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Activity Overlap and Risk of Mycobacterium bovis Transmission Between Wild European Mammals

Justus, William

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Yr wyf drwy hyn yn datgan mai canlyniad fy ymchwil fy hun yw'r thesis hwn, ac eithrio lle nodir yn wahanol. Caiff ffynonellau eraill eu cydnabod gan droednodiadau yn rhoi cyfeiriadau eglur. Nid yw sylwedd y gwaith hwn wedi cael ei dderbyn o'r blaen ar gyfer unrhyw radd, ac nid yw'n cael ei gyflwyno ar yr un pryd mewn ymgeisiaeth am unrhyw radd oni bai ei fod, fel y cytunwyd gan y Brifysgol, am gymwysterau deuol cymeradwy.

**Activity Overlap and Risk of *Mycobacterium bovis* Transmission Between Wild
European Mammals**

By William Justus



PRIFYSGOL
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Supervised by Graeme Shannon and Simon Valle

A dissertation submitted for the degree of Master of Science by Research in the School of
Natural Sciences

February 2023

Abstract

Understanding how disease moves through wildlife communities is essential to managing outbreaks of zoonotic diseases across the globe. Bovine tuberculosis is a disease caused by *Mycobacterium bovis* (*M. bovis*) that can threaten domestic and wildlife species. In Chapter I, I conducted a systematic review and meta-analysis on *M. bovis* prevalence rates in European wildlife species. I then used prevalence rates to identify Eurasian badgers (*Meles meles*), wild boar (*Sus scrofa*), fallow deer (*Dama dama*), and red foxes (*Vulpes vulpes*) as species of concern for the transmission of *M. bovis* and subsequently conducted a risk assessment for those species based on available literature. I found that research effort has primarily focused on badgers, wild boar, and red deer, but that the risk of community spread requires broader consideration. I also discovered that an in-depth spatiotemporal analysis was needed to better assess the risk of transmission between wildlife species. In Chapter II, I used camera traps to examine the activity of Eurasian badgers, fallow deer, and red foxes in North Wales as a means of evaluating how *M. bovis* may spread through a wildlife community. I calculated coefficients of overlap and selection ratios to quantify temporal overlap and time-period preference. Though activity varied by season, I found that badgers were generally nocturnal, foxes were nocturnal/crepuscular, and fallow deer were diurnal. Foxes and badgers had the largest degree of temporal overlap while badgers and deer had the least. All species spent at least a third of the diel cycle with overlapping activity. A high degree of connectedness between species has serious ramifications for wildlife diseases which may infect multiple host species. Efforts to manage these diseases should seriously consider broad sampling campaigns over species-specific efforts which may underreport the true scope of an outbreak.

Chapter One:

A Review of *Mycobacterium bovis* Transmission Risk in European Wildlife

Introduction

The importance of understanding disease ecology has become increasingly evident in recent years. The COVID-19 pandemic dramatically illustrated the destruction that disease can cause across all aspects of human life (Decerf et al, 2020). It was also a reminder that humanity remains inextricably linked with wildlife. The pandemic began with zoonotic transmission from wildlife, subsequently spread among and between human populations, and has now infected other wildlife species (Chandler et al, 2021; Shereen et al, 2020). Although the COVID-19 pandemic is now one of the most infamous examples of zoonotic disease, it is far from the only one to plague the modern world. Brucellosis, avian influenza, Ebola virus, rabies, plague, West Nile virus, toxoplasmosis, Hantavirus, anthrax, and numerous others are diseases which originate in animal species but are a threat to human life (Public Health England, 2019). Even if humans are not directly affected by a disease, the economic impact can be severe. In England, an outbreak of bovine tuberculosis costs farmers an average of £57,776 (Butler et al., 2010). The spread of disease may also threaten at-risk species and reduce global biodiversity (Thorne & Williams, 1988). Furthermore, it is likely that decreasing biodiversity will cause outbreaks of disease to become even more frequent and destructive (Pongsiri et al, 2009). Understanding how disease spreads among wildlife communities is essential to managing this threat.

Mycobacterium bovis (hereafter *M. bovis*) is the causative agent in bovine tuberculosis, a global disease that poses a risk to the health of cattle, humans, and wildlife (Hardie & Watson, 1992). Bovine tuberculosis is characterized by the progressive development of lesions in the

lungs, lymph nodes, or other organs and may spread through various bodily excretions (Ayele et al, 2004). The presence of *M. bovis* in wildlife species usually originates from infected livestock herds but may thereafter spread between wildlife species or back to livestock (O'Hare et al, 2020; Romero et al, 2008; Swift et al, 2021). An infected wildlife population may be either maintenance or spillover hosts (Corner, 2006). Maintenance hosts can sustain a baseline of infection in the population without additional inputs from another species, whereas spillover hosts cannot maintain infection in the population without re-infection from another species (Corner, 2006). Maintenance hosts are generally considered to be of greater concern, but both types of infected populations can further the spread of disease.

The mechanism by which *M. bovis* is spread between species is still poorly understood, though there is potential for both direct and indirect transmission. Infected animals may excrete *M. bovis* in their saliva, urine, or feces and bacilli may persist in the environment for up to eighty days (Corner et al, 2011; Fine et al, 2011; King et al, 2015; Sweeney et al, 2007). Animals may become infected through bites, aerosol transmission, or ingestion of infected materials (Corner, 2006, Corner et al, 2011; Richomme et al, 2020). Many wildlife species have been shown to persist with a latent form of infection characterized by no visible macroscopic lesions (NVLs), localization to the origin of infection, and little shedding of colony forming units (CFUs) (O'Brien et al, 2006; Payne et al, 2012; Delahay et al 2007; Gavier-Widén et al, 2009). It is only when the disease has become more advanced and systemic that high numbers of colony forming units may be shed through multiple routes (Corner, 2006; Michelet et al, 2018).

Whatever the details, the spread of *M. bovis* requires three basic conditions. There must be: a) an infected animal which is shedding bacilli in numbers large enough to infect another animal, b) opportunity for the two animals to come into contact (either directly or indirectly), and c) a behavior which facilitates the movement of the bacilli between them. An assessment of transmission risk must therefore evaluate each of these steps. The prevalence of *M. bovis*

infection in a population has been used as a general indicator of transmission risk in wildlife (Delahay et al, 2001, 2007). I conducted a meta-analysis of disease prevalence rates to identify species of concern and subsequently assessed those species for transmission risk using the criteria detailed above.

Materials and Methods

I conducted a series of systematic searches to collect bovine tuberculosis disease prevalence rates of European wildlife species, using broad search terms in the CAB, ProQuest, JSTOR, Science Direct, and Web of Science databases (Table 1, Appendix). I limited my results to peer-reviewed articles in academic journals, in English, for which a full text was readily available to Bangor University researchers, and which presented novel research. Papers which used generated data, case studies, captive populations, or pooled data from multiple regions were rejected. Furthermore, I did not include papers that duplicated a dataset which was already included in the analysis. I initially selected papers by title and abstract, then further refined them by reading the full text (Fig. 1). From an initial 1,830 publications, I selected 92 papers from my systematic search for inclusion in the final analysis.

Table 1. Details of the systematic searches conducted for the meta-analysis.

Database	Date	Search Terms	Fields Searched
Web of Science, JSTOR, CAB, Science Direct, ProQuest	16/11/2021	("Mycobacterium bovis" OR "bovine tuberculosis") AND (wildlife OR prevalence)	Title, All
ProQuest	20/01/2022	("Mycobacterium bovis" OR "M. bovis" OR "bovine tuberculosis") AND (wildlife OR ungulate OR carnivore OR predator OR mesocarnivore) AND (prevalence OR infection)	Abstract, Title, Key words
Web of Science	22/01/2022	("Mycobacterium bovis" OR "M. bovis" OR "bovine tuberculosis") AND (wildlife OR ungulate OR carnivore OR predator OR mesocarnivore) AND (prevalence OR infection)	Abstract, Title, Key words
JSTOR, CAB, Science Direct	24/01/2022	("Mycobacterium bovis" OR "M. bovis" OR "bovine tuberculosis") AND (wildlife OR ungulate OR carnivore OR predator OR mesocarnivore) AND (prevalence OR infection)	Abstract, Title, Key words

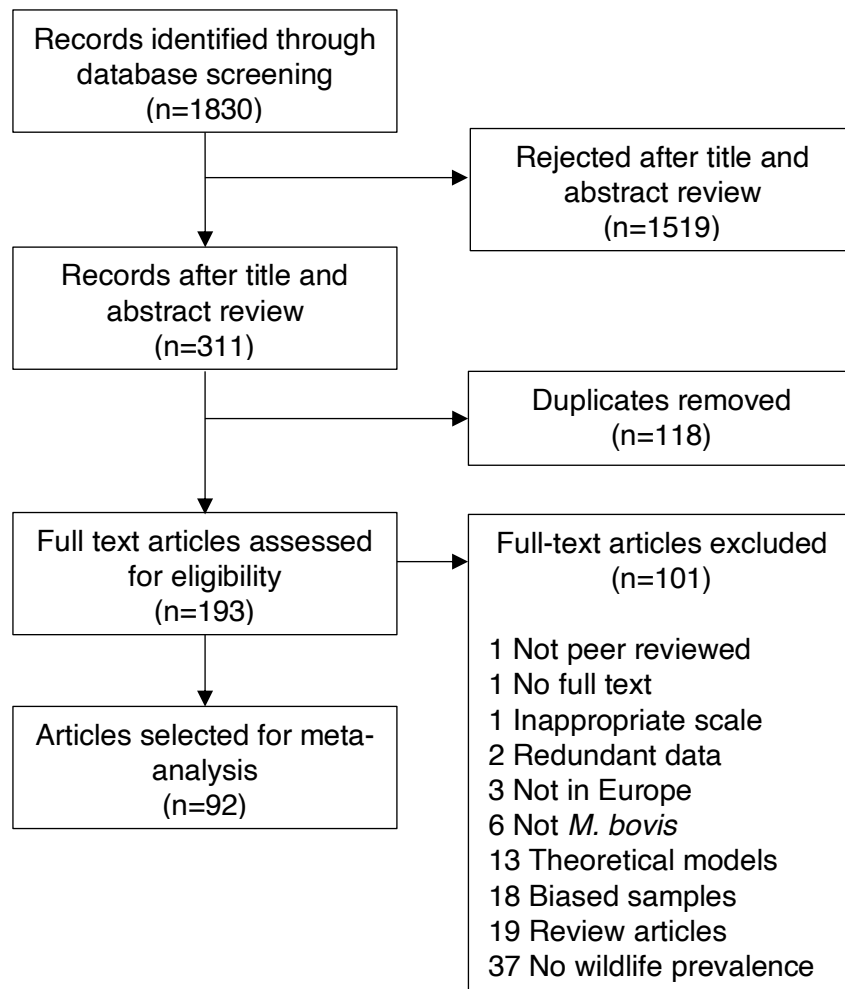


Figure 1. Paper selection process for the systematic review of *M. bovis* prevalence data.

Many of the selected papers contained multiple surveys of disease prevalence in wildlife, which provided a large database from which to conduct the meta-analysis. I collected data from 661 surveys of bovine tuberculosis prevalence rates in wildlife which covered about 50 species (some surveys did not identify beyond the genus). Though there were data from 12 nations included in the final analysis, most surveys were conducted in either Britain and Ireland or the Iberian Peninsula (Fig.2). The publication dates of selected papers ranged across a period of 36 years (Fig. 3). There were six different methods used to collect samples from animals and six different methods to test those samples for *M. bovis*. The animal sampling methods were

hunting, culling, live-trapping, collection of found-dead animals (includes road-traffic accidents), non-specific government collection (referred to as “Unknown”), and multiple sampling methods (using two or more methods). The *M. bovis* test methods used were serological assay, PCR, visible check for lesions, bacterial culture, unknown testing method, and combined methods (using two or more methods).

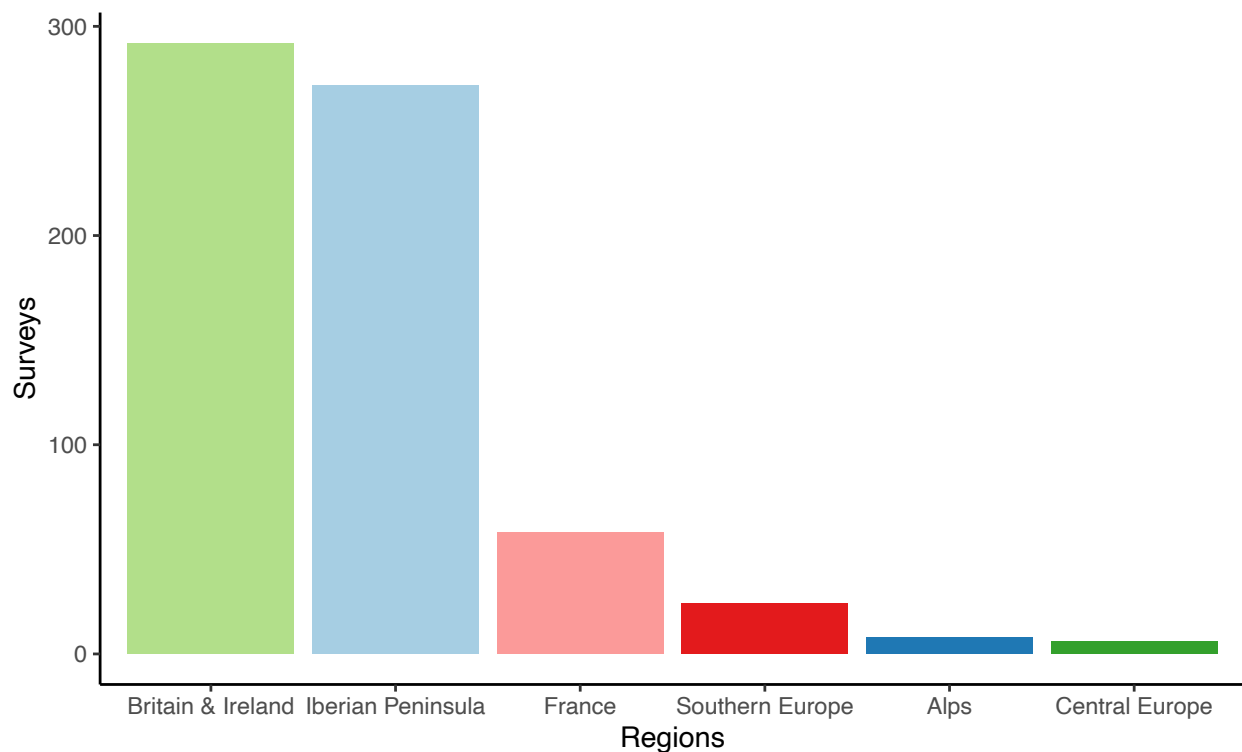


Figure 2. Study site locations of the studies included in the analysis. Region definitions are as follows: Alps = Switzerland, Austria, and Liechtenstein; Britain & Ireland = United Kingdom and Ireland; Central Europe = Germany, Poland, and the Netherlands; Iberian Peninsula = Portugal and Spain; Southern Europe = Italy and Slovenia

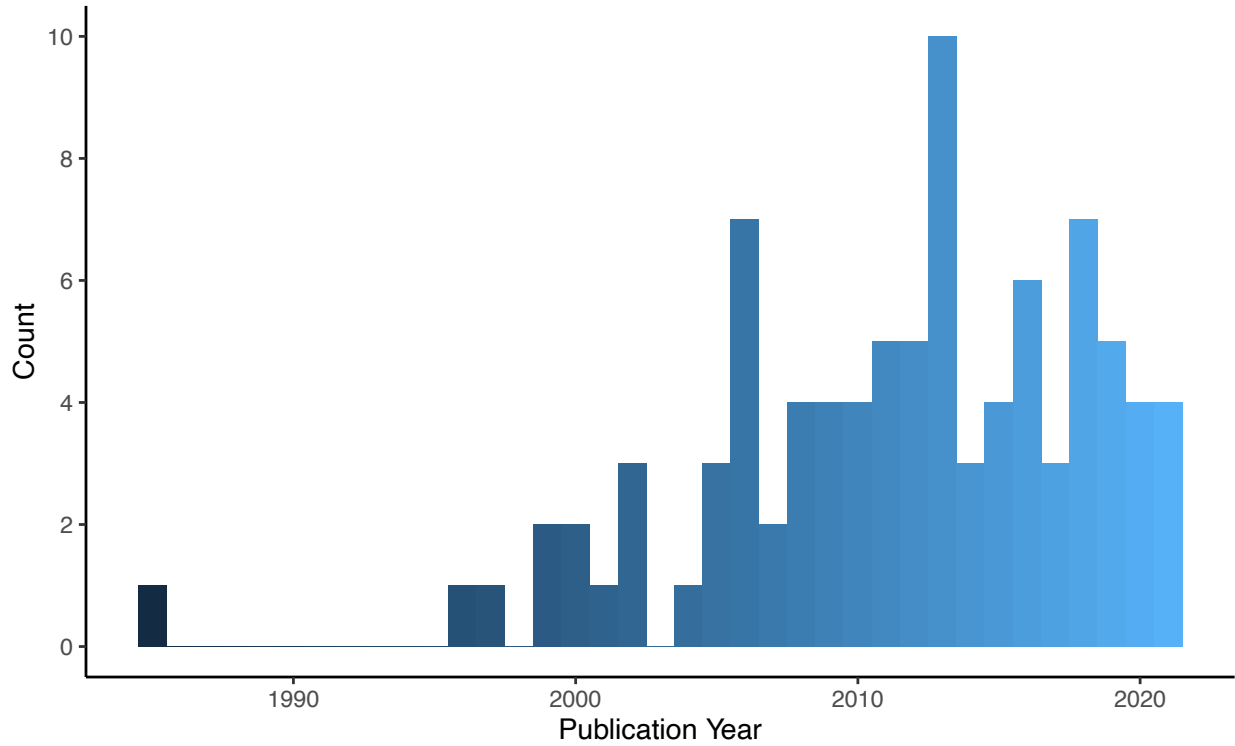


Figure 3. Distribution of the publication dates of the papers included in the analysis.

I calculated mean prevalence for species that had been surveyed at least ten times, since data from less studied species were thought to be insufficient to represent broader trends. Due to the large variability in sample size between surveys, I weighed mean prevalence by sample size so that surveys with larger sample sizes had a greater impact on the final prevalence estimates (Galmiche et al, 2019; O'Brien et al, 2019). I calculated weighted means using the following formula:

$$P_w = \frac{\Sigma(P_i \cdot N_i)}{N_t}$$

where P_w is weighted mean prevalence, P_i is the percent prevalence of an individual survey, N_i is the sample size of an individual survey, and N_t is the total number of animals sampled for a species. I restricted my calculations to surveys that contained at least one animal that tested positive for *M. bovis*. I only included those surveys that found a positive case of *M. bovis* in the weighted mean prevalence calculations. Following the meta-analysis results, I selected those species with high disease prevalence for a further analysis of transmission risk.

I analyzed the influence of animal sampling method and *M. bovis* testing method on reported disease prevalence rates by calculating each method's mean disease prevalence and weighted mean disease prevalence, including both positive and negative case results in my calculations. Since the data were not normally distributed, I used a Kruskal-Wallis test followed by a chi square test to determine if animal sampling method had a significant effect on mean prevalence rates and used similar methods to examine the effects of *M. bovis* test method and region of sampling. I then used a Dunn test with a Bonferonni correction to test for a significant difference between animal sampling methods and applied the same methodology to *M. bovis* testing methods.

Results

Fallow deer (*Dama dama*) had the highest weighted mean disease prevalence ($20.10 \pm 0.55\%$), followed by Eurasian badgers (*Meles meles*) ($11.04 \pm 0.12\%$), wild boar (*Sus scrofa*) ($8.96 \pm 0.22\%$), and red foxes (*Vulpes vulpes*) ($3.55 \pm 0.20\%$) (Fig. 4). The Iberian lynx had a high weighted mean disease prevalence but was excluded from the ranking due to a low total sample size (as low as 1 individual per survey), which likely skewed the result. Fallow deer also had the highest proportion (88.0%) of populations that were infected with *M. bovis*, followed by wild boar (87.3%), badgers (80.2%), foxes (60.6%), red deer (58.0%), and roe deer (53.3%).

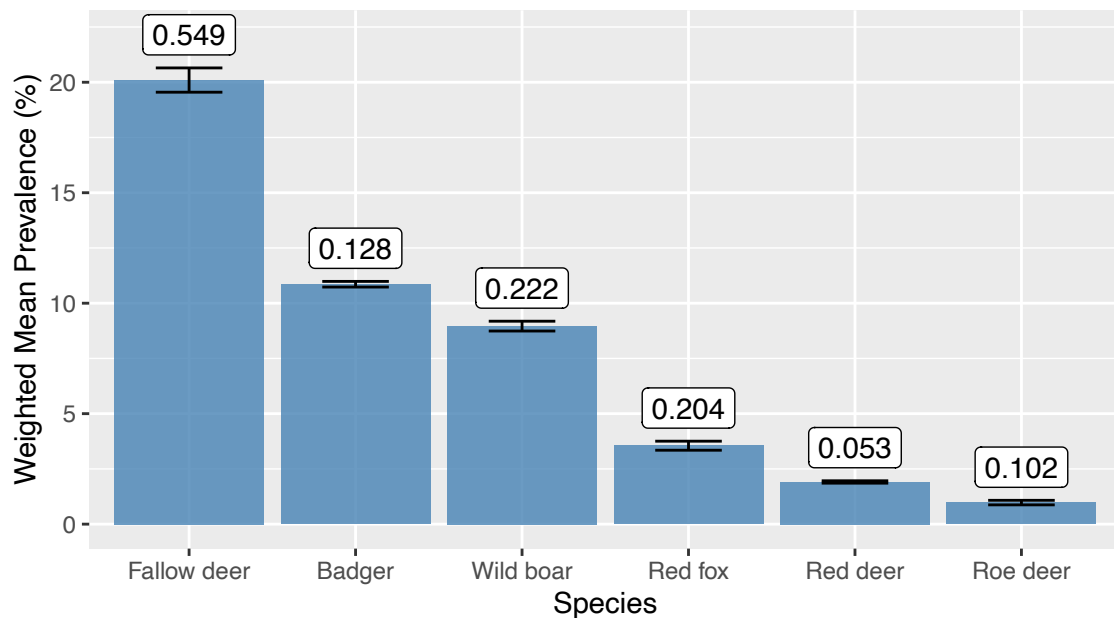


Figure 4. Weighted mean prevalence of wildlife populations infected with *M. bovis*. Error bars are standard deviation with the value above.

Hunting was the most commonly used method of animal sampling (used in 35% of studies), followed by live-trapping (used in 22% of studies, Fig. 5). Most surveys (55%) used combined methods to test for *M. bovis* (some combination of culture, PCR, physical exam, or serological assay) (Fig. 6). The majority of individuals sampled were red deer (100,685), followed by wild boar (64,037) and badgers (60,594), but badger populations were surveyed more frequently than other species (232 surveys, Fig. 7). Badgers were surveyed most often in Britain and Ireland, which accounted for 84.5% of all badger surveys (Fig. 8a). The Iberian Peninsula was the most common region for surveys of wild boar, red deer, red fox, and fallow deer (Fig. 8b-e). Roe deer were surveyed most frequently in France (Fig. 8f).

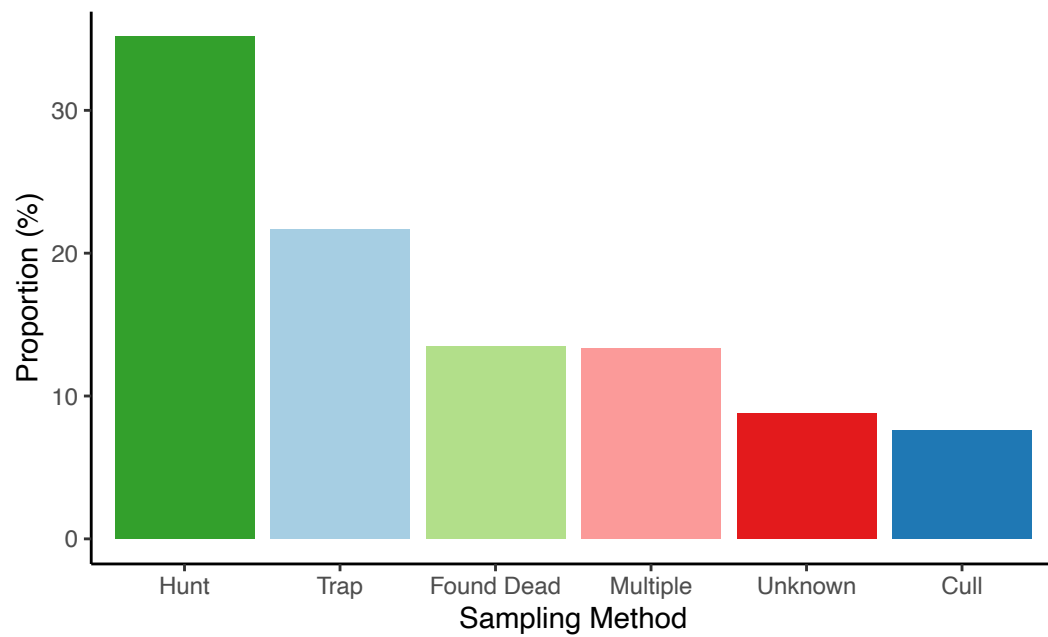


Figure 5. The relative use of each animal sampling method by studies in the meta-analysis.

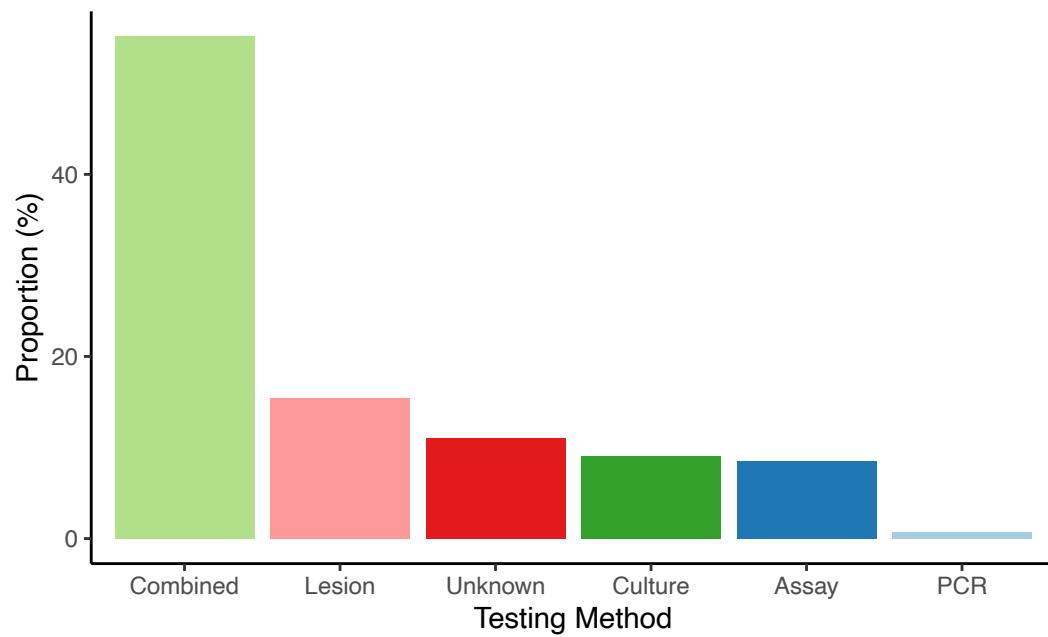
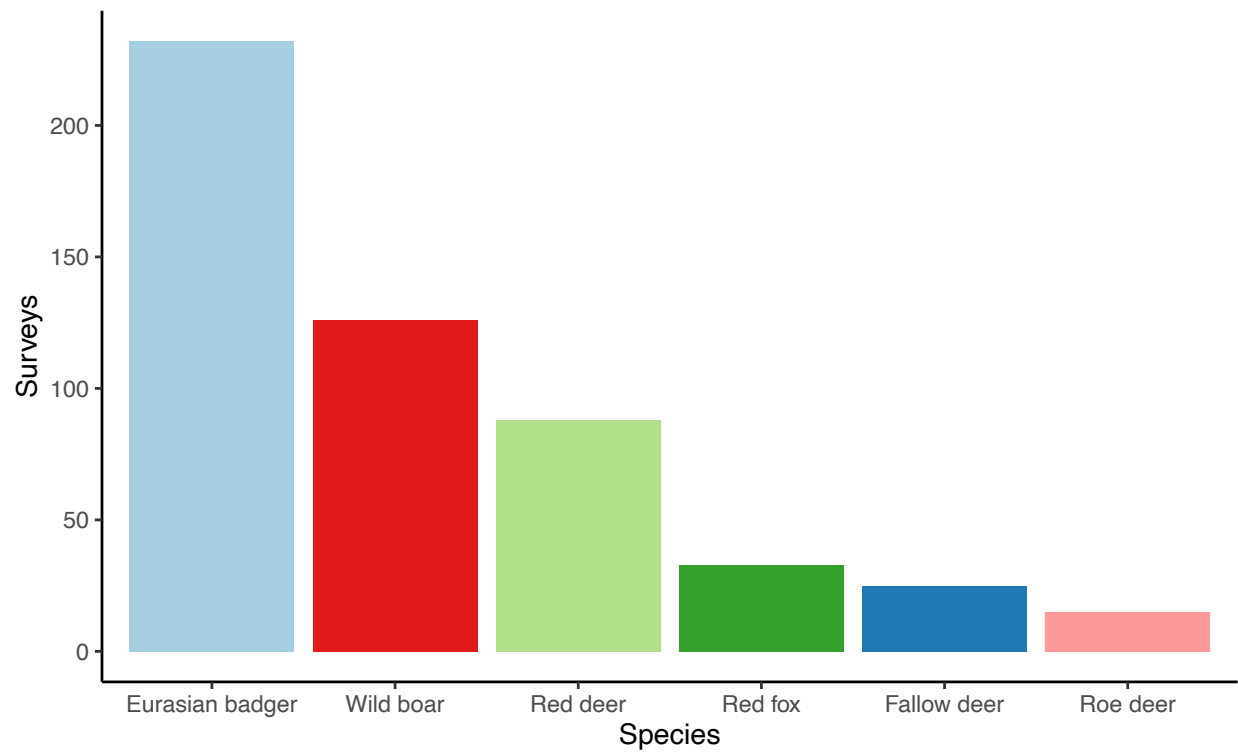


Figure 6. The relative use of each *M. bovis* testing method used by studies in the meta-analysis.

a)



b)

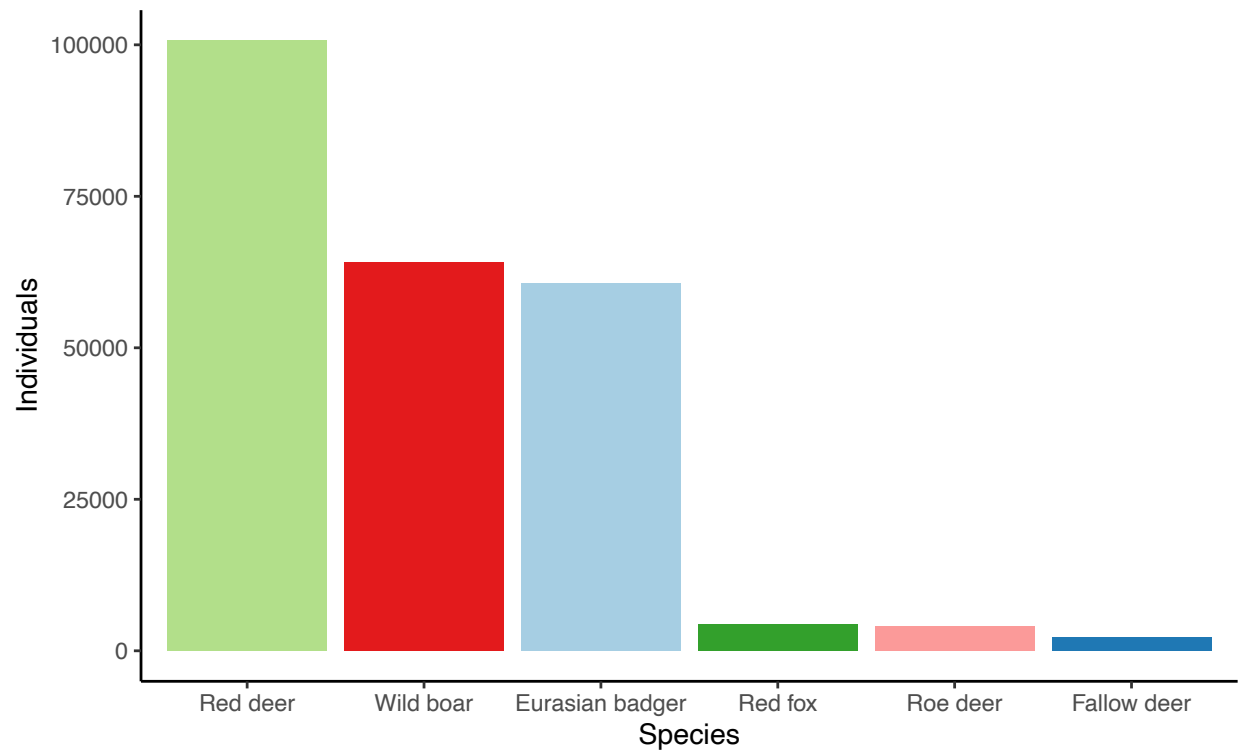


Figure 7. Numbers of a) surveys and b) individual animals sampled for the major species.

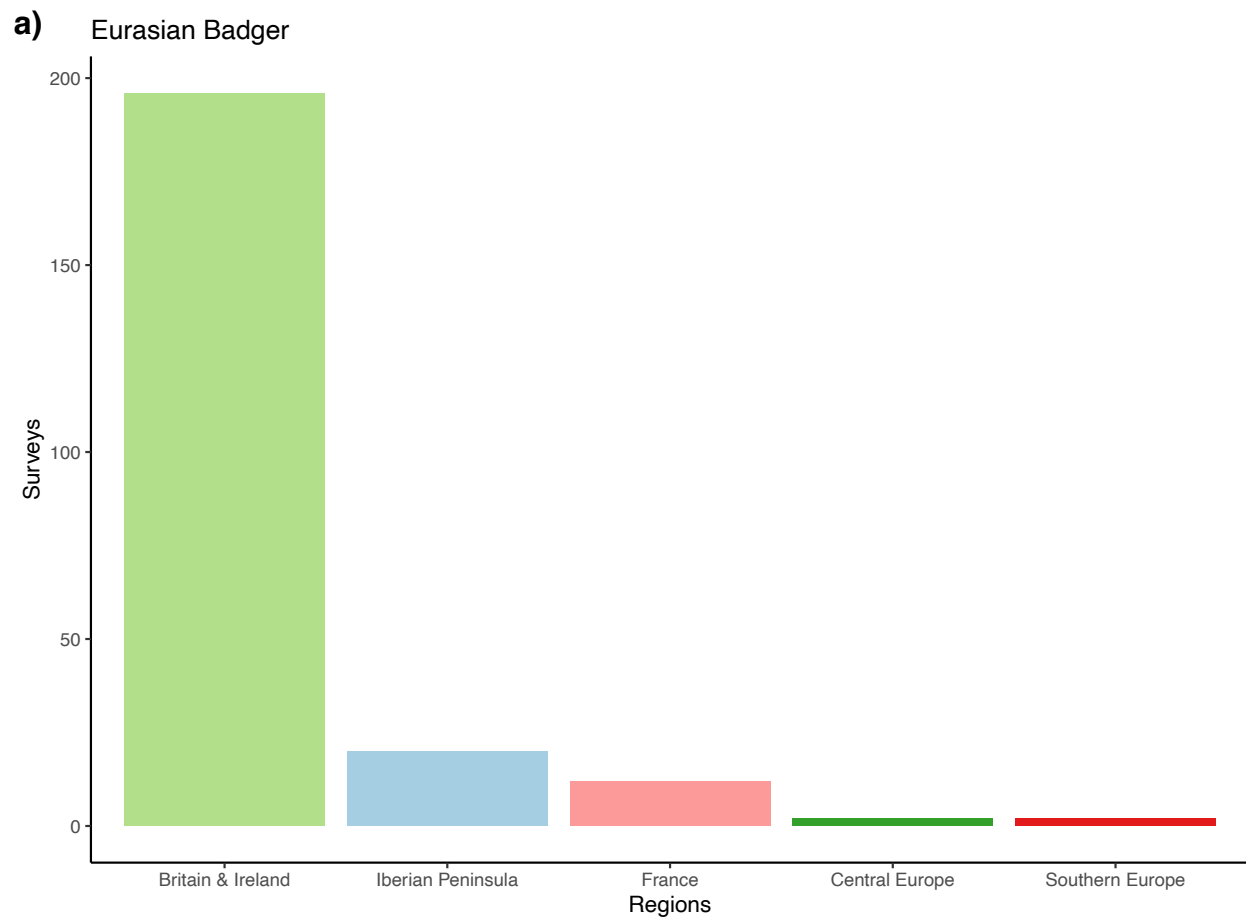
Animal sampling method (Kruskal-Wallis chi-squared, $h = 52.367$, $df = 5$, $p = 4.536 \times 10^{-10}$), *M. bovis* test method (Kruskal-Wallis chi-squared, $h = 13.235$, $df = 5$, $p = 0.02127$), and region (Kruskal-Wallis chi-squared, $h = 49.125$, $df = 5$, $p = 2.093 \times 10^{-9}$) were all related to a significant change in disease prevalence rates. Animal samples collected through culls had significantly higher (Dunn Test, $p < 0.0001$) disease prevalence rates than any other sampling method (Table 2). There was also a significant (Dunn Test, $z = 3.689$, $p = 0.0017$) difference between hunting and the unknown sampling methods. Culling was the animal sample method with the highest weighted mean prevalence (Table 2). Lesion examination resulted in significantly higher (Dunn Test, $z = -3.177$, $p = 0.0111$) disease prevalence rates than combined *M. bovis* test methods, but there were no other significant differences between testing methods. Serological assays found the highest weighted disease prevalence of the *M. bovis* testing methods, while lesion examination returned the lowest weighted disease prevalence results despite reporting high unweighted disease prevalence (Table 2).

Table 2. Disease prevalence associated with each animal sampling method. *** = $p < 0.001$

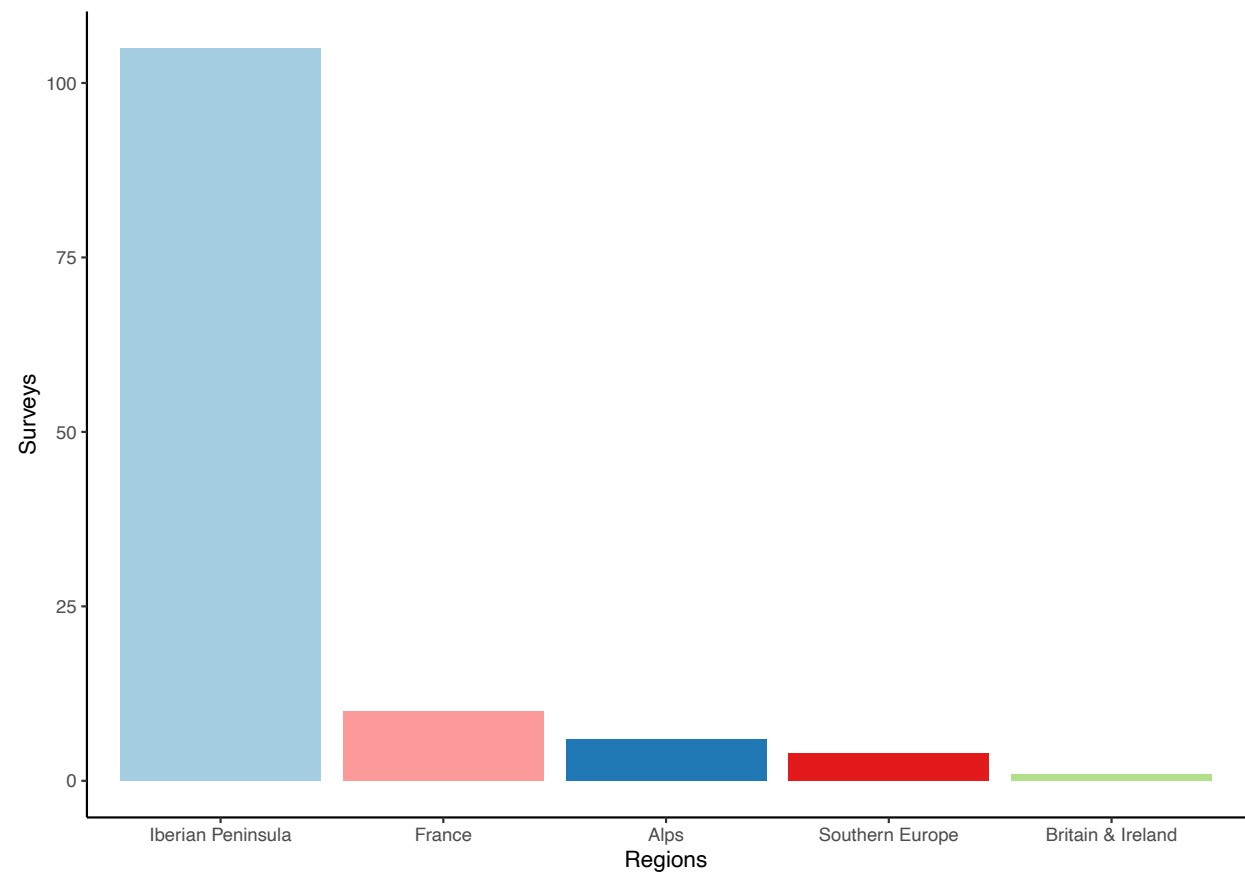
Sample Method	Mean Prevalence	Standard Error of Mean	Weighted Mean Prevalence	Standard Error of Weighted Mean
Hunt	16.63	1.59	4.03	0.00
Multiple	8.67	1.39	7.34	0.02
Unknown	4.39	0.99	6.58	0.05
Trap	11.53	1.35	8.31	0.01
Found Dead	11.05	1.83	7.99	0.03
Cull	24.02***	2.67	17.46	0.04

Table 3. Disease prevalence associated with each *M. bovis* testing method.

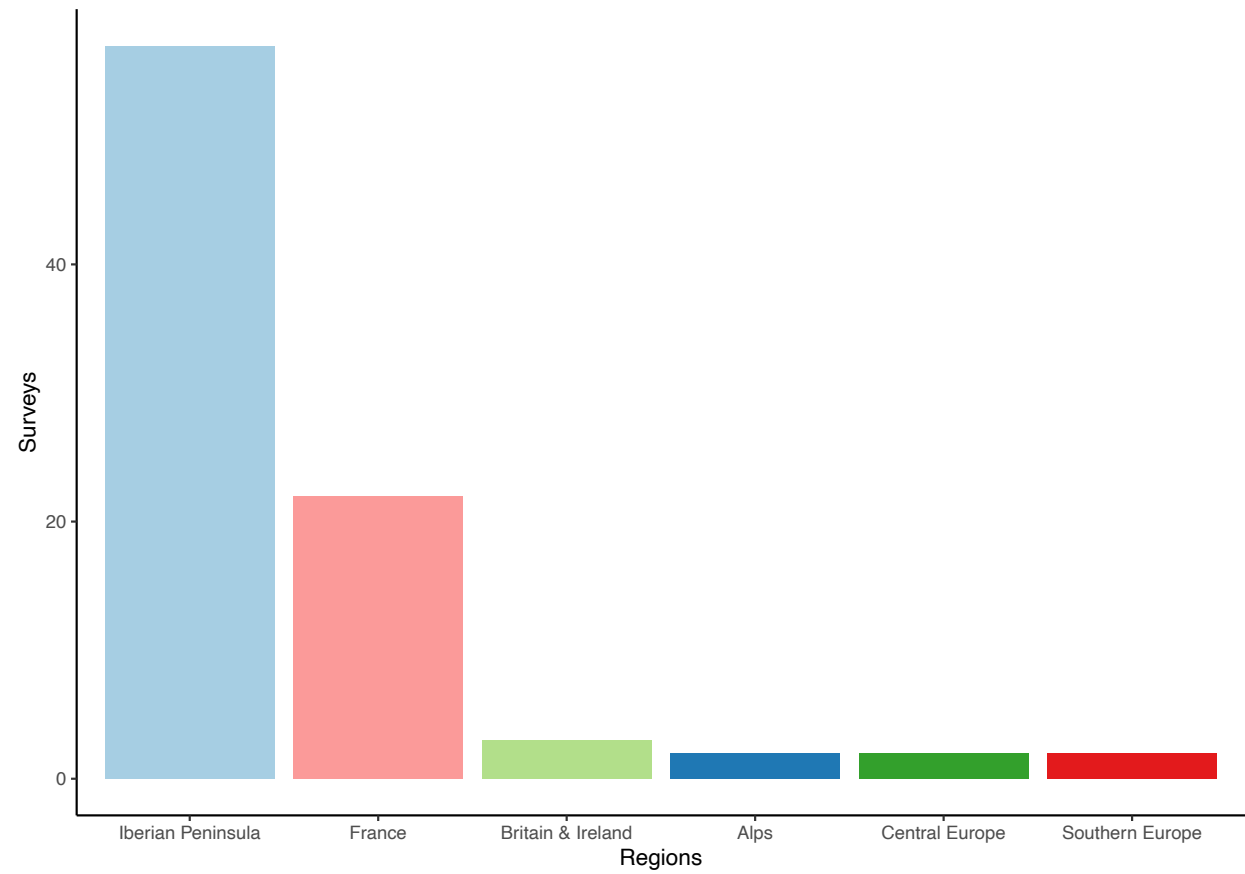
Test Method	Mean Prevalence	Standard Error of Mean	Weighted Mean Prevalence	Standard Error of Weighted Mean
Combined	12.29	0.97	7.52	0.00
Unknown	10.56	1.39	8.35	0.03
Assay	11.98	2.87	17.88	0.17
Culture	13.31	1.94	9.54	0.03
PCR	5.66	2.65	7.24	0.74
Lesion	19.28	2.64	3.08	0.01



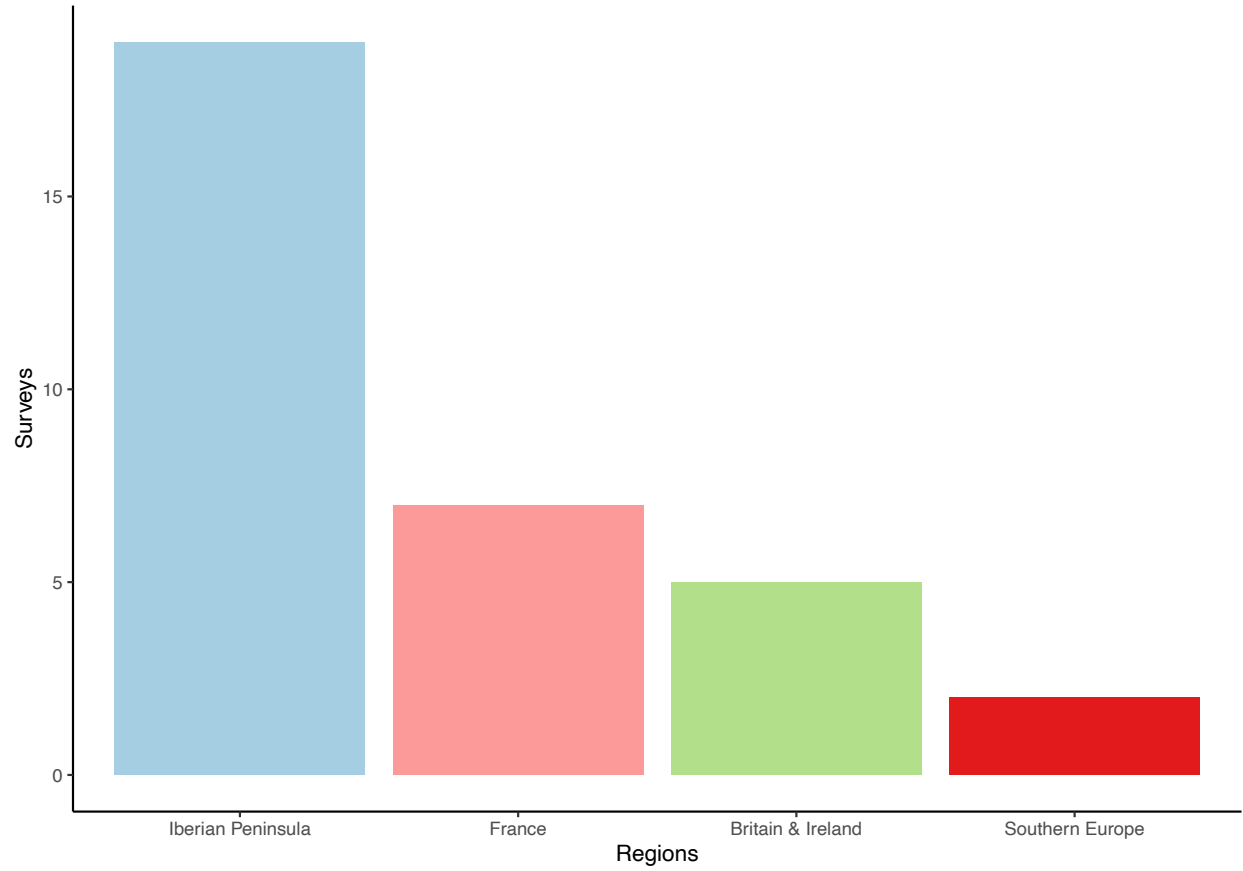
b) Wild Boar



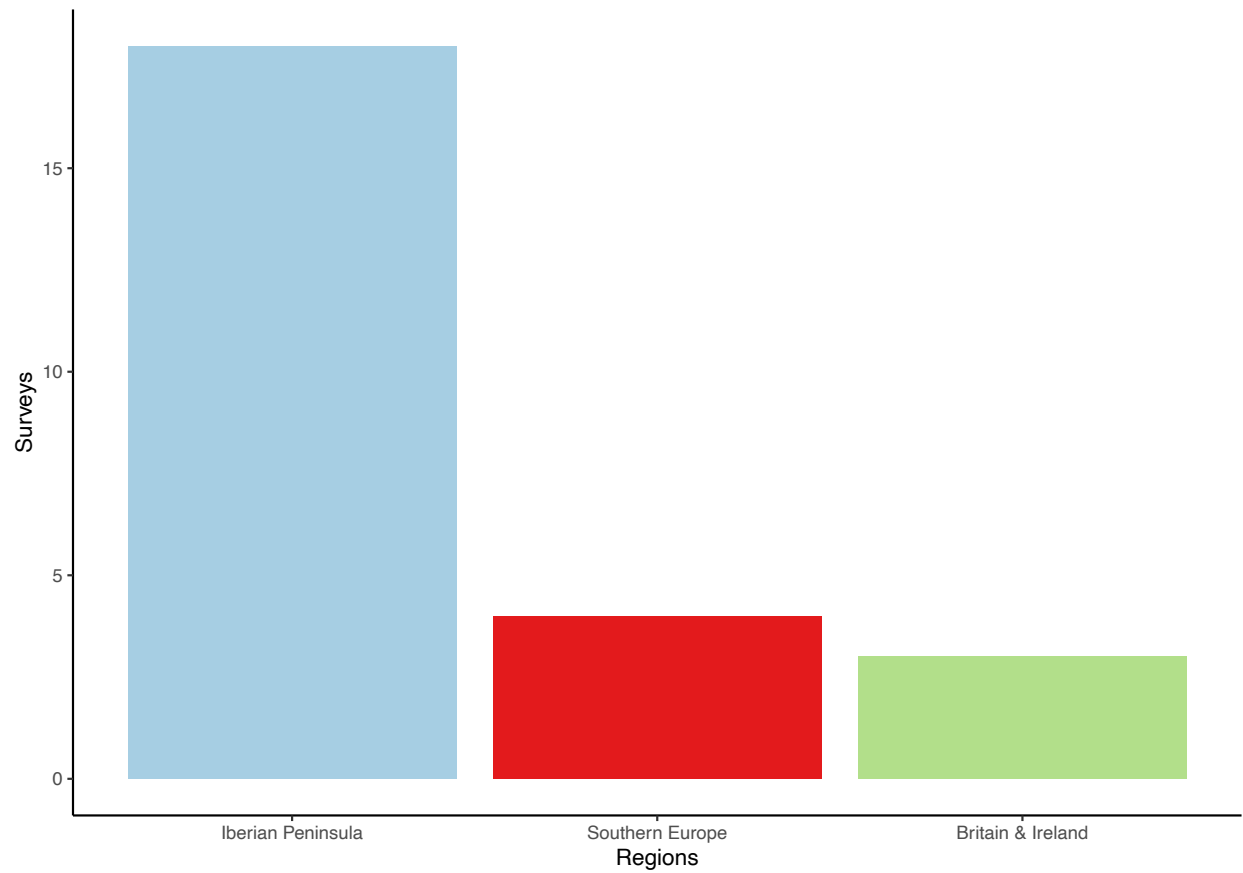
c) Red Deer



d) Red Fox



e) Fallow Deer



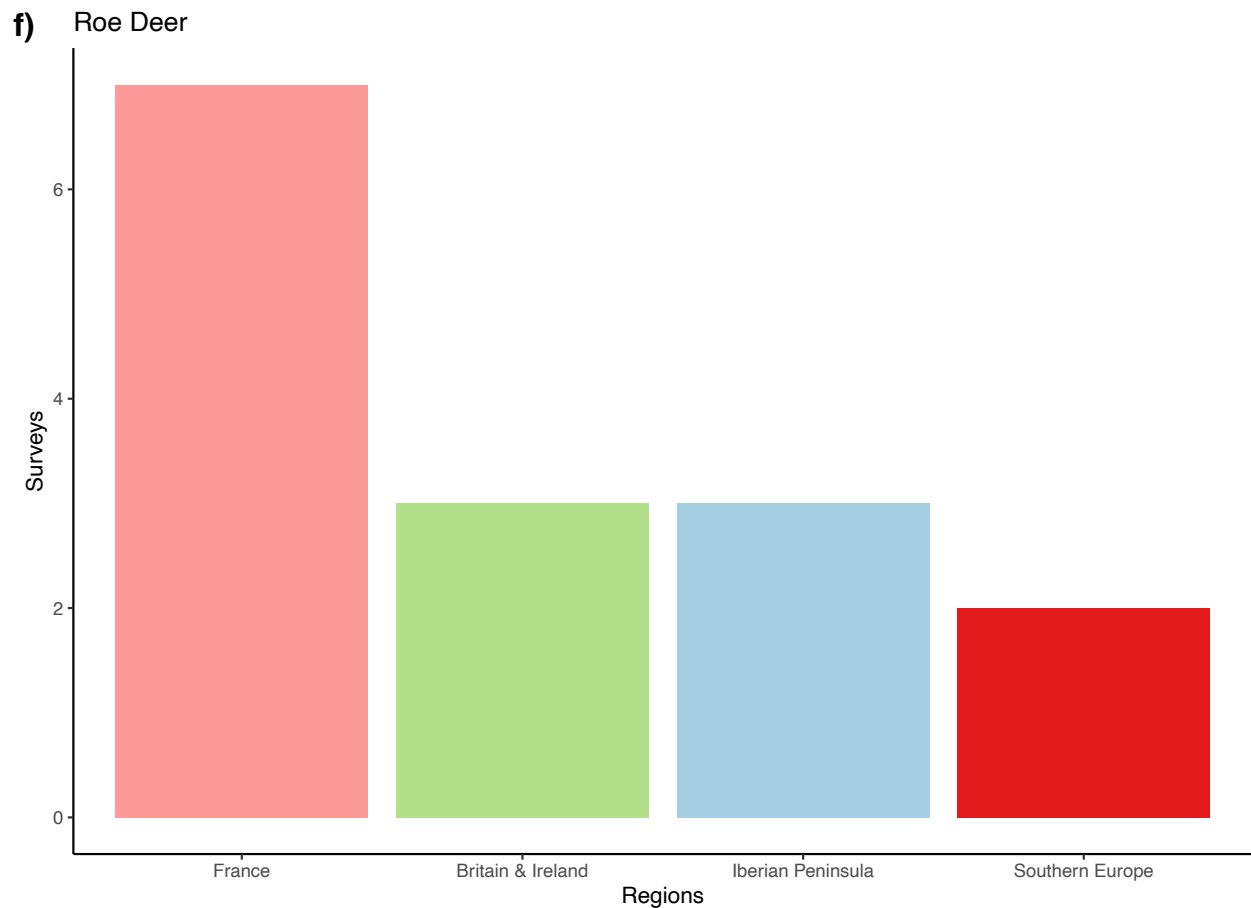


Figure 8. Species survey locations: a) badger, b) wild boar, c) red deer, d) red fox, e) fallow deer, and f) roe deer. Region definitions are as follows: Alps = Switzerland, Austria, and Liechtenstein. Britain & Ireland = United Kingdom and Ireland. Central Europe = Italy and Slovenia.

Discussion

The high prevalence of *M. bovis* in infected fallow deer populations is perhaps the most surprising result, as they have been understudied when compared to badgers, wild boar, or red deer. This result certainly calls for further research into the role fallow deer play in the disease ecology of bovine tuberculosis. Furthermore, the low disease prevalence found in red deer along with the relatively low percentage of infected populations may suggest that their contribution to the spread and maintenance of *M. bovis* is smaller than their large share of research effort might suggest. However, other factors like population size and pathophysiology contribute to the importance of a species in the ecology of a disease, and one should therefore

be careful to avoid extrapolating too far based on prevalence data alone (Corner, 2006; Delahay et al, 2001).

The contributions of the wildlife community at large to the disease ecology of bovine tuberculosis are likely underestimated due to a paucity of data. Though the scope of the pooled data included about fifty species, only six had sufficient data to allow for a meta-analysis. Thirteen species were only surveyed once, while badgers were surveyed 232 times. This unequal distribution of research effort may have reasonable founding, but it serves well to illustrate how myopic research trends may become. These trends may also be regionally specific. Of the surveys conducted in Britain and Ireland, 67.1% were of badgers. Comparatively, wild boar were the most surveyed species in the Iberian Peninsula but only accounted for 38.6% of the surveys in that region. Multi-species sampling should become a more frequent practice, especially in Britain and Ireland, if we are to better understand the disease ecology of bovine tuberculosis.

Disease prevalence rates varied according to animal sampling method and *M. bovis* test method, demonstrating the need for a standardization of methodology. Combined methods were used most frequently for *M. bovis* testing. The frequent occurrence of NVLs in wildlife species can make diagnosis difficult and combined methods may give a greater degree of precision (Gavier-Widén et al, 2009). The examination of gross lesions via necropsy may be an especially misleading *M. bovis* testing method when used alone. Finding gross lesions requires an advanced pathology, a thorough necropsy, and may easily be confounded by NVLs (Gavier-Widén et al, 2009). Limited sample sizes may also severely hinder the reliability of disease prevalence estimates from gross lesion examination. The large differences I found between lesion estimates for mean disease prevalence and weighted mean disease prevalence suggest that low sample sizes had a drastic effect on the calculations. The lack of a significant difference between *M. bovis* testing methods does not necessarily reflect an equal effectiveness but may

also be due to the effect of sample sizes on the calculations. The weighted mean, which accounts for differences in sample size, may better reflect trends in the effect of testing methods on disease prevalence. However, though provoking these trends may be, a true evaluation of testing method effectiveness should be tested experimentally.

Hunting was the most common method of gathering animal samples, probably because ungulates comprised four of the six examined species. Relying on hunters for sample collection could conceivably produce issues with non-random selection and carcass contamination, but other methods, like culling, may have similar biases. The high disease prevalence rates associated with culling relative to other sampling methods may be due to the circumstances in which culls are carried out, typically being a response to large outbreaks of disease rather than a standard monitoring practice. These findings may be affected by the categorical grouping of differing methods and should be viewed with caution. The significant effect of region on disease prevalence rates is likely due to the high geographic skew of studies included in the analysis, which reflects the regions most afflicted with bovine tuberculosis.

Though my analysis benefits from a breadth in scope, it is also limited by it. There was a large degree of variation in sample size of the surveys and the number of surveys per species. My analysis could not account for variation in the number of populations per survey or variation in ecosystems surveyed. There was also variation in animal sampling and testing methods that I represented with a few broad categories. Despite these concerns, the analysis effectively highlights trends in the literature and points to gaps in the research. Categorical groups allow for precision to be preserved despite individual variation, permitting confidence in the data trends, if not the values themselves.

I selected Eurasian badgers, wild boar, fallow deer, and red foxes for a more detailed analysis of transmission risk. The meta-analysis revealed that these species have high disease

prevalence rates, and they therefore warrant further investigation into their suitability as wildlife hosts of *M. bovis*.

Evaluation of Transmission Risk

Pathology and Epidemiology

Understanding the typical course of bovine tuberculosis in a species is of central importance for assessing risk of transmission (Corner, 2006; Delahay et al, 2001). The length of time during which an individual sheds bacilli, the number of bacilli shed, and the routes of excretion all contribute to the risk that an infected individual poses to the wildlife community (Delahay et al, 2001). The susceptibility of an individual to infection and subsequent transmission may be evaluated by understanding common routes of infection, lesion structure and location, and the routes and level of excretion (Corner, 2006). At the population level, disease prevalence and severity serve as factors which may affect transmission (Corner, 2006).

I found disease prevalence rates to be high (weighted mean of 11%) among infected populations of Eurasian badgers, and most of these animals will present with NVLs (Kelly et al, 2010; Murphy et al, 2010). Most badgers are infected by aerosol through the respiratory tract, though some may become infected from bites (Gallagher & Clifton-Hadley, 2000; Murphy et al, 2010). Badgers with latent infection can persist for years with limited sites of infection and low numbers of bacilli in infected tissues (Corner et al, 2011). The localized nature of infection, low bacilli count in infected tissues, and relatively small body mass of badgers suggests that badgers at this stage of infection will shed few CFUs. When the infection advances to a late-stage, badgers may exhibit macroscopic lesions, altered behavior, and shedding of bacilli through saliva, feces, and urine (Corner, 2006; Gallagher & Clifton-Hadley, 2000). It's at this

advanced stage that badgers are most likely to infect other species. Badger-to-badger infection may occur from constant exposure to individuals with latent infection, as prevalence rates get progressively higher with age (Woodroffe et al, 2005).

The relationship between fallow deer and *M. bovis* has been studied less than in badgers, but what research we have suggests that their populations exhibit relatively high disease prevalence rates (weighted mean of 20%) when infected (Amato et al, 2016, 2018; Aranaz et al, 2004; Delahay et al, 2001, 2002; García-Jiménez et al, 2012, 2013; Gortazar et al, 2008, 2011; Jaroso et al, 2010; Martín-Hernando et al, 2010; Romero et al, 2008). Lesions in deer species are usually associated with the respiratory tract and the lymph nodes of the head and may present as NVL or macroscopic lesions (Corner, 2006; Gavier-Widén et al, 2009). The common presence of respiratory lesions suggests aerosol as the usual route of infection. Infected fallow deer tend to develop thinly encapsulated granulomas filled with high counts of bacilli, which puts them at a high risk of excretion (Johnson et al, 2008). Granulomas in the lungs of fallow deer may be more widespread compared to more localized lesions in other deer species (Aranaz et al, 2004). Deer species have also been observed shedding *M. bovis* through multiple routes, including saliva and feces (Lugton et al, 1998). The higher body mass of fallow deer relative to smaller mammals like badgers may also contribute to a higher number of CFUs being excreted into the environment (Ward et al, 2009).

Wild boar have high disease prevalence rates (weighted mean of 9%) in infected populations. Though they can present with NVLs, visible lesions are common in infected animals, usually beginning in the mandibular lymph nodes and spreading into retropharyngeal and mesenteric lymph nodes as the disease progresses (García-Jiménez et al, 2013, 2015; Matos et al, 2016; Muñoz-Mendoza et al, 2013; Santos et al, 2009; Varela-Castro et al, 2021). The prevalence of lesions in the mandibular lymph nodes suggest that an oral origin of infection is common, though the results of Parra et al. (2006) demonstrate that a respiratory origin is also

possible. Lesions are typically multifocal and may range from small capsulated lesions to large granulomas with a calcified necrotic center (Matos et al, 2016; Parra et al, 2006; Santos et al, 2009). The capsulation of their lesions and a widespread immune response may reduce the number of bacilli shed by wild boar, but their large body mass and the frequency of large lesions suggests that they could shed substantial numbers of CFUs if the lesions open or the immune system fails (Parra et al, 2006). Open lesions draining into the salivary gland have been recorded in wild boar (Santos et al, 2009). In the advanced stages of infection when large lesions are widespread, wild boar have the potential to shed bacilli through multiple routes.

Though red foxes have lower disease prevalence rates (weighted mean of 4%) than Eurasian badgers, fallow deer, and wild boar, they do have high disease prevalence rates compared to many other mammal species (Martin-Atance et al, 2005; Matos et al, 2016; Millan et al, 2009). Infected foxes usually present NVLs which are typical of a latent stage of disease, a state in which they may persist for some time (Michelet et al, 2018). Foxes usually have an oral origin of infection, which suggests the ingestion of infected materials (Millan et al, 2008; Richomme et al, 2020). The mesenteric lymph nodes are the typical site of infection in foxes (Michelet et al, 2018; Richomme et al, 2020). Foxes have been known to shed bacilli in saliva, feces, and urine, even without exhibiting macroscopic lesions (Michelet et al, 2018). Foxes that shed bacilli through multiple routes while presenting NVLs may well be considered “super-shedders” since they are likely to shed many bacilli over a lengthy period (Michelet et al, 2018). However, the relatively small body mass of foxes may put a limit on the number of CFUs that can be excreted at any given time.

Space Use and Activity Patterns

To transmit *M. bovis*, an infected individual must encounter other animals. Shared space use is therefore necessary for disease transmission. If an infected animal overlaps spatially and temporally with another, then there is an opportunity for direct transmission and indirect transmission. If two animals are using the same space, but at different times, then there is only the chance for indirect transmission. I used habitat preference and population density as metrics of space use, while examining daily and seasonal activity patterns for the temporal use of those spaces.

Activity patterns are often plastic, with animals adjusting their active hours in response to predation, human presence, or season (Caravaggi et al, 2018; Johann et al, 2020; Lewis et al, 2021; Ohashi et al, 2013; Rossa et al, 2021). Despite this plasticity, determining typical activity patterns is still valuable for understanding the potential for temporal overlap. Badgers and foxes share similar activity patterns and co-occur spatially (Rossa et al, 2021; Torretta et al, 2016). Badgers are nocturnal while fox activity typically ranges from nocturnal to crepuscular (Caravaggi et al, 2018; Kammerle et al, 2020; Ogurtsov et al, 2018). When denning, badgers prefer rich clay soils in mixed woodland, probably for ease of sett construction and density of invertebrate prey, while foxes prefer denning in coniferous woodland and have no soil preferences (Kurek et al, 2014). Populations of foxes and badgers in upland habitats have larger ranges than is typical in resource-rich, lowland habitats (Parrott et al, 2012). Badgers live in large family units with as many as twenty-six individuals per group and a mean size of about five badgers. (Woodroffe et al, 2009). Red foxes are mostly solitary but do maintain long-term social relationships among a community that shares a territorial space (Dorning & Harris, 2019).

Thickets, meadows, and young forests are the most important habitat types for fallow deer, with meadows use increasing at night and during the winter (Borkowski & Pudelko, 2007). Fallow deer are typically diurnal, but there is evidence to suggest that fallow deer attempt to

avoid predation by adjusting their temporal activity patterns (Caravaggi et al, 2018; Ciuti et al, 2008; Rossa et al, 2021). Fallow deer populations are present in most European countries, though their distribution is often spotty and centered around areas where the animals were originally introduced (Chapman & Chapman, 1980; Esattore et al, 2022). Their populations are expanding rapidly in Britain with the potential for a range increase from 25,000 km² to 60,000 km², possibly aided by increased habitat provided by broadleaf tree plantings (Croft et al, 2019; Ward, 2005). Fallow deer may be solitary or associate in groups as large as 160 individuals, with group size highest in winter and spring (Thirgood, 1996).

Wild boar are nocturnal/crepuscular with a preference for woodland habitat (Carrasco-Garcia et al 2016; Fernandez-Llario et al, 2004; Haaverstad et al, 2014; Merli & Meriggi, 2006; Ogurtsov et al, 2018; Plhal et al, 2014). Wild boar may adjust their temporal activity patterns to avoid predation, as they have been shown to become increasingly nocturnal under hunting pressure (Johann et al, 2020; Ohashi et al, 2013). Their groups vary in size and composition with the seasons, ranging from large family units in excess of ten individuals in the summer to solitary or small mixed groups in the winter (Fernandez-Llario et al, 1996; Maselli et al, 2014). Wild boar populations have been growing and expanding across Europe for several decades but are only present at a few locations in Britain such as Gloucestershire, Dorset, and Kent/Sussex (Dutton et al, 2015; Goulding et al, 2003; Massei et al, 2015; Pittiglio et al, 2018; Wilson, 2003).

Denning, Foraging, and Movement

A considerable amount of research has been done examining the behavior of both wildlife and cattle in pastures and near farm buildings as a means of assessing risk of *M. bovis* transmission (Payne et al, 2016, 2017; Smith et al, 2008; Varela-Castro et al, 2021). Since

much of the research into wildlife and *M. bovis* has focused on badgers (35% of all surveys), our understanding of how disease transmission occurs in a wildlife community is rather limited (Macdonald, 2004; Sidorovich et al, 2011). Denning, foraging, and movement are behaviors which represent the key daily activities of a wildlife community and pose different threats of disease transmission.

Dens and setts are enclosed, moist spaces which offer an ideal environment for the spread and survival of aerosolized bacilli (Sweeney et al, 2007). Since other species, such as red foxes, are known to den in badger setts, the sett itself may be a significant route by which infection is spread between wildlife species (Nowakowski et al, 2020; Sidorchuk et al, 2015). Foxes are typically infected orally, so it seems unlikely that badgers are the common source of their infection (Millan et al, 2008; Richomme et al, 2020). However, foxes may be a source of infection for badgers. Foxes and badgers have also been observed spending time together near setts, even when foxes were not denning at that site, and appeared to leave the sett together (Macdonald, 2004). It is unknown what purpose this behavior might serve, but it is possible that badgers and foxes associate with one another for foraging, similar to the American badger (*Taxidea taxus*) and coyote (*Canis latrans*) in North America (Minta et al, 1992; Thornton et al, 2018). Under certain conditions, badger populations will use latrines where they routinely urinate and defecate. Badger latrines may be a source of indirect infection since they are visited by multiple species, but latrine use may also limit the amount of infectious material accidentally ingested by animals when foraging (Smith et al, 2008; Varela-Castro et al, 2021).

Foraging presents an opportunity for direct and indirect disease transmission since animals may forage in the same habitat. Ingestion of infected materials or aerosolized bacilli while foraging is likely a major origin of infection for all species. Both badgers and foxes are generalist predators that eat what is locally abundant (Cresswell et al, 1988; Díaz-Ruiz et al, 2013). In Britain, rabbits are the most important prey of foxes and earthworms are the primary

prey of badgers (Baker & Harris, 2006; Hofer, 1986; Kruuk & Parish, 1981; Webbon et al, 2006). Earthworms occur in high densities and are easiest for badgers to hunt in the short grass typical of grazed pasture (Kruuk & Parish, 1981). Rabbits may be found in high densities in bracken and in woodland that borders open habitats (Hofer, 1986). Wild boar have a generalist diet that is primarily comprised of plant matter, with animal matter occupying a low percentage of overall diet but one which is frequently present (Ballari et al, 2014). Forest fruits such as acorns and beech nuts are the most important elements of the wild boar diet during autumn and winter (Herrero et al, 2005). Where agricultural crops are available, they are heavily utilized by wild boar, especially in summer and autumn as crops reach maturity (Ballari et al, 2014). Badgers, foxes, and wild boar are all known scavengers, which may be a route of infection (Herrero et al, 2005; Kruuk & Parish, 1981; Sidorovich et al, 2011; Webbon et al, 2006). Fallow deer rely on grazing for graminoids for the bulk of their diet, though they also supplement with woodland browsing (Chapman & Chapman, 1975; Obidziński et al, 2013). Fallow deer may ingest infected materials while grazing, but the pathogenesis of infection in deer suggests that aerosol is of greater concern than ingestion (Corner, 2006). Their infected carcasses may also be a source of infection for scavenger species. Supplementary feeding has been linked to the spread of bovine tuberculosis in other deer species and feed sites may act as hotspots of disease transmission where they occur (Miller et al, 2003). Pastures and meadows seem to be likely sites of interaction between fallow deer, badgers, and foxes, since they are important foraging habitats for all three species.

The patterns of animal movement across a landscape are likely to predict the patterns of disease transmission. The usual range of disease transmission may be estimated using the home range size of an infected population, while dispersal distance could predict the maximum distance at which transmission may be considered a threat. Foxes tend to have large dispersal distances (21 km), while badgers seldom move beyond their neighboring groups with a mean

dispersal distance of 530 meters (Macdonald et al, 2008; Walton et al, 2021). The ineffectiveness of culls to reduce local fox densities suggests that foxes can disperse quickly and in substantial numbers (Baker & Harris, 2006). Badgers may have more variable home ranges in lower density populations and in habitats which require more generalist foraging (Cresswell et al, 1988). Wild boar also have home ranges that may vary in size according to resource availability, with larger home ranges occurring at higher altitudes (Fattebert et al, 2017). Dispersal distances in wild boar are moderate, with males dispersing significantly further than females (16 km and 4.5 km, respectively; Truvé & Lemel, 2003). Fallow deer sexually segregate, the different sexes commonly occupying different areas across the seasons (Ciuti et al, 2004). Male fallow deer have larger home ranges than females (9.75 km² compared to 2.06 km²) and travel furthest in the autumn (Borkowski & Pudelko, 2007). Males tend to be displaced by females into lower quality habitats with more disturbance and higher predation risk, which might help explain why their home ranges are larger than females (Ciuti & Apollonio, 2008). The sexual segregation of fallow deer might result in asymmetric disease prevalence within a population, with male deer at greater risk of infection and transmission. Since male fallow deer have larger home ranges and occupy more disturbed habitats than the females, it also seems probable that they would be more likely to come in contact with diseased animals and would be more likely to spread disease further afield. However, the smaller home range of female fallow deer is likely to result in higher population densities, making them more vulnerable to infection.

Risk Assessment

Successful disease transmission requires shared space use with an infected animal and a behavior to facilitate the transfer of infectious materials. The risk of successful disease transmission rises with a higher degree of exposure to infectious materials, whether through an

increasing concentration of CFUs or through a greater temporal overlap with infectious materials. An evaluation of disease transmission risk should therefore consider the pathology of the disease and the ecology of the animals in question. I evaluate disease transmission risk by examining risky behaviors, spatiotemporal overlap between species of concern, individual disease pathology, and population-level disease pathology.

Eurasian badgers can persist for years with a latent infection of *M. bovis*, during which time they are likely to shed few CFUs (Corner et al, 2011). Furthermore, most infected badgers in a population will be in this latent stage of infection (Kelly et al, 2010; Murphy et al, 2010). Though disease prevalence is high among infected populations, the pathology of bovine tuberculosis in badgers may mean that their threat of transmission is lower than the disease prevalence may otherwise suggest. Individuals exhibiting a late stage of infection are likely to shed significant numbers of CFUs and are of primary concern for disease transmission (Corner, 2006; Gallagher & Clifton-Hadley, 2000). The high disease prevalence rates, large group sizes, and the maintenance of bovine tuberculosis in badger populations is problematic because infected populations may produce a steady, if numerically small, supply of infectious individuals. The small home ranges and short dispersal distances of badgers may mean the threat of transmission they pose is more local, especially in lowland habitats (Cresswell et al, 1988; Macdonald et al, 2008). If the threat of disease transmission from badgers is primarily local, then large-scale culling schemes could be abandoned in favor of more targeted efforts. Furthermore, the large degree of research and management effort related to badger disease ecology may be disproportionate to the threat they pose to wildlife and agricultural communities.

The pathology of the bovine tuberculosis in fallow deer coupled with their large body mass make it likely that they shed many CFUs (Aranaz et al, 2004; Johnson et al, 2008; Ward et al, 2009). Fallow deer had the highest mean disease prevalence of the species I examined, which, combined with large group sizes, may mean that a given population could contain many

individuals capable of disease transmission. The large home range sizes of fallow deer, particularly in males, threatens a larger area with the possibility of disease transmission (Borkowski & Pudelko, 2007). Fallow deer probably pose a significant threat of disease transmission to those animals with which they come into direct contact, but their diurnal activity patterns may limit the occurrence of direct interactions. However, it seems likely that infected deer carcasses act as hotspots of disease (Miller et al, 2003).

Despite a solitary nature and low population density, infected populations of foxes have high disease prevalence rates relative to some more gregarious species (Dorning & Harris, 2019; Parrott et al, 2012). Infected foxes often present with NVLs that are typical of a latent stage of disease but may shed bacilli through multiple routes without exhibiting macrolesions (Michelet et al, 2018). These pathological traits may make foxes a serious threat for shedding *M. bovis*, as they can shed bacilli over a long period of time and potentially over a broad area, though their small body mass may limit the number of CFUs they shed until they reach an advanced stage of disease. Given that foxes spatially and temporally overlap with multiple species, they have potential for disease transmission in a wildlife community, though this is limited by their low population densities.

Wild boar have high disease prevalence rates and often associate in large groups, meaning many individuals may be infected with *M. bovis* in a given population (Fernandez-Llario et al, 1996; Maselli et al, 2014). Capsulated lesions and a widespread immune response common in wild boar may reduce the number of bacilli they shed, but their large body mass and the frequency they present with gross lesions suggests that they could shed substantial numbers of CFUs if the lesions open or the immune system fails (Parra et al, 2006). Short dispersal distances probably indicate that the threat of disease transmission from wild boar is primarily local (Truvé & Lemel, 2003). Wild boar spatially and temporally overlap with multiple species, so they have the potential to directly and indirectly transmit disease. Shared carcasses

or other feed sites are prime candidates for disease transmission both to and from wild boar, given the common presence of lesions in their mandibular lymph nodes (García-Jiménez et al, 2013, 2015; Matos et al, 2016; Muñoz-Mendoza et al, 2013; Santos et al, 2009; Varela-Castro et al, 2021).

Conclusions

All the species I examined utilize both woodland and open grassland habitats for resting or foraging, making it likely that they occupy the same spaces in regions in which they co-occur (Cagnacci et al, 2004; Escos et al, 1992; Merli & Meriggi, 2006; Meia & Weber, 1993; Morecroft et al, 2001; Plhal et al, 2014; Virgos et al, 1999; Woodroffe et al, 2016). Badgers, foxes, and wild boar shared similar nocturnal/crepuscular activity patterns, which increases their chances of encountering one another (Ogurtsov et al, 2018; Rossa et al, 2021; Torretta et al, 2016). Fallow deer are typically diurnal but may adapt their activity patterns in response to predation or human disturbance (Caravaggi et al, 2018; Ciuti et al, 2008; Rossa et al, 2021). Given the spatial overlap of these species, indirect transmission of bovine tuberculosis between them is possible. Since badgers, foxes, and wild boar share similar habitats and activity patterns, direct transmission of disease is a possible source of *M. bovis* infection. The lack of shared activity patterns between fallow deer and the other species makes direct transmission between them less likely. However, the precise degree of temporal overlap between these species may be underestimated by comparing categorical estimates of activity. A more detailed comparison of temporal activity is needed to reveal the connectedness between these species.

Ingestion and inhalation seem to be the most common routes of infection, pointing to scavenging, infected food sources, and shared airspace as the prime culprits of transmission (Corner, 2006; Gallagher & Clifton-Hadley, 2000; García-Jiménez et al, 2013, 2015; Millan et al, 2008; Murphy et al, 2010; Parra et al, 2006; Richomme et al, 2020; Gavier-Widén et al, 2009).

Shared airspace is probably a more common route of transmission between members of the same species than between species that are wary of each other, since aerosols can only travel a few meters (Tang et al, 2006). The close associations of badgers and foxes may be the exception to this trend, especially when sharing a sett.

Food sources become contaminated through exposure to infectious materials. Although urine and feces have been suspected as agents of infection, it seems unlikely these materials would be routinely ingested during foraging. A study in cattle found that feces were avoided during grazing and it's possible that this behavior is also found in other species (Smith et al, 2008). It seems more likely that shared food sources become contaminated via saliva or aerosol, especially at farms or supplementary feed sites. Maize and hay have both been shown to support *M. bovis* for about a month in favorable conditions and supplementary feed sites have been connected to bovine tuberculosis transmission among deer in Michigan (Fine et al, 2011; Miller et al, 2003). Though infected feed sites may be a prime candidate for a primary infection, there is likely another mechanism that facilitates disease transmission between species in the wild. The carcasses of animals in advanced stages of disease may serve as hot spots of disease transmission. Carcasses of uninfected animals could also become contaminated through the saliva or aerosol of an infected scavenger. Many species in a wildlife community may engage in scavenging behavior and hundreds of individuals may visit a single carcass (Häkkinen Siviä, 2021). Deer carcasses have been specifically noted as a common target of scavenging by wild boar, badgers, and foxes (Herrero et al, 2005; Kruuk & Parish, 1981; Sidorovich et al, 2011; Webbon et al, 2006). Given that three of the species often implicated with *M. bovis* transmission are scavengers, the role of infected carcasses in disease transmission in the wild may be worth further examination.

Though the precise mechanics of disease transmission between wildlife species remains unclear, scavenging and infected food sources seem likely candidates. More research should

be conducted to quantify how these sites may drive the disease ecology of a wildlife community. Despite the limited data on *M. bovis* infections in the wildlife community at large, badgers, fallow deer, wild boar, and red foxes appear to be species of concern for the transmission of bovine tuberculosis between wildlife species. Significant behavioral overlap between these species may signify that a wildlife community is at high risk of *M. bovis* infection should it be introduced to the area. In chapter two, I examine the behavioral overlap between these species and assess the likelihood of interspecies disease transmission.

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Chapter Two:

Activity Overlap of Three Woodland Mammals and Implications for *Mycobacterium bovis* Transmission in North Wales

Introduction

Disease transmission between animals can occur through many routes, but all require that the uninfected animal must encounter infected materials (Chapter I). Infected materials may be transmitted through direct contact with a disease host, such as through saliva or shared airspace, or may be indirectly transmitted, such as through feces deposited in the environment by a diseased animal (Corner, 2006; Delahay et al, 2001). If contact between animals, whether direct or indirect, is necessary for disease transmission, then it follows that there must also be a degree of shared space use. Animals which live in separate habitats cannot transmit diseases between them without an intermediate host. Assuming there is no intermediate host, it can therefore be assumed that a greater degree of spatiotemporal overlap between animals corresponds to a greater risk of disease transmission. The degree to which their habitat use overlaps temporally determines whether their contact with one another is primarily indirect or if there is a possibility of direct contact.

Wildlife species have been identified as carriers of *Mycobacterium bovis* (*M. bovis*) in outbreaks of bovine tuberculosis across Europe (Delahay et al, 2007; Hardstaff et al, 2014). In Britain, breakouts of *M. bovis* are primarily concentrated in the southwest, where populations of Eurasian badgers have long been known as reservoirs of the disease (Clifton-Hadley et al, 1995; Hardie & Watson, 1992; O'Hare et al, 2021; Vázquez et al, 2021). In other parts of Europe, fallow deer, wild boar, and red foxes have been identified as important hosts of *M.*

bovis (Aranaz et al, 2004; García-Jiménez et al, 2013, 2015; Richomme et al, 2020). In chapter one, I outlined why these animals may be considered species of concern for the transmission of *M. bovis*. Furthermore, these are species which commonly occur together in wildlife communities. The degree to which these species overlap spatially and temporally may be indicative of the vulnerability of a wildlife community to an outbreak of bovine tuberculosis.

Britain and Ireland primarily focus wildlife *M. bovis* survey effort on badgers despite the presence of other wildlife hosts (Chapter I). North Wales is home to three of the four species of concern identified in the previous chapter but has been understudied due to historically low rates of bovine tuberculosis and badger populations that are less dense than those in southern England (Parrott et al, 2012; Schroeder et al, 2020). However, there has been a recent outbreak of bovine tuberculosis among cattle in the area, with a 21% increase in new herd incidence from the previous year (Animal & Plant Health Agency, 2022). Understanding the behavioral overlap between badgers, foxes, and fallow deer in this region may be integral to predicting how bovine tuberculosis might spread through the wildlife community.

The analysis of camera trap data has proven to be a very effective method for estimating temporal patterns of animal activity (Di Bitetti et al, 2022; Foster et al, 2013; Lewis et al, 2021; Linkie & Ridout, 2011; Ogurtsov et al, 2018; Rossa et al, 2021). Activity is a measure of an animal's temporal use which can be measured at both gross and fine scales, revealing trends from years down to an hour. Animal activity is not a behavior-specific metric and may include foraging, traveling, mating, or other behaviors. Animals may alter their activity patterns in response to multiple factors including resource availability, season, disturbance, competition and predation (Chapter I). Quantifying animal activity allows researchers to predict when an animal will use a space in which it is present. Overlapping activity patterns are indicative of concurrent use of a shared space. In this study, I sought to explore how the overlapping activity and space use patterns of three woodland mammals might inform our understanding of disease

transmission in wildlife communities. I used camera traps to observe the activity badgers, fallow deer, and foxes and estimated temporal activity patterns from these data. I then examined the activity overlap of badgers, fallow deer, and foxes to assess the degree of interaction between them and discover any temporal factors which may affect such an interaction. I also tested how the activity of foxes and badgers may change with a relative absence of fallow deer. I then discussed the implications of species overlap for *M. bovis* transmission and the vulnerability of a wildlife community to an outbreak of bovine tuberculosis.

Materials and Methods

The Elwy Valley, Denbighshire, Wales, in the United Kingdom, is an area that has been marked by millennia of human habitation (Fig. 1). It is part of the Celtic broadleaf forests ecoregion within a temperate broadleaf and mixed woodland biome (Olson et al, 2001). The landscape is a mosaic of woodlands, pastures, and villages, crossed by hedges and roads. The global human footprint index varies from 20-40% in the countryside and 40-80% around the villages (Williams et al, 2020).

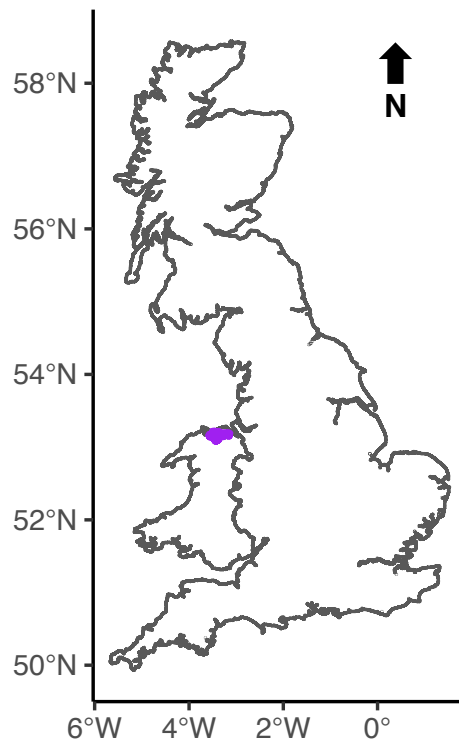


Figure 1. Map of Britain with the study area marked in purple.

Woodlands in the study area were divided into units based on aggregated woodland vectors from Ordnance Survey OpenMap (Ordnance Survey Ltd. 2017). A total of 40 units were chosen for sampling according to the proportion of total woodland area, with a minimum distance of 250m between sampling sites (Fig. 2). The realized distribution of sampled units was ultimately constrained by access restrictions. One camera trap was placed in each sampling unit and the cameras have collected year-round data through 2019 and 2020. Cameras were placed by Owain Barton with funding from and in conjunction with the Deer Initiative, a non-profit wildlife management organization. Once a month, the cameras were checked, images were downloaded, and the cameras were resupplied with batteries in the field.

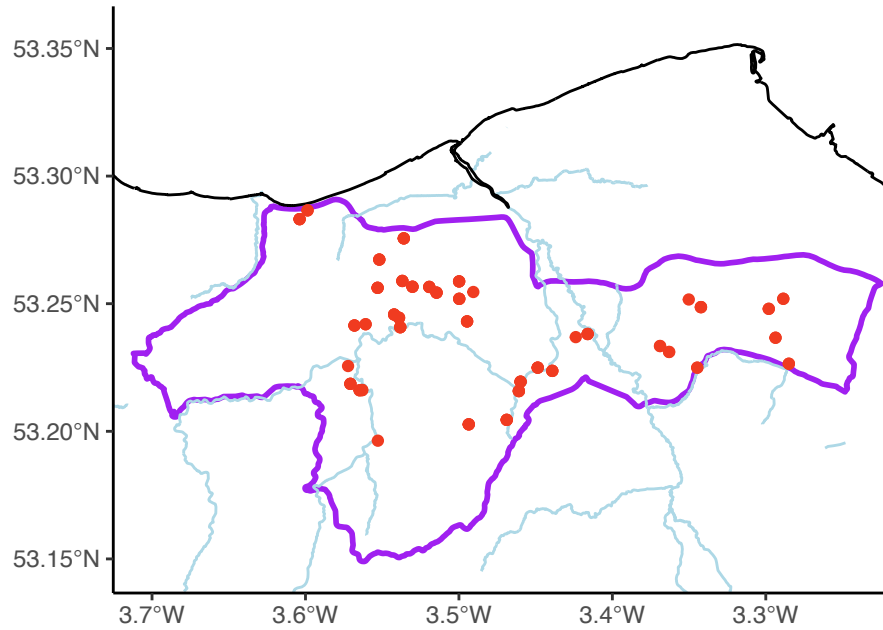


Figure 2. Map of unit placement within the study area. The study area is delineated in purple, the coastline in black, rivers in blue, and unit locations are marked in red.

I pooled the collected images before analysis and considered any variability in camera operation to be random. Initial processing was done with MegaDetector, which filtered out all images not containing wildlife. Image tagging was done with digiKam (7.6.0), and subsequent processing was conducted in R (R Core Development Team, 2022). Images more than 10 minutes apart were considered to be independent observations. I transformed image times using sunrise and sunset following the double-anchoring methods outlined by Vazquez et al. (2019) and implemented using the “activity” package in R (Rowcliffe, 2022). This transformation accounts for seasonal changes in daylight hours, allowing the activity estimates to be more representative of reality in the field. I used the “overlap” package to estimate activity overlap using kernel density estimation in a method developed by Ridout and Linkie (2009) (Meredith & Ridout; 2021). These methods have been used in similar studies (Di Bitetti et al, 2022; Foster et al, 2013; Lewis et al, 2021; Linkie & Ridout, 2011; Ogurtsov et al, 2018; Rossa et al, 2021).

I measured overlap between two distributions using an overlap coefficient which ranges from 0 (no overlap) to 1 (complete overlap), using the Dhat4 estimate (D_4) since my sample size was greater than 50 (Meredith & Ridout, 2021; Ridout & Linkie, 2009). The coefficient of overlap is defined as the minimum area under the curve of two overlapping probability density functions (Ridout & Linkie, 2009). I generated 10,000 bootstrapped estimates of overlap and took a mean corrected for bootstrap bias, then created 99% confidence intervals (CI) from my bootstrapped overlap estimates using the “basic” bias-corrected CI values (Meredith & Ridout, 2021). If the upper limit of the CI was < 0.90 , then I considered the overlapping activity patterns to be significantly different (Lewis et al, 2021). I examined the effect of year on overlap using the same method. Since almost all fallow deer observations were on the west side of the Clwyd River (99.85%), I was also able to test if fallow deer presence influenced badger or fox activity.

To test the effect of season on activity overlap, I divided the calendar year into four seasons: spring, summer, autumn, and winter. Each season was comprised of two months, with one month in between seasons to allow for independence. Spring was therefore defined as March and April, summer was June and July, autumn was September and October, and winter was December and January. I examined the spatial overlap of species by using a binary metric of presence/absence in each unit and applying the results to a map of the study area. I then calculated naïve occupancy as the mean ratio of days each species was sighted to the number of days (occasions) sampled in each woodland unit. Naïve occupancy does not account for imperfect detection and should therefore be treated with caution when analyzing complex space-use patterns, but it is a sufficient metric to measure simple spatial overlap.

I examined the preference of species for a specific time period by comparing use of each time period relative to its availability, following the practice outlined in similar studies (Bu et al, 2016; Gerber et al, 2012; Ogurtsov et al, 2018). I first divided the 24-hour diel cycle into four activity periods: morning twilight, evening twilight, day, and night. I defined morning twilight as

the period one hour before sunrise to one hour after sunrise, evening twilight as the period one hour before sunset to one hour after sunset, day as the period from the end of morning twilight to the beginning of evening twilight, and night as the period from the end of evening twilight to the beginning of morning twilight. I then pooled the hours of morning and evening twilight into one twilight period. I considered nocturnal activity to occur at night, diurnal activity during the day, and crepuscular activity to occur during twilight. To determine if species preferred being active in a certain period, I calculated selection ratios following Manly et al. (2002) using the following formula:

$$w_i = o_i / \pi_i$$

where w_i is the selection ratio for the period, o_i is the proportion of observations in the period, π_i is the proportion of the lengths in period i to the lengths of all periods. A selection ratio > 1 indicates that the period is preferentially used more than it is available, while a selection ratio < 1 indicates that the period is avoided (Bu et al, 2016). I created selection ratios for all species overall, by season, and by year. I then calculated selection ratios for badgers and foxes by fallow deer presence. Finally, I conducted chi-square tests ($df = 2$, $\alpha = 0.05$) on the detections in each period to determine if the selection ratios were due to random chance or species preference (Bu et al, 2016).

Results

The camera traps captured 12,019 images of fallow deer, 6,413 images of badgers, and 2,103 images of foxes over the 731 days the cameras remained in the field. Total survey effort was 17,548 camera days with a detection rate of 1.17 detections per camera day (Table 1). Despite having higher survey effort (2933 relative to 2900 camera days), detection rate was lower in 2019 relative to 2020 (0.96 and 1.41 detections per camera day, respectfully, Table 1).

Survey effort (3,002 camera days) and detection rate (0.87 detections per camera day) were lowest in summer (Table 1). Winter had the greatest survey effort (9322 camera days) and detections (11674), while spring had the highest detection rate (1.31 detections per camera day, Table 1). There was random variation in camera operation between units, but since the images from all units were pooled before the analysis, I considered this variation to have a negligible effect.

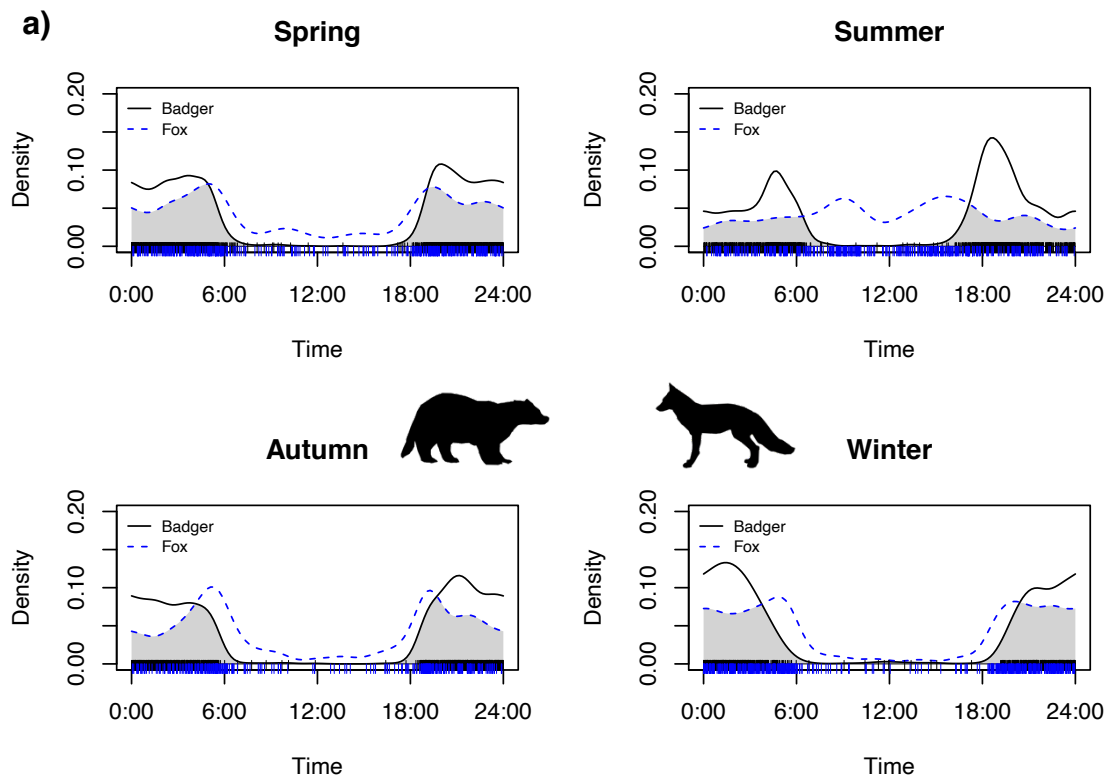
Table 1. Survey effort, detections, and detection rate for period in the study.

Data Subset	Survey Effort (Camera days)	Detections (n)	Detection Rate (Detections per camera day)
All	17548	20539	1.17
2019	2933	2817	0.96
2020	2900	4084	1.41
Spring	3165	4151	1.31
Summer	3002	2608	0.87
Autumn	8251	8865	1.07
Winter	9322	11674	1.25

All species demonstrated overlapping activity (Fig. 3). The degree of temporal overlap was highest between badgers and foxes (Δ_4 : 0.73) and lowest between badgers and fallow deer (Δ_4 : 0.42) (Table 2). The upper CI of all overlap coefficients was < 0.90 , so the activity patterns of the species may be considered substantially different (Table 2). Fallow deer and foxes maintained their temporal patterns across years, but there was a difference in badger activity between 2019 and 2020 (Appendix 1). There was also a significant difference between years for badger/deer overlap and badger/fox overlap. I found that the difference between badger activity in 2019 and 2020 was due to increasing crepuscular behavior among badgers in 2020 (Appendix 3).

Table 2. Overlap coefficients (Δ_4) between species with 95% confidence intervals in parentheses. An upper CI < 0.90 indicates a significant difference between overlapping activity patterns.

Badger		
0.42 (0.41-0.43)	Fallow Deer	
0.73 (0.71-0.75)	0.68 (0.66-0.70)	Fox



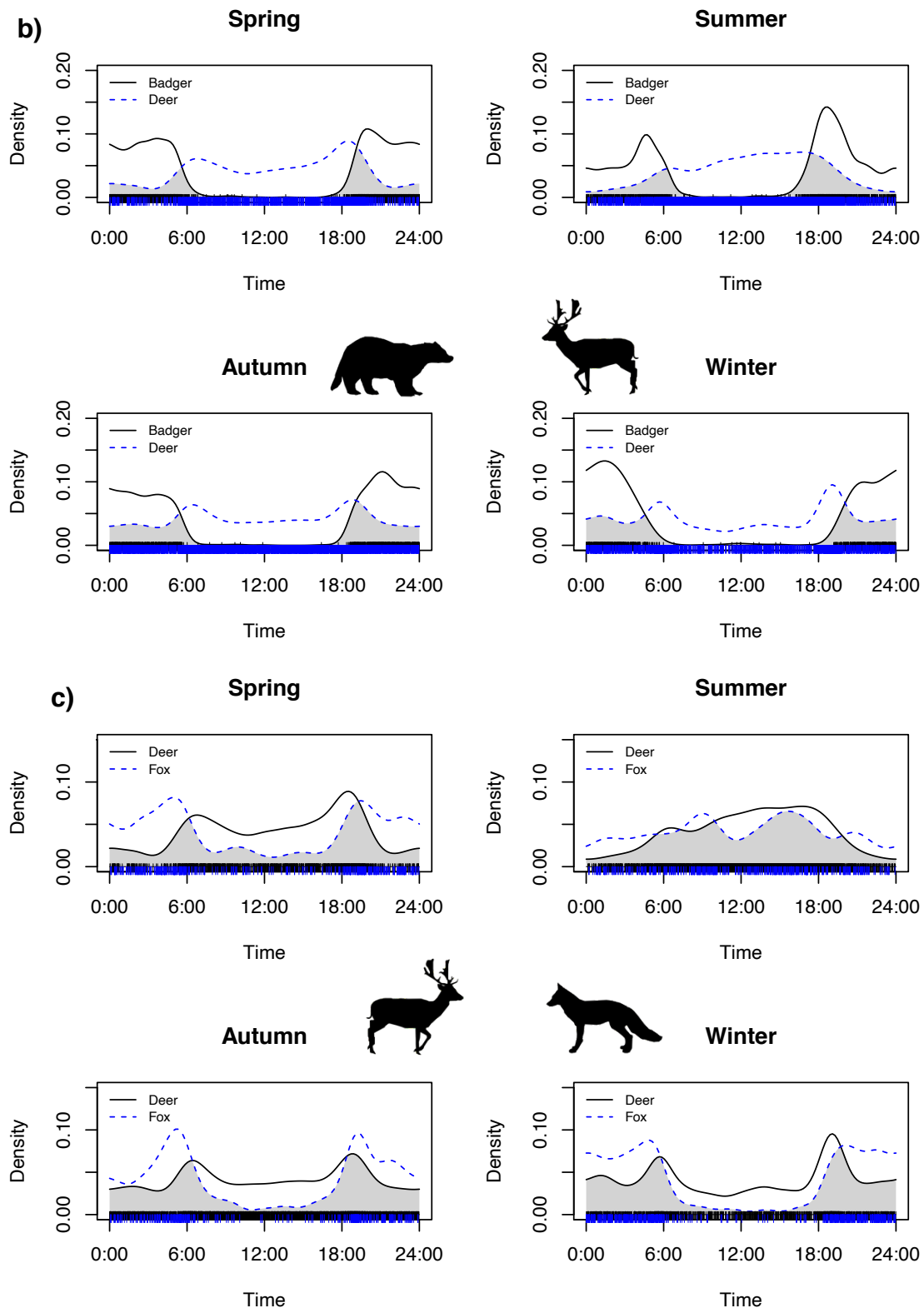


Figure 3. Overlap of the daily activity season for a) badgers and foxes b) badgers and fallow deer c) fallow deer and foxes. Sunrise and sunset are anchored to 6:00 and 18:00 respectively. Solid and dashed lines represent species kernel densities, while the shaded area represents overlap.

Overlap between deer and foxes was highest in summer (Δ_4 : 0.80) and lowest in spring (Δ_4 : 0.60) (Fig. 3, Appendix 1). Badger and deer overlapped the most in autumn (Δ_4 : 0.43) and the least in spring (0.32), while badgers and foxes overlapped the most in spring (Δ_4 : 0.73) and the least in summer (Δ_4 : 0.49) (Fig. 3, Appendix 1).

Table 3. Overlap coefficients (Δ_4) of species activity by season with 95% confidence intervals in parentheses. An upper CI < 0.90 indicates a significant difference between activity patterns.

Badger			
Spring			
0.69 (0.66-0.73)	Summer		
0.94 (0.91-0.97)	0.65 (0.62-0.69)	Autumn	
0.79 (0.75-0.82)	0.50 (0.50-0.53)	0.82 (0.78-0.86)	Winter
Fallow Deer			
Spring			
0.82 (0.79-0.85)	Summer		
0.88 (0.85-0.91)	0.75 (0.73-0.78)	Autumn	
0.76 (0.72-0.79)	0.62 (0.60-0.88)	0.86 (0.83-0.88)	Winter
Fox			
Spring			
0.60 (0.54-0.66)	Summer		
0.92 (0.87-0.97)	0.55 (0.49-0.61)	Autumn	
0.87 (0.82-0.97)	0.48 (0.42-0.53)	0.83 (0.78-0.89)	Winter

Badgers and deer both had activity patterns that varied seasonally save for between spring and autumn (Table 3). Foxes maintained similar activity patterns in autumn, winter, and spring, while their activity in summer varied from the other seasons (Table 3). Badgers were mostly nocturnal but became nocturnal/crepuscular in the summer (Table 4-5). Foxes were primarily nocturnal/crepuscular but became diurnal/crepuscular in the summer. Fallow deer were usually diurnal but became nocturnal/crepuscular in winter. The presence of fallow deer affected the overlap of foxes and badgers, but only affected the activity of foxes (Appendix 2). Foxes increased their nocturnal activity in the absence of fallow deer, with decreasing crepuscular and diurnal activity (Appendix 4).

Table 4. Manly selection ratios (w_i) of day periods for each species. Green values ($w_i > 1$) signify preference while orange values ($w_i < 1$) indicate avoidance.

	Nocturnal	Crepuscular	Diurnal	X ²	p-value
Badger	2.05	0.76	0.04	5483.82	p < 0.05
Deer	0.68	1.56	1.09	1175.87	p < 0.05
Fox	1.38	1.30	0.50	382.60	p < 0.05

Table 5. Manly selection ratios (w_i) of day periods for each species by season. Green values ($w_i > 1$) signify preference while orange values ($w_i < 1$) indicate avoidance.

Badger					
	Nocturnal	Crepuscular	Diurnal	χ^2	p-value
Spring	2.16	0.56	0.02	183.81	$p < 0.05$
Summer	1.51	1.99	0.09	679.42	$p < 0.05$
Autumn	2.17	0.54	0.02	201.47	$p < 0.05$
Winter	2.33	0.10	0.03	148.80	$p < 0.05$
Fallow Deer					
	Nocturnal	Crepuscular	Diurnal	χ^2	p-value
Spring	0.57	1.78	1.12	183.81	$p < 0.05$
Summer	0.40	1.43	1.42	679.42	$p < 0.05$
Autumn	0.84	1.58	0.93	201.47	$p < 0.05$
Winter	1.09	1.60	0.67	148.80	$p < 0.05$
Fox					
	Nocturnal	Crepuscular	Diurnal	χ^2	p-value
Spring	1.46	1.37	0.39	97.10	$p < 0.05$
Summer	0.78	1.03	1.21	14.90	$p < 0.05$
Autumn	1.46	1.60	0.30	104.88	$p < 0.05$
Winter	1.78	1.11	0.17	246.40	$p < 0.05$

All three species were recorded in 63% of units, with 88% of units recording two species or more (Fig. 4). One unit did not record any observations fallow deer, badgers, or foxes. Badgers and foxes had a 98% overlap in unit presence. The mean naïve occupancy of foxes was low (0.07) compared to fallow deer (0.20) and badgers (0.15). Naïve occupancy at each unit varied from 0.00-0.20 for foxes, 0.00-0.85 for fallow deer, and 0.00-0.59 for badgers.

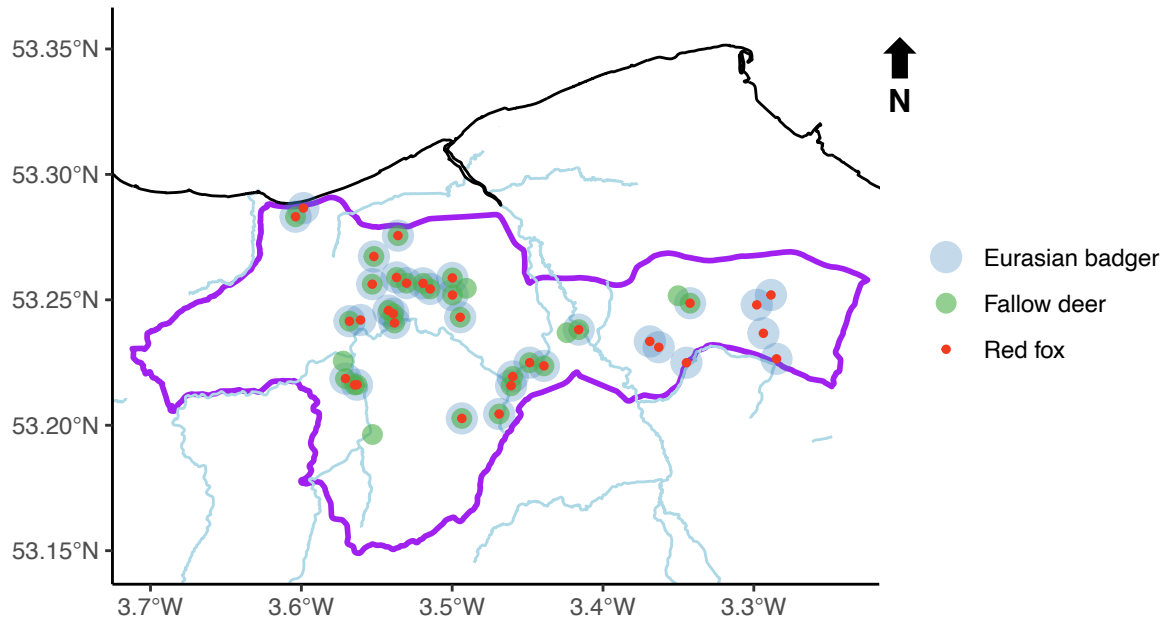


Figure 4. Map of species presence in woodland units with the study area outlined in purple. Rivers are shown in light blue and the coastline is shown in black.

Discussion

Transmission of *M. bovis* requires some degree of behavioral overlap between species. My study revealed that badgers, fallow deer, and foxes commonly co-occurred in woodlands and overlapped temporally. Direct transmission between these species is therefore a strong possibility. Since at least one of these species is active throughout the diel cycle, an encounter between them and other wildlife or domestic species seems likely. Furthermore, there is no time period which may be considered “safe” from disease transmission if all three of these species are infected with bovine tuberculosis.

All species had seasonal shifts in activity, generally using more daylight hours in summer and more nighttime hours in winter. One might initially assume this trend was caused by relative availability of darkness versus daylight, but the analysis accounts for changes in availability and thus demonstrates true preference. The seasonal changes in preference may be in response to

seasonal changes in human disturbance and/or resource availability (Caravaggi et al, 2018; Lewis et al, 2021). It may be that humans are more active during daylight hours in winter and therefore push wildlife to use more nighttime hours. Fallow deer have been noted for utilizing meadow habitat more at night and in winter, which may be an attempt to avoid contact with livestock (Borkowski & Pudelko, 2007). Foxes and badgers may also seasonally shift their use of meadows to avoid livestock (Chapter I). Many of the woodland units were adjacent to livestock pasture, so it's possible that the camera traps were able to detect movement between woodland and meadow habitats. There were also seasonal changes in overlap, though species had a high degree of overlap regardless of season. These results suggest that these species experience one another as a frequent presence in shared woodland spaces.

The results of the spatial analysis suggest a high degree of spatial overlap between all species. Badgers and foxes had a 98% overlap in their unit presence. This high degree of spatial overlap supports a close association of badgers and foxes (Chapter I). The naïve occupancy results supported known trends in population density for these species but must be viewed with caution since the occupancy analysis did not account for imperfect detection (Chapter I). Both foxes and badgers have been noted for having larger home ranges in upland habitat relative to lowland habitats, which may explain the low occupancy in the study area (Parrott et al, 2012). Fallow deer were the only animal present at five sites, a result which may be due to their larger range size relative to badgers and the removal of foxes through hunting (Chapter I).

Badgers were the only species that varied their activity from 2019 to 2020, demonstrating increased crepuscular activity. This increase in crepuscular activity caused an increase in overlap with both deer and badgers relative to 2019. It's unknown whether this shift in badger activity was caused by an external stimulus and further research is needed to observe how badger activity may change in subsequent years. The COVID-19 pandemic lowered human

disturbance on wildlife and caused a shift in the activity of some species, a trend which may also explain the variation in badger activity (Manenti et al, 2020). If badgers were more crepuscular when human disturbance was lowered, then the strong preference of badgers for nocturnal hours may be an attempt to avoid humans. This result suggests that animal activity patterns that appear typical may be a behavioral response to the constant threat of human disturbance. The effects of human disturbance on wildlife activity are well documented and should contribute to our understanding of how diseases might spread through a wildlife community (Caravaggi et al, 2018; Ohashi et al, 2013). Combined effects of human disturbance, such as habitat loss and increased nocturnality, could drive animals to increasing levels of spatiotemporal overlap and seriously contribute to the spread of disease. Stress related to human disturbance can also cause weakened immune function in animals, possibly increasing their vulnerability to infection and worsening the severity of the disease (Brearley et al, 2012).

Both nocturnal and nocturnal/crepuscular activity has been reported in badgers (Caravaggi et al, 2018; Ogurtsov et al, 2018). My findings that foxes are nocturnal/crepuscular and fallow deer are diurnal/crepuscular also reflect what was found in similar studies (Caravaggi et al, 2018; Kammerle et al, 2020). Though general activity patterns are useful shorthand for animal behavior, the overlap results demonstrate that species with disparate activity patterns may still have substantial temporal overlap. Even species which are generally active at different hours are still likely to come into direct contact. It should also be remembered that animal activity is plastic and could change in response to variation in predation or disturbance (Chapter I). Future research is needed to examine how disease and disease management efforts (such as culling) may affect animal activity patterns.

I found that fox activity varied between the east and west sides of the river, possibly as a result of the relative absence of fallow deer on the east bank (Appendix 2). Foxes had increased nocturnal activity while decreasing their diurnal and crepuscular activity when fallow deer were

absent, which also caused an increase in overlap with badgers. It's possible that the difference in fox behavior was caused by a factor other than fallow deer presence, but the possibility of an interaction between foxes and fallow deer should not be discounted. Foxes have been known to predate the fawns of roe deer and there is some evidence that they may also predate fallow deer fawns (Kjellander et al, 2012; Panzacchi et al, 2009, 2010). Foxes in the presence of fallow deer may increase their diurnal activity in the summer to predate on deer fawns while the mothers are off foraging.

Badgers consistently had a high degree of overlap with foxes, as foxes did with fallow deer, but the degree of overlap between badgers and fallow deer was relatively low. Badgers and foxes are known to associate closely and share similar behavioral patterns, but a connection with fallow deer is less clear (Chapter I). Foxes may serve as an intermediary between badgers and fallow deer that could potentially increase disease transmission between all three species. Foxes are usually considered to be spillover hosts for bovine tuberculosis, so their role in disease transmission is probably confined to active outbreaks rather than serving as a reservoir that could spark new outbreaks of disease (Delahay et al, 2007; Richomme et al, 2020). I outlined in Chapter I the efficacy of foxes as hosts for *M. bovis*, but further research should be done to examine what role they may play in outbreaks of bovine tuberculosis. Foxes and certain other wildlife species have sometimes been discounted in discussions of bovine tuberculosis disease ecology because of their relatively limited interactions with cattle (Delahay et al, 2007; Payne et al 2017). However, my findings suggest that multiple species may participate in the spread of a disease through a wildlife community and species such as the red fox may have a strong indirect effect on disease prevalence in cattle, despite a relatively low incidence of direct contact.

Research into bovine tuberculosis in wildlife has largely been focused on one or two species despite the evidence for community-wide disease transmission (Chapter I). The large

degree of overlap between badgers, foxes, and fallow deer supports the likelihood of transmission between wildlife species. Bovine tuberculosis research and management efforts should endeavor to consider disease transmission at the community level and to broaden animal sampling to include multiple species. Future research should examine the space-use of these species on a fine scale so that we can better quantify the degree of their interaction. It would be particularly interesting to use GPS-collars to examine individual movements and proximity to other animals. It would also be worthwhile to test how differing habitats, resource availability, and level of disturbance or predation may affect their activity patterns.

Understanding how other species, such as wild boar, fit into this activity framework may also be helpful for understanding how disease may spread through a wildlife community. Collecting detailed spatiotemporal information alongside a systematic *M. bovis* testing campaign with spoligotyping of *M. bovis* strains would be a particularly useful study in elucidating the disease ecology of bovine tuberculosis in a wildlife community.

Wildlife species exist as part of a community of species with which they share time, space, and disease. The unexpected connection between foxes and fallow deer serves as a cautionary tale about selection bias; if we only search for relationships that we expect, then we will miss unexpected connections between species that we thought disparate. It seems likely that most medium and large wildlife species at least experience each other as a common presence in shared spaces, if not as a more regular and entangled part of their lives. This theory is borne out by studies with a broader scope in recorded species, which found similarly high degrees of temporal overlap between them (Foster et al, 2013; Ogurtsov et al, 2018). A high degree of connectedness between species has serious ramifications for wildlife diseases which may infect multiple host species, such as bovine tuberculosis, chronic wasting disease, or rabies. Efforts to manage and prevent these diseases should seriously consider broad sampling campaigns over species-specific efforts which may underreport the true scope of an outbreak.

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Chapter I Appendices

Search on Web of Science, JSTOR, CAB, and Science Direct, 16 November, 2021:

(TI=("Mycobacterium bovis" OR "bovine tuberculosis")) AND ALL=(wildlife AND prevalence)

ProQuest Search, 16 November, 2021: ti("Mycobacterium bovis" OR "bovine tuberculosis")

AND (wildlife AND prevalence)

ProQuest Search, 20 January, 2022: (AB(("Mycobacterium bovis" OR "M. bovis" OR "bovine tuberculosis") AND (wildlife OR ungulate OR carnivore OR predator OR mesocarnivore) AND (prevalence OR infection))) OR (TI(("Mycobacterium bovis" OR "M. bovis" OR "bovine tuberculosis") AND (wildlife OR ungulate OR carnivore OR predator OR mesocarnivore) AND (prevalence OR infection))) OR (IF(("Mycobacterium bovis" OR "M. bovis" OR "bovine tuberculosis") AND (wildlife OR ungulate OR carnivore OR predator OR mesocarnivore) AND (prevalence OR infection)))

Web of Science Search, 22 January, 2022: ((TI(("Mycobacterium bovis" OR "M. bovis" OR "bovine tuberculosis") AND (wildlife OR ungulate OR carnivore OR predator OR mesocarnivore) AND (prevalence OR infection))) OR AB(("Mycobacterium bovis" OR "M. bovis" OR "bovine tuberculosis") AND (wildlife OR ungulate OR carnivore OR predator OR mesocarnivore) AND (prevalence OR infection))) OR KP(("Mycobacterium bovis" OR "M. bovis" OR "bovine tuberculosis") AND (wildlife OR ungulate OR carnivore OR predator OR mesocarnivore) AND (prevalence OR infection)))

JSTOR Search, 24 January, 2022: (((("Mycobacterium bovis" OR "M. bovis" OR "bovine tuberculosis") AND (wildlife OR ungulate OR carnivore OR predator OR mesocarnivore)) AND (prevalence OR infection))

CAB Search, 24 January, 2022: title:("Mycobacterium bovis" OR "M. bovis" OR "bovine tuberculosis") AND (wildlife OR ungulate OR predator OR carnivore OR mesocarnivore) AND (prevalence OR infection)) OR ab:("Mycobacterium bovis" OR "M. bovis" OR "bovine tuberculosis") AND (wildlife OR ungulate OR predator OR carnivore OR mesocarnivore) AND (prevalence OR infection))) OR subject:("Mycobacterium bovis" OR "M. bovis" OR "bovine tuberculosis") AND (wildlife OR ungulate OR predator OR carnivore OR mesocarnivore) AND (prevalence OR infection)))

Science Direct Search, 24 January, 2022: title, abstract, or keyword: ("Mycobacterium bovis" OR "M. bovis" OR "bovine tuberculosis") AND (wildlife OR ungulate OR predator OR carnivore) AND (prevalence OR infection)

Chapter II Appendices

Appendix 1. Overlap coefficients (Δ_4) by season and year with 95% confidence intervals. An upper CI < 0.90 indicates a significant difference between overlapping activity patterns.

Year Overlap (2019/2020)			
	Overlap (Δ_4)	Lower CI	Upper CI
Fallow Deer	0.99	0.98	1.00
Fox	0.92	0.88	0.95
Badger	0.84	0.82	0.86
Deer/Fox Overlap			
	Overlap (Δ_4)	Lower CI	Upper CI
2019	0.65	0.62	0.68
2020	0.71	0.68	0.74
Spring	0.60	0.55	0.65
Summer	0.80	0.75	0.84
Autumn	0.68	0.64	0.73
Winter	0.68	0.64	0.72
Badger/Deer Overlap			
	Overlap (Δ_4)	Lower CI	Upper CI
2019	0.36	0.35	0.37
2020	0.48	0.47	0.50
Spring	0.32	0.29	0.35
Summer	0.41	0.39	0.43
Autumn	0.43	0.41	0.46
Winter	0.41	0.38	0.44
Badger/Fox Overlap			
	Overlap (Δ_4)	Lower CI	Upper CI
2019	0.68	0.65	0.71
2020	0.76	0.73	0.79
Spring	0.73	0.69	0.77
Summer	0.49	0.45	0.54
Autumn	0.68	0.62	0.73
Winter	0.70	0.66	0.75

Appendix 2. Overlap coefficients (Δ_4) between and within species by fallow deer presence with 95% confidence intervals. An upper CI < 0.90 indicates a significant difference between overlapping activity patterns.

Badger/Fox Overlap			
	Overlap (Δ_4)	Lower CI	Upper CI
Deer Present	0.71	0.69	0.74
Deer Absent	0.82	0.78	0.86
Fallow Deer Present/Absent Overlap			
	Overlap (Δ_4)	Lower CI	Upper CI
Badger	0.95	0.93	0.97
Fox	0.83	0.80	0.87

Appendix 3. Manly selection ratios (w_i) of day periods for each species by year. Green values ($w_i > 1$) signify preference while orange values ($w_i < 1$) indicate avoidance.

Badger					
	Nocturnal	Crepuscular	Diurnal	X ²	p-value
2019	2.23	0.41	0.01	3617.38	p < 0.05
2020	1.87	1.12	0.08	2089.04	p < 0.05
Deer					
	Nocturnal	Crepuscular	Diurnal	X ²	p-value
2019	0.70	1.55	1.08	410.72	p < 0.05
2020	0.68	1.57	1.09	765.87	p < 0.05
Fox					
	Nocturnal	Crepuscular	Diurnal	X ²	p-value
2019	1.44	1.36	0.41	271.20	p < 0.05
2020	1.32	1.24	0.59	122.47	p < 0.05

Appendix 4. Manly selection ratios (w_i) for foxes and badgers by fallow deer presence. Green values ($w_i > 1$) signify preference while orange values ($w_i < 1$) indicate avoidance.

Fox					
	Nocturnal	Crepuscular	Diurnal	X ²	p-value
Deer Present	1.31	1.34	0.56	184.15	p < 0.05
Deer Absent	1.71	1.15	0.23	237.23	p < 0.05
Badger					
	Nocturnal	Crepuscular	Diurnal	X ²	p-value
Deer Present	2.04	0.77	0.05	1614.30	p < 0.05
Deer Absent	2.09	0.74	0.02	3871.53	p < 0.05