An Integrative Approach to the Management of Disease in Mixed Wildlife-Livestock Systems

Catherine Elizabeth Cowie

PhD

University of York

Environment Department

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Abstract

Livestock diseases have a significant impact on human health and economic activity. Where multi-host pathogens are present in mixed wildlife-livestock systems, wildlife reservoirs of disease may prevent eradication in livestock. Management of such diseases requires an understanding of the biological processes governing their transmission, and also of the socio-economic factors influencing stakeholder's management decisions. This thesis aims to identify risk factors for the presence of single and multiple diseases in livestock, quantify direct and indirect interactions between multiple wildlife and livestock species and evaluate the effectiveness and practicality of the resulting possible management interventions.

The study mainly uses the example of tuberculosis in south-central Spain, a region which suffers high prevalence in both cattle and wildlife. Risk factors for disease were evaluated with questionnaires and participatory mapping. Potential opportunities for disease transmission through direct and indirect interactions between different host species were measured using proximity logging and GPS collars attached to multiple wildlife and livestock species, and base stations placed at resource and control points on a cattle farm. The resultant possible disease management interventions were ranked by stakeholders using best-worst scaling.

Risk factors for tuberculosis in cattle were the presence of wildlife, the number of streams per hectare on a farm and the provision of cattle food on the ground. Intra- and inter-herd contacts between cattle were risk factors for multiple livestock diseases. Direct interactions between species happened so rarely that they are not likely to account for all disease transmission. Indirect interactions, particularly between cattle, red deer and pigs, warrant further investigation. These results were used to identify possible management interventions. An expert panel ranked a ban on supplementary feeding of game species as the most effective intervention. Different stakeholder groups varied in which interventions they considered practical. Management of livestock diseases in mixed systems requires the targeted prevention of indirect interactions between livestock and wildlife, using interventions that are effective, practical and supported by relevant stakeholders.

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Author's Declaration

I declare that, except where explicit reference is made to the contributions of others, this thesis is the result of my own work. The work was conducted and the text was produced by me, and where co-authors are listed it is due to their support of the study, shown in preparation for publication in peer reviewed journals. This work is being submitted for the degree of Doctor of Philosophy at the University of York and has not been submitted for any other degree or examination.

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Chapter 1: An introduction to disease management in mixed wildlife-livestock systems

Livestock disease

Impacts of livestock disease on human health

Livestock rearing has many benefits to the human species. It occupies approximately 30% of the planet's ice free terrestrial surface, and represents a global asset of at least $1.4trillion (Thornton 2010). It provides energy dense food sources, high in important protein and micronutrients and is especially valuable for young, pregnant or immunosuppressed people (Murphy & Allen 2003). In developing countries these health benefits often far outweigh the risks (Perry & Grace 2009) and farming continues despite the presence of zoonotic livestock diseases. These can have a significant impact on human and animal health and welfare. Many diseases are shared between livestock and humans, and over 77% of livestock diseases can infect multiple hosts (Cleaveland, Laurenson, & Taylor 2001). These diseases can become widespread in human populations once introduced, for example pandemics such as the recent outbreaks of avian and swine influenza virus (Capua & Munoz 2013). They can also have a major impact in places where they spillover from domestic species, e.g. rabies or sleeping sickness (Perry & Grace 2009). Furthermore, the associated losses of productivity, livestock mortality, reduced food security, trade restrictions, and impaired economic development have a considerable impact on human wellbeing in both the industrialized and developing world (Schley *et al.* 2012; Kleinlützum, Weaver, & Schley 2013).

Economic impacts of livestock diseases

Aside from the direct effects on human health, livestock diseases also have significant direct and indirect socio-economic impacts (Gramig & Horan 2011). Direct costs from the reduction in productivity, loss of consumer demand and control efforts can be significant, for example the cost of epidemics of Nipah virus in pigs in

Malaysia (£71 million), foot-and-mouth disease in the United Kingdom (£10 billion), and contagious bovine pleuropneumonia (CBP) in Botswana (£186 million) (Chua 2003; Ward *et al*. 2007). The impact of even minor livestock diseases has increased over recent decades, as higher production costs and variable output prices have reduced profit margins for farmers (Thornton 2010; van der Voort *et al.* 2013).

Transboundary livestock diseases have significant economic, trade and/or food security importance for a considerable number of countries. These diseases tend to easily spread to other countries, can reach epidemic proportions and control requires cooperation between several nations (Otte, Nugent, & McLeod 2004). These diseases have the biggest economic impacts. For example, by 2004 CBP had spread to 27 countries in Africa at an estimated annual cost of £1.2 billion per year (Otte, Nugent, & McLeod 2004), and Knight-Jones & Rushton (2013) estimate an annual global cost of foot-and-mouth disease of over £1 billion per year. Indirectly, livestock diseases also have intangible costs that are difficult to quantify. These may include loss of farmer reputation, the effects on tourism and other industries, the disutility of ill health on individuals and their friends and families, reduced land value or the impact of changing to another livelihood (Thompson *et al.* 2002; Brook & McLachlan 2006; Perry & Grace 2009).

Livestock disease prevention and control

Prevention or control of livestock diseases is heavily dependent on how each pathogen is transmitted. Over the last 25 years, livestock disease management has shifted in focus from treatment of individuals to herd-level prevention (van der Voort *et al.* 2013). Depending on the disease this may involve putting animals in isolation (e.g. for animals infected with parasitic nematodes) until the infection has passed with time and/or treatment, or slaughter of animals that show clinical signs or test positively for a disease that cannot be treated (for biological or economic reasons). Many countries across the globe, particularly in the developed world, have implemented livestock disease eradication schemes based on the test-and-slaughter method (e.g. Alvarez *et al.* 2012). This involves testing animals for the disease in question and removing infected individuals from the population to prevent further spread of the disease. In some cases this method has been successful in reducing disease levels (e.g. foot-and-mouth disease in the UK (Davies, 2002)), especially alongside other measures such as biosecurity. However, Moda *et al.* (1996) identified six reasons why test-and-slaughter schemes are not always successful, and these are summarised below:

1. There is the risk of reintroduction of the disease from unmanaged neighbouring areas.
2. The presence of various domestic and wild animal species can act as alternate reservoirs of the disease.
3. The transmission of disease from humans to livestock.
4. The presence of immuno-compromised humans and animals can help the disease to spread.
5. Veterinary professionals may disbelieve that the disease can be contracted by humans and therefore ignore the risks.
6. Frequent lack of communication between veterinary and medical clinicians results in failure to identify the source of infection.

This highlights that disease control is not purely an epidemiological problem, but is influenced by the presence of multiple hosts and the social and economic factors influencing stakeholder's decision making about disease management.

Preventative control presents one alternative or parallel approach to test and slaughter schemes. This aims to protect susceptible animals from becoming infected, and this can also be achieved by vaccination. Livestock vaccination has achieved the successful reduction of many diseases such as the zoonotic *Escherichia coli* infection (Varela, Dick, & Wilson 2013) and rinderpest (Roeder, Mariner & Kock 2013) in cattle (*Bos primigenius*), or porcine circovirus in pigs (*Sus scrofa domesticus*) (Shen, Halbur, & Opriessnig 2012). Furthermore, livestock vaccination has also been shown to benefit human health, in the case of brucellosis (Roth *et al.* 2003). However, vaccination can be expensive so careful cost-benefit analyses need to be undertaken. Furthermore, in some cases surveillance can only be achieved economically by using tests that are presently unable to differentiate between infected and vaccinated individuals (Waters *et al.* 2012; Pfeiffer 2013), as seen with bovine tuberculosis.

Diseases that can be transmitted indirectly may also require control of the disease vector or fomite. For example, this may include control of the midges that transmit the Bluetongue virus between cattle and wildlife (Maclachlan & Mayo 2013). Preventing livestock access to infected areas will reduce the transmission of pathogens that can survive in the environment. Biosecurity on farms is important for this reason, particularly as many livestock diseases are primarily transmitted within herds (e.g. Menzies & Neill 2005). Surveillance of livestock populations is a pre-requisite for good prevention and management. Surveillance can be continuous (Kuiken *et al.* 2011; Boadella *et al.* 2011a), or reactive in areas where there is a risk of infection (e.g. Alexandrov *et al*. 2013), depending on the type of disease, the current disease prevalence and the resources available for surveillance.

Livestock disease prevention and control is likely to become yet more important in the future. Environmental change will pose a threat to livestock disease management in the future, and some diseases are already being shown to increase their range with increasing temperatures (e.g. Daszak *et al*. 2013). Furthermore, agricultural intensification increases the risk of the emergence of new zoonotic livestock diseases (Jones, Jones, & Cross 2013). It is imperative that surveillance, prevention and control of livestock diseases maintain disease prevalence at levels that ensure food and economic security.

Disease reservoirs

Haydon *et al*. (2002) define a disease reservoir as “one or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the defined target population". A wildlife population may have high disease prevalence without being a reservoir – it is transmission to the target species that warrants this definition (Corner 2006). This persistence in multiple hosts is clearly a selective advantage for a pathogen, making it difficult to control or eradicate. The reservoir could be another infected species or individual in which persistence is obtained by long term chronic infection, or through free living stages by showing prolonged survival in the environment (e.g. Young *et al*. 2005; Pickup *et al*. 2006; Fine *et al*. 2011a). There are many examples of diseases that are maintained in a wildlife reservoir that re-infects humans and our pet or livestock populations. Humans are most commonly exposed to wildlife diseases through the infection of domestic species (Böhm *et al.* 2007). For example, rabies is maintained by wild populations including many bat species and the European fox (*Vulpes vulpes*), and can be transmitted to domestic dogs, which may infect humans (Chomel 1993). Diseases that affect livestock also have wildlife reservoirs, such as chronic wasting disease which passes from white-tailed deer (*Odocoileus virginianus*) to cattle (Wasserberg *et al.* 2009). Badgers (*Meles meles*) have been identified as a reservoir of tuberculosis (TB) for cattle in the UK. In Europe, the European rabbit (*Oryctolagus cuniculus*) is thought to be a reservoir for Johne's disease (paratuberculosis) in cattle (Daniels *et al.* 2003; Judge *et al.* 2005). Already difficult to manage, the presence of wildlife reservoirs of transboundary diseases can further complicate disease eradication (Siembieda *et al.* 2011).

Compared to human and livestock hosts, relatively less attention is paid to humans or pets forming a reservoir of disease that infects wildlife hosts, yet this has been shown to be the case with the endangered Iberian lynx (*Lynx pardinus*) being infected with feline leukaemia virus or parvovirus, likely transmitted by the domestic pet population (Millán *et al.* 2009). Whilst much work has been conducted identifying single species disease reservoirs, the reality is that many pathogens are able to infect multiple hosts, with one or more of these species acting as reservoirs for multiple other species in the system. It is important to consider the concept of a disease reservoir community, with infection maintained in all species even though certain species could not host the disease permanently without re-infection from others (Haydon *et al*. 2002; Anderson *et al*. 2013a).

Control of disease reservoirs

It is important to maintain awareness of the distinction between ‘wildlife control’ and ‘pathogen or disease control’ (Hone 2007). If control of a pathogen in a wildlife disease reservoir is the true objective, three main routes of control are available: population size and/or density reduction, fertility control, vaccination against the disease or implementing biosecurity measures to prevent transmission between the reservoir and host species. Reductions in population size have often been implemented by culling. This has been shown to be effective in some certain circumstances, for example culling of white-tailed deer in the USA achieved a significant reduction in the livestock prevalences of chronic wasting disease (Mateus-Pinilla *et al.* 2013) and TB (Carstensen, Brien, & Schmitt 2011). In the Randomised Badger Culling Trial (RBCT) in south-west England, proactive culling that achieved a badger population reduction of 70% or more resulted in a significant reduction in bovine TB prevalence (Donnelly *et al.* 2007a).

There are however negative aspects to population reduction for the control of wildlife reservoirs. Boadella *et al*. (2012) showed that culling can have different effects for different diseases, with reduced wild boar (*Sus scrofa*) densities resulting in reduced livestock TB prevalence, but Aujeszky’s disease prevalence remaining unaffected in an area where both diseases were endemic. Furthermore, culling may have unexpected effects such as perturbation of the wildlife species being targeted. Changes in their behaviour may lead to increased disease transmission to cattle, as was revealed in the RBCT in an area of high bovine TB prevalence (Macdonald, Riordan, & Mathews 2006; Carter *et al.* 2007; Jenkins, Woodroffe, & Donnelly 2010; Riordan *et al.* 2011). Culling may not be viable in areas where the wildlife reservoir species are numerous, of conservation importance and/or have high economic value, such as buffalo (*Syncerus caffer*) in southern Africa (Renwick, White, & Bengis 2007). Culling may also have indirect effects on species of conservation concern. For example, paratuberculosis has been identified European rabbits on the Iberian peninsula, and these could be a reservoir for the disease in cattle (Maio *et al.* 2011). However, rabbits are an integral part of the food chain here, and are particularly important for the survival of the critically endangered Iberian lynx (Gortázar *et al.* 2010).

Conflict over management objectives such as the need to reduce population densities of lucrative game species may also prevent population reductions (Cowie *et al.* 2014, chapter 2). The support of rural inhabitants is often vital for their expertise and access to their land (e.g. Donnelly *et al*. 2007b). Wild animals are often highly valued by the general public. Culling is an emotive topic and social or political opposition may also prevent successful disease reduction in this way (Artois *et al.* 2001). Animal welfare concerns are also an important factor and should always be considered at all stages of a culling based management strategy (e.g. Woodroffe *et al*. 2005). Finally, reducing natural populations can be very expensive, and cost-benefit analyses need to be conducted and carefully considered (Bolzoni & De Leo 2007).

Most alternatives to population reduction are still in experimental stages of development (Artois *et al.* 2011). One of these is the use of non-surgical fertility control of overabundant disease reservoir species. Interest in this method is steadily increasing in order to address feral cat and dog populations that may cause problems in the conservation of wild carnivores (Massei & Miller 2013) or to reduce overabundant populations of wild horses or urban deer (Kirkpatrick, Lyda, & Frank 2011). If fertility control agents could be administered in oral baits, welfare and perturbation issues would be addressed and population size would gradually decline (Swinton *et al.* 1997). The GnRH immunocontraceptive agent “GonaCon” has been developed for use in wildlife and appears to be successful at reducing fertility in most mammals (Massei *et al.* 2008). Models have indicated that a combination of disease vaccination and fertility control may achieve equivalent results to culling in the control of wildlife reservoirs of disease (Smith & Cheeseman 2002), but would avoid many of its disadvantages. However, as wildlife are free-ranging, the challenge of achieving bait consumption by only the target species remains problematic. Some tools have been developed to achieve this, such as the Boar-Operated-System feeder that only wild boar can open (Campbell, Long, & Massei 2011), but further research is required to ensure the safety and specificity of fertility control.

Vaccination of the wildlife reservoir is another option. This has been shown to be the most effective method for the control and elimination of rabies in foxes in Europe. An estimated 17 million oral vaccination baits have been distributed across the continent, achieving dramatic reductions or the elimination of the disease (Cliquet *et al*. 2013; Rabies Bulletin for Europe 2012). Vaccination may have a role to play in reducing wildlife disease reservoirs, but will need to be delivered by oral bait in order to achieve sufficient uptake (Anderson *et al.* 2013b). As with fertility control, achieving specificity to the target population will be a significant challenge. Such baits may not be licensed for widespread use quickly, and cost-benefit analysis will also be a factor in the implementation of this method.

For transmission from a wildlife disease reservoir to occur, wildlife must have direct or indirect interactions with livestock. Biosecurity has long been recommended for the reduction of wildlife:livestock disease (Horan *et al.* 2007), though few studies have put forward practical suggestions (Ward, Judge, & Delahay 2010). To control badger to cattle disease transmission, suggestions include altering cattle grazing regimes, habitat management, badger latrine management, and excluding badgers from cattle housing and feed stores (Tolhurst *et al.* 2009; Ward, Judge, & Delahay 2010; Judge *et al.* 2011). Indeed, relatively simple measures such as using metal panels, electric fencing and metal food bins to prevent badger access to cattle or stored food have been shown to be 100% effective (Judge *et al.* 2011). To control indirect interactions between cattle and wildlife in south central Spain, Barasona *et al*. (2013) developed “bump gates” that allowed trained cattle to push open gates to access water resources from which wildlife were excluded. Wildlife were given access to separate water supplies that were fenced to exclude cattle. This separation prevented indirect interaction at a key resource on the farm, and cattle TB levels were significantly reduced following the study (Barasona *et al.* 2013a).

<u>Management of disease in mixed systems: knowledge gaps</u>

Risk factors are variables that are associated with an increased risk of disease or infection. These factors are not necessarily causal. For example, in human being young does not cause chicken pox (caused by infection with varicella zoster virus), but young people have a higher risk of infection because they are less likely to have previously encountered the virus. Being young is therefore a risk factor for the chicken pox in humans. This concept is often used in human health epidemiology.

Knowing the risk factors for a disease can be used to focus disease management effort, without needing to know the detailed epidemiological mechanisms involved. They may be specific to particular systems and so should be investigated on local or regional scales. Novel techniques such as participatory mapping can be used to generate spatial data on risk factors to complement information on farm management practices, environmental conditions and the presence of wildlife hosts. Furthermore, wildlife diseases have previously been investigated individually, but looking for shared risk factors for multiple diseases might improve the efficiency of livestock disease management.

In epidemiologically complex multi-species disease communities, disease eradication may not be possible without more detailed information about the mechanisms of disease transmission in the region. One key knowledge gap about disease transmission is the quantification of direct and indirect interactions within and between multiple species in a disease community. Direct interactions occur where there is physical contact between two individuals, of the same or different species. Indirect interactions are when two individuals occupy the same space or use the same resource, at different times. The time difference between their use of the location can be related to the critical time window for the environmental survival of an infective dose of the pathogen in question. Knowledge of the spatial and temporal nature of these interactions may reveal opportunities for targeted, and therefore more efficient, disease management.

Finally, the social and economic aspects of livestock disease management are increasingly recognised as critical factors in disease eradication (Reed 2008; Munyeme *et al.* 2010; de Garine-Wichatitsky *et al.* 2013a). Support from a range of stakeholders can be essential, and disease management interventions need to be appropriate for the socio-cultural environment in which they are applied. Socio-economic research methods are increasingly being applied to biological problems alongside more traditional biological research (Pfeiffer 2013). Consultation with stakeholders at all scales of disease management is important, and presently it is often the local stakeholders who execute disease management directly who are least consulted. Incorporating local knowledge into an interdisciplinary approach to disease management will result in more effective interventions that are readily implemented and more likely to achieve disease reduction or eradication.

Disease transmission in livestock and reservoir populations

Contact rates

Models of the dynamics of many diseases in many species have been developed, and are generally based on the principles of a simple 'SIR' model developed by Anderson & May (1979). Individuals are considered to be Susceptible, Infected or Recovered and Immune, and individuals join the system through births and leave through natural or disease induced mortality. Arguably the most important parameter in any such equation is the disease transmission coefficient, which describes the rate at which susceptible individuals become infected. Disease transmission coefficients are difficult parameters to quantify, and are usually estimated in models that consider various host population and pathogen characteristics (Caley & Ramsey 2001). This coefficient governs the rate of disease spread within a population and therefore may influence the rate at with other species are also infected. With directly transmitted diseases the coefficient is a function of the rate of contact between individuals. Accurate knowledge of contact rates would therefore vastly improve models of disease transmission.

Social behaviour plays a highly important role in the transmission of a pathogen between hosts as it governs contact rates between groups and individuals, in turn affecting the rate of disease spread (Woodroffe *et al.* 2009). These behaviours may include mating, grooming or territory defence (Böhm *et al.* 2007) and therefore may vary considerably between individuals and species, depending on social organisation structures (Bansal, Grenfell, & Meyers 2007). In the past, contact rates had been assumed to increase with population density, for example in a study on foxes where contact rates were based on encounter rates at a known population density (White & Harris 1994), and extrapolated to other densities by assuming a linear relationship between home range size and density (White, Harris, & Smith 1995). The same assumption has often been made in epidemiological models of wildlife contact rates (Smith *et al.* 2009). However, Ji *et al*. (2005) found a non-linear relationship between contact rates and population density in brush-tail possums (*Trichosurus* *vulpecula*), where the highest contact rates were seen in medium density populations. This indicates that a linear relationship should not be assumed, which has significant implications for disease control strategies. Reduction of the densities of wildlife disease hosts may not result in reduced disease prevalence in the target livestock species (Carter *et al.* 2007; Riordan *et al.* 2011).

Estimating contact rates

In the past, contact rates have been estimated for inclusion in wildlife disease models based on consensus expert opinion (e.g. Smith & Wilkinson 2002). Radio tracking has also been used to estimate contact rates (e.g. with red foxes by White & Harris (1994)) but this is labour intensive and provides only limited data. Direct observation has the advantage of accuracy (Totton *et al.* 2002; Hamede *et al.* 2009), but relies on observing individuals only at certain locations, and is not suitable for the study of elusive or cryptic species. GPS collars were used by Schauber *et al*. (2007) to investigate contact rates between white-tailed deer, but these have been found to lack the fine scale detail required to determine contact between individuals (Ganskopp & Johnson 2007), though certain overlap indices give better approximations than others (Robert, Garant, & Pelletier 2012).

Proximity data loggers address many of the limitations of other methods (Smith 2001; Prange *et al.* 2006; Walrath 2011; Drewe, Weber, & Carter 2012). All captured individuals are identified, and the collars remove the need for the presence of researchers in the field, thereby minimising disturbance (Tuyttens *et al.* 2002; Böhm *et al.* 2008), as well as reducing sampling effort. The loggers record the start time and duration of each contact between collared animals and allow for continuous recording over time (Ryder 2012). One notable limiting factor is that the species must be readily trappable, in order to fit the collars to a sufficient number of animals in the study population (Goodman 2007). Measuring the relationship between pairs of individuals (dyadic relationships) with proximity data loggers leads to a wealth of information about social structure (Whitehead 2008), and many of the limitations of incomplete data sets such as unrecorded contacts between study animals or observational error are avoided (James, Croft, & Krause 2009).

Proximity loggers have previously been used to investigate animal contact networks with reference to disease transmission. Ji *et al*. (2005) demonstrated a non-linear contact rate-population density relationship in brush-tailed possums, a reservoir for tuberculosis in New Zealand, and Marsh *et al*. (2011) revealed the complexities of wild rabbit social structures over time in Australia, relating to the spread of rabbit haemorrhagic disease. To date, only two studies have attempted to include multiple species - both studying cattle and badger contacts with reference to TB transmission in the UK. Böhm *et al*. (2009) identified individual heterogeneity in contact rates that may be important in disease management interventions. Drewe *et al*. (2013) found that indirect contacts on pasture are likely to be far more important in disease transmission than direct contacts.

Indirect interactions

Indirect interaction refers to shared space use, where individuals visit the same location at different times. Transmission through indirect interaction requires the pathogen to survive prolonged exposure to the environment. Awareness of the environmental survival time of a disease provides a critical time window (CTW) for disease transmission between the visits of two individuals to a single location. This would be valuable information in the development of disease management strategies, but in reality is often highly variable depending on the environmental conditions (Kukielka *et al.* 2013, appendix 1). Both GPS and proximity logger technology can reveal information about indirect interactions. GPS loggers can be used to estimate home range overlaps (e.g. Robert *et al*. 2012). As the data are also temporally explicit this could help to identify when and where indirect interactions occur. The use of proximity logging base stations (which use the same technology as the collars worn by the study animals) allows for the quantification of direct contacts between each individual and the location of the base station, which might be placed at a resource such as a feeding station (Drewe *et al.* 2013). Overlaps in the time spent by different individuals at this location indicate shared space use, and the time between their visits can be related to the CTW of environmental survival of the pathogen.

<u>Socio-economic aspects of livestock disease control</u>

It is increasingly recognised that stakeholders make disease management decisions based on a broad range of social, cultural and economic considerations as well as the scientific evidence. Wildlife disease reservoirs may be particularly difficult to manage as the species themselves may have cultural and/or economic value to local stakeholders and/or wider society. The eradication of fox rabies in Europe is an example of successful wildlife disease reservoir management. Freuling *et al.* (2013) stated that it "would not have been possible without the motivation, continuous effort, support and assistance of the responsible veterinary and public health authorities, hunters and other involved stakeholders". As foxes have little economic value and are not of conservation concern, this illustrates how vital consideration of socio-economics is in the management of wildlife disease reservoirs in more complex systems. Biologically focussed research is vital for the development of effective management strategies, but stakeholder tolerance for the interventions required will likely determine their success (White & Ward 2010). Land managers also have detailed local knowledge that can be highly important in the development of appropriate interventions. Furthermore, participation in the development of management strategies may reduce marginalisation of small stakeholder groups, enable the development of strategies well suited to local socio-cultural and environmental conditions, generate high quality data and feedback and establish common ground for learning and conflict resolution (Dougill *et al.* 2006; Reed 2008; Austin *et al.* 2010). As a result these methods are increasingly used in mitigation of wildlife-agriculture conflict mitigation (Selin, Schuett, & Carr 2000; Brook & McLachlan 2006).

Social research methods

Social research methods, many initially generated for market research purposes, can be usefully applied to efforts to control livestock diseases in complex systems. Questionnaires provide a simple and accurate way to obtain quantified comparable data from different individuals (White *et al.* 2005), and have previously been used to learn about management practices in relation to TB in the UK (Ward, Judge, &

Delahay 2010) and Spain (Cowie *et al.* 2014). Also, participatory mapping (pGIS) allows respondents to annotate maps, accessing knowledge that could not otherwise be conveyed. This is increasingly used as an effective means of encouraging engagement and knowledge exchange (Sandström *et al.* 2003; Irvine *et al.* 2009), and can complement data derived in more traditional ways (Austin *et al.* 2009).

In order to assess land managers' attitudes or preferences for a range of management options such as livestock disease management interventions, a range of techniques are available. Choice experiments, where respondents choose a preferred combination of possible management outcomes in a range of scenarios, have been used identify conflicts and opportunities for collaboration in the management of wild deer (Austin *et al.* 2010). Q methodology presents participants with a list of quotes or opinions sourced from similar stakeholders or the literature, and assesses the extent to which they agree with each one. This has been used to identify stakeholders' distinct viewpoints on the introduction of alternative policies for pollution control in agriculture in Thailand (Bumbudsanpharoke *et al.* 2010). Adaptive conjoint modelling, which assesses the relative importance of different attributes of something (i.e. a management intervention), has been used to identify the range of opinions that veterinarians and farmers have about mitigation strategies for bluetongue in the UK (Cross *et al.* 2009). Best-worst scaling, previously mainly used in healthcare research, has been used to assess scientist's opinions about priorities for the management of global biodiversity (Rudd 2011) and to inform decision making about greenhouse gas mitigation in agriculture, based on both expert and farmer opinions (Jones, Jones, & Cross 2013).

Examples of social research methods in livestock disease management

The application of social research methods to livestock disease management remains limited. Detailed expert opinion and consensus was quantified using adaptive conjoint analysis on the international designation of foot-and-mouth disease status (Garabed *et al.* 2009). Looking at a range of respondents, van Schaik *et al.* (1998) quantified farmers, veterinarians and technician's perceptions of the risk of the introduction of bovine herpes virus (BHV1) to a farm during an eradication campaign. They found few differences between these stakeholders, indicating a good level of awareness of the risk factors for the disease. However, in Cross *et al*. (2009)'s similar study regarding strategies for bluetongue mitigation, differences between veterinarians and farmers were observed. This allowed for greater understanding of which mitigation strategies might be adopted by each of these groups, but also indicates that knowledge exchange between stakeholders could be improved. Finally expert opinion was again consulted, using best-worst scaling to assess both the practicality and effectiveness of *E. coli* management interventions in cattle. Considering efficacy and practicality separately identified the best mitigation strategies (Cross, Rigby & Edwards-Jones 2012). Management effort can then focus on introducing, maintaining or expanding on these strategies in order to reduce livestock disease prevalence. These studies have made valuable contributions to decision-making about livestock disease management, and are often not difficult to implement thanks to the use of market research software. The socio-economic aspects of disease management need to be considered in future disease interventions, to mitigate and prevent conflicts over management and therefore attain the social and economic benefits of livestock disease reduction or eradication.

Thesis objectives

In order to study the management of livestock diseases in mixed wildlife:livestock systems, interdisciplinary use of social and biological research methods have been employed. These were used to address the main objectives of the thesis, which were:

1. To identify the risk factors for the presence of disease in livestock in a mixed wildlife and livestock agro-ecological system.

General hypothesis: Disease prevalence will be associated with one or more distinct farm management or environmental risk factors.

Specific hypothesis: In South-central Spain cattle herd level TB prevalence will be positively or negatively associated with one or more distinct farm management or environmental factors.

Purpose: Identification of the factors associated with higher or lower disease prevalence could lead to improvements in livestock disease management practice.

2. To identify shared risk factors for the presence of multiple livestock diseases.

General hypothesis: The presence of multiple diseases will be associated with one or more distinct farm management or environmental factors.

Specific hypothesis: In northern Spain, cattle herd level TB and brucellosis prevalence will be positively or negatively associated with one or more farm management or environmental risk factors.

Purpose: Identification of shared risk factors for multiple diseases could lead to the more efficient management of both diseases.

3. To quantify direct and indirect interactions between wildlife and livestock in a mixed agroecological system, with reference to disease transmission.

General hypothesis: In a missed wildlife and livestock system, differences in some species or individuals interactions with other species or individuals will be observed.

Specific hypothesis: In South-central Spain, individuals from different species of wildlife and livestock on a cattle farm will show different rates of direct and indirect interaction with other individuals.

Purpose: Understanding when, where and between which individuals direct and indirect interactions occur could lead to improve targeting of disease management activities.

4. To understand different stakeholder groups' views of the effectiveness and practicality of possible disease management interventions in a mixed wildlife-livestock system with conflicting management objectives.

General hypothesis: Stakeholder groups will rank different effective management activities with different levels of practicality.

Specific hypothesis: In South-central Spain, different stakeholder groups (hunters, cattle farmers and veterinarians) will rank different suggested bovine TB management strategies with different levels of practicality.

Purpose: Knowing the similarities and differences of opinion of different stakeholders about the practicality of possible interventions will help to determine who might be willing and able to implement or further develop these disease management activities.

The example of livestock disease in mixed wildlife-livestock systems in Spain

This thesis uses the example of livestock disease management in Spain to address the study objectives. Factors affecting livestock TB and brucellosis are studied in two key regions, including disease reservoirs in wild boar and red deer (*Cervus elaphus*) populations. These diseases and the regions and species of Spain that are affected are described in the following sections.

Bovine tuberculosis

The bovine tuberculosis problem

The bacterium *Mycobacterium bovis* is one of several forms of the *Mycobacterium tuberculosis* complex which can give rise to a TB infection. In humans, the status of this transboundary disease appears to have deteriorated over the last two decades, and is now considered an epidemic in the developing world (van Zyl Smit *et al.* 2010). In the 1980s TB treatment comprised a relatively small part of the public health effort in most developing counties. However, the spread of HIV/AIDS infection led to increased TB infections, which were harder to diagnose and treat and led to a five-fold increase in mortality from TB infection.

Whilst most clinical cases of TB in humans are caused by *M. tuberculosis* it is thought that the number of *M. bovis* infections in humans has previously been underestimated, and could account for an average of 2.1% of pulmonary cases and 9.4% of extra-pulmonary cases around the globe (Cosivi *et al.* 1998). The clinical symptoms of infection are the same as those of infection with *M. tuberculosis*. Given that 1.8 million people were reported to have died of TB in 2008, and 9.4 million new cases were reported, *M. bovis* infection could account for nearly 1 million new cases per year, and many deaths (WHO, 2009). *M. bovis* is transmitted to humans from animals, usually livestock, and evidence for human to human transmission is limited (Moda *et al.* 1996).

Aside from direct human infection with *M. bovis*, TB also has a major effect on many animal species, often affecting farming and conservation efforts. Different species are affected in different ways and the bacterium is difficult to detect and untreatable in animals (Radostits *et al.* 2007). The disease therefore causes major problems for the conservation of species that may be affected, particularly those at higher trophic levels which are likely to encounter the disease in their prey (Gortázar *et al.* 2008). Species of conservation concern affected by bTB include the Iberian lynx in Spain (Gortázar *et al.* 2008), bison (*Bison bison*) in North America and Canada (Himsworth *et al.* 2010), and buffalo (*Syncerus caffer*) in South Africa (de Garine-Wichatitsky *et al.* 2010).

Infection with TB occurs in cattle around the world (Kaneene *et al.* 2002; Gilbert *et al.* 2005), and has also been identified in pigs, goats and sheep (Gutierrez & Marin 1999; Gaspar *et al.* 2008; Di Marco *et al.* 2012; Bailey *et al.* 2013; Napp *et al.* 2013). This carries high social and economic costs for farmers, both through reduced productivity and as a result of disease eradication schemes where infected animals or whole herds may have to be slaughtered to prevent disease spread. Whilst financial compensation may be provided, farming communities still bear high emotional and indirect financial costs (Brook & McLachlan 2006).

Tuberculosis transmission

Animals infected with TB generally show the formation of gross legions in the lymph nodes and/or lungs (Fitzgerald & Kaneene 2013). Other symptoms include weight loss, and bronchial, thoracic and/or mesenteric lymph nodes are also frequency affected (Martín-Hernando *et al.* 2007). Some species such as European badgers can remain latently infected with the disease for many years, whereas others such as brush-tail possums normally die within 3-14 months of becoming clinically ill (Fitzgerald & Kaneene 2013). There may be no fixed duration of infection, and natural infection results in little or no immunity to future exposure to the disease (Vicente *et al.* 2013). Transmission routes include the faecal-oral route (Benham & Broom 1991; Hutchings & Harris 1999; Judge *et al.* 2005), inspection of infected carcasses (Barron *et al.* 2011), fomite movement where the pathogen is moved on another object such as farm equipment (Kleinlützum, Weaver, & Schley 2013), aerosol transmission (especially over short distances where the bacterium may be expelled from the lungs during coughing or sneezing (Pritchard 1988)), vertical transmission from mother to offspring through maternal milk (Neill *et al.* 1994; Porphyre, McKenzie, & Stevenson 2011) and very rarely through cutaneous infection (Neill *et al.* 1994). The respiratory route is thought to be the most important. It appears that the inhalation of only a few of the bacterium is sufficient to initiate lesions, though the viability of these bacilli may rapidly deteriorate in the environment (Neill, Bryson, & Pollock 2001).

Environmental survival of M. bovis

Studies on the environmental survival of *M bovis* in the environment are limited and variable in their study goals. Estimates of environmental survival time have ranged from no survival to the recovery of genetic material up to 300 days later (Young, Gormley, & Wellington 2005; Michel *et al.* 2007), and do not always focus on the survival of a minimum infective dose. Survival has been demonstrated in faeces and slurry, soil, bronchial pus, foodstuffs, water and infected tissues (Young, Gormley, & Wellington 2005; Palmer & Whipple 2006; Michel *et al.* 2007; Fine *et al.* 2011a, b; Kukielka *et al.* 2013, appendix 1), highlighting the potential importance of indirect transmission routes.

Risk factors for bovine tuberculosis

Risk factors for bovine TB are farm management or environmental factors that allow disease persistence and/or spread in the target population. Identifying risk factors may allow for improved disease management without the need for detailed biological evidence of the processes involved. However, risk factors may be different in different systems. Researchers have identified risk factors for TB across the globe (Humblet, Boschiroli, & Saegerman 2009). Within a farm, large herd sizes are an important risk factor (Griffin 1996; Ramirez-Villaescusa *et al.* 2010; Mill *et al.* 2012) and farm biosecurity influences intra-herd direct and indirect transmission (O'Brien *et al.* 2011; Brennan & Christley 2012). Between farms, contact with other herds (Johnston *et al.* 2011), the occurrence of TB in contiguous herds (Skuce, Allen, & Mcdowell 2012) and cattle movement between farms (Marangon *et al.* 1998; Gilbert *et al.* 2005) are important factors. TB prevalence has also been linked to the existence and level of the disease in nearby wildlife (Rodríguez-Prieto *et al.* 2012; Martínez-López *et al.* 2013) and to a lack of biosecurity measures aimed at reducing direct or indirect contact with wildlife on the farm itself (Ward, Tolhurst, & Delahay 2006; Tolhurst *et al.* 2009; Ward, Judge, & Delahay 2010; Judge *et al.* 2011). Furthermore, increased rainfall has been associated with reduced TB prevalence, possibly because of the increased availability of water and therefore the reduction in aggregation at waterholes (Vicente *et al.* 2013).

Examples of wildlife reservoirs of bovine tuberculosis

There are four main cases of true wildlife reservoirs of tuberculosis affecting domestic livestock. Adapted from information summarised by Fitzgerald & Kaneene (2013), the details of these reservoirs are shown in table 1. In all cases the reservoir species reach high densities and are able to utilise the same habitats as domestic species. Although brush-tailed possums are considered a pest species in New Zealand, wild boar, red deer and white-tailed deer have economic value as game species in the places they form a disease reservoir and badgers are a protected species in the UK.

Table 1: Summary of the main wildlife reservoirs for bovine tuberculosis

Wildlife Reservoir Species	Geographic location	Livestock species affected	Contributing factors	References
White-tailed deer and elk	North America	Domestic cattle	High population densities, shared habitat use with cattle	Schauber, Storm, & Nielsen 2007; Atwood *et al.* 2007; O'Brien *et al.* 2011; Miller & Sweeney 2013
European badger	England, Wales and Ireland	Domestic cattle	High population densities, species protected by law, opposing social and political pressures	Garnett, Delahay, & Roper 2002; Phillips *et al.* 2003; Vicente, Fernández de Mera, & Gortázar 2006; Donnelly *et al.* 2007b; Carter *et al.* 2007; Sobrino *et al.* 2008; Sleeman *et al.* 2009; Jenkins, Woodroffe, & Donnelly 2010; Martin *et al.* 2011; Vicente *et al.* 2013
Wild boar and Red deer	Spain	Domestic cattle, goats, deer and pigs	Increasing populations of reservoir species, conflicting management objectives as game species	Vicente *et al.* 2006; Gortázar *et al.* 2008; Naranjo *et al.* 2008; Acevedo *et al.* 2008; Meng, Lindsay, & Sriranganathan 2009; Martin *et al.* 2011; Gortázar *et al.* 2011a,b; Marreros, Gortázar, & Balseiro 2012; Boadella *et al.* 2012
Brush-tailed possum	New Zealand	Domestic cattle, deer and ferrets	Introduction of the reservoir species to New Zealand	Phillips *et al.* 2003; Porphyre, Stevenson, & McKenzie 2008; Porphyre, McKenzie, & Stevenson 2011; Fitzgerald & Kaneene 2013; Barron, Nugent, & Cross 2013

Current tuberculosis management strategies

In cattle, test-and-slaughter schemes are the primary method used to control TB (Alvarez *et al.* 2012; Fitzgerald & Kaneene 2013). The intra-dermal cervical tuberculin skin test is used to ascertain if an individual has antibodies for the *M. bovis* bacterium. More accurate tests are also available, such as the gamma interferon test for *M. bovis*, but are not yet considered cost-effective in most cases (Schiller *et al.* 2011). Positive reactors are removed from the herd and slaughtered to prevent the further spread of the disease. This method has successfully reduced bovine TB levels in many countries, but there are concerns that uncertain test results could inhibit total eradication of the disease with this method alone (Szmaragd *et al.* 2012). Test-and-slaughter can also be very expensive and may not immediately be considered cost effective, particularly in developing countries, though consideration of the indirect effects of the disease may mean the benefits still outweigh the costs (Mwacalimba, Mumba, & Munyeme 2013).

Control of wildlife reservoirs of TB have focussed on preventing contact between the reservoir species and livestock (Fitzgerald & Kaneene 2013). Depending on the wildlife species, wildlife-proof fencing at the wildlife-livestock interface can be used to reduce these contacts (e.g. Artois *et al*. 2011; Brook 2010; Barasona *et al*. 2013a), and prevent wildlife access to livestock food resources (Delahay *et al.* 2005; Nishi, Shury, & Elkin 2006), where indirect transmission may occur. The banning of supplementary feeding of game species has also been suggested as beneficial (Artois *et al.* 2001, 2011; Zanella *et al.* 2008; Fitzgerald & Kaneene 2013). Culling to reduce the reservoir species population density, and therefore reduce the level of disease and risk of transmission can also be effective. Examples include the culling of brush-tailed possums in New Zealand and badgers in the UK and Ireland, where it has been shown that culling can achieve reductions in bovine TB prevalence (Donnelly *et al.* 2007b; Porphyre, Stevenson, & McKenzie 2008; Nugent 2011). However, there can be significant issues with achieving and maintaining sufficient culling intensity (Jenkins, Woodroffe, & Donnelly 2010), the cost-benefits of a culling programme (Smith *et al.* 2007), animal welfare concerns (Woodroffe *et al.* 2005) and political and public pressure to identify alternative strategies (O'Connor, Haydon, & Kao 2012).

Vaccination of cattle against the disease confers some protection (indeed, it was tested and proven effective in cattle before use with humans (Waters *et al.* 2012)), but is not 100% protective and eliminates the ability to test for active infection (Lopez-Valencia *et al.* 2010; Waters *et al.* 2012). Research is underway on the development of vaccines for TB in wildlife species that act as reservoirs of the disease. The BCG vaccine is effective when injected (Carter *et al.* 2012), though it does require high doses in some species (Lesellier *et al.* 2011). A heat-killed *M. bovis* vaccine has been shown to confer similar protection to wild boar in Spain (Beltrán-Beck *et al.* 2012). Practical vaccine delivery to wild mammals requires the use of oral baits (Ballesteros *et al.* 2011). Research has focused on the development of oral baits for badgers (Kelly *et al.* 2011) and wild boar (Ballesteros *et al.* 2010) in Europe, and brush-tailed possums in New Zealand (Gormley & Corner 2009; Tompkins *et al.* 2013). Models indicate that vaccination could be a valuable part of an integrated disease management strategy for TB in badgers in the UK (Hardstaff *et al.* 2013) and wild boar in Spain (Anderson *et al*. 2013b).

<u>Brucellosis</u>

Brucellosis is an infectious disease that is now considered the most common zoonosis in the world. Infection results from ingestion or inhalation of the organism (*Brucella abortus, B. melitensis* or *B. suis*) and tends to be chronic (Franco *et al.* 2007; Dasari, Naha, & Prabhu 2013). In livestock as with humans, infection affects the reproductive tract, causing abortions and/or retention of the placenta after birth (Godfroid & Käsbohrer 2002; Schumaker, Peck, & Kauffman 2012). Risk factors for bovine brucellosis include large herd sizes (Makita *et al.* 2011; Serrano *et al.* 2011; Sanogo *et al.* 2012), a history of abortions in a herd (Serrano *et al.* 2011), the purchase of breeding stock (Dias *et al.* 2009; Gonçalves *et al.* 2009) and pasture rental practices (Dias *et al.* 2009). Wildlife are thought to be a reservoir of bovine brucellosis in the United States (Schumaker, Peck, & Kauffman 2012), though not in other countries with high wildlife densities, such as Spain (Muñoz *et al.* 2010). Brucellosis is currently managed by test-and-slaughter conducted with serological testing. Biosecurity measures such as managing the feeding of wild animals that may carry the disease are also recommended (Miller & Sweeney 2013). Roth *et al.* (2003) found that mass vaccination of livestock in Mongolia resulted in a significant decrease in human infections that was also found to be cost effective economically.

Study site

Research to address the objectives of this thesis was conducted in Spain, in south-western Europe. Spain is a major livestock producer in the European Union, with a national herd of over 6 million cattle (RASVE, 2013), producing beef and dairy products. They are also the second largest pork producer in the European Union (after Germany), and the third largest in the world (Simon-Grifé *et al.* 2013). The country has a variety of climates and habitats that were classified into five different bio-regions by Muñoz *et al*. (2010), ranging from mountainous Atlantic areas with high precipitation to dry savannah-like regions.

South-central Spain

The work for this thesis was conducted mainly in the South-central bio-region, considered to have a Mediterranean climate. Extensive livestock farming in this region is typically practiced in agroforestry systems called "dehesa" (Gaspar *et al.* 2009). Dehesa is characterised by the presence of *Quercus spp* Oak trees on pasture, commonly in close proximity to forests and scrubland which livestock can also access. Average farm sizes are very variable, and stocking densities are much lower than other extensive farming systems in Europe (Plieninger *et al*. 2004; Milan *et al*. 2006; Gaspar *et al*. 2008). Multiple livestock species such as domestic sheep, goats, and pigs are often mixed with cattle. Many land owners have shifted to focussing on large game hunting, leading to an interspersed distribution of different land uses (Delibes-Mateos *et al.* 2009). Indeed, a property may be divided into farm and game rearing areas without separation with a fence. The annual mean temperature in this bio-region is 14.5°C but this is highly variable throughout the year, ranging from below zero to over 40°C (Kukielka *et al.* 2013, appendix 1 and 2).

Over the last decade, researchers from many disciplines have investigated the epidemiologically complex TB situation in this region (Vicente *et al.* 2013). Despite national control measures bovine TB prevalence remains persistently high here (Allepuz *et al.* 2011). Cattle herd TB prevalence was 5.35% in 2011, amongst the highest in the country (RASVE 2013). The region has high density populations of both red deer and wild boar (Acevedo *et al.* 2007, 2008). Both species have been found to have high levels of TB (Vicente *et al.* 2006), with evidence from testing by culture that prevalence is as high as 52% in wild boar and 27% in red deer is some areas (Gortázar *et al.* 2008). Mean prevalences over the last decade have been found to be 59% in wild boar and 9.4% in red deer when estimated by the presence of TB-like lesions. This is probably the highest prevalence of TB in wildlife to have been reported in the scientific literature worldwide (Vicente *et al.* 2013).

Regarding the management of bovine TB, south-central Spain has multiple stakeholders with some conflicting land management objectives that affect bovine TB management. Whilst all stakeholders would like to eliminate TB, certain management interventions such as culling wildlife or cattle movement restrictions would have negative economic implications. Those that rear game species at high densities for hunting may worsen the risk of TB transmission, but the economic incentives to do so are high, and hunting is an established traditional pastime in this region. Furthermore, the tradition and economic benefits of game rearing are such that many farmers will rear game on the same land as their cattle, providing supplementary food to encourage breeding and aggregation (Vicente, Fernández de Mera, & Gortázar 2006; Rodríguez-Prieto *et al.* 2012; Kukielka *et al.* 2013, appendix 1). Livestock disease management in this region is a complex biological and socio-economical problem.

Atlantic-northern Spain

Research was also conducted in Cantabria and Asturias, regions found on the north coast of Spain. Habitats in this mountainous region include pastures and deciduous woodlands. Wild boar and roe deer (*Capreolus capreolus*) are abundant, with red deer also numerous in some locations (Muñoz *et al.* 2010). Cattle farming in this region is dependent on the seasons. Between April and November most cattle herds are kept grazing at high altitudes in the mountains, with different herds often sharing grazing. In winter, cattle are moved to lowland areas and kept in barns that may have access to small outdoor yards or fields.

In this region, TB levels in cattle are relatively low, with a herd level incidence of 0.14% in Asturias and 0.74% in Cantabria in 2011 (RASVE 2013). Brucellosis infection levels vary, with herd incidence officially 0% in Asturias, and 0.53% in Cantabria in 2011. Prevalence in Cantabria is amongst the highest in the country (RASVE 2012). Both diseases can be found in wildlife here, but evidence to date suggests only TB has wildlife reservoirs (Muñoz *et al.* 2010; Gortázar *et al.* 2011). TB prevalence in trapped and road-killed badgers here can be as high as 12.4% (Balseiro *et al.* 2011) and in a study with wild boar in Asturias was 10.3% (Marreros, Gortázar, & Balseiro 2012). Regarding brucellosis, Muñoz *et al.* (2010) concluded, after extensive sampling across the Iberian Peninsula, that the disease could not be maintained in wild populations alone. However, both diseases persist in the cattle population here.

Current livestock disease management strategies

In Spain, culling is used as part of TB management to reduce wild boar and red deer population densities in some places. However, hunting of these species is a popular recreational activity, meaning that many land managers deliberately feed and manage wildlife to maintain high densities. Biosecurity measures have been introduced to regulate the wild animal translocations often associated with hunting (Royal Decree 1082/2009) and on hunted carcass disposal (both locally and through

EU law). A wildlife disease surveillance scheme has also been established which will identify the spatial and temporal trends of TB in wildlife (Kuiken *et al.* 2011; Boadella *et al.* 2011a; Gortázar *et al.* 2011a). Although some improvements to biosecurity are being made in Spain, the opposing wildlife management objectives influence the likelihood of TB transmission between livestock and wildlife.

Study species

Cattle

Domestic cattle (*Bos primigenius*) are widespread on every continent except Antarctica. They are reared for meat and dairy production, and produce other products such as skin for leather and dung for fertilizer or fuel. Cattle carry many diseases that are pathogenic to humans, including tuberculosis, brucellosis, cryptosporidosis, Creutzfeldt-Jakob disease, haemorraghic colitis (caused by *Escherichia coli* 0157 infection), or food poisoning caused by *Salmonella spp*. infection (Gortázar *et al.* 2007). Because of their economic importance, cattle are considered the host species of interest in this study.

Pigs

Domesticated pigs (*Sus scrofa domesticus)* are farmed by humans for their meat and leather. Evidence is emerging that they may act as a reservoir for TB in cattle populations (Di Marco *et al.* 2012). Swine influenza has long been recognised as a zoonotic disease (Easterday 1980) and concerns have increased recently as the first human influenza pandemic of this century emerged from pig populations (Capua & Munoz 2013). The pigs examined in this study were Iberian pigs, prized for the quality of their meat, known as "*Jamon Iberico*", and have a high economic and cultural value.

Red Deer

Distributed throughout Europe and Asia, the red deer is a ruminant species, abundant throughout most of its range. Red deer are hunted as a game species for recreation and for their meat. In some parts of their range they have suffered from overhunting and habitat loss, though in other places they are overabundant and can be considered a pest species (Gortázar *et al.* 2006; Lovari *et al.* 2008; Acevedo *et al.* 2008; Tanentzap, Kirby, & Goldberg 2012). They have considerable economic value. Red deer are farmed for their meat and other products, and in China these are particularly valuable as some products are used in traditional medicines. In Europe they also generate revenue through sport, hunting and tourism. They are susceptible to a range of zoonotic diseases including TB, anthrax, West Nile virus or Lyme disease (Böhm *et al.* 2007; Lovari *et al.* 2008).

Wild Boar

Wild boar are natural inhabitants of Europe and Asia and have been introduced to the USA, Australia and New Zealand where they have hybridised with local pig breeds and are known as feral pigs or hogs. As with red deer, wild boar are a valuable game species and are also hunted and farmed for their meat. In Europe, a reduction in hunting pressure in the second half of the 20th Century has led to an increase in population densities, leading to concern about vegetation and agricultural damage, and the increasing prevalence of infectious diseases and parasites (Gortázar *et al.* 2006; Acevedo *et al.* 2007; Ruiz-Fons, Segalés, & Gortázar 2008). Wild boar share infectious diseases with livestock, particularly Aujeszky's disease, classical swine fever and certain biovars of brucellosis (Boadella *et al.* 2012). They are also susceptible to zoonotic diseases including TB, hepatitis E, and trichinellosis (Meng, Lindsay, & Sriranganathan 2009). In some regions population densities are increasing again to supply commercial hunting industries (Acevedo *et al.* 2006).

<u>Thesis structure</u>

In order to achieve the objectives set out in this thesis, an interdisciplinary approach drawing on socio-economic and biological research techniques was undertaken. In chapter 1, the background and justification for the work is established. The problem of livestock disease is explained and the effect of wildlife reservoirs of some of these diseases. Methods for the study of both the biological and socio-economic aspects of livestock disease are assessed. The example of tuberculosis is explained in more detail, and the study sites and study species are introduced.

In chapter 2, risk factors for presence of a single disease – bovine tuberculosis - on cattle farms in South-central Spain are assessed. Cattle farmers participated in a questionnaire and participatory GIS mapping exercise in order to quantify the natural and managed features of their farms and their cattle and wildlife management activities. This identified factors that could be used to test the hypothesis that certain farm management or environmental risk factors would be associated with herd level bovine TB prevalence. They were also questioned on their opinions about bovine tuberculosis in their area in order to assess their knowledge and attitude towards wildlife and disease management. Information theoretic modelling was used to identify the most important risk factors for disease on farms in the study area. The implications of these risk factors for future management are discussed.

In chapter 3, a similar risk factor-based questionnaire was repeated in Atlantic-northern Spain, in a region affected by multiple diseases – TB and brucellosis. This is used to test the hypothesis that multiple diseases share one or more risk factors for herd level prevalence. Joint management programmes for any shared risk factors for multiple livestock diseases could reduce costs and increase the efficiency of disease management interventions. Beef and dairy farmers completed a questionnaire and their responses were ground-truthed where possible with camera traps used around the farm buildings. The benefits of understanding shared risk factors for multiple diseases are discussed.

Chapter 4 focuses on the biological aspects of livestock diseases by quantifying the direct and indirect interactions between multiple wildlife and livestock species on a cattle farm in south central Spain. This tests the hypothesis that differences in the direct and indirect interactions between different individuals and species will be observed. Wild boar, red deer, domestic pigs and cattle were collared with proximity data and GPS positioning loggers. Along with base stations placed around the farm these allowed for the collection of spatially and temporally explicit data on space use, direct contacts and overlaps in space and resource use. The relative importance of direct and indirect interactions is discussed for each species. This is valuable information for focussing research effort on the development of practical and effective management interventions to control diseases maintained by a wildlife reservoir.

In chapter 5, the findings from chapters 2 and 4 are drawn upon, along with a literature review, to create a list of possible bovine tuberculosis management interventions in south-central Spain. The efficacy of these interventions is assessed by an expert panel, and the practicality is ranked by key stakeholders in the disease management system – veterinarians, hunters and farmers. This tests the hypothesis that different stakeholder groups will rank different management activities with different levels of practicality. Best-worst scaling is used to generate balanced rankings of these interventions by these stakeholders. Those interventions ranked highly for both practicality and efficacy should be considered as potentially valuable interventions in the control of tuberculosis.

In chapter 6 the aims of the thesis are revisited and evaluated along with the key findings presented in the previous chapters. The implications of our findings and their importance for the management of livestock diseases in Spain and worldwide are discussed.

Chapter 2: Risk factors for the detected presence of *Mycobacterium bovis* in cattle in south central Spain

Preface

The investigation of disease management in mixed wildlife-livestock systems begins by looking at the risk factors for the presence of disease. Over the last 25 years, the focus of livestock disease management has shifted from the treatment of individuals to the to herd level prevention efforts (van der Voort *et al.* 2013). It is therefore appropriate to investigate herd level risk factors for the presence of disease as this will be most relevant to policy makers. These risk factors can be identified using disease surveillance data (often pre-existing) and quantifying the potential factors that influence it. Risk factors can take many forms, from the more obvious biological, environmental and farm management related, to genetic, political, social, economic or cultural factors (Humblet, Boschiroli, & Saegerman 2009). Although they may be complex, many risk factors can be relatively easily identified through consultation with relevant stakeholders.

Recent studies have called for the integration of social science approaches with more traditional bioscientific research on bovine tuberculosis (Pfeiffer 2013). As I am taking an integrated approach to investigating risk factors for disease, this chapter will also take to opportunity to consult stakeholders on their opinions of current disease management. Consultation can reveal detailed local knowledge that is essential in order to plan effective and socio-culturally appropriate disease management interventions. Furthermore, the process of consultation itself can engage stakeholders and increase their support for the resulting management interventions (Dougill *et al.* 2006; Reed 2008; Austin *et al.* 2010).

This chapter addresses thesis objective one, has been published in the *European Journal of Wildlife Research* (2014) 60, p113-123, with co-authors Beatriz Beltran-Beck, Christian Gortázar, Joaquin Vicente, Michael Hutchings and Piran White. This chapter is my own work.

Abstract

Tuberculosis (TB) is a chronic bacterial disease of livestock and wildlife, which has major social and economic costs. In Spain, cattle test-and-slaughter schemes have dramatically reduced TB levels, but a wildlife reservoir of the disease is thought to be preventing total eradication. I aim to identify the risk factors for the presence of TB in cattle in Spain.

In this case-control study, we combined a farmer-based questionnaire and participatory (pGIS) mapping with government records in Almodovar, Spain. Data were collected from a mixture of TB-free and infected farms, yielding a total sample of 73 farms. Generalised linear modelling and information theory were used to identify the risk factors strongly associated with TB, and farmers were also asked their opinions on TB and wildlife management.

The risk factors most strongly associated with TB on a farm were the presence of wildlife, the number of streams per hectare and feeding volume foods (e.g. hay) on the ground. Farmers' opinions about TB were influenced by their experience of the disease and their interactions with wildlife.

The results highlight the complexities of managing TB, and demonstrate the need for a system-level understanding of the inter-relationships among epidemiological, ecological, environmental, social and political risk factors.

Introduction

Tuberculosis (TB), caused by *Mycobacterium bovis* infection, is a chronic bacterial disease of livestock and wildlife around the world. Globally, 3.5 million people die from tuberculosis each year, with *M. bovis* thought to be responsible for 3% of these deaths. Even in industrialised countries the disease causes human illness and death (Diez *et al*. 2002). Another cause for concern is the impact of *M. bovis* infection on livestock, particularly cattle (*Bos primigenius*). In livestock the bacterium can be difficult to detect, even when animals are infectious, and there is currently no economically viable treatment (Radostits *et al.* 2007). The disease therefore carries high social and economic costs for farmers, both through reduced productivity and as a result of disease eradication schemes where infected animals or whole herds may have to be slaughtered to prevent disease spread. Whilst financial compensation may be provided, farming communities still bear high emotional and indirect financial costs (Brook & McLachlan, 2006). There is therefore an urgent need to identify the key risk factors associated with the disease, so that management strategies can be improved.

Spain is a major livestock producer within the European Union, rearing over 6 million cattle. Since test-and-slaughter campaigns became compulsory, TB in cattle in Spain has declined from 12% herd prevalence in 1987 to 1.5% in 2010 (RASVE, 2013). However, there are still regions in central and southern Spain where prevalence remains persistently high – as much as 5.35% in 2011 (Allepuz *et al.* 2011, RASVE 2013). Extensive cattle grazing at low stocking densities is common in this area, often overlapping with traditional large species game rearing (Gortázar *et al.* 2011b). Wild ungulates in this region are widespread and abundant (Acevedo *et al.* 2008) and have high prevalences of TB (Vicente *et al*. 2006), estimated from culture to be as high as 52% in wild boar (*Sus scrofa*) and 27% in red deer (*Cervus elaphus*) in some areas (Gortázar *et al*. 2008). These species have been shown to maintain the disease in the absence of livestock (Gortázar *et al*. 2005), and thus form a likely reservoir for the disease (Corner, 2006). Recent research suggests that Eurasian badgers (*Meles meles*), which are currently at low densities but increasing in abundance in some areas of Spain, also contract the disease here (Sobrino *et al*. 2008). Since badgers play a key role in the dynamics of TB in other countries such as Britain and Ireland (Sleeman *et al.* 2009; Ramirez-Villaescusa *et al*. 2010), there is also a possibility that they may act as a host in the TB cycle in Spain (Balseiro *et al.* 2011).

Whilst TB levels in cattle have been reduced by test-and-slaughter schemes, there are concerns that maintenance of the disease in wildlife populations will prevent total eradication (Phillips *et al*. 2003). The management of the disease in wildlife tends to focus on biosecurity measures and culling to reduce wildlife densities. Culling can be effective, but the extent of population reduction required to eradicate disease may be considerable, especially where the disease exhibits frequency-dependent transmission (Ramsey & Efford, 2010). Moreover, maintaining low population levels requires considerable long-term investment, and may not be feasible (Donnelly *et al*., 2007; Jenkins *et al*. 2010). Furthermore, in some species the process of culling can disturb social behaviour and movements in a way that may increase the transmission of disease (Carter *et al.* 2007). Modification of farming practices to enhance biosecurity and reduce transmission risks is increasingly used as part of livestock disease management (Ward *et al*. 2010). Alternative methods such as vaccination, either of cattle or of wildlife (Gormley & Corner, 2009; Lopez-Valencia *et al*., 2010), or fertility control to reduce wildlife numbers (White *et al*., 1997; Massei *et al*., 2008) may also have a role to play in reducing TB.

In Spain, culling is used as part of TB management to reduce wild boar and red deer population densities in some places. However, hunting of these species is a popular recreational activity, meaning that many land managers deliberately feed and manage the wildlife to maintain high densities, possibly negating other efforts to reduce numbers. Biosecurity measures have been introduced recently to regulate the wild animal translocations often associated with hunting (Royal Decree 1082/2009) and hunted carcass disposal (both locally and through EU law). Research is identifying practical methods such as targetted fencing of key resources to prevent direct or indirect contact between wildlife and livestock (Barasona *et al*. 2013a). A wildlife disease surveillance scheme has also been established which will identify the spatial and temporal trends of TB in wildlife (Gortázar *et al*. 2011).

Although some improvements to biosecurity are being made in Spain, it is possible that farm management practices may influence the likelihood of TB transmission between livestock and wildlife. The identification of links between certain management practices and TB in cattle is the first step in the development of cost-effective and sustainable TB management solutions. However, for effective disease management, it is important that any proposed modifications to farming practice are actually implemented. The importance of including stakeholders in the identification of risks and the development of effective management solutions is increasingly recognised (Reed, 2008; Austin *et al*. 2009; 2010), but the mechanism to promote this engagement is also important. Questionnaires provide a simple and accurate way to obtain quantified, comparable data from different individuals (White *et al*. 2005) and have been previously used to investigate TB risk factors elsewhere in Europe (e.g. Griffin, 1993; Marangon *et al*. 1998). Moreover, the process of consultation itself may provide a platform for discussion (Dougill *et al*. 2006). Participatory Geographical Information Systems (pGIS), where participants annotate maps of study areas with data and observations, are used increasingly as an effective means of encouraging engagement and knowledge exchange (Sandström *et al*. 2003; Irvine *et al*. 2009), and can complement data derived in other more traditional ways (Austin *et al*. 2009).

In this case-control study we collect data on potential risk factors for bovine TB in south central Spain, using farmer interviews and pGIS mapping alongside government TB records. Using a generalised linear modelling and information theoretic approach, we aim to identify the key risk factors for TB in the area, in order to inform further research. This tests the hypothesis that one or more factors will be significantly associated with herd level TB prevalence We expect wildlife to be associated with TB in the study region, but other risk factors may be identified that differ on this local scale from those seen in the general literature. The effects of farmers' perceptions concerning TB and its management are also evaluated.

Methods

Study area

The study was conducted in south central Spain, in the Almodovar region of Castilla La-Mancha (figure 1). This area has high population densities of red deer and wild boar (Acevedo *et al*. 2008), and a high prevalence of TB in both wildlife and cattle (Vicente *et al*. 2006). Extensive livestock farming in this region is typically practiced in an agroforestry system called "dehesa" (Gaspar *et al*. 2009). This is characterised by the presence of woody pasture, commonly in close proximity to forests and scrubland, through which cattle, sheep, goats and Iberian pigs often mix freely. Farm sizes are highly variable, and stocking densities are much lower than other extensive farming systems in Europe (e.g. Plieninger *et al*. 2004; Milan *et al*. 2006; Gaspar *et al*. 2008). Many landowners have shifted to focussing on game hunting (principally deer and wild boar), leading to an interspersion of different land uses.

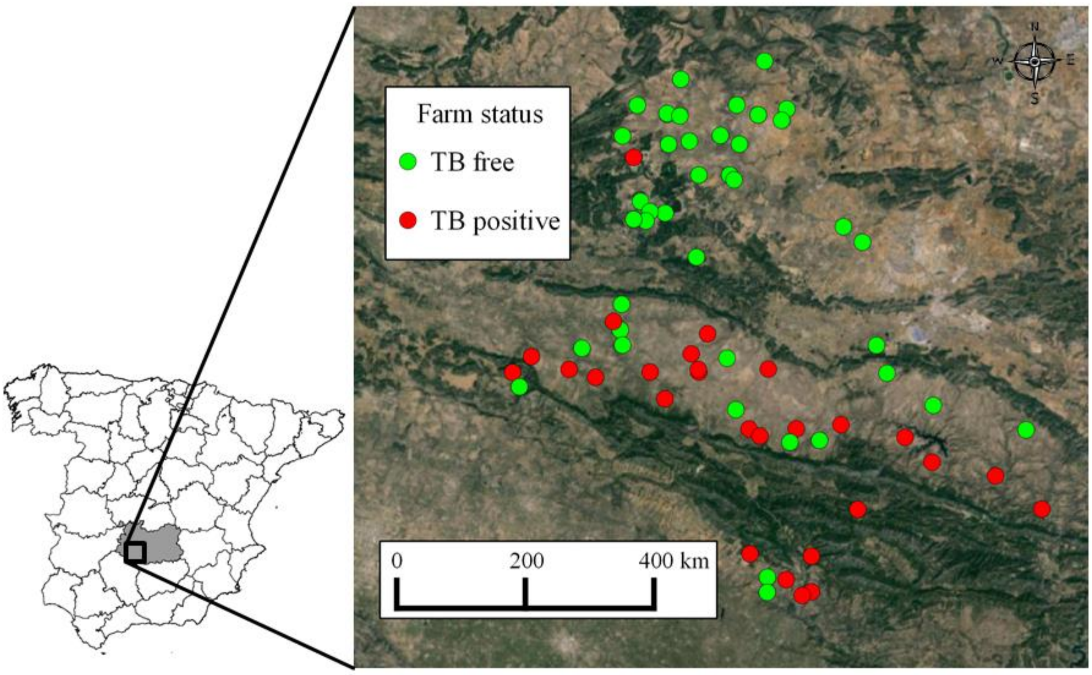


Figure 1: A map of Spain showing the Almodovar del Campo region of Castilla-La Mancha (shaded) in South-central Spain. The square box shows the location and TB status of the study farms within this area.

Questionnaires

All cattle farmer contact details within the region were obtained from government TB testing records. Efforts were made to evenly sample farms of TB-infected and free status, and within these groups farmers were selected at random. Participant recruitment and questionnaires were conducted by a single interviewer between March and May 2010. All respondents received information about the purpose of the work and our intended use of the data, and oral consent to participate was then obtained. Questions were developed following a review of the literature on wildlife-livestock disease transmission, and also addressed possible management practices that might lead to livestock TB infection (table 2, appendix 3). As part of every questionnaire, we also asked farmers to quantify their opinions on a 1-10 scale concerning wildlife and disease management in the local area (table 3).

Prior to analysis, variables were standardised as required to make them more comparable between farms, for example the number of each type of water body was divided by farm area to give the number of water sources per hectare. Furthermore, variables relating to animal food or water resources on the farm were divided by the number of cattle on the farm to standardise these variables to a ‘per animal’ level. Where farmers had not given specific information, variables were categorised to increase the number of responses that could be included in the analysis. Nine additional variables were added from other sources to better understand the observed data, including all the information from the government’s cattle TB testing scheme from 2006 to 2009 (with information such as farm area and herd sizes), and total numbers of red deer and wild boar hunted on each farm. The Spanish TB testing scheme tests all cattle over six weeks old at least once a year with the single cervical tuberculin test. Animals in high prevalence areas are tested up to three times per year, and at over six months old are also eligible for additional gamma interferon testing for improved accuracy. TB positive animals are slaughtered and the farmer compensated, and farms are classified as TB free after three years without a positive test result.

Participatory mapping

Using a participatory GIS approach, data were also collected by farmers drawing on maps. A large colour aerial photograph of each farm was taken to the interview, along with maps of the surrounding area to show the farm in context. Farmers were asked to annotate the map with the boundaries of their farm (and other fencing), the land uses of the surrounding properties and the location of key features, including water sources and any feeding stations. This information was then input into arcGIS (v9.3) over layers of detailed maps and aerial photographs of the region. The perimeter of each farm was split and characterised by the surrounding land uses to enable the calculation of the percentage of bordering land use types. The area of wildlife habitat, defined as dense scrub, was measured from the aerial photographs for each farm. Dense scrub was easily identified in this mainly dehesa landscape as continuous vegetation cover, clearly visible on aerial maps. Distances between water troughs, farm buildings and areas of wildlife habitat were also measured. All of these data were extracted for each farm and added to the data set for further analysis.

Table 2: Summary of the main questions and response types in the risk factors section of the questionnaire.

No		Question	Response Type
1		Is the respondent also a hunter	Yes/No
2		Responsibility for cattle on the farm	Owned/Contracted
3		Number of cattle on the farm	Numeric (count)
4		Length of time land used for cattle rearing	Numeric (Years)
5		Type of cattle rearing	Dairy/Beef/Bulls/Fattening for beef
6		How the cattle are reared	Extensive/Semi Extensive/Intensive
7		Area of the farm	Numeric (Hectares)
8	i	Adjoining properties changed from game to cattle farming	Yes/No
	ii	Adjoining properties changed from cattle to game rearing	Yes/No
9	i-ii	Number of (i) rivers and (ii) permanent rivers	Numeric (count)
10	i-ii	Number of (i) ponds and (ii) permanent ponds	Numeric (count)
11	i-ii	Number of (i) streams and (ii) permanent streams	Numeric (count)
12		Number of water troughs	Numeric (count)
13		Pasture use	Owned/Shared/Both
14	i-iv	Separation from other livestock species[1]	Yes/No
15	i-ii	How (i) concentrated animal feeds and (ii) hay are stored	Outside/Behind fence/Behind wall/Within open shed/Within closed shed
16	i-ii	Where cattle are offered (i)concentrates and (ii)hay	On the ground/Low feeders/High feeders
17		Cattle purchased within the region in the last year	Numeric (count)
18		Cattle purchased outside the region in the last year	Numeric (count)
19		How manure is disposed of on the farm	Removed from farm/Removed but stored on the farm/Stored and used to fertilise farm/Not removed
20		How many people live on the farm	Numeric (count)
21		How many people work on the farm	Numeric (count)
22		Other domestic species living on the farm[2]	Yes/No
23		How dogs are kept when on the farm	Loose/Tied/Kept inside
24		Types of fencing used	None/Wire/Barbed wire/Electric fence/Hunting mesh/Wall
25		Types of gates used	None/Cattle grid/Single bar/Full gate
26	i-v	Max number of each wildlife species seen in one day in the last year[3]	Numeric (count)
27	i-vii	Effect of damage experienced by each wildlife species[4]	Scale from 1 (very little problem) to 10 (big problem)
28		Hunting on the land	Small game/Big and Small game/No
29	i-ii	How many (i) red deer and (ii) wild boar shot on the farm in the last year	Numeric (count)
30	i-vii	Opinion of effect of diseases that can be transmitted to cattle being present in each wildlife species[4]	Scale from 1 (very little problem) to 10 (big problem)

[1] Cattle, Goats, Sheep, Pigs
[2] Cats, Guard dogs, Other dogs, Goats, Sheep, Pigs
[3] Red deer, Roe deer, Wild boar, Badgers, Rabbits
[4] Red deer, Roe deer, Wild boar, Badgers, Rabbits, Foxes, Pigeons

Data analysis

As over one-hundred variables were collected, initial screening was required to identify those variables which were the most important to explain the TB status of the farms. All statistical analyses were conducted in R (R Team, 2013). Univariate binary generalised linear models (GLMs) were conducted for each potential predictor variable with the detected presence or absence of TB on each farm between 2006 and 2009 as the response variable. This means that if TB had been detected at any time in those years the farm was considered TB positive. Variables that were considered significant (p<0.1) were then tested for collinearity using Spearman's Rank correlations, Pearson's correlations, Chi squared tests and/or plotting the variables as appropriate for the continuous, categorical or binary variables. Collinearity was determined in Spearman's Rank tests as a correlation of over 0.7, and in Chi Squared tests and Pearson's correlations as a p value of <0.1. Where significant correlations between predictor variables were observed, the variable most strongly associated with the presence of TB was selected. Potentially confounding variables (location, testing protocol, number of times each farm is tested per year) were also retained.

The resulting variables were entered into a binary GLM. This formed the 'global model' for information theoretic analysis, where a set of models with different combinations of the terms in the global model are generated (Symonds & Moussalli, 2011). The AICc (Second-order Akaike Information Criterion), recommended for use with small sample sizes by Burnham & Anderson (2002) was calculated for every model. If the overdispersion coefficient was greater than 1, the QAICc would have been employed. The Akaike weight was also calculated for each model. The models were ranked from 'best' (highest) downwards by this value, and the models that contribute to a cumulative sum of Akaike weights of over 0.95 formed the 'confidence set'. We were therefore 95% confident that the best approximating model was within this set, and rejected the other models. Full model averaging was then used to produce parameter estimates for each variable, based on all of the models in the confidence set. The 'importance' of each variable was calculated for each variable by summing the Akaike scores of each model that contains the variable in question. The evidence ratio shows how many times better the 'best' model is than the model in question. The R^2 goodness of fit of each model was also calculated, and each model's residuals assessed for normality and independence using Q:Q plots. Separate analyses of the farmers' opinions were conducted in GLMs, and again the distribution and independence of the model residuals were examined.

Table 3: Summary of the opinion based questions farmers were asked about wildlife and disease management in their local area.

		Responses	
Question	Response Type	Range	Mean
What is your opinion of large game hunting in your area?	1=very negative, 10=very positive	1 - 10	6.58
What is your opinion of wildlife management in your area?	1=very negative, 10=very positive	1 - 10	4.87
How important do you think TB is in your area?	1=not important, 10=very important	1 - 9	2.65
Do you believe wildlife transmit TB to cattle?	1=not likely, 10=very likely	1 - 10	5.74

Results

Sixty-seven cattle farmers were approached, with sixty-three of these agreeing to participate, leading to a response rate of 94%. As some farmers owned more than one farm, data were collected for a total of 73 farms (all separate cattle herds), covering over 35% of the farms in the study area. Of these, 27 had tested positive for TB at least once between 2006 and 2009, and 46 had been TB-free during this time. Thus, 30% of TB positive and 37% of TB-free farms were sampled. Mean farm area was 430 (SD=402) hectares, and most farms kept at least one other species of livestock, with some also keeping sheep, goats and Iberian pigs.

Data extracted from the questionnaire, maps and government records were compiled into a single data set of 128 variables (table 17, appendix 4). Screening with univariate binary GLMs identified 19 distinct variables significantly associated with the presence or absence of TB to $p<0.1$. In correlations, the percentage of adjoining land used as hunting estates was strongly positively correlated with large game hunting within the farm, and the presence of red deer, wild boar, roe deer and badgers. The 'presence of red deer' variable was retained to represent the presence of wildlife, as the presence of all species was highly correlated. After removal of correlated variables, eight variables remained for inclusion the 'global model'. Data from farms with any missing values were removed prior to analysis to ensure valid model comparisons. The overdispersion coefficient was 0.59, so the AICc was used to compare the resulting set of models. The confidence set of 6 models is shown in table 4. All models conformed to the assumptions of normality and independence of residuals. The sum of the Akaike weights for these models is equal to 1, indicating that the best available model lies within this set. These models have adjusted R^2 values between 0.75 and 0.76, demonstrating that they explain a good amount of the variance in the data.

Table 4: Summary of the 'confidence set' of models generated using the information theoretic approach, showing the model estimates (S.E.) for each variable in each model.

	(Intercept)	V1*	V2*	V3*	V4*	V5*	V6*	V7*	V8*	Res. d.f.	logLik	AICc	delta	Akaike weight	Evidence Ratio	Adjusted R^2
Model 1	653.53 (163.5)	2.87 (1.05)	-3.71 (1.61)	-0.02 ($3.82e^{-5}$)	-286.06 (107.4)	-	-	-	-	62	-18.4	47.8	0	0.382		0.746
Model 2	673.80 (164.1)	2.94 (1.05)	-3.68 (1.67)	-0.02 ($3.83e^{-5}$)	-312.78 (113.4)	-1.08 (1.15)	-	-	-	61	-18.0	49.3	1.49	0.181	2.16	0.754
Model 3	695.71 (185.0)	2.95 (1.08)	-3.78 (1.61)	-0.02 ($4.31e^{-5}$)	-304.71 (114.5)	-	-0.20 (0.33)	-	-	61	-18.3	49.9	2.05	0.137	2.85	0.749
Model 4	649.07 (163.8)	2.87 (1.05)	-3.61 (1.66)	-0.02 ($3.82e^{-5}$)	-281.73 (108.4)	-	-	-0.22 (0.88)	-	61	-18.4	50.2	2.35	0.118	3.31	0.746
Model 5	664.07 (181.2)	2.83 (1.08)	-3.73 (1.62)	-0.02 ($4.22e^{-5}$)	-285.90 (107.4)	-	-	-	-0.16 (1.16)	61	-18.4	50.2	2.39	0.116	3.38	0.746
Model 6	736.13 (198.1)	3.05 (1.10)	-3.81 (1.65)	-0.02 ($4.62e^{-5}$)	-344.58 (128.3)	-1.17 (1.15)	-0.25 (0.35)	-	-	60	-17.7	51.3	3.47	0.067	5.79	0.758
Model averages	**669.22 (172.9)**	**2.90 (1.06)**	**-3.71 (1.63)**	**-0.02 ($4.04e^{-5}$)**	**-296.85 (112.4)**	**-1.10 (1.18)**	**-0.22 (0.34)**	**-0.22 (0.90)**	**-0.16 (1.18)**							
Relative importance		**1.00**	**1.00**	**1.00**	**1.00**	**0.25**	**0.20**	**0.12**	**0.12**							

*V1=Presence of wildlife, V2=Feeding cattle volume food on the ground, V3=Latitude of the main farm building, V4=Number of streams per hectare, V5= Goat separation from cattle, V6=Number of times each farm was tested for TB within the study period, V7=Cattle separation from other cattle herds, V8=Cattle testing protocol used

The evidence ratio indicates that the ‘best’ model is over twice as good as the next closest. The relative importance scores show that the potential risk factor of the presence of wildlife is positively associated with the presence of TB. Feeding volume foods to cattle outside and the number of streams per hectare were negatively associated with TB and also highly important in explaining the variance in the data. Latitude is an important confounding variable. Separation of cattle from goats and other cattle herds were found to be much less important. Cattle testing protocols and routines, which may have confounded the TB data, were relatively unimportant.

Answers to the other opinion-based questions were often related to each other, and a wide range of responses were observed (table 3). Though they had opinions, some farmers expressed difficulty in answering opinions based questions, resulting in some missing data in the following models. All data from farms with missing data points was removed prior to analysis. All models meet the assumptions of normality and independence of model residuals. In GLMs, farmers having favourable opinions of hunting in the area were also more likely to have favourable opinions of wildlife management (t=2.233, d.f.=51, p=0.035). Farmers expressing a favourable opinion of hunting were less likely to believe that wildlife could transmit TB to livestock (t=-2.64, d.f.=49, p=0.011), and farmers who gave higher ratings to the belief that wildlife might transmit TB to livestock also rated TB as a more important issue (t=2.447, d.f.=55, p=0.018).

Discussion

Data reliability

Our mixed-methods approach, employing government data, questionnaires and participatory GIS has identified potential risk factors for bovine TB in cattle in south-central Spain. In some situations, farmer responses to questionnaires can be considered unreliable, although when they concern matters of high personal importance, responses concerning management practices and observations of wildlife have been shown to be accurate in similar situations (Brook, 2010; Brook & McLachlan, 2006). For the development of TB management strategies these results indicate that it may be very important to consult a wider range of stakeholders and consider any conflicts or conflicting objectives (Gortázar *et al.*, 2010). In the study region, bovine TB control needs to take both hunting and farming activities into consideration. Despite our relatively small sample size, the very high response rate we recorded and our random sampling method will have helped to reduce bias, and shows that farmers were generally willing to be consulted and keen to contribute information to help reduce TB in their area. Experimenter bias was reduced as one person conducted all of the interviews. The effects of recall bias were decreased by avoiding asking respondents for information that relied on memory of events beyond the previous year. Some missing values were observed where farmers were uncertain of the number of streams on their farms, and in the opinion based data where respondents' felt unable to classify their thoughts on the scale provided. As the presence and number of streams varies by year and season, this is unlikely to have introduced bias to the data. The classification of questions may need to be revised in any future studies to facilitate communication of opinions. As little is known about the risk factors for TB in cattle in this region of Spain, the explorative statistical methods used are appropriate for generating information on which further research can be based.

Confounding variables

The number of times the cattle on each farm were tested for TB had the potential to strongly influence the TB response variable, especially as the cervical tuberculin skin test is known to be an imperfect test. Both this and the cattle testing protocol followed were not strongly associated with TB in the confidence set of models (table 4). This is important as it indicates that the TB data were not strongly influenced by differences caused by regulatory factors. These factors are often overlooked in the analysis of epidemiological data, yet accurate testing is vital for accurate scientific assessment of disease management. Continuing the current strict and independent TB testing (including pre-movement tests) in the future will be very important in reducing the spread of bovine TB.

One significant confounding variable was the latitude of the farm (see figure 1, table 4). TB was more frequently observed towards the south of our study region. This variable was not correlated with the presence of wildlife, the proximity to another TB positive farm, or any map-derived features such as the presence or proximity of wildlife suitable habitat. The other potential risk factors in the model are still strongly related to TB, so we must conclude that latitude is explaining a different aspect of the variance in the models. Research on a larger scale may reveal the reason for this effect.

Information-theoretic analysis

Care must be taken with using the information-theoretic framework, as it compares a model set regardless of the quality of those models. However, used appropriately it overcomes many of the limitations of other model selection methods, not least the overreliance on one ‘best’ model (Richards *et al.*, 2011), and is increasingly recommended for use in ecology (Hegyi & Garamszegi, 2011). In this study the variables with high relative importance also show strong estimates and generate models with a low overdispersion coefficient value and relatively high adjusted R^2 values, indicating goodness of fit. As not all of the variance is explained by the model, further research into the other aspects of TB disease transmission in this region of Spain may be beneficial. In particular, knowledge of *M. bovis* survival in this environment and quantification of indirect interactions between livestock and wildlife may tell us more about the relative importance of the risk factors identified in this study.

Wildlife-related risk factors

The presence of wildlife was an important variable in all of the final models, showing a strong positive relationship between wildlife on the farm and bovine TB. This is consistent with the evidence that wildlife are persistent sources of TB risk to livestock (Gortázar *et al*. 2005; Vicente *et al*. 2006; Naranjo *et al*. 2008). The management of red deer and wild boar is complicated by their importance as game species. In Spain, management for hunting involves keeping these species at high densities on hunting estates, which can lead to wildlife spillover onto farming land (which may be equally attractive to wildlife), or may even be encouraged on the same land. Regarding badgers, only one clinical case of TB has been reported in a badger in southern Spain to date (Sobrino *et al*. 2008), though this individual showed lesions consistent with being an excretor of *M. bovis*. The significant association of the presence of badgers with the presence of TB, both in binary models and through being correlated with the presence of wildlife variable, indicates that badgers may have the capacity to contribute more to the dynamics of TB in Spain than was previously accounted for by disease managers.

Maintaining potential TB hosts such as red deer and wild boar at high densities for hunting purposes is clearly at odds with achieving effective TB control through wildlife population reduction. Furthermore, the relatively high levels of hunting on farms close to hunting estates suggest a conflict of interests that needs to be better understood. Research is being conducted currently into vaccination of wild boar against *M. bovis* using oral baits, which may provide a partial solution that would not require major management changes (Beltrán-Beck *et al*. 2012). Reducing aggregation of wildlife and livestock around key resources would reduce risk of transmission of TB between these groups. Strategies such as excluding large game wildlife species from farms by fencing, reducing the attractiveness of the farm to wildlife by improving food storage security or reducing wildlife population densities are most likely to be successful, though the long term sustainability of these needs to be considered. Policy makers and land managers should work together to resolve the conflict caused by large game overabundance in an area with such an animal health problem.

Environmental risk factors

The final models are consistent in indicating that TB was detected more frequently on farms with a lower number of streams per hectare. This is likely to be due to the reduced number of water sources forcing more animals, both livestock and wildlife, to visit the same locations for drinking water. This could lead to more opportunities for direct and indirect transmission of TB between wildlife and livestock, and has been identified previously as a risk factor for TB in wild boar in Spain (Acevedo *et al.* 2007). This would be particularly important as most streams in this region are seasonal and therefore not available throughout the year. No other types of water (e.g. ponds, rivers or water troughs) were associated with TB in our analysis. It is possible that streams are a preferred water source when available, as the water does not stagnate. Providing access to other water sources, perhaps those to which access can be controlled such as raised water troughs that cattle can reach but wild boar cannot, might limit this effect.

Social and political influences on disease risk

Analysis of the farmers' opinions of wildlife and disease management in the area revealed some factors that may improve ensuing management strategies. The hunting respondents' satisfaction with current wildlife management suggests that game rearing is currently the predominant wildlife management objective, even amongst cattle farmers. Furthermore, farmers who hunted were inherently more sympathetic to wildlife, to the extent that they did not believe that wildlife played a significant role in TB transmission. However, farmers who had direct experience of TB on their farm were much more aware of the risks posed by wildlife, and placed a far greater emphasis on the importance of effective TB management. The range of responses (table 3) shows a large heterogeneity of knowledge of the role of wildlife in the TB disease cycle, and also a wide range of management priorities. In order for farmers to learn about the risk factors for disease transmission without having to experience it first hand, education will be a valuable tool. Further consultation concerning farmer perspectives and concerns would be useful in helping to determine the potential opportunities for more effective reduction of transmission of TB. The involvement of farm residents in education about TB may also improve the likelihood of success of any management actions. Furthermore, given that farmer responses seemed to be influenced by their hunting activities there is a need to seek compromises, establish the management priorities for the region, and to pursue social management strategies alongside biological ones. Indeed, it has been shown in North America that areas where hunters do not support TB management, prevalence in wildlife and livestock is higher (Carstensen *et al*., 2011). Here we have questioned only one key stakeholder group in TB management in this region of Spain, and in order to develop well supported management plans all major stakeholder groups, including veterinarians, hunters and other land owners, would need to be consulted.

Farm management-based risk factors

Regarding farm management practice, the final models identified only the provision of volume food (e.g. hay) on the ground as negatively associated with bovine TB. This factor requires further investigation, as it may be related to feeding volume instead of concentrate foods. Though feeding on the ground may allow wildlife to share the food, it may also cause less aggregation between cattle than concentrated foods, reducing intra-herd disease transmission. More details on the feeding regime are required to draw any conclusions about this risk factor. The lack of other farm management related risk factors contradicts similar assessments in other places where these were the main risk factors (e.g. Griffin, 1996; Kaneene *et al*., 2002; Phillips *et al*. 2003), highlighting the different approaches that may be required in different systems and that the scale of a study is important to target management efforts accurately. It is possible that some of the farm management variables may have explained similar variance in the data to the stronger presence of wildlife variable, as they often affect contact between wildlife and livestock. These variables may still influence the disease cycle, especially as management of the primary risk factors improves. For example, TB has been identified in pigs and goats in Spain (Gutierrez & Marin, 1999; Parra *et al.*, 2003; Gomez-Laguna *et al*., 2010; Gortázar *et al*., 2011b), suggesting that other livestock may also be contributing to the disease system. Indeed, stocking goats or different cattle herds together were risk factors that did explain some variance in some of the final models. As well as addressing the factors more strongly associated with TB, advising farmers to maintain separation between livestock types, which very few farmers currently do, could prevent transmission amongst livestock species becoming a problem in the future.

Conclusions

The integrative approach of this study, combining data on experiences, perceptions and management practices from farmers with data on disease incidence from government sources has allowed us to identify risk factors affecting TB in the Almodovar region of Spain, which should now be investigated further. The results highlight the multi-faceted nature of TB, and emphasise that progress in the management of the disease can only be made through a system-level understanding of the epidemiological, ecological, environmental, social and political factors involved. The management of TB is clearly a complex problem, and further research is necessary to evaluate the risk factors identified in this study, and the feasibility of achieving the management interventions that would be required to address them. This risk-based approach should be complemented with economic assessments, and further consultation with farmers, veterinarians and local authorities. Consultation with the relevant stakeholders at all stages of the process of developing new management strategies will help to reduce any conflicts and increase the likelihood that problems are identified and addressed early in the process.

Chapter 3: Shared risk factors for multiple livestock diseases: a case study of bovine tuberculosis and brucellosis

Preface

In chapter two, simple methods were used to identify specific risk factors for tuberculosis. As disease management policies focus on herd level prevention, this is a very practical scale on which to investigate disease risk factors (van der Voort *et al.* 2013). In this chapter this idea is expanded upon. Drawing on the techniques used in chapter two to determine risk factors, questions are asked and disease surveillance data taken that was relevant to two bovine diseases – brucellosis and tuberculosis.

Knowledge of significant shared risk factors between important diseases would enable the development of combined management strategies. In the past, disease management plans have been developed for individual diseases. However, different diseases may still share the same transmission routes, and therefore it could be possible to manage these with one management strategy.

Economics affects disease management at local, national and international scales (Perry & Grace 2009; Gramig & Horan 2011). Both mitigation of infections and disease prevention strategies can have significant costs (e.g. Otte, Nugent, & McLeod 2004; Knight-Jones & Rushton 2013). Funding may also fluctuate depending on other economic factors. For example, at the time of writing the recent global economic downturn is constraining funding for bovine tuberculosis management in Spain (Vicente *et al.* 2013). As a result, disease management strategies need to demonstrate substantial benefits at minimal costs. Combining the management of multiple diseases is an as yet unexplored way to achieve this at lower costs than the separate management of each disease. Significant shared risk factors, if known, could be targeted in this way. This chapter aims to identify shared risk factors for two bovine diseases of high global importance.

As with chapter two, this also provided an important opportunity to assess stakeholders' views on the diseases studied and how they are currently managed. This chapter addresses thesis objective two and at the time of writing has been submitted to *Research in Veterinary Science* for publication along with co-authors Nelson Marreos, Christian Gortázar, Racquel Jaroso, Piran White and Ana Balseiro. This chapter is my own work.

Abstract

Livestock diseases can result in reduced farm productivity. The bacterial diseases tuberculosis (TB) and brucellosis may share some transmission characteristics which, if managed in common, would result in more cost-effective management. Here, we identify risk factors shared between these diseases using a case-control approach and information theoretic modelling. One-hundred cattle farmers in Atlantic Spain were interviewed about farm characteristics and management practices. The risk factor shared between both diseases was intra- and inter-herd contact between cattle. Disease-specific risk factors were the presence of wildlife for TB, and cattle movement between farms for brucellosis. An integrated approach to disease management needs to consider cattle movement and farm biosecurity, reinforced by an education campaign to increase farmer awareness. This would be likely to bring benefits in reducing both diseases and improve the efficiency of any interventions.

Introduction

Bovine diseases cause a great deal of damage to cattle (*Bos primigenius*) farming communities (Brook & McLachlan, 2006; Gortázar *et al.*, 2007). Many of these diseases need to be controlled not only because of their impact on farm productivity but also because of their effect as zoonoses (Palmer, 2011). Disease management plans usually tackle livestock diseases individually. However, different diseases often share the same transmission routes or vectors, and therefore may share one or more risk factors for infection. Identifying and managing these risk factors across different diseases could achieve disease control at reduced cost and effort compared to when they are considered individually.

Two diseases that may benefit from this approach are bovine TB and brucellosis. Both are bacterial diseases, and both share a similar global distribution of infection in domestic livestock (World Health Organisation for Animal Health, 2013). Bovine TB is caused by infection with *Mycobacterium bovis*, and closely related members of the *M. tuberculosis* complex. It is a chronic disease which can be difficult to detect even when animals are infectious, and for which there is no treatment (Radostits *et al.*, 2007). Brucellosis is caused in cattle by *Brucella abortus* or *Brucella melitensis*. Infection causes abortions and/or retention of the placenta, and also cannot be treated (Godfroid & Käsbohrer, 2002).

'Risk factors' refer to aspects of a system that influence the introduction of a disease, or disease persistence in a cattle population. The identification of risk factors could allow for effective disease management, even if the epidemiology is poorly understood (Pfeiffer, 2013). Known risk factors for brucellosis include larger herd sizes (Makita *et al.*, 2011; Serrano *et al.*, 2011), a history of abortions in the herd (Serrano *et al.*, 2011), the purchase of breeding stock (Dias *et al.*, 2009; Gonçalves *et al.*, 2009), and pasture rental practices (Dias *et al.*, 2009). For TB, some risk factors are similar, such as large herd sizes (Griffin, 1996; Mill *et al.*, 2012; Ramirez-Villaescusa *et al.*, 2010), contact with other cattle herds (Johnston *et al.*, 2011) and cattle movements between farms

(Gilbert *et al*., 2005; Marangon *et al*., 1998). However, TB prevalence has also been linked to the existence and level of disease in wildlife (Rodríguez-Prieto *et al*., 2012), and poor farm biosecurity (Judge *et al*., 2011). As many of the known risk factors for these diseases are similar, combining management efforts for these diseases where they occur concurrently could achieve effective management at reduced cost.

Both of these diseases cause heavy economic losses to cattle farming in Europe, and Spain provides an example of a country were the impact of these diseases has been considerable. Spain is a major livestock producer within the European Union and the Spanish cattle population is still affected by both TB and brucellosis despite national disease eradication schemes (RASVE, 2013, 2012). Until now, these diseases have largely been managed in isolation of each other. Test-and-slaughter schemes have reduced TB prevalence from 12% of cattle herds in 1987 to only 1.33% in 2011 (RASVE, 2013), but complete eradication has not been achieved and the disease remains present in cattle in 48% (242) of 505 veterinary districts (Rodriguez-Campos *et al*., 2012). A separate national eradication scheme has reduced brucellosis prevalence from 2.32% in 2000 to 0.12% in 2011 (RASVE, 2012), progressing more successfully than TB eradication. Nevertheless, Spain still suffers amongst the highest prevalences of brucellosis in domestic livestock in Europe (EFSA, 2012; Godfroid & Käsbohrer, 2002).

Brucellosis in Spain can be controlled quite effectively by test-and-slaughter schemes to detect and remove infected cattle (EFSA, 2012; Radostits *et al*., 2007). However, effective control of TB is more complicated, since it is sometimes hindered by the existence of wildlife reservoirs for infection. TB prevalence can reach as high as 52% in wild boar (*Sus scrofa*) and 27% in red deer (*Cervus elaphus*) in Mediterranean Spain (Vicente *et al*., 2006). It is thought that a wildlife reservoir has enabled TB to persist here, and that wild ungulate overabundance contributes to this problem (Gortázar *et al*., 2011b; Muñoz *et al*., 2010).

In Atlantic Spain (figure 2), TB levels in cattle are relatively low, with a herd level prevalence of 0.14% in Asturias and 0.74% in Cantabria in 2011 (RASVE, 2013). Brucellosis infection levels vary, with herd level prevalence officially 0% in Asturias, and 0.53% in Cantabria in 2011. Prevalence in Cantabria is amongst the highest in the country (RASVE, 2012). Both diseases can be found in wildlife here, but evidence to date suggests only TB has wildlife reservoirs (Gortázar *et al*., 2011b; Muñoz *et al*., 2010). TB prevalence in trapped and road-killed badgers can be as high as 12.4% (Balseiro *et al*., 2011) and in a study with wild boar in Asturias was 10.3% (Marreros *et al*., 2012). Regarding brucellosis, Muñoz *et al*. concluded, after extensive sampling across the Iberian Peninsula, that ruminant brucellosis could not be maintained in wild populations alone (Muñoz *et al*., 2010).

The failure to eradicate these diseases in cattle suggests that disease management could be improved, and further interventions may be required. In this study we aim to identify shared risk factors for the detected presence of TB and/or brucellosis on cattle farms in Atlantic Spain. This tests the hypothesis that the prevalence of multiple diseases will be associated with one or more distinct farm management or environmental risk factors. As farmers would be key stakeholders involved in implementing any management interventions, their perceptions regarding TB and brucellosis are also examined.

Methods

Study area

Study sites were located in the Valle de Nansa in Cantabria and various districts in Asturias, covering an area of approximately 8,000km^2 within the Atlantic bio-region of Spain as defined in Muñoz *et al* (2010). This is a mountainous region composed of pastures, deciduous forests and mountain habitats (Muñoz *et al.*, 2010). Livestock farming practices here are representative of many mountainous regions around the world. Furthermore, despite over 25 years of test-and-slaughter both diseases are still present in this cattle population.

Cattle farming in Cantabria and Asturias is characterised by seasonal management. Between April and November most cattle herds are kept grazing at high altitudes in the mountains, with different herds often sharing grazing. In winter, cattle are moved to lowland areas and kept in barns that may have access to small outdoor yards or fields. As conditions in each location are so different, the risk factors for disease need to be considered separately for summer and winter farms.

Figure 2: Map to show the study regions of Asturias and Cantabria in Spain. Map data provided by the Spanish National Centre of Geographic Information.

Study design

A literature review of the known risk factors for TB and brucellosis was conducted in Web of Knowledge and PubMed, using search terms 'risk' and 'risk factor' in combination with the common name for each disease, or the scientific names for the bacteria. Questionnaires were developed to cover a range of farm characteristics, farm management practices, and external factors that might affect cattle on the farm, including the presence of wildlife (table 5, appendix 6), and included the risk factors identified in the literature review.

Respondent contact details and the disease status of all farms in the study areas were obtained from government records. Each disease was considered separately and farms were considered positive if they had one or more positive test results in the five years prior to the interviews. TB tests used the single intradermal cervical tuberculin test and were always confirmed by culture (RASVE, 2013). Brucellosis was diagnosed with serological tests (RASVE, 2012). The target population of all cattle farmers in the study area were classified by their farms' infected or disease-free status for each disease, after which respondents were selected at random within these groups.

Questionnaires

All interviews were conducted in person to avoid any bias caused by respondent illiteracy, generally *in situ* at the respondents' farms. Respondents received information about the purpose of the work and our intended use of the data, and oral consent to participate was then obtained (appendix 6). All interviewers were qualified vets engaged in full time scientific research and were aware of the importance of reducing bias. Questionnaires (table 18) were conducted in August and September 2010 in Cantabria (n=63 farms) and between October 2011 and March 2012 in Asturias (n = 37 farms). As many participants as possible were interviewed in the time available. In total 114 farmers were approached and 100 participated, giving a response rate of 88% and data for 100 different cattle herds.

Table 5: Summary of the main questions and response types in the risk factors section of the questionnaire. Based on a review of relevant literature, variables with an *a priori* expectation of being associated with the presence of TB in cattle are marked with an asterisk(*), and those expected to be associated with Brucellosis are marked with a circumflex(^). Most questions required a separate response regarding summer and winter cattle pastures. Full variable list and analyses can be seen in table 19, appendix 7.

No		Question	Response Type
1		Is the respondent also a hunter	Yes/No
2		Number of cattle on the farm (males, females, yearlings) ^*	Numeric (count)
3		Breed of cattle kept on the farm	Categorical
4		Length of time land used for cattle rearing	Numeric (Years)
5		Type of cattle rearing	Dairy/Beef/Bulls/Fattening for beef
6		Farm land owned or rented by the respondents^	Owned/Rented
7		Area of the farm (all areas used by cattle)	Numeric (Hectares)
8		Percentage of adjoining land used for cattle/sheep/goat/pig farming/natural habitat/urban areas/agriculture/water	Percentage (%) for each land use type
9	i-ii	Number of (i) non-permanent and (ii) permanent water bodies^*	Numeric (count)
10	i-ii	Number of streams^*	Numeric (count)
11		Number of artificial watering points (e.g. water troughs)^*	Numeric (count)
12		How many other cattle herds share pastures	Numeric (count)
13	i-iv	Separation from other livestock species[1]^*	Yes/No
14	i-ii	How (i) concentrated animal feeds and (ii) hay are stored	Outside/Behind fence/Behind wall/Within open shed/Within closed shed
15	i-ii	Where cattle are offered (i)concentrates and (ii)hay	On the ground/Low feeders/High feeders
16		Cattle purchased within the region in the last year^	Numeric (count)
17		Cattle purchased outside the region in the last year*	Numeric (count)
18		How manure is disposed of on the farm	Removed from farm/Removed but stored on the farm/Stored and used to fertilise farm/Not removed
19		Types of fencing used*	None/Wire/Barbed wire/Electric fence/Hunting mesh/Wall
20		Other domestic species living on the farm[2]	Yes/No and count for each species
21		How dogs are kept when on the farm	Loose/Tied/Kept inside
22		Cattle race (used for restraining cattle for examination) ownership	Shared/Not shared
23		What shelter do the cattle have access to on the farm	Loose in closed barn/Tied in closed barn/Open barn/Open air corral/None provided
24	i-iv	Max number of each wildlife species seen in one day in the last year[3]*	Numeric (count)
26		Hunting on the land	Small game/Big and Small game/No
27	i-ii	How many (i) red deer and (ii) wild boar hunted in the last year*	Numeric (count)

[1] Cattle, Goats, Sheep, Pigs
[2] Cats, Guard dogs, other dogs, Goats, Sheep, Pigs
[3] Red deer, Roe deer, Wild boar, Badgers, Rabbits

Data validation

Heat and motion sensitive infrared-triggered camera traps (IR-3BU® and IR-5®, Leaf River Outdoor Products, Taylorsville, Mississippi, USA or NightTrakker NT50, Uway Outdoor Products, Atlanta, GA, USA) were placed on the winter barns and pastures of 18 of the study farms, in Cantabria (n=7) and Asturias (n=11), whilst cattle were present. Up to eight cameras were placed on each farm as required to cover farm resources (food and water), buildings and control areas, each for a minimum of 15 days. These were used to observe wildlife visits to the farm, and provided an opportunity to verify the respondents' reports of the presence or absence of wildlife.

Statistical analyses

In the final database, some variables were created by standardising data by farm or herd size – for example herd size divided by the farm size became the stocking density (appendix 7). All analyses were conducted in R, version 2.15.3. As so many variables were collected, univariate binomial generalized linear models (GLMs) were used to screen which variables were the most important predictors for the presence of each disease. Those predictor variables that were considered significant were then tested for collinearity with each other using an appropriate correlation test. Collinearity was tested using Spearman's Rank tests as a correlation of over 0.7, and in Chi Squared tests and Pearson's correlations as a p value of <0.1, as appropriate for the variables concerned. Where significant correlations between predictor variables were observed, the variable most strongly associated with disease presence was chosen. The selected variables were then entered into a multivariable GLM for each disease. Known risk factor variables identified in the literature review were also included and together these formed the 'global model' for information theoretic analysis. The literature review identified TB risk factors of herd size, cattle movement activities, the presence of wildlife and contact between cattle herds. For brucellosis they were herd size, cattle movements and the use of rented pastures. This allowed for understanding of the relative importance of the questionnaire variables. Biologically meaningful interactions between terms in each global model were also included.

Information theoretic analysis generates a set of models made from different combinations of the terms in the global model (Burnham *et al*., 2010). The AICc (Second-order Akaike Information Criterion), recommended for use with smaller sample sizes, is calculated for each of these models and used to compare them. Models with a delta value (the difference between the lowest AICc scoring model and each other model) of less than 6 were selected to form the 'confidence set', following Symonds and Moussalli (2010)'s recommendation that models with a delta score of less than 6 should not be discounted. Full model averaging gives estimates for each variable derived from all models in the confidence set. The adjusted R^2 goodness of fit is shown for each model, and model residuals were assessed for normality and independence. The evidence ratio shows how many times better the 'best' model is than each other model. Experimenter bias was investigated in a multivariable GLM including all variables that were used in each global model. For the analysis of data on farmer opinions, and to compare camera trap data with respondent reports of the presence of wildlife, univariate GLM models were constructed to complement descriptive statistics.

Results

Of the 100 farms where interviews were conducted, 35 had tested positive for TB in the five years prior to when the questionnaire was conducted, with 11 cases in Cantabria and 24 cases in Asturias. No significant difference between these regions was detected. Brucellosis was only detected in Cantabria, where 13 (21%) positive cases were observed. Only the data from Cantabria were analysed with regard to brucellosis.

Mean reported farm sizes were 32 (95% CI ±6.27) hectares in winter pastures and 398 (95% CI ±96.04) hectares in summer pastures. Stocking densities were higher where cattle spend the winter (63.8 animals/ha) than in summer pastures (2.01 animals/ha), though summer pastures were often shared with other herds so true summer stocking densities may be higher. The majority of farms were rearing cattle for beef production (n=79), and though not finally a risk factor due to autocorrelation, in a univariate binomial GLM dairy farming (n=21) was significantly associated with the detection of TB in the herd (z=3.235, d.f.=98, p=0.001). The questionnaire generated a total of 94 variables suitable for testing as risk factors, and a further 28 were generated by standardising those (full list and results of univariate tests shown in appendix 7).

Data validation

GLMs showed that none of the variables used in the global models, or the responses to the opinions questions, were significantly associated with any interviewer. This analysis was not conducted on the brucellosis data as only one interviewer conducted the sixty-three questionnaires in Cantabria. No significant differences were identified between farmer reports of the presence of wildlife and those observed in the camera traps for red deer (z=1.113, d.f.=16, p=0.265), wild boar (z=0.521, d.f.=16, p=0.602) or badgers (z=-0.813, d.f.=16, p=0.416).

Table 6: Estimates (standard error shown in brackets) for the confidence set of models where the presence of **TB** is the response variable. The models are ranked by their AICc scores. 'Delta' shows the difference between the AICc of the 'best' and current model.

	(Intercept)	V1*	V2*	V3*	V4*	V5*	V6*	Adjusted R^2	df	AICc[1]	Delta[2]	Evidence Ratio[3]
Model 1	-2.573	2.233	1.843	-0.013	0.002	-1.342	0.834	0.635	93	107.6	0.00	
	(1.131)	(0.860)	(0.848)	(0.005)	(0.370)	(0.673)	(0.400)					
Model 2	-2.274	2.065	2.157	-0.013	-0.321	-	0.752	0.593	94	109.6	1.96	3.08
	(1.089)	(0.840)	(0.824)	(0.006)	(0.319)		(0.382)					
Model 3	-2.324	2.131	1.788	-0.012	-	-1.184	0.644	0.588	94	109.8	2.20	3.48
	(0.940)	(0.816)	(0.832)	(0.006)		(0.575)	(0.346)					
Model 4	-2.103	2.213	1.562	-0.009	0.288	-1.210	-	0.589	94	110.0	2.40	3.86
	(1.085)	(0.861)	(0.822)	(0.005)	(0.334)	(0.647)						
Model 5	-2.668	1.966	1.340	-	0.013	-1.400	0.551	0.578	94	111.1	3.52	6.73
	(1.105)	(0.842)	(0.769)		(0.357)	(0.665)	(0.354)					
Model 6	-1.486	1.984	1.445	-0.009	-	-0.903	-	0.551	95	111.2	3.57	6.89
	(0.801)	(0.806)	(0.792)	(0.005)		(0.533)						
Model 7	-2.267	1.991	1.241		0.210	-1.322		0.552	95	111.3	3.74	7.54
	(1.059)	(0.839)	(0.767)		(0.325)	(0.643)						
Model 8	-1.783	1.979	1.900	-0.010	-0.034			0.551	95	111.4	3.83	7.87
	(1.028)	(0.820)	(0.802)	(0.005)	(0.027)							
Model 9	-1.856	2.015	1.935	-0.010				0.520	96	111.9	4.27	9.82
	(0.776)	(0.796)	(0.751)	(0.005)								
Model 10	-2.566	2.162	2.266	-0.013			0.454	0.542	95	112.0	4.41	10.54
	(0.943)	(0.811)	(0.804)	(0.006)			(0.318)					
Model 11	-1.809	1.841	1.172			-1.087		0.515	96	112.3	4.71	12.22
	(0.801)	(0.806)	(0.792)			(0.533)						
Model 12	-1.977	2.241		-0.009	-0.195	-1.481	0.721	0.560	94	112.7	5.11	14.90
	(1.067)	(0.840)		(0.005)	(0.335)	(0.625)	(0.380)					
Model 13	-2.437	1.906	1.318			-1.281	0.416	0.534	95	112.7	5.14	15.16
	(0.910)	(0.809)	(0.005)			(0.538)	(0.159)					
Model Averages	-2.163	2.056	1.664	-0.011	-0.005	-1.246	0.625					
	(0.980)	(0.827)	(0.734)	(0.005)	(0.295)	(0.604)	(0.334)					
Relative Importance		1.00	0.97	0.89	0.80	0.77	0.74					

*V1 = the presence of wild boar on winter farms, V2 = barns where cattle movement is not restricted, V3 = herd size, V4 = cattle herd contacts in winter, V5 = the use of a communal race, V6 = cattle herd contacts in summer

Table 7: Estimates (standard error shown in brackets) for the confidence set of models where the presence of **brucellosis** is the response variable. The models are ranked by their AICc scores. 'Delta' shows the difference between the AICc of the 'best' and current model.

	(Intercept)	V1*	V2*	V3*	V4*	V5*	V6*	Adjusted R^2	df	AICc[(1)]	Delta[(2)]	Evidence ratio[(3)]
Model 1	-2.611 (0.637)	1.357 (0.558)	1.691 (0.935)					0.52	58	57.6		
Model 2	-2.124 (0.784)	1.440 (0.575)	1.871 (0.981)	-0.008 (0.010)				0.57	57	58.3	0.98	2.16
Model 3	-2.988 (0.774)	1.358 (0.565)	1.745 (0.931)		0.721 (0.720)			0.55	57	58.9	1.91	2.83
Model 4	-2.327 (0.731)	1.325 (0.555)	1.732 (0.930)			-0.01 (0.011)		0.53	57	59.4	2.73	3.71
Model 5	-2.612 (0.637)	1.353 (0.571)	1.694 (0.937)				0.002 (0.055)	0.52	57	59.9	3.45	4.89
Model 6	-2.458 (0.906)	1.445 (0.583)	1.923 (0.985)	-0.008 (0.010)	0.620 (0.727)			0.60	56	60.0	3.56	4.89
Model 7	-2.084 (0.748)	1.401 (0.580)	1.952 (0.980)	-0.009 (0.009)			0.044 (0.082)	0.58	56	60.4	4.20	6.07
Model 8	-2.022 (0.806)	1.404 (0.578)	1.850 (0.972)	-0.007 (0.010)		0.00 (0.012)		0.58	56	60.6	4.44	6.57
Model 9	-2.716 (0.869)	1.330 (0.564)	1.764 (0.924)		0.688 (0.724)	-0.01 (0.011)		0.56	56	60.9	4.92	7.00
Model 10	-3.030 (0.794)	1.320 (0.575)	1.777 (0.936)		0.782 (0.752)		0.018 (0.057)	0.55	56	61.2	5.34	7.71
Model Average	-2.47 (0.769)	1.373 (0.570)	1.800 (0.951)	-0.008 (0.010)	0.703 (0.731)	-0.006 (0.011)	0.021 (0.065)					
Relative Importance		1.000	1.000	0.360	0.290	0.290	0.178					

*V1 = number of farmers sharing summer pastures, V2 = purchase of cattle from within the veterinary district in the last year, V3 = herd size, V4 = the presence of free ranging dogs, V5 = use of rented pasture, V6 = the purchase of cattle from outside the veterinary district in the last year.

[1]The AICc shows the second order Akaike Information Criterion score for each model.
[2]The delta value shows the difference in AICc scores between the model with the lowest AICc score and the model in question.
[3]The evidence ratio indicates how many times better the model with the lowest AICc score is than the model in question.

Risk factors for TB

In univariate GLMs, seven variables were significantly associated with TB, with *p* values <0.01. This strict selection policy generated a manageable number of variables for the next stage of modelling. After testing for autocorrelation three variables remained: barns where cattle movement is not restricted, the presence of wild boar in winter and the use of a communal cattle race. These were entered into the global model along with the variables we had *a priori* expectation of being risk factors for cattle TB (table 6). The interaction terms, communal race use:herd size (d.f.=92, z=0.901, *p*=0.367) and cattle contact in summer:cattle contact in winter (d.f.=92, z=0.405, *p*=0.686), were not significant and were removed to increase the power of the models. The presence of wild boar on winter farms (highly correlated with the presence of badgers on winter farms) and the use of barns the cattle can move freely within were the best supported positively associated risk factors. Herd size, and cattle herd contact (in summer and winter) were also positively associated, whilst the use of a communal race showed a negative associated. These remained important in explaining the variance in the data. A model containing all six of these variables was best supported by the evidence ratio.

Risk factors for Brucellosis

Using only data from Cantabria (n=61), three variables were significantly positively associated with brucellosis (*p*<0.1) in the initial univariate binomial GLMs; the number of farmers sharing summer pastures, if cattle were purchased in the area within the last year and the presence of free ranging dogs. The presence of dogs may influence contact between livestock and wildlife as they may reduce the use of farm resources by wildlife. None of these variables were autocorrelated, and were therefore put in the global model along with variables already known to be associated with brucellosis. The interaction term land rental:herd size, was not significant (d.f.=55, z=0.637, *p*=0.524) in any model and was therefore removed. The best supported model includes the number of farmers sharing summer pastures and the purchase of cattle from within the veterinary area as risk factors for brucellosis, both positively associated with the presence of brucellosis, whilst the other variables were of low relative importance (table 7).

Responses to opinion-based questions

None of the stated opinions about either disease were statistically related to the respondents' experience of disease on their farm. The biggest difference was observed when respondents were asked how important they thought the disease was in their area (on a scale of 1→10 where 1 was 'not important and 10 was 'very important'). For TB the mean response was 7.87, and 60.2% of the respondents gave a score of 10. For brucellosis the mean score was 8.84, and 58.7% of respondents selected 10. Regarding transmission of each disease to cattle from wildlife (on a scale of 1→10 where 1 was 'not likely' and 10 was 'very likely'), the mean response for TB was 7.45, and for brucellosis was 7.78, with more respondents selecting 10 for brucellosis (47.5%) than for TB (38.1%). Finally, there was little agreement about the trends of these diseases in the area. For TB, 38% believed the disease to be increasing, 45% think it is stable and 17% believe it is decreasing. For brucellosis 46% believe the disease to be increasing, 48% think it is stable and only 6% feel it is decreasing.

Discussion

Significant risk factors

This study has identified shared and disease-specific risk factors for TB and brucellosis in cattle in Atlantic Spain. Shared risk factors were a positive association between inter- and intra-herd contacts between cattle and the presence of both diseases. For TB, the presence of wildlife in winter was a positive association, whilst cattle movement between herds was positively associated with only brucellosis. The methods used could be replicated with other diseases and/or livestock types worldwide. Identifying shared risk factors for multiple diseases will allow for the integration of management effort, potentially increasing the efficiency and effectiveness of management interventions.

Data validation

The large number of variables generated from the questionnaire maintained the detail necessary to identify possible management interventions. This type of research relies heavily on the accuracy of farmers responses to questions. Although we could not assess the reliability of all of these responses, answers concerning management practices and wildlife observations have been shown to be accurate (using ground-truthing) in similar circumstances, where the respondents believe the matter is of high personal importance (Brook & McLachlan, 2006; Brook, 2010). Despite time constraints limiting the number of potential participants approached, the high response rate and levels of concern about the diseases in their area indicate that this was a matter of interest to our respondents. Although we could only attempt ground-truthing for variables relating to the presence of wildlife, the consistency observed indicates that respondents were knowledgeable about their farms. The effects of recall bias were reduced by only asking respondents for information relating to the previous year. Experimenter bias does not appear to have influenced the risk factors or opinions identified in this study.

Non-significant risk factors

Before we examine the risk factors, it is also important to note the variables that were not associated with these diseases. For example, herd size, repeatedly mentioned in the literature as a risk factor for both brucellosis and TB (where larger herds are more likely to have the disease detected), was a relatively unimportant factor for brucellosis here. The presence or type of water was also not related to either disease, despite being associated with the maintenance of bacterial colonies in the environment (Fine *et al.*, 2011a,b). These differences highlight that local risk factors may differ from those identified elsewhere, and therefore local management will benefit from localised research prior to disease management interventions.

Shared risk characteristics

Until now, livestock disease management has largely focussed on managing one disease individually. However, identification of shared risk characteristics between multiple diseases could help to justify and effectively target management effort, and improve the cost effectiveness of any interventions. In this study, a common risk factor for TB and brucellosis was contact between cattle. For TB, risk factors were the use of barns where cattle movement is not restricted and contact with other cattle herds. Of the respondents that reported keeping cattle in barns this way, 11 of the 13 were dairy producers. Concentration of animals makes direct and indirect cattle-to-cattle transmission more likely as the animals are sharing resources and remain in close proximity to each other (Menzies & Neill, 2005). In this study the practice of tying animals up within the barn was not significantly associated with TB detection, so this could be a worthwhile management change for dairy farmers where possible. For both diseases, Brennan & Christley (2012) identified that the implementation of biosecurity measures is often not achieved, so further research to provide evidence of efficacy for users, as well as reducing costs (in time and money) would be necessary in this region.

The number of farmers sharing the summer pastures, and therefore the number of herds sharing the same space, was a significant risk factor for brucellosis. In this case, 51 of the 53 respondents that reported sharing summer pastures were beef farmers. Separating these herds to prevent direct and indirect spread of the disease should be a beef cattle management priority, whilst further research would determine if this also applies to dairy farming. This requires more investigation into the feasibility of achieving herd separation in this very traditional cattle management system.

Disease-specific risk characteristics

Differences in the risk factors for these two diseases were also identified. No cattle movement between herds variables were associated with TB, indicating that movement control protocols may be sufficient for this disease. However, movements within Cantabria were an important risk factor for brucellosis, supporting previous findings (Dias *et al*., 2009; Gonçalves *et al*., 2009). The significance of local cattle movements in Cantabria, rather than movements from outside the area, is not unexpected as Cantabria suffers higher brucellosis prevalence than most other regions of Spain (RASVE, 2012). Management efforts could focus on improving testing prior to cattle movements, or introducing a period of isolation after movement. A complete cattle movement restriction in areas with infected cattle herds would also be effective.

Although no wildlife variables were associated with brucellosis, the presence of wild boar or badgers was a risk factor for TB. For effective TB management it appears that most effort needs to go into wildlife management in winter. Evidence that wild boar are a disease reservoir for TB in cattle in Mediterranean Spain has already been established (e.g. Gonçalves *et al*., 2009; Naranjo *et al*., 2008) and research is underway into wild boar vaccination projects that could be used to improve the success of TB eradication schemes (Beltrán-Beck *et al*., 2012). Badgers are already associated with TB in cattle in Atlantic Spain and parts of France (Payne *et al.*, 2012), as well as widely in England, Wales and Ireland (Sleeman *et al*., 2009). Further research on the badgers' impact as a TB host in the Atlantic bio-region could be beneficial to TB prevention and eradication schemes in many similar parts of Europe. The exclusion of wildlife from barns is also important as wildlife visits occur because of the concentration of resources (food and water). Simple biosecurity measures have been shown to be 100% effective at preventing badgers access to barns (Judge *et al*., 2011), and ungulate access to fenced areas (Barasona *et al*., 2013a) when appropriately deployed.

The use of a communal cattle race (for restraining animals) appears to show a negative relationship with TB. This could be due to the practice of disinfecting races moved between farms, whereas those kept on a farm may be cleaned less regularly, possibly allowing intra-herd TB transmission. Furthermore, the quality and condition of privately owned races may be variable, and those in bad condition may result in reduced testing accuracy or missed tests. Further research would be required to understand the importance of this risk factor.

Social influences on disease risk

The opinions questions revealed high levels of concern about these diseases in Atlantic Spain. This, along with the high response rate, indicates that farmers may be likely to accept feasible management changes to achieve eradication of these diseases. Respondents gave similar scores for the likelihood of wildlife transmitting these two diseases to cattle. This indicates a lack of knowledge about these diseases, as in reality of the two only TB is known to spread in this way (Gortázar *et al*, 2007, Muñoz *et al*, 2010). This misunderstanding of the differences between these infections could be improved with education, and this awareness may then positively impact on farm management. Prevalence of both diseases is currently decreasing in the study region (RASVE 2013, 2012), yet the majority of respondents believed it was stable or increasing. Awareness of current disease prevalence and trends may incentivise farmers to work with officials to achieve eradication in their area.

Conclusions

This study identifies for the first time shared risk factors for two diseases in the same region. In Atlantic Spain, management should focus on reducing intra- and inter-herd cattle contacts. Furthermore, wildlife management on winter farms may reduce TB prevalence and improved control over cattle movement between herds may reduce brucellosis prevalence. Farmers' knowledge about both of these diseases indicated that an education programme may be highly beneficial.

Risk factors for disease may vary spatially, with environment or management practices, and temporally, particularly as disease eradication schemes are undertaken. As disease prevalence reduces, currently relatively unimportant risk factors may emerge as critical barriers to achieving disease eradication. Indeed, our findings for TB risk factors differ from a similar study conducted in an area of higher TB prevalence (Cowie *et al.*, 2014). Similar methodology could be repeated to identify how risk factors change in different locations, and hence as the basis for developing more targeted and locally-specific disease management strategies. Disease-specific studies of transmission and risk remain important for building epidemiological understanding, but adopting an integrative approach to management across multiple diseases provides the possibility of large improvements in the effectiveness and efficiency of management interventions.

Chapter 4: Direct and indirect interactions within and between four species in a mixed livestock-wildlife community

Preface

Chapters two and three identified herd level risk factors for disease in cattle. For tuberculosis, the main factors identified were the presence of wildlife, the density of water resources on the farm, the use of enclosed barns and contact between cattle herds. This is valuable information that will help determine disease management policies and direct future research, and could be applied in the shared disease risk factors management approach. However, sufficient knowledge of both the biological and non-biological processes that affect risk mitigation measures is necessary for effective disease management (Pfeiffer 2013). More information is required on the mechanisms by which these risk factors affect the transmission and maintenance of disease, which happens on a smaller scale between individual animals.

Disease transmission is determined by many factors, including the characteristics of the pathogen, the ecology, physiology and behaviour of the hosts, and environmental conditions (Anderson & May 1979; Martin *et al.* 2011; Craft *et al.* 2011; Vander Wal, Paquet, & Andrés 2012). Social behaviour determines direct and indirect interactions between individuals of the same and different species, and therefore is a critical determinant of disease transmission (Böhm *et al.* 2008; Drewe 2010). However, in the past contact rates have been difficult to quantify, and it is only with the increased availability of proximity logging technology that direct and indirect interaction rates are being accurately quantified (Böhm, Hutchings, & White 2009; Drewe *et al.* 2013).

The quantification of contact rates allows us to investigate in more detail the risk factors observed in chapters two and three. As the technology used generates data on an individual animal scale, in depth information about interactions can be quantified. This will improve our understanding of the mechanisms behind the risk factors we previously observed. For example, water was identified as an important risk factor, and now we are able to quantify direct and indirect interactions at water sites. Knowing when and where our study individuals and species interact will significantly improve the design and efficiency of management interventions that aim to reduce disease transmission. This chapter addresses the third objective of the thesis.

Abstract

Livestock diseases can have considerable negative effects on human health and economic activity. Wildlife reservoirs often inhibit disease eradication in sympatric livestock populations. Understanding these inter-species interactions is therefore an important factor in understanding the disease cycle. In this study, we quantified both direct interactions and indirect interactions in a mixed livestock-wildlife community. Direct interactions occur when close proximity is recorded between known individuals, recorded by proximity loggers. Indirect interactions occur when individuals share the same space and/or resources, and can be quantified by proximity loggers or GPS fixes.

Our study was conducted on a cattle farm in south-central Spain, where multiple wildlife and livestock species are considered to be part of a tuberculosis (TB) host community, maintaining bovine TB in the area. Proximity loggers recorded over 55,000 direct interactions and 12,500 indirect interactions at base stations placed in the environment were recorded by proximity loggers, as well as over 40,000 GPS logger fixes, used to estimate home ranges and daily activity patterns for each species. Of the direct interactions, only 0.38% were between wildlife and livestock. Connectivity analysis and network visualisation showed that pigs had strong connectivity to cattle and the full social network of all species.

Over 90% of indirect interactions between cattle and wildlife occurred within the estimated three day critical time window for the environmental survival of *Mycobacterium bovis*. Red deer home ranges and daily activity patterns revealed significant potential for indirect interactions, particularly in autumn. Pigs and deer also cross the farm boundary regularly, and may thus pose a between-farm interaction risk. Most direct and indirect inter-species interactions occurred at water points.

The infrequent occurrence of direct interactions between individuals from different species suggests that they are unlikely to be the sole mode of disease transmission. Indirect interactions may therefore play an important role, and more research on the

environmental survival of pathogens is necessary. Nevertheless, our study has identified that interactions may differ between the species pairs, and vary with the availability of food and/or water resources. In the study area, greater emphasis needs to be placed on the roles of red deer and domestic pigs in the TB host community in order to achieve eradication.

Introduction

Livestock diseases pose a significant threat to human health, social wellbeing and economic activity (Martin *et al*., 2011). Over 77% of the pathogens affecting domestic mammals can infect multiple hosts (Cleaveland *et al*., 2001). This can result in epidemiologically connected multi-species communities in which a pathogen persists (Haydon *et al*., 2002). Disease transmission depends on the characteristics of the pathogen, such as the minimum infective dose, the virulence of the pathogen and how long it can survive in the environment. It is also dependent on the ecology, susceptibility and pathogen excretion rates of each host species, existing disease prevalence, environmental conditions and contact between infected and susceptible animals (Anderson & May, 1979; Craft *et al*., 2011; Martin *et al*., 2011; Vander Wal *et al*., 2012). Social behaviour strongly influences interactions between individuals of the same and different species (Böhm *et al*., 2008; Drewe, 2010). This study focusses on quantifying the contacts between individuals from multiple species in a disease community. Identifying the different roles each species plays in the transmission of a disease will improve the evidence base for making decisions about strategies for effective disease management.

Inter-species transmission of disease may occur through direct or indirect interactions between individuals. Direct interaction refers to direct physical contact or very close proximity between individuals. Indirect interaction refers to shared space use, where individuals visit the same location at different times. Transmission through indirect interaction requires the pathogen to survive exposure to the environment. Both interaction types are more probable at commonly used watering or feeding stations (resource points) that cause animals to aggregate.

Proximity data loggers and GPS technology allow for the quantification of direct and indirect interactions (Drewe *et al*., 2012; Prange *et al*., 2006; Walrath, 2011), but to date only two studies have attempted to quantify interactions between multiple species; both studying contact between badgers (*Meles meles*) and cattle (*Bos primigenius*) in the UK with reference to tuberculosis (TB) transmission. Böhm *et al*. (2009) identified considerable individual heterogeneity in contact rates and found that direct contacts between badgers and cattle did occur although they were infrequent. Drewe *et al.* (2013) found that indirect interactions between species were much more frequent overall than direct interactions.

TB is one of the most widespread examples of disease that is prevalent in both wildlife and livestock (Fitzgerald & Kaneene, 2013). Wildlife reservoirs of the disease have been identified in brush-tailed possums (*Trichosurus vulpecula*) in New Zealand (Anderson *et al*., 2013a), badgers in the England, Wales and Ireland (O'Connor *et al*., 2012), wild deer in the USA and Canada (Nishi *et al*., 2006; O'Brien *et al*., 2011), buffalo and wildebeest in South Africa (Renwick *et al*., 2007) and wild boar (*Sus scrofa*) in Europe (Gortázar *et al*., 2011, 2008; Naranjo *et al*., 2008). Primarily caused in cattle by *Mycobacterium bovis* infection, this bacterial disease can spill over into human and wildlife populations. In an infected animal, *M. bovis* can be excreted in all bodily fluids, meaning transmission is possible directly through close contact (aerosol transmission, meat or milk ingestion) or indirectly via contact with the blood, saliva or excreta of the infected individual (Neill *et al*., 2001; Radostits *et al*., 2007).

Control of tuberculosis is particularly complicated where there are multiple livestock and wildlife hosts. This is the case in south-central Spain, an area where relatively low density cattle and pig (*Sus scrofa domesticus*) rearing occurs alongside red deer (*Cervus elaphus*) and wild boar hunting activities (Cowie *et al*., 2014; Gortázar *et al*., 2011; Kukielka *et al*., 2013, appendix 1). There is therefore significant potential for interactions between large game and domestic species. Bovine TB outbreaks occur in distinct clusters within the region, and are significantly positively associated with wild boar TB prevalence and hunting activities (Martínez-López *et al*., 2013; Rodríguez-Prieto *et al*., 2012). The presence of wildlife has also been shown to be a significant risk factor for bovine TB at the individual farm level (Cowie *et al*., 2014). Both cattle and wildlife display high TB prevalences in South-central Spain, and levels in wild boar and red deer have increased over the last twelve years (Vicente *et al*., 2013). It is thought that the presence of these wildlife reservoirs is inhibiting eradication in the cattle population despite a long standing test-and-slaughter scheme (Diez *et al*., 2002; Gortázar *et al*., 2011b; Naranjo *et al*., 2008).

In this study we aim to quantify direct and indirect interactions between cattle, pigs, red deer and wild boar in a mixed community infected with TB. This tests the hypothesis that differences in the direct and indirect interactions between individuals and species in a mixed system will be observed. Based on recent literature, we expect to observe more indirect than direct interactions. To our knowledge, this is the first study to quantify close interactions between known individuals from multiple wildlife and livestock species.

Methods

Study area

The landscape in south-central Spain is characterised by the agroforestry system "dehesa", a savannah-like habitat with low densities of oak trees (*Quercus* spp), commonly adjacent to areas of forest and scrubland (Gaspar *et al.*, 2009). Extensive livestock rearing of beef cattle, small ruminant (sheep and some goats) and Iberian pigs takes place here at low stocking densities (Gaspar *et al*., 2008; Milan *et al*., 2006; Plieninger *et al*., 2004). Farm sizes are highly variable, with a recent study identifying a mean size of 467ha (range 37-2040ha) (Cowie *et al*., 2014). The area has high densities of red deer and wild boar (Acevedo *et al*., 2008) which are managed for recreational hunting activities, often on the same or adjacent land as livestock farming (Herruzo & Martinez-Jauregui, 2013) . TB is prevalent in this area despite test-and-slaughter schemes, affecting 5.35% of cattle herds in the area in 2011 (RASVE, 2013). Large scale sampling studies have also revealed high prevalences in wild boar (59.0%) and red deer (9.4%) in the region (Vicente *et al*., 2013).

Environmental conditions vary throughout the year, with temperature ranging from below zero to over 40°C. A wet season starts in autumn and typically contributes most of the annual rainfall within 3 months. As a result, water and food for animals is often limited in the peak of the dry season (from June to September) and livestock receive supplementary food and water from artificial sources (Kukielka *et al*., 2013, appendix 1). At the end of the dry season, acorns fall from the oak trees (an event called "*Montanera*") providing food for animals, especially pigs and wildlife species.

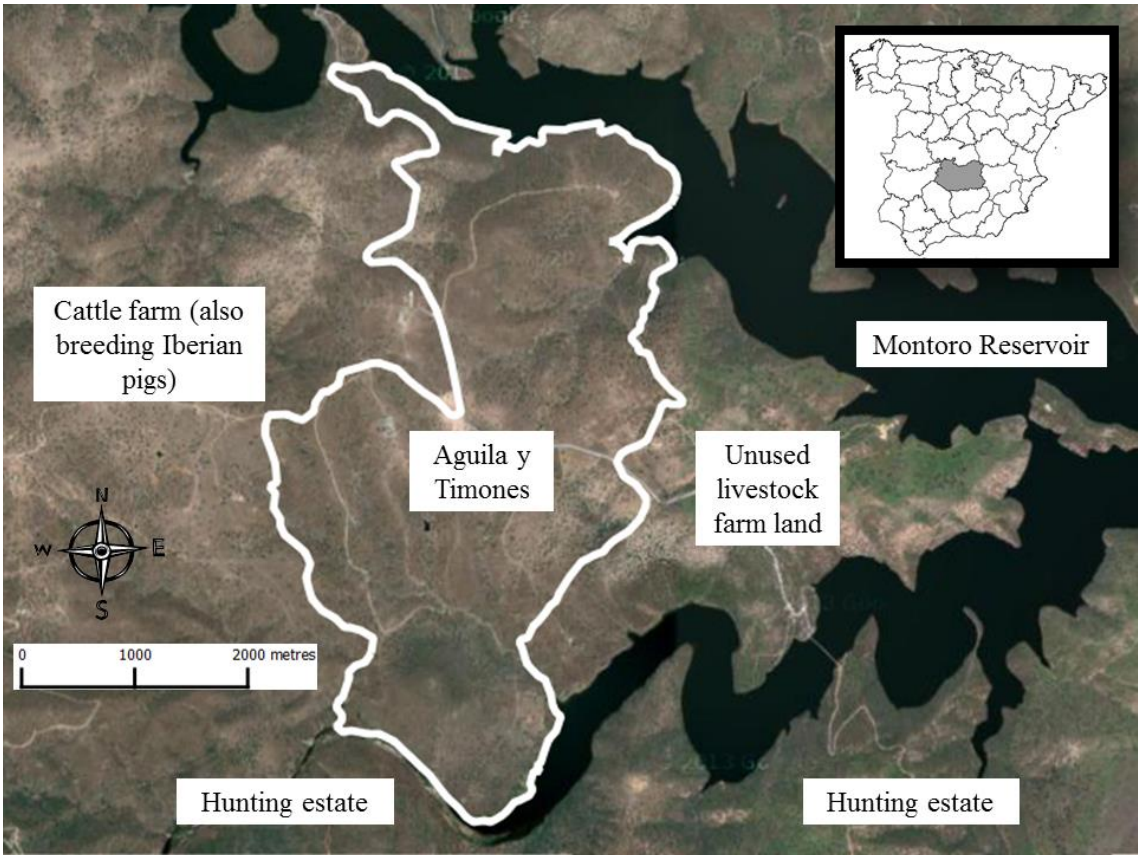


Figure 3: Map showing the region of Castilla-La Mancha (shaded grey) in mainland Spain (insert), and the '*Aguila y Timones'* farm perimeter (white line) and surrounding features.

Study farm

This study was conducted on a traditionally managed beef cattle rearing farm which also conducts hunting activities, called "*Aguila y Timones*", located in Ciudad Real province (figure 3). The farm covers 300ha, and was rearing an average of 90 "Retinta" cattle and 5 adult Iberian pigs during the study period. The cattle were often separated into two herds. Cattle and pigs received supplementary food in the summer months, when pigs were also kept in a piggery to prevent loss of condition. Simple wire fencing was used at the farm boundaries. This contained cattle but allowed pigs and wildlife species to cross into and out of the farm.

The farm is considered representative of cattle farms in the region because of typical management practices, stocking densities, environmental conditions and TB prevalence.

One difference is that parts of the farm border the Montoro reservoir (figure 4), meaning that wildlife and livestock had greater access to water year round than is often available on other farms in the region. Within the farm, water was available from seasonal streams and stream flow was managed by two small reservoirs.

Wildlife densities are moderate compared to hunting estates in the area, but representative of cattle farms, with night-time spotlight transects revealing no more than 15-20 red deer, 10 roe deer and 10 wild boar. Government sampling of the farm's cattle with skin tests confirmed by culture showed a mean TB prevalence of 8.65% during the study period. Wildlife shot on the farm and the neighbouring hunting estates were tested for TB following methods used by Vicente *et al*. (2006). This revealed mean local prevalences of 84% in wild boar and 30% in red deer. Samples from the Iberian pigs on the farm were also tested, with seropositivity indicating that 36% (n=25, including juveniles) had antibodies against a member of the *M. tuberculosis* complex (Aurtenetxe *et al*., 2008).

Data collection

Data collection required the deployment of proximity data loggers (Sirtrack Tracking Solutions, New Zealand) and GPS location loggers (Microsensory Systems, Spain) on the livestock and wildlife species, as well as at base stations around the farm. Prior to collaring any animals, base stations with proximity loggers were placed at the site of key resources on the farm – food points, water sources and control points (figure 4). Food points are places where concentrated cattle feed was provided, either in permanent raised troughs or on the ground. Control points were placed at random within areas with no other resources and no animal paths that lead to these resources. Base stations were set to record contacts with animals wearing collars at UHF30, triggering recording at an estimated radius of 3.1m around the base station (Goodman, 2007). Base stations were placed 1-2 metres above the ground, hung from natural features such as dead trees where possible, and were left in place for 1 month before recording began to avoid bias caused by animal investigation of the novel objects.

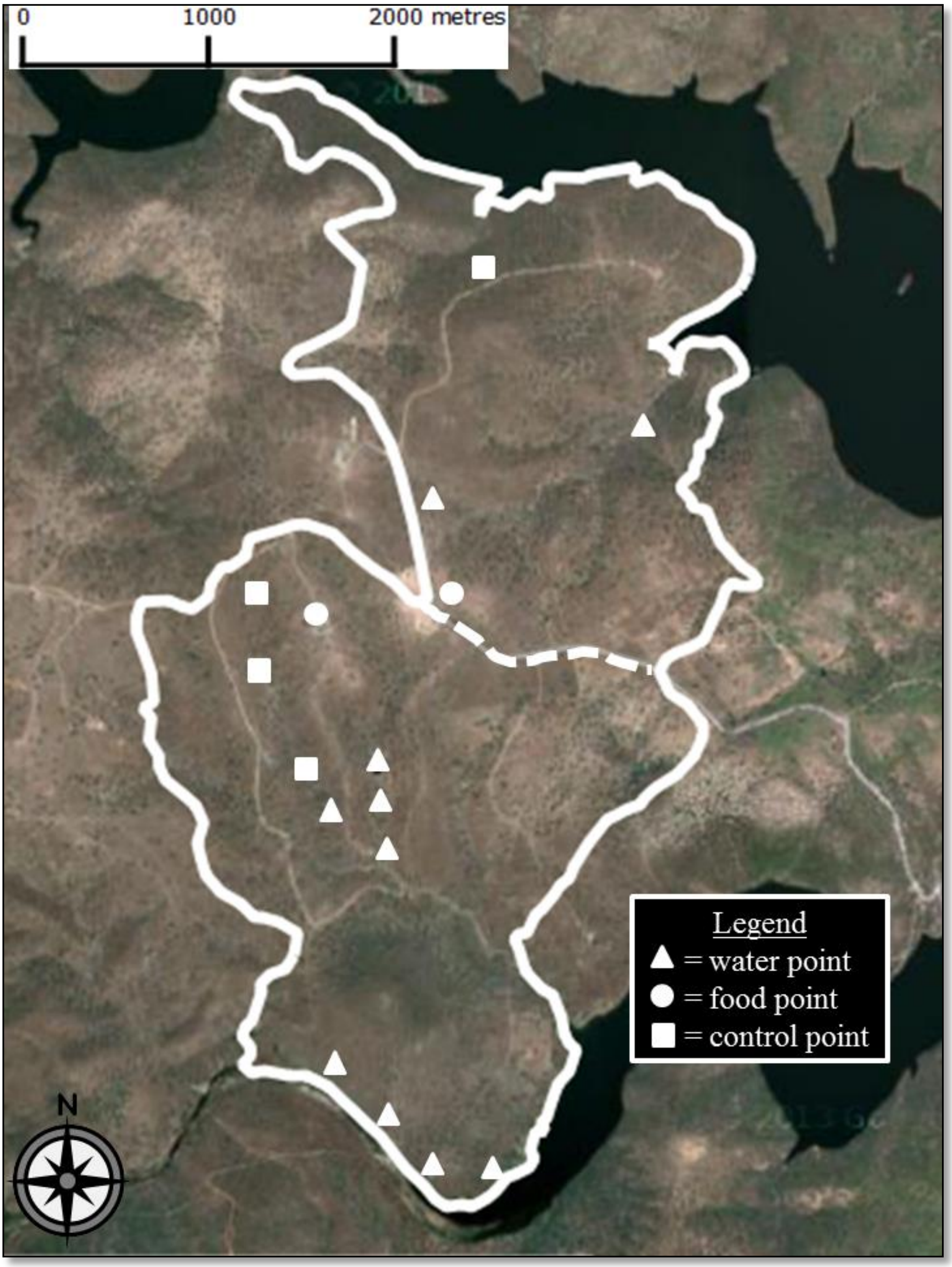


Figure 4: Map of *Aguila y Timones* farm showing the location of proximity data logger base stations. The dashed white line indicates a fence that often used to separate the two cattle herds kept on the farm.

Cattle collaring took place during routine veterinary inspections of the cattle, whilst they were restrained in the farm's own cattle race. Pigs were captured individually and held by hand during collaring. Collaring wildlife species required the use of traps. All work met the Spanish and European standards for animal trapping and research. Two corral and four cage traps were deployed at appropriate locations on the farm after detailed observations of typical wildlife movement and resource use on the farm. They were set only on days when a qualified veterinarian could attend to captured animals and were checked early every morning. The traps, trapping and handling procedures are described in greater detail by Barasona *et al.* (2013b). Red deer and wild boar were anaesthetised, weighed, ear tagged, assessed for condition, age and sex, and had a collar fitted if the weight of the collar was less than 5% of the weight of the animal. The collaring strategy was therefore opportunistic and dependent on the animals captured. All animal proximity loggers were set to a UHF setting of 45, triggering recording if another collared animal comes within a radius of 1.5m (Goodman, 2007). Four GPS loggers were available and these were deployed to maximise information from wildlife species. As these transmitted locations back through the mobile network hourly they are also helped in the monitoring and return of collars. Where collars did not fall off, they were recovered during routine wildlife hunting activities.

Data processing

Data were downloaded from the collars and information from the 24 hours after collaring and before collar retrieval were removed from the data set. Any erroneous records, where letters are recorded instead of numbers, were also deleted. Each dyad of animals that contacted each other should have two reciprocal data sets. In reality they often differ slightly, so the longest data set for each dyad was retained for further analysis. One-way ANOVA tests were used to compare the means of the used and rejected datasets for each dyad.

The collars recorded two contacts with an interval between them of up to 17 seconds as one long continuous contact. This is known as the separation time. Short separation times allow for fine scale data on direct contacts, but have been shown to increase the likelihood of broken contacts being recorded, where one long contact is recorded as multiple shorter contacts (Prange *et al*., 2006). These broken contacts are often recorded as contacts of one second duration, which are not considered valid contacts when evaluating possible disease transmission. Using Drewe *et al*. (2012)'s 'contactweld' function in R (version 2.15.3, R Core Development Team, 2012) contacts with a separation time of 60 seconds or less were merged into single longer contacts. After this, any remaining one second contacts were removed from the data set.

Direct interactions data analysis

Initially, the raw data were arranged in contact frequency and mean contact duration matrices. Seasonal variation in when these contacts occurred was examined with generalised linear models (GLMs). However, these do not account for the amount of time each individual, and all the individuals it could contact, were available for contact (i.e. collared with a proximity logger). The connectivity measures C_{freq} and C_{dur} were therefore calculated to allow for comparisons between species dyads. These were calculated for each dyad of individuals, following the methods of Böhm *et al*. (2009), as:

C_{freq}:

$$\frac{\left(\frac{Number\ of\ contacts}{Number\ of\ days\ individual\ was\ collared}\right)}{Daily\ number\ of\ other\ individuals\ also\ collared}$$

C_{dur}:

$$\frac{\left(\frac{Total\ duration\ of\ contacts}{Number\ of\ days\ individual\ was\ collared}\right)}{Daily\ number\ of\ other\ individuals\ also\ collared}$$

Another way of measuring direct contact was when two or more animals were recorded in close proximity at a base station at the same time. This showed that they were within the 3m radius detection zone at the same time at a particular site on the farm. These contacts are described, and standardised by the number of base stations at each resource type. Seasonal differences were assessed in GLMs. Finally, network visualisation (conducted in Gephi, version 0.8.2) allowed for interpretation of the direct contact structure between all individuals collared. A node in a network can have high network reach by having a large number of connections or very strong connections, or both. Simple standardised network measures of the contact frequency per individual per day and the number of connections per individual were calculated.

Indirect interactions data analysis

GPS data for each species allowed for the calculation of home range areas, and the overlaps between these home ranges. Using the 'adehabitat' package in R, the kernel density estimation was used to calculate the utilisation distribution (UD) with least-squares cross validation for each individual. This method does not rely on predetermined distributions, allows for the incorporation of various centres of activity and can be used where limited data are available. Core and home ranges were set as 50% and 95% UD respectively, and were calculated for each season. GPS data also provided information about the study animals' movements, both on and off the farm. Using the spatial analysis programme QGIS (version 1.8.0), fixes were classified as inside or outside of the farm boundary, and the number of times each individual crossed the boundary was calculated, and standardised by the number of days each animal was collared.

Using the proximity logger data, visits to base stations at key resources were quantified. Standardised connectedness measures C_{freq} and C_{dur} were calculated for each species at each base station type, standardised by the number of days the base station was in place and the daily number of base stations available for contact on each of those days.

Finally, the intervals between visits to base stations by two individuals of the same or different species were classified into a range of critical time windows (CTWs). Thirteen CTWs were selected, ranging from 30 seconds to 12 days. The number and percentage of contacts and the number divided by the number of base stations at each resource were calculated for each dyad of species which both contacted base stations at each resource.

Results

Data returns

Proximity data loggers returned over 400,000 direct contacts between 17 cows, eight pigs, two red deer and two wild boar. This represented 24%, 63%, 13% and 20% of the estimated available population of each species respectively. Data were collected continuously for 2 years starting in summer 2010, though there were only 202 consecutive days where all four species types were collared at the same time.

After removing reciprocal contacts from each dyad of individuals, significant levels of agreement between the used and rejected datasets were identified. Tests were calculated for each contact dyad and p values ranged from 0.02 – <0.0001, with R^2 values from 0.61 – 0.97. The data contained large proportions of one second contacts. To provide an example with the cattle contact data, the raw data contained 43.1% (95% CIs = 35.7 – 50.4) one second contacts. Merging contacts with a separation time of less than 60 seconds resulted in a mean 50.3% (95% CIs = 42.4 – 58.1) reduction in the number of these contacts. However, the percentage of one second contacts remained high after merging, at 47.0% (95% CIs = 44.5 – 49.5). All remaining one second contacts where then deleted from the dataset. Similar patterns were observed in data for all species and the same processes were applied, resulting in a direct contacts dataset of 57,188 contacts. This dataset allowed us to investigate all possible species dyads except wild boar-wild boar and pig-red deer.

Proximity loggers at base stations returned over 75,000 raw contacts from four control, two food and 10 water points around the farm (figure 4). As base stations were set to record at a different detection distance, the data were always taken from the base station records and reciprocal contacts on the collars were discarded. After merging contacts to a separation time of 60 seconds and removing one second contacts, 12,628 contacts remained for further analysis.

GPS loggers were attached to two cows, one pig, two red deer and two wild boar. The herd behaviour of the pigs and cattle means that the livestock GPS data normally represent the position of the majority of the animals of that species. After the removal of any erroneous records, 43,595 fixes were used in further analysis. Most fixes were recorded from cattle (43%) and red deer (44%), whilst pigs (5%) and wild boar (8%) returned fewer because they more frequently lost satellite reception and tended to lose their collars. All species recorded adequate fixes to exceed the minimum of 50 fixes required for kernel home range estimation (Seaman *et al*., 1999).

Direct interactions

Of the 57,188 direct contacts recorded, only 875 (1.53%) contacts were observed between different species, and only 216 (0.38%) were between wildlife and livestock (table 8). Variation was observed over the seasons for both contact frequency and mean contact duration. Cattle-pig and cattle-red deer contacts occurred more frequently in autumn and winter. Considerable variation was observed in the duration of contacts. Cattle-cattle and pig-pig contacts were each significantly longer in winter (GLM, both $p<0.001$) and cattle-pig contacts had significantly longer durations in autumn, during the acorn mast (GLM, $p<0.001$).

Table 8: Contact matrices showing (a) the number of contacts and (b) mean contact durations in seconds (SD) between each species type.

(a)	Cattle	Pig	Wild Boar	Red Deer
Cattle	31582	613	21	193
Pigs		18389	2	-
Wild Boar			-	46
Red Deer				6342

(b)	Cattle	Pig	Wild Boar	Red Deer
Cattle	165 (507)	307 (749)	343 (524)	89 (152)
Pigs		239 (561)	3 (1)	-
Wild Boar			-	1486 (2350)
Red Deer				102 (135)

Direct interactions – connectedness

For intra-species dyads, measures of connectedness were markedly higher for the pig:pig dyad, and this was also the only dyad for which C_{freq} and C_{dur} were significantly correlated. In all other dyads these measures were independent of each other (table 9). Cattle:cattle connectedness may be lower in our sample population than normal as the herd were sometimes separated into two groups on different parts of the farm.

Table 9: Connectedness values (C_{freq} and C_{dur}) for each dyad, with the standard error (S.E.) shown in brackets.

Dyad Type	Dyad	Number of contacts	Mean C_{freq}(±S.E.)	Mean C_{dur}(±S.E.)
Intra-species	Cattle-Cattle	31583	1.58 (±0.84)	238.95 (±15.58)
	Pig-Pig	18389	48.62 (±6.70)	4444.81 (±104.90)
	Red Deer-Red Deer	6342	7.07 (±0.77)	931.39 (±15.19)
Inter-species	Cattle-Pig	613	9.16 (±2.03)	1385.26 (±37.51)
	Cattle-Red Deer	193	6.58 (±1.72)	995.35 (±31.80)
	Cattle-Wild Boar	21	11.40 (±2.26)	1724.40 (±41.85)
	Pig-Wild Boar	2	50.38 (±6.82)	4605.23 (±106.78)
	Red Deer-Wild Boar	46	8.76 (±0.81)	1023.17 (±15.92)

Direct interactions at base stations

Over 86% of the 2441 direct contacts at food, water or control points occurred between cattle, and only 0.73% of these occurred between different species, always between cattle and either pigs, red deer or wild boar. Most contacts occurred at food (52%) and water (43%) points, with only 111 (5%) logged at control points. Within species, there was significantly more cattle-cattle overlap at food and water points in summer than in autumn or winter (GLM, $p<0.01$), but 93% of red deer-red deer contacts occurred in autumn. Of those that did occur between species, over 80% (15/18) occurred at water points and 72% (12/18) occurred in summer. When standardised by the number of base stations, most inter-species contacts occurred at water points (table 10).

Table 10: The number of shared space use events divided by the number of base stations at each resource across the farm for each dyad.

		Resource Point		
Contact type	Dyad	Control	Food	Water
Intra-species	Cattle-cattle	36.33	636	90.75
	Pig-pig	0	0	0.25
	Red deer-red deer	0	0	39.25
Inter-species	Cattle-pig	0	0.50	0.88
	Cattle-red deer	0	0	0.63
	Cattle-wild boar	0.67	0	0.38

Direct interactions – network analysis

The network of contacts between collared individuals (figure 5) shows that most contacts were concentrated within species. Inter-species contacts occurred much less frequently. Whilst red deer had many contacts with each other, wildlife species did not appear to have frequent contact with livestock species.

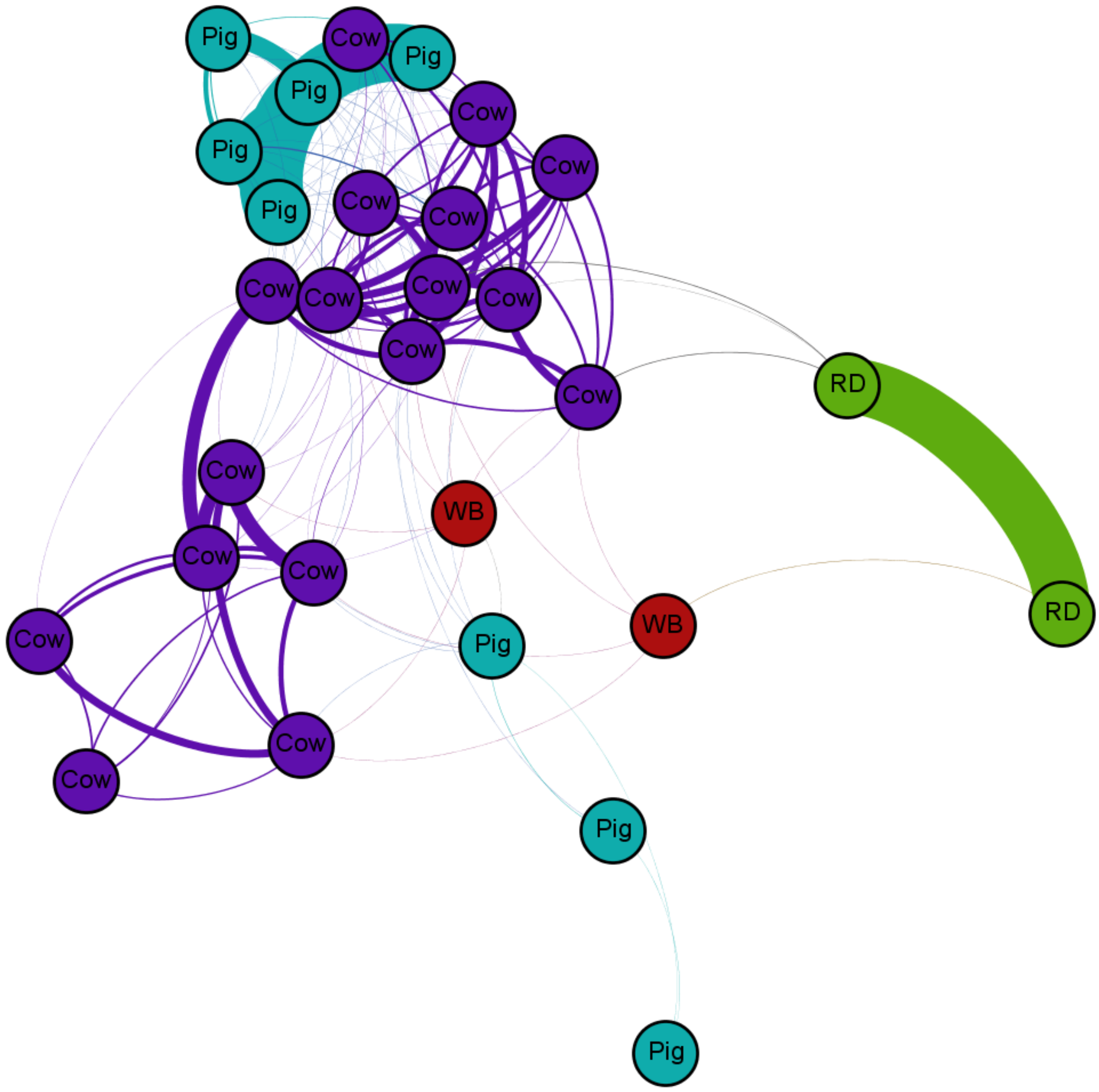


Figure 5: Network visualisation of all direct contacts recorded by proximity loggers. ‘WB’ denotes wild boar and ‘RD’ denotes red deer. The thickness of the lines between the nodes is proportionate to the contact frequency between each dyad of individuals. The visualisation is arranged by the Yifan Hu’s multi-level algorithm (Hu 2005).

The network measures (table 11) show that pigs had a higher standardised number of contacts and network connections than cattle. These pigs are contacting cattle and wild boar (figure 5). Of the wildlife, red deer had a higher contact rate, although the majority of these contacts were with each other. Despite far fewer daily contacts, wild boar had a higher mean number of connections.

Table 11: Species level summary of network measures

	Mean number of contacts per individual per day	Mean number of network connections per individual
Cattle	0.67	0.74
Pig	7.60	1.17
Red Deer	7.09	1.50
Wild Boar	0.23	3.00

Indirect interactions – home range overlaps

Mean livestock home ranges (95%UD) were 2.08km^2 in cattle and 4.21km^2 in pigs. Mean home ranges for wildlife were 7.25km^2 in red deer and 1.96km^2 in wild boar. Home range overlaps were high, particularly between red deer and cattle, whose combined home ranges overlapped by over 4.0km^2(27% and 95% of the total range for each species respectively) throughout the year. Overlaps between individual red deer, cattle and pigs, and pigs and red deer all peaked considerably in autumn. Core (50%UD) ranges were much smaller, with only red deer and cattle maintaining a core range overlap of over 0.5km^2(3% and 12% respectively) throughout the year. Cattle-wild boar overlaps were comparatively low, with no core range overlap recorded.

Indirect interactions – farm boundary crossings

The GPS data also allowed us to investigate animal movements across the farm boundary. Cattle were unable to cross farm boundaries, but pigs showed the highest rate of boundary crossings at 1.4 crossings per day. Red deer showed far more frequent crossings (1.19/day) than wild boar (0.61/day).

Indirect interactions – daily activity patterns

Similarities were observed in the daily activity patterns of red deer and cattle (figure 6). Coupled with similar diets, boundary crossings and high core and home range overlaps throughout the year, red deer may be exposed to pathogens and then return to the farm.

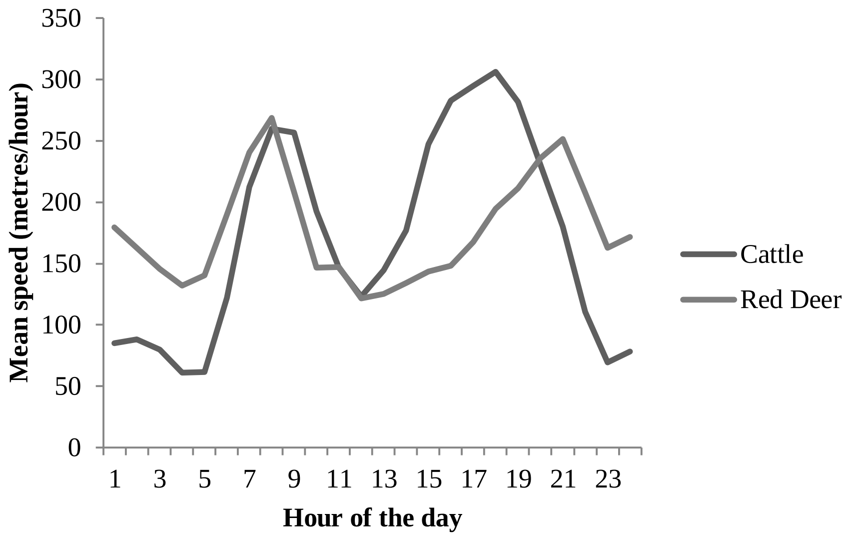
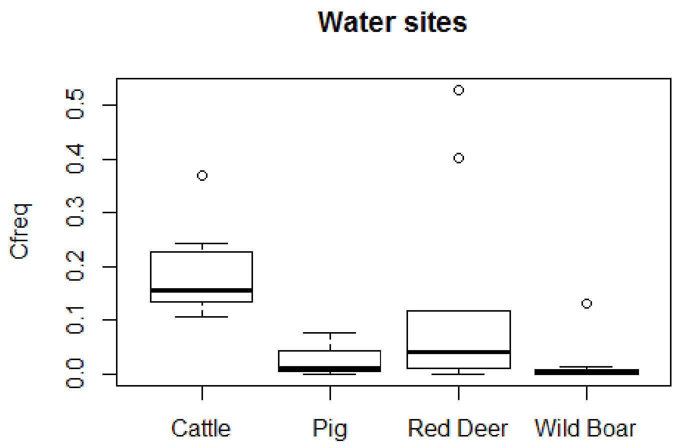


Figure 6: Daily activity patterns of cattle and red deer recorded concurrently by GPS logger collars at '*Aguila y Timones*' farm.

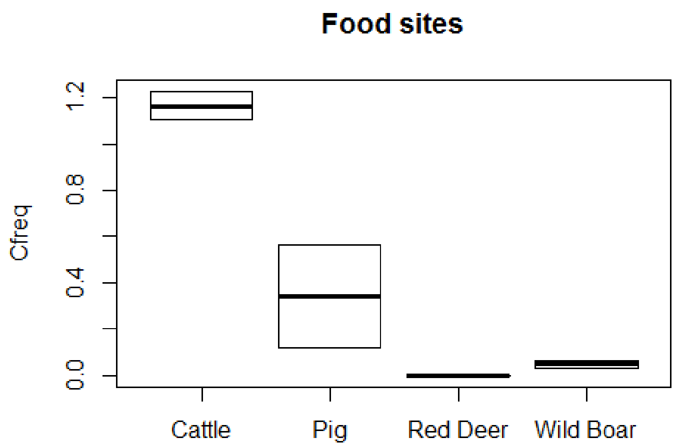
Indirect interactions – connectedness to resources

Over 50% of all individuals of each species were detected at resource locations, with every individual except one red deer being detected at both food and water points. The same C_{freq} and C_{dur} measures of connectedness were calculated between each individual and each resource type. C_{freq} (figure 7) varied considerably between species and resources. Livestock showed the highest connectedness to food locations. Wildlife appeared to use these resources much less, with wild boar showing some connection to control points. These wild boar contacts all occurred at a single base station, and the presence of some long duration contacts here indicates that they may have used this site as a resting place. C_{dur} measures (figure 8) display very similar patterns to C_{freq}, showing that cattle are the only species to stay for longer durations at resource points.

(a)



(b)



(c)

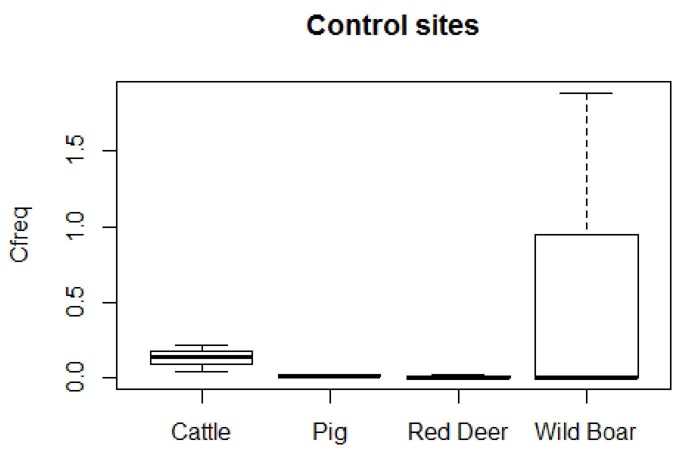
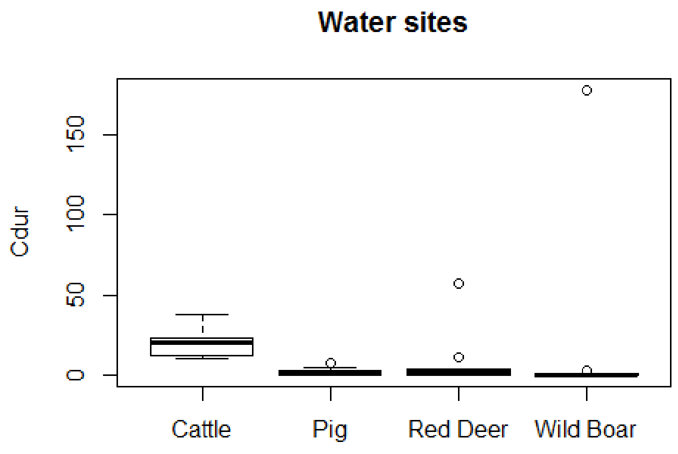
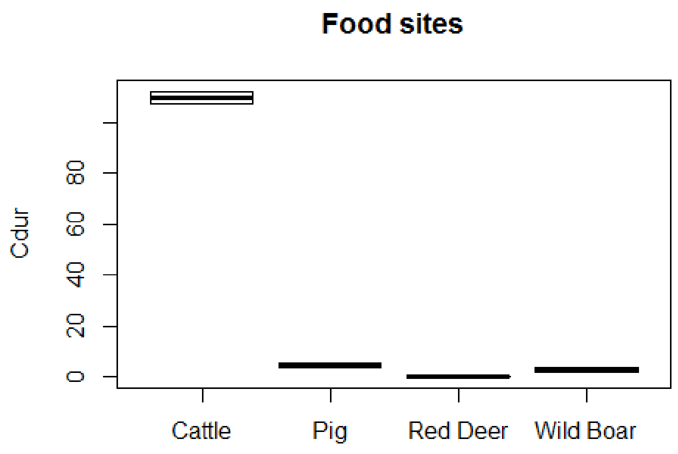


Figure 7: Box plots showing the C_{freq} of each species to (a) water, (b) food, (c) control sites on the farm. Note the scales are different for each resource.

(a)



(b)



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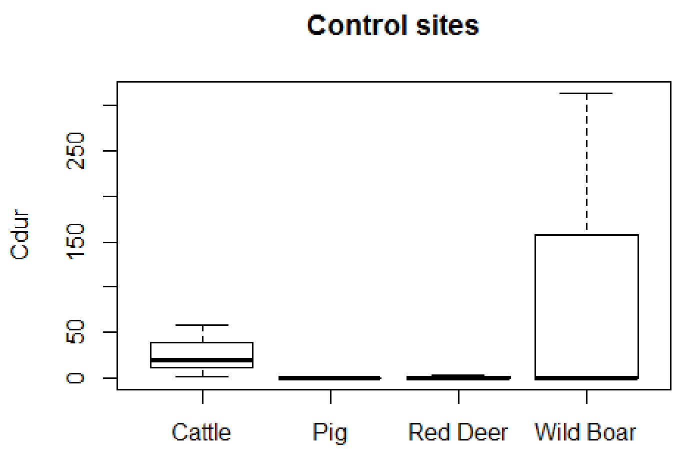


Figure 8: Box plots showing the C_{dur} at (a) water, (b) food and (c) control points on the farm. Note the scales are different for each resource.

Critical time window (CTW) analysis

The number of indirect interactions (where one animal visits a resource and then another of the same or a different species visits the same location at a later time) that occurred with intervals less than the range of CTWs were calculated for intra- (figure 9) and inter-species (figure 10) interactions. Most intra-species indirect interactions occurred within short CTWs, with 50% or more occurring within five minutes at all resource types. When standardised by the number of base stations, the majority of these interactions occurred at food resource points. Pigs were also more likely to have indirect interactions at food sites, but these were more spread out, with 50% of these interactions occurring within a three hour interval. No indirect interactions were recorded between red deer at food sites, but 50% of indirect interactions at water points fell within the five minute CTW. Few indirect interactions between different species were observed, and most interactions fell within longer CTWs.

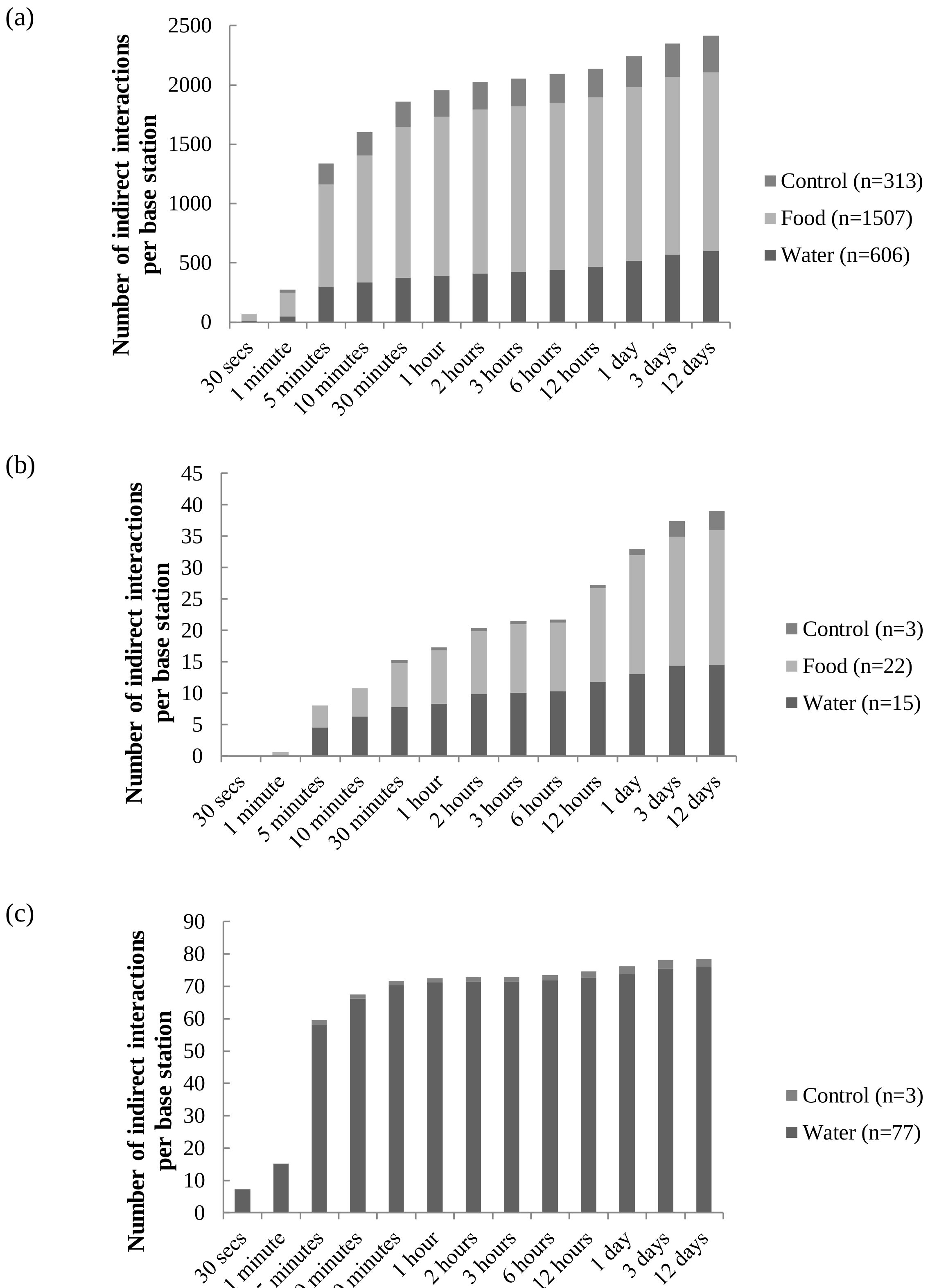


Figure 9: The standardised, cumulative number of intra-species indirect interactions per base station that fell within each critical time window at each resource type for (a) cattle, (b) pigs and (c) red deer. Legends on each graph show the total number of indirect interactions recorded during the study period. No data were available for wild boar as individuals were not collared concurrently. Where resource types are missing no indirect interactions were recorded at these locations. Note the different scales in each graph.

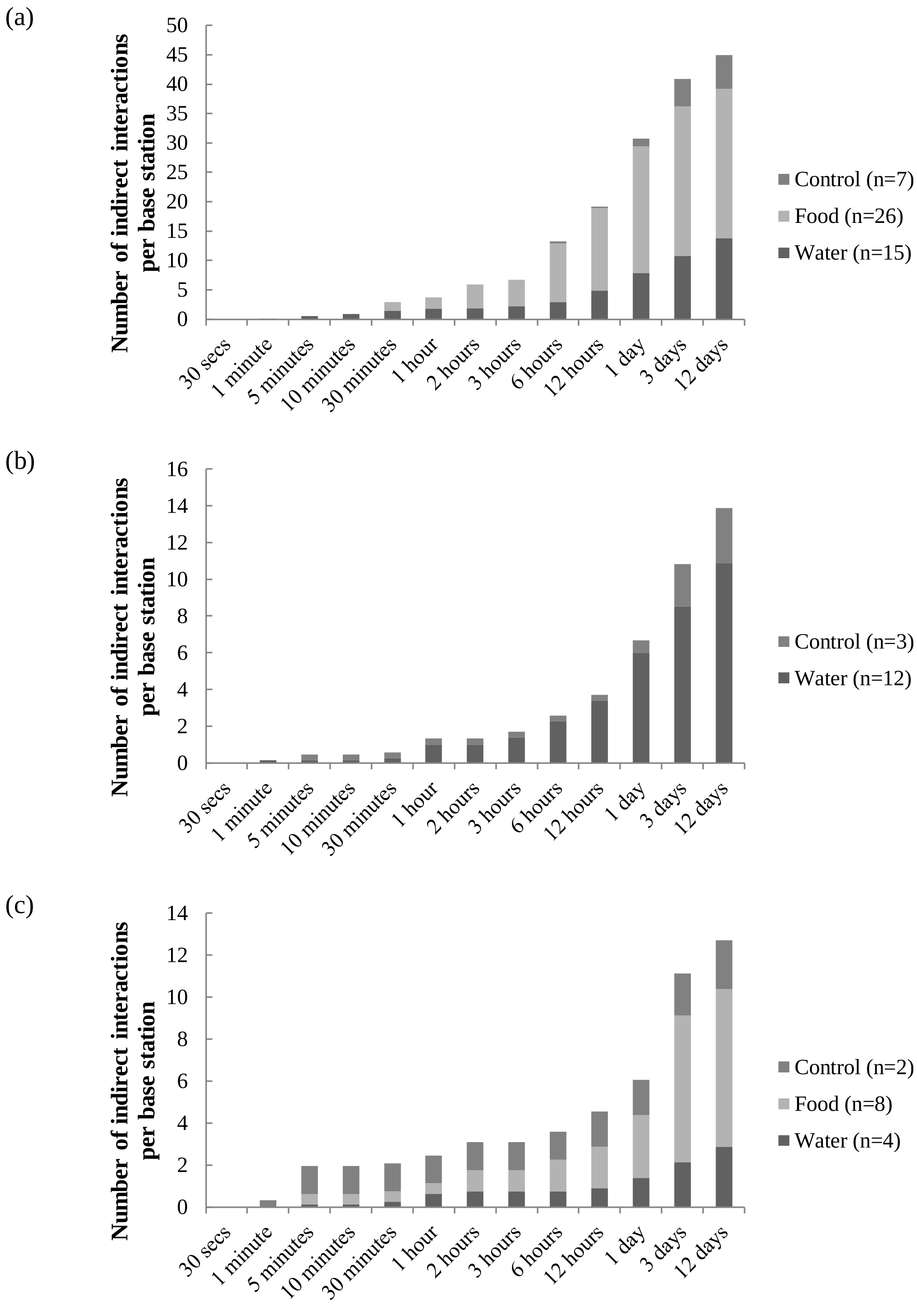


Figure 10: The standardised, cumulative number of inter-species indirect interactions per base station that fell within each critical time window at each resource type for (a) cattle-pigs, (b) cattle-red deer and (c) cattle-wild boar. Legends on each graph show the total number of indirect interactions recorded during the study period. Where resource types are missing no indirect interactions were recorded at these locations. Note the different scales in each graph.

Discussion

This study has quantified direct and indirect interactions between individuals in a disease community, and is the first such study to include multiple livestock and wildlife species. As with similar research (Drewe *et al.*, 2013), data on direct interactions were very limited, despite the long-term continuous sampling. Direct intra-species contacts were frequent, but direct inter-species interactions were relatively rare, identifying more opportunity for disease transmission within species than between species. Futhermore, this highlights the possible importance of the environmental survival of pathogens and indirect interactions between individuals in maintaining disease. In our host community, red deer displayed the most spatial and temporal overlap with cattle. Both red deer and pigs cross farm boundaries frequently, possibly contacting other wildlife or livestock directly or indirectly, and maybe bringing contracted pathogens back onto the stuy farm. Wild boar showed contact with a relatively high number of individuals in the community. Regarding other livestock species, pigs on our study farm displayed strong connectedness to cattle and the resources they use, as well as the whole direct contact network. Pigs may be an important part of the disease cycle, as has been previously identified in another Mediterranean system in Sicily (Di Marco *et al*., 2012). Food sites were mainly used by livestock, but cattle may have indirectly shared water resources with red deer. Both direct and indirect interactions between wildlife and livestock were more likely at water sites in summer (when water is limited) or on pastures at the end of the summer when acorns begin to provide a new food source.

Data evaluation

This study measured inter- and intra-species interactions, which should not be interpreted as measures of disease transmission. Nevertheless, these interactions provide indications of the potential for disease transmission, and the combination of GPS and proximity logger technology provides the best available spatially and temporally explicit data on animal movements and social contacts (e.g. Drewe *et al.*, 2012). However, including more wildlife individuals would allow more significant conclusions to be drawn. Problems were encountered with keeping collars on wild boar and pigs, with pigs occupying traps intended for wild boar, and attracting deer into traps. Wildlife was found at only moderate densities on the farm (Acevedo *et al*., 2008, 2007), which limited trapping success but was considered important as this is representative of cattle farms in the study area. Proximity logger derived data has great potential for informing animal social networks and models (e.g., Marsh *et al*., 2010), but for detailed analysis it is important to include as much of the total population of each study species as possible. Furthermore, if social interactions are being studied in relation to disease transmission and the disease is known to survive in the environment, it is also very important to investigate when and where indirect interactions occur (Kukielka *et al*., 2013, appendix 1).

Direct interactions

The low number of direct inter-species interactions suggested behavioural avoidance of contact between livestock and wildlife The direct contacts that were recorded do indicate that cattle contacts with both red deer and pigs are most frequent in autumn (at the end of the dry season, during the acorn mast). Furthermore, cattle-pig contacts were significantly longer at this time. This relates to previous work on the study farm that showed the number of visits to farm resources increased through the dry season and peaked during the acorn season in autumn (Kukielka *et al*., 2013, appendix 1).

The standardised connectedness measures need to be considered carefully as the number of direct inter-species interactions was so low. The data provide a basis for comparison to future studies. Cattle-cattle connectivity was likely lower than it would have been for one herd as the cattle were separated into two herds at times. Pigs showed very high intra-species C_{freq} and C_{dur}, confirming visual observations that they stay together in a groups, including for long durations whilst sleeping.

The low direct interaction frequency at base stations indicated behavioural avoidance of inter-species contacts at resource points. Those that did occur between cattle and each other species largely occurred at water points and in the summer. In the dry summer season water availability within the farm becomes limited, concentrating animals' use of this resource and leading to contacts that may otherwise have been avoided (Vicente *et al.*, 2013).

The importance of an individual's position in a social network can be measured by the strength of their connections (in this case measured by contact frequency), the number of connections they have, or both. Red deer and pigs both had very high mean contact frequencies. However, network visualisation showed that red deer had very high contact frequency between the two individuals collared, and it is pigs that have more frequent contacts with the whole network. Although wild boar had very few contacts with the network, they did have the highest mean number of network connections, showing no particular avoidance of other species types. Studies have shown that food alone may not be sufficient stimulus for inter-species interaction by wild boar (Wyckoff *et al.*, 2009). However, male feral swine in Texas and wild boar in Switzerland were found to be significantly more attracted to sows in oestrus than to food (Wu *et al.*, 2012; Wyckoff *et al.*, 2009). As interbreeding has been observed in the study region this may be a factor influencing wild boar behaviour here also. Though relatively infrequent, irect interactions may still be an important part of disease transmission. Further data on the likelihood of transmission of a pathogen during a direct contact event would allow for the more accurate assessment of their importance.

Indirect interactions

In our data set, year round core and home range overlaps were highest for cattle and red deer, with all overlaps increasing in autumn (during the acorn mast). This agrees with the results of Kukielka *et al*. (2013) and indicates that pathogens could be shared between these species (appendix 1). This is particularly true as foraging behaviour in the same area may increase the likelihood of transmission of pathogens between different individuals. Furthermore, foraging may be taking place at similar times for cattle and red deer as their daily activity levels follow such similar patterns. Red deer also crossed the farm boundary more than one time per day on average, increasing the risk of encountering disease outside the farm and then transmitting it to livestock within the farm. However, pigs also showed moderate levels of home range overlap with cattle, and the highest rate of boundary crossings. Further research and testing of pig and red deer movements and disease in this area may be important for a fuller understand of disease maintenance and spread. In terms of disease transmission, the relative importance of each species will depend on the dyad and the pathogen. For instance, red deer share more viral pathogens with cattle, whilst wild boar have more pathogens in common with pigs.

Most indirect interactions between individuals from different species occurred at water points. Red deer and cattle also showed the highest connectivity to water, though red deer generally stayed for shorter durations. Water points should therefore be considered a potential hotspot for disease transmission between wildlife and livestock is south-central Spain. Vicente *et al.* (2007a) described a positive association between wildlife aggregation at water and ground level feeding sites and tuberculous-like lesions on large game hunting estates, suggesting that host aggregation could drive disease transmission. Pathogen control strategies should focus on water site management. Additionally, the density of water points on farms has been shown to be related to be negatively associated with TB risk in cattle (Cowie *et al*., 2014 (chapter 2); Kaneene *et al*., 2002). This means that having less water sites is associated with an increased risk of bovine TB on the farm. The availability of water on our study farm from the reservoir introduces bias into our results. However, as water causes aggregation even when other sources were available, this suggests that water may be a very important risk factor for disease in places where it is more limited. In combination with the known TB prevalence data in the area and the knowledge that wildlife here support the disease in the absence of cattle (Corner, 2006; Gortázar *et al*., 2005; Naranjo *et al*., 2008; Vicente *et al*., 2006), it seems likely that indirect interactions are more important than direct contact in the transmission of TB in this area.

Critical time windows

Intra-species indirect interactions occurred much more frequently in short CTWs than inter-species interactions. Cattle and pigs had most of these contacts at food sites, though red deer never utilised the same food sites and instead showed most indirect interactions at water points. All species showed more indirect interactions at resource sites compared to control sites. It is clear that inter-species contacts are avoided, with less than 50% of use of the same resources by different species occurring within a 6 hour CTW. Indirect interactions between cattle and pigs or wild boar happened mostly at food sites, whereas red deer used the same water resources. A review of the literature on the survival of *M. bovis* in the environment applied to this region suggests that on average there could be a 3-day CTW in the dry season, and a 12-day CTW in the wet season (Kukielka *et al*., 2013, appendix 1). Over 90% of intra-species indirect interactions at food or water resources occurred within a 3-day CTW. In the same region, Barasona *et al*. (2013a) demonstrated that separating wildlife and livestock access to water resources appears to have reduced TB prevalence in cattle. As pigs and cattle were more closely connected to food resources it may also be important to consider separation of livestock at feeding stations. This is currently especially important in the study region as pigs are not subject to test and slaughter controls for TB.

Management implications

As most direct and indirect interactions occur within species, livestock disease testing and management (such as test-and-slaughter schemes) remain important, as does large game disease management. Pigs were well connected to resources and the direct animal social network on this study farm. At present, pigs are not monitored for TB in Europe, though *M. bovis* infection is increasingly reported in several countries (e.g. Bailey *et al*., 2013; Di Marco *et al*., 2012). In south-central Spain, pigs are increasingly being refused by slaughterhouses due to visible TB-compatible lesions (Christian Gortázar, *pers comms*). The TB seroprevalence in pigs of over 30% identified in this study suggests further research into the significance of pigs as a TB host could improve our understanding of the disease cycle on these traditionally managed farms. Larger scale prevalence testing would indicate if a test-and-slaughter scheme would be beneficial, to reduce disease prevalence in pigs and other species.

Red deer showed more direct and indirect interactions with cattle than between wild boar and cattle. Thus far, research efforts in this area have often focussed on wild boar as the main wildlife disease reservoir. Our data showed that red deer cross farm boundaries frequently, moving into hunting estates and/or other livestock farms. Within the farm, they had large home range overlaps with cattle year round, with direct contacts with cattle occurring significantly more often in autumn and winter. Furthermore, their daily activity patterns were notably similar to those of cattle, particularly in the morning. Interactions aside, this also suggests they could be part of any vector-borne disease cycles (e.g. bluetongue). These results suggest that the possible role of red deer in the disease cycle in this region of Spain should receive further investigation. However, TB prevalence in the region has recently been shown to be stable at around a mean of 9.4%, whilst mean wild boar TB prevalence is 59.0% and has been increasing over the last decade (Vicente *et al*., 2013). Further research into direct and indirect interactions between wild boar and livestock is also necessary.

All data from the proximity loggers suggested that the study animals avoid direct inter-species interactions. Those that were recorded may still have epidemiological significance, yet indirect interactions also emerged as important. Understanding the environmental survival time of a minimum infective dose of a disease would show which of the CTWs proposed in this study were important. Regarding TB, a recent literature review identified only 15 studies published between 1930 and 2011, that estimated *M. bovis* environmental survival times. Survival times ranged from no *M. bovis* recovery to recovery of genetic material after up to 300 days, on a wide range of substrates. In our study, intra-species indirect interactions occurred within short CTWs, suggesting they would also be a potential route for infection with viral diseases which tend to have shorter environmental survival times (Kukielka *et al.*, 2013, appendix 1). The role of indirect interactions in disease transmission within and between species needs to be explored further.

Management strategies that reduce shared wildlife and livestock space use are likely to achieve greater reductions in inter-species disease transmission. In our study, most direct and indirect inter-species interactions occurred at water. This is especially noteworthy given the presence of the reservoir as a water source on our study farm. Typical farms in the area do not have this additional water source and therefore more interactions may occur at water points, particularly when water becomes limited in the dry season. Further research should focus on reducing opportunities for indirect interactions at water points, possibly by using species specific fencing (e.g. Barasona *et al.*, 2013a).

Conclusions

Direct interactions were concentrated within species, and were relatively less frequent than indirect interactions, suggesting that indirect interactions could also play an important role, depending on the movement and environmental survival of pathogens. Future research and management needs to focus on indirect interactions and the environmental survival of pathogens. In south-central Spain, greater emphasis needs to be placed on the roles of red deer and domestic pigs in the TB disease cycle in order to achieve eradication. Temporally, management should focus on preventing interactions at water points in summer and around oak trees during the acorn mast in autumn. This study has contributed to our understanding of multi-species host communities, quantifying direct and indirect interactions between multiple species and showing how they are influenced by the seasonal and spatial distribution of key resources.

Chapter 5: Effective and practical interventions for bovine tuberculosis management

Preface

In chapters two and three disease risk factors were identified at the farm level, and these were explored in greater detail in chapter four as individual level direct and indirect interactions between multiple hosts in a disease community were quantified. This generated a wealth of information that can be used to determine the most effective management interventions for the study region. However, the success of these disease management interventions is determined by the extent to which stakeholders enact the necessary controls or changes on local scales (Munyeme *et al.* 2010; de Garine-Wichatitsky *et al.* 2013a). Their ability to implement disease management interventions, and their tolerance of recommended interventions needs to be carefully considered before these are implimented (White & Ward 2010; Benjamin *et al.* 2010). Furthermore, they possess detailed local knowledge that could assist in the development of environmentally, economically and socio-culturally appropriate disease management strategies (Cowie *et al.* 2014). Whilst experts might determine which interventions are likely to be effective (based on known risk factors, chapters two and three), it is the stakeholders who would have responsibility for implementing the controls who would know whether the changes required are practical. Effectiveness and practicality are both important in determining which interventions should be introduced.

Recent studies have called for the integration of social science approaches with more traditional bioscientific research on bovine tuberculosis (Pfeiffer 2013). This chapter investigates the views of different stakeholder groups that manage the wildlife and livestock in these study systems. Often, especially in mixed systems, these stakeholders will have different and sometimes opposing management objectives. This can lead to conflict, reducing dialogue between stakeholders, escalating disease management costs and increasing the time to disease eradication (Gortázar *et al.* 2010). By consulting all relevant stakeholder groups on the same possible interventions, differences are likely to emerge about which of these they consider appropriate. This may lead to different stakeholders undertaking different interventions to contribute to disease management.

This chapter addresses the fourth thesis objective, and at the time of writing has been submitted to the *Veterinary Journal* for publication, along with co-authors Michael Hutchings, Christian Gortázar, Piran White and Joaquín Vicente. This chapter is my own work.

Abstract

Livestock disease control strategies are usually determined at national and international levels, yet their successful implementation is determined by the stakeholders effecting them on local scales. Such stakeholders may also have detailed knowledge that would contribute to the development of disease controls that are suited to the socio-cultural and environmental conditions where management is undertaken. In this study we aim to evaluate a list of potential bovine tuberculosis (TB) management interventions for south-central Spain. This area has high TB prevalence in wildlife and livestock, so veterinarians, livestock farmers and hunters are all key stakeholders in TB management. A literature review identified possible management activities. The effectiveness of each intervention was ranked by local experts, and practicality was ranked by hunters, cattle farmers and veterinarians, using a best-worst scaling exercise. The most effective intervention, the banning of supplemental feeding of game species, was not considered practical by stakeholders. The most effective and practical interventions were the separation of wildlife and livestock access to waterholes, testing cattle every three months on farms with a recent positive TB case and removing gut-piles from the land after hunting events. Although all three of these options were well supported, each stakeholder group supported one of them more strongly, suggesting that it might be effective to promote different disease management contributions in different stakeholder communities. This integrated approach will allow for the identification of the optimum combination of management tools that can be delivered efficiently.

Introduction

Livestock diseases have a significant impact on human health and economic activity. Many are existing or emerging zoonoses (Jones *et al*. 2013), and still more can infect wildlife species, creating reservoirs of disease that make eradication difficult (Haydon *et al.* 2002). The cost of the resulting loss of productivity, reduced reproductive rates, mortality, reduced food security, trade restrictions, impaired economic development, disease control and surveillance strategies have significant impacts in both the developed and developing world (Perry & Grace 2009; Schley *et al.* 2012; Kleinlützum, Weaver, & Schley 2013).

Disease management interventions are often determined on national and international scales, yet are implemented by stakeholders on local scales (De Garine-Wichatitsky *et al.* 2013a). Diverse management objectives and broader social, economic and cultural factors influence stakeholders' support for disease management strategies. The history of the management of a disease will have a bearing, for example if there have been scientific uncertainties at any stage of understanding the disease system (e.g. Woolhouse & Wood 2013), or if one or more groups of stakeholders are strong opponents of the required changes to land, livestock or wildlife management (Olmstead & Rhode 2007). Stakeholder tolerance for a management intervention is likely to determine its success (White & Ward 2010; Benjamin *et al.* 2010), and involving stakeholders in the development of interventions appears to help to gain their support (Brook 2010). Farmers play a critical role in the success of livestock disease control programmes (Munyeme *et al.* 2010; De Garine-Wichatitsky *et al.* 2013b). Furthermore, stakeholders are likely to have detailed local knowledge that will be important in the development of strategies that are well suited to local socio-cultural and environmental conditions. Well supported interventions would generate high quality data and feedback, and provide a platform for learning, communication and conflict resolution between different stakeholder groups (Dougill *et al.* 2006; Reed 2008; Austin *et al.* 2010).

Socio-economic research methods

Though they have not often been applied to biological problems, a wealth of socio-economic research methods exist, that could help to inform the development of wildlife management strategies, many of which have been developed initially for use in market research or medical decision making. These can be applied to livestock disease, assessing stakeholders' attitudes and preferences for a range of management interventions. Techniques include choice experiments, Q methodology, adaptive conjoint analysis and best-worst scaling (Cross *et al.* 2009; Austin *et al.* 2010; Bumbudsanpharoke, Moran, & Hall 2010; Rudd 2011). Best-worst scaling (BWS) can be used to rank items such as possible management interventions or priorities by criteria such as importance, effectiveness or practicality. In environmental science, this technique has been used to quantify scientists' priorities for global biodiversity management (Rudd 2011) and to inform decision making about greenhouse gas management in agriculture (Jones *et al*. 2013). The method has only once been used to assess livestock disease interventions, investigating management interventions for *Escherichia coli* infection in cattle. Experts were consulted on the effectiveness and practicality of 30 different potential interventions. The ranked practicality and effectiveness of each intervention was then plotted to identify which could be effective (Cross, Rigby, & Edwards-Jones 2012). Such studies have the potential to contribute valuable information to the decision-making process, and are not difficult to implement using software designed for market research.

Bovine tuberculosis

One of the most important livestock diseases worldwide is bovine tuberculosis (TB), caused by *Mycobacterium bovis* infection. Thought to cause over one million new human cases of TB each year (Cosivi *et al.* 1998; Torres-Gonzalez *et al.* 2013), the multi-host nature of the pathogen means that not only cattle (*Bos primigenius*) are affected. Other wildlife and livestock can also become infected (Gortázar *et al.* 2007; Di Marco *et al.* 2012; Fitzgerald & Kaneene 2013; Napp *et al.* 2013). These populations can form wildlife reservoirs of the disease for cattle, as has been found with goats and pigs (Bailey *et al.* 2013; Napp *et al.* 2013), Eurasian badgers (*Meles meles*) in the UK and Ireland, and wild boar (*Sus scrofa*) on the Iberian peninsula (Naranjo *et al.* 2008; Jenkins, Woodroffe, & Donnelly 2010).

In livestock, the disease carries high social and economic costs for farmers, through reduced productivity and movement restrictions as a result of disease eradication schemes, where infected animals or herds may be slaughtered to prevent disease spread. Although financial compensation may be provided, farming communities still bear high emotional and indirect financial costs (Brook & McLachlan 2006). Management in cattle has focussed on test-and-slaughter schemes, the culling of wildlife reservoirs and improving farm biosecurity (Artois *et al.* 2001, 2011; Fitzgerald & Kaneene 2013), but the disease is still prevalent worldwide. The importance of social support for management has been demonstrated in two states in North America where there have been outbreaks of TB in cattle and free-ranging white-tailed deer (*Odocoileus virginianus*). In Minnesota, a small isolated outbreak of TB in cattle and deer was contained with proactive deer culling and total bans on baiting and feeding deer, which would not have been possible without support from farmers, hunters, the general public and politicians (Carstensen, O'Brien, & Schmitt 2011). However, in Michigan recreational hunters manage deer populations to encourage high densities, whilst farmers are seeking to control TB using a management strategy that includes culling. After over a decade of disease control effort these conflicts and the disease still persist, and there was little public support for the interventions required (Carstensen, O'Brien, & Schmitt 2011; O'Brien *et al.* 2011). Conflicting management objectives also affect wildlife TB reservoirs in game species in Spain, and TB in badgers and cattle in the UK. Disease management will be optimised if stakeholders collaborate in their disease management efforts despite different or even conflicting management objectives. To facilitate this, it has been recommended that improvements to management should begin by targeting stakeholders that are most likely to adopt (Baumgart-Getz *et al*. 2012). Therefore, stakeholders' opinions of the practicality of management interventions has to be assessed.

Aims

Recent research (chapters 2, 3 and 4) and local experience has revealed a number of potential management interventions that could reduce TB levels in cattle in an area of high prevalence. In order to guage likely support for their implementation, it is important to evaluate their perceived efficacy, and how practical they are for stakeholders to implement. This tests the hypothesis that different stakeholder groups will rank different potential management activities as practical. Consensus or differences of opinion between stakeholders will help to identify the interventions that re most likely to be accepted, and who can implement them. In this study we use a best-worst scaling approach to rank a list of potential interventions for bovine TB management, by effectiveness and practicality. Effectiveness is assessed by an expert panel and practicality by the stakeholders who would implement such changes.

Methods

Study area

Here, we focus on an area of south-central Spain (Ciudad Real province, Castilla-La Mancha) where a complex epidemiological scenario for TB is formed by the presence of mixed wildlife and livestock populations (Allepuz *et al.* 2011; RASVE 2013). Rural land use in this region is composed of heterogeneously mixed livestock agriculture and game rearing activities. Low stocking density livestock farming takes place in a typical agroforestry system called "dehesa" (Plieninger, Pulido, & Schaich 2004), characterised by the presence of *Quercus spp*. oak trees on pasture and scrubland, through which cattle and other livestock species often mix freely. Farm sizes are highly variable and game rearing activities often take place on the same or adjacent land (Delibes-Mateos *et al.* 2009; Cowie *et al.* 2014; Kukielka *et al.* 2013, appendix 1). Cattle herd TB prevalence in the region in 2011 was 5.35%, amongst the highest in Spain (Rodríguez-Prieto *et al.* 2012; RASVE 2013; Martínez-López *et al.* 2013). Currently, cattle farms in high prevalence areas with a recent positive case are tested two times a year, with a minimum 4-6 month gap between tests (RASVE, 2013). Large game species wild boar and red deer (*Cervus elaphus*) are widespread and prevalent (Acevedo *et al.* 2007, 2008) and have high levels of TB prevalence; 59% in wild boar and 9.4% in red deer on hunting estates in the region (Vicente *et al.* 2013). The presence of disease in both wildlife and livestock makes farmers, hunters and veterinarians all stakeholders in bovine TB management, although hunters are not currently formally involved in cattle TB control.

Literature review

A literature review of known management interventions for TB in mixed wildlife:livestock systems was undertaken in order to generate a list of options that could be presented to stakeholders in the study area. Web of Knowledge and Science Direct were searched with multiple combinations of relevant terms including "bovine tuberculosis", "bTB", "intervention", "management", "strategy", "livestock", "tools" and “wildlife reservoir”. The relevance of the resulting papers to our study area was considered, and government reports about TB management in the region were also evaluated. At this stage, interventions were included based on experimental or theoretical evidence. Management interventions that addressed the risk factors identified in chapters two (Cowie *et al.* 2014) and four were incorporated. This resulted in a list of 40 possible management interventions to reduce TB in cattle in south-central Spain.

Expert ranking of effectiveness

In order to select the most likely subset from a large number of potential interventions and to evaluate their effectiveness, a panel of six experts was consulted. All experts were qualified veterinarians engaged in full time academic research into wildlife and livestock disease management in the study area. The objectives of the study and their role in it were explained to them. They were presented with the list of 40 interventions, and asked to categorise each one based on its effectiveness for reducing TB prevalence in cattle (table 12). It was strongly stressed that this needed to be a measure of effectiveness, and that practicality or cost should not be considered at this stage. The total score for each intervention ranked them by effectiveness, and the top twenty were used in the questionnaire stage of the study.

Table 12: Categories and scores for ranking interventions based on expert opinion

Category	Score
Priority retain	+2
Retain	+1
Reject	-1
Don’t Know	0

Questionnaire design

The BWS questionnaire was designed in Sawtooth (version 7.0.26, Sawtooth Software Inc, USA), and was accessible online or in printed paper form. The front page of the paper or website explained the purpose and aims of the study, the use of the data and confirmed the respondent's anonymity in all data analyses. Respondents were informed about the estimated time of completion of 15 minutes, and provided with an email address to contact if they had any questions or concerns. Consent to participate was then obtained by respondents ticking a consent box. The questionnaire had four sections (table 13), and 'skip logics' were used to ensure that respondents only answered appropriate questions based on their primary involvement with TB management (as hunters, farmers or veterinarians). It is worth noting that some farmers may also have been hunters or veterinarians, and respondents were classified by their primary source of income.

Table 13: Summary of the questions and BWS scaling exercise completed by respondents in Ciudad Real province

Part	Section	Estimated time to complete	Questions	Response options
1	Respondent details	1 minute	A. How old are you?	<20/ 21-30/ 31-40/ 41-50/ 50+
			B. Are you a land owner?	Yes/No
			C. What is your main source of income?	Hunting/Veterina-ry work/Cattle farming
2	Knowledge and experience of TB in livestock and wildlife (*veterinarians*)	2 minutes	A. What do you think of the density of wildlife in your area?	Low/ Average/ Abundant/ Overabundant/ I don't know
			B. Have you tested cattle for TB in the last 5 years?	Yes/ No
			C. Do you inspect hunted wildlife for signs of disease?	Yes/No
			D. Have you identified TB in wildlife?	Yes/No
			E. In positive identification of TB in wildlife, was this achieved with field inspections or laboratory diagnosis?	Field inspections/Labo-ratory diagnosis

Part	Section	Estimated time to complete	Questions	Response options
3	Knowledge and experience of TB in livestock and wildlife (*hunters*)	2 minutes	A. Do you think that wildlife are carriers of TB?	Yes/ No/ I don't know
			B. In your opinion, are cattle the only carriers of TB?	Yes/ No/ I don't know
			C. What do you think of the density of wildlife in your area?	Low/ Average/ Abundant/ Overabundant/ I don't know
			D. Do you provide supplementary food to wildlife?	Yes/ No
			E. Do you use baits for wildlife prior to hunting events?	Yes/ No
4	Knowledge and experience of TB in livestock and wildlife *(cattle farmers*)	2 minutes	A. Have you had a positive TB result in any of your cattle in the last 3 years?	Yes/ No
			B. Do you own the farm where you rear cattle?	Yes/ No
			C. What types of livestock are present on the cattle farm (please, select as many as necessary)?	Cattle/ Pigs/ Goats/ Sheep
			D. What do you think of the density of wildlife in your area?	Low/ Average/ Abundant/ Overabundant/ I don't know
5	Best-worst scaling exercise	10 minutes	See example best-worst scaling exercise in figure 11.	Four TB management interventions are shown in each of 15 pages
6	Contact details and comments	0-2 minutes	Respondents are shown their individual ranking of the 20 management options, and given the opportunity to comment on each individually, and then make general comments in the following section. Respondents were asked for their contact details (email address and/or telephone number) if they wished to participate in future research.	General and specific comments boxes

The BWS exercise presents the respondent with 15 sets of 4 of the 20 possible interventions (table 13). The exercise was balanced so that each option appeared the same number of times across the 15 sets, but sets were selected at random. In each set, the respondent was asked to select the least and the most practical option out of the 4 presented, and to repeat this for each set (figure 11). The importance of considering the practicality was explained and emphasised, and repeated at the top of each page. Respondents were also informed that this would generate a ranking of their responses that would be presented to them upon completion of the exercise. In this ranking they had the opportunity to comment on individual interventions, and in the final section they were able to make any more general comments.

Below there are four potential interventions that could reduce TB levels in your area. Considering for now just these four interventions, which do you think are the least and most practical to implement? Please select one of each.

At this stage, please consider only the practicality of each option, there is the opportunity later to provide your opinion about their effectiveness.

Least Practical		Most Practical
○	Vaccinate 70% of wild boar population using an oral bait	○
○	Maintain cattle separately from pigs	○
○	Use 'wildlife proof' stores for concentrated cattle feeds	○
○	Cull wild boar on farms to reduce densities by 50%	○

Figure 11: An example of one page of the best-worst scaling exercise, translated from an original Spanish version.

Questionnaire implementation

Respondents were recruited opportunistically, using appropriate means of communication for each group of stakeholders. Veterinarians were contacted through the professional and government organisations which they were associated with, and through providing private veterinary surgeries in the area with leaflets and paper copies of the questionnaire. Paper copies were provided in an addressed envelope so that they could be returned directly as well as through distributors. Hunters were recruited by distributing leaflets at hunting events, and through distributing paper copies and leaflets for the website through the regional branch of a national hunters association. Farmers were recruited through veterinary and government offices, and through advertising the questionnaire during other research activities. For all stakeholders, snowball sampling, where participants inform other potential respondents about the questionnaire, was encouraged. A clear criteria for participants was set in all advertising.

Data analysis

A ranking of the efficiency of the 20 suggested interventions was already achieved by the expert panel process. Further data analysis was conducted in Sawtooth. Most and least practical choices were treated as maximising or minimising utilities respectively, following Random Utility (RU) theory. RU choice models retrieve estimates of the trade-offs made by each respondent that best explain the observed pattern of most-least choices. A matrix of most-least offsets is then modelled using a single multinomial logit. Hierarchical Bayes modelling was then used to estimate each respondent’s weight for each intervention, using 20,000 preliminary iterations and 10,000 more after this point. Convergence was checked in a graphical plot that showed the scores generated in each iteration. Finally, the scores were rescaled into positive values that show likelihood of the item having been chosen by each respondent, so that the sum of the 20 rankings is equal to 100 (Sawtooth Software, 2013).

The quality of the responses was evaluated using a ‘fit statistic’. In complex questionnaires such as this, respondents may tend to give random answers. The fit statistic, calculated by multiplying the root likelihood for each respondent by 1000, ranges from 0 to 1000. Given the number of possible interventions and the number of sets completed, respondents with a fit statistic over 282 were considered to have estimated scores that perform considerably differently from random responses (Sawtooth Software 2013).

Statistical analyses were conducted in R (version 2.15.3, R Core Development Team 2012). The most effective and practical interventions were selected based on graphical visualisations of the rankings. The practicality ranking data were tested to see if they met the assumptions of a normal distribution with Shapiro-Wilks tests. One-way

ANOVAs were used to assess the differences in these rankings between different stakeholder groups, and specific differences were explored with Tukey's honest significant difference (HSD) post hoc tests.

Results

Expert panel rankings

The expert panel identified the top 20 most effective interventions (table 14). Every intervention in this list had a rank score of over six, meaning either all experts stated that the item should be retained, or some marked it as 'priority retain'. The interventions were ranked by their total scores to give an order of perceived effectiveness.

Table 14: The top twenty management interventions, shown in order of perceived effectiveness, as determined by the expert panel.

Rank	TB Management Intervention	Shortened description	Reference(s)
1	Stop the supplemental feeding and baiting of all wildlife on cattle farms	Stop supplementary food	Miller *et al.* 2003; Vicente, Fernández de Mera, & Gortázar 2006; Palmer & Whipple 2006; Acevedo *et al.* 2008; Ramirez-Villaescusa *et al.* 2010; Fine *et al.* 2011a; O'Brien *et al.* 2011; Gortázar *et al.* 2011; Schoepf *et al.* 2012; Anderson *et al.* 2013a
2	Separate wildlife and livestock access to waterholes to prevent shared use	Control waterhole access	Michel *et al.* 2007; Munyeme *et al.* 2010; Cowie *et al.* 2014; Barasona *et al.* 2013a, Chapter 2, Chapter 4
3	Test cattle every 3 months on farms where cattle have recently tested positive	Quarterly TB testing	Cattle test-and-slaughter schemes often increase testing frequency with positive results, though normally to a maximum of 3 times a year so this intervention represents an increase in bovine TB surveillance (e.g. RASVE, 2013). Hadorn & Stark 2008; Humblet, Boschiroli, & Saegerman 2009
4	Remove gut piles from the land after hunting and use to feed vultures	Gut-piles to vultures	Pozio *et al.* 2001; Jennelle *et al.* 2009; Vicente *et al.* 2011; Dupont *et al.* 2011; Moreno-Opo *et al.* 2012; Zanella *et al.* 2012; Gortázar *et al.* 2010
5	Use wildlife proof fencing to prevent wildlife access to the farm	Wildlife proof fencing	Ward, Tolhurst, & Delahay 2006; Ward, Judge, & Delahay 2010; Brook 2010; Judge *et al.* 2011; Barasona *et al.* 2013a
6	Cull wild boar on farms to reduce densities by 50%	Cull wild boar	Donnelly *et al.* 2007; Gortázar *et al.* 2008; Carstensen, O'Brien, & Schmitt 2011; Boadella *et al.* 2012; García-Jiménez *et al.* 2013

Rank	**TB Management Intervention**	**Shortened description**	**Reference(s)**
7	Always use interferon test in addition to skin test to improve accuracy of cattle TB testing	Use interferon test	Gormley *et al.* 2006; de la Rua-Domenech *et al.* 2006
8	Introduce zonification – accept that an area has a high disease prevalence & prevent spread beyond this area	Zonification	Renwick, White, & Bengis 2007, Gortázar, C. *pers comms*
9	Maintain cattle separately from goats	Separate goats	Gutierrez & Marin 1999; Napp *et al.* 2013
10	Maintain cattle separately from pigs	Separate pigs	Di Marco *et al.* 2012; Bailey *et al.* 2013
11	Feed cattle in high feeders that wildlife cannot access	Use high feeders	Kaneene *et al.* 2002; Roper, Garnett, & Delehay 2003; Palmer, Waters, & Whipple 2004; Humblet, Boschiroli, & Saegerman 2009
12	Vaccinate 70% of wild boar population using an oral bait	Vaccinate wild boar	Cross, Buddle, & Aldwell 2007; Ballesteros *et al.* 2010, 2011; Beltrán-Beck *et al.* 2012; Tompkins *et al.* 2013
13	Manage water to prevent build up of areas of mud (where the latest research suggests *M. bovis* may survive in the soil)	Manage mud	Vicente, J., unpublished data from Doñana National Park in southern Spain.
14	Maintain newly acquired animals away from existing stock for 90 days	Quarantine new stock	Marangon *et al.* 1998; Gilbert *et al.* 2005; Gopal *et al.* 2006
15	Introduce TB testing and culling scheme for the Iberian pig	Pig test-and-slaughter	Di Marco *et al.* 2012; Bailey *et al.* 2013
16	Make TB testing and culling of the goats obligatory in all cases	Goat test-and-slaughter	Gutierrez & Marin 1999; Napp *et al.* 2013
17	Remove gut piles from the land after hunting and destroy them	Destroy gut-piles	Pozio *et al.* 2001; Jennelle *et al.* 2009; Zanella *et al.* 2012
18	Use 'wildlife proof' stores for concentrated cattle feeds	Concentrate food storage	Garnett, Delahay, & Roper 2002; Ward, Tolhurst, & Delahay 2006; Ward, Judge, & Delahay 2010; Judge *et al.* 2011
19	Use 'wildlife proof' stores for hay type cattle feeds	Bulk food storage	Garnett, Delahay, & Roper 2002; Ward, Tolhurst, & Delahay 2006; Ward, Judge, & Delahay 2010; Judge *et al.* 2011
20	Prevent contact with neighbouring livestock by double fencing	Use double fencing	Kaneene *et al.* 2002; Menzies & Neill 2005

Response to the questionnaire

Questionnaire distribution and advertisement began in January 2013 and continued until November 2013. Fifty-six questionnaires were completed online and seventy-three respondents answered paper-and-pencil versions. Some cases where participants did not complete the best-worst scaling process were observed (online n=16, 29%, paper n=11, 15%), leaving 102 respondents with complete responses. All of these had a fit statistic of over 282 (mean = 538, range 320 – 787) and so were included in further analyses.

Respondents

Thirty-six of these respondents were cattle farmers, thirty-two were hunters and thirty-four were veterinarians. The majority of veterinarians responded online (n=29, 85%), whilst the majority of hunters (n=23, 72%) and farmers (n=33, 92%) responded using paper versions. All age groups (Question 1A, table 13) were represented, though only those aged 40+ were land owners (Question 1C, table 13).

Most hunters (87%) believed that wildlife could transmit TB. Most also stated that they provide supplementary food for wildlife (87%) and put down baits prior to a hunting event (87%). Veterinarians were involved in TB management either through the testing of cattle for the disease (68%) or the inspection of wildlife carcasses (41%). Of those that carry out inspections of hunted wildlife carcasses (n=14), all actively looked for characteristic TB lesions, and 13 (93%) have identified TB. In 94% of cases TB was identified by field inspections of carcasses – looking for characteristic lesions in the lymphoid tissues of the respiratory and digestive systems. Most of the farmers that responded were the owner of the land on which they farmed (n=31, 86%) and most farmed only cattle (81%), though two respondents also kept pigs (6%), and another four also farmed sheep (11%).

All respondents gave their opinion on the density of wildlife in the study area (figure 12). The majority of all stakeholders' responses were that it was average or abundant, with only vets and farmers considering it to be overabundant.

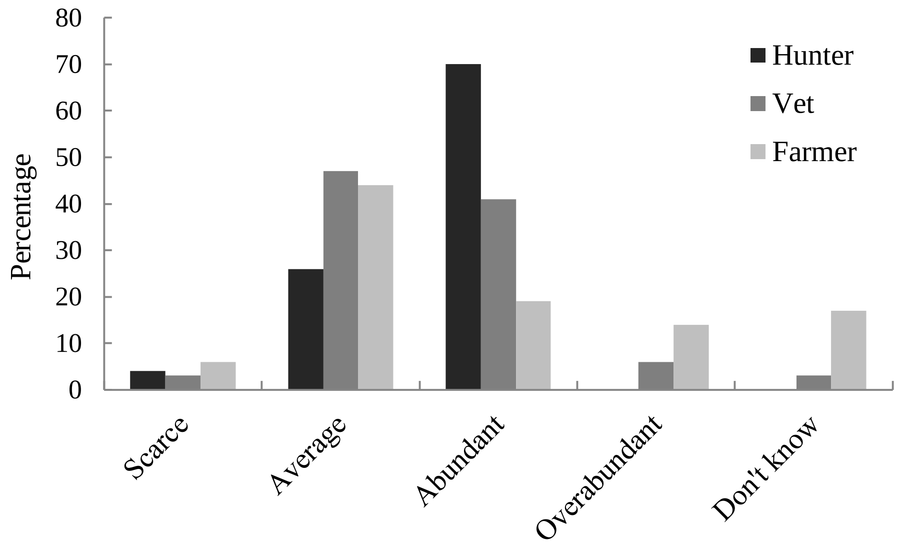


Figure 12: Percentage of each stakeholder group that selected each category of their opinion of wildlife density (question 2A, 3C and 4D in table 13 as appropriate for each stakeholder)

Farmers and hunters displayed the lowest difference of opinion about the practicality of interventions (figure 13), with a total absolute mean score difference of 18.83. By comparison, farmers and veterinarians were less likely to agree (mean score difference = 43.54) and hunters and veterinarians displayed the least agreement (mean score difference = 51.57).

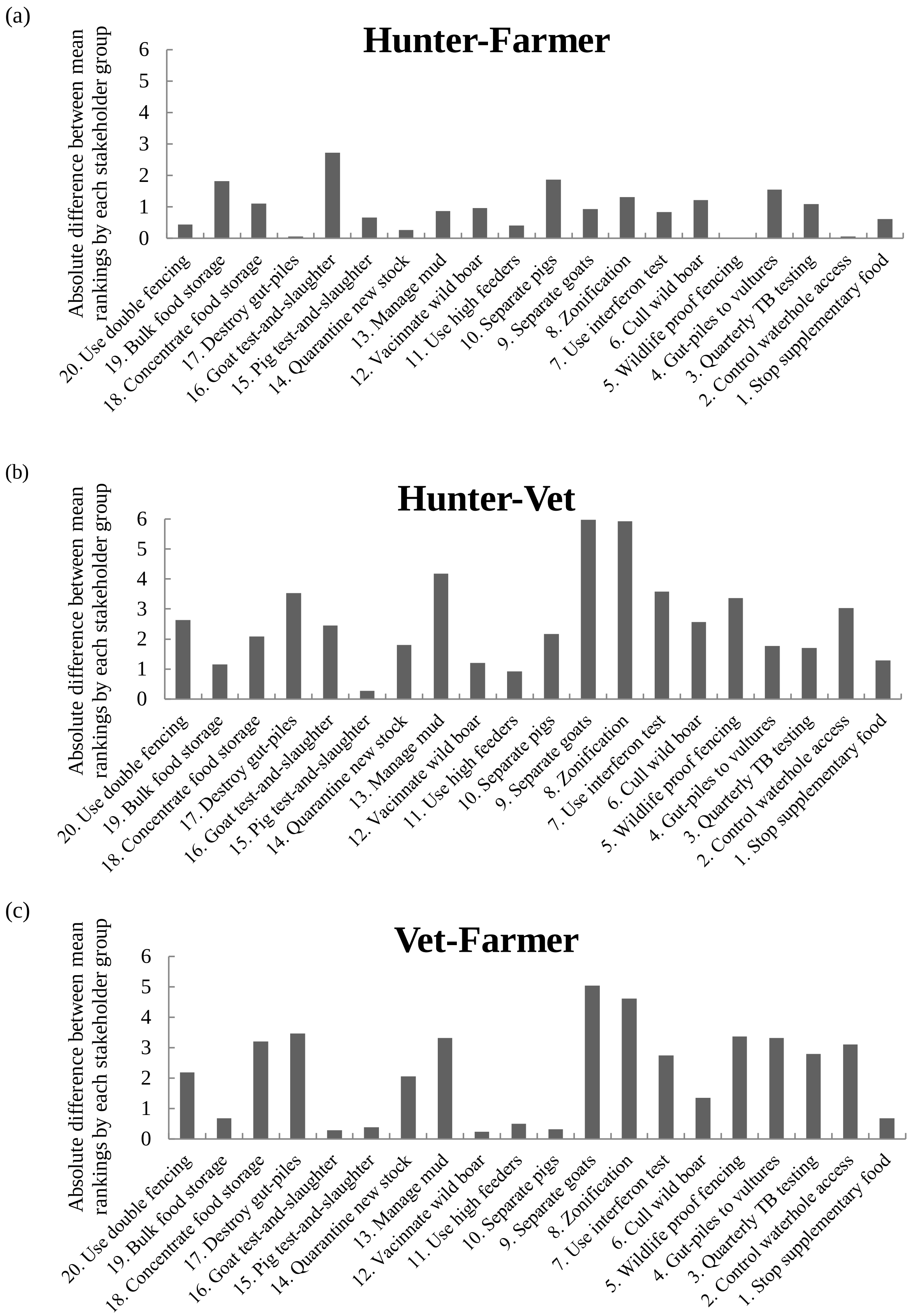


Figure 13: The absolute differences in the mean practicality rankings given for each dyads (a) Hunters and Farmers, (b) Hunters and Vets and (c) Vets and Farmers for each intervention.

Effectiveness and practicality

Considering both effectiveness and practicality rankings (figure 14), the three best interventions to pursue were the separation of access to waterholes by wildlife an cattle (table 14, item 2), testing cattle every three months on farms with a recent TB positive case (item 3) and removing gut-piles from the land after hunting to feed to vultures (item 4). Furthermore, each of these was more strongly supported by a different stakeholder group (figure 15). Despite being considered the most effective intervention, stopping supplementary feeding was not considered highly practical by any stakeholder groups (item 1).

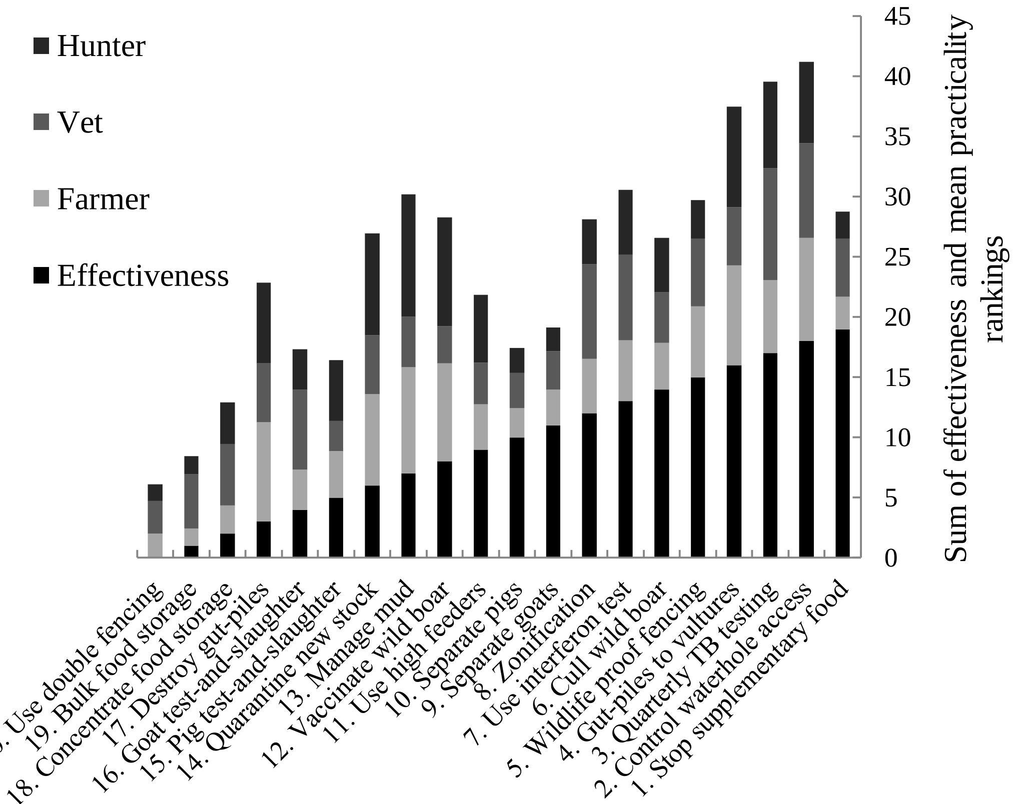


Figure 14: Sum of the effectiveness and mean practicality rankings for each possible TB management intervention. The intervention numbers correspond to the list in table 14.

Stakeholders did display good levels of agreement over certain interventions, including relatively low practicality rankings for the culling of wild boar (table 14, item 6), maintaining cattle away from goats or pigs (items 9 and 10) and using double fencing to prevent contact between livestock on adjacent farms (item 20). Other possible interventions divided opinion. Hunters gave high rankings to wild boar vaccination, management of muddy areas and maintaining newly acquired stock away from existing cattle, yet these were received some of the lowest rankings by veterinarians (items 12-14). Veterinarians ranked interventions involving increased TB disease testing highly (items 3, 7, 16), except for testing the Iberian pigs (item 15). Farmers always ranked the removal of hunted gut-piles as highly practical (items 4 and 17), yet also favoured farm-based management such as mud management and the quarantine of newly acquired stock (items 13 and 14), which were less well supported by veterinarians.

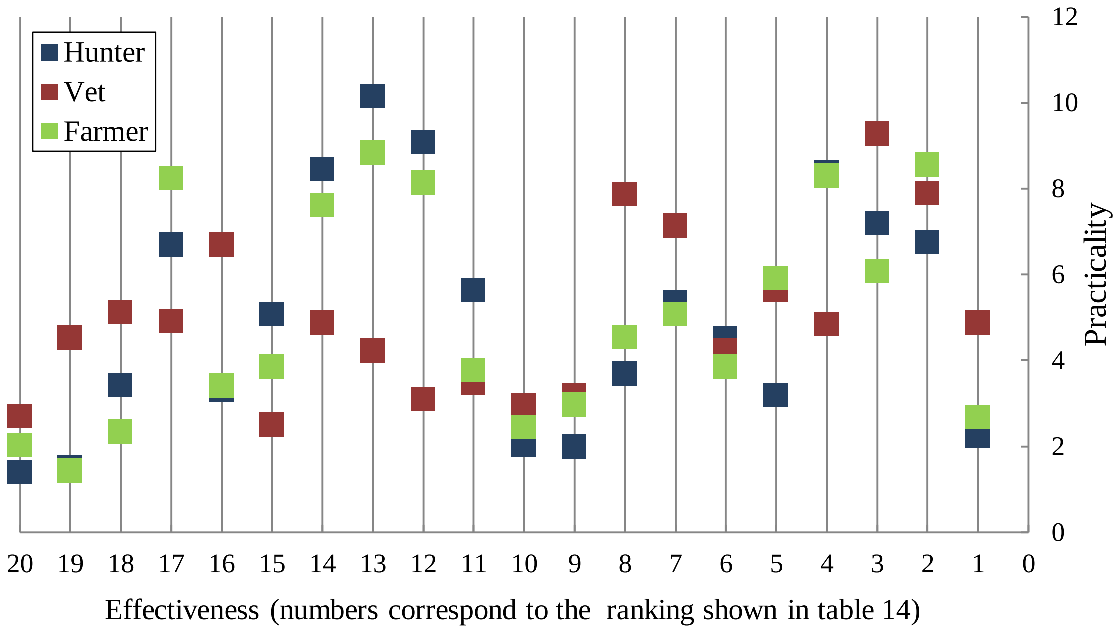
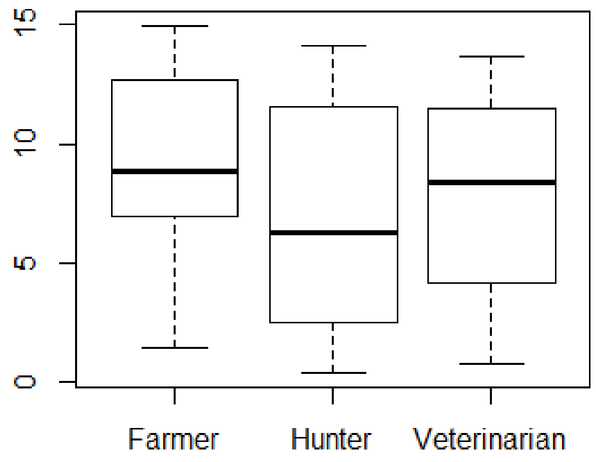


Figure 15: A graph to show the rankings for effectiveness and practicality of the 20 TB potential disease management interventions. Practicality is shown on a ratio scale, so that a ranking of 10 is considered ten times more practical than a ranking of one.

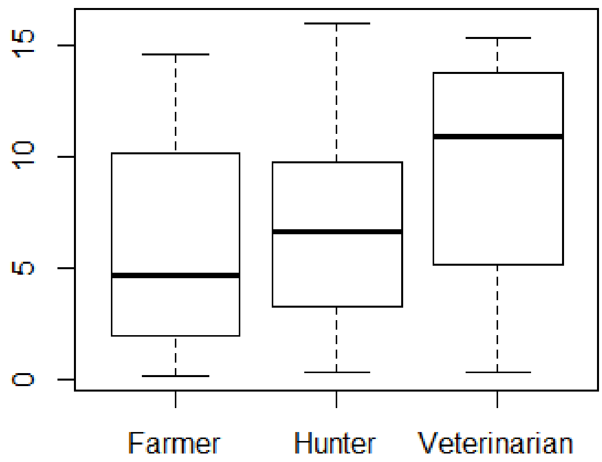
Most effective and practical interventions

The differences between stakeholders' rankings of the most effective and practical interventions were examined further. In statistics on the three best interventions (items 2, 3 and 4), the data were found to be normally distributed, and ANOVA models indicated differences between the stakeholders (d.f. = 99. item 2: F=2.18, *p*=0.119; item 3: F=3.91, *p*=0.023; item 4: F=6.501, *p*=0.002). Post hoc tests revealed no significant differences between stakeholders regarding the separation of wildlife and livestock at waterholes (item 2, figure 16(a)). Veterinarians were significantly more likely to favour three-monthly testing on farms with a recent TB positive case (estimate=3.15, p=0.02*, item 3, figure 16(b)). Both farmers and hunters thought the removal of hunted gut-piles for vulture feeding was more practical than veterinarians did (farmers; estimate=3.24, p=0.009** and hunters; estimate=3.24, p=0.005**, item 4, figure 16(c)).

(a) Separation of wildlife and livestock access to waterholes to prevent shared use



(b) Test cattle every three months on farms with a recent positive TB results



(c) Remove gut-piles from the land after hunting and use to feed vultures

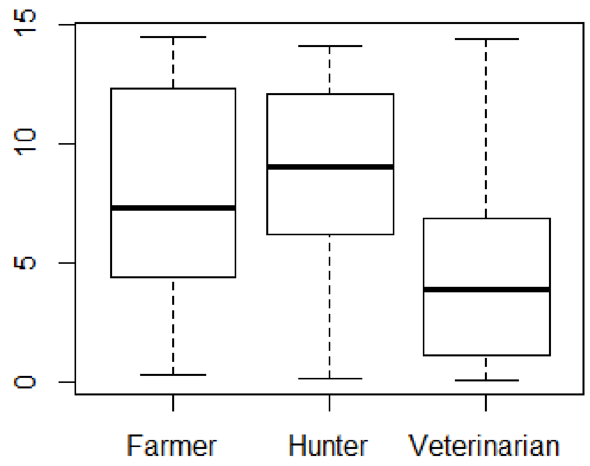


Figure 16: Boxplots of the practicality rankings by respondents each stakeholder group, for each of the best disease management interventions. Boxplots show the median of the data in the thick bar, the first and third quartiles within the box and the full range of the data within the whiskers.

Discussion

In this study we have ranked the effectiveness and practicality of potential disease management interventions, according to different stakeholders. At the time of writing, this is the first time this approach has been applied to evaluate bovine TB management. The results suggest that despite different management objectives, different stakeholder groups may be willing to undertake certain interventions that have been identified as potential control options. In the study region the interventions deemed most effective by experts, stopping the supplementary feeding of games species, was not considered practical by farmers or hunters. All stakeholders supported separating wildlife and livestock access to waterholes as an effective and practical option. Veterinarians ranked three-monthly cattle TB testing as highly practical, whilst hunters and farmers supported the removal of gut-piles after hunting activities. If economically viable, these interventions may contribute to the reduction of bovine TB prevalence. Furthermore, the identification of which stakeholders consider which tasks practical provides the opportunity to develop a combined management strategy, where each stakeholder implements the controls they are able to undertake most easily. An integrated approach, where the most appropriate management tools are utilised by the most appropriate stakeholders, could be very beneficial for disease control. This also compliments previous findings that the integration of a variety of tools will be necessary to achieve significant reductions in disease prevalence (Miller & Sweeney 2013; Pfeiffer 2013).

Methodology

Respondent-driven “snowball” advertising and distribution of the questionnaire was utilised, which has been used in previous BWS exercises (Cross, Rigby, & Edwards-Jones 2012). In rural communities this has the important advantage of including otherwise hard-to-reach participants, and it has also been shown to be economical, efficient and effective in various studies (Sadler *et al.* 2010; Goodman, 2011). Despite the complexity of the BWS exercise a good proportion of respondents completed all 15 pages of the exercise. Paper versions of the questionnaire may have suffered a reduced response rate due to the extra work involved in returning it to the distributor or research institute. Nevertheless, they were essential to reach participants that may not have had internet access.

The BWS exercise generates quantified yet readily comprehensible results that can be easily conveyed to all stakeholders. This provides a platform through which to continue a dialogue with stakeholders about the disease management strategies, Indeed, engagement with stakeholders through the process means the intervention is more likely to be supported, and any conflicts will be identified promptly (Dougill *et al.* 2006; Cowie *et al.* 2014).

Stakeholder agreement on practicality

Hunters and farmers showed notably greater agreement over the practicality of interventions with each other than either group did with veterinarians. In the study region, hunter and farmer management objectives are often aligned. However, persuing both may result in decreased disease transmission opportunities. The difference of opinion between these groups and veterinarians is concerning, as veterinarians are often the main source of wildlife and livestock disease information for both stakeholders and policy makers (Cross *et al.* 2009; Simon-Grifé *et al.* 2013). As farmers and hunters would be the stakeholders primarily responsible for the execution of the majority of our list of possible interventions, veterinarians will need to provide appropriate information and support, as well as listen to what is practical. In turn, scientific researchers need to provide veterinarians with good evidence to support assertions of the effectiveness of the best interventions.

Banning supplementary feeding of wildlife on cattle farms

Stopping the supplementary feeding and baiting of wildlife on cattle farms was rated as the most effective TB management intervention by experts. Supplementary feeding is often used in the region to boost wildlife densities and aggregate animals prior to hunting events (Vicente *et al.* 2006; Acevedo *et al.* 2008; Schoepf *et al.* 2012; Anderson *et al.* 2013a), yet has been associated with wildlife and livestock disease (Gortázar *et al.* 2006). This highlights a conflict of interest between stakeholders. Considerable evidence exists that supplementary feeding is a risk factor for TB (see table 13, item 1). However, bans on supplementary feeding have previously been implemented in similar systems in France (Zanella *et al.* 2008), with limited short term disease reduction success, possibly because they were not well supported, because of similar management conflicts. Both farmers and hunters ranked this practice as relatively impractical and it is important to identify why. In rural communities the economic and social benefits of hunting are considerable, and may be perceived as benefits that outweigh the costs of TB. Veterinarians ranked this option more highly, and are well placed to provide education and evidence to other stakeholders to increase their acceptable of this intervention.

Separation of wildlife and livestock access to waterholes

Watering points have previously been identified as a risk factor for TB in cattle (Acevedo *et al.* 2007; Cowie *et al.* 2014), probably because it causes aggregation of both wildlife and livestock species (chapter 4). Water provides favourable conditions for *M. tuberculosis complex* (MTBC) bacteria to survive in the environment (for example, MTBC have been detected at 50% of the waterholes at Doñana National Park in southern Spain (Joaquin Vicente, unpublished data)). The separation of wildlife and livestock at watering holes was recently demonstrated in our study region by Barasona *et al.* (2013a). These authors trained cattle to operate ‘bump’ gates, allowing them access to water that was otherwise fenced off to wild ungulates. At the same time, wildlife was given access to water that was fenced to prevent cattle access (100% effective) during the dry season. Within the first year after this system was implemented a significant reduction in cattle TB prevalence was observed on the study farm, in combination with continued test and slaughter. Furthermore, gates and fencing are readily available and are relatively inexpensive to install and maintain. As this option was considered effective and practical by all stakeholders, and is therefore likely to be well supported, further implementation is recommended in the study region.

Three-monthly cattle test and slaughter on farms with a recent positive case

Veterinarians strongly supported improvement to the test-and-slaughter strategy. Quarterly testing represents an increase in surveillance from current methods, likely to identify infected animals more quickly, enabling prompt removal of these animals before further disease transmission occurs. This was considered highly practical by veterinarian participants; significantly more so than by hunters or farmers. The feasibility of introducing this control measure therefore needs to be carefully considered. Farmers may be less likely to support it because in the short-term they bear the indirect negative effects of a positive reactor being identified (e.g. Knight-Jones & Rushton 2013). They may also be concerned about the increased risk of false positive results with increased sampling (Humblet, Boschiroli, & Saegerman 2009). Indeed, veterinarians also supported the use of the interferon test (item 7), which has greater sensitivity and allows for earlier detection of recently infected animals (Alvarez *et al.* 2012). Both methods are more expensive than the currently used skin test at less frequent intervals, and the country may not be able to afford the increased cost that would be incurred. However, this could still prove worthwhile in cost-benefit analysis if a rapid reduction in TB prevalence could be achieved. As we know wildlife disease reservoirs have an effect in this region (Cowie *et al.* 2014), this strategy may have to be implemented alongside control of the wildlife-livestock interface.

Remove gut-piles from the land after hunting and use to feed avian scavengers

Avian predators provide valuable environmental services in south-central Spain, and include small populations of Cinereous vultures (*Aegypius monachus*) and Spanish Imperial Eagles (*Aquila adalberti*) which are of conservation concern in the region and have globally decreasing populations (Birdlife International 2012, 2013). Such predators have suffered from previous legislation that required the removal of all carcasses from the land, but at present EU legislation allows for some carcasses to be left in-situ (Moreno-Opo *et al.* 2012). On high density hunting estates up to 15 gut-piles (approximately 400kg) can be generated per km^2 in one hunting event (Vicente *et al.* 2011). Unfortunately, non-target species may also scavenge carcasses, and wild boar in particular are frequent scavengers, to the extent that they become the predominant scavengers in the absence of vultures (Vicente *et al.* 2011). This contributes to the maintenance of disease in the wildlife-livestock community. Carcass and gut-pile removal is therefore important in this region. In our study, veterinarians ranked gut-pile removal as less practical than farmers and hunters did, possibly because of concerns about the further spread of disease. However, the experts strongly supported the method as effective, and recent research has demonstrated that non-target consumption can be effectively and affordably controlled when carcasses are provided to avian predators in controlled locations (Moreno-Opo *et al.* 2012).

Implications for current research and management

Test-and-slaughter schemes have played a valuable role in the reduction of TB in cattle (RASVE 2013), and this work suggests that key stakeholders consider this a practical option. Testing frequency could be increased where appropriate if the necessary costs could be met. Research has recently shown that direct and indirect interactions between cattle account for the majority of their contacts (chapter 4). Stakeholders' high practicality rankings for biosecurity measures that address these contactssuch as separating access to water and managing the build-up of mud (figure 15, items 2 and 13) indicates that they might be willing to undertake interventions that would reduce these interactions.

Current research is focussed on some interventions that were not ranked as highly practical. Research on TB control in the region is currently also focussed on vaccination of wildlife, particularly in wild boar (Beltrán-Beck *et al.* 2012; Anderson *et al.* 2013b). Vaccination is a very promising option for disease control in mixed wildlife-livestock disease communities (Cross, Buddle, & Aldwell 2007; Buddle *et al.* 2011; Tompkins *et al.* 2013). Both hunters and farmers ranked this option as highly practical, indicating it would be well accepted if other factors such as cost and availability did not limit its use. However, experts and veterinarians did not rank it as particularly effective or practical, perhaps because that they consider the 70% population vaccination goal (following a recent modelling study based on vaccination in the region by Anderson *et al.* (2013b)) to be difficult to achieve, or because effectiveness of an oral bait has not yet been demonstrated in the field. It would be valuable to re-evaluate the veterinarians' opinions on vaccination in more detail in order to understand their reservations and identify what needs to be addressed.

Wider implications

Communication between policy makers and stakeholders will be critical to the success of disease control efforts. It has been shown that veterinarians are the main source of livestock disease information for hunters and farmers (Cross *et al.* 2009; Simon-Grifé *et al.* 2013), so veterinarians will need to provide consistent and updated advice on disease management in their area. The participation of communities in disease management and surveillance is highly recommended (de Garine-Wichatitsky *et al.* 2013a), and provides a platform for communication between stakeholder groups (Dougill *et al.* 2006). Hunting activity in Spain is increasing (Herruzo & Martinez-Jauregui 2013), leading to the conflicting management objectives with livestock farming observed in our study region. However, hunters and farmers demonstrated reasonable agreement over wildlife abundance and the most practical and effective management interventions. As their activities often overlap in this region there could be common ground for consensus building. Disease control schemes need to explicitly include local consultations regarding the application of national and internationally determined recommendations and legislation appropriately to localised socio-economic and environmental conditions (de Garine-Wichatitsky *et al.* 2013a). If successful, the benefits of the adoption of new practices would be communicated amongst stakeholders, encouraging further management improvements (Baumgart-Getz *et al*. 2012).

Conclusions

In south-central Spain, the most effective, practical and economically viable option for bovine TB control is the separation of wildlife and livestock access to water resources. The economic viability of quarterly testing of herds with a recent positive case and removal of all parts of the carcass after hunting should be further evaluated. Further social research should be conducted to identify the factors influencing stakeholders' considerations of what was practical. Greater dissemination of the scientific evidence and promotion of the benefits of stopping supplementary feeding of game species will be beneficial.

This study demonstrates the use of a simple ranking exercise to identify what experts and key different stakeholders consider effective and practical disease management interventions. The stakeholders that consider these interventions practical were also identified, suggesting who might be willing or able to undertake these interventions. We have generated readily comprehensible results that can be used to start further dialogue with stakeholders, including introducing them to what experts believe are the most effective interventions. Focussing on effective interventions executed by those stakeholders that consider them practical will help to ensure that benefits are realised quickly.

Chapter 6: Discussion

This study has used multiple approaches to explore and quantify the risk factors for the presence of single and multiple diseases, the direct and indirect interactions that occur between multiple species and the effectiveness and practicality of the resulting possible disease management interventions.

Environmental, farm management and wildlife-related risk factors for TB were identified in chapter 2. Disease management recommendations focussed on resolving conflicting objectives in game rearing and managing wildlife and livestock access to water and food resources. Education and communication emerged as important factors in an integrative approach to disease management.

In chapter 3, the research extended to the identification of shared risk factors for multiple diseases. Direct and indirect cattle-to-cattle interactions were revealed as a risk factor for both brucellosis and tuberculosis. For TB the presence of wildlife in winter was a positive association, whilst for brucellosis cattle movement between herds was strongly related. Targeting management at shared risk factors would reduce the costs of disease management compared to interventions aimed at individual diseases.

The high relative importance of indirect interactions, both within and between species, for possible disease transmission was identified in chapter 4. Pigs showed strong direct connectivity to cattle and the resources they use. Red deer showed indirect interactions through shared space use, and both species moved across the farm boundary, increasing the possibility of introducing disease. Further research into the concept of a disease reservoir community, and the environmental survival of pathogens, will lead to improved disease management.

Finally, stakeholder's views on the efficiency and practicality of possible management interventions were ranked in chapter 5. The separation of wildlife and livestock access to waterholes, increased frequency of cattle testing and the removal of hunted gutpiles from the land were identified as effective and practical TB management interventions in the study system. This study demonstrated the use of a simple ranking exercise to quantify stakeholders' views on disease management strategies, which are critical for the success of any interventions.

Together, these chapters have evaluated disease risk and management on multiple scales, and determined effective and practical interventions to reduce disease prevalence in a mixed wildlife-livestock host community. Integrated management, where appropriate stakeholders make use of one or more effective and relevant management tools, is recommended.

Methodology

The socio-economic and ecological research methods used in this study have contributed information on how to proceed with disease management in a mixed wildlife-livestock system. In the assessment of risk factors for disease, interviews structured by the questionnaires and participatory mapping exercise yielded detailed information about farms and their management. Questionnaires are a simple and effective way to generate quantified and comparable data from participants (White *et al.* 2005), whilst participatory mapping accesses information that cannot otherwise easily be conveyed (Austin *et al.* 2009). In our study, the participatory mapping exercise was well received by participants, and quickly understood. However, the task was time consuming for researchers and participants, and so may be difficult to implement on larger scales. The use of computer hardware to collect data electronically would significantly improve the accuracy and efficiency of this method.

The use of best-worst scaling to rank disease management interventions has successfully evaluated the practicality and effectiveness of a range of possible disease management interventions. This process allows for the involvement of a range of stakeholders, was available through different media and was well supported by research software. As well as directing future disease management in the region, the method generates easily interpretable results that can be used to stimulate further discussion with stakeholder groups and policy makers (Cross, Rigby, & Edwards-Jones 2012). One caveat for the use of best-worst scaling is that it is cognitively demanding. Despite using the lowest possible number of sets and options in each set to get balanced results, some cases of incomplete sets or erroneous responses (for example selecting multiple best and worst options) were observed. Unfortunately, the snowball sampling method used means that we did not know how many respondents started but did not complete the exercise. This method could be used on any scale but participants need to be carefully informed about the exercise and warned that it will take time and concentration, in order to get the best results. As information on disease management increases, the method can be adapted to include more quantified information, for example by replacing expert opinion of effectiveness with measured disease reduction capabilities for each intervention. Along with the each stakeholder group's rankings of the practicality this could then be used to estimate which combination of stakeholders engaging in which interventions would be enough to achieve significant disease reduction or even eradication of a disease. Where there is enough information available on disease management interventions, this method could be repeated anywhere where multiple stakeholders managing a disease.

The use of proximity data loggers and GPS trackers on collars attached to both wildlife and livestock species gave detailed spatially and temporally explicit information about the direct and indirect interactions between known individuals. Important lessons were learned about the use of proximity loggers to assess interactions between individuals. In order to generate data that can be used for network analyses and support the modelling of disease transmission, the study design must incorporate the collaring of the majority of individuals of each species in the study population(s). Individuals need to be readily trapped, and re-trapped if required for collar recovery (Goodman 2007). Only then can individual variation and the presence of un-collared individuals be sufficiently accounted for (James, Croft, & Krause 2009; Ryder *et al.* 2012). Detailed contact data for a population would allow for social network analysis. This increasingly popular tool would then allow for the identification of high risk individuals or contact bottlenecks, which might then be targeted for management (Christley *et al.* 2005; Hamede *et al.* 2009). The data also provide an excellent basis for the development of disease transmission models, if sufficient information is also available about the transmission rate, virulence and environmental survival of the pathogen, and the susceptibility and ecology of the hosts (Perkins *et al.* 2009; Drewe 2010). Spatially and temporally explicit contact data would improve existing SIR model by allowing for the inclusion of accurate probabilities of direct and indirect contact. Along with quantified characteristics about the host and pathogen this improves our understanding of the transmission rate – a vital part of the SIR models.

As proximity data logging technology continues to improve and expand, the industry must also realise the requirement for 'base stations' that can be deployed in the field without causing significant disturbance to the environment or the study species. These can monitor indirect interactions between individuals, increasingly recognised as an important part of disease transmission if the pathogen can survive in the environment (Drewe *et al.* 2013; Pfeiffer 2013). In our study, standard cattle loggers were adapted for this purpose, but logging stations designed for the task may be less conspicuous and be able to measure a better defined area. Furthermore, individual variation between loggers has been identified and needs to be addressed to ensure the variation observed is due to the individual and not the collar (Boyland *et al.* 2013). Technology that allows for the transmission and remote download of data from the logger while it is still attached to the animal would be useful to ensure maximum data collection and monitor study animals during the study period and collar retrieval. Finally, though expensive, the integration of GPS and proximity logger technology into one collar would provide temporally and spatially explicit data that would be highly valuable in the study of contact networks and pathogen transmission.

Recommendations for the management of TB in south-central Spain

The management of TB in south-central Spain will require an integrated approach, where each stakeholder group contributes to disease management in ways they find acceptable and feasible to achieve and that have been shown to be effective. Chapter 2 identified wildlife reservoirs of the disease as the main factor determining the success or failure of disease eradication schemes, with other risk factors possibly relating to direct or indirect contacts between livestock and wildlife, as well as intra-herd cattle contacts. The likely importance of indirect interactions was identified in chapter 4. The long-standing conflict of interest caused by the cultural importance and economic value of game rearing means that significant wildlife population reductions are not achievable. However, chapter 5 identified that different stakeholder groups may be willing to adopt different interventions that would contribute to livestock disease reduction. A list of the specific recommendations for south-central Spain now follows:

1. The management of indirect interactions, both within and between species, was identified as likely to be relatively more important than direct interactions, which were infrequently observed. Chapters 2 and 4 indicate that water sources may be important, and evidence suggests that is possible to manage access to waterholes in this region (Barasona *et al.* 2013a). Furthermore, emerging data indicates that *M. bovis* survival times in the region may be longest in muddy conditions (J. Vicente, *pers comms*). Water management to reduce these areas may therefore reduce the critical time window in which the disease can be transmitted indirectly between individuals. Chapter 5 revealed that key stakeholders in the region also thought that waterhole management was a practical option, so the next step is to ensure this is economically viable and introduce this method on a wider scale.

2. The connectivity of pigs to livestock, both directly and indirectly through the shared use of resources indicate that they could transmit disease to cattle, if infected. Regarding TB, the 30% *Mycobacterium tuberculosis* complex (MTC) seroprevalence and visible lesions in some carcasses indicates that this may currently be a concern. Further research, including wider seroprevalence testing will ascertain the extent to which pigs may act as reservoirs for TB in cattle. As with wildlife, management would focus on reducing interactions with cattle. A test-and-slaughter scheme would also be important as pig products are used for human consumption.

3. Red deer emerged as significant potential reservoirs of disease in cattle. Research is currently focussed on the management of *M. bovis* reservoirs in wild boar (e.g. Beltrán-Beck *et al.* 2012), yet red deer can also act as a reservoir (Gortázar *et al.* 2011a). This study suggests increased direct and indirect contact between red deer and cattle during the '*Montanero*', when acorns fall from the oak trees found in south-central Spain's 'dehesa' habitat in autumn. It may be valuable to investigate the benefits of preventing these interactions. This could still be achieved by enclosing cattle within a smaller area of the farm during autumn, ideally with wildlife proof fencing. The costs of any supplementary feeding required may be mitigated by the reduced incidence of TB. Leaving some areas of the farm accessible to wildlife, where they also suffer no competition with livestock for food, may reduce direct and indirect interaction frequencies.

4. Biosecurity for the management of disease transmission remains important in this region. As feeding cattle on the ground emerged as a risk factor, a relatively simple transition like providing food troughs could make an important difference. Hunters need to be made aware that they too can make a difference by reporting infected wildlife, and collaborating in the hygienic removal of infected animals and carcasses from missed wildlife-livestock habitats (Aranaz *et al.* 2004). Formally including hunters in wildlife and livestock disease management projects is likely to be beneficial.

5. As levels of awareness about TB and its management varied among stakeholders, education of the relevant stakeholders about the disease and its prevalence in the region may significantly improve management and co-operation with disease control efforts. The results of studies such as chapter 5 provide easily interpretable information with which to start a dialogue with stakeholders. The provision of social support for the implementation of the recommended disease management interventions could be crucial to their success. This could take the form of regular community meetings, volunteer TB management ambassadors for each stakeholder group, and the provision of

regular feedback from researchers and veterinarians to the stakeholder community.

Implications for future research and disease management

As well as the specific applications for disease management in the study areas, this thesis reveals some valuable general insights into the management of any livestock disease in mixed wildlife-livestock systems. Many of the recommendations regarding TB control in south-central Spain apply to disease management in any mixed wildlife-livestock system.

Farm biosecurity is an over-looked, often cost-effective means of reducing interactions between individuals (Judge *et al.* 2011). This can refer to contact within livestock herds (e.g. Menzies & Neill 2005), between herds (e.g. chapter 3, Johnston *et al*. 2013) or between livestock and wildlife (e.g. chapter 4, Cooper *et al.* 2010; Kukielka *et al*. 2013). Biosecurity measures would need to be tailored to local management goals, but need to prevent interactions between individuals in time (e.g. allowing access to common resources at different times of the day or year, removing substrates that allow for the environmental survival of pathogens) or space (e.g. fencing to prevent interactions between individuals, or the use of high feeders for cattle that wildlife cannot access). If a disease causes chronic infection and is contracted by long-lived species, these measures must be undertaken for long enough to account for ‘temporal vectors’, where an individual may carry the disease through time (Nugent 2011; Palmer 2013).

The importance of multiple disease management was highlighted in chapter 3. Most livestock populations face the threat of multiple diseases, and new diseases emerge frequently, particularly with increasing agricultural intensification and environmental change (Daszak *et al.* 2013; Jones *et al.* 2013). Livestock disease managers tend to identify the best management strategies for a single disease and pursue one or more of these. Cost-benefits and socio-economic factors are considered accordingly. However, identifying and managing common risk factors for multiple diseases would allow increase the efficiency of management and would likely improve the cost-benefits beyond what could be achieved when diseases are controlled individually. For example, Bennett & IJpelaar (2005) identified 34 different endemic livestock diseases that cause production and economic losses in the UK alone. It is likely that some of these have shared risk factors that could be managed in common.

This study, and others using proximity logging technology with multiple species, has identified behavioural avoidance of direct interactions between individuals of different species (Chapter 4, Böhm, Hutchings, & White 2009; Drewe *et al.* 2013). Although this may not be the case in all systems, it highlights the likely importance of indirect interactions in disease transmission, which must be considered when researching and managing pathogens that survive environmental exposure in infective doses. Though the technology currently available encourages focus on direct contacts, future studies must consider indirect interaction, if it is known that the relevant pathogen demonstrates survival in the environment. This can be achieved by placing proximity logging base stations at resource and control points in the study area. Knowledge of the survival times of infective doses in the environmental conditions of the region of interest will greatly improve our understanding of disease transmission (Kukielka *et al*. 2013, appendix 1). Used in combination with critical time window analysis (such as in chapter 4), this could help to quantify the spatial and temporal patterns of indirect disease transmission. The resultant data could then form the basis of the biological information required as part of accurate decision support modelling.

The categorisation of the stakeholders responding to the questionnaire and exercise in chapter 5 identified differences between stakeholder groups. These groups tended to respond differently to the possible disease management interventions presented to them due to different and sometimes conflicting livestock and wildlife management objectives. These views are often long held and can be a determining factor in the success of disease control efforts (Benjamin *et al.* 2010). An understanding of these differences, and the use of interventions that each group is willing to accept, will encourage each group to improve management in ways that are acceptable to them

(Cross *et al*. 2012). Consideration of the social and economic framework in which stakeholders make decisions will be beneficial for livestock disease management.

Researchers must continue to incorporate the socio-economic aspects of management into wildlife and livestock disease control research (Pfeiffer 2013). Many appropriate social research techniques have been developed for market research and healthcare decision making, and so are readily available (Cross *et al*. 2012). They are also often easy to implement and generate readily comprehensible results. The integration of these methods with more traditional biological and epidemiological research will give a fuller understanding of the whole disease community, including the stakeholders themselves, allowing for more targeted and appropriate disease management interventions.

Awareness and knowledge exchange between stakeholders in our study region was found to be limited, and this highlighted that the results of scientific research do not always quickly get through to stakeholders managing disease on local scales. Knowledge exchange with stakeholders should become a formal part of the scientific process in these situations, undertaken alongside local, regional and national animal health authorities. Providing simple and balanced information to stakeholders will increase the likelihood of their acceptance of appropriate interventions Benjamin *etal*. 2010; Brook 2010; Pfeiffer 2013), make them aware of the results of their contributions to consultations and hasten the implementation of any necessary disease management interventions.

Livestock diseases result have significant economic impacts (Gramig & Horan 2011). The effects on farming communities are increasingly severe as higher production costs and fluctuating output prices reduce profit margins (Thornton 2010; van der Voort *et al.* 2013). It was beyond the scope of this thesis to explore the economics of disease management, yet it is clear that the interventions recommended will need to be evaluated economically. Techniques such as cost-benefit analysis and decision support modelling exist to facilitate these decisions (e.g. Smith *et al.* 2007; Bennett, McClement, & McFarlane 2012; Mwacalimba, Mumba, & Munyeme 2013). Recently, advances have been made in applying economic performance measurements to disease management interventions as part of interdisciplinary frameworks (Bennett, McClement, & McFarlane 2012). Modelling can incorporate farm-specific details, a range of management options and the impacts of regulatory requirements imposed on farmers to support the selection of controls that could achieve disease reduction or eradication at minimum costs (Gramig & Horan 2011; van der Voort *et al.* 2013). Stakeholder perceptions may differ from strategies based only on epidemiological considerations (Kerebel *et al*. 2013). Models must therefore integrate information from biological, economic, social and environmental sources (Lynch 2000). This would be a logical continuation of the interdisciplinary study of livestock disease management.

Conclusions

An integrative approach to disease management in mixed systems is necessary in order to achieve disease reduction or eradication (Wilkinson *et al*. 2011). The identification of risk factors for disease can be achieved relatively easily, and used to direct both management and further research. Shared risk factors for multiple diseases provide the opportunity to effectively and efficiently manage multiple diseases. Finer scale information about interactions between the multiple species in a disease community provides more detail about how a disease might be transmitted, and therefore what management strategies might be effective. Indirect interactions may be relatively more important than direct contacts between individuals, and need to be considered when a pathogen demonstrates survival in the environment. In order to ensure the implementation of any resultant suggested management interventions, stakeholder consultation can identify the optimum combinations of measures that can be introduced. Focusing on effective interventions, executed by the most appropriate stakeholder groups, will maximise disease management success. This interdisciplinary approach could be applied to any biological problem that involves human management of the environment.

Appendix 1: Spatial and temporal interactions between livestock and wildlife in South Central Spain assessed by camera traps

E. Kukielka[1, 2*], J. A. Barasona[3], C. E. Cowie[3, 4], J. A. Drewe[5], C. Gortázar[3], I.Cotarelo[3], J.Vicente[3]

[1]*Royal Veterinary College, Royal College Street, London, NW1 0TU, UK*

[2]*Zoological Society of London, Regent's Park, London, NW1 4RY, UK*

[3]*Instituto de Investigación en Recursos Cinegéticos (IREC – CSIC – UCLM), Ronda de Toledo s/n 13071, Ciudad Real, Spain.*

[4]*Environment Department, University of York, Heslington, York, YO10 5DD, UK*

[5]*Royal Veterinary College, Hawkshead Lane, North Mymms, Herts, AL9 7TA, UK*

**Corresponding author: Tel.: +44(0)7774788731. E-mail address: ekukielka1@gmail.com (E.Kukielka)*

This manuscript shows work written by Miss Esther Kukielka during her MSc research project which was co-supervised by the author of this thesis, along with Dr. Julian Drewe from the Zoological Society of London and Prof. Christian Gortázar from IREC, Spain’s national wildlife research institute. The research was conducted on the same cattle farm as was used in chapter four, and the camera trap sampling was conducted by the thesis author alongside other data collection on the farm. This manuscript has been published in *Preventive Veterinary Medicine* (2013) vol. 112, issue 3-4, pages 213-221.

Abstract

The diversification of livestock farms into hunting estates in South Central Spain (SCS) may impede the success of *Mycobacterium bovis* eradication programmes by facilitating transmission between wildlife and livestock. In this observational study we aimed to provide information of relevance about the nature and frequency of interactions (observed visits to study points) between livestock (cattle and domestic pigs) and wildlife (wild boar and red deer). The study was conducted in an extensive cattle farm in SCS where the land is also used for game hunting. During a period of one year, camera traps (n=16) were placed at *a priori* risk points for interspecies interactions: water (natural and artificial troughs), food placed on the ground for baiting wildlife, and pasture. To define indirect interspecies interactions, a critical time window for *M. bovis* to survive in the environment was selected based on the literature. Results suggest that wildlife frequented food and pasture points more often than water points, and that the number of visits increased through the dry season, peaking during the acorn season (October- January) and the deer breeding season (June-July). Direct interactions were rare (n=10), as opposed to indirect interactions (n=8992). Wildlife-followed-by-livestock interactions (n=7714) occurred much more often than livestock-followed-by-wildlife (n=1278) and were frequent at water points (66% water points, 17% food, 17% pasture). Results also suggest that water points are a hotspot for indirect interactions and might therefore be a source of infection at the wildlife-livestock interface in the territory covered, particularly for *M. bovis*, as it is around water where the bacteria seem to survive the longest. Preventing aggregation and therefore reducing contact rates between domestic and wild animals especially at water points may be valuable for disease control in South Central Spain.

Introduction

Pathogens shared by livestock and wildlife are considered an increasing problem worldwide (Daszak, 2000; Cleaveland *et al*., 2001; Gortázar *et al*., 2007). There are implications in areas such as public health (Michel *et al*., 2010), economic loss (WorldBank, 2012), conservation of endangered species (Gortázar *et al*., 2008) and animal welfare (Sainsbury *et al*., 1995). Understanding infection dynamics and disease transmission is key to establishing multi-host pathogen control measures.

Mycobacterium tuberculosis complex (MTC) bacterial pathogens (including *M. bovis and M. caprae*) cause tuberculosis (TB) and are important because they are transmitted within and between livestock and wildlife populations through direct and indirect routes of infection (Morris *et al*., 1994; Palmer *et al*., 2001; Palmer *et al*., 2004; Hermoso de Mendoza *et al*., 2006). *M. bovis* causes chronic TB in cattle and is able to affect a wide range of hosts, including humans (Morris *et al*., 1994; O'Reilly and Daborn, 1995; Delahay *et al*., 2001). It is considered a public health and economic concern both in developing and developed countries (Cosivi *et al*., 1998; Diez *et al*., 2002; Zinsstag *et al*., 2006; EFSA, 2011). Eradication of TB in cattle has been attempted by test and slaughter campaigns in several countries. However, it is thought that the existence of wildlife reservoirs (see Haydon *et al*. (2002)) compromises the success of this strategy (Corner, 2006; Gortázar *et al*., 2008).

In Spain, cattle TB prevalence has reduced since the introduction of a compulsory National eradication programme in 1987. However, in some areas herd prevalence is as high as 7.22%, although a negative trend in time in prevalence has been observed overall (RASVE, 2013). TB in South Central Spain (SCS), where hunting is part of popular tradition, is highly prevalent not only in cattle (2011 herd prevalence: 5.35% (RASVE, 2013)), but also in wild boar (*Sus scrofa)* (mean prevalence on SCS hunting estates: 44.5-52.4%) and red deer (*Cervus elaphus)* (13.7-18.5%) (Vicente *et al*., 2007b; Gortázar *et al*., 2008; Garcia-Bocanegra *et al*., 2012). Wild boar are a known TB reservoir for cattle in Spain (Naranjo *et al*., 2008) and a maintenance host in Portugal

(Santos *et al*., 2009), whilst red deer are highly suspected of being also a true reservoir (Vicente *et al*., 2007b). Prevalence data for domestic pigs is lacking and therefore their potential contribution to the maintenance of TB, as shown by Di Marco *et al.* (2012) in Sicily, is unknown (Parra *et al*., 2005). In SCS, there has been a tendency for private livestock farms to diversify into hunting estates during recent decades, thereby encouraging shared space use between wild and domestic species. Furthermore, translocations of trophy species, fencing of wildlife populations and supplementary feeding have increased aggregation and shared space use between domestic and wild animals (Gortázar *et al*., 2006; Vicente *et al.*, 2007a), which could lead to indirect transmission of disease at the interface (Palmer *et al*., 2004) if the necessary conditions occur (Corner 2006). Survival times of *M. bovis* in the environment, although highly variable, may be as much as several months, e.g. Fine *et al*. (2011a), giving rise to the possibility of indirect transmission through shared space use. Disease control at the wildlife-livestock interface requires mitigation of contact between wildlife and cattle, through adequate biosecurity, husbandry measures and pasture management (Judge *et al*., 2011). Therefore, the description of the spatial and temporal patterns of interactions at the interface is a necessary first step.

This study aims to provide information of relevance about the nature and frequency of interactions between livestock (cattle and domestic pig) and wildlife (wild boar and red deer) in an extensive beef cattle farm in Ciudad Real province, SCS. It refers exclusively to interspecies interactions and therefore should not be taken as a transmission model. We tested the hypothesis that water and food points in dry Mediterranean conditions are more appealing than pasture points and therefore are a hotspot for livestock and wildlife interactions. We also evaluated the aggregation potential on wildlife of food baiting on the ground, a practice commonly performed on large game hunting estates in SCS.

Materials and Methods

Literature review

The study included a literature review (see appendix 2) to identify estimations of survival times in the environment for *M. bovis* in different areas of the world.

Study area

The study was conducted at an extensive beef cattle farm called "Aguila y Timones" (38°32'51.49''N; 4°9'23.73''W, UTM Grid zone 30S), in Ciudad Real province, Castilla La Mancha region, SCS (figure 17). The farm territory covered 300ha and contained an average of ninety cows and five domestic pigs. Livestock extensive farming in south-western Iberian Peninsula is practiced in typical agroforestry systems called "dehesas" (savannah-like habitats) characterised by the presence of wooded pasture (predominantly oak trees *Quercus* spp. (Diaz *et al*., 1997).

Figure 17: Map of Spain showing the study area of Castilla-La Mancha in dark grey.

Farm sizes can be highly variable depending on the suitability of the land for livestock rearing. Large game hunting is a secondary use of this land and has become the predominant source of income, resulting in a heterogeneous distribution of land uses. Territories adjacent to the study farm consist of hunting estates (separated by wire fencing), a water reservoir and, partially, by a river which seasonally becomes dry. Telemetry data shows that wildlife and pigs go through the wire fence and can move between and across fenced territories (Vicente, 2010). Food baiting has been in place at the farm for several years as part of the popular tradition of game hunting, typical in SCS where game and extensive farming practices physically overlap and/or border. Wild boar, red and roe deer (*Capreolus capreolus*) on the farm occur in moderate densities compared to the rest of the region (no more than 15-20 red deer, 10 roe deer and 10 wild boar (Vicente, J. *personal communication*)). On our study farm, government test-and-slaughter activities during 2008-2011 (based on skin test, confirmed by culture after sampling at the slaughterhouse) shown mean cattle herd prevalence to be 8.65%. Regarding wildlife, animals shot on the farm and in the neighbouring hunting estates during the hunting season 2010-2011 shown a 83.9% and a 29.7% TB prevalence (based on the criteria following by Vicente *et al*. (2006)) in wild boar and red deer respectively (Vicente, personal communication). This farm is considered representative of others in the area based on similar management practices, environmental conditions, similar mean prevalence of tuberculosis-like lesions (Vicente *et al*., 2006)) and MTC (Boadella *et al*., 2011b; Garcia-Bocanegra *et al*., 2012)) in the area (Bioregion 3, (Muñoz *et al*., 2010)), and farm size (SCS mean farm area=430 hectares (SD=402) (Cowie *et al*. 2014)).

Ciudad Real environmental conditions are variable throughout the year, with temperatures ranging from below zero to over 40 °C. The wet season typically starts in September-October and contributes most of the annual rainfall. The dry season (from June to September) is when food and water resources become limited for ungulates. Water is available at artificial water holes and at a water reservoir (bordering the North and the South of the farm) throughout the year and at seasonal streams during the wet season. Cattle are not accommodated indoors at any time and their diet is supplemented during the dry season with concentrated feed and hay, provided in an elevated trough. By contrast, pigs are accommodated indoors during the dry season.

Camera trap surveys

Camera trap (CT) surveys were conducted between October 2010 and December 2011 in order to include all the seasons of the year. Heat and motion infrared-triggered CTs were used (IR-3BU®, Leaf River Outdoor Products, Taylorsville, Mississippi, USA). The infrared source works through its connection to the movement sensor and presents a maximum scope of 6 metres. Cameras were set to take up to three pictures per minute, and are able to record continuously. Date and time were displayed for each picture.

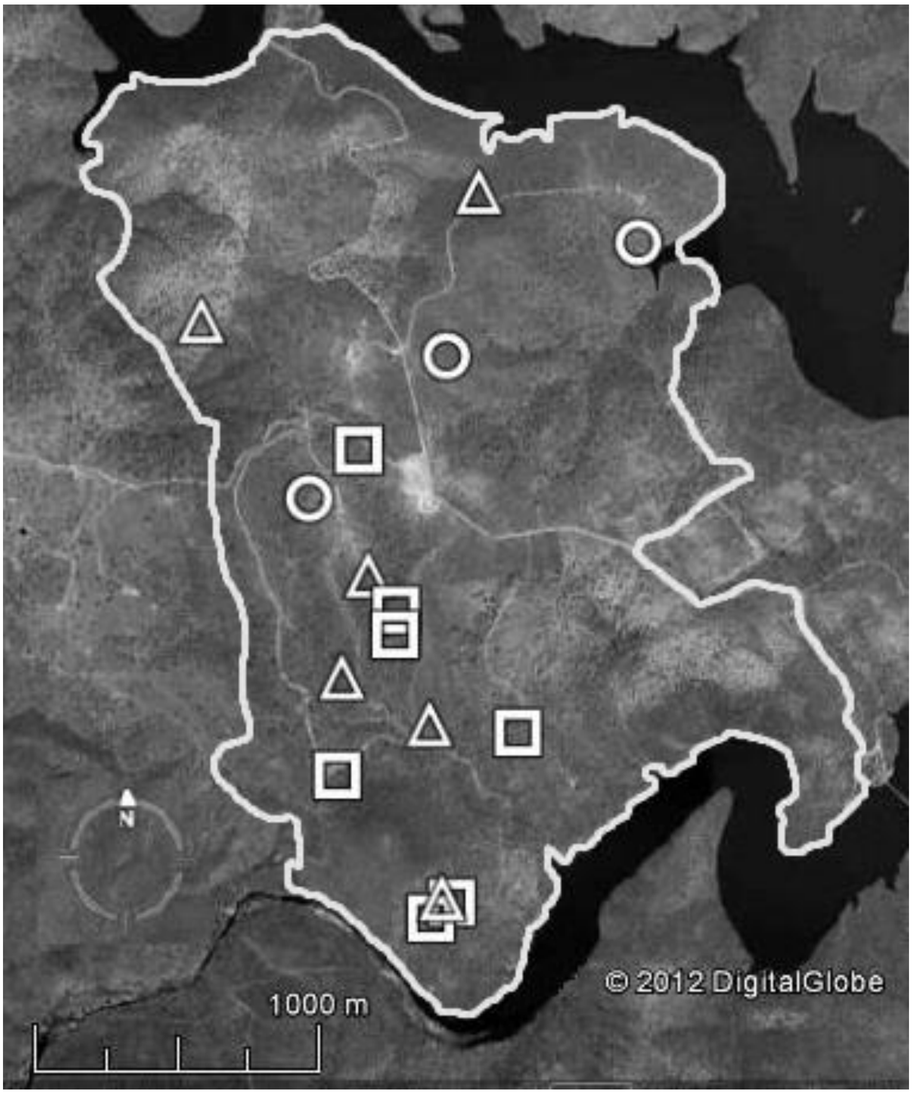


Figure 18: CT distribution at Águila y Timones farm territory (delimited by a continuous white line). Camera location type (water, pasture and supplementary food points) is represented by figures (circles, triangles and squares, respectively)

Sixteen cameras were placed on the farm with the aim of covering all the water and artificial food resources, and covering pasture areas to provide control data points (under the hypothesis of food and water being aggregation points). As a result, cameras were slightly more concentrated towards the south-west of the farm, which contains more resources and wildlife habitat (figure 18). Pasture units (n=6) were placed in order to avoid water and food points, as well as wildlife and/or cattle paths between these resources, in order to consider them control points. Food units (n=7) were placed at supplementary wildlife feeding points (n=6) (where maize was placed simulating hunting food baiting on the ground at natural roaming places) and at an elevated cattle-feeding trough (n=1). Water units (n=3) were placed at natural and artificial water points. The number of active CT stations varied during the study period due to conditions that were beyond the control of the research team, such as theft and flooding of cameras. Therefore, camera-days (number of days a camera unit was operational) varied between CTs.

Camera trap stations consisted of a single camera placed 30-50 cm above the ground (detection distance average: 3.5m, detection arc: 90 degrees). All CTs were considered independent (unable to record the same area patch) as they were separated from each other at least 37 metres, at least six times the CT activation distance.

Data coding

Secure digital memory cards were collected and replaced from the CTs, and batteries replaced as required in the field, at least every two weeks. Pictures recorded by each camera unit were stored in unique folders which were studied individually by visual observation at the time of constructing the Excel data base (Microsoft Excel, version 2007; Microsoft Coorporation). Photograph details were stored in Excel files containing the following variables: Camera location type, camera unit identifier, date (dd/mm/yy), start and finish time of each single series of pictures (hours, minutes and seconds) and animal species (cattle, pig, wild boar and red deer). As animals were not individually identified, assumptions had to be made to when classifying animals as separate individuals.

Variable definitions

Independent variables were Camera location type (water, food and pasture) and Season (dry, June to September; and wet, October to May, according to environmental conditions of Ciudad Real).

Dependent variables were direct interactions and indirect interactions. A direct interaction was defined as simultaneous presence of two or more species in the same photograph. An indirect interaction was defined as visit of one species to a CT unit following the visit of another species within a specific Critical Time Window (CTW). A conservative CTW was assessed to be twelve days during the wet season and three days during the dry season, determined by the literature review (appendix 2).

Statistical analysis

Descriptive analyses of livestock (cow and pig) and wildlife (wild boar, red deer and roe deer) activity patterns were conducted in Microsoft Excel and R software (version 2.15.0; Bell laboratories, Auckland). Activity patterns were described as: a) Daily activity profile (proportion of visits to CT by Hour of the day and Season for each species), b) Number of visits by Month of the year and species, and c) Number of visits to all CTs according to Season and CT type regarding livestock and wildlife. Since cameras registered variable numbers of days per month and season, corrections were applied as follows: the observed number of monthly visits (multiplied by the monthly camera-days) was divided by the minimum-camera-days recorded in a month (which was September with 305 camera-days); whereas the observed number of visits to all CTs according to Season and CT type (multiplied by the camera-days for the specific season and camera type) was divided by the minimum-camera-days recorded by "Season and Camera type" combination (Dry-Water=327 days).

Given count data and overdispersion, a negative binomial model was selected to estimate indirect interactions between livestock (cattle and pigs) and known or highly suspected TB wildlife reservoir species (wild boar and red deer) by season and CT (Zuur, 2009). Eight separate models were fitted (four models for livestock using an area after wildlife and four models for wildlife using an area after livestock). Unit of analyses were the 15 CTs (due to absence of detection of any wildlife species of interest at the elevated cattle feeding trough (food point), this camera was removed from the analysis). Independent variables were CT type and season. Outcomes were the number of indirect interactions at each CT in each season. The log of the number of camera-days at each CT was used as indicator of effort. Letting μ_{ij} represent the expected number of indirect interactions at CT type j in season i, the equation was:

$$Log(\mu_{ij}) = \beta_1 * Season_i + \beta_2 * CT\ type_j + Effort$$

Results of the model were the estimated mean number of indirect interspecies interactions for each season and CT type with their 95% confidence interval. To obtain the estimated monthly average number of indirect interactions, the number of indirect interactions of each season was divided by the number of months the season covered (dry = four months, wet = eight months). Results are presented as monthly average number of indirect interactions per season by each camera type. SPSS Statistics (version 19; Inc. Surrey, UK) was used to fit all negative binomial models. All reported *p*-values are 2-sided and *p*-value <0.05 was considered statistically significant.

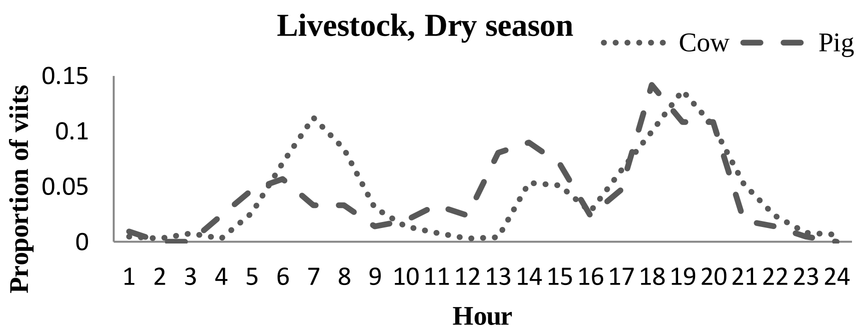
Results

Due to absence of detection of any wildlife species of interest at the cattle elevated feeding trough, the pool of pictures from food CT units belongs exclusively to wildlife supplementary feeding points (n=6).

Activity patterns: Diurnal activity profile

Cattle were found to be a diurnal species with two peaks of activity between 6-8h and 17-21h. The domestic pig was also found to be a diurnal species but with a long bout of activity during daytime (figure 19(a) and (b)). Wild boar are a crepuscular/nocturnal species with a peak of activity between 23h-5h. Red deer are a mainly crepuscular/nocturnal species, presenting two peaks of activity between 5-8h and 19-22h, although some diurnal activity is observed in the wet season. Roe deer are a mainly nocturnal species, although they also have a marked bout of activity throughout the day (figure 20).

(a)



(b)

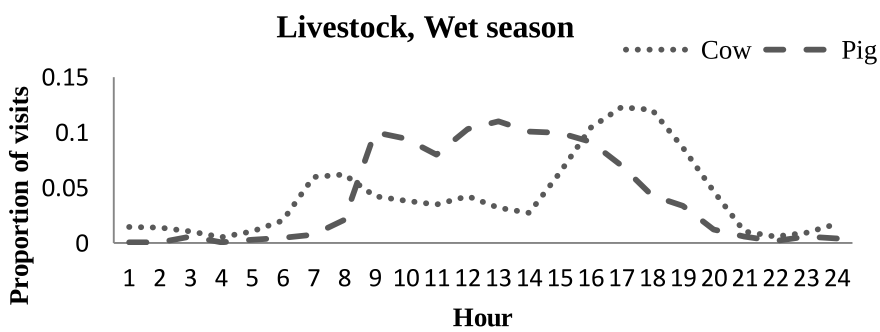
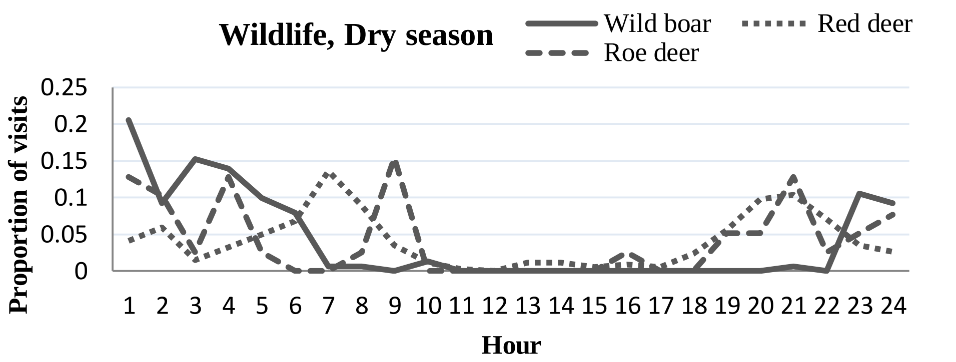


Figure 19: Livestock daily activity profile at Águila y Timones farm during the study period assessed as proportion of visits to all CT by Hour of the day and Season

(a)



(b)

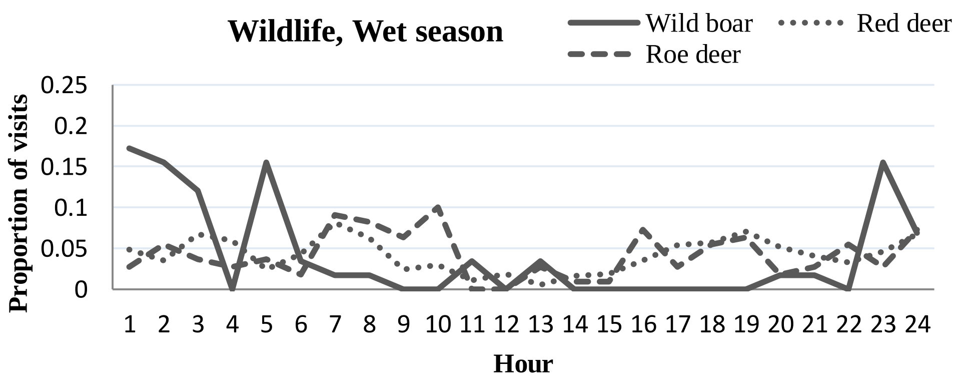


Figure 20: Wildlife daily activity profile at *Águila y Timones* farm during the study period assessed as proportion of visits to all CT by Hour of the day and Season

Number of visits by Month

Cattle visits were most frequent from April to August, whereas domestic pig visits were most frequent during October-December (figure 21). Wild boar visits were more frequent during July-August with a second peak during October, and red deer visits were more frequent during June-August, with a second peak during November-December. Finally, roe deer visits were more frequent during September-December (figure 21).

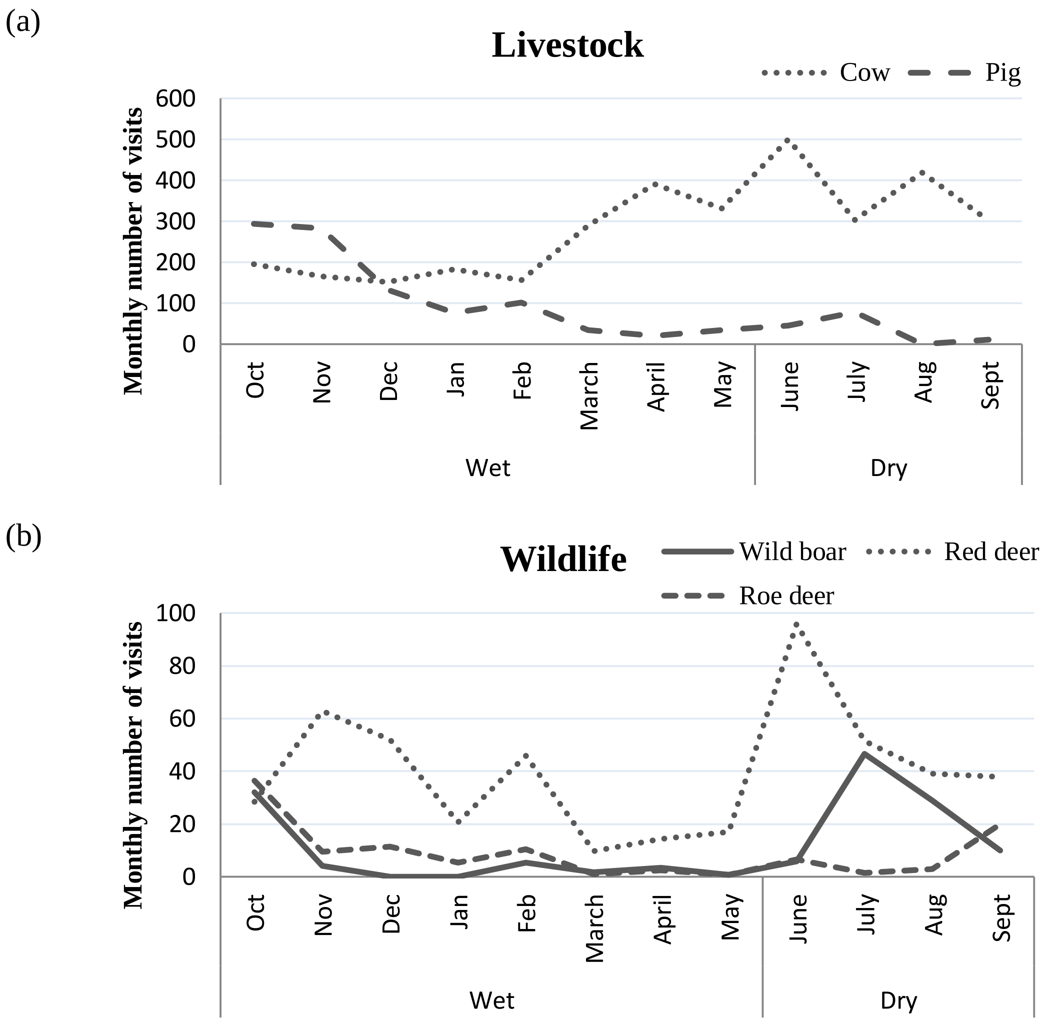


Figure 21: Number of visits to all CT by Month of the year and species (cow, domestic pig, wild boar, red deer and roe deer) corrected by the minimum-camera days recorded in a Month (305 days)

Number of visits according to Season and CT type regarding livestock and wildlife

Livestock visits were more frequent at water CTs than at food or pasture, especially during the dry season. Visits by pigs were less frequent at food and pasture CTs during the dry season than at water CTs. Wildlife visits at water CTs were scarce across all seasons. They were also scarce at pasture CTs, except for red deer throughout the year and roe deer during the wet season. Regarding wild boar, visits were more frequent at food CTs, especially during the dry season, whereas roe deer visits were more frequent at pasture and food CTs, during the wet and dry seasons respectively (table 15).

Table 15: Corrected number of visits according to Season (dry and wet) and Camera type (water, food and pasture) of livestock and wildlife during the study period (Number of CT=15; Águila y Timones, Ciudad Real, Spain. 2010-2011)

Camera type	Season	Cow	Pig	Wild boar	Red deer	Roe deer
Water	Dry	1062	168	2	12	10
	Wet	539	143	0	6	1
	Total	1601	311	2	18	11
Food	Dry	326	8	58	61	11
	Wet	120	166	12	16	4
	Total	446	174	70	77	15
Pasture	Dry	155	0.6	3	90	2
	Wet	211	103	3	68	20
	Total	366	104	6	158	21

Direct interactions

Direct livestock-wildlife interactions were very rare (n=10) and only occurred between wild boar and pigs (n=8), and between cattle and red deer (n=2). Only one of these occurred in the wet season, with all others observed in the dry season.

Indirect interactions

Indirect interactions (n=8992) occurred more frequently than direct interactions. Using a critical time window of twelve days during the wet season and three days during the dry season, we observed 1278 indirect interactions of livestock followed by wildlife and 7714 indirect interactions of wildlife followed by livestock.

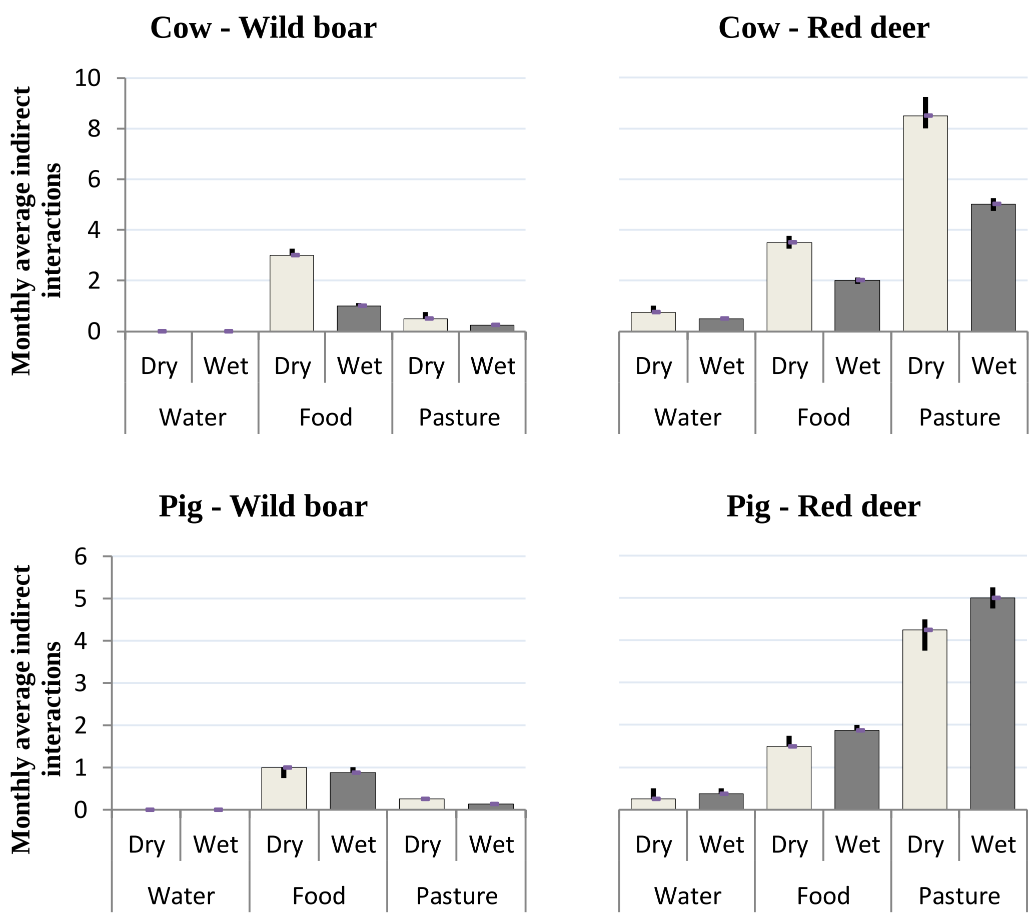


Figure 22: Indirect interactions of livestock followed by wildlife displayed as predicted monthly average number of indirect individual interactions (and therefore corrected by CT effort)

Results from fitting the negative binomial model (figures 22 and 23) predicted monthly average number of indirect interactions per season by each camera type (and therefore corrected by CT effort) with confidence intervals of 95% (error bars). In all models, associations between number of indirect interactions with camera type and season were statistically significant ($p<0.001$) when both variables were entered simultaneously in the equations. All post –hoc tests of differences in number of indirect interactions by camera type were also statistically significant ($p<0.001$).

Indirect interactions of livestock followed by wildlife according to season were more frequent during the dry months, except for pigs followed by red deer interactions. According to camera location type, predicted indirect interactions of livestock followed by wild boar were more frequent at food points, whereas interactions of livestock followed by red deer were more common at pasture points (figure 22).

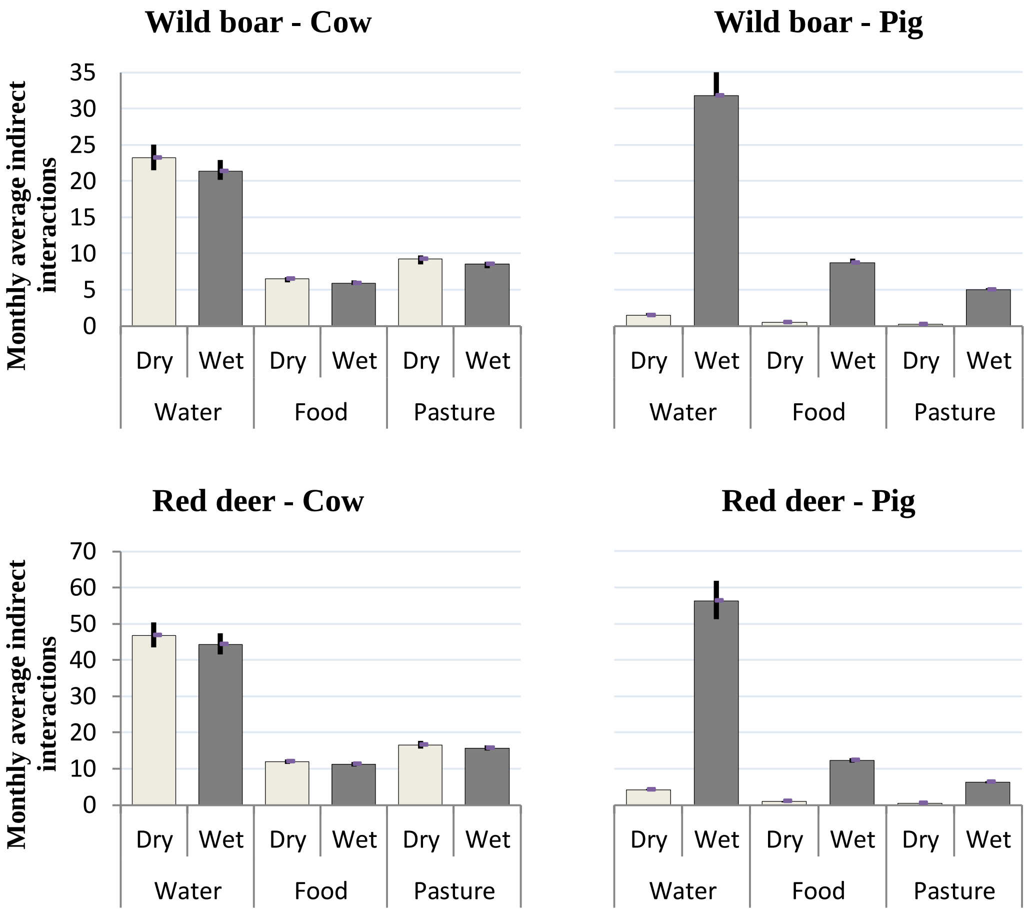


Figure 23: Indirect interactions of wildlife followed by livestock displayed as predicted monthly average number of indirect individual interactions (and therefore corrected by CT effort)

According to season, predicted indirect interactions of wildlife followed by cattle were more frequent during the dry months, whereas indirect interactions followed by pig were more frequent during the wet months. According to camera location type, water points had the highest number of wildlife followed by livestock predicted indirect interactions compared with food and pasture (66% water points, 17% food, 17% pasture) (figure 23).

Discussion

The aim of this research was to increase our understanding of spatial and temporal interactions between livestock and wildlife. Our observations at *a priori* risk points for interactions show that 1) direct interactions occurred very rarely, 2) indirect interactions of wildlife followed by livestock were more frequent than the opposite and occurred mostly at water points, and 3) indirect interactions of livestock followed by wildlife occurred mostly at supplementary feeding points for wild boar interactions and at pasture points for red deer interactions, especially during the dry season for cattle interactions and wet season for domestic pig interactions.

Data validation

CT is a non-invasive method useful for estimating interactions as it is able to record data continuously, it does not affect animal behaviour and causes minimal disturbance of the study animals, therefore reducing observer bias caused by the presence of a surveyor (O'Connell, 2010) when compared with other methods. Regarding measurement errors, one of the main problems found with the use of CTs are “false absences”, defined as failure to detect an animal when present in the area (MacKenzie *et al*., 2004). Detection probability should be assessed in future studies in order to address underestimation of animal presence and interactions due to this problem. An important factor to bear in mind when detection probability is studied, especially with regard to disease transmission, is the effect of terminally ill animals. When terminally ill, infected animals may present behavioural changes. On the one hand, lack of elusive behaviour leading to closer or direct contact with individuals of other species has been reported (Paterson & Morris, 1995; Sauter & Morris, 1995): cattle showed an increased interest and physical approach towards simulated sick possums, which could mean an increase of interactions in appealing points such as food or water reservoirs where CT are placed. On the other hand, terminally-ill animals could have a reduced range of movement in the area and may therefore be less likely to move as much as they normally do. This could negatively affect the likelihood of detection by CTs. Nevertheless, a recent study (Drewe *et al.*, 2013) suggests that infection status of badgers naturally infected with tuberculosis does not affect the behaviour of their visits to latrines located in areas shared with cattle. Consequently, it is unclear if or how detection is affected by infected animal behaviour.

CTs were placed to maximise coverage while assuring independence of territories covered by camera. All supplementary feeding points were covered; however, due to the ample water extension area, theft and flooding of some water CTs, some water points were not covered.

Activity patterns

Camera traps have been recently used in research to assess overlapping of activity patterns (Harmsen *et al*., 2009; Ridout and Linkie, 2009; Linkie and Ridout, 2011), which could lead to direct interactions between different species. Daily activity patterns suggest that diurnal species such as cattle, pig and to some extent red deer may be more likely to interact directly. Following the same reasoning, crepuscular or nocturnal species such as wild boar and roe deer could be more likely to interact directly with each other. However, in agreement with Böhm *et al*. (2009), few direct interactions were observed during the study (n=10), all of them taking place at wildlife feeding points. The scarce number of direct interactions suggests that indirect interactions may have a more important role in the *M. bovis* transmission in SCS than direct interactions. Future work should focus on indirect interactions as the same spoligotypes have been found in both wildlife and livestock populations in the area (Vicente, personal communication), suggesting evidence of interface pathogen transmission most likely due to indirect interactions.

Regarding the number of visits per species to the different CT location types, the study showed that livestock more often frequented water and food points compared to pastures. Wild boar visited food points more often compared to other points, whereas deer species visited pasture points relatively more frequently. These results could be a reflection of the limited water and food availability in this region, especially in summer time, and suggest aggregation of animals around these resources. However, as this farm is bordered by a river and natural water sources are readily available, water-related events must be interpreted with caution regarding application of the results to other farms. Vicente *et al*. (2007b) described a positive association between wildlife aggregation at water and feeding points (at the ground level) and tuberculous-like lesions in large game hunting estates, thus suggesting that aggregation could drive the risk of *M. bovis* transmission. Additionally, Kaneene *et al*. (2002) and Castillo *et al*. (2011) demonstrated a positive correlation between the number of watering points in farms and increased risk of TB in Michigan and South Western Spain, respectively (a case control study carried out in the Republic of Ireland refutes this idea (Griffin *et al.*, 1993), however, this may be due to different management practices). Considering these results, food and especially water points should be considered potential hotspot for *M. bovis* transmission in farms, and therefore pathogen control methods should focus on feeding and watering sites management. Our results suggest that food baiting for hunting, although in place for short periods of time, could be considered a multi species aggregation point, and therefore a potential disease transmission point.

Seasonal patterns

According to season, there is a generalised increase in the number of wildlife visits during the dry season. We speculate that this could be a result of an increase of movement due to the scarcity of natural food and water resources. In addition, the strikingly high number of red deer visits mainly due to hinds (data not shown) during June-July could be explained by the natural species breeding period (Carrascal and Salvador, 2002). Regarding the autumn increase of visits, we speculate that large game move seasonally from hunting grounds to surrounding farms during the hunting season (October- February). Most of the hunting season coincides with the *montanera* season (October- January) when *Quercus* acorns fall from the trees, contributing to a large proportion of the animal's seasonal diet. The domestic pig's yearly activity pattern reflects the traditional management strategy related to *montanera*: due to harsh environmental conditions and to avoid weight loss, pigs are housed and fed during the dry season. Once the *montanera* season arrives, they are released back onto the farm to obtain their highest weight at the end of this period, before being slaughtered.

Therefore, most pig visits were recorded during the wet season. As pigs from nearby farms are able to pass through the wire fence surrounding the farm, some pigs were still recorded during the dry season.

The literature review conducted in this study reveals a dearth of knowledge about environmental *M. bovis* persistence and infectivity, especially in hot and dry conditions with high levels of solar radiation such as those of Ciudad Real. Research is currently underway in Doñana National Park, South of Spain; nevertheless, there is still a need for better *M. bovis* detection methods in the environment (Fine *et al*., 2011b). This would increase the accuracy of CTWs in studies such as ours and therefore help in the development of efficient disease management strategies.

Predicted interactions

In order for transmission to occur, several factors must be addressed, with interactions between species being only one of them (Corner, 2006). This study refers exclusively to interspecies interactions; and should therefore not be taken as a transmission model. Interactions were only measured between livestock and known or highly suspected *M. bovis* reservoirs (wild boar and red deer) as there is not enough evidence to consider roe deer a reservoir species in Spain. According to our CTW selection, indirect interactions of livestock followed by wildlife were less frequent than interactions of wildlife followed by livestock. They occurred more often at food points for wild boar and pasture points for red deer and during the dry season for cattle and wet season for pigs. These results could be explained by the higher number of livestock individuals than those of wildlife on this farm. We speculate that, if conditions of indirect transmission took place, the main direction of transmission would be from wildlife to livestock. Nevertheless, once *M. bovis* is maintained in both wild and domestic animal populations it is probable that the direction of transmission is bidirectional. More research is needed regarding *M. bovis* transmission dynamics and environmental survival in the area to fully assess the implications of these results. A specific example of transmission through habitat sharing has been seen in Kruger National Park between Cape buffalo and cattle, where the same dominant strain of *M. bovis* has been isolated in both species

(Michel *et al*., 2009). Avoidance measures such as species specific feeder devices could be implemented (e.g., Long (2010)) in order to avoid food sharing and potentially disease transmission (Palmer *et al*., 2001; Palmer *et al*., 2004) if hunting food baiting is to be continued.

Consistent with the activity patterns above, indirect interactions of wildlife followed by pigs occurred more often during the wet season, probably because they are kept indoors during the dry season. Indirect interactions of wildlife followed by cattle occurred more often during the dry season and would occur more frequently at water points. Regarding indirect disease transmission at water points, *M. bovis* has shown long survival times at high humidity in sites with close climatological conditions to Ciudad Real (Duffield & Young, 1985; Tanner & Michel, 1999). Further studies are encouraged to establish *M. bovis* survival time and risk of disease transmission in water points in this region.

In order to control disease at the interface there is a need to implement biosecurity measures in extensive farming systems in SCS (Judge *et al*., 2011), in conjunction with the continued efforts of livestock disease eradication schemes, and the application of wildlife disease control methods (such as oral *M. bovis* vaccination in wild boar (Garrido *et al*., 2011; Beltran-Beck *et al*., 2012)).

Conclusions

The wildlife-livestock interface in SCS does not usually involve direct interactions, but indirect interactions. Our findings suggest that water points are a hotspot for indirect interactions and might therefore be a source of infection for livestock in the territory covered, particularly for *M. bovis* as it is in water where the bacteria seems to survive the longest.

Appendix 2: Literature review on *M. bovis* survival time

This appendix shows the supplementary material for Appendix 1, and details a literature review conducted by Esther Kukielka during her MSc research project, co-supervised by the author of this thesis. This is thought to be the best available review of the literature on the environmental survival of *M. bovis*, and the results were applied to environmental conditions at the study farm used in Chapter 4 and Appendix 1.

Literature review

The literature search on environmental survival times of *M. bovis* was conducted through Web of Knowledge and Pubmed covering the period from 1980 to May 2012 in order to establish a critical time window (CTW) during which survival of viable *M. bovis* in the environment could lead to exposure of the bacterium to other species. Search terms were*: "survival AND Mycobacterium bovis AND environment", "persistence AND Mycobacterium bovis AND environment" and "epidemiology AND Mycobacterium bovis AND environment".* In total, 132 citations were listed and four articles selected because they offered estimations of *M. bovis* survival times in the environment. Relevant references cited in these four articles were traced and 11 additional research reports were found. Among them, one doctoral dissertation and two review papers (Morris *et al*., 1994; O'Reilly and Daborn, 1995) covered survival times established in the literature. Therefore, 15 papers were retrieved and critically reviewed. The environmental conditions of the research sites described in the 15 papers were examined in order to assess the external validity of their results in Ciudad Real weather conditions.

External validity of previous M. bovis survival studies in Ciudad Real

Monthly maximum average temperatures and average precipitation during 2011 were investigated through ForecaLtd. (2012). Global Horizontal Radiation (a measurement of solar radiation) was extracted from the European Photovoltaic Geographical Information System (PVGIS).

Temperature and precipitation levels on most of the stated research sites differ greatly when compared to Ciudad Real (appendix 2). Ciudad Real is the hottest of all research sites of the Northern (showed) and Southern hemispheres (not shown because of inversion of seasons). Monthly maximum average temperatures in the Southern Hemisphere research sites (Australia, New Zealand and South Africa) vary between 15 and 30^{o}C (ForecaLtd., 2012). Ciudad Real has the lowest rate of precipitations for every month, except for Nelspruit in May-June (only research sites of the Northern hemisphere shown). It is important to consider that inter-annual variability in the precipitation regime is very high in Mediterranean climates. In addition, solar radiation differs greatly when compared to Ciudad Real. As an illustration, the yearly total Global Horizontal Radiation is 1800 kWh/m^2 in Ciudad Real, compared with 1000 kWh/m^2 in Southern England (PVGIS).

Ciudad Real weather was herein described by two periods: wet season (October-May, with monthly precipitations of more than 2 cm/m^2, less than 23ºC as average monthly maximum temperature and less than 6000 Wh/m^2 average daily solar radiation) and dry season (June-September, with monthly precipitations of less than 2 cm/m^2, over 23ºC and over 6000 Wh/m^2)

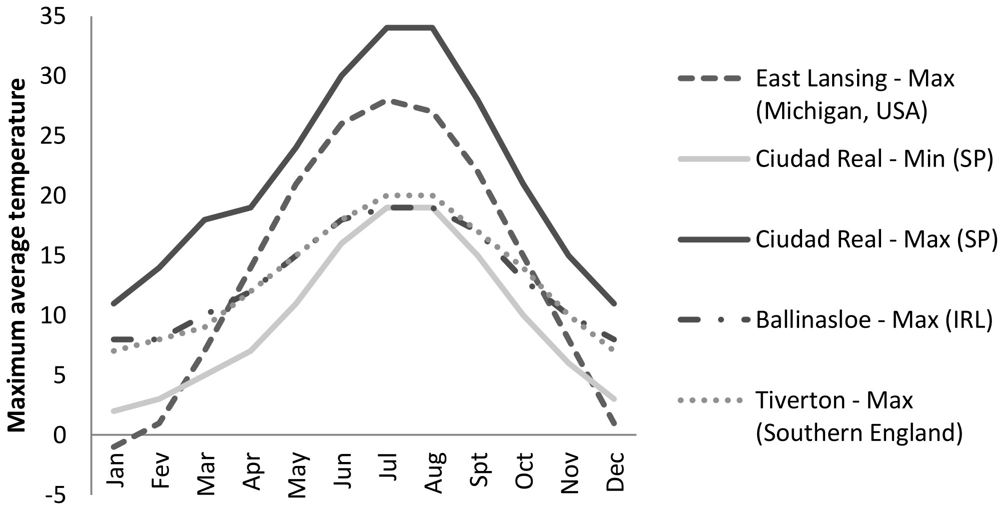


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Figure 24: Monthly maximum average daily temperature during 2011 in research sites from relevant papers retrieved in the literature review on *M. bovis* survival in the environment.

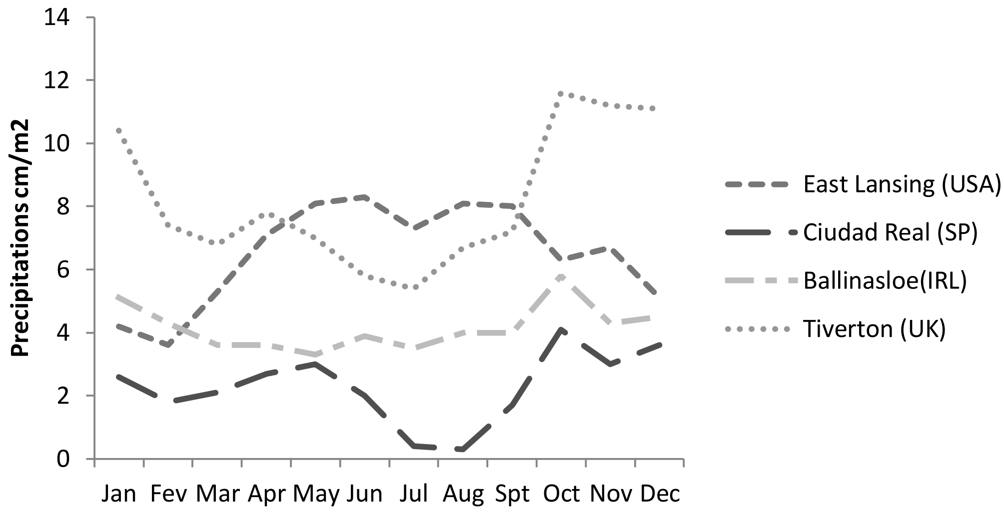

Figure 25: Precipitation average during 2011 in research sites from relevant papers retrieved in the literature review on *M. bovis* survival in the environment.

Survival times of *M. bovis* in the environment were found to be highly variable dependant on substrate studied, *M. bovis* detection methodology and environmental factors related to study site, varying between zero and 730 days with a median of 49 days. Table 15 (Appendix 2) summarizes reported maximum survival times, reflecting its variability.

The most extensive paper to establish *M. bovis* survival was by Fine *et al*. (2011a). In East Lansing (Michigan, USA) *M. bovis* survived up to 88 days in soil, 55 days in hay and water and 43 days in corn. Survival was significantly shorter in spring/summer compared with fall/winter: up to 37 days survival in soil, 58 days in hay and water, and 43 days in corn in fall/winter while only 20 days in soil, 3 days in hay, 53 days in water and 11 days in corn in spring/summer.

Two studies were carried out in Mediterranean-like climates and are therefore relevant to Ciudad Real. Tanner and Michel (1999) reported that *M. bovis* could survive in experimentally contaminated faeces up to 28 days and a minimum of 5 days at Kruger National Park, South Africa. Maximum survival times were 28 days under sunlight conditions and during winter and moist conditions, when temperatures range between 0-

32 ºC. Minimum survival times were two days under sunlight conditions and during spring time, when temperatures range between 20-40 ºC. Duffield and Young (1985) reported an *M. bovis* environmental survival of less than a week under sunlight conditions, at a mean temperature of 43 ºC and up to 28 days under 80% shade conditions, with a mean temperature of 34 ºC. These results indicate that temperature, humidity and sunlight influence *M. bovis* survival times in the environment.

Due to climatological conditions, *M. bovis* in Ciudad Real will probably have lower survival rates than those previously reported, although maybe similar to those carried out at Kruger National Park and probably also in Australia. Ciudad Real weather was herein described by two periods according to Vicente *et al*. (2007) and to average monthly temperature, precipitation and solar radiation variation: wet season (October-May), and dry season (June-September). Sensitivity analyses with different CTW were carried out for this study without considerable changes in model estimates of associations between number of indirect interactions, CT types and seasons (we used firstly, seven days during the whole year; and secondly, three days during the dry season and seven days during the wet season). Therefore, conservatively CTW was assessed to be twelve days during the wet season and three days during the dry season.

Table 16: Reported M. bovis maximum survival times from relevant papers retrieved in the literature review on M. bovis survival in the environment.

Reference	Maximum *M. bovis* survival	Sample type
Williams and Hoy (1930)	180 days during Autumn	Faeces
Maddock (1933)	49 days during Summer	Soil artificially infected with infected lung tissue
Maddock (1936)	42 days	Faeces
Genov (1965) cited in Morris *et al*. (1994)	730 days	Mix of faeces, blood and urine buried 5 cm deep
Anon (1979) cited in Morris *et al*. (1994)	70 days during Winter	Bronchial pus
Duffield & Young (1985)	28 days	Soil under 80% shade and moist conditions
Jackson *et al*. (1995)	Between 14 and 28 days in Spring and Winter	Soil
Tanner & Michel (1999)	42 days during Winter	Infected lung tissue under moist and shade conditions
Scanlon & Quinn (2000)	180 days	Slurry under shade conditions
Young *et al*. (2005)	Up to 300 days	Genes found in soil
Palmer & Whipple (2006)	112 days	Foodstuff
Fine (2006)	No *M. bovis* recovery	Soil, hay, corn and water
Michel *et al*. (2007)	No *M. bovis* recovery	Water
Fine *et al*. (2011a)	88 days	Soil during Winter/Spring
Fine *et al*. (2011b)	No *M. bovis* recovery	Hay, grain, silage, pasture grass, soil, faeces, blood, water

Appendix 3: English translation of the questionnaire used in Chapter 2 as part of interviews with cattle farmers in south-central Spain

Question	**Response Type**
Farmer name and telephone number	Name/Tel. no.
Is the respondent also a hunter?	Yes/No
REGA number (Farm government registration number)	Number
Coto number (Hunting permit for the land, if applicable)	Number
Who owns the cattle on this farm?	Farm owner/ A contractor
How long has this farm been used for cattle farming?	Number (years)
If the farm has been used for cattle farming for less than 10 years, what was the previous land use?	Text
What type of cattle farming is conducted on the farm?	Dairy/Meat/Breeding animals for bull fighting/Fattening for beef
How are the cattle kept on this farm?	Extensive/Semi-extensive/Intensive
What is the total area of the farm?	Number (hectares)
In the last 10 years, has any adjacent farm changed from cattle farming to being a hunting estate?	Yes/No
Or the inverse?	Yes/No
Is shelter always available to cattle?	Permanent/Not permanent
What types of shelter are available to the cattle?	Closed building(s)/Open building(s)/Yard/None
Is there a river, pond or other natural body of water on your farm?	Yes/No, if no skip to next section
If there is water, is it permanent or non-permanent?	Permanent/Non-permanent
What is the length of the shoreline of the largest body of water?	Number (meters)
How many pools can cattle access?	Number
How many permanent pools are there on the farm?	Number
Are there other drinking places which wildlife can access?	(provide details)
Are the pastures that you use...	Owned/Leased/Communal

Question	Response Type
If you own the pastures, are they divided between...	Other livestock cattle (no/ sometimes/always) Goats (no/ sometimes/always) Sheep (no/ sometimes/always) Pigs (no/sometimes/always)
If the pastures you use are leased, are they divided between...	Other livestock cattle (no/ sometimes/always) Goats (no/ sometimes/always) Sheep (no/ sometimes/always) Pigs (no/sometimes/always)
If the pasture you use are communal, are they divided between...	Other livestock cattle (no/ sometimes/always) Goats (no/ sometimes/always) Sheep (no/ sometimes/always) Pigs (no/sometimes/always)
Regarding the storage of animal feed for the cattle, Are concentrates/volume foods stored...	Outside without restrictions to wildlife access/outside protected by a fence or low wall/outside protected by a high wall or wildlife proof fencing/stored inside a shed that wildlife could access/stored in a sealed warehouse or shed inaccessible to wildlife
Whereabouts are the cattle offered the animal feed? (Concentrates/volume feeds separately)	On the ground with unrestricted access by wildlife/in a low feeder relatively accessible to wildlife/in a high feeder relatively inaccessible to wildlife/offered to cattle only within pens that are inaccessible/other places (please provide details)
How many cattle were purchased and/or entered the farm from within your veterinary district in the last year?	Number

Question	Response Type
How many cattle were purchased and/or entered the farm from outside your veterinary district in the last year?	Number
How many cattle were purchased and/or entered the farm from within your veterinary district in the 5 years?	Number
How many cattle were purchased and/or entered the farm from within your veterinary district in the 5 years?	Number
What happens to the manure from the pens?	Removed from any pens but kept on the land/removed from the pens but stored elsewhere on the farm/stored and used to fertilise the farm/Not removed
How many people live on the farm?	Number
How many people work but do not sleep on the farm?	Number
How many individuals of the following species exist on the farm?	Number of ... Cats, Guard/working dogs, other dogs, goats, sheep, pigs in extensive farming, pigs in intensive farming.
If you have dogs, how are they maintained?	Number kept loose/tied up/free ranging
What type of fencing do you have in the area cattle are kept, and on the whole farm?	Wire fence/Barbed wire/Electric fence/Mesh fencing (height 1.5m or less)/Wildlife proof fencing/Walls/Other (please specify)
If you do have fences, what entrances exist on the area cattle are kept, and the whole farm?	Gate(always closed)/Gate (not always closed)/Single bar/No gates/Other (please specify)
Indicate the maximum number of individuals of each of these species you have seen in one day on the whole farm and in the cattle occupied areas in the last year: Red Deer/Roe Deer/Wild Boar/Badger/Rabbits	Number (count) for each species
Please indicate if you have seen signs of the following species in the last year on the whole farm and in the cattle occupied areas: Red Deer/Roe Deer/Wild Boar/Badger/Rabbits/Foxes	Presence/Absence of observed signs

Question	Response Type
What is your opinion of each of the following species in relation to the damage they have caused on your farm? Red Deer/Roe Deer/Wild Boar/Badgers/Foxes/Rabbits/Pigeons	1-10 scale where 1=very little problem and 10=big problem.
What kinds of damage by wildlife have you experienced on your farm?	Please specify
If damage has been experienced, what actions have you taken to avoid/prevent further damage?	Please specify
What is your opinion of the following wildlife species, in relation to the diseases that they could pass to cattle? Red Deer/Roe Deer/Wild Boar/Badgers/Foxes/Rabbits/Pigeons	1-10 scale where 1=very little problem and 10=big problem.
If you hunt on your farm, do you hunt large or small game, or both?	Large game/ Small game/ both
How many wild boar and/or red deer were hunted on the farm in the last year?	Number (count)
What is your opinion of large game hunting in your area?	Scale from 1-10 where 1=very negative and 10=very positive
What is your opinion of wildlife management in your area?	Scale from 1-10 where 1=very negative and 10=very positive
Are there any other aspects of game management that concern you regarding diseases shared with cattle?	Please specify
How important do you think TB is in your area?	Scale from 1-10 where 1=not important and 10=highly important
Do you believe wildlife transmit TB to cattle?	Scale from 1-10 where 1=very unlikely and 10=very likely
How do you think TB is changing in your region?	Increasing/Staying the same/Decreasing
What organisations, associations, groups or professionals have given you information about how to control TB?	Please specify
Please tell us any general comments you may have	Comments

Appendix 4: List of all potential TB risk factors identified in Chapter 2

Table 17: This table shows all the variables generated by the questionnaire, participatory mapping and the Spanish government's cattle TB testing information. The p values reported are the result of the initial univariate binary GLM models of each variable with the response variable of the detected presence or absence of TB in cattle on each farm. Models where p<0.10 were considered for the final model and are marked with a "*" symbol. This table is accepted as online materials for publication in the *European Journal of Wildlife Research*.

Variable name	Variable description	Response type	Data summary	d.f.	*p* value	Notes
Variables generated from the questionnaire						
Farmer name	As some farmers had more than one farm, each farmer name forms a new category	Category (names)	67 different respondents gave answers about 73 different farms as some owned more than one farm	71	0.130	
Hunter status	Does the respondent farmer also engage in hunting activity	Binary (Yes/No)	N Yes=29 N No=44	71	0.394	
Cattle Owner	Who owns the cattle that are kept on this farm?	Binary (Farm owner/A contractor	N Owner=39 N Contractor=34	71	0.241	
Farm cattle rearing duration	How long each farm has been used for cattle rearing	Continuous (years)				Almost all cattle farms had been in this land use for as long as respondents could remember and there was insufficient data for statistical analysis.
10 year land use change	What the previous land use was if this land was converted to cattle farming within the last 10 years	Categorical – grouped by responses				Almost all cattle farms had been in this land use for as long as respondents could remember and there was insufficient data for statistical analysis.
Cattle type	What type of cattle farming was is conducted on the farm. "Fattening for beef" refers to seasonal short term grazing of cattle to fatten them before slaughter, rather than engaging in breeding and rearing cattle. Normal "beef" rearing refers to maintaining a herd and breeding from them to generate stock for slaughter.	Categorical – Dairy/Beef/Breeding for bull fighting/Fattening for beef	N Beef = 49 N Fattening for beef = 21 N Breeding for bull fighting=1 N Beef and fattening for beef mixed=2	71	Beef=0.066* Fattening for beef = 0.63 Bull fighting = 0.99 Mixed = 0.70	In categorical analysis Beef farming was significantly negatively associated with the presence of TB. A variable called 'beef farming' was later generated to examine this further, shown later in this table

Variable name	Variable description	Response type	Data summary	d.f.	*p* value	Notes
Cattle management	How the cattle are kept on the farm	Categorical – Extensive/Semi extensive/Intensive				All respondents said that the cattle were farmed extensively- i.e. they were able to range throughout the farm year round
Respondent stated farm area	The respondent's estimate of the area of the farm	Continuous (hectares)	Range 20 – 2100 Ha Mean 437 Ha	71	0.341	There is also a variable for the area of each farm based on the farm perimeters drawn by respondents
Neighbour change from cattle to hunting	Have any neighbouring cattle farms converted to being hunting estates in the last 10 years	Binary – Yes/No	N Yes = 8 N No = 65	71	0.114	
Neighbour change from hunting to cattle	Have any neighbouring hunting estates converted to cattle farming in the last 10 years	Binary – Yes/No	N Yes = 1 N No = 72			As only one respondent had a neighbouring estate that had converted to cattle farming there were insufficient data to conduct statistics on this variable
Cattle shelter permanency	The permanency of the shelter available to the cattle on the farm	Binary - Permanent/Non-permanent				All farmers responded that there were no shelters, and therefore any were non-permanent and there was insufficient data to conduct statistics on this variable
Cattle shelter availability	What type of shelter is available to cattle on the farm	Categorical – Closed building(s)/Open building(s)/Yard/None				As with variable 12, most respondents replied that there were no shelters and therefore there was insufficient data to conduct statistics on this variable

Variable name	**Variable description**	**Response type**	**Data summary**	**d.f.**	***p* value**	**Notes**
Natural water	Presence of a river, pond or other natural body of water on the farm	Binary – yes/no				All respondents answered ‘yes’ to this question so it was not possible to conduct statistics on this data
Permanence of natural water	Permanence of the natural water bodies found on the farm – are they present year round?	Binary – Permanent/Not permanent				Respondents replied that at least one source of water was permanent and therefore this variable was not analysed statistically
Shoreline of largest water body	Respondents estimated the length of the shoreline of the longest body of water on their farm	Continuous (metres)	Range 0 – 100,000m Mean 1581m	62	0.941	Eight missing data here where respondents were unable to estimate the length
Number of rivers	The total number of rivers on the farm	Categorical based on responses– Zero, One or Two	N 0 = 40 N 1 = 31 N 2 = 2	71	Zero rivers = 0.345 One river = 0.747 Two rivers = 0.992	
Number of permanent rivers	The number of rivers on the farm that contain water year round	Binary based on responses – Zero or One	N 0 = 61 N 1 = 12	71	0.775	

Variable name	Variable description	Response type	Data summary	d.f.	*p* value	Notes
Number of streams	The number of streams on each farm	Categorical based on responses	N 0 = 41 N 1 = 18 N 2 = 2 N 3 or more = 13	71	Zero streams =0.436 One stream =0.448 Two streams =0.992 Three or more streams =0.815	Nine missing values here where respondents were uncertain of the number of streams on their farms
Number of permanent streams	The number of permanent streams on each farm	Binary based on responses	N 0 = 70 N 1 = 3			As only three respondents reported permanently flowing streams there was insufficient data here for statistical analysis
Number of springs	The number of natural springs on each farm	Categorical based on responses	N 0 = 59 N 1 = 9 N 2 = 2 N 3 = 0 N 4 or more = 2			Due to the low numbers of springs in each category this variable was reclassified as a binary variable called 'presence of springs' which can be seen later in this table
Number of ponds	The number of natural ponds on each farm	Count	Range 0-17 ponds Mean 2.85 ponds	70	0.998	One missing value here where a respondent was uncertain of the number of ponds on their farm
Number of permanent ponds	The number of natural ponds on the farm that are present all year round	Count	Range 0-14 ponds Mean 1.77 ponds	67	0.514	Four missing values here where respondents were uncertain of the number of permanent ponds on their farms

Variable name	**Variable description**	**Response type**	**Data summary**	**d.f.**	***p* value**	**Notes**
Number of water troughs	The number of artificial water troughs on the farm	Count	Range 0-50 troughs Mean 4.95 troughs	61	0.680	Ten missing values here where respondents were uncertain of the number of water troughs on their farm
Cattle accessible	How many water sources can cattle access on the farm	Count	Range 0-60 sources Mean 8.23 sources	71	0.786	This is a sum of all the water sources available to cattle on the farm. This was verified with the respondents during participatory mapping where possible
Pastures	The status of ownership of the farm	Categorical – Owned/Leased/Part owned and part leased	N Owned = 54 N Leased = 14 N Combination = 5	71	Owned = 0.176 Leased = 0.407 Combined= 0.413	
Shared pastures	Were pastures shared with other cattle herds	Binary - Yes/No	N Yes = 19 N No = 53	71	0.571	
Cattle separation	Were cattle herds maintained in contact with other cattle herds (including neighbours' herds)	Categorical - Never/Sometimes/Always	N Never = 71 N Sometimes = 2 N Always = 0			As only two respondents even sometimes kept their cattle in contact with other herds there was insufficient data for statistical analysis
Goat separation	Were cattle herds maintained in contact with goats (including neighbours' goats)	Categorical - Never/Sometimes/Always	N Never = 62 N Sometimes = 5 N Always = 6	71	Never = 0.024* Sometimes = 0.084* Always = 0.696	A variable called 'binary goat separation' was generated to further investigate the effects of separation of cattle and goats, and can be seen later in this table

Variable name	**Variable description**	**Response type**	**Data summary**	**d.f.**	***p* value**	**Notes**
Sheep separation	Were cattle herds maintained in contact with sheep (including neighbours' sheep)	Categorical - Never/Sometimes/Always	N Never = 37 N Sometimes = 20 N Always = 16	71	Never = 0.037* Sometimes = 0.101 Always = 0.721	A variable called 'binary sheep separation' was generated to further investigate the effects of separation of cattle and sheep, and can be seen later in this table
Pig separation	Were cattle herds maintained in contact with goats (including neighbours' goats)	Categorical - Never/Sometimes/Always	N Never = 60 N Sometimes = 5 N Always = 8	71	Never = 0.073* Sometimes = 0.992 Always = 0.181	A variable called 'binary pig separation' was generated to further investigate the effects of separation of cattle and pigs, and can be seen later in this table
Concentrate feed storage	How 'hard' concentrated cattle feed is stored on each farm	Categorical - Outside without restrictions to wildlife access/outside protected by a fence or low wall/outside protected by a high wall or wildlife proof fencing/stored inside a shed that wildlife could access/stored in a sealed warehouse or shed inaccessible to wildlife/Don't feed concentrates/Stored away from the farm	N Don't feed concentrates = 5 N Stored away from the farm = 5 N Stored in shed accessible to wildlife = 20 N Stored in shed inaccessible to wildlife = 43	71	Don't use = 0.215 Off farm = 0.497 Wildlife accessible shed = 0.527 Wildlife inaccessible shed = 0.320	

Variable name	Variable description	Response type	Data summary	d.f.	*p* value	Notes
Volume food storage	How volume food (for example hay) is stored on each farm	Categorical - Outside without restrictions to wildlife access/outside protected by a fence or low wall/outside protected by a high wall or wildlife proof fencing/stored inside a shed that wildlife could access/stored in a sealed warehouse or shed inaccessible to wildlife/Don't feed volume foods/Stored away from the farm	N Outside without restrictions to wildlife access = 12 N Outside protected by a fence or low walls = 1 N Stored in shed accessible to wildlife = 37 N Stored in shed inaccessible to wildlife = 19 N Stored away from the farm = 4	71	Outside = 0.038* Protected by fence = 0.992 Wildlife accessible shed = 0.086* Wildlife inaccessible shed = 0.152 Off farm = 0.203	Two binary variables called 'volume foods stored outside' and 'volume foods stored in wildlife accessible sheds' were generated to further investigate the significant results observed here. These can be seen later in the table

Variable name	Variable description	Response type	Data summary	d.f.	*p* value	Notes
Concentrate food offer	Where cattle are offered their concentrated food	Categorical - On the ground with unrestricted access by wildlife/in a low feeder relatively accessible to wildlife/in a high feeder relatively inaccessible to wildlife/offered to cattle only within pens that are inaccessible to wildlife/Don't offer concentrate foods	N Don't offer concentrates = 5 N On the ground = 53 N In low feeders = 10 N In high feeders = 4	71	Don't offer = 0.215 On ground = 0.299 Low feeders = 0.682 High feeders = 0.858	
Volume food offer	Where cattle were offered their volume (for example hay) food	Categorical - On the ground with unrestricted access by wildlife/in a low feeder relatively accessible to wildlife/in a high feeder relatively inaccessible to wildlife/offered to cattle only within pens that are inaccessible/Other	N On the ground = 34 N In low feeders = 9 N In high feeders = 29	71	0.111	
Cattle within district last year	The number of cattle that entered the farm from elsewhere within the local veterinary district within the last year	Count	Range 0 – 208 cattle Mean 6.09 cattle	69	0.069*	Two missing values occurred where farmer's were unable to recall or retrieve information about the number of cattle that entered the farm

Variable name	**Variable description**	**Response type**	**Data summary**	**d.f.**	***p* value**	**Notes**
Cattle beyond district last year	The number of cattle that entered the farm from elsewhere beyond the local veterinary district within the last year	Count	Range 0 – 800 cattle Mean 30.46 cattle	69	0.954	Two missing values occurred where farmers were unable to recall or retrieve information about the number of cattle that entered the farm
Manure disposal	What happened to manure that was produced on the farm	Categorical - Removed from any pens but kept on the land/removed from the pens but stored elsewhere on the farm/stored and used to fertilise the farm/Not removed	N Removed from farm = 5 N Used to fertilise the cattle pastures = 48 N Not removed = 20	71	Removed = 0.657 Used to fertilise pasture =0.986 Not removed = 1.00	
Farm residents	The number of human residents on the farm	Count	Range 0-12 people Mean 1.45 people	71	0.643	
Farm workers	The number of regular farm workers who do not reside on the farm	Count	Range 0-5 people Mean 1.23 people	71	0.282	
Number of cats	The number of cats on the farm	Count	Range 0 – 20 cats Mean 3.24 cats 46 farms had cats	69	0.361	Two values missing due to respondent uncertainty about the number of cats on their farm
Number of guard/working dogs	The number of guard or working dogs kept on the farm	Count	Range 0 – 10 dogs Mean 1.55 dogs 36 farms had guard dogs	71	0.888	

Variable name	**Variable description**	**Response type**	**Data summary**	**d.f.**	***p* value**	**Notes**
Number of other dogs	The number of non-working or guard dogs kept on the farm	Count	Range 0 – 63 dogs Mean 2.34 dogs 29 farms had non-working dogs	71	0.760	This includes two farms who keep packs of dogs that are used for hunting (around 30 dogs per pack). The mean number of non-guard dogs not including these packs was 1.11 dogs (range 0 – 15 dogs)
Number of goats	The number of goats on the farm	Count	Range 0 – 280 goats Mean 11.32 goats 14 farms had goats	71	0.838	
Number of pigs in extensive farming	The number of pigs kept free ranging on the farm	Count	Range 0 – 180 pigs Mean 7.83 pigs 17 farms had free ranging pigs	70	0.715	One missing value here due to uncertainty over the number of free ranging pigs on the farm
Number of pigs in intensive farming	The number of pigs kept contained on the farm	Count	Range 0 – 120 pigs Mean 0.89 pigs 3 farms had intensively farmed pigs			As only three respondents reported keeping pigs in this way so there were insufficient data for statistical analysis
Number of sheep	The number of sheep that are kept on the farm	Count	Range 0 – 4000 sheep Mean 375.64 sheep 40 farms had sheep	70	0.817	One missing value here due to uncertainty over the number of sheep on the farm
Dog maintenance	How any dogs kept on the farm are kept	Categorical – kept inside/tied up outside/free ranging	N No Dogs = 21 N Loose = 50 N Tied outside = 2	71	No dogs = 0.023* Loose dogs = 0.087* Tied dogs = 0.773	This data was also made into a ‘presence of dogs’ variable that can be seen later in this table

Variable name	Variable description	Response type	Data summary	d.f.	*p* value	Notes
Fencing	What type of fencing exists on the farm	Categorical - Barbed wire/Electric fence/Wire fencing (height 1.5m or less)/Mesh fencing/Wildlife proof fencing/Walls/Other	N Barbed Wire = 9 N Wire Fence = 58 N Mesh fence = 6	71	Barbed wire = 0.327 Wire Fence = 0.718 Mesh Fence = 0.521	
Fencing entrances	What type of gates exist on the farm, and how they are maintained	Categorical based on responses - Gate(always closed)/Gate (not always closed)/Single bar/No gates/Other	N Gate always closed = 43 N Single bar = 27 N No gates = 1	69	Gate always closed = 0.054* Single bar = 0.696 No gates = 0.994	Two missing variables here where answers we not provided regarding gates. A separate binary variable called ‘gates closed’ was generated to further investigate the significant result observed here and can be seen later in this table
Max red deer	The maximum number of red deer observed on the farm by the respondent at one time in the last year	Count	Range 0 – 106 deer Mean 8.81 deer 41 respondents had observed red deer	71	0.025*	
Max roe deer	The maximum number of roe deer observed on the farm by the respondent at one time in the last year	Count	Range 0 – 15 deer Mean 1.60 deer 35 respondents had observed roe deer	70	0.851	One missing value here where the respondent was unsure of what roe deer looked like
Max wild boar	The maximum number of wild boar observed on the farm by the respondent at one time in the last year	Count	Range 0 – 40 wild boar Mean 3.44 wild boar 41 respondents had observed wild boar	71	0.611	

Variable name	**Variable description**	**Response type**	**Data summary**	**d.f.**	***p* value**	**Notes**
Max rabbits	The maximum number of rabbits observed on the farm by the respondent at one time in the last year	Count	Range 0 – 20 rabbits Mean 1.68 rabbits 32 respondents had observed rabbits	63	0.103	Eight missing values here where respondents were unable to determine the number of rabbits
Max badgers	The maximum number of badgers observed on the farm by the respondent at one time in the last year	Count	Range 0 – 4 badgers Mean 0.42 badgers 17 respondents had observed badgers	71	0.890	
Hunting	Does game hunting take place on the farm?	Categorical – Large game/Small game/ Small and large game/No hunting	N Large game = 12 N Small game = 28 N Mixture = 21 N No hunting = 11	71	Large game = 0.306 Small game = 0.075* Mixture = 0.933 No hunting = 0.782	Three separate variables called 'hunt small game', 'hunt large game' and 'hunting both' were generated to further investigate the significant effects seen here and can be seen later in this table
Red deer hunted	The number of red deer the respondent reports having been hunted on the farm in the last year	Count	Range 0 – 87 deer Mean 10.03 deer Deer hunted on 24 farms	71	0.221	
Wild boar hunted	The number of wild boar the respondent reports having been hunted on the farm in the last year	Count	Range 0 – 118 wild boar Mean 9.86 wild boar Wild boar hunted on 28 farms	71	0.946	
Variables generated through participatory mapping and GIS analysis						
Map farm area	The total area of the farm when calculated based on the perimeter of the farm that was drawn on the aerial map by the respondent	Continuous - hectares	Range 32.6 – 2040.4 Ha Mean 466.5 Ha	71	0.997	

Variable name	Variable description	Response type	Data summary	d.f.	*p* value	Notes
Hunting estate distance	The distance along the perimeter of the respondent's farm that borders land used for rearing and hunting game species (wild boar and/or red deer and possibly other ungulate species)	Continuous – metres	Range 0-29713m Mean 3510m	66	0.020*	Five missing values as five respondents were unable or unwilling to name the land uses surrounding their farm
Hunting estate percentage	The percentage of the perimeter of the respondent's farm that borders land used for rearing and hunting game (wild boar and/or red deer, and possibly other ungulate species)	Percentage	Range 0-100% Mean 30.12%	66	0.022*	Five missing values as five respondents were unable or unwilling to name the land uses surrounding their farm
Cattle farm distance	The distance along the perimeter of the respondent's farm that borders land used for cattle farming	Continuous - metres	Range 0-14040m Mean 4708m	66	0.526	Five missing values as five respondents were unable or unwilling to name the land uses surrounding their farm
Cattle farm percentage	The percentage of the perimeter of the farm that borders land used for cattle farming	Percentage	Range 0-100% Mean 47.37%	66	0.165	Five missing values as five respondents were unable or unwilling to name the land uses surrounding their farm
Sheep farm distance	The distance along the perimeter of the respondent's farm that borders land used for cattle farming	Continuous - metres	Range 0-16320m Mean 4754m	66	0.321	Five missing values as five respondents were unable or unwilling to name the land uses surrounding their farm
Percentage sheep farming	The percentage of the perimeter of the farm that borders land used for sheep farming	Percentage	Range 0-100% Mean 42.63%	66	0.733	Five missing values as five respondents were unable or unwilling to name the land uses surrounding their farm
Pig farm distance	The distance along the perimeter of the respondent's farm that borders land used for pig farming	Continuous - metres	Range 0-3815m Mean 318m	66	0.758	Five missing values as five respondents were unable or unwilling to name the land uses surrounding their farm

Variable name	**Variable description**	**Response type**	**Data summary**	**d.f.**	***p* value**	**Notes**
Percentage pig farming	The percentage of the perimeter of the farm that borders land used for pig farming	Percentage	Range 0-52.74% Mean 3.70%	66	0.997	Five missing values as five respondents were unable or unwilling to name the land uses surrounding their farm
Other land use distance	The distance along the perimeter of the respondent's farm that borders land used for other land uses. These were non-animal rearing uses such as arable farming, olive plantations or residential areas.	Continuous - metres	Range 0-5763m Mean 254m	66	0.926	Five missing values as five respondents were unable or unwilling to name the land uses surrounding their farm
Percentage other land use	The percentage of the perimeter of the farm that borders land under non-animal use. This includes uses such as arable farming, olive plantations or residential areas.	Percentage	Range 0-71.02% Mean 3.97%	66	0.804	Five missing values as five respondents were unable or unwilling to name the land uses surrounding their farm
Wildlife habitat area	The area of dense scrub on the farm. This was defined as continuous shrub cover and was easily identified on aerial maps in the dehesa landscape.	Continuous - hectares	Range 0-626 Ha Mean 64 Ha	71	0.138	
Farm buildings distance	The distance from the farm's main buildings to the nearest wildlife habitat – defined as continuous cover of dense scrub. The wildlife habitat could be within or beyond the perimeter of the farm.	Continuous – metres	Range 100-10279m Mean 2209m	71	0.997	

Variable name	**Variable description**	**Response type**	**Data summary**	**d.f.**	***p* value**	**Notes**
Water troughs habitat distance	Distance from the closest water trough to wildlife habitat – defined as continuous cover of dense scrub. The wildlife habitat could be within or beyond the perimeter of the farm.	Continuous - metres	Range 59-9731m Mean 1752m	71	0.175	
Presence of wildlife habitat	Derived from the maps, this is a binary variable simply stating the presence or absence of wildlife habitat in the maps. This was defined as continuous shrub cover and was easily identified on aerial maps in the dehesa landscape.	Binary – Presence/Absence	N Present = 45 N Absent = 28	71	0.581	
Percentage wildlife habitat	The percentage of the total farm area that was classified as wildlife habitat.	Percentage	Range 0-64.8% Mean 10.23%	71	0.114	
<u>Variables obtained from official government records of farms and cattle TB testing</u>						
TB	The detected presence of TB in cattle in any of the years 2007,2008 or 2009	Binary – Detected Presence/Absence	N Present = 27 N Absent = 46			This is the response variable for all of the univariate GLM tests reported in this table
Herd Size	The number of cattle on the farm at last inspection in 2009	Count	Range 5 – 1268 cattle Mean 157 cattle	71	0.153	
Number of times tested	The number of times each farm was tested for TB within the study period	Count	Range 2 – 6 times Mean 2.88 times	71	0.476	

Variable name	**Variable description**	**Response type**	**Data summary**	**d.f.**	***p* value**	**Notes**
Number tested 2008	The number of cattle on each farm that were tested for TB in 2008. This may include multiple visits to the farm within the year.	Count	Range 8-1107 cattle Mean 157 cattle			These data were not tested statistically the number tested is always associated with previous detection of TB on each farm and therefore any effect of testing is autocorrelated with the previous presence of TB
Number tested 2009	The number of cattle on each farm that were tested for TB in 2009. This may include multiple visits to the farm within the year.	Count	Range 5-1286 cattle Mean 158 cattle			These data were not tested statistically the number tested is always associated with previous detection of TB on each farm and therefore any effect of testing is autocorrelated with the previous presence of TB
Cattle testing protocol 2008	The protocol used to test cattle for TB in 2008	Categorical – 12 groups	N Protocol 1 = 19 N Protocol 2 = 1 N Protocol 2 = 3 N Protocol 4 = 2 N Protocol 5 = 2 N Protocol 6 = 2 N Protocol 7 = 1 N Protocol 8 = 3 N Protocol 9 = 2 N Protocol 10 = 1 N Protocol 11 = 1 N Protocol 12 = 1	72	Protocol 1 = 0.004* Protocol 3 = 0.025* Protocol 8 = 0.003*	No other protocols were significantly associated with TB detection. Individual binary variables were generated to further investigate these significant effects and can be seen later in this table

Variable name	**Variable description**	**Response type**	**Data summary**	**d.f.**	***p* value**	**Notes**
Cattle testing protocol 2009	The protocol used to test cattle for TB in 2009	Categorical – 10 groups	N Protocol 1 = 19 N Protocol 2 = 5 N Protocol 3 = 17 N Protocol 4 = 2 N Protocol 5 = 4 N Protocol 6 = 2 N Protocol 7 = 1 N Protocol 8 = 19 N Protocol 9 = 3 N Protocol 10 = 1	72	Protocol 1 = 0.005* Protocol 2 = 0.016* Protocol 3 = 0.001* Protocol 5 = 0.044* Protocol 6 = 0.098* Protocol 8 = 0.007*	No other protocols were significantly associated with TB detection. Individual binary variables were generated to further investigate these significant effects and can be seen later in this table
Percentage positive 2008	The percentage of cattle that tested positive to TB on each study farm in 2008	Percentage	Range 0-17.40% Mean 1.32%			These data were not tested with the TB response variable as they are also a measure of TB levels
Percentage positive 2009	The percentage of cattle that tested positive to TB on each study farm in 2009	Percentage	Range 0-9.10% Mean 0.49%			These data were not tested with the TB response variable as they are also a measure of TB levels
Official Farm Area	The official area of the farm as measured in detail in government records	Continuous - hectares	Range 22 – 2134 Ha Mean 415 Ha	71	0.303	This varies slightly from the farmer stated farm areas. These data were later used for standardising other variables by farm area

Variable name	**Variable description**	**Response type**	**Data summary**	**d.f.**	***p* value**	**Notes**
<u>Variables redefined or derived from data collected in the questionnaire and participatory GIS exercise</u>						
Beef farming	Cattle are being reared for beef (this excludes shorter term 'fattening for beef' rearing	Binary – Yes/No	N Yes = 49 N No = 24	71	0.456	As beef farming was significantly associated with TB in categorical analysis along with other cattle rearing types this binary variable was generated
Stocking density	The number of cattle on each farm divided by farm area	Continuous	Range 0.003 – 2.976 cows/Ha Mean 0.55 cows/Ha	71	0.222	
Protocol 1 in 2008	The use of cattle testing protocol 1 to test cattle for TB in 2008	Binary – Used/Not used	N Used = 19 N Not Used = 54	71	0.005*	
Protocol 8 in 2008	The use of protocol 8 to test cattle for TB in 2008	Binary – Used/Not used	N Used = 38 N Not Used = 35	71	0.001*	
Protocol 1 in 2009	The use of protocol 1 to test cattle for TB in 2009	Binary – Used/Not used	N Used = 19 N Not Used = 54	71	0.005*	
Protocol 3 in 2009	The use of protocol 2 to test cattle for TB in 2009	Binary – Used/Not used	N Used = 17 N Not Used = 56	71	0.005*	
Protocol 8 in 2009	The use of protocol 8 to test cattle for TB in 2009	Binary – Used/Not used	N Used = 19 N Not Used = 54	71	0.185	
Presence of streams	The reported presence or absence of streams on the farm	Binary – Presence/Absence	N Present = 21 N Absent = 41	71	0.410	This variable was created as there were low numbers in some categories of the 'number of streams' variable
Presence of springs	The reported presence or absence of springs (springs, wells, or fonts) on the farm	Binary – Presence/Absence	N Present = 14 N Absent = 59	71	0.790	This variable was created as there were low numbers in some categories of the 'number of springs' variable
Rivers per hectare	The reported number of rivers on the farm divided by the total farm area in hectares	Continuous	Range 0 – 0.03 rivers/Ha Mean 0.003 rivers/Ha	71	0.291	

Variable name	Variable description	Response type	Data summary	d.f.	*p* value	Notes
Permanent rivers per hectare	The reported number of permanent rivers on the farm divided by the total farm area in hectares	Continuous	Range 0 – 0.02 permanent rivers/Ha Mean 0.001 permanent rivers/Ha	71	0.791	
Streams per hectare	The reported number of streams on the farm divided by the total farm area in hectares	Continuous	Range 0 – 0.02 streams/Ha Mean 0.003 streams/Ha	62	0.062*	
Permanent streams per hectare	The reported number of permanent streams on the farm divided by the total farm area in hectares	Continuous	Range 0 – 0.02 permanent streams/Ha Mean 0.0003 permanent streams/Ha	71	0.578	
Springs per hectare	The reported number of springs on the farm divided by the total farm area in hectares	Continuous	Range 0 – 0.1 springs/Ha Mean 0.002 springs/Ha	69	0.398	
Ponds per hectare	The reported number of ponds on the farm divided by the total farm area in hectares	Continuous	Range 0 – 0.06 ponds/Ha Mean 0.009 ponds/Ha	70	0.715	
Permanent ponds per hectare	The reported number of permanent ponds on the farm divided by the total farm area in hectares	Continuous	Range 0 – 0.06 permanent ponds/Ha Mean 0.005 permanent ponds/Ha	68	0.632	
Water troughs per hectare	The reported number of water troughs on the farm divided by the total farm area in hectares	Continuous	Range 0 – 0.08 water troughs/Ha Mean 0.02 water troughs/Ha	61	0.517	
Number of water sources per hectare	The total reported number of water sources of any kind on the farm divided by the total farm area in hectares	Continuous	Range 0.003 – 0.2 water sources/Ha Mean 0.03 water sources per Ha	71	0.714	This measure needs to be considered with caution as it does not account for the size of each water body – a river may in reality provide much more water availability per hectare than smaller water sources

Variable name	**Variable description**	**Response type**	**Data summary**	**d.f.**	***p* value**	**Notes**
Rivers per cow	The reported number of rivers on the farm divided by the herd size	Continuous	Range 0 – 0.001 rivers per cow Mean 0.00006 rivers per cow	71	0.661	
Ponds per cow	The reported number of ponds on the farm divided by the herd size	Continuous	Range 0 – 0.004 ponds per cow Mean 0.0002 ponds per cow	70	0.527	
Water troughs per cow	The reported number of water troughs on the farm divided by the herd size	Continuous	Range 0 – 0.007 water troughs per cow Mean 0.0003 water troughs per cow	61	0.411	
Number of water sources per cow	The total reported number of water sources of any kind on the farm divided by the herd size	Continuous	Range 0.007 – 0.54 water sources per cow Mean 0.09 water sources per cow	71	0.693	
Volume foods stored outside	Where volume foods (such as hay) are stored on the farm	Binary – outside or elsewhere	N outside = 12 N elsewhere = 61	71	0.091*	This variable is as a result of outside food storage having been significant in analysis of the categorical variable 'volume food storage' above in this table.
Volume foods stored in wildlife accessible sheds	Whether volume foods such as hay are stored in sheds that the respondent stated are accessible to wildlife	Binary – wildlife accessible shed or elsewhere	N shed = 37 N elsewhere = 36	71	0.273	This variable is as a result of shed storage that's accessible to wildlife having been significant in analysis of the categorical variable 'volume food storage' above in this table.

Variable name	Variable description	Response type	Data summary	d.f.	*p* value	Notes
Volume offered in low feeders	Whether volume foods were offered in low feeders or not	Binary – low feeders or other methods of offering volume foods to cattle	N low = 9 N other = 64	71	0.251	This variable is as a result of the use of low feeders to offer volume foods to cattle having been significant in analysis of the categorical variable 'volume food offer' above in this table.
Volume offered in high feeders	Whether volume foods were offered in high feeders or not	Binary – high feeders or other methods of offering volume foods to cattle	N high = 29 N other = 44	71	0.007*	This variable is as a result of the use of high feeders to offer volume foods to cattle having been significant in analysis of the categorical variable 'volume food offer' above in this table.
Gates closed	Whether gates on the farm are always kept closed, or are at least occasionally left open. These might be gates within or around the perimeter of the farm	Binary – closed or at least sometimes open	N closed = 44 N open = 27	69	0.097*	This variable was created due to a significant effect in analysis of the categorical variable 'fencing entrances' above in this table
Presence of dogs	The reported presence of dogs of any kind on the farm	Binary – Presence/Absence	N Present = 52 N Absent = 21	71	0.084*	
Presence of pigs	The reported presence of pigs on the farm	Binary – Presence/Absence	N Present = 20 N Absent = 53	71	0.613	
Presence of goats	The reported presence of pigs on the farm	Binary – Presence/Absence	N Present = 14 N Absent = 59	71	0.145	
Presence of sheep	The reported presence of sheep on the farm	Binary – Presence/Absence	N Present = 40 N Absent = 33	71	0.138	
Goat stocking density	The number of goats divided by the farm size in hectares to give an estimate of the stocking density of goats on each farm	Continuous	Range 0 – 1.3 goats/Ha Mean 0.03 goats/Ha	71	0.234	

Variable name	Variable description	Response type	Data summary	d.f.	*p* value	Notes
Pig stocking density	The number of pigs divided by the farm size in hectares to give an estimate of the stocking density of pigs on each farm	Continuous	Range 0 – 1.5 pigs/Ha Mean 0.05 pigs/Ha	71	0.405	
Sheep stocking density	The number of sheep divided by the farm size in hectares to give an estimate of the stocking density of sheep on each farm	Continuous	Range 0 – 4 sheep/Ha Mean 0.79 sheep/Ha	71	0.398	
Binary goat separation	The separation of goats from the cattle on the farm	Binary – Separated/Not separated	N Separated = 62 N Not separated = 11	71	0.089*	This variable was generated as a result of a significant result in the variable 'Goat separation' above in this table
Binary sheep separation	The separation of sheep from the cattle on the farm	Binary – Separated/Not separated	N Separated = 36 N Not separated = 37	71	0.199	This variable was generated as a result of a significant result in the variable 'Sheep separation' above in this table
Binary pig separation	The separation of pigs from the cattle on the farm	Binary – Separated/Not separated	N Separated = 13 N Not separated = 60	71	0.602	This variable was generated as a result of a significant result in the variable 'Pig separation' above in this table
Hunt small game	Exclusively hunting small game on the farm (i.e. hunting, but not large game)	Binary – hunt small game only, yes or no	N hunt small game = 28 N don't hunt small game exclusively = 45	71	0.007*	This variable was created as the result of a significant result in the 'hunting' variable above in this table
Hunt large game	Exclusively hunting large game on the farm (i.e. hunting, but not small game)	Binary – hunt large game only, yes or no	N hunt large game = 12 N don't hunt large game exclusively = 61	71	0.046*	This variable was created as the result of a significant result in the 'hunting' variable above in this table

Variable name	**Variable description**	**Response type**	**Data summary**	**d.f.**	***p* value**	**Notes**
Hunting both	Whether both small and large game hunting is conducted on the farm	Binary – Yes or No	N Yes = 21 N No = 52	71	0.383	This variable was created as the result of a significant result in the 'hunting' variable above in this table
Presence of red deer	Whether sightings or signs of red deer having visited the farm have been observed in the last year	Binary – Presence/Absence	N Present = 41 N Absent = 32	71	0.009*	
Presence of roe deer	Whether sightings or signs of roe deer having visited the farm have been observed in the last year	Binary – Presence/Absence	N Present = 37 N Absent = 35	70	0.051*	One missing value here where the respondent was uncertain about what roe deer looked like
Presence of wild boar	Whether sightings or signs of wild boar having visited the farm have been observed in the last year	Binary – Presence/Absence	N Present = 57 N Absent = 16	71	0.063*	
Presence of rabbits	Whether sightings or signs of rabbits having visited the farm have been observed in the last year	Binary – Presence/Absence	N Present = 41 N Absent = 32	71	0.009*	
Presence of badgers	Whether sightings or signs of badgers having visited the farm have been observed in the last year	Binary – Presence/Absence	N Present = 24 N Absent = 49	71	0.026*	

Appendix 5: Camera trapping data to support the groundtruthing undertaken in Chapter 3

Location	Farm Number	TB status	Brucellosis status	Wild Boar	Red Deer	Roe Deer	Badgers
Asturias	1	Negative	Negative	Not recorded	Not recorded	Not recorded	Not recorded
	2	Positive	Negative	Present	Present	Present	Present
	3	Negative	Positive	Present	Present	Present	Not recorded
	4	Negative	Negative	Present	Present	Not recorded	Not recorded
	5	Positive	Negative	Not recorded	Present	Present	Present
	6	Negative	Positive	Not recorded	Not recorded	Present	Not recorded
	7	Positive	Negative	Not recorded	Not recorded	Not recorded	Not recorded
Cantabria	8	Negative	Negative	Present	Not recorded	Not recorded	Not recorded
	9	Negative	Negative	Present	Not recorded	Present	Present
	10	Positive	Negative	Present	Not recorded	Present	Not recorded
	11	Positive	Negative	Present	Not recorded	Not recorded	Not recorded
	12	Positive	Negative	Present	Not recorded	Present	Not recorded
	13	Positive	Negative	Not recorded	Present	Present	Present
	14	Negative	Negative	Present	Not recorded	Not recorded	Not recorded
	15	Positive	Negative	Present	Not recorded	Not recorded	Not recorded
	16	Positive	Negative	Not recorded	Not recorded	Not recorded	Not recorded
	17	Negative	Negative	Present	Not recorded	Present	Present
	18	Negative	Negative	Present	Not recorded	Present	Not recorded

Appendix 6: Participant recruitment protocol and full English translation of the questionnaire used in chapter 3

Participant recruitment protocol

Farmers were approached with a telephone call from the interviewers who later conducted the questionnaire. Although no strict protocol was followed, interviewers ensured the potential participant understood that the questionnaire was aimed at establishing if their management practices, wildlife on the farm, surrounding land use or other factors were related to the presence, or absence, of either disease on their farm. They were assured that their farms and their answers would be anonymous if/when the results were published. They were informed that the interview may take as long as 1½ hours and that the interviewer would travel to meet them at their farm. Infected and disease-free farms received the same information.

Full questionnaire

Table 18: English translation of the full questionnaire used in chapter 3 (overleaf)

Question	Response Type
The Farm	
Farmer name and telephone number	Name/Tel. no.
Is the respondent also a hunter?	Yes/No
REGA number (Farm government registration number)	Number
Farm name	Name
How long has this farm been used for cattle farming?	Number (years)
If the farm has been used for cattle farming for less than 10 years, what was the previous land use?	Text
In what municipality is the farm located?	Municipality name
What type of cattle farming is conducted on the farm?	Dairy/Beef/Other
How many adult females/bulls/juveniles <2 years old and juveniles <1 year old are there in the herd?	Number (count)
What breed are the cows and the bulls on the farm?	Categorical based on responses – name of breed
What is the total area of the winter pastures/ summer pastures/ area where cattle move between these sites?	Number (hectares)
What is the altitude of the winter pastures/ summer pastures/ area where cattle move between these sites?	Number (metres above sea level)
In winter, what type(s) of shelter are available to the cattle?	Closed building(s), with cattle loose inside/ Closed building(s) with cattle tied inside/Open building(s)/Yard/None
Is there a river, pond or other natural body of water on your farm?	Yes on the winter farm/ Yes on summer pastures/No, if no skip to next section
Regarding the winter farm, if there is water, is it permanent or non-permanent?	Permanent/Non-permanent
Regarding the winter farm, what is the length of the shoreline of the largest body of water?	Number (meters)
Regarding the winter farm, how many pools can cattle access?	Number
Regarding the winter farm, how many permanent pools are there on the farm?	Number
Regarding the winter farm, are there other drinking places which wildlife can access?	(provide details)
Regarding the summer farm, if there is water, is it permanent or non-permanent?	Permanent/Non-permanent

Regarding the summer farm, what is the length of the shoreline of the largest body of water?	Number (meters)
Regarding the summer farm, how many pools can cattle access?	Number
Regarding the summer farm, how many permanent pools are there on the farm?	Number
Regarding the summer farm, are there other drinking places which wildlife can access?	(provide details)
What is the percentage of the pastures that you use that are owned (by the respondent)/ rented/ communal	Percentage (separately for winter and summer pastures)
Do the winter pastures have barriers between you cattle and…	Other livestock cattle (no/ sometimes/always)
	Goats (no/ sometimes/ always)
	Sheep (no/ sometimes/ always)
	Pigs (no/sometimes/ always)
Do the summer pastures have barriers between you cattle and…	Other livestock cattle (no/ sometimes/ always)
	Goats (no/ sometimes/ always)
	Sheep (no/ sometimes/ always)
	Pigs (no/sometimes/ always)
How many other farmers share the same winter pastures as you?	Number (count)
How many other farmers share the same summer pastures as you?	Number (count)
Regarding the storage of animal feed for the cattle, Are concentrates/volume foods stored...	Outside without restrictions to wildlife access/outside protected by a fence or low wall/outside protected by a high wall or wildlife proof fencing/stored inside a shed that wildlife could access/stored in a sealed warehouse or shed inaccessible to wildlife/ other (please provide details)
Where are the cattle offered the animal feed? (Concentrates/volume feeds separately)	On the ground with unrestricted access by wildlife/in a low feeder relatively accessible to wildlife/in a high feeder relatively inaccessible to wildlife/offered to cattle only within pens that are inaccessible/other places (please provide details)

Environment	
What percentage of the perimeter of the winter farm is made up of the following land use types?	Cattle farming/Sheep farming/ Goat farming/ Equine activities/ Natural Areas/ Agricultural land not used for grazing/ Agricultural land used for grazing/ Urban or industrial activities/ Water courses/ Other (please detail)
What percentage of the perimeter of the summer farm is made up of the following land use types?	Cattle farming/Sheep farming/ Goat farming/ Equine activities/ Natural Areas/ Urban or industrial activities/ Water courses/ Other (please detail)
Other Risk Factors	
How many cattle were purchased and/or entered the farm from within your veterinary district in the last year?	Number (count)
How many cattle were purchased and/or entered the farm from outside your veterinary district in the last year?	Number (count)
How many cattle were purchased and/or entered the farm from within your veterinary district in the 5 years?	Number (count)
How many cattle were purchased and/or entered the farm from within your veterinary district in the 5 years?	Number (count)
Have there been any herd breakdowns/compulsory slaughters in the cattle on your farm?	Indicate YES or NO, and if yes the disease that caused it.
What was the last year that there was a compulsory slaughter of any cattle on your farm?	Number (Year)
What happens to the manure from the pens?	Removed from any pens but kept on the land/removed from the pens but stored elsewhere on the farm/Stored and used to fertilise the farm/Not removed
Regarding the management of cattle races used to restrain cattle for testing, are they owned (by the respondent), or does the respondent use communal cattle races?	Owned/Communal (details provided if relevant)
Biosecurity	
Which of the following domestic animals are present on the (a) winter and (b) summer farm?	Presence or Absence of Cats/ Guard Dogs/ Non-working Dogs/ Horses/ Goats/ Sheep/ Pigs

If you have dogs, how are they maintained?	Categorical – Loose/ Tied up outside/ Inside
What type of fencing do you have in the area cattle are kept, on the summer and winter farms?	No fencing used/ Hedges / Barbed wire/ Electric fencing/ Wire fence (height 1.5m or less)/ Mesh fence/ Walls/ Other (please specify)
Wildlife	
Please indicate the maximum number of the following species you have seen on your farm in one day within the last year on the (a) winter and (b) summer farms…	Number (count) of… Red Deer/ Roe Deer/ Wild Boar/ Badgers/ Rabbits/ Foxes
Please indicate if you have seen signs of the following species in the last year on the (a) winter and (b) summer farms…	Presence/Absence of observed signs of… Red Deer/Roe Deer/Wild Boar/Badger/Rabbits/Foxes
What is your opinion of each of the following species is relation to the damage they have caused on your farm?	1-10 scale where 1=very little problem and 10=big problem. Red Deer/ Roe Deer/ Wild Boar/ Badgers/ Foxes/ Rabbits/ Pigeons
What kinds of damage by wildlife have you experienced on your farm?	Please specify
If damage has been experienced, what actions have you taken to avoid/prevent further damage?	Please specify
What is your opinion of the following wildlife species, in relation to their ability to transmit TUBERCULOSIS to cattle?	1-10 scale where 1=very little problem and 10=big problem. Red Deer/Roe Deer/Wild Boar/Badgers/Foxes/Rabbits/Pigeons
What is your opinion of the following wildlife species, in relation to their ability to transmit BRUCELLOSIS to cattle?	1-10 scale where 1=very little problem and 10=big problem. Red Deer/ Roe Deer/ Wild Boar/ Badgers/ Foxes/ Rabbits/ Pigeons
Hunting	
Do you hunt on your summer or winter farms?	Yes (winter farm)/ Yes (summer pastures)/ Yes (both)/ No (skip to next section)
How many wild boar /red deer/ other species were hunted on the (a) winter and (b) summer farm in the last year?	Number (count)
What is your opinion of large game hunting in your area?	Scale from 1-10 where 1=very negative and 10=very positive

What is your opinion, from the point of view of transmitting diseases to cattle, of management of hunting on game farms nearby?	Scale from 1-10 where 1=very negative and 10=very positive
Are there any other aspects of game management that concern you regarding diseases shared with cattle?	Please specify
About Tuberculosis	
How important do you think TB is in your area?	Scale from 1-10 where 1=not important and 10=highly important
Do you believe wildlife transmit TB to cattle?	Scale from 1-10 where 1=very unlikely and 10=very likely
How do you think TB is changing in your region?	Increasing/Staying the same/Decreasing
About Brucellosis	
How important do you think Brucellosis is in your area?	Scale from 1-10 where 1=not important and 10=highly important
Do you believe wildlife transmit Brucellosis to cattle?	Scale from 1-10 where 1=very unlikely and 10=very likely
How do you think Brucellosis is changing in your region?	Increasing/Staying the same/Decreasing
General Information	
What organisations, associations, groups or professionals have given you information about how to control TB?	Please specify
Please tell us any general comments you may have	Comments
Animal Health Information	
How was your farm ranked in the latest official bovine tuberculosis classifications?	T1, 2, 3 or 4.
How many of your cattle tested positive for TB in the last round of testing?	Number (count)
How many of your cattle tested positive for Brucellosis in the last round of testing?	Number (count)
With what frequency do you administer anthelminthic drugs to you cattle herd?	Number of times per year
In what season do you administer anthelminthic drugs?	Categorical - Spring/Summer/Autumn/Winter
How frequently have you observed diarrhoea in your adult cows?	Categorical – Often, often in certain animals, frequently, occasionally, never
How frequently have you observed diarrhoea in your juvenile cows (<2years)?	Categorical – Often, often in certain animals, frequently, occasionally, never

How often do you observe miscarriages in your female cows?	Categorical – Often, often in certain animals, frequently, occasionally, never
How many miscarriages did you observe in your cows in the last year?	Number (count)
How many animals were sent for slaughter in the last year?	Number (count)

Appendix 7: List of all potential TB and Brucellosis risk factors identified in Chapter 3

Table 19: This table shows the potential risk factors for TB and Brucellosis identified in interviews with cattle farmers in Asturias and Cantabria in northern Spain, and the results of univariate binary GLMs conducted between each variable and the detected presence data for Tuberculosis and Brucellosis. ‘OR’ denotes the odds ratio, and ther confidence intervals are calculated based on this number. The significance of the *p* values is also shown by a mark next to significant values, where “^” = <0.1, “*” = <0.05, “**” = <0.01 and “***” = <0.001. The maximum d.f. for TB models is 98, and for Brucellosis models is 59. The reduced d.f. for Brucellosis is due to using only data from Cantabria (n=63), and having two missing values for the Brucellosis status of two farms there.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
Variables generated directly from participants' responses to the questionnaire																	
1	Respondent a hunter	Does the respondent farmer also engage in hunting activity (Binary – Yes/No)	Hunting may alter a respondents' knowledge of wildlife on the farm, management practices and/or opinions about wildlife and disease	N Yes = 13 N No = 87	98	0.280	0.779	1.19	-2.43	4.81	59	0.746	0.456	2.00	-2.96	6.96	
2	Number of female cows	The number of female cows on the farm	May be more related to disease than overall herd size, and would indicate in more detail where management may be required	Range 0-250 cows Mean 42 cows	97	-1.194	0.232	1.01	-0.97	2.98	58	0.293	0.769	1.00	-0.97	2.98	One farm stated they had zero female cows as they recently experienced culling and at that time had no cattle on their farm. This data was therefore excluded from this model.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
2	Number of yearling cows	The number of cows on the farm that are one years old	May be more related to disease than overall herd size, and would indicate in more detail where management may be required	Range 0 – 118 yearlings Mean 14 yearlings	97	-0.655	0.513	1.01	-0.98	-0.98	57	0.569	0.569	1.02	-1.00	3.03	One farm stated they had zero yearling cows as they recently experienced culling and at that time had no cattle on their farm. This data was therefore excluded from this model.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
2	Number of bulls	The number of bulls on the farm	May be more related to disease than overall herd size, and would indicate in more detail where management may be required	Range 0 – 10 bulls Mean 1.5 bulls	97	-2.521	0.012 *	1.59	-0.77	3.94	58	1.108	0.268	1.18	-1.10	3.47	One farm stated they had zero bulls as they recently experienced culling and at that time had no cattle on their farm. This data was therefore excluded from this model. Based on the data available, this model suggests that having less bulls increases the probability of detection of TB on a farm. This may be because farms with less bulls may share them, or because the few bulls there contact more females and therefore create a greater contact network for the spread of any disease.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
2	Number of calves	The number of calves (cows under one year old) on the farm	May be more related to disease than overall herd size, and would indicate in more detail where management may be required	Range 0 – 90 calves Mean 10 calves	97	-1.283	0.200	1.03	-0.97	3.03	58	0.102	0.918	1.00	-1.00	3.01	One farm stated they had zero calves as they recently experienced culling and at that time had no cattle on their farm. This data was therefore excluded from this model.
2	Herd size	The total number of cattle kept on the farm	This is a known risk factor for both brucellosis and TB in cattle [references 15, 16, 19-21]	Range 5 – 410 animals Mean 70.5 animals	95	-1.149	0.257	1.01	-0.96	2.97	57	0.228	0.819	1.08	-0.86	2.70	One farm stated they had zero animals at this time as they recently experienced culling and at that time had no cattle on their farm. This data was therefore excluded from this model. A further 2 respondents did not give the number of cattle on their farm.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
3	Breed of female cows	Categorical: 1=Holstein Friesian 2=Asturiana de los Valles 3=Limousin 4=Tudanca 5=Charolais 6=Limousin x Charolais 7=Limousin x Swiss brown 8=Charolais x other 9=Limousin x other 10=Crossbreed without specification	Certain breeds may be more susceptible to disease	N 1 = 20 N 2 = 24 N 3 = 6 N 4 = 8 N 5 = 1 N 6 = 3 N 7 = 1 N 8 = 5 N 9 = 14 N 10 =16	87	1) 1.736 3) -2.049 4) -2.377 9) -2.976 10) -2.873	1) 0.082^ 3) 0.041* 4) 0.017* 9) 0.02* 10) 0.04*	1) 2.33 3) 7.00 4) 11.67 9) 2.33 10) 3.33	1) -0.86 3) 0.13 4) 5.16 9) 0.70 10) 2.16	1) 5.53 3) 13.87 4) 18.17 9) 5.97 10) 6.50	42						All other breeds were not significantly associated with the detected presence of TB. No breeds were significantly associated with the detected presence of Brucellosis. The baseline variable for the statistic was the first category, the Holstein Friesian breed. This breed was positively associated with TB detection, whereas the other breeds were negatively associated with detection of the disease, particularly the crossbred animals.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
3	Breed of bulls	Categorical: 1=Holstein Friesian 2=Asturiana de los Valles 3=Limousin 4=Tudanca 5=Charolais 6=Limousin x Charolais 7=Limousin x Swiss brown 8=Charolais x other 9=Limousin x other 10=Crossbreed without specification	Certain breeds may be more susceptible to disease	N 1 = 3 N 2 = 22 N 3 = 19 N 4 = 4 N 5 = 5 N 6 = 5 N 7 = 1 N 8 = 3 N 9 = 3 N 10 = 6	57						42						No breed of bull was significantly associated with the detected presence of either TB or Brucellosis

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
4	Farm age	The number of years the farm land has been used for cattle farming	Longer durations of cattle farming on the same land may be associated with elevated disease levels (personal observations)	Range 6 months – 150 years Mean 26 years	78	2.11	0.035 *	1.02	-1.17	3.21	58	-0.553	0.580	1.02	-1.01	3.05	Missing values here due to 20 farmers responding that the farm had always been a farm. This did not allow for statistical analysis, and it is important to note that the mean number of years the land has been used for cattle farming is likely much higher. Based on the data available, increased farm age is associated with increased probability of detection of TB.
5	Production type	Binary based on responses – 1=Dairy farming 2= Beef farming	Methods associated with different production types may influence disease introduction or persistence	N Dairy Farming = 21 N Beef farming = 79	98	-3.235	0.0012**	5.52	2.20	8.85	59	-0.186	0.852	1.25	-5.25	7.75	This model shows that dairy farms are significantly more likely to have TB detected in their cattle.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
6	Percentage winter pastures owned	Percentage of the winter pastures that cattle can access that are owned by the respondent	Rental practices have been associated with brucellosis [reference 17] and the shared space use could also lead to TB transmission	Range 0 – 100% Mean 63%	95	0.270	0.787	1.00	-0.97	2.97	59	0.674	0.500	1.01	-0.97	2.98	Three missed values here where respondents were unsure of the percentage owned
6	Percentage winter pastures leased	Percentage of the winter pastures that cattle can access that are leased by the respondent	Rental practices have been associated with brucellosis [reference 17] and the shared space use could also lead to TB transmission	Range 0 – 100% Mean 35%	95	-1.167	0.243	1.01	-0.97	2.98	59	-0.478	0.632	1.00	-0.97	2.98	Three missed values here where respondents were unsure of the percentage leased
6	Percentage winter pastures communal	Percentage of the winter pastures that cattle can access that are shared with other farmers	Rental practices have been associated with brucellosis [reference 17] and the shared space use could also lead to TB transmission	Range 0-80% Mean 1.5%													As only two respondents reported using communal pastures in winter there were insufficient data for statistical analysis

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
6	Percentage summer pastures owned	Percentage of the summer pastures that cattle can access that are owned by the respondent	Rental practices have been associated with brucellosis [reference 17] and the shared space use could also lead to TB transmission	Range 0 – 100% Mean 29%	97	1.629	0.103	1.01	-0.96	2.98	59	-0.006	0.994	1.87	-3.34	7.08	One missing value here where the respondent was unsure of the percentage of summer pastures owned
6	Percentage summer pastures leased	Percentage of the summer pastures that cattle can access that are leased by the respondent	Rental practices have been associated with brucellosis [reference 17] and the shared space use could also lead to TB transmission	Range 0 – 100% Mean 16%	97	0.994	0.320	1.01	-0.97	2.98	59	-1.087	0.277	1.02	-0.98	3.02	One missing value here where the respondent was unsure of the percentage of summer pastures leased

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
6	Percentage summer pastures communal	Percentage of the summer pastures that cattle can access that are shared with other farmers	Rental practices have been associated with brucellosis [reference 17] and the shared space use could also lead to TB transmission	Range 0 – 100% Mean 54%	97	-2.188	0.029 *	1.01	-0.96	2.98	59	0.764	0.445	1.01	-1.11	3.12	This model suggests that the percentage of summer pastures that are managed communally may be negatively associated with the detection of TB. One missing value here where the respondent was unsure of the percentage of communal summer pastures used
7	Winter pasture area	Continuous – Surface area (Hectares). The area of the land that cattle have access to in winter.	May be important as contact with wildlife and other cattle may increase with farm size. Allows for the calculation of stocking densities	Range 0.01 – 139 Ha Mean 32.3 Ha	81	0.951	0.342	1.01	-0.97	2.98	50	-0.123	0.902	1.00	-0.98	2.98	Seventeen missing values here where respondents were unsure of the area that cattle had access to.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
7	Summer pasture area	Continuous – Surface area (Hectares). The area of the land that cattle have access to in summer.	May be important as contact with wildlife and other cattle may increase with farm size. Allows for the calculation of stocking densities	Range 0.5 – 2040 Ha Mean 398.5 Ha	80	-1.904	0.057^	1.00	-0.98	2.98	43	-0.827	0.408	1.00	-0.98	2.98	This model suggests that smaller summer pasture areas may be positively associated with detection of TB. Eighteen missing values here where respondents were unsure of the area that cattle had access to.
8	Percentage of winter pastures' adjoining land used for cattle farming	Percentage of winter pastures' adjoining land used for cattle farming	Adjoining land use activities may affect disease in cattle on the study farm	Range 0 – 100% Mean 35.76%	97	0.333	0.739	1.00	-0.97	2.97	59	0.433	0.665	1.00	-0.97	2.98	One missing value here where the respondent was unable to give a figure for the percentage of winter pastures surrounding the winter farm
8	Percentage of winter pastures' adjoining land used for sheep farming	Percentage of winter pastures' adjoining land used for sheep farming	Adjoining land use activities may affect disease in cattle on the study farm	Range 0 – 25% Mean 0.38%													Insufficient data for statistical analysis as only 2 respondents stated sheep farming was conducted adjacent to their winter farm

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
8	Percentage of winter pastures' adjoining land used for goat farming	Percentage of winter pastures' adjoining land used for goat farming	Adjoining land use activities may affect disease in cattle on the study farm														No respondents reported goat farming adjacent to their winter farm.
8	Percentage of winter pastures' adjoining land used for keeping horses	Percentage of winter pastures' adjoining land used for keeping horses	Adjoining land use activities may affect disease in cattle on the study farm	Range 0 – 50% Mean 0.86%	98	1.066	0.286	1.07	-0.97	2.97							Insufficient data for statistical analysis with the Brucellosis response variable as no respondents reported adjoining land used for horses in Cantabria.
8	Percentage of winter pastures' adjoining land that is natural habitat	Percentage of winter pastures' adjoining land that is natural habitat	Adjoining land use activities may affect disease in cattle on the study farm	Range 0 – 100% Mean 6.20%	98	2.275	0.023 *	1.04	-0.95	3.03							Insufficient data for statistical analysis with the Brucellosis response variable. This TB model indicates that a higher percentage of adjoining land (to the winter farm) that is natural habitat increases the probability of TB detection in cattle.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
8	Percentage of winter pastures' adjoining land used for agriculture	Percentage of winter pastures' adjoining land used for agriculture	Adjoining land use activities may affect disease in cattle on the study farm	Range 0 – 100% Mean 5.78%	98	-0.180	0.857	1.18	-185.05	187.40	59	-0.320	0.749	1.36	-126.50	129.22	
8	Percentage of winter pastures' adjoining land that is water	Percentage of winter pastures' adjoining land that is water	Adjoining land use activities may affect disease in cattle on the study farm	Range 0 – 20% Mean 0.42%													As only 3 respondents reported water on the boundary of their winter pastures there were insufficient data for statistical analyses
8	Percentage of summer pastures' adjoining land used for cattle farming	Percentage of summer pastures' adjoining land used for cattle farming	Adjoining land use activities may affect disease in cattle on the study farm	Range 0 – 100% Mean 56.36%	42	-1.503	0.133	1.01	-0.96	2.99							Missing values here as data was not always collected for this variable on the study sites in Cantabria.
8	Percentage of summer pastures' adjoining land used for sheep farming	Percentage of summer pastures' adjoining land used for sheep farming	Adjoining land use activities may affect disease in cattle on the study farm	Range 0 – 25% Mean 1.12%	32	0.672	0.502	1.13	-1.22	3.49							Missing values here as data was not always collected for this variable on the study sites in Cantabria

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
8	Percentage of summer pastures' adjoining land used for horses	Percentage of summer pastures' adjoining land used for horses	Adjoining land use activities may affect disease in cattle on the study farm	Range 0 – 100% Mean 5.74%	32	0.686	0.193	1.02	1.00	3.04							Missing values here as data was not always collected for this variable on the study sites in Cantabria
8	Percentage of summer pastures' adjoining land that is natural habitat	Percentage of summer pastures' adjoining land that is natural habitat	Adjoining land use activities may affect disease in cattle on the study farm	Range 0 – 100% Mean 32.69%	33	-0.016	0.987	1.00	-0.98	2.98							Missing values here as data was not always collected for this variable on the study sites in Cantabria
8	Percentage of summer pastures' adjoining land used for agriculture	Percentage of summer pastures' adjoining land used for agriculture	Adjoining land use activities may affect disease in cattle on the study farm	Range 0 – 100% Mean 12.77%	33	-1.419	0.156	1.02	-0.97	3.01							Missing values here as data was not always collected for this variable on the study sites in Cantabria
8	Percentage of summer pastures' adjoining land used for urban dwellings	Percentage of summer pastures' adjoining land used for urban dwellings	Adjoining land use activities may affect disease in cattle on the study farm	Range 0 – 15% Mean 0.71%													As only 3 respondents reported urban area on the boundaries of their summer pastures there was insufficient data for statistical analysis

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
8	Percentage of summer pastures' adjoining land that is water	Percentage of summer pastures' adjoining land that is water	Adjoining land use activities may affect disease in cattle on the study farm	Range 0 – 50% Mean 1.82%													As only 2 respondents reported water on the boundary of their summer pastures there were insufficient data for statistical analyses
9(i)	Natural winter water	Binary – The presence of natural water sources in the winter pastures	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence of water will help to identify specific high risk water types	N Water present = 48 N Water absent = 52	98	-0.336	0.737	1.15	-1.83	4.14	59	-0.736	0.462	1.60	-2.11	5.31	

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
9(ii)	Natural winter water permanence	Binary – is the natural water source on winter pastures permanent or non-permanent (seasonal)	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence of water will help to identify specific high risk water types	N Permanent = 45 N Non Permanent = 55	98	-0.736	0.461	1.37	-1.63	4.37	59	-0.605	0.545	1.47	-2.24	5.19	

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
9(i)	Natural summer water	Binary – the presence of natural water sources on the summer pastures	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence of water will help to identify specific high risk water types	N Present = 84 N Absent = 15	98	-2.092	0.037 *	3.35	0.15	6.84	59	0.009	0.993	4.62	-20.75	20.84	This model shows that the presence of water on summer pastures was negatively associated with the detected presence of TB. Therefore, having less water increases the probability of detection of TB in cattle. This is thought to be caused by increased interactions with other cattle and with wildlife due to aggregation at available water sources.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
9(ii)	Natural summer water permanence	Binary – is the natural water source on summer pastures permanent or non-permanent (seasonal)	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence of water will help to identify specific high risk water types	N Permanent = 81 N Not Permanent = 18	96	-2.614	0.009 **	4.35	0.91	7.79	59	-0.514	0.608	1.92	-5.04	8.88	This model shows that the presence if permanent streams is negatively associated with detection of TB in cattle.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
10	Winter streams	Binary - The presence of streams (of all sizes) in the winter pastures	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence of water will help to identify specific high risk water types	N Present = 42 N Absent = 58	98	-0.721	0.471	1.36	-1.65	4.38	59	-0.838	0.402	1.75	-2.07	5.57	
10	Summer streams	Binary – the presence of streams on the summer cattle pastures	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence of water will help to identify specific high risk water types	N Present = 78 N Absent = 22	98	-1.313	0.189	1.93	-1.30	5.16	59	-0.273	0.785	1.27	-3.47	6.02	

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
11	Managed winter water sources	Binary – the presence of water troughs or other artificial sources of water for cattle on winter pastures	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence of water will help to identify specific high risk water types	N Present = 44 N Absent = 54	95	1.722	0.085^	2.12	-0.02	5.15	59	0.849	0.396	1.71	-1.98	5.41	Four missing values here where respondents did not provide an answer to this question
11	Managed summer water sources	Binary – the presence of water troughs or other maintained sources of water for cattle on summer pastures	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence of water will help to identify specific high risk water types	N Present = 45 N Absent = 51	93	1.658	0.097^	2.08	-0.97	5.13	59	0.490	0.624	1.38	-2.38	5.13	Five missing data here where five respondents did not provide an answer to this question. This model suggests that the presence of maintained water sources may be positively associated with the detection of TB in cattle

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
12	Number of farmers sharing summer pastures	Count - the number of other farmers the respondent shares summer pastures with	Contact between cattle herds is a known risk factor for brucellosis [reference 17, 18] and TB [reference 22]	Range 0 – 30 other farmers Mean 5.2 other farmers	92	-1.513	0.130	1.06	-0.98	3.10	58	2.168	0.030 *	1.12	-0.95	3.18	This Brucellosis model indicated that the probability of Brucellosis detection in cattle increases with the number of farmers sharing summer pastures.
12	Number of farmers sharing winter pastures	Count - the number of other farmers the respondent shares winter pastures with	Contact between cattle herds is a known risk factor for brucellosis [reference 17, 18] and TB [reference 22]	Range 0 – 100 other farmers Mean 3.4 other farmers	96	-0.744	0.457	1.06	-0.98	3.10	59	0.454	0.650	1.05	-1.14	3.24	Two missing values here where respondents were uncertain of the number of other farmers sharing winter pastures.
13	Winter pasture shared with cattle	Categorical variable stating if cattle herds are kept on pastures shared with other cattle herds in winter	Contact between cattle herds is a known risk factor for brucellosis [reference 17, 18] and TB [reference 22]	N Never = 69 N Sometimes = 9 N Always = 22	98	Never = -1.857 Sometimes = -0.170 Always = -0.377	Never = 0.274 Sometimes = 0.865 Always = 0.706	Never = 1.75 Sometimes = 1.14 Always = 1.22	Never = -0.76 Sometimes = -3.01 Always = -2.08	Never = 4.26 Sometimes = 5.30 Always = 4.52	59	Never = -1.860 Sometimes = 1.375 Always = 0.907	Never = 0.102 Sometimes = 0.168 Always =0.364	Never = 6.40 Sometimes = 8.53 Always = 1.97	Never = 3.23 Sometimes = 3.70 Always = -2.17	Never = 9.57 Sometimes = 13.37 Always = 6.11	The baseline variable of these statistics was the ‘Never’ category.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
13	Winter pasture shared with sheep	Categorical variable stating if cattle herds are kept on pastures shared with sheep in winter	Sheep can contract both brucellosis and TB and therefore might be part of the disease cycle	N Never = 89 N Sometimes = 5 N Always = 6	98	0.021	0.984	1.07	-3.72	5.86	59	-0.323	0.747	1.23	-2.48	4.94	As the 'sometimes' and 'always' categories had too few data points for statistical analysis these were grouped and statistics were conducted on a binary variable of cattle separated (n=11) or not separated (89) from sheep.
13	Winter pasture shared with goats	Categorical variable stating if cattle herds are kept on pastures shared with goats in winter	Goats can contract both brucellosis and TB and therefore might be part of the disease cycle	N Never = 89 N Sometimes = 4 N Always = 7	98	0.273	0.785	1.77	-0.90	4.45	59	-0.521	0.603	1.38	-2.28	5.05	As the 'sometimes' and 'always' categories had too few data points for statistical analysis these were grouped and statistics were conducted on a binary variable of cattle separated (n=11) or not separated (n=89)from goats.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
13	Winter pasture shared with horses	Categorical variable stating if cattle herds are kept on pastures shared with horses in winter	Horses can contract both brucellosis and TB and therefore might be part of the disease cycle	N Never = 36 N Sometimes = 9 N Always = 18	60	Never = -2.254 Sometimes = 0.826 Always = 1.461	Never = 0.061^ Sometimes = 0.409 Always = 0.144	Never = 1.22	Never = -1.51	Never = 3.95	58	Never = -2.424 Sometimes = 1.200 Always = 0.397	Never = 0.110 Sometimes = 0.230 Always = 0.691	Never = 4.66 Sometimes = 2.80 Always = 1.33	Never = 1.59 Sometimes = -1.82 Always = -2.71	Never = 7.74 Sometimes = 7.42 Always = 5.38	Missing values here as compartmentalisation of pastures between cattle and horses was not recorded at study farms in Asturias. The baseline variable of these statistics was the 'Never' category.
13	Summer pastures shared with other cattle	Categorical variable stating if cattle herds are kept on pastures shared with other cattle herds in summer	Contact between cattle herds is a known risk factor for brucellosis [reference 17, 18] and TB [reference 22]	N Never = 43 N Sometimes = 2 N Always = 53	95	-2.435	0.015 *	3.13	0.94	2.78	58	0.612	0.540	1.51	-1.26	4.28	As the 'sometimes' and 'always' categories had too few data points for statistical analysis these were grouped and statistics were conducted on a binary variable of cattle separated (n=55) or not separated (n=43) other cattle herds in summer. This TB model suggests that separation is negatively associated with detection of TB – if cattle are separated from other herds, the probability of TB detection is reduced.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
13	Summer pastures shared with sheep	Categorical variable stating if cattle herds are kept on pastures shared with sheep in summer	Sheep can contract both brucellosis and TB and therefore might be part of the disease cycle	N Never = 70 N Sometimes = 4 N Always = 23	95	-2.346	0.019 *	1.80	0.23	4.12	58	0.908	0.364	1.62	-1.15	4.39	Insufficient data for analysis of every category, so statistics were conducted on a binary variable of separated (n=27) or never separated (n=70). Three missing variables were participants did not provide a response. This TB model indicates that sheep separation from cattle is negatively associated with detection of TB in cattle – increased separation may reduce TB.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
13	Summer pastures shared with goats	Categorical variable stating if cattle herds are kept on pastures shared with goats in summer	Goats can contract both brucellosis and TB and therefore might be part of the disease cycle	N Never = 71 N Sometimes = 3 N Always = 22	95	-1.967	0.049 *	1.24	0.05	6.63	57	0.861	0.389	1.45	-1.32	4.22	Insufficient data for analysis of every category, so statistics were conducted on a binary variable of separated (n=25) or never separated (n=71). Three missing variables were participants did not provide a response. This TB model indicates that goat separation from cattle may be negatively associated with detection of TB in cattle – increased separation may reduce TB.
13	Summer pastures shared with horses	Categorical variable stating if cattle herds are kept on pastures shared with horses in summer	Horses can contract both brucellosis and TB and therefore might be part of the disease cycle	N Never = 19 N Sometimes = 0 N Always = 42	60	-0.591	0.554	1.07	-1.02	3.15	58	0.790	0.427	1.48	-1.32	4.28	Missing values here as compartmentalisation of pastures between cattle and horses was not recorded at study farms in Asturias. Binary analysis was conducted here for always or never separated as there were no values in the ‘sometimes’ category.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
14(i)	Store concentrated cattle food	Categorical – 1 = Outside 2 = Outside, fenced 3 = Outside, well covered 4 = In an open sided shed 5 = In a closed shed	Food stored where it is accessible to animals (livestock or wildlife) may become contaminated with brucellosis or TB bacterium	N 1 = 2 N 2 = 2 N 3 = 1 N 4 = 3 N 5 = 84	86						84						Where respondents used more than one method for storing concentrated foods they were allocated as using the method that was most accessible to livestock or wildlife as this is thought to be of most concern for disease transmission. Three missing values here where data could not be categorised, and five where farmers did not feed concentrated food. No concentrated food storage methods were significantly associated with the detected presence of either TB or Brucellosis

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
14(ii)	Store volume cattle food	Categorical – 1 = Outside 2 = Outside, fenced 3 = Outside, well covered 4 = In an open sided shed 5 = In a closed shed	Food stored where it is accessible to animals (livestock or wildlife) may become contaminated with brucellosis or TB bacterium	N 1 = 12 N 2 = 3 N 3 = 0 N 4 = 6 N 5 = 73	86						57						There are six missing values here where respondents did not store volume foods such as hay on their farm. No volume food storage methods were significantly associated with the detected presence of either TB or Brucellosis

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
15(i)	Offer concentrated cattle food	Categorical – 1 = On the ground 2 = Low feeders (feed troughs that are low in height) 3 = High feeders (feed troughs or containers that are accessed by cattle high above the ground 4 = Open barn (A cattle barn which allows access to other animals such as cats and dogs or wildlife species 5 = Closed barn (An enclosed cattle barn that does not allow access to other species	Food that is offered to cattle where it is accessible to wildlife may become contaminated with brucellosis or TB bacterium. Intra herd cattle disease transmission could also occur this way	N On the ground = 3 N Low feeders = 13 N High feeders = 7 N Open barn = 46 N Closed barn = 26	90						57						Five missing values here due to five respondents not providing concentrated foods to their cattle. No methods of offering cattle concentrated food were associated with the detected presence of Brucellosis or TB

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
15(ii)	Offer volume cattle food	Categorical – 1 = On the ground 2 = Low feeders (feed troughs that are low in height) 3 = High feeders (feed troughs or containers that are accessed by cattle high above the ground 4 = Open barn (A cattle barn which allows access to other animals such as cats and dogs or wildlife species 5 = Closed barn (An enclosed cattle barn that does not allow access to other species)	Food that is offered to cattle where it is accessible to wildlife may become contaminated with brucellosis or TB bacterium. Intra herd cattle disease transmission could also occur this way	N On the ground = 4 N Low feeders = 15 N High feeders = 9 N Open barn = 50 N Closed barn =18	90	Open barn = -1.789	Open barn = 0.074^	Open barn = 8.54	Open barn = 2.04	Open barn = 15.04	56						There are four missing values here where respondents did not feed volume foods such as hay to their cattle. No methods were significantly associated with the detected presence of Brucellosis. This model indicates that offering food in an open barn may be negatively associated with the detection of TB in cattle. The baseline variable for these statistics was the first category – volume foods offered on the ground

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
16	Number purchased from within local veterinary district in the last year	Count – number of cattle brought onto the farm from elsewhere within the local veterinary district in the last year	Cattle purchases (and therefore movement between farms) are known to be associated with brucellosis [reference 17, 18] and TB [reference 22]	Range 0 – 25 animals Mean 0.7 animals	97	0.845	0.398	1.06	-1.04	3.15	59	1.534	0.125	3.67	-0.08	7.41	One missing value here where a respondent could not give a figure for the number of cattle purchased from within the veterinary district in the last year.
17	Number purchased from outside local veterinary district in the last year	Count – number of cattle brought onto the farm from outside the local veterinary district in the last year	Cattle purchases (and therefore movement between farms) are known to be associated with brucellosis [reference 17, 18] and TB [reference 22]	Range 0 – 39 animals Mean 1.7 animals	96	0.928	0.353	1.03	-0.94	3.00	59	0.357	0.721	1.02	-1.05	3.08	Two missing values here where respondents could not give a figure for the number of cattle purchased from outside of the local veterinary district in the last year

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
18	Manure disposal	Categorical based on where manure is disposed of on the farm – 1 = outside the farm 2 = Outside of the cattle farmed area but within the total area of the farm 3 = Spread on grasslands 4 = Used to fertilize agricultural areas	Manure disposal practices may affect farm biosecurity and either reduce or cause the spread of brucellosis or TB	N used to fertilize cattle pastures = 61 N used to fertilize agricultural areas = 1													As every respondent used manure to fertilize cattle pastures there was insufficient data for statistical analysis
19	Winter fencing – no barrier	Binary variable – use of this type of external farm barrier in winter	The type of barriers at farm perimeters will affect contact between the cattle herd and other cattle herds and/or wildlife	N Use = 33 N Don't use = 64	96	2.092	0.036 *	2.81	-0.40	6.02	59	-0.273	0.785	1.39	-2.29	5.07	This TB model indicates that not having fencing is positively associated with detection of TB. Two missing values here where respondents did not provide an answer to this question

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
19	Winter fencing - hedge	Binary variable – use of this type of external farm barrier in winter	The type of barriers at farm perimeters will affect contact between the cattle herd and other cattle herds and/or wildlife	N Use = 9 N Don't use = 27	35	0.130	0.896	1.11	-3.30	5.52							Data for this variable were only collected in Asturias, so it was not possible to test this variable in relation to Brucellosis
19	Winter fencing – barbed wire	Binary variable – use of this type of external farm barrier in winter	The type of barriers at farm perimeters will affect contact between the cattle herd and other cattle herds and/or wildlife	N Use = 68 N Don't use = 29	96	-1.938	0.053^	2.40	-0.68	5.48	57	-1.205	0.228	2.25	-1.59	6.09	This TB model indicates that barbed wire fencing is negatively associated with detection of TB, possibly because it prevents cattle contact with other cattle herds. Two missing values here where respondents did not provide an answer to this question
19	Winter fencing – electric fencing	Binary variable – use of this type of external farm barrier in winter	The type of barriers at farm perimeters will affect contact between the cattle herd and other cattle herds and/or wildlife	N Use = 51 N Don't use = 46	96	0.753	0.452	1.38	-1.62	4.37	57	1.413	0.645	2.49	-1.25	6.23	Two missing values here where respondents did not provide an answer to this question

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
19	Winter fencing - walls	Binary variable – use of this type of external farm barrier in winter	The type of barriers at farm perimeters will affect contact between the cattle herd and other cattle herds and/or wildlife	N Use = 38 N Don't use = 59	96	-0.247	0.805	1.11	-1.91	4.14	57	0.745	0.456	1.61	-2.10	5.31	Two missing values here where respondents did not provide an answer to this question
19	Winter fencing - wire	Binary variable – use of this type of external farm barrier in winter	The type of barriers at farm perimeters will affect contact between the cattle herd and other cattle herds and/or wildlife	N Use = 12 N Don't use = 85	96	0.458	0.647	1.33	-2.34	5.01	57	-0.01	0.991	5.18	-317.56	327.92	Two missing values here where respondents did not provide an answer to this question
19	Summer fencing – no barrier	Binary variable – use of this type of external farm barrier in summer	The type of barriers at farm perimeters will affect contact between the cattle herd and other cattle herds and/or wildlife	N Use = 53 N Don't use = 40	94	2.027	0.043 *	2.42	-0.61	5.46	59	0.033	0.974	1.02	-2.83	4.88	This TB model indicates that not having fencing is positively associated with detection of TB. Four missing values here where respondents did not provide an answer to this question.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
19	Summer fencing – hedges	Binary variable – use of this type of external farm barrier in summer	The type of barriers at farm perimeters will affect contact between the cattle herd and other cattle herds and/or wildlife	N Use = 8 N Don't use = 24	31	1.195	0.232	3.94	-2.23	10.11							Data for this variable were only collected in Asturias, so it was not possible to test this variable in relation to Brucellosis
19	Summer fencing – barbed wire	Binary variable – use of this type of external farm barrier in summer	The type of barriers at farm perimeters will affect contact between the cattle herd and other cattle herds and/or wildlife	N Use = 31 N Don't use = 64	93	1.371	0.170	1.85	-1.22	4.93	59	-1.562	0.118	5.45	-0.35	11.26	Five missing values here were respondents did not provide an answer to this question
19	Summer fencing – electric fence	Binary variable – use of this type of external farm barrier in summer	The type of barriers at farm perimeters will affect contact between the cattle herd and other cattle herds and/or wildlife	N Use = 37 N Don't use = 58	93	2.122	0.034 *	2.55	-0.50	5.59	59	-1.105	0.269	2.50	-1.99	6.99	Five missing values here were respondents did not provide an answer to this question. This TB model indicates that the use of electric fence in summer is positively associated with the detection of TB in cattle.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
19	Summer fencing - walls	Binary variable – use of this type of external farm barrier in summer	The type of barriers at farm perimeters will affect contact between the cattle herd and other cattle herds and/or wildlife	N Use = 15 N Don't use = 80	93	1.550	0.121	2.42	-1.05	5.88	59	-0.291	0.770	1.40	-4.75	7.54	Five missing values here were respondents did not provide an answer to this question
19	Summer fencing - wire	Binary variable – use of this type of external farm barrier in summer	The type of barriers at farm perimeters will affect contact between the cattle herd and other cattle herds and/or wildlife	N Use = 7 N Don't use = 88	93	1.211	0.226	2.62	-1.72	6.97	59	-0.009	0.993	4.62	-760.43	769.67	Five missing values here were respondents did not provide an answer to this question
20	Number of guard dogs at winter pastures	Count – the number of guard dogs living on the winter farm	Guard dogs may affect contacts between the cattle herd and other cattle and/or wildlife	Range 0 – 4 guard dogs Mean 0.3 guard dogs 16 respondents used guard dogs	98	-1.659	0.097^	2.41	-0.42	5.75	59	0.423	0.672	1.23	-1.98	4.44	This TB model indicates that having more guard dogs on the farm may be associated with a reduced probability of TB detection in cattle. This may be due to the dogs reducing wildlife visits to the farm

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
20	The number of goats on winter pastures	Count - the number of goats living on the winter farm	Goats can contract brucellosis and TB and therefore may be a part of the disease cycle. This variable also allows us to calculate stocking density	Range 0 – 20 goats Mean 0.6 goats	98	-0.933	0.351	1.16	-1.14	3.46	59	0.315	0.753	1.03	-1.09	3.14	
20	The number of sheep on winter pastures	Count – the number of sheep living on the winter farm	Sheep can contract brucellosis and TB and therefore may be a part of the disease cycle. This variable also allows us to calculate stocking density	Range 0 – 70 sheep Mean 1.3 sheep	95	0.899	0.369	2.27	-2.45	6.99	57	1.214	0.225	1.13	-1.04	3.31	Three missing values here where respondents were not able to state the number of sheep on their winter farm

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
20	The number of pigs on winter pastures	Count – the number of pigs living on the winter farm	Pigs can contract TB and therefore may be a part of the disease cycle. This variable also allows us to calculate stocking density	Range 0 – 5 pigs Mean 0.3 pigs 12 respondents kept pigs	98	0.511	0.610	1.11	-1.29	3.51	59	1.060	0.289	1.44	-1.32	4.20	
20	Number of guard dogs at summer pastures	Count – the number of guard dogs living on the summer pastures	Guard dogs may affect contacts between the cattle herd and other cattle and/or wildlife	Range 0 – 4 guard dogs Mean 0.2 guard dogs	33	-0.843	0.399	1.55	-1.74	4.84							Data for summer pastures was only collected in the Asturias part of the survey so n=35 for this data.
20	The number of goats on summer pastures	Count – the number of goats living on the summer pastures	Goats can contract brucellosis and TB and therefore may be a part of the disease cycle. This variable also allows us to calculate stocking density	Range 0 – 4 goats Mean 0.1 goats	33	0.007	0.995	52.30	-737.47	842.07							Data for summer pastures was only collected in the Asturias part of the survey so n=35 for this data. As only one respondent reported keeping goats there were insufficient data for statistical analyses

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
20	The number of sheep on summer pastures	Count – the number of sheep living on the summer pastures	Sheep can contract brucellosis and TB and therefore may be a part of the disease cycle. This variable also allows us to calculate stocking density	Range 0 – 70 sheep Mean 2.4 sheep	33	0.007	0.995	1.77	-14.32	17.86							Data for summer pastures was only collected in the Asturias part of the survey so n=35 for this data.
20	The number of pigs on summer pastures	Count – the number of pigs living on the summer pastures	Pigs can contract TB and therefore may be a part of the disease cycle. This variable also allows us to calculate stocking density	Range 0 – 5 pigs Mean 0.5 pigs	33	0.337	0.736	1.13	-1.67	3.92							Data for summer pastures was only collected in the Asturias part of the survey so n=35 for this data.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
21	How dogs are kept	Categorical – Loose, Tied or Inside buildings	May affect contacts between the cattle herd and other cattle, livestock or wildlife. Loose dogs may deter wildlife.	N Loose = 49 N Tied = 8 N Inside = 2	57	Loose = -1.834	Loose = 0.066^	Loose = 1.72	Loose = -0.91	Loose = 4.35	28	Loose = -2.391	Loose = 0.017 *	Loose = 2.85	Loose = -0.19	Loose = 5.90	59 respondents kept dogs and responded to this question. As there was insufficient data for statistical analysis of the 'tied' and 'inside' categories statistics were conducted on a binary variable of 'loose' or 'not loose'. For both diseases, the presence of loose dogs is negatively associated with the detection of disease. Loose dogs may repel wildlife or other livestock visits to the farm.
22	Cattle race shared	Binary – Is the cattle race used on this farm communally owned, and therefore shared for use with other herds) – Yes or No	Sharing cattle races represents a farm biosecurity risk as disease fomites may move between farms with the equipment.	N Shared = 46 N Not Shared = 54	98	-2.907	0.004 **	3.11	0.00	6.21	59	-0.203	0.839	1.14	-2.60	4.88	This model shows that shared use of cattle races is negatively associated with the detection of TB. This may be due to higher levels of hygiene being applied to shared races.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
23	Shelter – loose in closed shed	Binary – Is this type of shelter used on the farm – Yes or No	The type of shelter available to cattle may affect intra-herd contacts and shared space use, potentially affecting intra-herd disease transmission	N Yes = 13 N No = 87	98	2.995	0.0027**	8.13	4.19	12.08	59	-0.010	0.992	4.17	-182.03	190.37	This model shows that the use of closed barns with the cattle loose inside is positively associated with detection of TB on farms
23	Shelter – tied in closed shed	Binary – Is this type of shelter used on the farm – Yes or No	The type of shelter available to cattle may affect intra-herd contacts and shared space use, potentially affecting intra-herd disease transmission	N Yes = 74 N No = 26	98	-1.982	0.0474*	2.56	-0.59	5.71	59	-0.012	0.990	1.01	-3.11	5.13	This model shows that the use of closed barns with the cattle tied inside is negatively associated with the detection of TB on farms.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
23	Shelter – open shed	Binary – Is this type of shelter used on the farm – Yes or No	The type of shelter available to cattle may affect intra-herd contacts and shared space use, potentially affecting intra-herd disease transmission	N Yes = 10 N No = 90	98	1.656	0.097^	3.10	-0.78	6.99	59	0.495	0.620	1.56	-3.27	6.39	This model shows that the use of open sheds for cattle shelters is positively associated with the detection of TB on cattle farms.
23	Shelter – open air corral	Binary – Is this type of shelter used on the farm – Yes or No	The type of shelter available to cattle may affect intra-herd contacts and shared space use, potentially affecting intra-herd disease transmission	N Yes = 38 N No =62	98	0.244	0.807	1.11	-1.90	4.13	59	-0.447	0.655	1.35	-2.48	5.18	

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
23	Shelter – none provided	Binary – Is this type of shelter used on the farm – Yes or No	The type of shelter available to cattle may affect intra-herd contacts and shared space use, potentially affecting intra-herd disease transmission	N Yes = 2 N No = 98													With only two positive responses to this variable there were insufficient data for statistical analysis
24(i)	Max red deer in a day in winter	Count data of the maximum number of red deer that the respondent had observed together in the last year at the winter farm	Red deer are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 25]	Range 0-50 red deer Mean 3.20 red deer	93	-0.203	0.839	1.09	-1.23	3.41	56	-0.313	0.755	1.01	-1.02	3.04	Five missing values here were respondents did not provide an answer to this question

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
24(i)	Max red deer in a day in summer	Count data of the maximum number of red deer that the respondent had observed together in the last year on the summer pastures	Red deer are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 25]	Range 0 – 100 red deer Mean 12.67 red deer	89	2.090	0.037 *	6.17	1.53	10.80	52	0.391	0.164	1.02	-0.97	3.01	This TB model indicates that the number of red deer on the summer pastures farm is positively associated with the detection of TB in cattle. Nine missing values here where respondents were unsure of the maximum number of deer they had seen in a day
24(iii)	Max wild boar in a day in winter	Count data of the maximum number of wild boar that the respondent had observed together in the last year at the winter farm	Wild boar are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 25]	Range 0 – 14 wild boar Mean 2.16 wild boar	84	2.333	0.020 *	1.19	-0.92	3.31	47	-0.375	0.708	1.07	-1.28	3.42	This TB model indicates that the number of wild boar on the winter farm is positively associated with the detection of TB in cattle. Fourteen missing values where respondents were uncertain of the number of wild boar they had seen in one day

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
24(iii)	Max wild boar in a day in summer	Count data of the maximum number of wild boar that the respondent had observed together in the last year at the summer pastures	Wild boar are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 25]	Range 0 – 14 wild boar Mean 3.28 wild boar	81	1.306	0.192	1.09	-1.00	3.18	45	-0.224	0.823	1.03	-1.22	3.28	Seventeen missing values here where respondents were uncertain how many wild boar they had seen at once on the summer pastures
24(iv)	Max badgers seen in a day in winter	Count data of the maximum number of badgers that the respondent had observed together in the last year at the winter farm	Badgers are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 13]	Range 0 – 5 badgers Mean 0.63 badgers	89	1.682	0.093^	3.74	-0.55	8.02	53	-0.932	0.352	1.74	-1.82	5.31	This TB model indicates that the number of badgers seen on the winter farm may be positively associated with the detection of TB in cattle. Nine missing values here where respondents were uncertain about the maximum number of badgers they had seen together in the last year on winter pastures

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
24(iv)	Max badgers seen in a day in summer	Count data of the maximum number of badgers that the respondent had observed together in the last year at the summer pastures	Badgers are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 13]	Range 0 – 5 badgers Mean 0.63 badgers	87	1.051	0.293	1.51	-1.84	4.85	52	-0.130	0.897	1.06	-2.00	4.12	Eleven missing values here where respondents were uncertain about the maximum number of badgers they had seen together in the last year on summer pastures
25	Hunting at winter farm	Binary variable – is large game hunting conducted on the winter farm	Hunting on the farm may involve different management objectives than for only cattle farming, such as encouraging elevated wildlife population densities which could contribute to cattle TB	N Hunt = 95 N Don't hunt = 4	98												Insufficient data in the different categories for statistical analyses

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
25	Hunting in summer pasture	Binary variable – is large game hunting conducted on the winter farm	Hunting on the farm may involve different management objectives than for only cattle farming, such as encouraging elevated wildlife population densities which could contribute to cattle TB	N Hunt = 96 N Don't hunt = 3													Insufficient data in the different categories for statistical analyses
26(i)	Number of red deer shot on the winter farm in the last year	Count variable of the number of red deer hunted on the winter farm in the last year before the questionnaire was conducted	This variable can be used to calculate an estimate of wildlife population density	Range 0 – 50 red deer Mean 4.68 red deer	51	0.884	0.377	2.67	-3.99	9.33	22	1.140	0.254	1.04	-0.99	3.06	Only data from farms where red deer hunting is conducted (n=53) were used for this model

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
26(i)	Number of red deer shot on summer pastures in the last year	Count variable of the number of red deer hunted on the summer pastures in the last year before the questionnaire was conducted	This variable can be used to calculate an estimate of wildlife population density	Range 0 – 50 red deer Mean 4.06 red deer	50	-0.394	0.694	1.01	-1.00	3.02	22	1.140	0.254	1.04	-0.99	3.06	Only data from farms where red deer hunting is conducted (n=52) were used for this model
26(ii)	Number of wild boar shot at winter farm in last year	Count variable of the number of wild boar hunted on the winter farm in the last year before the questionnaire was conducted	This variable can be used to calculate an estimate of wildlife population density	Range 0 – 300 wild boar Mean 27.85 wild boar	53	2.072	0.038*	13.38	1.59	25.18	25	0.856	0.392	1.02	-0.98	3.01	This TB model indicates that the number of wild boar shot on the winter farm is positively associated with the detection of TB in cattle. This may be due to the farmers shooting large numbers encouraging wildlife such as wild boar, and they are contributing to maintaining the disease in the cattle population. Only data from farms where wild boar hunting was conducted (n=55) was used for this model

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
26(ii)	Number of wild boar shot on summer pastures in the last year	Count variable of the number of wild boar hunted on the summer pastures in the last year before the questionnaire was conducted	This variable can be used to calculate an estimate of wildlife population density	Range 0 – 100 wild boar Mean 22.72 wild boar	51	2.008	0.045 *	1.03	-0.96	3.03	25	0.856	0.392	1.02	-0.98	3.01	This TB model indicates that the number of wild boar shot on the summer pastures is positively associated with the detection of TB in cattle. This may be due to the farmers shooting large numbers encouraging wildlife such as wild boar, and they are contributing to maintaining the disease in the cattle population. Only data from farms where wild boar hunting was conducted (n=53) was used for this model
Variables obtained from government information																	
	Region	Binary – Cantabria or Asturias	This is a potential confounding variable	N Asturias = 37 N Cantabria = 63	98	-1.69	0.067^	1.85	-0.92	4.61							

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Farmer	Farmer name	This is a potential confounding variable	100 different farmer responded about the 100 different farms													No statistics conducted on this data
	TB status	Binary – Positive or Negative	This forms the response variable for TB models	N Positive = 35 N Negative = 65							59	-1.046	0.296	3.16	-2.73	9.04	A farm is considered positive if it had at least one positive intradermal tuberculin test result in the last five years
	Brucellosis status	Binary – Positive or Negative	This forms the response variable for brucellosis models	N Positive = 13 N Negative = 87	96	-1.95	0.051^	8.00	2.32	13.68							A farm was considered positive if it had at least one positive case, diagnosed by serology, in the last 5 years
	Winter X location	UTM Zone 30 longitudinal co-ordinates for location of each winter farm	This is a potential confounding variable	Range 199921 - 395934	94	-2.047	0.071^	1.41	-0.99	3.81	58	1.543	0.123	2.83	-16.48	16.54	Co-ordinates for winter farms were not collected for 4 of the farms (3 in Asturias and 1 in Cantabria). This model indicates that there may have been slightly more TB at study sites in the East of our study area.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Winter Y location	UTM Zone 30 latitudinal co-ordinates for location of each winter farm	This is a potential confounding variable	Range 4770727 - 4839467	94	-0.922	0.356	9.36	-86.03	104.74	58	0.065	0.949	8.98	-49.87	67.83	Co-ordinates for winter farms were not collected for 4 of the farms (3 in Asturias and 1 in Cantabria)
Variables created by standardising other variables																	
	Winter stocking density	The herd size divided by the farm area in winter pastures	This is related to herd size and may relate to intra-herd contact rates and disease transmission	Range 0.06 – 4800 animals/Ha Mean 63.84 animals/Ha	78	0.434	0.664	1.54	-2.26	5.35	48	0.026	0.979	1.00	-1.22	3.23	As some herds share pastures with unknown numbers of other cattle this variable may not indicate true stocking densities. The highest stocking density is remarkably high as these animals remained inside in a barn for the whole winter.
	Summer stocking density	The herd size divided by the farm area in winter pastures	This is related to herd size and may relate to intra-herd contact rates and disease transmission	Range 0.006-30.5 animals/Ha Mean 2.01 animals/Ha	80	0.374	0.709	1.45	-2.53	5.44	41	-0.326	0.744	1.04	-1.19	3.28	As some herds share pastures with unknown numbers of other cattle this variable may not indicate true stocking densities

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Number of cattle purchased in the last year	Count – number of cattle brought onto the farm from elsewhere in the last year	Cattle movements and purchases are known risk factors for TB and brucellosis [reference 17, 18, 22]	Range 0 – 64 animals Mean 2.4 animals	96	1.017	0.309	1.03	-0.99	3.04	59	1.110	0.267	1.05	-1.00	3.10	Two missing values here where respondents could not give a figure for the number of cattle purchased from outside of the local veterinary district in the last year
	Cattle purchased within the area in the last year	Binary variable showing if cattle were purchased within the veterinary district in the last year or not	Cattle movements and purchases are known risk factors for TB and brucellosis [reference 17, 18, 22]	Yes = 32 No = 68	98	0.970	0.332	2.59	-3.93	9.11	61	3.011	0.004 **	20.29	15.55	20.55	
	Presence of red deer in winter	Binary variable – presence of red deer or signs of red deer, or absence of any signs of red deer	Red deer are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 25]	N Present = 42 N Absent = 55	96	1.690	0.091^	2.11	-0.94	5.17	58	1.024	0.306	2.00	-1.86	5.86	This TB model indicates that the presence of red deer on the winter farm is positively associated with the detection of TB in cattle. Two missing values here where respondents were not sure if they had seen red deer on their winter farm

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Presence of red deer in summer	Binary variable – presence of red deer or signs of red deer, or absence of any signs of red deer	Red deer are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 25]	N Present = 61 N Absent = 35	95	2.998	0.0027**	3.83	0.77	6.90	58	1.384	0.166	4.53	-1.31	10.37	This TB model indicates that the presence of red deer on summer pastures is positively associated with the detection of TB in cattle. Three missing values here where respondents were uncertain about having seen red deer on their farm
	Presence of wild boar in winter	Binary variable – presence of wild boar or signs of wild boar, or absence of any signs in winter	Wild boar are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 25]	N Present = 75 N Absent = 22	96	2.042	0.004 **	8.25	3.99	12.51	58	-1.696	0.090^	3.08	-0.72	6.88	These models indicates that the presence of wild boar on the winter farm is positively associated with the detection of TB in cattle, and may be negatively associated with the detection of Brucellosis in cattle. Two missing values here where respondents were uncertain if they had seen wild boar on their winter farm

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Presence of wild boar in summer	Binary variable – presence of wild boar or signs of wild boar, or absence of any signs in summer	Wild boar are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 25]	N Present = 82 N Absent = 14	95	1.943	0.052^	4.39	0.20	8.59	58	-0.313	0.754	1.27	-2.91	5.44	This TB model indicates that the presence of wild boar on the summer pastures may be positively associated with the detection of TB in cattle. Four missing values here where respondents were uncertain if they had seen wild boar on their summer pastures
	Presence of badgers in winter	Binary variable – presence of badgers or signs of badgers, or absence of any signs in winter	Badgers are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 25]	N Present = 49 N Absent = 48	96	2.288	0.022 *	2.73	-0.31	5.77	58	-0.525	0.599	1.43	-2.44	5.29	This TB model indicates that the presence of badgers on the winter farm may be positively associated with the detection of TB in cattle
	Presence of badgers in summer	Binary variable – presence of badgers or signs of badgers, or absence of any signs in summer	Badgers are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 25]	N Present = 50 N Absent = 46	95	0.405	0.685	1.19	-1.81	4.18	58	1.147	0.251	2.17	0.21	4.13	Three missing values here where respondents were uncertain about having seen badgers on their summer pastures

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Summer water bodies per HA	The total number of natural water bodies on the summer pastures, divided by the summer farm area.	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence and density of water available will help to identify specific high risk water types	Mean 0.055 natural water sources per HA Range 0-2 natural water sources per HA	65	-0.362	0.717	1.51	-4.66	7.69	34	-0.043	0.965	3.39	-7040.70	7047.48	33 values missing here due to uncertainty about the number of water bodies on summer farms (they can be highly variable)

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Winter water bodies per HA	The total number of natural water bodies on the winter pastures, divided by the winter farm area.	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence and density of water available will help to identify specific high risk water types	Mean 0.059 natural water sources per HA Range 0-2 natural water sources per HA	78	-0.417	0.677	1.69	-5.24	8.62	51	-0.611	0.541	2.02	-4.20	8.24	20 observations missing here due to uncertainty about the number of water bodies on winter farms (they can be highly variable)

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Summer streams per HA	The total number of summer streams divided by the size of the summer farm in hectares	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence and density of water available will help to identify specific high risk water types	Mean 0.025 streams per HA Range 0 – 0.5 streams per HA	68	0.880	0.379	24.83	-50.51	100.16	34	-0.854	0.393	2.03	-2.43	6.49	30 observations missing here due to uncertainty about the number of streams on summer farms (they can be highly variable)

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Winter streams per HA	The total number of winter streams divided by the size of the winter farm in hectares	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence and density of water available will help to identify specific high risk water types	Mean 0.027 streams per HA Range 0 – 0.5 streams per HA	81	**0.278**	0.781	2.54	-53.50	58.57	51	-0.421	0.674	1.75	-5.67	9.17	17 observations missing here due to uncertainty about the number of streams on winter farms (they can be highly variable)

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Artificial water sources per HA in summer	The total number of water sources created and maintained by the farmer (e.g. water troughs) on the summer pastures, divided by the total area of the summer pastures in hectares	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence and density of water available will help to identify specific high risk water types	Mean 0.026 farmer managed water sources per HA Range 0-0.5 farmer managed water sources per HA	68	0.886	0.376	25.76	-50.86	102.39	34	0.267	0.789	2.12	-30.73	34.97	30 observations missing here due to uncertainty about the number of water sources on summer farms (they can be highly variable)

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Artificial water sources per HA in winter	The total number of water sources created and maintained by the farmer (e.g. water troughs) on the winter pastures, divided by the total area of the winter pastures in hectares	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence and density of water available will help to identify specific high risk water types	Mean 0.036 farmer managed water sources per HA Range 0 – 0.5 farmer managed water sources per HA	81	-0.163	0.870	1.65	-40.66	43.96	51	-0.348	0.728	1.45	-4.24	7.13	17 observations missing here due to uncertainty about the number of water sources on winter farms (they can be highly variable)

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Artificial water sources per cow in summer	The total number of water sources created and maintained by the farmer on the summer pastures, divided by the number of cows	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence and density of water available will help to identify specific high risk water types	Mean 0.015 farmer managed water sources per cow Range 0-0.167 farmer managed water sources per cow	91	0.858	0.391	957.1	-4867.9	6782.36	59	-0.993	0.321	1.47	-1.43	4.37	7 observations missing here due to uncertainty about the number of water sources on summer farms (they can be highly variable)

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Artificial water sources per cow in winter	The total number of water sources created and maintained by the farmer on the winter pastures, divided by the number of cows	Water sources encourage congregation of livestock and wildlife, affecting direct contact and shared space use. Knowing the type and permanence and density of water available will help to identify specific high risk water types	Mean 0. farmer managed water sources per cow Range 0 – farmer managed water sources per cow	89	0.834	0.401	1.95	-2.45	6.36	59	0.412	0.680	2.70	-19.27	24.68	9 observations missing here due to uncertainty about the number of water sources on winter farms (they can be highly variable)
	Pig stocking density in summer	The total number of pigs on the summer farm divided by the total area of the farm in hectares	Pigs can contract TB and therefore may be a part of the disease cycle	Mean 0.026 pigs/HA Range 0 – 0.6 0pigs/HA	31	0.508	0.612	2.46	-9.04	13.95							Only 31 observations here as the pig stocking density question was only asked in Asturias

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Pig stocking density in winter	The total number of pigs on the winter farm divided by the total area of the farm in hectares	Pigs can contract TB and therefore may be a part of the disease cycle.	Mean 0.018 pigs/HA Range 0 – 0.6 pigs/HA	81	0.861	0.389	10.20	-18.91	39.30	51	-0.006	0.995	21.14	-216.79	259.06	17 observations missing here as 17 farmers did not keep pigs
	Goat stocking density in summer	The total number of goats on the summer farm divided by the total area of the farm in hectares	Goats can contract TB and brucellosis and therefore may be a part of the farm's disease cycle	Mean 0.024 goats/HA Range 0 – 0.8 goats/HA	31	0.007	0.995	7.31	-32.02	46.64							Only 31 observations here as the goat stocking density question was only asked in Asturias
	Goat stocking density in winter	The total number of goats on the winter farm divided by the total area of the farm in hectares	Goats can contract TB and brucellosis and therefore may be a part of the farm's disease cycle	Mean 0.052 goats/HA Range 0 - 2 goats/HA	81	-0.674	0.500	2.21	-4.15	8.57	51	-0.007	0.994	1.29	-24.09	26.66	17 observations missing here as 17 farmers did not keep goats

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Sheep stocking density in summer	The total number of sheep on the summer farm divided by the total area of the farm in hectares	Sheep can contract TB and brucellosis and therefore may be a part of the farm's disease cycle	Mean 0.178 sheep/HA Range 0 – 4.12 sheep/HA	31	0.007	0.995	2.72	-6.36	11.80							Only 31 observations here as the sheep stocking density question was only asked in Asturias
	Sheep stocking density in winter	The total number of sheep on the winter farm divided by the total area of the farm in hectares	Sheep can contract TB and brucellosis and therefore may be a part of the farm's disease cycle	Mean 0.146 sheep/HA Range 0 – 4.12 sheep/HA	81	0.484	0.628	1.19	-2.49	4.86	49	-0.006	0.995	1.17	-20.42	22.75	17 observations missing here as 17 farmers did not keep sheep
	Winter Wild Boar Density	An estimate of wild boar population density derived from dividing the number of wild boar shot on the farm by the farm area in winter	Wild boar are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 25]	Mean 2.42 wild boar/hectare Range 0 – 42.86 wild boar/hectare	50	0.217	0.083^	1.34	-0.98	3.66	24	0.946	0.344	1.21	-1.19	3.61	

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Winter Red Deer Density	An estimate of red deer population density derived from dividing the number of deer shot on the farm by the farm area in winter	Red deer are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 25]	Mean 0.378 red deer//hectare Range 0 – 5.71 red deer/hectare	47	-1.108	0.268	2.38	-1.91	6.67	22	0.982	0.326	1.46	-1.42	4.35	
	Summer Wild Boar Density	An estimate of wild boar population density derived from dividing the number of wild boar shot on the farm by the farm area in summer	Wild boar are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 25]	Mean 0.143 wild boar/hectare Range 0 – 0.80 wild boar/hectare	39	1.755	0.079^	4.08	-0.29	8.45	15	1.201	0.229	13.48	-3.60	30.56	Many missing values here as most farmers did not hunt on their summer pastures.

					TB						Brucellosis						
Question in table 5	**Variable Name**	**Response Type**	**Reason for inclusion in the study**	**Data summary**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**d.f.**	**z**	***p* value**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**Comments**
	Summer Red Deer Density	An estimate of red deer population density derived from dividing the number of deer shot on the farm by the farm area in summer	Red deer are known hosts for TB, and so may contribute to a wildlife reservoir of the disease and therefore a risk factor for TB. [reference 25]	Mean 0.01 red deer//hectare Range 0 – 0.05 red deer/hectare	37	0.802	0.422	2.47	-3.58	8.52	10	0.974	0.330	37.37	-43.42	118.17	Many missing values here as most farmers did not hunt on their summer pastures.

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