. 77, 103-108

Monaghan, M.L., Doherty, M.L., Collins, J.D., Kazda, J.F., Quinn, P.J. (1994). The tuberculin test. Vet. Microbiol. 40, 111–124.

Myers, J.A., Steele, J.H., 1969.Bovine tuberculosis control in man and animals. Warren H Green, inc, Missouri, USA. 403.

Muñoz Mendoza, M., de Juan, L., Menéndez, S., Ocampo, O., Mourelo, J., Sáez, J.L., Domínguez, L., Gortázar, C., García Marín, J.F., Balseiro, A. (2012). Tuberculosis due to *Mycobacterium bovis* and *Mycobacterium caprae* in sheep. Vet. J. 191, 267–269

Napp, S., Allepuz, A., Mercader, I., Nofrarías, M., López-Soria, S., Domingo, M., Romero, B., Bezos, J., Pérez de Val, B. (2013). Evidence of goats acting as domestic reservoirs of bovine tuberculosis. Vet Rec. doi: 10.1136/vr.101347.

Naranjo, V., Hofle, U., Vicente, J., Martin, M. P., Ruiz-Fons, F., Gortazar, C., Kocan, K. M. and Fuente, J. de la. (2006). Genes differentially expressed in oropharyngeal tonsils and mandibular lymph nodes of tuberculous and nontuberculous European wild boars naturally exposed to *Mycobacterium bovis*. Immunol. Med. Microbiol. 46, 298-312.

Neill, S. D., O'Brien, J. J., McCracken, R. M.(1988). *Mycobacterium bovis* in the anterior respiratory tracts in the heads of tuberculin-reacting cattle. Vet. Rec. 122: 184-186.

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, Pollock, J. M., Bryson, D. B., Hanna, J. (1994). Pathogenesis of *Mycobacterium bovis* infection in cattle. Vet. Microbiol. 40, 41-52.

Neill, S.D., Bryson, D.G., Pollock, J.M.(2001). Pathogenesis of tuberculosis in cattle. Tuberculosis. 81, 79–86.

Nerlich, A. G., Haas, C. J., Zink, A., Szeimies, U., Hagedorn, H. G. (1997). Molecular evidence for tuberculosis in an ancient Egyptian mummy. Lancet. 350, 1404.

Nerlich, A.G., Lösch, S., 2009. Paleopathology of Human Tuberculosis and the Potential Role of Climate. Interdiscip. Perspect. Infect. Dis. doi:10.1155/2009/437187.

O'Brien, D. J., Schmitt, S. M., Fitzgerald, S. D., Berry, D. E., Hickling, G.J. (2006). Managing the wildlife reservoir of *Mycobacterium bovis*: the Michigan, USA, experience. Vet. Microbiol. 112, 313–323

Ocepek, M., Pate, M., Zolnir Dovc, M., Poljak, M. (2005). Transmission of *Mycobacterium tuberculosis* from human to cattle. J. Clin. Microbiol. 43, 3555–3557.

O'Neil, B.D., Pharo, H.J., 1995. The control of bovine tuberculosis in New Zealand. N.Z. Vet J. 43, 249–255.

OIE.(2006). Manual of diagnostic tests and vaccines for terrestrial animals. Bovine tuberculosis available at: http://www.oie.int/eng/normes/mmanual/ A_00054.htm (Accessed 15 May, 2012).

OIE.2009. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals .available at: http://web.oie.int/eng/normes/MMANUAL/2008/pdf/2.04.07_BOVINE_TB.pdf (accessed 26, October, 2012).

Okafor, C. C., Grooms, D.L., Bruning-Fann, C.S., Averill, J.J., Kaneene, J.B. (2011). Descriptive Epidemiology of Bovine Tuberculosis in Michigan (1975–2010): Lessons Learned. Vet. Med. Int. doi:10.4061/2011/874924.

Olea-Popelka, F., Butler, D., Lavin, D., McGrath, G., O'Keeffe, J., Kelton, D., Berke, O., More, S., Martin, W., (2006). A case study of bovine tuberculosis in an area of County Donegal, Ireland Irish Vet. J. 59, 683-690.

Olea-Popelka, F.J., Costello, E., White, P., McGrath, G., Collins, J.D., O'Keeffe, J., Kelton , D.F., Berke, O., More, S., Martin, S.W. (2008). Risk factors for disclosure of additional tuberculous cattle in attested-clear herds that had one animal with a confirmed lesion of tuberculosis at slaughter during 2003 in Ireland. Prev. Vet. Med. 85, 81–91.

Olmstead, A.L., Rhode, P. W. (2004). An Impossible Undertaking: The Eradication of Bovine Tuberculosis in the United States. J. Econ. Hist. 64, 734-72.

Palmer, M.V., Waters, W.R., Whipple, D.L. (2002). Aerosol delivery of virulent Mycobacterium bovis to cattle. Tuberculosis 82, 275–282.

Parra, A., Fernández-Llario, P., Tato, A., Larrasa, J., Garc´ıa, A., Alonso, J.M., Hermoso de Mendoza, M., Hermoso de Mendoza, J. (2003). Epidemiology of *Mycobacterium bovis* infections of pigs and wild boars using a molecular approach. Vet. Microbiol. 9, 123-133.

Parra, A., Garcia, A., Inglis, N. F., Tato, A., Alonso, J. M., Hermoso de Mendoza, M., Hermoso de Mendoza, J., Larrasa, J. (2006). An epidemiological evaluation of *Mycobacterium bovis* infections in wild game animals of the Spanish Mediterranean ecosystem. Res. Vet Sci. 80, 140-146.

Pavlik, I., Bures, F., Janovsky, P., Pecinka, P., Bartos, M., Dvorska, L., Matlova, L., Kremer, K., van Soolingen, D. (2002). The last outbreak of bovine tuberculosis in cattle in the Czech Republic in 1995 was caused by *Mycobacterium bovis subspecies caprae*. Vet. Med. – Czech, 47, 251–263.

Perez AM, Ward MP, Ritacco V. (2002). Simulation-model evaluation of bovine tuberculosis-eradication strategies in Argentine dairy herds. Prev. Vet. Med. 30, 351-60.

Phillips, C.J.C., Foster, C.R.W., Morris, P.A., Teverson, R. (2003). The transmission of *Mycobacterium bovis* infection to cattle. Res. Vet. Sci. 74, 1-15.

Pollock, J.M, Rodgers, J.D, Welsh, M.D, McNair, J. (2006). Pathogenesis of bovine tuberculosis: The role of experimental models of infection. Vet. Microbiol. 112, 141–150.

Porphyre, T., McKenzie, J., Stevenson, M. (2007). A descriptive spatial analysis of bovine tuberculosis in intensively controlled cattle farms in New Zealand. Vet. Res. 38, 465-479.

Porphyre, T., Stevenson, M. A., McKenzie, J. (2008). Risk factors for bovine tuberculosis in New Zealand cattle farms and their relationship with possum control strategies. Prev. Vet. Med. 86, 93–106.

Pritchard, D. G. (1988). A century of bovine tuberculosis 1888–1988: Conquest and Controversy. J. Comp. Pathol. 99, 357–99.

Quinn, P.J., Markey, B.K, Carter, M.E, Donnelly, W.J., Leonard, F.C. (2002). Veterinary microbiology and microbial disease, 1st ed. Oxford: Blackwell Science. 97-105.

Radostits, O.M., Gay, C.C., Blood, D.C., Hinchcliff. K.W.(2000). Veterinary Medicine a Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses.9th ed. London, GB: WB Saunders Co.

Ratnapradipa, D., Brown, S.L., Wodika, A.B. (2011). Examining the breadth and depth of environmental health through a modified Delphi technique. Am. J. Health Educ. 42, 50-57.

Reviriego-Gordejo, F.J., Vermeersch, J.P. (2006). Towards eradication of bovine tuberculosis in the European Union. Vet. Microbiol. 112, 101–109.

Ramırez-Villaescusa, A.M., Medley, G.F., Mason, S., Green, L.E. (2010). Risk factors for herd breakdown with bovine tuberculosis in 148 cattle herds in the south west of England. Prev. Vet. Med. 95, 224–230.

Regassa, A., Medhin, G., Ameni, G. (2008). Bovine tuberculosis is more prevalent in cattle owned by farmers with active tuberculosis in central Ethiopia. Vet. J. 178, 119–125

Reilly, L. A., Courtenay, O. (2007). Husbandry practices, badger sett density and habitat composition as risk factors for transient and persistent bovine tuberculosis on UK cattle farms. Prev. Vet. Med. 80, 29–142.

Rodríguez, E., Sánchez, L.P., Pérez, S., Herrera, L., Jiménez, M. S., Samper, S., Iglesias, M.J. (2009). Human tuberculosis due *to Mycobacterium bovis* and *M. caprae* in Spain, 2004–2007. Int. J. Tuberc .Lung Dis. 13, 1536–1541.

Rodríguez-Prieto, V., Martínez-López, B., Barasona, J. A., Acevedo, P., Romero, B., Rodriguez-Campos, S., Gortázar, C., Sánchez-Vizcaíno, J.M., Vicente, J. (2012). A Bayesian approach to study the risk variables for tuberculosis occurrence in domestic and wild ungulates in South Central Spain. Vet. Res. 8:148

Romero, B., Rodríguez, S., Bezos, J., Díaz, R., Copano, M.F., Merediz, I., Mínguez, O., Marqués, S., Palacios, J.J., García de Viedma, D., Sáez, J.L., Mateos, A., Aranaz, A., Domínguez, L., de

Juan, L. (2011). Humans as source of Mycobacterium tuberculosis infection in cattle, Spain. Emerg. Infect. Dis., 17, 2393-2395.

Rothschild, B. M., Martin, L. D. G. Lev, et al. (2001). Mycobacterium tuberculosis complex DNA from an extinct bison dated 17,000 years before the present. Clin. Infect. Dis. 33, 305–311.

Salo, W.L., Aufderheide, A.C., Buikstra, J., Holcomb, T.A. (1994). Identification of *Mycobacterium tuberculosis* DNA in a pre-Columbian Peruvian mummy. Proc. Natl. Acad. Sci. 91, 2091–2094.

Schiller, I., Waters, W.R., Vordermeier, H.M., Jemmi, T., Welash, M., Keck, N., Whelan, Gormley, E., Boschiroli, M.L., Moyen, L.G., Vela, C., Cagiola, M., Buddle, M.B., Palmer, M., Thacker, T., Oesch, B. (2011). Bovine tuberculosis in Europe from the perspective of an officially tuberculosis free country: Trade, surveillance and diagnostics. Vet. Microbiol. 151, 153-159

Schoembaum, M.A., Espe, B.H., Behring, B. (1992). Epidemic of bovine tuberculosis cases originating from an infected beef herd in Oklahoma, USA. Prev Vet Med 13, 112-120.

Shitaye, J.E., Tsegaye, W., Pavlik, I. (2007). Bovine tuberculosis infection in animal and human populations in Ethiopia: a review. Vet. Med. 52, 317–332

Skuce, R.A., Allen, A.R., McDowell, S.W. J. (2011). Herd-Level Risk Factors for Bovine Tuberculosis: A Literature Review. Vet. Med. Int. 2012:621210. doi:10.1155/2012/621210.

Smith, I. (2003). Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. Clin. Microbiol. Rev. 463-496.

Smith, N.H., Dale, J., Inwald, J., Palmer, S., Gordon, S.V., Hewinson, R. G., Smith, J.M. (2003). The population structure of *Mycobacterium bovis* in Great Britain: clonal expansion. Proc. Natl. Acad. Sci. U. S. A. 100, 15271–15275.

Smith, N.H., Gordon, S.V., de la Rua-Domenech, R., Clifton-Hadley, R.S., Hewinson, R.G. (2006). Bottlenecks and broomsticks: the molecular evolution of *Mycobacterium bovis*. Nat. Rev. Microbiol. 4, 670 – 681.

Stamp, J. T. (1948). Bovine pulmonary tuberculosis. J. Comp. Pathol. 58, 9-23.

Steger, G. (1970). Rindertuberkulose in Tierschauen [Bovine tuberculosis at cattle shows]. Tierarzliche Umschau 22, 416–421.

Tanner, M., Michel, A.L. (1999). Investigation of the viability of *M. bovis* under different environmental conditions in the Kruger National Park. Onderstepoort. J. Vet. Res.66, 185–190.

Thoen, C., Lobue P., de Kantor, I. (2006). The importance of *Mycobacterium bovis* as a zoonosis. Vet. Microbiol. 112, 339–345.

Une, Y., Mori, T. (2007). Tuberculosis as a zoonosis from a veterinary perspective. Comp. Immunol. Microbiol. Infect. Dis. 30, 415–425.

Viera, A. J., Garrett, J.M. (2005). Understanding Inter observer Agreement: The Kappa Statistic. Fam. Med. 37, 360-363.

Vordermeier, M., Whelan, A., Ewer, K., Goodchild, T., Clifton-Hadley, R., Williams, J. and Hewinson, R. G. (2006). The BOVIGAM® assay as ancillary test to the tuberculin skin test. Gov. Vet. J. 16, 72-80.

Vordermeier, H.M., Whelan, A.O., Hewinson, R.G. (2008). The scientific case for the gamma-interferon Bovigam assay. Gov. Vet. J. 19, 38–43.

Whipple, D. L., Bolin, C. A., Miller, J.M. (1996). Distribution of lesions in cattle infected with *Mycobacterium bovis*. J. Vet. Diag. Invest. 8, 351-354.

Wilesmith, J.W. (1983). Epidemiological features of bovine tuberculosis in cattle herds in Great Britain. J Hygiene 90, 159-176.

Wilesmith, J.W., Williams, D.R. (1986). Tuberculosis lesions in reactor cows. Vet. Rec. 119, 51.

Wolfe, D.M., Berke, O., Kelton, D.F., White, P.W., More, S.J., O'Keeffe, J., Martin, S.W. (2010). From explanation to prediction: a model for recurrent bovine tuberculosis in Irish cattle herds. Prev. Vet. Med. 94, 170-177.

Young, J. S., Gormley, E., Wellington, E. M.H. (2005). Molecular detection of *Mycobacterium bovis* and *Mycobacterium bovis* BCG (Pasteur) in soil. Appl. Environ. Microbiol.71, 1946-1952.

Zanella, G., Durand, B., Hars, J., Moutou, F., Garin-Bastuji, B., Duvauchelle, A., Ferme, M., Karoui, C., Boschiroli, M.L.(2008). *Mycobacterium bovis* in wildlife in France. J. Wildl. Dis. 44, 99–108.

7. ANEXES

Annex 1. National epidemiological bTB questionnaire

MINISTERIO DE MEDIO
AMBIENTE Y MEDIO RURAL Y
MARINO

DIRECCIÓN GENERAL DE RECURSOS
AGRÍCOLAS Y GANADEROS

SUBDIRECCIÓN GENERAL DE
SANIDAD DE LA PRODUCCIÓN
PRIMARIA

ENCUESTA EPIDEMIOLÓGIA REDUCIDA PARA LA OBTENCIÓN Y GRABACIÓN DE DATOS EPIDEMIOLÓGICOS EN BROTES DE TUBERCULOSIS BOVINA, BRUCELOSIS BOVINA Y BRUCELOSIS OVINA Y CAPRINA

Programa nacionales de erradicación de enfermedades 2012

MINISTERIO DE MEDIO
AMBIENTE Y MEDIO RURAL Y
MARINO

DIRECCIÓN GENERAL DE RECURSOS
AGRÍCOLAS Y GANADEROS

SUBDIRECCIÓN GENERAL DE
SANIDAD DE LA PRODUCCIÓN
PRIMARIA

INTRODUCCION

La investigación epidemiológica constituye una herramienta esencial para el control de enfermedades como la brucelosis y la tuberculosis, sea cual sea la calificación sanitaria de la zona considerada y la estrategia de control que se practique. Permite, a nivel de rebaño, confirmar o descartar la sospecha de infección, la determinación de su origen, la búsqueda de otros rebaños epizootiológicamente relacionados con el infectado y la evaluación del perfil de transmisión de la enfermedad para así decidir la estrategia más adecuada de control y erradicación.
La encuesta debe realizarse con preferencia en el conjunto de la unidad epidemiológica, entendida ésta como el agregado de individuos (y por extensión de rebaños) sometidos a similar riesgo sanitario.

Los principales objetivos de la investigación epidemiológica son:

1º Determinar el origen del brote mediante investigación retrospectiva: está diseñada para identificar la fuente probable del brote. Como paso previo, es necesario excluir la posibilidad de re-infección en el rebaño infectado. Para ello es necesario tener en cuenta:

- Todas las entradas de los animales de la unidad epidemiológica en estudio y los periodos de pastoreo común de varios rebaños durante dicho periodo.
- Posibilidad de transmisión debida al préstamo, intercambio de materiales o intervención humana.
- Investigar los diferentes lugares de tránsito de los animales, en caso de existir un periodo de tiempo importante entre la salida de animales de la explotación de origen y la entrada en la de destino.
- Considerar todos los riesgos relacionados con el comercio y transporte de animales.

2º Identificar los factores de riesgo: sobre el terreno para la transmisión dentro del rebaño, evolución del brote, las posibles rutas de diseminación del agente y los riesgos para la salud humana.

Este protocolo de investigación debería desarrollarse en todos los rebaños con sospecha/confirmación de brucelosis/tuberculosis como resultado de una prueba de diagnóstico directo o indirecto con resultado positivo, y siempre se realizará, de acuerdo con lo establecido en los Programas Nacionales de Erradicación de la Tuberculosis Bovina, Brucelosis Bovina y Brucelosis Ovina y Caprina, en **todos los nuevos rebaños positivos en que se confirme la enfermedad.**

Así mismo, y a partir del año 2012, los Programas Nacionales aprobados por la Comisión Europea para su co-financiación contemplan la necesidad de incluir datos epidemiológicos equivalentes para explotaciones libres (controles) relacionadas con las explotaciones positivas (casos) en el sistema informático BRUTUB, por lo que es necesario realizar esta misma encuesta epidemiológica en dichas explotaciones libres seleccionadas baja unos criterios mínimos.

BASE LEGAL

Ley 8/2003, de sanidad animal

Directiva 64/432/CEE y modificaciones, del Consejo, relativa a problemas de policía sanitaria en materia de intercambios intracomunitarios de animales de las especies bovina y porcina: Anexo A

Real Decreto 1716/2000, sobre normas sanitarias para el intercambio intracomunitario de animales de las especies bovina y porcina

Directiva 91/68/CEE y modificaciones, del Consejo, relativa a las normas de policía sanitaria que regulan los intercambios intracomunitarios de animales de las especies ovina y caprina

Real Decreto 1941/2004, por el que se establecen las normas de policía sanitaria que regulan los intercambios intracomunitarios y las importaciones de terceros países de animales de las especies ovina y caprina

Directiva 77/391/CEE, del Consejo, por la que se establece una acción de la Comunidad para la erradicación de la brucelosis, de la tuberculosis y de la leucosis en los bovinos

Directiva 78/52/CE, del Consejo, por la que se establecen los criterios comunitarios aplicables a los planes nacionales de erradicación acelerada de la brucelosis, de la tuberculosis y de la leucosis enzoótica de los bovinos

Real Decreto 2611/1996 y modificaciones, por el que se regulan los programas nacionales de erradicación de enfermedades de los animales

Decisión 2011/807/UE, por la que se aprueban los programas anuales y plurianuales y la contribución financiera de la Unión para la erradicación, el control y la vigilancia de determinadas enfermedades de los animales y zoonosis, presentados por los Estados miembros para 2012 y años sucesivos.

Recomendaciones FVO y de los subgrupos de la Task Force para los Programas de Erradicación. En concreto, la Misión DG(SANCO)/2007-7367 recomendó adoptar el modelo de estudio epidemiológico de una de las Comunidades Autónomas visitadas, que había sido adaptado para facilitar el registro de la información obtenida con el fin de identificar factores de riego asociados. Este hecho ha vuelto a ser puesto de manifiesto por la Misión DG(SANCO)/2008/7792

Programas Nacionales de Erradicación de Enfermedades www.rasve.mapya.es

MOTIVOS QUE ORIGINAN LA REALIZACIÓN DE LA ENCUESTA

1. Aparición de síntomas o lesiones en animales de una explotación (vigilancia pasiva).
2. Aparición de lesiones en matadero (vigilancia activa).
3. Detección de reaccionantes positivos en pruebas de laboratorio (programa de calificación) o en pruebas de campo.
4. Relación epidemiológica con un brote de infección previamente identificado y con el que puede existir una probabilidad de contacto, directo o indirecto.
5. Realización de la encuesta en explotaciones libres seleccionadas como controles de una explotación infectada o caso, que permitan la realización de estudios caso-control para identificar factores de riesgo.

ACTUACIONES PRELIMINARES

1. **Formación:** Los encuestadores deben ser adiestrados específicamente en la metodología para la realización de la encuesta, con la que estarán familiarizados antes de emplearla en el campo. El objetivo básico es evitar los sesgos en la captura de información imputables al encuestador o a la sistemática de encuesta.
2. **Recopilación de datos:** Antes de visitar cada explotación se obtendrán de las distintas fuentes de información (REGA, SITRAN, SIMOGAN....) los antecedentes relativos a:
    a. Datos de la explotación
    b. Historial sanitario (pruebas, confirmaciones...).
    c. Movimiento hacia la explotación.
    d. Explotaciones colindantes, espacios naturales, granjas cinegéticas...

OBJETIVOS DE LA INVESTIGACIÓN

1. **INVESTIGAR LAS FUENTES DE CONTAMINACIÓN E INFECCIÓN**.
    a. Animales infectados (enfermos, infectados crónicos o portadores inaparentes; latentes (posibilidad de transmisión vertical en brucelosis).
    b. Productos del aborto o fallo reproductivo (fetos y anejos fetales) en brucelosis
    c. Cadáveres.
    d. Productos de origen animal, secreciones de los animales.
    e. Deyecciones (heces y orina)
    f. El medio, incluyendo posibles vectores mecánicos (perros, gatos, alimentos, pastos y agua contaminados…), reservorios silvestres y reservorio humano (en el caso de tuberculosis).

2. EVALUAR LA RECEPTIVIDAD Y REACTIVIDAD (DETERMINANTES DE ENFERMEDAD)

a. Factores intrínsecos (determinantes del hospedador): raza, edad, estado productivo, inmunización previa…
b. Factores extrínsecos (determinantes ambientales): alojamiento, manejo, alimentación, estrés...

3. INVESTIGACIÓN DEL ORIGEN Y DE LAS FORMAS DE TRANSMISIÓN PREDOMINANTES:

Transmisión horizontal

a. <u>Fuentes de Contagio directo:</u>
    i. Animales infectados (entradas, cesión reproductores, animales de explotaciones colindantes) durante la gestación, lactación y contacto venéreo durante la monta
    ii. Portadores humanos (TBC).
    iii. Animales silvestres (que comparten alimentación y agua, a veces reproducción).
b. <u>Fuentes de Contagio Indirecto:</u>
    i. Acceso a pastos o caminos comunes contaminados por animales de la propia explotación u otra.
    ii. Material contaminado en alojamientos: especialmente durante los partos, alimentación y agua con deyecciones.
    iii. Alimentos contaminados desde fuera: finca drena a otra o recibe drenaje de otra.

Transmisión vertical: en el caso de brucelosis

4. RECOPILACION DE DATOS EQUIVALENTES EN EXPLOTACIONES CONTROLES

Para seleccionar las explotaciones “control” relacionadas con las explotaciones infectadas “casos” se seguirán los siguientes criterios mínimos:

a. Se seleccionará, para realizar la encuesta epidemiológica, un mínimo de 1 explotación control por cada explotación caso, si bien idealmente y en la medida de lo posible el número mínimo de encuestas control por cada caso seria de 2, con el fin de contemplar la posibilidad de que alguno de los rebaños controles pueda infectarse con posterioridad a la realización de la encuesta.
b. Las explotaciones control deberán llevar calificadas sanitariamente un mínimo de 2 años consecutivos.
c. Las explotaciones control deberán estar situadas en la misma ubicación geográfica que la explotación caso, en la misma comarca o unidad veterinaria local.
d. Las explotaciones control deberán tener un censo parecido al de la explotación caso y una aptitud productiva y manejo similares.

<u>DEFINICIONES Y CÓDIGOS UTILIZADOS EN LA ENCUESTA</u>

1. Encabezado:

Código CA-AA-000: 2 dígitos de la CCAA; 2 últimos dígitos del año; número de la encuesta correlativo en el año de referencia. Este número será asignado automáticamente por el sistema informático cuando esté disponible la aplicación.
Especie (ES): 01 Bovino 02 Ovino 03 Caprino 04 Ovino y caprino
Calificación anterior: tuberculosis: T1, T2-, T2+,T3,Ts, Tr Brucelosis bovina: B1, B2-, B2+, B3, B4, Bs, Br
Brucelosis ovina y caprina: M1, M2-, M2+, M3, M4, Ms, Mr
2. Antecedentes de laboratorio:

- motivo: 01 vigilancia pasiva; 02 vigilancia activa; 03 programa de calificación; 04 relación epidemiológica 05 pruebas previas o posteriores al movimiento
- tipo veterinario: 01 ADS; 02 empresa privada; 03 empresa pública; 04 SVO

- LABº: 01 Provincial de Sanidad Animal 02 Regional de Sanidad Animal 03 Nacional de Referencia 04 Comunitario de Referencia 05 Otros

3. Antecedentes investigación etiológica:

- MOTIVO: 01 reactores positivos 02 sospecha en matadero 03 sospecha clínica 04 relación epidemiológica
- TIPO: 01 cultivo 02 biotipificación 03 PCR 04 spoligotyping 05 VTNR-MLVA
- Especie bacteriana: *B. abortus, B. melitensis B. suis, M. bovis, M. caprae, M. tuberculosis, M. africanum*

4. Investigación epidemiológica:

(*) Sólo en el caso de brucelosis:
Posible latente: animal de reposición nacidos de una hembra sero-positiva
Pasto posiblemente contaminado: aquel en el que no se ha respetado el periodo mínimo de cuarentena de 60 días tras la retirada de animales positivos.
(**) Sólo en el caso de tuberculosis

5. Fuentes de contagio: últimas entradas de animales

- Animales de riesgo: en general, se consideran entradas de riesgo aquellas que proceden de un rebaño calificado durante menos de 3 años.
- NRE Origen: Código R.E.G.A. de la explotación de origen
- Nº y tipo: 01 reproductoras 02 reposición 03 sementales

MINISTERIO DE MEDIO
AMBIENTE Y MEDIO RURAL Y
MARINO

DIRECCIÓN GENERAL DE RECURSOS
AGRÍCOLAS Y GANADEROS

SUBDIRECCIÓN GENERAL DE
SANIDAD DE LA PRODUCCIÓN
PRIMARIA

ENCUESTA EPIZOOTIOLÓGICA: código: CA-AA-000

OBJETO DE LA ENCUESTA: TUBERCULOSIS BRUCELOSIS

TIPO DE ENCUESTA:
CASO ☐
CONTROL ☐ **COD REGA EXPLOTACIÓN CASO RELACIONADA ____________**

COD REGA.____________PROVINCIA: ___________(COMARCA/UVL):_______________________

MUNICIPIO__________________ TITULAR DE LA EXPLOTACIÓN:________ _______ESPECIE (ES): ___

CALIF ANTERIOR _______Y FECHA DE OBTENCIÓN: TUBER: _____/____/_____ BRUCE ____/____/____

COORDENADAS GEOGRÁFICAS: X: Y: FECHA ENCUESTA:_____/_____/_____

MOTIVO DE LA ENCUESTA:

Síntomas o lesiones en animales de la explotación (Vigilancia pasiva) ☐ Lesiones en matadero (Vigilancia activa) ☐

Reaccionantes positivos en laboratorio (programa de calificación) o prueba de campo ☐ Explotación control ☐

¿TIENE RELACIÓN EPIDEMIOLÓGICA CON BROTE? SI ☐ NO ☐

ENCUESTADOR:____________________________________ TELF. ________________

CARACTERÍSTICAS DE LA EXPLOTACIÓN

- **I- CENSOS**

		Bovinos	Ovinos	Caprinos	Otras_______________
Adultos	Machos				
	Hembras				
Reposición					

- **II- MANEJO**
- A) APTITUD: Leche ☐ Carne ☐ Mixto ☐ Lidia ☐ Otros ☐
- B) MANEJO: Intensivo ☐ Semi-extensivo ☐ Extensivo ☐
- C) TRASHUMANCIA/TRASTERMINANCIA: Si ☐ No ☐

  Municipio de destino______________________

- Forma parte de una unidad epidemiológica con antecedentes recientes (desde el penúltimo control): : Si ☐ No ☐
- Prevalencia del municipio: ______% (ÚLTIMA CAMPAÑA DE SANEAMIENTO)
- Linda con espacio natural: : Si ☐ No ☐
- Distancia a área forestal/matorral (en metros): ______
- Linda con finca actividad cinegética de caza mayor: Si ☐ No ☐ Nº DE EVENTOS EN LA ÚLTIMA TEMPORADA: _____
- Linda con granja cinegética: : Si ☐ No ☐ Censo medio: ________
- Se ha detectado presencia de las siguientes especies silvestres de riesgo: JABALÍES ☐ CÉRVIDOS ☐ ZORROS ☐ OTROS CARNÍVOROS ☐ TEJONES ☐

ANTECEDENTES LABORATORIO/PRUEBAS CAMPO (3 AÑOS)

Fecha	LABº	MOTIVO	TIPO VET	Especie animal	investigados	Positivos	% Positivos

ANTEDEDENTES DE INVESTIGACIÓN ETIOLÓGICA (4):
¿SE HA REALIZADO INVESTIGACIÓN ETIOLÓGICA? SI ☐ NO ☐

Fecha	LABº	MOTIVO	Especie animal	Investigados	Positivos	Especie bacteriana	TIPO ANÁLISIS

INVESTIGACIÓN EPIDEMIOLÓGICA

FUENTES DE CONTAMINACIÓN E INFECCIÓN

EN LA EXPLOTACIÓN HAY: Seropositivos-Reactores: Si ☐ No ☐ Posibles Latentes*: Si ☐ No ☐

EN EL CASO DE LA TUBERCULOSIS**, EN EL CASO DE HABER REACTORES, SON: A IDTB ☐ A GAMMA-INF ☐ A AMBAS ☐

¿HA HABIDO ABORTOS, NACIDOS DÉBILES O MUERTOS, DESDE LA ÚLTIMA ACTUACIÓN*? Si ☐ No ☐

TASA DE ABORTOS/NACIDOS DÉBILES QUE MUEREN EN LA 1ª SEMANA ESTIMADA (ÚLTIMOS 12 MESES): <2% ☐ 2-10% ☐ >10% ☐

¿SE OBSERVÓ ORQUITIS EN MACHOS*? Si ☐ No ☐

EXISTE UN SISTEMA DE DESTRUCCIÓN DE ABORTOS, SECUNDINAS*: Si ☐ No ☐ ¿Tienen acceso a ellos perros o gatos*?: Si ☐ No ☐

¿EXISTEN PUNTOS DE AGUA ESTANCADA DENTRO DE LA EXPLOTACIÓN? : Si ☐ No ☐ CUÁNTOS? ______

¿PUEDEN CONTAMINARSE DE FORMA PROLONGADA LOS COMEDEROS Y ABREVADEROS CON DEYECCIONES ANIMALES?: Si ☐ No ☐

¿DRENA LA EXPLOTACIÓN A OTRAS?: Si ☐ No ☐

Relacionar:____

¿RECIBE EL DRENAJE DE OTRAS EXPLOTACIONES? : Si ☐ No ☐

Relacionar____

¿PUEDE ACCEDER A PASTOS POSIBLEMENTE CONTAMINADOS*? : Si ☐ No ☐

¿UTILIZA EN LA FERTILIZACIÓN DE LOS PASTOS DEYECCIONES ANIMALES O AGUAS RESIDUALES? SI ☐ NO ☐

¿Uso de ESTIÉRCOL COMO ABONO? No ☐ De la propia granja ☐ De otra granja ☐

Realizar un listado de explotaciones de contacto cercano y su calificación sanitaria::

RECEPTIVIDAD Y REACTIVIDAD DE LOS ANIMALES (DETERMINANTES DE ENFERMEDAD)

INDICAR FECHA: / / Y ESTADO DE PARIDERA EN EL MOMENTO DEL CHEQUEO:

Mayoritariamente en el último 1/3 gestación: ☐ mayoritariamente en los primeros 2 meses de gestación ☐ mayoritariamente vacías ☐

¿SE REALIZÓ LA ÚLTIMA VACUNACIÓN EN LA EDAD CORRECTA*? Si ☐ No ☐

TIPO DE VACUNA UTILIZADA: B19 ☐ RB51 ☐ REV1 ☐

ESTIMACIÓN TASA DE COBERTURA VACUNAL SOBRE EL CENSO TOTAL (EN %): < 50% ☐ 50-80% ☐ >80% ☐

¿AISLAMIENTO DE LAS HEMBRAS PRÓXIMAS AL PARTO? SI ☐ NO ☐

¿INTALACIONES Y MATERIAL ESPECÍFICO PARA EL MANEJO DE LA PARIDERA? SI ☐ NO ☐

¿CAMBIO DE INDUMENTARIA E HIGIENE CORPORAL DESPUÉS DE ATENDER A LAS HEMBRAS PARIDAS? SI ☐ NO ☐

GESTIÓN DEL CALOSTRO ¿SE ALMACENA Y CONGELA PARA CASOS DE NECESIDAD? SI ☐ NO ☐

¿ORDEÑA? SI ☐ NO ☐ TIPO: MANUAL ☐ MECÁNICO ☐

¿SE DETECTAN PROBLEMAS EVIDENTES DE ALIMENTACIÓN ESTACIONAL INSUFICIENTE? Si ☐ No ☐

¿SE DETECTAN PROBLEMAS DE PARASITACIÓN QUE AFECTEN A LA RESISTENCIA? Si ☐ No ☐

¿SE TIENEN RESULTADOS SEROLÓGICOS O EVIDENCIAS CLÍNICAS DE PARATUBERCULOSIS?** SI ☐ NO ☐

FUENTES DE CONTAGIO (FORMAS DE TRANSMISIÓN)

1.EXÒGENAS:

1.1- ENTRADA DE ANIMALES:

ULTIMAS ENTRADAS DE ANIMALES DE RIESGO EN LA EXPLOTACIÓN DURANTE LOS DOS ÚLTIMOS CONTROLES

Fecha Entrada	N.R.E. ORIGEN	Nº	TIPO	CALIFICACIÓN	¿CUARENTENA DE ENTRADA?	FECHA DE LAS PRUEBAS 30 DÍAS	Nª POSITIVOS QUE PROCEDÍAN DE ESE MOVIMIENTO

Nº TOTAL DE MOVIMIENTOS DE ENTRADA: _______ Nº TOTAL DE ANIMALES ENTRANTES: _________

¿HAN ENTRADO O CONVIVEN ANIMALES DOMÉSTICOS DE OTRA EXPLOTACIÓN EN SUS PASTOS Si ☐ No ☐

1.2- CONTACTO CON FAUNA SILVESTRE:

¿ACCEDEN LOS ANIMALES SILVESTRES DE RIESGO A LAS FUENTES DE ALIMENTACIÓN? Si ☐ No ☐

¿ACCEDEN LOS ANIMALES SILVESTRES DE RIESGO A LAS FUENTES DE AGUA? Si ☐ No ☐

¿HAN ENTRADO O CONVIVEN ANIMALES SILVESTRES O CINEGÉTICOS DE RIESGO EN SUS PASTOS? Si ☐ No ☐

¿DE QUÉ TIPO? SUIDOS ☐ CÉRVIDOS ☐ CARNÍVOROS ☐ TEJONES ☐

¿HA VISTO RESTOS DE VÍSCERAS, PROCEDENTES DE ACTIVIDAD CINEGÉTICA? : Si ☐ No ☐

Estado del CERCADO: Completo ☐ Parcial ☐ Sin cercado ☐

1.3- CONTACTO (DIRECTO O INDIRECTO) CON OTROS ANIMALES DOMÉSTICOS:

¿REALIZA PASTOREO EN COMÚN Y/O CONVIVENCIA CON ANIMALES DE OTROS REBAÑOS SUSCEPTIBLES? : Si ☐ No ☐

¿SE COMPARTEN PUNTOS DE AGUA CON OTROS REBAÑOS? SI ☐ NO ☐

¿SE COMPARTEN CAMINOS DE DESPLAZAMIENTO CON OTROS REBAÑOS? SI ☐ NO ☐

¿HA OBSERVADO SÍNTOMAS DE LA ENFERMEDADEN OTRAS ESPECIES DOMÉSTICAS??: Si ☐ No ☐

¿EN CUALES? BOVINOS ☐ OVINOS ☐ CAPRINOS ☐ CERDOS ☐ PERROS ☐ GATOS ☐

¿ACCEDEN OTROS ANIMALES DOMÉSTICOS A LAS FUENTES DE ALIMENTACIÓN O AGUA? Si ☐ No ☐

2. ENDÓGENAS:

¿HIZO REPOSICIÓN CON HIJOS DE ANIMALES POSITIVOS DEL AÑO ANTERIOR*? : Si ☐ No ☐

¿RESULTÓ ALGUNO DE LOS HIJOS, POSITIVO EN LAS ÚLTIMAS PRUEBAS*? : Si ☐ No ☐

¿SE LE CONCEDIÓ PRÓRROGA DE SACRIFICIO? : Si ☐ No ☐ PLAZO EN MESES: ____________________

¿HA HABIDO CASOS HUMANOS ENTRE LAS PERSONAS QUE FRECUENTAN LA EXPLOTACIÓN Si ☐ No ☐

¿HA TENIDO POSITIVIDADES INTERMITENTES, CONTINUAS O ALTERNAS, EN LOS ÚLTIMOS 3 AÑOS? : Si ☐ No ☐

¿PUEDEN ACCEDER LOS ANIMALES DE COMPAÑÍA (PERROS Y GATOS) AL ALMACÉN DE PIENSO?: SI ☐ No ☐

CONCLUSIONES DEL ENCUESTADOR SOBRE EL POSIBLE ORIGEN (NO CUMPLIMENTAR EN ENCUESTAS CONTROL)

☐ CONVIVENCIA CON OTRAS ESPECIES DOMÉSTICAS
☐ CONVIVENCIA CON OTRAS ESPECIES SILVESTRES
☐ INTRODUCCIÓN DE ANIMALES INFECTADOS
☐ NO SACRIFICIO DE POSITIVOS
☐ FUENTES DE ALIMENTACIÓN O AGUA CONTAMINADAS
☐ AUSENCIA DE BIOSEGURIDAD/DEFICIENCIAS EN INSTALACIONES O EQUIPO
☐ TRASHUMANCIA/PASTOS COMUNALES
☐ SISTEMÁTICA DE ORDEÑO DEFICIENTE
☐ CONTACTO HUMANO PORTADOR
☐ RECIRCULACIÓN
☐ MOVIMIENTOS CLANDESTINOS
☐ POSIBLES FALSOS NEGATIVOS
☐ CONDICIONES HIGIÉNICAS DEFICIENTES
☐ INSUFICIENTE COBERTURA VACUNAL
☐ MANEJO DEFICIENTE DE LA PARIDERA
☐ OTRAS (especificar posible causa) ____

____

____

NOTAS :

De lo anterior se concluye que el origen más probable de la infección sea: EXÓGENO ☐ ENDÓGENO ☐

EL VETERINARIO ENCUESTADOR
Fecha realización: de de 2.009

EL VETERINARIO OFICIAL RESPONSABLE
Ha sido informado el de 2.009

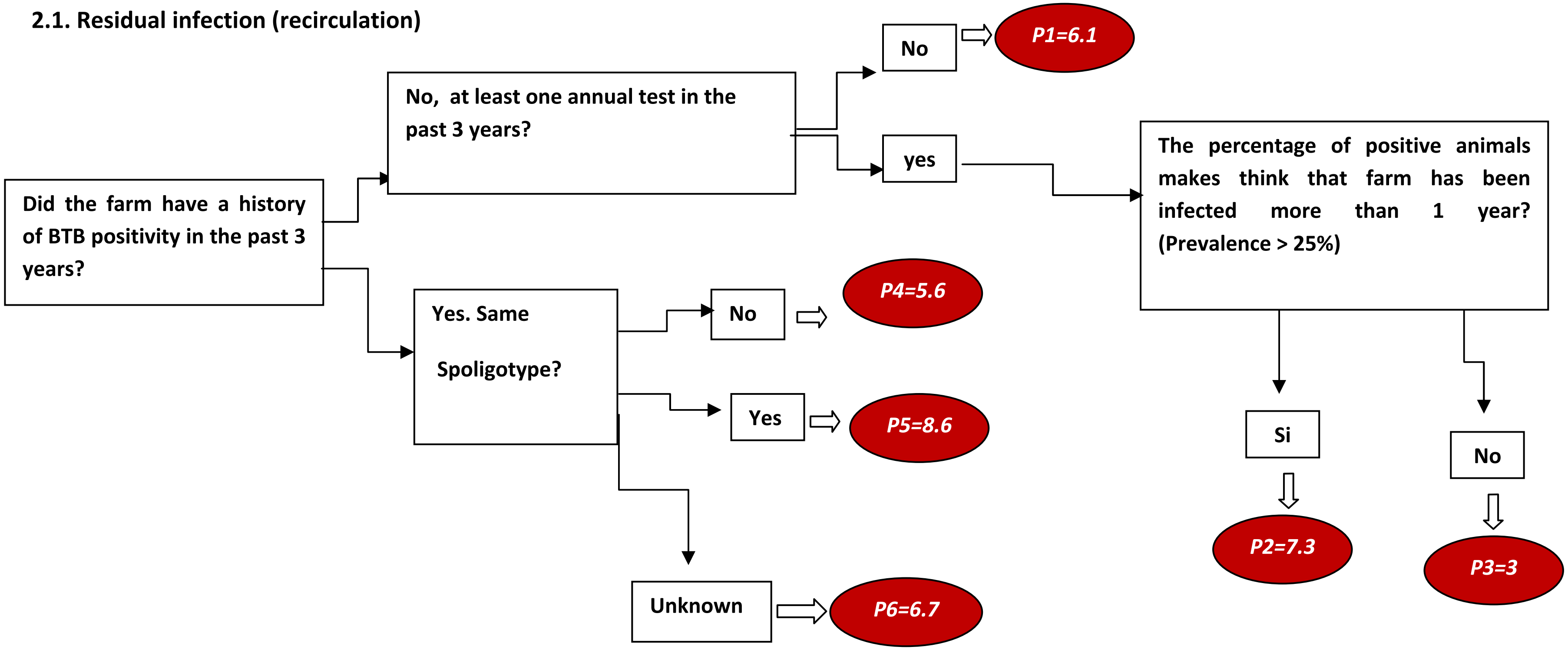
Fdo.______________________________

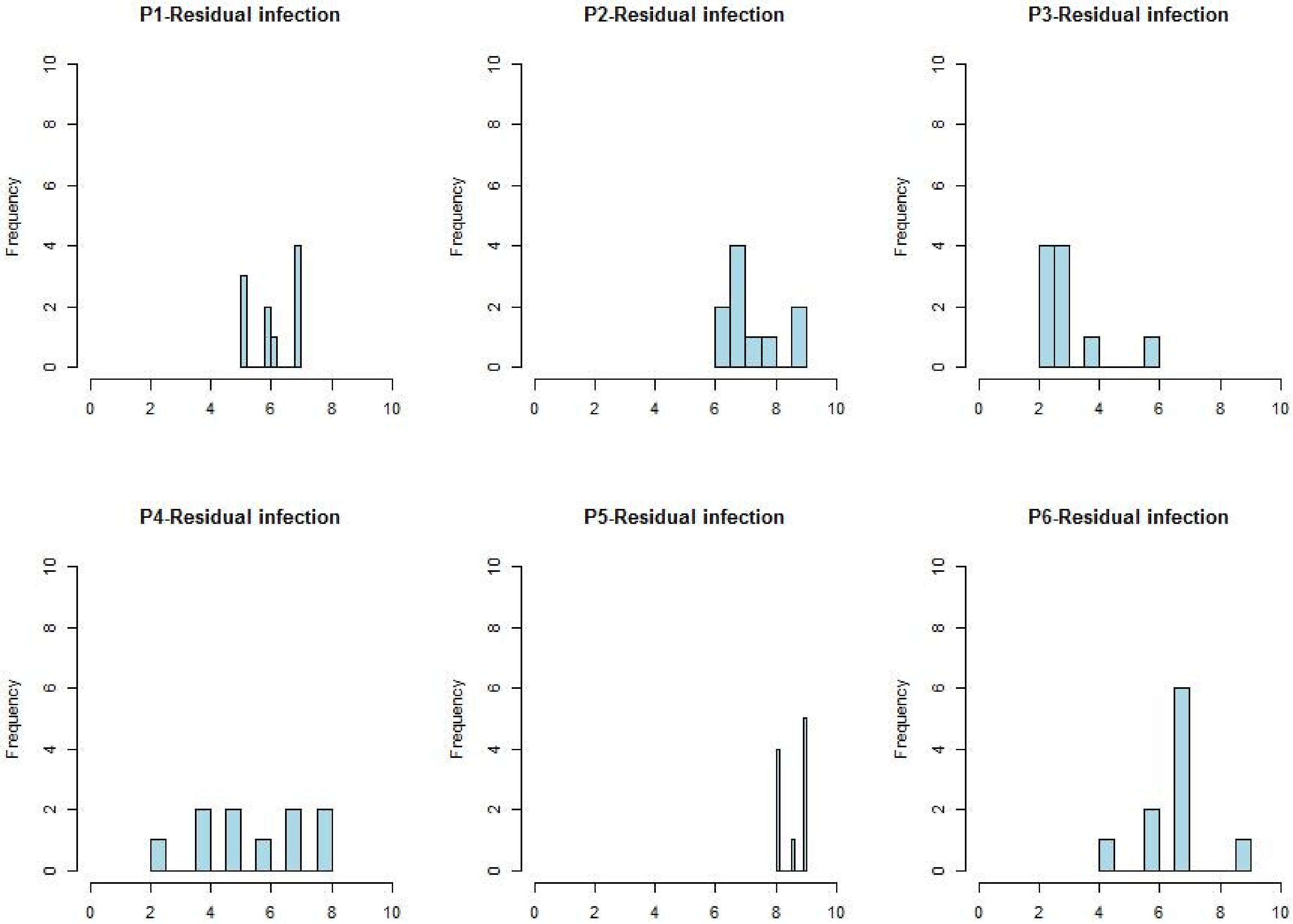
Fdo.____________________________________

GRABADA EN EL SISTEMA INFORMÁTICO EL / / POR ______________________________

Annex 2: Decision tree diagrams and distribution of values assigned by experts' opinion for each event.

2.1. Residual infection (recirculation)





P1-Residual infection

P2-Residual infection

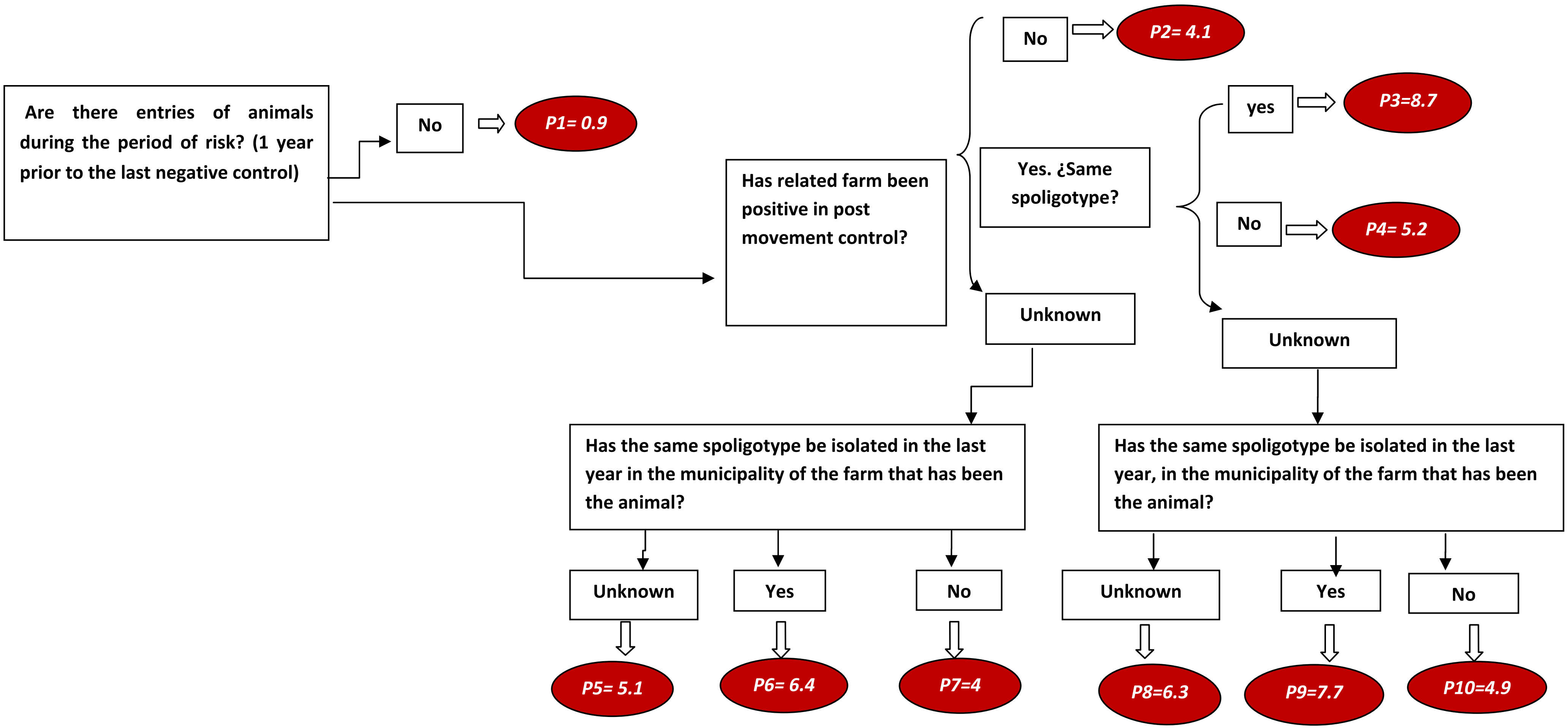
P3-Residual infection

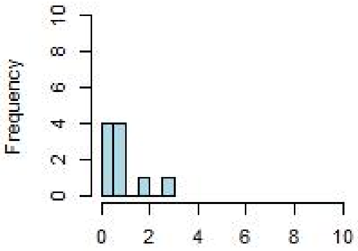
P4-Residual infection

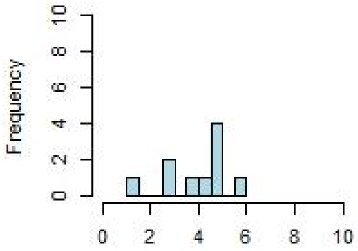
P5-Residual infection

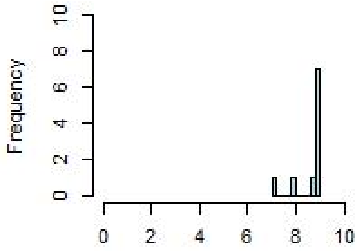
P6-Residual infection

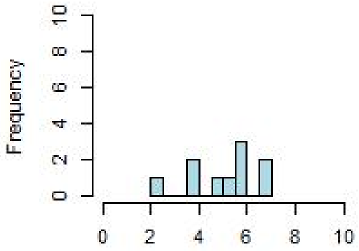
2.2. Entry of infected animals from other farms of Spain (the possibility of illegal entries are not considered)

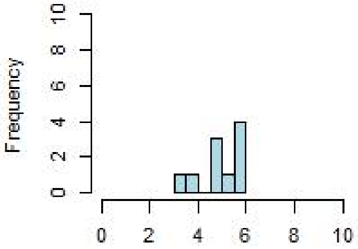


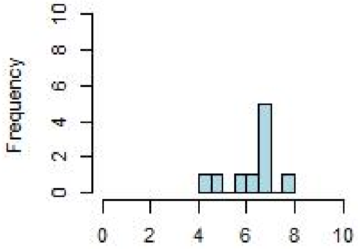

P1-Introduction animals

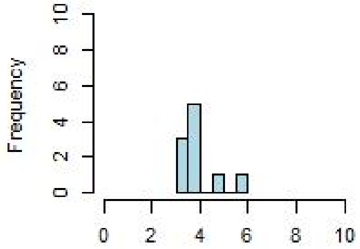

P2-Introduction animals

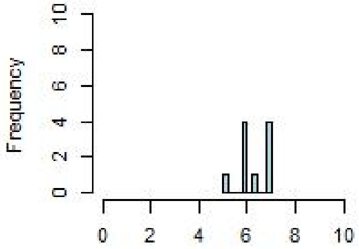

P3-Introduction animals

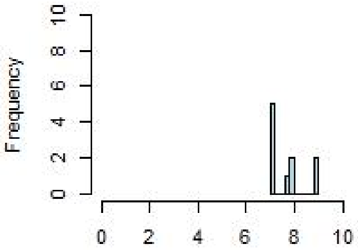

P4-Introduction animals

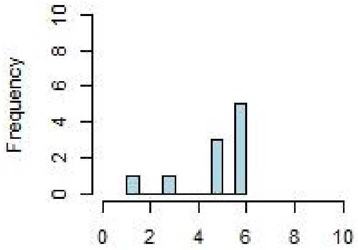

P5-Introduction animals

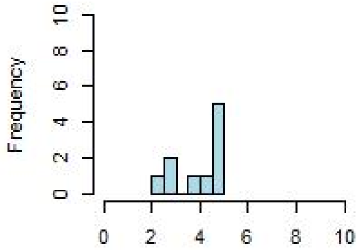

P6-Introduction animals


P7-Introduction animals

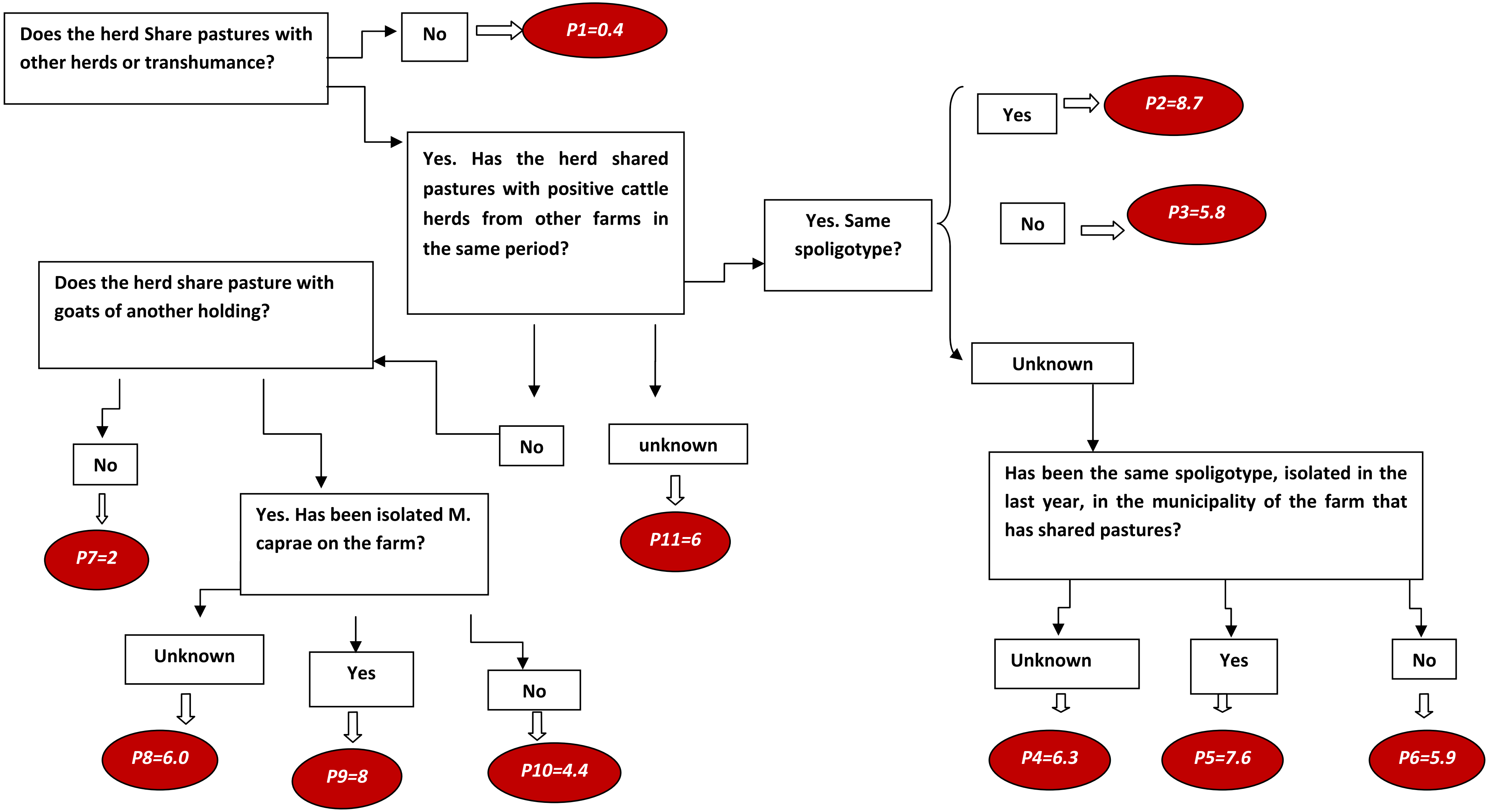

P8-Introduction animals

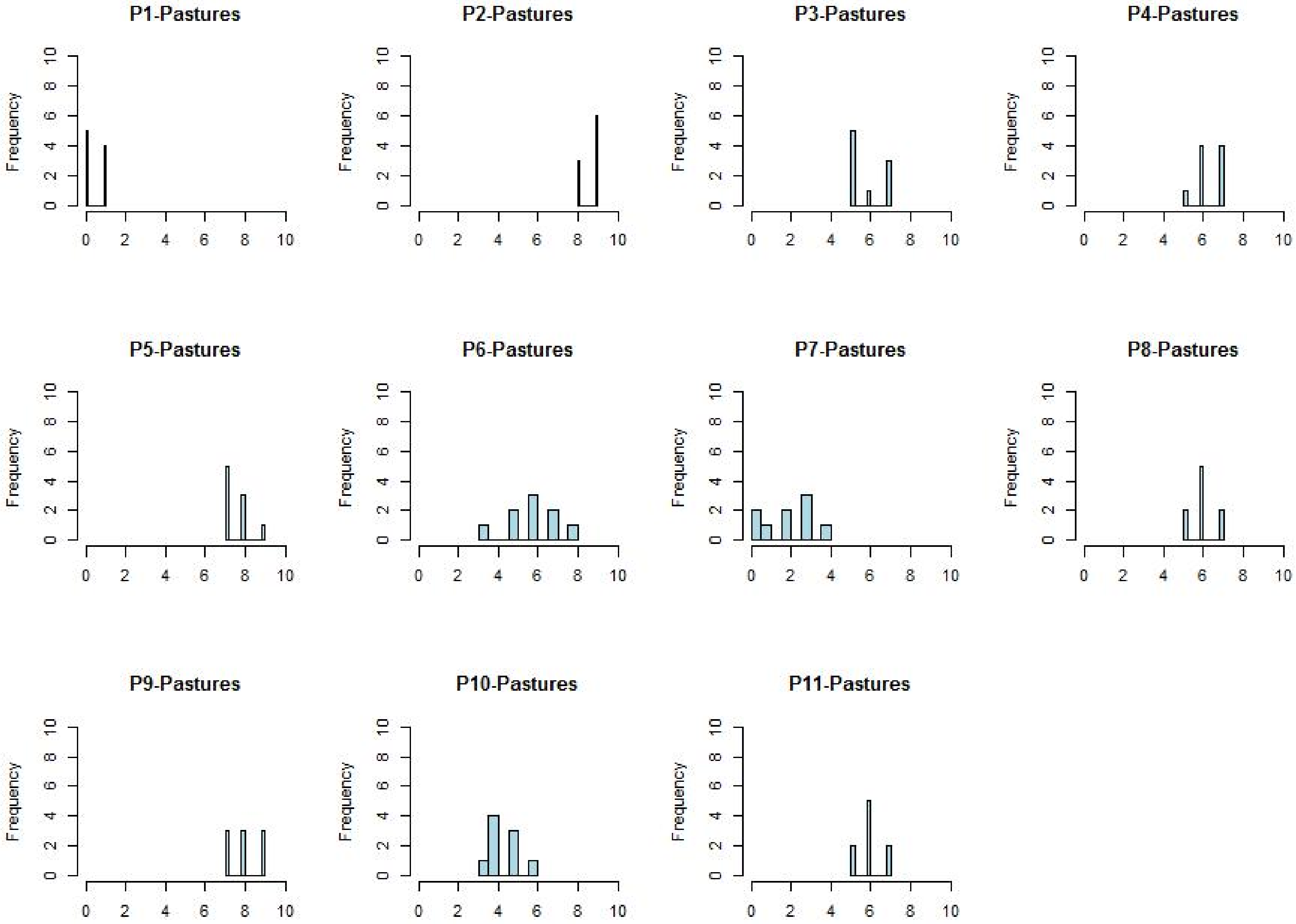

P9-Introduction animals


P10-Introduction animals

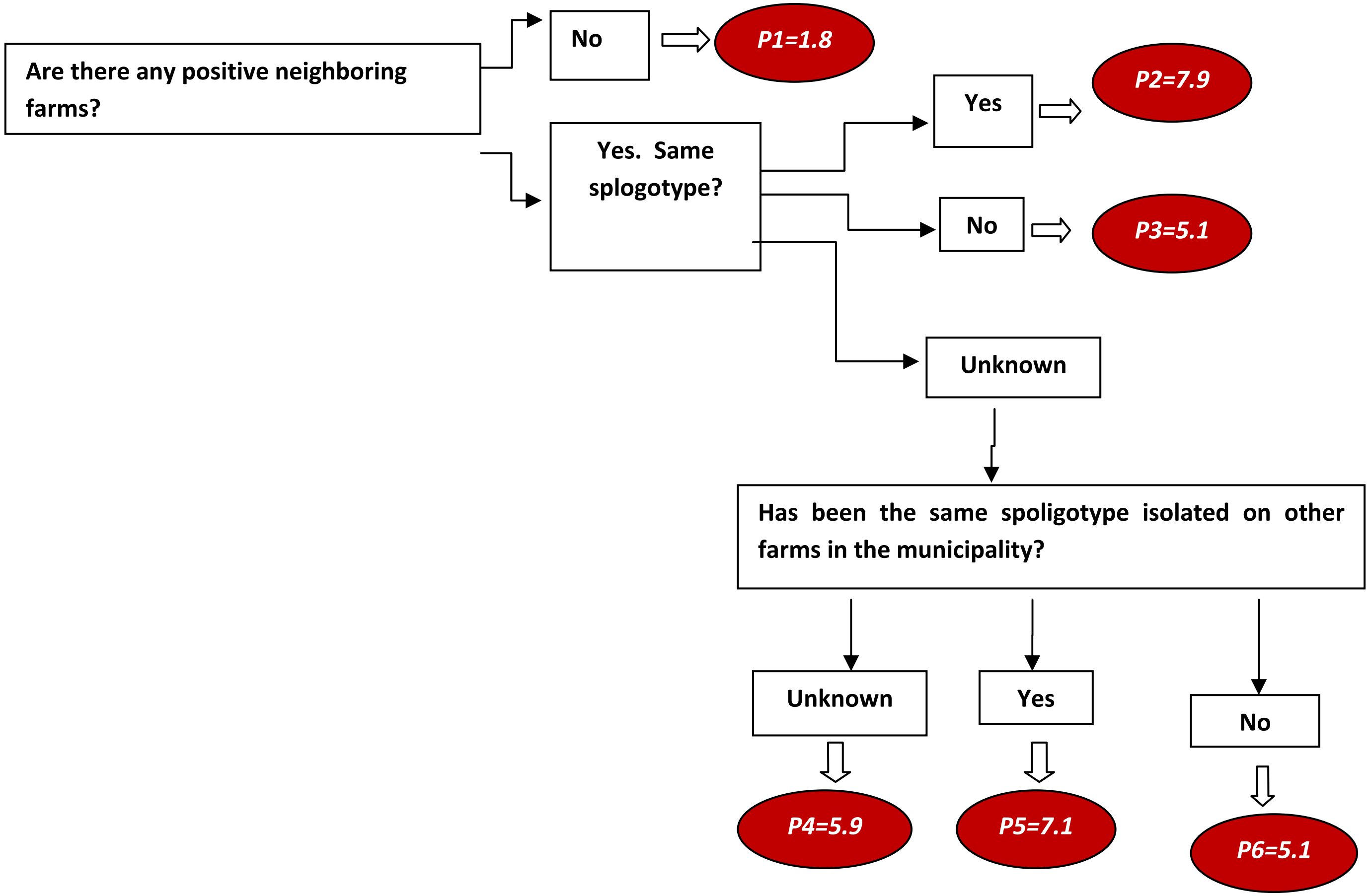

P11-Introduction animals

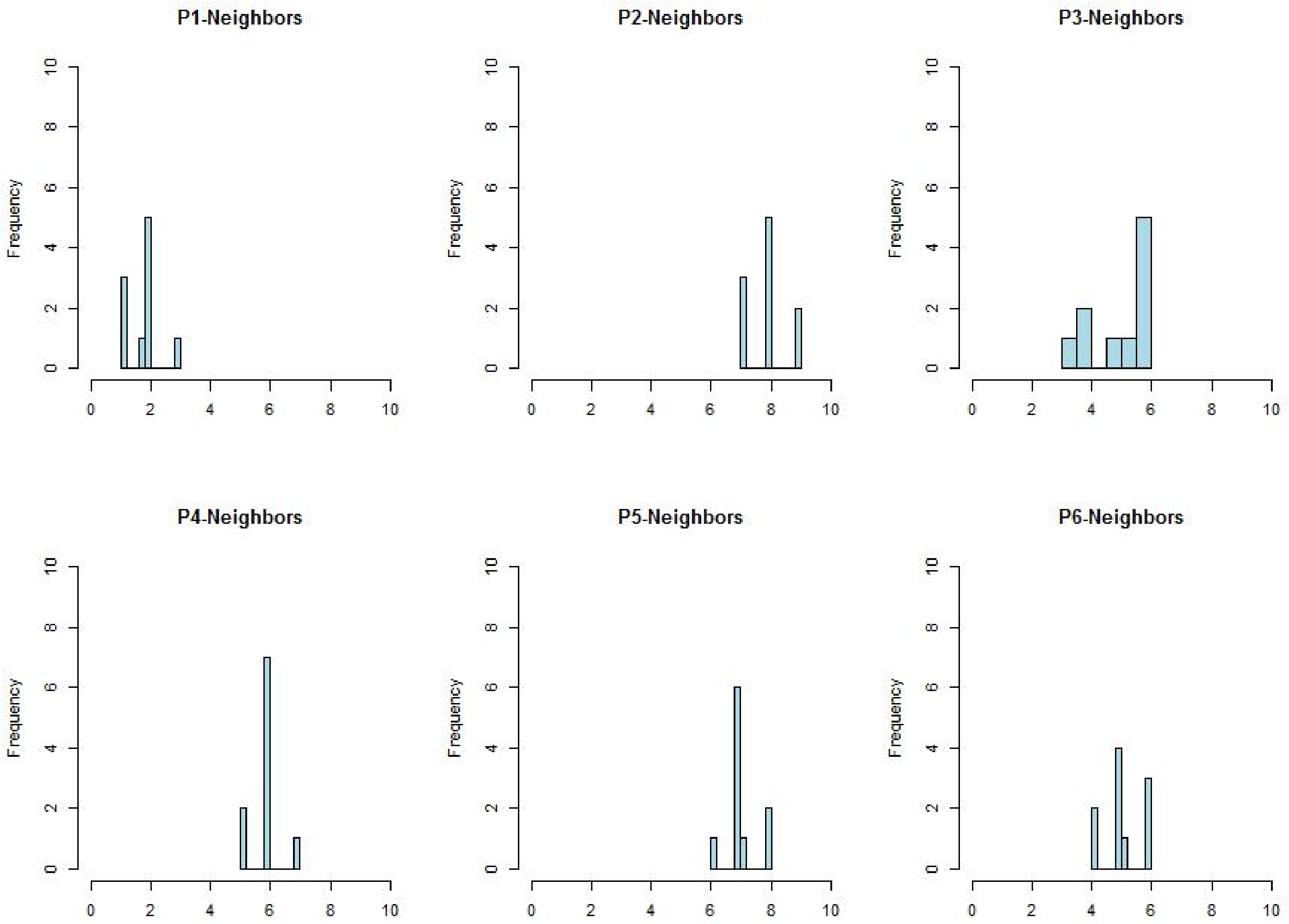
2.3. Communal Pastures or transhumance



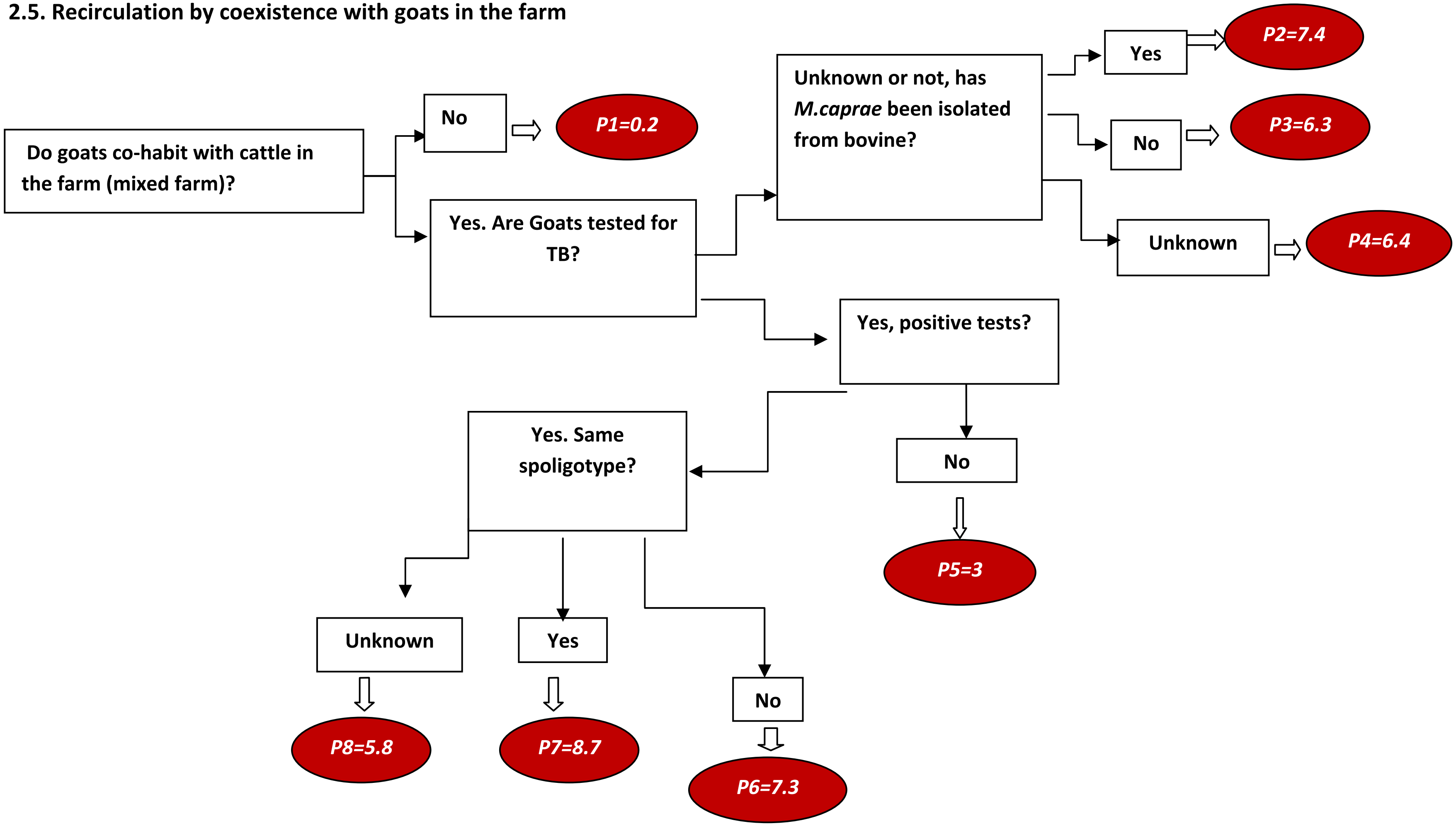

P1-Pastures
P2-Pastures
P3-Pastures
P4-Pastures
P5-Pastures
P6-Pastures
P7-Pastures
P8-Pastures
P9-Pastures
P10-Pastures
P11-Pastures
Frequency
0
2
4
6
8
10

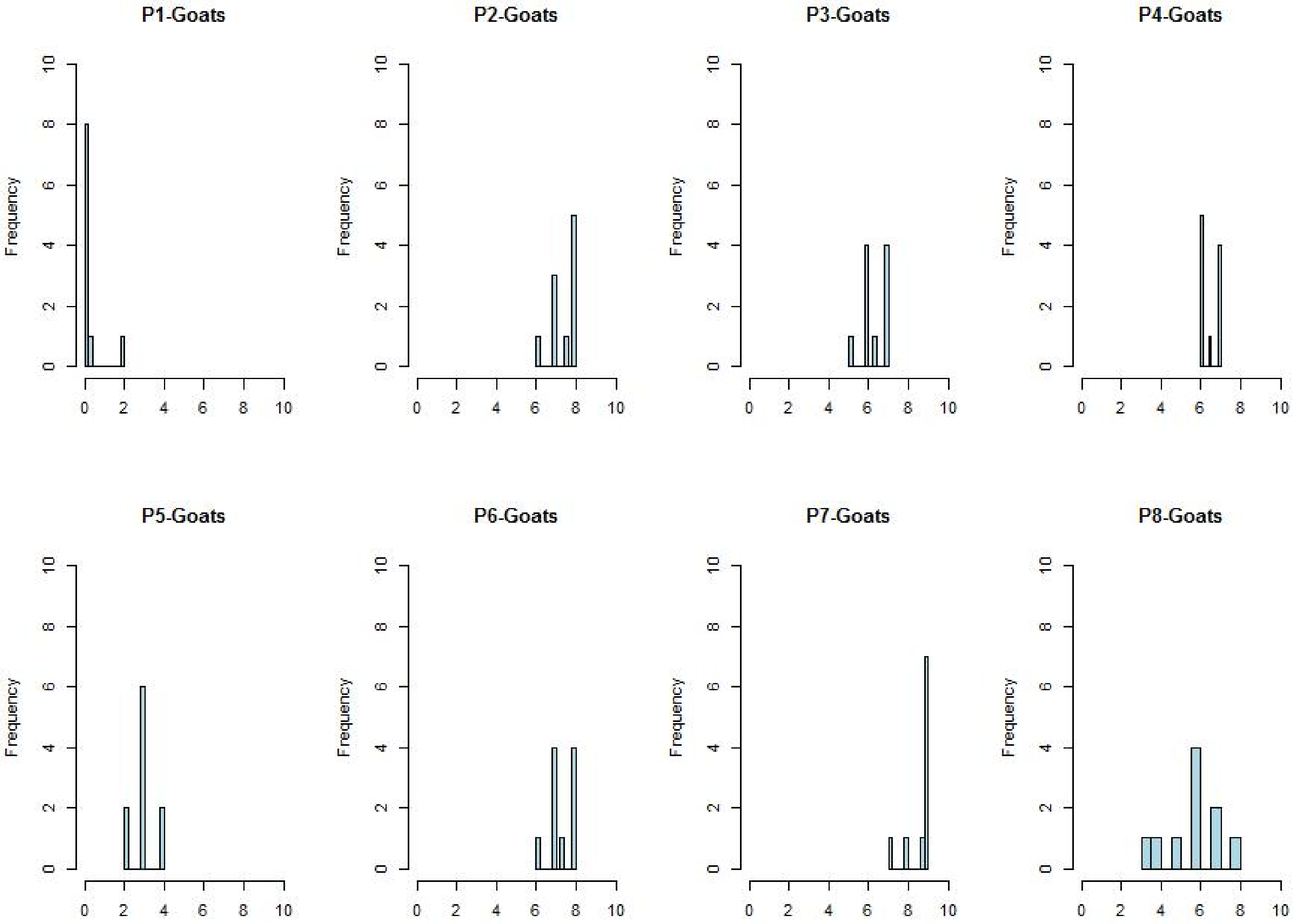
2.4. Contiguous spread from infected herds (Neighborhood spread)



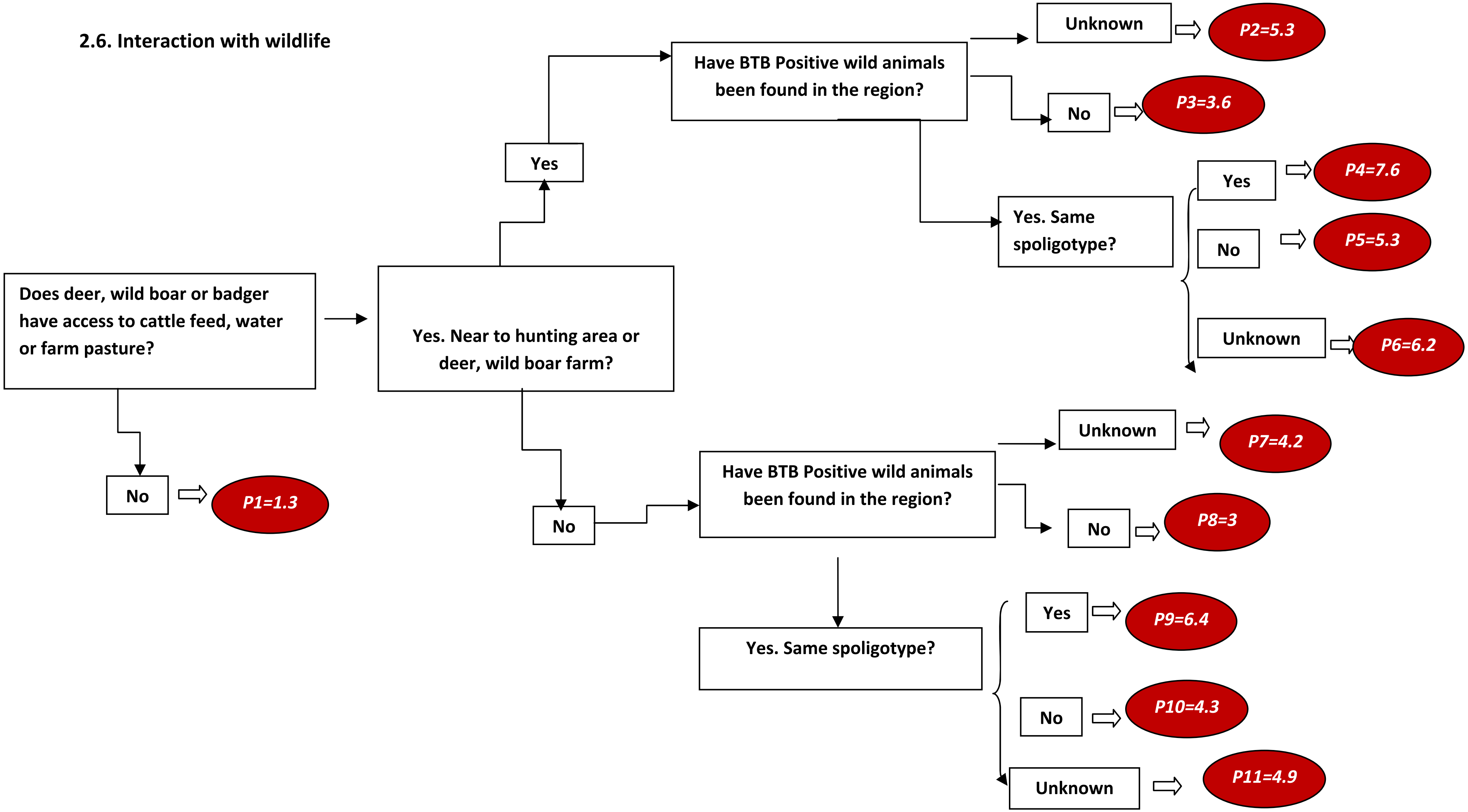

P1-Neighbors
P2-Neighbors
P3-Neighbors
P4-Neighbors
P5-Neighbors
P6-Neighbors
Frequency
0
2
4
6
8
10

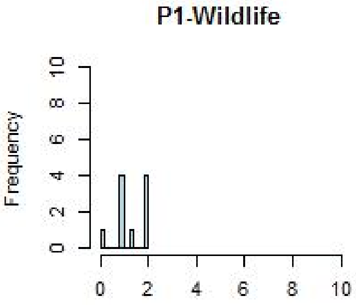
2.5. Recirculation by coexistence with goats in the farm

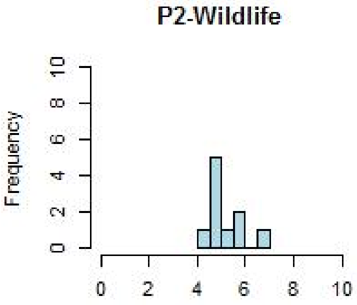


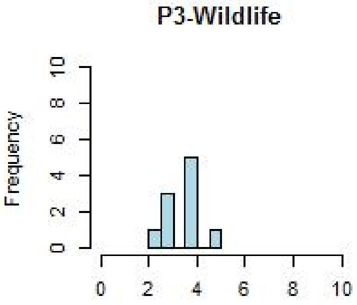

P1-Goats
P2-Goats
P3-Goats
P4-Goats
P5-Goats
P6-Goats
P7-Goats
P8-Goats
Frequency
0
2
4
6
8
10

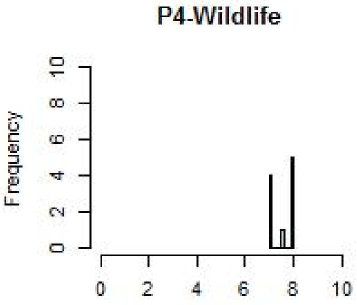
2.6. Interaction with wildlife

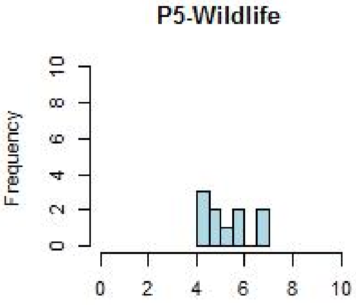


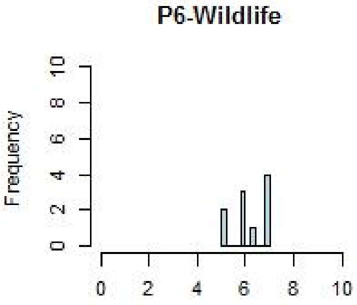

P1-Wildlife
Frequency
0 2 4 6 8 10
0 2 4 6 8 10

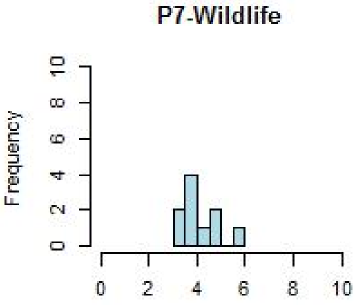

P2-Wildlife
Frequency
0 2 4 6 8 10
0 2 4 6 8 10

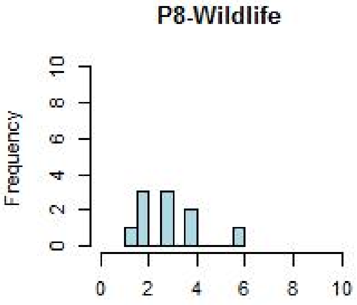

P3-Wildlife
Frequency
0 2 4 6 8 10
0 2 4 6 8 10

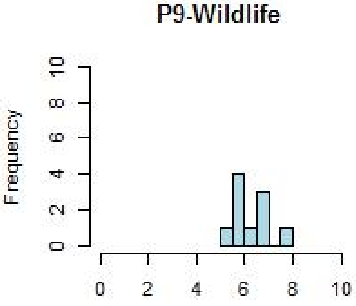

P4-Wildlife
Frequency
0 2 4 6 8 10
0 2 4 6 8 10

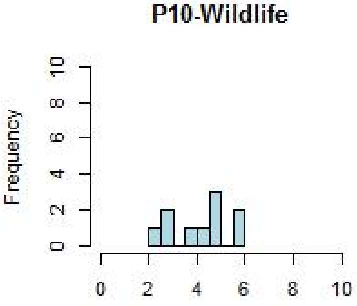

P5-Wildlife
Frequency
0 2 4 6 8 10
0 2 4 6 8 10

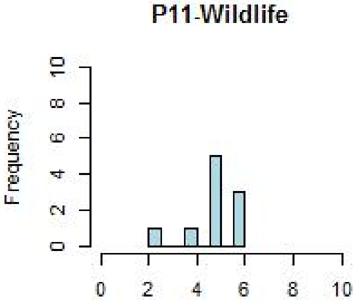

P6-Wildlife
Frequency
0 2 4 6 8 10
0 2 4 6 8 10


P7-Wildlife
Frequency
0 2 4 6 8 10
0 2 4 6 8 10

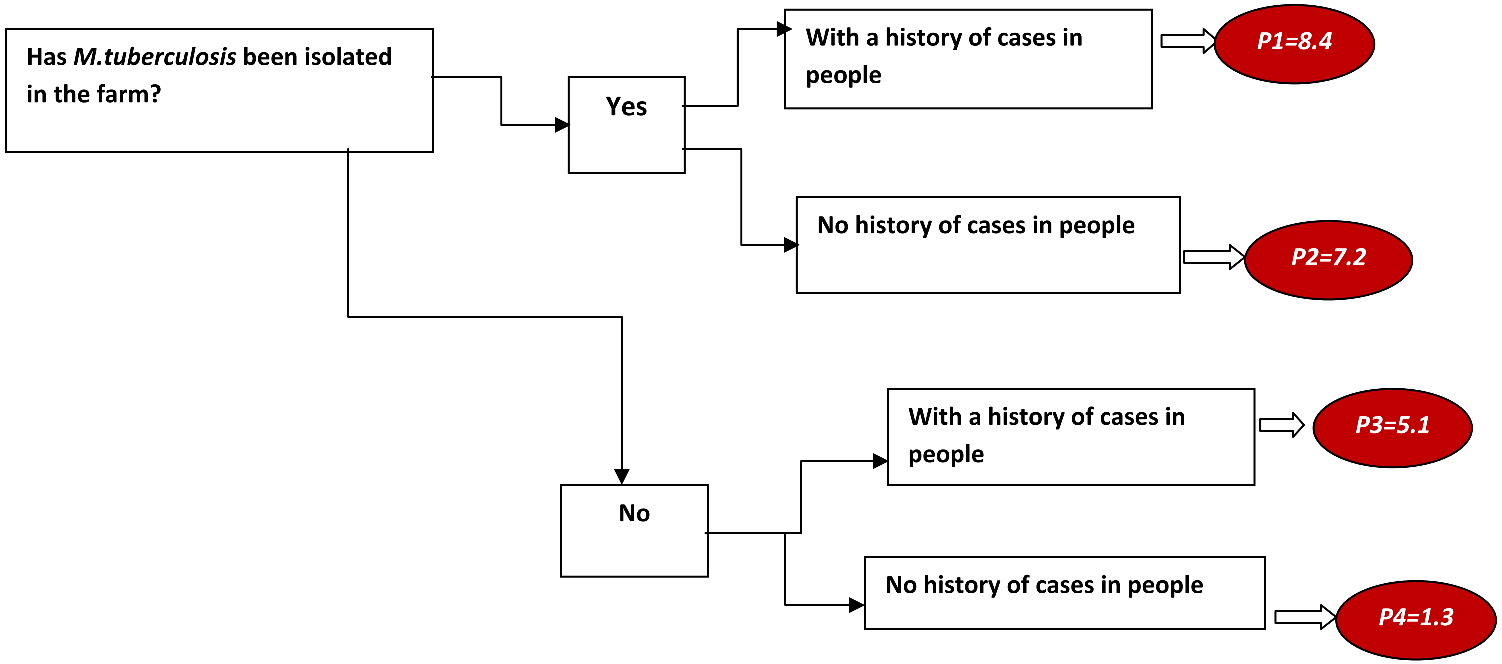

P8-Wildlife
Frequency
0 2 4 6 8 10
0 2 4 6 8 10

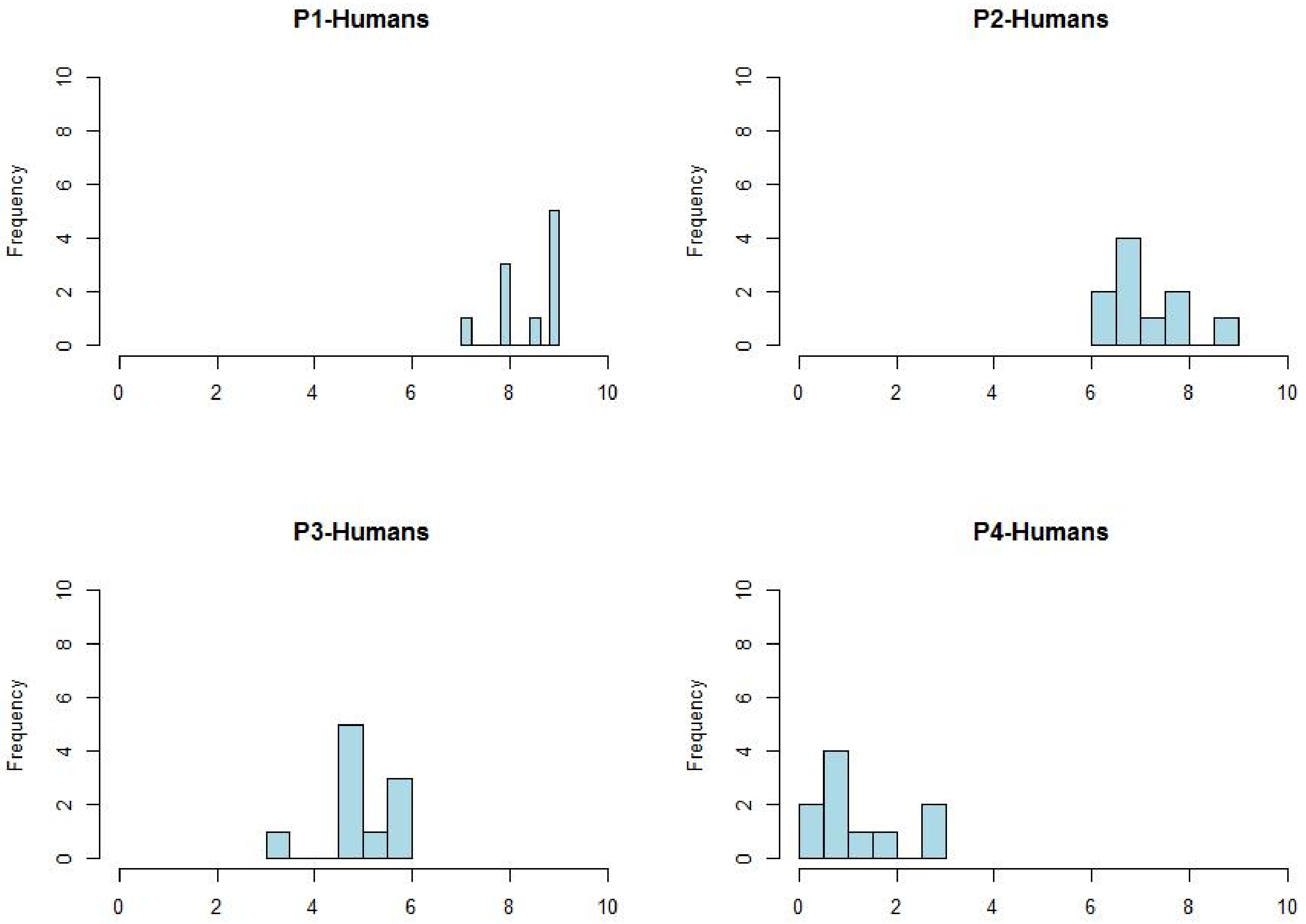

P9-Wildlife
Frequency
0 2 4 6 8 10
0 2 4 6 8 10


P10-Wildlife
Frequency
0 2 4 6 8 10
0 2 4 6 8 10


P11-Wildlife
Frequency
0 2 4 6 8 10
0 2 4 6 8 10

2.7. Contact with infected Human (Human antecedent of TB)




P1-Humans
P2-Humans
P3-Humans
P4-Humans
Frequency
0
2
4
6
8
10

Annex 3: epidemiological questionnaire case-control study

Nº ENCUESTA: **FECHA DE ENCUESTA______/______/______**

ESTUDIO DE LAS CAUSAS DE PERSISTENCIA TUBERCULOSIS BOVINA EN GRANJAS DE CARNE

DATOS GENERALES DE LA GRANJA

INFORMACION DE CONTACTO

ENCUESTADOR________________________

TELÉFONO_______________MÓVIL______________CORREO ELECTRÓNICO________________

LOCALIZACIÓN DE LA GRANJA

COD. REGA_________________________ NOMBRE DE LA GRANJA_________________________

PROVINCIA________________________ TITULAR DE LA EXPLOTACIÓN___________________

COMARCA/UVL____________________ TELÉFONO DE CONTACTO________________________

MUNICIPIO________________________

COORDENADAS GEOGRÁFICAS: X__________________________ Y________________________

CAMPAÑA DE SANEAMIENTO DE TUBERCULOSIS

- CALIFICACIÓN ACTUAL_____________
- FECHA DE OBTENCIÓN:_____/_____/________/
- ¿LA EMPRESA/ADS RESPONSABLE DEL SANEAMIENTO EN LOS ÚLTIMOS 10 AÑOS HA SIDO SIEMPRE LA MISMA? SI ☐ NO
- EN CASO DE QUE SI, ¿EL VETERINARIO QUE HACE EL SANEAMIENTO SUELE SER EL MISMO? SI ☐ NO ☐
- EN CASO DE QUE NO, NOMBRAR LAS DISTINTAS EMPRESAS/ADS RESPONSABLES DEL SANEAMIENTO EN LOS ÚLTIMOS 10 AÑOS

NOMBRE	REALIZA OTROS SERVICIOS VETERINARIOS (SI/NO)	PERÍODO	
		AÑO INICIO	AÑO FINAL

- ¿CÓMO SE SUELE REALIZAR LA LECTURA DE LAS PRUEBAS DE TB?
  CUTÍMETRO ☐ PALPACIÓN ☐ CUTÍMETRO Y PALPACIÓN ☐ OTROS:___________

CARACTERÍSTICAS DE LA GRANJA

- APTITUD: CARNE ☐ MIXTA ☐
- RAZA:___________________________
- CENSO DE ANIMALES

EDADES	HEMBRAS	MACHOS
Total de animales		
Recría		
De 2 a 5 años		
De 5 a 10 años		
De 10 a 15 años		
>15 años		

- ¿TIENE ALGUNA OTRA ESPECIE ANIMAL DE PRODUCCIÓN/COMPAÑÍA EN LA GRANJA? SI ☐ NO

	OVINO	CAPRINO	OTROS RUMIANTES	PORCINO	ÉQUIDOS
Recría y/o engorde					
Adultos					

- ¿LAS ESPECIES ANTERIORES TIENEN POSIBILIDAD DE CONTACTO DIRECTO CON EL BOVINO DE LA GRANJA?

	OVINO	CAPRINO	OTROS RUMIANTES	PORCINO	ÉQUIDOS
Si / No					

- TIPO DE INSTALACIONES
  NAVE CERRADA ☐ NAVE ABIERTA ☐ SIN NAVES (SÓLO PASTOREO)
- NÚMERO DE NÚCLEOS DE LA GRANJA _________________

MANEJO

- TIPO DE MANEJO
  INTENSIVO ☐ SEMI-INTENSIVO ☐ EXTENSIVO ☐
- ¿ES UNA GANADERÍA DE PRODUCCIÓN ECOLÓGICA? SI ☐ NO ☐
- EN EL TIEMPO QUE HA SIDO POSITIVA, EL ORIGEN DE LA REPOSICIÓN ERA:
  INTERNO ☐ EXTERNO ☐
- EN EL CASO DE ORIGEN EXTERNO, ¿RESULTÓ ALGUNA VACA POSITIVA?
  SI ☐ NO ☐
- ¿Y EL ORIGEN DE LOS MACHOS?: INTERNO ☐ EXTERNO ☐

- EN EL CASO DE ORIGEN EXTERNO, ¿RESULTÓ ALGÚN MACHO POSITIVO?
  SI ☐ NO ☐
- ¿HA HECHO REPOSICIÓN CON HIJAS DE VACAS DE SU PROPIA GRANJA QUE HAN RESULTADO SER POSITIVAS? SI ☐ NO ☐
- ¿RESULTÓ ALGUNA DE ELLAS POSITIVA A LAS PRUEBAS DE DIAGNÓSTICO?
  SI ☐ NO ☐

PASTOREO

- MESES DE PASTOREO AL AÑO: ________________
- ÁREA TOTAL DE PASTOREO (Ha):____________
- ¿REALIZA TRANHUMANCIA? SI ☐ NO
- EN CASO AFIRMATIVO:
  DISTANCIA RECORRIDA(Km):____________________
  LUGAR DE DESTINO:____________________________
- ¿PODRÍAN TENER CONTACTO CON ANIMALES DE OTROS REBAÑOS (GRANJA VECINA)? SI ☐ NO
  ESPECIES: BOVINO ☐ CAPRINO ☐ OVINO ☐ EQUINO ☐
- ¿ALGUNA DE ÉSTAS GRANJAS HA SIDO POSITIVA EN LOS ÚLTIMOS AÑOS?
  SI ☐ NO
- EN EL CASO DE QUE ALGUNA DE ÉSTAS HAYA SIDO POSITIVA EN LOS ÚLTIMOS 10 AÑOS, ¿HA RECIBIDO DRENAJE DE RESIDUOS DE DICHA GRANJA?
  SI ☐ NO ☐
- ¿COMPARTE ÁREAS DE PASTOREO CON ANIMALES DE OTROS REBAÑOS SUCEPTIBLES? SI ☐ NO ☐

ESPECIES	NÚMERO DE ANIMALES

- ¿UTILIZA SUPLEMENTOS DE VITAMINAS Y SALES MINERALES DURANTE EL PASTOREO? SI ☐ NO ☐
- ¿UTILIZA ALIMENTACION SUPLEMENTARIA (ENSILADO, ALFALFA,…) DURANTE EL PASTOREO? SI ☐ NO ☐
  PERIODO: DE_________ HASTA:__________

BIOSEGURIDAD

- ¿LA GRANJA ESTÁ VALLADA?
  COMPLETO ☐ PARCIAL ☐ SIN VALLA ☐
- TIPO DE VALLA: ☐ PASTOR ELÉCTRICO ☐ ENREJADO OTROS:______________
- ¿COMPARTE MAQUINARIA (ESTIÉRCOL, ETC.) CON OTRAS GRANJAS?
  SI ☐ NO ☐
  ¿EN CASO DE QUE SI, ¿ALGUNA DE ÉSTAS GRANJAS ES POSITIVA? SI ☐ NO ☐
- ¿UTILIZA ESTIÉRCOL COMO ABONO DE LA PROPIA GRANJA? SI ☐ NO ☐
- ¿DE QUE ESPECIE ES EL ESTIÉRCOL?:___________
- EN CASO DE QUE SEA DE OTRA GRANJA (BOVINO O CAPRINO), ¿HA SIDO ÉSTA POSITIVA EN LOS ÚLTIMOS 10 AÑOS? SI ☐ NO ☐

- UTILIZA FORRAJE DE LA PROPIA GRANJA ☐ DE OTRA GRANJA
- ¿SE REALIZA O REALIZABA AISLAMIENTO INMEDIATO Y EFECTIVO DE LOS POSITIVOS Y LOS DUDOSOS HASTA SU SACRIFICIO? SI ☐ NO ☐
- ¿TIEMPO DE PERMANENCIA DE LOS POSITIVOS DESDE LA REALIZACIÓN DE LA PRUEBA DE TUBERCULINA HASTA EL SACRIFICIO (DÍAS)?:

  Media: _________ Mínimo: _________ Máximo:_______

- ¿SE HAN PODIDO HACER SIEMPRE LAS PRUEBAS DE SANEAMIENTO A TODOS LOS BOVINOS DE LA GRANJA?
  SI ☐ A VECES NO ☐ A MENUDO NO ☐ NO SABE ☐

  EN CASO DE QUE NO, NÚMERO APROXIMADO POR SANEAMIENTO:_________
  MOTIVO:_______________________________

FACTORES AMBIENTALES Y CONTACTO ANIMALES SILVESTRES

- ÁREA BOSQUE DENTRO DE LA GRANJA SI ☐ NO ☐
- DISTANCIA DEL ÁREA DE BOSQUE A LOS BOVINOS:_______
- LINDA CON FINCA CINEGÉTICA SI ☐ NO ☐
  CENSO MEDIO:________ ESPECIES:__________________________ DISTANCIA:________
- ¿SE HAN DETECTADO RESTOS DE VÍSCERAS PROCEDENTES DE ACTIVIDAD CINEGÉTICA EN LA GRANJA? SI ☐ NO ☐
- ¿SE HA DETECTADO LA PRESENCIA DE ANIMALES SILVESTRES EN LA GRANJA?
  NO (0) ☐ EXCEPCIONALMENTE (1) ☐ FRECUENTEMENTE (2) ☐

ESPECIES	FRECUENCIA (0,1,2)	SE HAN INCREMENTADO EN LOS ÚLTIMOS 10 AÑOS
JABALÍES		
CIERVOS		
GAMOS		
CORZO		
ZORROS		
TEJONES		
REBECOS		

ASPECTOS SANITARIOS

DURANTE LOS AÑOS QUE HA SIDO POSITIVA:

- ¿HA TENIDO PROBLEMAS POR FASCIOLA?
  SI ☐ NO ☐
- ¿SE HA DIAGNOSTICADO VIRUS DE LA DIARREA VÍRICA BOVINA (BVD)?
  SI ☐ NO ☐
- EN EL CASO DE QUE TENGA CAPRINO, ¿SE REALIZAN PRUEBAS DE TB?
  SI ☐ NO ☐
- EN CASO DE QUE SI, ¿HAN DADO ALGUNA VEZ RESULTADO POSITIVO?
  SI ☐ NO ☐

ANTECEDENTES DE TBB DEL PERSONAL DE CONTACTO

- ¿HA HABIDO ANTECEDENTES DE TUBERCULOSIS CONFIRMADA ENTRE LAS PERSONAS QUE FRECUENTAN LA EXPLOTACIÓN?

  SI ☐ NO ☐

  ¿CUÁNDO?: _____________

- OBSERVACIONES:

____
____
____
____