

TECHNICAL REPORT submitted to EFSA

Scientific review on Tuberculosis in wildlife in the EU¹

Prepared by Wilson Gavin^a, Broughan Jennifer^b, Chambers Mark^b, Clifton-Hadley Richard^b, Crawshaw Tim^b, de la Fuente José^c, Delahay Richard^a, Gavier-Widen Dolores^d, Gortazar Christian^c, Hewinson Glyn^b, Jackson Vicky^a, Martín-Hernando^c, Maria Paz^c, Neimanis Aleksija^d, Salguero Francisco Javier^b, Vicente Joaquin^c, Ward Alastair^a, McDonald Robbie^a

^aThe Food and Environment Research Agency, United Kingdom

^bVeterinary Laboratories Agency, United Kingdom

^cConsejo Superior Investigaciones Cientificas (Instituto de Investigación en Recursos Cinegéticos), Spain

^dStatens Veterinärmedicinska Anstalt, Sweden

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Executive Summary

1. Bovine TB (bTB) in livestock has been controlled or eradicated across most of Europe with the application of strict testing and controls of disease in cattle. However, in some areas bTB has proven difficult to eradicate, at least in part, because of the persistence of wildlife reservoirs of infection. We have undertaken a general review of the current state of knowledge of bTB in wildlife and the implications of disease, principally for livestock, but also for conservation and public health. We have sought to provide an accessible account that will help formulate directions for research and management of the disease.
2. Badgers are the best-understood wildlife reservoir for bTB in Europe. bTB is a chronic infection in badgers, with a relatively minor impact on survival and fertility. In Britain and Ireland, badgers live at relatively high density and often make contact with livestock at pasture and in farm buildings. Although their role in disease dynamics is relatively well understood, management remains challenging, because of the risks of disrupting social stability and increasing disease transmission. Outside of Britain and Ireland, knowledge of badger populations and of their role in disease is relatively scant.
3. Wild boar are highly susceptible to infection and bTB is widespread in Europe and can reach high prevalence, particularly in parts of the Iberian peninsula, where boar are maintenance hosts. Spatial aggregation and between-group contact, and hence disease transmission risks, are exacerbated where supplementary feeding (e.g. for hunting) takes place. Boar also appear to become infected by scavenging infected carcasses.
4. In most cases, deer are thought to be spill-over, end hosts. Localised exceptions occur in SW Britain, where fallow deer live at high density and commonly interact with cattle, and in parts of Spain and France where management practices and high population density mean that red deer are probably maintenance hosts.
5. Few other species are significant bTB hosts in terms of the risks they present to livestock. Semi-domesticated cats may present a potential zoonotic risk. The conservation status of critically endangered Iberian lynx is further threatened by the disease.
6. While culling can be effective in tractable populations, it is generally problematic for extensive control of disease in wildlife. The ecology of wild animal populations means that culling can be ineffective and in some circumstances may exacerbate disease. In particular, culling badgers has been shown experimentally to reduce bTB incidence in cattle in culling areas, but to temporarily increase incidence in neighbouring areas.
7. Improving biosecurity by reducing wildlife activity around farm buildings, limiting practices such as feeding and watering wild animals in proximity to livestock, and safely disposing of animal waste, represent good approaches to husbandry, but the benefits in terms of reducing disease incidence in livestock have not been evaluated.
8. Vaccination is a promising avenue for bTB control in complex wildlife reservoirs. The use of BCG has been evaluated in several wildlife species. A large-scale field safety trial of BCG vaccination of badgers is underway in the UK, with a view to large-scale deployment in 2010. The development of oral formulations for a BCG vaccine for wildlife faces major challenges, and a 5-year programme of work is underway in Britain and Ireland. Similar work is well advanced in boar and may also be appropriate for deer.

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9. Co-ordinated surveillance of bTB in wildlife and of host populations across the EU, using similar methodology and reporting systems would be valuable for sharing knowledge and research efforts across countries with similar and re-emerging bTB problems.
10. Specific research requirements for better understanding and management of bTB in wildlife include: improved trap-side diagnostics, the existence/role of superspreaders, mechanisms of excretion, means of bTB transmission between wildlife and livestock, risk management in husbandry, and the responses of host populations to management, including culling and vaccination.

Contents

Executive summary	2
Preface	5
10 Key questions	7
1. What problems are caused by bTB in wildlife?	7
2. What is the prevalence of bTB in wildlife?	8
3. What methods allow us to detect bTB in wild animals?	9
4. How do we monitor bTB in wildlife?	10
5. What is the evidence of transmission of bTB from wildlife to livestock?	11
6. Which wildlife hosts are important and what do we know about their populations?	12
7. How can culling wildlife contribute to bTB control?	13
8. What are the prospects for vaccinating wildlife?	14
9. What other options are there for bTB control in wildlife?	15
10. What are the important unknowns?	16
Technical reviews	
1. Badgers	17
2. Wild boar	29
3. Deer	43
4. Other species	63
Glossary of technical terms	82
Questionnaire respondents	84
References	87

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Preface

Bovine tuberculosis (bTB) is caused by *Mycobacterium bovis*, a member of the *M. tuberculosis*-complex (MTBC). This pathogen has an extensive host range including bovines, other livestock including small ruminants such as goats and sheep, and a wide range of wildlife species and humans.

Bovine tuberculosis is enzootic in cattle in some European countries, with herd prevalence that ranges from 1.1 to 12.1%, while in others sporadic outbreaks are detected. Eradication programmes based on the test-and-slaughter policy in the EU have proved successful in some countries but have failed to eradicate disease in other member states due, at least in part, to the presence of reservoirs of bTB in wildlife. The best documented of these in the EU are the Eurasian badger (*Meles meles*) in the UK and the Republic of Ireland (RoI), and the wild boar (*Sus scrofa*) in the Iberian Peninsula. As the results of more wildlife surveys become available, it is clear that several deer species may also be hosts of *M. bovis* infection although their role as wildlife reservoirs for bTB in livestock is less clear. Infected wildlife is a threat to the progress of bTB eradication campaigns and may potentially have additional impacts on wild species of conservation value and on human public health.

Over recent years several detailed wildlife studies have been conducted in those EU member states that have been unable to control bTB using current cattle testing and control policies. In addition, badger culling trials, using different experimental approaches, have been conducted in the UK and the RoI. These studies have given rise to unprecedented insights into the biology of bTB infection in wildlife and how this influences bTB incidence in livestock.

Our aspiration was that a broad ranging review of the main wildlife hosts would assist in clarifying those factors that may contribute to the role of wildlife in perpetuating bTB in livestock. Knowledge of these factors and their impact will help in the design of large-scale strategic approaches and implementation of targeted control to reduce infection transmission and contribute to improvements in animal health and welfare. To our knowledge these data have neither been captured, nor synthesized in one review to give a general description of host ecology and pathology in those wildlife species that could be important in the epidemiology of tuberculosis in livestock in EU member states. Nor has there been an attempt to describe the distribution and frequency of bTB in wildlife species across the EU. Since the identification of wildlife reservoir hosts is crucial for the implementation of effective control measures, our review will underpin the development of such control measures by identifying the potential risks of transmission of tuberculosis from wildlife to livestock in the EU and review control measures that may be available to prevent such spread.

We have tackled this broad topic in two ways. To provide a general and accessible synthesis of the state of knowledge, we have posed and answered 10 key questions. This has been done in “informed-layman” terms and covers the role of wildlife in the widest sense. Second, we have provided a more technical and referenced collation of knowledge for four groups of wildlife. There is a reasonably well-developed body of literature on bTB in badgers, boar and deer so we have compiled a section on each of these, and a further section on all other wild species for which the available knowledge is relatively limited and localized.

We have adopted a flexible approach to defining the scope of the review. We have considered all animals that are free-living in Europe as within scope, including native and naturalized species, but have drawn on limited literature from captive and domesticated animals where this is helpful. We have mostly used information from Europe, but have included some international research, particularly from the US and New Zealand, where it is helpful. Questionnaires were sent to the CVOs of EU member states and TB and wildlife

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researchers throughout Europe, asking them to describe the degree and characteristics of bTB infection in wildlife in their countries. The results of this were used to augment the published literature. The list of respondents is given in Appendix 1.

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10 Key questions

1. What problems are caused by bTB in wildlife?

Tuberculosis is a chronic granulomatous infection caused by bacteria of the *Mycobacterium tuberculosis* complex. *Mycobacterium bovis*, the aetiological agent of bovine tuberculosis, and its close relative *M. caprae*, can infect a wide range of domestic and wild animals. The infection of domestic animals presents important economic, environmental and health risks.

The risks to humans and other animals posed by reservoirs of infection in wildlife vary widely, depending on the specific epidemiological situation of the wild host and the local environment. The consequences of infection in wild animals fall into three areas: reservoir of infection for livestock, morbidity and mortality in wildlife hosts (particularly in protected and endangered species) and the impact on public health.

The role of wild animals in the maintenance and spread of *M. bovis* infection in livestock represents the greatest economic impact of the disease in wildlife in Europe. The disease is of particular importance in countries where eradication programmes have substantially reduced the incidence of bovine tuberculosis but where disease persists and new outbreaks occur. The best-known European examples of wildlife reservoirs of bTB are the Eurasian badger (*Meles meles*) in the UK and RoI, and the wild boar (*Sus scrofa*) in Spain. Other examples are the brush-tailed possum (*Trichosurus vulpecula*) in New Zealand, white-tailed deer (*Odocoileus virginianus*) in the USA and Cape buffalo (*Syncerus caffer*) in Africa.

In certain cases bTB has an impact on biodiversity conservation by affecting the survival of endangered species. In Europe, small populations of the Critically Endangered Iberian lynx (*Lynx pardina*) may be at particular risk because of the population's vulnerability to additional sources of mortality. These carnivores may become infected through consumption of tuberculous carcasses.

Tuberculosis is a zoonosis, hence wild animals may act as a source of infection for human beings. There is a danger of transmission of infection by direct contact between infected animals and handlers as well as indirect contact, potentially from infected food. Regarding direct contact, people most at risk are handlers of sick animals or infected carcasses through aerosol contamination when the carcass is open and cut, or through entry of organisms via cuts in the skin or oral routes with poor hygiene. Furthermore, hunted wild animals can be used for human consumption. Post-mortem inspection to detect lesions, condemnation of the affected organs or whole carcasses and cooking markedly reduce the danger of infection. Infection of semi-domesticated cats and domesticated cats and dogs may present direct zoonotic potential.

2. What is the prevalence of bTB in wildlife?

Mycobacterium bovis infection has been detected in many wild and domestic animals, often in countries where bovine tuberculosis in cattle is widespread. However, wild species do not reach the status of maintenance host for *M. bovis* in all countries where cases have been recorded and few systematic surveys for bTB have been undertaken. Therefore, disease recording often relies on limited observations or passive surveillance and is subject to the inherent sources of bias associated with carcasses obtained from pest and game management, road kills and veterinary and wildlife hospitals. Notable exceptions to this include some estimates of prevalence in the better-known wildlife reservoirs, though all of these require consideration of variation in the sensitivity of different means of disease detection.

Badgers

Badgers are recognised as the principal wildlife reservoir in the UK and RoI, and prevalence estimates exist for these countries only. Infection has also been identified in badgers in Switzerland and Spain. Bovine tuberculosis in badgers has been recorded most often towards the south and west of the UK mainland. By contrast, in areas of the UK where the risk of cattle herd breakdown is low, there are very few data on bTB in badgers. The prevalence in badgers removed from ten bTB hotspot areas in south west England ranged from 2% to 37% and in the RoI, the prevalence of bTB in four large removal areas was 19.5%.

Wild boar

M. bovis infection in Eurasian wild boar is widespread in Europe, being reported in both officially TB-free and non-OTF countries. In the last ten years reports of confirmed infection based on more than 20 animals have originated from Croatia, France, Italy, Portugal, Slovakia and Spain. Prevalence figures range from 1 to 52%. Most reports came from Mediterranean countries and the highest prevalence was recorded in the southern part of the Iberian Peninsula.

Deer

M. bovis infection in wild deer is widespread in Europe, and has been reported in both officially TB-free and non-OTF countries. In the last ten years reports of confirmed infection based on more than 20 animals have originated from the United Kingdom, Spain and Ireland. From the limited number of reports it is clear that infection is highly clustered within certain localities. In red deer, prevalence estimates, again based on 20 or more animals, range from 1% to 27%; in roe deer, from 0% to 3%; in fallow deer, from 3% to 21%; in muntjac there was a single estimate of 5%; and in sika and sika crosses another single estimate of 4%.

Other species

In carnivores, such as the fox, domestic cat and Iberian lynx, prevalence estimates based on 20 or more animals range from 1% to 17%; similarly in mustelids (excluding badgers) from 1% to 4%; in rodents, from 1% to 3%; in insectivores (the mole), 1%; in herbivores (the chamois), less than 1%.

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3. What methods allow us to detect bTB in wildlife?

Accurate diagnosis of *Mycobacterium bovis* infection in wildlife is an important component of the development of strategies to control TB. Despite its limitations, the gold standard for the detection of *M. bovis* in wildlife remains the isolation and culture of the organism from infected tissues obtained *post mortem*.

Detection rates are highest where visible lesions (VL) are present but often *M. bovis* may be isolated from tissues with no visible lesions (NVL). Histopathology can help to improve detection rates by excluding tissue changes caused by other parasites but cannot differentiate between infections caused by *M. bovis* and infections caused by other mycobacteria. Isolation of mycobacteria from clinical samples taken from live animals (e.g. urine, faeces, tracheal aspirates) is particularly insensitive, in part because of the intermittent nature of bacterial excretion amongst some infected animals.

Bacterial culture is an expensive and lengthy process and can take up to 12 weeks to ensure a sample is positive. The use of genetic probes can be used to reduce this time considerably but most are only *M. tuberculosis*-complex group specific. PCR offers the promise of faster and more specific detection of *M. bovis* from tissue, live animals and the environment. However, despite widespread use, a standardized, validated procedure for PCR detection of *M. bovis* does not yet exist and culture has proved more sensitive than PCR for the detection of *M. bovis* from *post-mortem* samples. *M. bovis* isolates obtained by culture are amenable to molecular typing by spoligotyping and Mycobacterial Interspersed Repetitive Units - Variable Number Tandem Repeats (MIRU-VNTR) typing which may allow greater understanding of the epidemiology of the infection. The ability to perform molecular typing on samples taken from live animals and the environment would represent a significant advance in understanding the epidemiology of bTB in wildlife.

Culture of *M. bovis* is a labour-intensive procedure and so diagnosis frequently relies on the detection of an immune response to *M. bovis* infection. The principal immunological response of the host to infection with *M. bovis* is the acquired cellular immune response, exemplified by the proliferation of lymphocytes and the production of cytokines such as gamma interferon (IFN γ). The mainstay of diagnosis of bTB in cattle, the tuberculin skin-test is a method of detecting the cellular response in *M. bovis* infected animals, but is impractical for free-ranging wild animals because of the need to examine animals for any cutaneous reaction 24-72 hours after the injection of tuberculin.

A variety of immunological tests are now available for the diagnosis of bTB in wildlife. For greatest sensitivity of detection, the IFN γ enzyme immunoassay (EIA) is the most appropriate test and is available for badgers and deer. In some situations it may not be feasible to operate the IFN γ EIA especially if low cost, simple, rapid tests are required or where blood samples have been stored or subject to delay in processing. In such cases, serological tests such as ELISA or a lateral-flow rapid test (e.g. STAT-PAK®) are available for badgers, deer and wild boar, although their relatively low sensitivity may be problematic. That said, the sensitivity of the STAT-PAK® appears to be higher for wild boar than for other wildlife species. Serological tests appear particularly suitable for detecting animals with advanced disease. Such animals have more extensive bTB pathology, and by inference are more likely to excrete *M. bovis* and have an increased propensity for onward transmission of infection.

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4. How do we monitor bTB in wildlife?

Monitoring is the systematic recording of epidemiological data, with no other specific purpose than detecting temporal trends. Ideally this should include or integrate with data on host abundance and distribution. Monitoring the prevalence of infection in juvenile (≤ 1 year old) hosts can be a proxy to incidence, since these individuals could only have become infected during the preceding year.

Unfortunately, there is a lack of long time series of data on bTB prevalence in wild host populations, other than badgers. Such information would be valuable aid to the development of policy on bTB control.

Ideally the monitoring of bTB prevalence in wildlife hosts in EU member states should be carried out using comparable methods for each species. Monitoring is of greatest value when based on active random sampling of wildlife, rather than on passive surveillance, though in countries where the expected prevalence is low it can be difficult to achieve meaningful results at reasonable cost. A sensitive and cost-effective approach is to combine cheaper methods used at large geographical scales, such as lesion recording and serology, with targeted application of more expensive tools such as culture and PCR-confirmation.

Although the presence of bTB-compatible lesions is not a perfect tool for estimating prevalence of disease, such information is considered to be valuable for exploring the magnitude and general distribution of infection in wildlife, provided a large enough sample size is obtained from an extensive area. Ideally, lesion identification should be carried out by trained staff in a systematic manner, and the presence of MTBC infection at the local level should later be confirmed by culture.

Alternatively, newly developed serological tools can be used to describe the trends and distribution of wildlife hosts in contact with MTBC. For instance, ELISA tests based on bPPD can easily be applied to wild boar sera collected for classical swine fever monitoring in the European Union.

MTBC infection is best confirmed by culture and molecular identification of the causative agent. Samples for culture should include a range of tissues in badgers; tonsils and mandibular lymph nodes in wild boar; and at least tonsils and medial retropharyngeal lymph nodes in deer. Ideally, deer samples should also include the left bronchial and mediastinal lymph nodes, and the mesenteric and ileocaecal lymph nodes. The culture of clinical samples (e.g. faeces, urine, sputum) is of limited sensitivity in live animals.

It is particularly important that survey methods and the reporting of results is standardised, and that methods employed for bTB monitoring are described in detail and that disease incidence and prevalence rates are considered in the light of the characteristics of the diagnostic methods used.

5. What is the evidence of transmission of bTB from wildlife to livestock?

Mycobacterium bovis has been detected in a wide range of wildlife species within the EU. Although presenting a theoretical risk to livestock, factors such as the nature of pathology, prevalence of infection and host ecology and behaviour require evaluation before any particular species can be considered to pose a significant risk to livestock. Currently, information linking wildlife to livestock as sources of infection is mainly associative and robust evidence of bTB transmission from wildlife to livestock is only available for a limited number of species.

Badgers

The evidence that badgers transmit bTB to cattle is compelling. Associative evidence includes descriptions of bTB in badger carcasses, isolation of the causative organism, surveys where the badger was the only or the principal infected species, road traffic accident (RTA) surveys and statutory badger removal operations. Laboratory transmission experiments have confirmed that badgers can infect cattle, and badgers are known to excrete *M. bovis* in faeces, sputum, urine and from open abscesses. Molecular typing results have demonstrated that badgers and cattle generally share the same spoligotypes in the same geographical locations.

Intervention studies have provided stronger evidence of the direction of transmission between the two species. Where badgers have been largely removed from areas of persistent cattle bTB infections, the cattle reactor rate has been markedly reduced for a sustained period subsequent to culling. In recent, scientifically controlled trials, cattle incidence declined in areas where badgers were removed relative to comparable uncultured areas.

Wild boar

Locally, high bTB prevalences have been reported in wild boar with evidence that is consistent with this species being a maintenance rather than spillover host for *M. bovis*, although this is yet to be confirmed. There is also associative evidence linking bTB in wild boar and livestock, particularly the spatial correlations between genotypes in wild boar, cattle, goats and deer.

Deer

TB has been recorded in various species of deer but there is little direct evidence from EU countries that they present a serious risk to domestic stock. However, in the USA, the case of white-tailed deer is more persuasive. Here, increases in deer numbers due to supplementary winter feeding and changes in feeding behaviour have provided greater opportunity for bTB to spread within the deer population. Evidence so far supports an indirect pathway through contaminated food to cattle.

Prevalence and ecology of fallow deer, red deer and to a lesser extent Reeves' muntjac suggest a possible role for these species as maintenance hosts and in bTB transmission to cattle in some localized areas of the UK and Spain.

6. Which wildlife hosts are important and what do we know about their populations?

Mycobacterium bovis has an exceptionally wide host range, including humans. A variety of wild and domestic mammals including bovids, deer, goats, pigs, and a wide range of rodent and carnivore species may become infected.

The importance of a species as a source of bTB transmission to cattle depends on a combination of factors. Potential risk factors in wild hosts include endemic infection in relatively high-density populations, the persistence of infection in individuals over time, the potential to excrete high numbers of bacilli, and host behaviour and ecology consistent with transmission to cattle. Different host species and populations will vary widely in the extent to which they exhibit these characteristics and so disease risks will vary markedly across regions of Europe.

In general there has been little proactive surveillance for bTB infection in the majority of wildlife hosts in Europe, and what work has been carried out has focused on areas where there is a known wildlife reservoir of infection, or infection is endemic in cattle. Prevalence has often been estimated from passive surveillance of farmed or hunted species. In a small number of studies, more systematic methods have been used to estimate bTB prevalence in wildlife including collation and analysis of existing data, systematic trapping and *post mortem* examination, or live-sampling of animals for culture of *M. bovis* or the estimation of seroprevalence.

The Eurasian badger has long been implicated as the main wildlife reservoir of bTB in the UK and RoI, and their lethal control has formed an integral part of strategies to reduce bTB in cattle. Badger abundance in the UK tends to be relatively high in areas where bTB in cattle is a problem. National badger sett surveys suggested that in some parts of the UK there was a substantial increase in badger abundance between the 1980s and 1990s. Research has revealed considerable detail about the ecology, behaviour and population demographics of badgers. Elsewhere in Europe where badger population densities are considered to be generally lower than those in the bTB affected parts of the UK and Republic of Ireland, there have been few confirmed reports of bTB in badgers. Hence, although the risks badgers may pose for onward transmission of bTB to domestic animals elsewhere in Europe are unknown, the evidence to date suggests that they are likely to be lower than in the UK or Republic of Ireland.

M. bovis infection has been reported in wild boar from a number of European countries, with the highest prevalence reported from the Iberian Peninsula. In Spain, correlation between wild boar density and cattle bTB incidence is one of several factors suggesting that wild boar may be important as a reservoir of bTB for domestic animals. Current increases in the geographical range and abundance of wild boar in the Iberian Peninsula, and recent indications of an increasing trend in bTB prevalence in affected areas, emphasise the need for further research.

Infection in wild deer also appears to be widespread and has been recorded in several European countries. Studies have indicated spatial associations between common strains of *M. bovis* among deer, and between deer, cattle and other species suggesting that transmission occurs between these hosts. Deer densities are spatially variable, and at high densities there may be a significant risk of bTB transmission to domestic animals. For example, the risk to domestic cattle from fallow deer and red deer was estimated to be comparable to that of the badger in certain localities in the UK. Across Europe, many countries collect cull returns that can provide crude indices of deer abundance and population trends. There have also been a number of recent developments in methods to

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produce precise and accurate estimates of deer density at the local level, which will become increasingly valuable in monitoring deer populations in bTB affected areas.

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7. How can culling wildlife contribute to bTB control?

Culling is used to reduce the size of a host population in order to reduce host density, disease prevalence and the absolute number of infectious individuals, such that spillover of infection to other hosts such as domestic animals either ceases or remains at a tolerable level. The aim can be to eradicate a species from a defined area, or to reduce and maintain numbers below a certain level. Eradication is likely to only be a favoured option if the host is an introduced species, such as the brushtail possum (*Trichosurus vulpecula*) in New Zealand. However, *Mycobacterium bovis* infects a wide range of animal hosts, and the important wildlife reservoirs of bTB in Europe are native species, therefore culling is only likely to be considered as a means to reduce host population size.

A number of factors must be carefully considered in order to determine if culling is appropriate. Resource availability, the size of the infected area, the ecology of the wildlife host, and the period over which culling is required will all influence whether this is a cost-effective approach. Culling can have potentially negative and sometimes unpredictable consequences. Culled populations may respond by increasing productivity, so that culling may have to be repeated at regular intervals, with cost and logistical implications. Such compensatory reproduction may also have counter-productive effects such as increasing the proportion of young, susceptible individuals in the population. Culling may also promote increased dispersal by surviving animals, and increased immigration into the culled area. Such behavioural effects were observed when badgers were experimentally culled in the UK and the RoI. Finally, culling wild animals can invoke strong public reactions, particularly when native species are targeted. For example, badgers are an iconic symbol of nature conservation in the UK and culling them has been the subject of deeply contentious debate.

The outcomes of culling wildlife to control bTB in domestic animals are mixed. In Australia, systematic culling of the introduced Asian water buffalo (*Bubalus bubalis*) made a significant contribution to the near-complete elimination of bTB from Australian livestock. The control of possums is thought to have contributed to a reduction in bTB infection in cattle herds in New Zealand by over 84% between 1994 and 2008. Both programmes required sustained financial support, and were only one component amongst a range of measures implemented to reduce disease levels, including a strict test and slaughter regime for cattle. Various forms of badger culling have supplemented cattle-based controls in the UK and the RoI for 25 years. During this period, in the UK there has been a nationwide increase in the incidence and geographical extent of bTB in cattle. Large field experiments in the UK and the RoI demonstrated that widespread, proactive badger culling reduced the incidence of bTB in cattle within culled areas. However, in the UK, the same experimental work also identified increases in bTB incidence in immediately adjacent uncultured areas, which then diminished with time after culling ceased. Localised reactive culling in response to recent cattle bTB outbreaks was also associated with increased incidence of bTB in cattle, although this finding is the subject of ongoing scientific debate.

In Europe, widespread indiscriminate culling of the important wildlife hosts of bTB is unlikely to offer an effective solution on its own. However, targeted culling may still have a role under certain circumstances if employed together with other measures such as vaccination and improved biosecurity of domestic animals alongside cattle testing and controls. The potential for targeted culling to be used successfully may be enhanced as a result of ongoing developments in diagnostic testing and improved understanding of bTB dynamics in wildlife.

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8. What are the prospects for vaccinating wildlife?

Vaccination of wildlife reservoirs to either eradicate *M. bovis* infection or reduce it to a level where transmission to livestock is prevented, offers a potential strategy for bTB control in cattle.

Whilst considerable efforts are being made to develop new vaccines against human and bovine TB, *Mycobacterium bovis* strain bacille Calmette-Guerin (BCG) is currently the only candidate that could be available for use in wildlife in the near future. It is one of the most widely used (100 million children receive the vaccine annually) and safest human vaccines available. Moreover, a number of human clinical trials have shown that there is no persistent or long term harmful effects of BCG vaccination among patients with pulmonary tuberculosis or among strong reactors to the tuberculin skin test and that BCG vaccination does not reactivate latent bTB or increase bacteriological breakdown rates of suspected cases.

In humans, BCG protects against severe forms of primary progressive bTB in children but has proved inconsistent in protecting against pulmonary disease in adults. BCG has been used extensively for vaccine studies in laboratory animals and is currently being developed for use in a variety of domestic and wild animals. BCG vaccination via subcutaneous and mucosal routes has been shown to have a clear protective effect against experimental challenge with *M. bovis* in a number of wildlife species including badgers (*Meles meles*), captive and wild brush-tail possums (*Trichosurus vulpecula*), white-tailed deer (*Odocoileus virginianus*) and farmed red deer (*Cervus elaphus*). Duration of BCG-induced immunity up to one year has been reported for brushtail possums and vaccinated deer harbouring low numbers of virulent *M. bovis* organisms did not succumb to disease activation over time.

A major obstacle to effective BCG vaccination of wildlife is the identification of a practical means of delivering a stable vaccine preparation to target species in the field, since oral baiting is generally considered the only feasible means of vaccine delivery for large-scale disease management in wildlife populations. An edible lipid matrix has been developed which allows BCG bacilli to be maintained in a viable state suitable for oral delivery. Recent experimental infection studies in a range of wildlife species including badgers, brushtail possums and white-tailed deer have shown that oral vaccination with lipid-formulated BCG can induce levels of protection against *M. bovis* infection which are comparable to those induced by injecting the vaccine. More importantly, when delivered orally to a wild possum population, the vaccine was shown to protect against natural disease exposure. Specific baits for the selective vaccination of wild boar piglets have recently been developed.

To obtain a licence to use BCG in wildlife it is necessary, among other things, to show that the vaccine protects animals against *M. bovis* (usually in an experimental setting) and that it is safe for use in a natural setting. Such studies are in progress in the UK for the injectable form of BCG in badgers and an application for a licence will be submitted in 2009 with a view to initiating field deployment in 2010. It will take a number of years to generate the data required for a licence application for oral BCG in wildlife and one of the major challenges is to identify suitable delivery matrices for effective vaccine deployment to each target species.

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9. What other options are there for bTB control in wildlife?

Targeting the host or pathogen with culling or vaccination remain the principal tools available for bTB control in wildlife. However, there are other potential approaches that could contribute to the reduction of bTB transmission from wildlife to domestic animals.

Biosecurity – reducing contact between livestock and wildlife

Theoretically, bTB transmission between wildlife and domestic animals could be reduced without culling or vaccination if the two could be physically separated. Infectious wild hosts may infect domestic animals directly as a result of close contact, or indirectly via contamination of food or the environment with faeces, urine or sputum. The most obvious means to prevent contact between wild and domestic mammals is physical exclusion using fencing. Although fences can be successfully used to control movements of larger mammals such as deer, the costs and logistics of construction and maintenance at an appropriate scale may limit the range of potential applications. Exclusion of small animals is more difficult. Also, consideration must be given to any potentially detrimental effects of fencing on other wildlife.

Badgers in the UK are known to forage on farmland grazed by cattle, and in farmyards and buildings where cattle and feed is housed. Badgers are known to defaecate and urinate while foraging in these areas, and therefore may pose a risk of bTB transmission via both direct and indirect routes. Keeping badgers away from cattle and cattle feed in farm buildings may be possible using badger proof barriers and feed containers, and electric fencing. Keeping cattle away from areas of pasture where there is a high risk of contamination with badger excreta may also be possible with fencing. However, these measures will have cost implications to farmers, and the benefits are currently unknown. In a field trial in the USA, white-tailed deer (*Odocoileus virginianus*), considered to be the main reservoir of bTB infection for local cattle, were successfully deterred from accessing and contaminating cattle feed by using dogs encouraged to remain within the cattle pasture.

In game species, such as deer and wild boar, the management of spatial aggregations at supplementary feeding sites or waterholes, and the safe disposal of viscera by hunters, could contribute to reducing bTB transmission risks.

Fertility control

Fertility control offers opportunities to manage wildlife populations by reducing rates of recruitment. The basic principle involves administering an immunocontraceptive vaccine that renders individuals infertile, which in turn reduces population growth rates. In terms of controlling disease in wild hosts, the aim may be to reduce population density to a level at which infection either cannot be maintained, or is prevented from spilling over into livestock. Potential advantages of this method over culling would include greater public acceptability and reduced animal welfare concerns. It may also cause less disruption to the social structure of wild host populations than culling, and so avoid the associated and potentially counter-productive epidemiological consequences. However, much more research is required on immunocontraceptives, the demographic consequences of fertility control, and methods of delivery before its potential can be realised.

These approaches should not be considered in isolation, as their greatest value may be if used in combination. For example, the effectiveness of a vaccination program could be increased by the addition of effective fertility control to curtail the recruitment of susceptible young animals in a population that may have been released from disease-induced mortality.

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10. What are the important unknowns?

The technical reviews highlight that knowledge of bTB in wildlife across Europe is patchy and relates to local experience of the problem in livestock. There is a great deal of information on the role of badgers in bTB dynamics and on badger culling in Britain and Ireland, although the merits of other approaches such as vaccination and managing badger-cattle interactions are poorly understood. In Spain there is a considerable body of knowledge available for wild boar and to a lesser extent deer, but similar gaps exist in understanding of the implications of management options. There is a great deal of information on bTB pathology and the mechanisms of diagnostics for badgers, boar and deer, which is of generic value to other wild host species. However, pathology, immune responses and diagnostic test performance do vary widely amongst species, and for many other wild hosts there is little or no information available. Nevertheless there is a degree of “read-across” of knowledge and understanding among host species and among countries. The scale of investment and resulting depth of knowledge in pathology and diagnostics is in contrast to the generally broad and shallow coverage of the ecological aspects of bTB dynamics in wildlife in Europe.

At the most basic level, there is a clear need to develop co-ordinated surveillance and monitoring of wildlife bTB across Europe, using consistent methodology and reporting mechanisms and incorporating reliable host population data. Similarly, a general sharing of knowledge about host populations and livestock management systems and better co-ordination of research programmes will provide a cost-effective means of implementing, evaluating and improving management.

Considering more detailed and technical advances, further development of sensitive “trap-side” tests for detecting infection in live animals that are rapid and simple to deploy, might open up research and management options that have as yet been unavailable.

A greater understanding of the strains of *M. bovis* infecting wildlife is also required to determine whether the organism is becoming adapted to its wildlife host.

While knowledge of pathology is relatively advanced for some hosts, there is little information on the process and sources of bacterial excretion in infected hosts or on the role of latency in wildlife species. What governs intermittent excretion, what determines progression of infection to the point at which excretion takes place and when is this likely to occur?

Improvements in diagnostics and understanding of excretion might help us to identify “superspreader” hosts, i.e. those animals that are responsible for a disproportionate amount of disease transmission. This would potentially allow us to target the management of wildlife hosts more effectively.

The precise mechanism for bTB transmission from wildlife to domestic livestock remains a conspicuous gap in knowledge. Investigating how and where this occurs is an extremely technically challenging area of research. The relative importance of direct and indirect exposure, via environmental contamination, remains unclear. However, identifying proxies for transmission risk, such as contact behaviours has been made more achievable by employing technology such as proximity collars. Small-scale studies of this type have been initiated on cattle and badgers. The extent to which domestic animals become infected due to contact with the contaminated environment is unknown and evaluating this will remain difficult.

Better understanding of the relationship between livestock husbandry practices (including management of deer and wild boar for hunting) and transmission risks from wildlife would allow the identification of specific practices that are risky or protective.

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Semi-quantitative frameworks for assessing risk posed by different wildlife hosts are now available and could be applied to a range of wildlife hosts and livestock systems across Europe. Specifically, sensitivity analysis of the factors affecting risk could provide a means of prioritising investigations. Equally, simulation modelling provides a means of better understanding the outcomes of a range of management options for host populations.

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Technical reviews

1. Badgers (*Meles meles*)

1.1 Prevalence & distribution

Although bTB is a recurring problem in cattle in several countries in the EU, badgers are recognised as the principal wildlife reservoir in only the UK and the RoI (Caffrey 1994). Hence, the majority of research relating to bTB in badgers has been carried out there, and prevalence estimates exist only for these countries. Elsewhere in Europe, bTB has also been isolated from badgers in Switzerland (Bouvier et al., 1957) and Spain (Sobrino et al., 2008).

Research on bTB in badgers in the UK and RoI has produced a range of prevalence estimates. However, the reliability of these estimates depends to a large extent on the method of detection of infection employed, the sample size and, since the disease can be highly spatially aggregated in badgers (Delahay et al., 2000), on the spatial scale of the sampling. There is also considerable geographical bias as the majority of samples have originated from areas where the bTB problem in cattle is most severe, and often arose as a result of operations to cull badgers in these areas. Hence bTB in badgers has been most frequently reported in the south and west of the UK. By contrast, in areas of the UK where the risk of cattle herd breakdown is low, there are scant data on bTB in badgers.

Recently the UK government conducted the Randomised Badger Culling Trial (RBCT), a large-scale field experiment to assess the effects of badger culling on bTB incidence in cattle. As part of this study (see section 1.4 for further details), badgers were removed from ten 100km² bTB hotspot areas. The prevalence of bTB in badgers in these areas, as determined by microbiological culture of tissue following *post mortem* examination, showed considerable variation amongst areas, with values ranging from 2% to 37% (Bourne et al., 2007). These are likely to be underestimates of true prevalence given the limited sensitivity (55%) of standard post mortem and culture detection relative to extended post-mortem and culture (Crawshaw et al., 2008). In a similar study in the RoI, the prevalence of bTB in badgers in four large removal areas was 19.5% (Griffin et al., 2005). During a 22 year period of a long-term study of a wild badger population in a bTB hotspot area in south west England, annual bTB prevalence ranged from 1% to 11%, although this was based on the less sensitive approach of microbiological culture of clinical samples (i.e. faeces, urine, sputum, wound and abscess swabs) from live badgers (Delahay et al., 2000; Vicente et al., 2007a).

1.2 The disease in badgers

Pathogenesis

Badgers appear to become infected most often via inhalation of aerosols containing *M. bovis* (Nolan and Wilesmith, 1994; Gallagher et al., 1998; Gallagher and Clifton-Hadley, 2000). A primary infection is established in the lungs and thereafter is spread to mediastinal and tracheobronchial lymph nodes. This is followed by lympho-haematogenous dissemination, which results in new foci of infection in the lungs and associated lymph nodes, and in extra-thoracic organs and lymph nodes (Gallagher 1998; Gallagher and Clifton-Hadley, 2000; Gavier-Widen et al., 2001). Badgers may also become infected via bites by tuberculous individuals (Clifton-Hadley et al., 1993; Gallagher 1998). This causes a local tuberculous reaction in wounded tissues, followed by dissemination to the lungs (Gallagher et al., 1976).

Disease progression varies in its manifestations. Lesions may grow chronically to result in more severe disease after a prolonged period, often causing large parts of the lungs to be replaced by granulomatous inflammation and necrosis, or there may be widespread infection of many tissues. However, the majority of infected badgers are able to control the progression of disease, and develop mild forms with small lesions. Badgers are often confirmed as infected by culturing *M. bovis* from tissues (usually a pool of lymph nodes), but show no gross lesions. This is known as 'no visible lesion' (NVL) tuberculosis, and has been reported as affecting up to 80% of infected badgers, although the proportion varies between studies. This form of infection is accompanied by very small lesions, which can only be observed microscopically (Corner et al., 2007; Gallagher 1998, Gavier-Widen et al., 2009).

The relationship between pathology and the dose of infection has been studied experimentally. Endobronchial infection with <10 cfu of *M. bovis* resulted in infection in all three inoculated badgers (Corner et al., 2008a), indicating high susceptibility to bovine tuberculosis by this route. These animals had 1-2 mm lesions in the lungs and caseous lesions in the draining lymph nodes at 6 weeks post infection (p.i.). Microscopic lesions were observed in extra-thoracic sites, such as the hepatic lymph node at 6 weeks p.i. Subsequently (at 18 to 24 weeks p.i.), disseminated disease occurred, including miliary 1 mm foci in the lungs, and lesions in mesenteric, hepatic and popliteal lymph nodes.

Clinical signs

The majority of infected badgers develop mild forms of non-progressive or slowly progressing tuberculosis, surviving for several years without showing signs of disease (Clifton-Hadley et al., 1993). Badgers with end-stage tuberculosis show emaciation, lethargy and occasionally subcutaneous oedema (Corner et al., 2008a). Bite wounds in the skin or purulent exudates draining from them may be visualized grossly, but they may or may not be tuberculous.

Gross pathology

The spectrum of tuberculous lesions include 1 mm white foci which vary in number but can be numerous (i.e. miliary), larger nodules (a few mm to several cm) often with caseous necrosis and mineralization, and areas of lung consolidation and necrosis of various proportions, sometimes replacing large parts of the lungs. Affected lymph nodes may be enlarged and with white solid or necrotic areas. Chronic lesions may consist of small fibrotic and calcified foci without enlargement of the lymph node. Tuberculous bite wounds become purulent and may form fistulas into subjacent tissues (Gavier-Widen et al., 2001; Sobrino et al., 2008). Many of the tuberculous lesions in badgers are very small, and detailed *post mortem* examination increases the lesion detection considerably (Crawshaw et al., 2008).

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Histopathology further increases the likelihood of detection of lesions in badgers (Corner et al., 2008; Crawshaw et al., 2008).

The lungs and thoracic lymph nodes are the most frequently recorded locations of lesions in badgers, being recorded in 60% of animals with gross lesions (Gallagher et al., 1976; Gallagher and Clifton-Hadley, 2000; Gavier-Widen et al., 2001; Jenkins et al., 2008b; Sobrino et al., 2008). Lymphatic and haematogenous dissemination leads to infection in other lymph nodes and organs. Only 1-5% of tuberculous badgers show severe generalized disease (Gallagher 1998; Jenkins et al., 2008b). Approximately 5% of tuberculous badgers have bite wounds, accompanied by a higher prevalence of gross tuberculous lesions (Jenkins et al., 2008b). Young badgers apparently develop more severe forms of disease. Macroscopic lesions were present in 65% of cubs, while the proportion of gross lesions in adult badgers was 35% (Nolan, 1991). Moreover, under-detection of tuberculosis was more frequent in adult badgers than in cubs (Crawshaw et al., 2008).

The severity of disease in an infected individual is related to the infectious dose. Experimental endobronchial infection of badgers showed that 17 weeks p.i. with a high dose of *M. bovis* (3 x 10³ cfu), disease was more widely disseminated than with medium (10² cfu) or low (<10 cfu) doses. The lowest dose produced lesions in one of three badgers, but *M. bovis* was recovered from them all. In this experiment, the most frequent site of extra-thoracic lesions was the hepatic lymph node (Corner, et al., 2007).

Histopathology

The earliest form of histological lesion, which is rarely found, consists of roughly round to oval, loose clumps of randomly arranged, round to polyhedral macrophages and intact neutrophils. Fully developed tuberculous granulomas can be variable in size but are reasonably consistent in histological architecture. These granulomas display variable amounts of a central necrotic area containing neutrophilic debris surrounded by epithelioid cells. Peripheral lymphocytic rims vary from thin to moderately thick and often contain prominent clusters of plasma cells and lymphocytes. The deposition of collagen around granulomas is usually sparse. The central area of necrosis can show mineralization (Sobrino et al., 2008). Often, granulomas of various stages of development are observed in the same organ (Sobrino et al., 2008). Very often, acid-fast bacilli (AFB) cannot be detected in the granulomas (Crawshaw et al., 2008). In an experimental study, high doses of *M. bovis* produced lesions with more extensive necrosis and caseation, and higher numbers of AFB in the lungs and thoracic lymph nodes than low dose infection (Corner et al., 2007).

In one study, histological lesions were observed on average in 7.6 tissues per badger when a standard protocol was used. Milder forms of disease, identified by a more detailed protocol, revealed lesions histologically in 4.4 different tissues. The most frequent sites of lesions were the bronchial and mediastinal lymph nodes, hepatic lymph nodes and liver, mesenteric and rectocolic lymph nodes, lymph nodes of the head (retropharyngeal, parotid, mandibular), superficial lymph nodes (prescapular, popliteal, axillary), renal lymph nodes and kidneys (Crawshaw et al., 2008).

Routes of bacterial shedding

The predominance of lesions in the respiratory tract indicates that most transmission occurs by the respiratory route (Gavier-Widen et al., 2001; Jenkins et al., 2008b). However, the limited progression of infection in most badgers is probably accompanied by little or no shedding as mycobacterial cultures of tracheal aspirates are often negative (Corner 2008a). On the other hand, experimentally infected badgers showed respiratory excretion at 3 weeks post infection despite only having early lung lesions. This was in agreement with

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observations that badgers with early lung lesions can be infectious (Gallagher and Clifton-Hadley, 2000; Gavier-Widen et al., 2001). Mucosal lesions may become a source of *M. bovis* owing to their superficial location and potential to ulcerate. Badgers may shed *M. bovis* into luminal spaces of the respiratory, gastrointestinal and urinary systems, and from tuberculous skin wounds (Corner et al., 2008a; Gavier-Widen et al., 2001) and draining abscesses (Cheeseman et al., 1985). Respiratory excretion may be intermittent (Clifton-Hadley et al., 1993; Corner et al., 2008a). Badgers with severe pathology and high bacterial loads may shed high numbers of bacilli (Gallagher et al., 2000) and urine can be a particularly prolific source in affected animals (MAFF, 1979). Bacterial counts have been reported from a range of badger excretions including 75 to 200 x 10³ cfu/ml⁻¹ in purulent exudates, 217 to 250 x 10³ cfu/ml⁻¹ in urine, and 68 to 75 x 10³ cfu/g⁻¹ in faeces (Gallagher, 1998).

1.3 Diagnostics

Accurate diagnosis of *M. bovis* infection in badgers is an important component of the development of strategies to control bTB in this species. Culture isolation of *M. bovis* is still considered the 'gold-standard' diagnostic test. However, culture confirmation of bTB in the badger is particularly insensitive using clinical samples obtained from live animals (Chambers et al., 2002). The sensitivity of post mortem tissue culture was recently estimated at around 55% using a standard necropsy protocol (Crawshaw et al., 2008). More infected animals, particularly adults and those with non-visible lesions (NVL) were detected using an extended protocol that included many more tissues, sampled individually, and submitted to an extended culture regime. Against this background, alternative, sensitive *in vitro* diagnostics that can be used to test live animals are required.

The first genuine immunological test for bTB infection in the live badger was the Brock Test, a serum antibody ELISA test directed to a single antigen of *M. bovis*, MPB83 (Goodger et al., 1994; Nolan, 1991). The Brock Test has a sensitivity of 40-53%, depending on the source (Dalley et al., 2008; Clifton-Hadley et al., 1995b; Greenwald et al., 2003, Sawyer et al., 2007). Mahmood et al. (1987) demonstrated T-cell proliferative responses in badgers experimentally infected with *M. bovis*. The assay was subsequently modified to use bovine and avian tuberculin to show *M. bovis* specific T-cell responses in badgers naturally infected with *M. bovis* (Dalley et al., 1999) and in wild badgers vaccinated with BCG (Southey et al., 2001). Although considerably more sensitive than the Brock Test, the assay is time-consuming, technically demanding, and requires the use of radioisotopes. Hence it could not easily be used on a large scale. Nonetheless, it demonstrated that badgers infected with *M. bovis* were competent to mount a cellular immune response, opening the door for the development of more suitable tests, such as the assay of (IFN γ).

Subsequent developments in badger immunology have resulted in a lateral flow serum antibody test (BrockTB STAT-PAK®) (Greenwald et al., 2003) and most recently, a quantitative real-time PCR (Sawyer et al., 2007) and an enzyme immunoassay (EIA) to detect the antigen-specific production of IFN γ (Dalley et al., 2008). The Brock Test and both IFN γ tests require specialist laboratory facilities and equipment, and take 3 and 48 hours to complete, respectively. The BrockTB STAT-PAK® can be performed anywhere and produces a result in less than 20 minutes.

BrockTB STAT-PAK®

The BrockTB STAT-PAK® is no more sensitive than the Brock Test (Greenwald et al., 2003) but is cheaper, quicker, and easier to perform. At present, the need to anaesthetise the badger in order to obtain a blood sample limits the potential of the BrockTB STAT-PAK® to be used animal-side in the field. With a sensitivity of 49% when compared to the 'gold standard' of necropsy tissue culture (VLA, unpublished data), the BrockTB STAT-PAK® is unlikely to be sufficiently sensitive for routine bTB surveillance. However, sensitivity was significantly higher in animals with more severe TB, classified by more frequent excretion of *M. bovis* or the presence of visible lesions at necropsy (Chambers et al., 2008). Therefore the BrockTB STAT-PAK® could be of potential use where a simple tool is required to detect badgers more likely to be at advanced stages of disease.

Interferon-gamma (IFN γ) assays

Currently, the most accurate method for diagnosis of bTB in the live badgers is based on the stimulation of lymphocytes in whole-blood culture and the subsequent measurement of IFN γ production, either using a quantitative real-time PCR method for measurement of badger

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IFN γ mRNA (Sawyer et al., 2007) or detection of the protein by sandwich ELISA (Dalley et al., 2008). The latter IFN γ EIA was reported to have a sensitivity of 80.9% and a specificity of 93.6% (Dalley et al., 2008) and was more sensitive than the PCR-based method. The comparative levels of IFN γ produced following stimulation with bovine and avian tuberculin is used as the basis of determining the bTB status of badgers, resulting in a more sensitive test than the use of defined *M. bovis* antigens ESAT6 and CFP10. During evaluation of the test, only one of nine culture positive badgers missed by the IFN γ EIA was correctly diagnosed by the Brock Test, suggesting that the combination of both a T-cell and serological test has little diagnostic advantage (Dalley et al., 2008).

Factors affecting the performance of immunodiagnostic tests

Previous studies using the Brock Test in badgers suggested that it may give a higher rate of false positive reactions (i.e. lower specificity) in cubs or juveniles compared with adults (Clifton-Hadley et al., 1995b; Newell et al., 1997). The sensitivity and specificity of the Brock Test, BrockTB STAT-PAK® and IFN γ EIA in badgers of different age groups were recently evaluated (Chambers, submitted). The sensitivity of the two serological tests was found to be no different between cubs and adults and was within the ranges reported previously (Dalley et al., 2008; Clifton-Hadley et al., 1995; Greenwald et al., 2003; Sawyer et al., 2007). In contrast, the sensitivity of the IFN γ EIA was lower in cubs (57.1%, 95% CI 18.4 - 90.1%) compared with adults (84.6%, 95% CI 69.5 - 94.1%). This difference was not statistically significant, due to the low numbers of infected cubs available for testing (n = 7) and hence large confidence intervals are associated with the estimate of test sensitivity. Nonetheless, this result suggests that the cut off value used to determine positivity in the test might need to be adjusted when applied to cubs.

Immunological test positivity is more likely in badgers at more advanced stages of TB, especially in the case of serological tests. The Brock Test is more sensitive in detecting badgers with a history of excreting *M. bovis* (Chambers et al., 2002) and its sensitivity was found to increase significantly, the longer the badger had been detected as tuberculous on the basis of live sampling for culture (Chambers, 2009). Similarly, the sensitivity of the BrockTB STAT-PAK® was significantly higher in animals with more severe TB, as classified by more frequent excretion of *M. bovis* or the presence of visible lesions at necropsy (Chambers et al., 2008). For both the IFN γ EIA and the Brock Test, sensitivity was also significantly higher in badgers with visible bTB lesions, compared with those with NVL at *post-mortem* examination (Dalley et al., 2008). In general, specificity of all tests was higher in cubs, although only significantly so in the case of the Brock Test. Taking age into account, sensitivity of the Brock Test was significantly lower at first culture positive event (based on live sampling) (58%), but increased to >80% as infection progressed (Chambers, 2009).

Conclusions

A variety of immunological tests are now available for the diagnosis of bTB in the live badger. The most appropriate test to use will depend on the objectives of the application and the resources available. For greatest sensitivity of detection, the IFN γ EIA is the most appropriate test. An optimal cut off for use in badger cubs should be determined as data from a larger number of tuberculous cubs become available, so that the high sensitivity reported for this test is achieved for all age groups. In field situations, the BrockTB STAT-PAK® presents a viable alternative given its speed and simplicity, although its low sensitivity limits its applications. Badgers with more extensive bTB pathology and those that excrete *M. bovis* are likely to have increased propensity for onward transmission of infection, and serological tests may be particularly suitable for detecting such animals.

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1.4 Epidemiology

Early investigations into possible wildlife sources of infection revealed that prevalence of *M. bovis* infection appeared to be higher in badgers than in other species (Little et al., 1982b; Barrow & Gallagher 1981). In addition, relatively high prevalence of infection in badgers was associated with areas of high cattle herd breakdown incidence (Muirhead et al., 1974). Further studies have confirmed that *M. bovis* infection tends to be spatially clustered in both badgers (Delahay et al., 2000; Olea-Popelka et al., 2003) and cattle (Krebs et al., 1997), and that where they occur together they usually share common strains of *M. bovis*, consistent with transmission between the two species (Woodroffe et al., 2005a). Direct evidence for transmission of *M. bovis* from badgers to cattle was demonstrated experimentally (Little et al. 1982c), although under highly artificial conditions.

Evidence for bTB transmission from badgers to cattle

A number of studies conducted in the UK and Ireland have presented compelling evidence for the field transmission of *M. bovis* from badgers to cattle, particularly where badger culling operations had been associated with changes in cattle herd breakdown rates (Krebs et al., 1997; Clifton-Hadley et al., 1995b; Eves 1999; Griffin et al., 2005). The RBCT provided conclusive experimental evidence that under field conditions badgers contributed to the incidence of bTB in cattle herds (Donnelly et al., 2003, 2006, 2007). This experiment, conducted between 1998 and 2005, compared cattle herd breakdown rates in 100km² areas where badgers were culled either proactively (annual culling on all accessible land) or reactively (localised culling in response to cattle bTB outbreaks), with experimental control areas where no culling took place (Bourne et al., 2007).

Culling has been shown to have both positive and negative effects on the incidence of bTB in cattle. Where badger densities were reduced sufficiently, proactive culling caused a significant reduction in the incidence of bTB breakdowns in cattle herds (Griffin et al., 2005; Bourne et al., 2007; Donnelly et al., 2007; Woodroffe et al., 2008). However, where densities were reduced to a lesser extent, such as in reactively culled areas or around the periphery of proactively culled areas, there was an increase in cattle herd bTB breakdown incidence (Donnelly et al., 2003; Donnelly et al., 2006). Further monitoring of the RBCT areas showed that after culling ceased, cattle herd breakdown rates within proactively culled areas decreased further and the elevated risks around their periphery diminished (Jenkins et al., 2008a). The negative impact of badger culling on herd breakdown rates in the RBCT was attributed to the ensuing disruption of badger social structure. Previous field studies had shown that such disturbance was associated with enhanced movements of badgers (Carter et al., 2007) and that movement might be related to the incidence of infection in badger populations (Rogers et al., 1998). Enhanced ranging by badgers during the RBCT (Woodroffe et al., 2005b) was associated with increased bTB prevalence (Woodroffe et al., 2006), and may have also led to increased rates of contact between badgers and cattle. The impacts of badger culling on cattle herd breakdown rates observed during the RBCT and in Ireland provide robust field evidence of inter-specific bTB transmission.

Potential routes of transmission

Tuberculosis in the badger can affect virtually all organ systems, but the distribution of lesions in badgers at *post mortem* examination is consistent with transmission via the respiratory route (Gallagher et al., 1976). Open lesions in the lungs may be associated with excretion of bacilli in sputum and suggest that the primary route of transmission between badgers is by respiratory aerosol, most probably within the confines of the sett (Krebs, 1997). The mode of bTB transmission between badgers and cattle is not fully understood, and

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remains among the most conspicuous gaps in knowledge. TB in cattle has been demonstrated to be a disease of the respiratory system (Bourne et al., 2007), and it is likely that most animals become infected via inhalation of aerosol droplets containing *M. bovis*. Menzies & Neill (2000) reported that a single bacillus transported in a droplet was sufficient to establish infection within the bovine lung, and Dean et al. (2005) showed that a single colony forming unit (CFU) was sufficient to cause the same severity of pathology as doses of up to 1000 CFU. Hence, cattle could potentially acquire bTB infection by coming into close contact with infected wild animals such as the badger, or material contaminated by badger excretory products (e.g. grass, soil and cattle feed).

Given that badger excretions can contain large numbers of bacilli (Gallagher, 1998; Gallagher & Clifton-Hadley, 2000), which may persist in the environment under certain conditions (King et al., 1999; Gallagher, 1998), this constitutes a potentially important source of infection. Badgers regularly mark territorial boundaries with faeces, urine and other secretions (Kruuk, 1989; Brown, 1993; Delahay et al., 2007b), and these boundaries are often found in pasture grazed by cattle. Therefore, the opportunities for disease transmission from excretory products on pasture may be substantial. Although cattle generally avoid grazing in the vicinity of badger excreta, they have been observed to graze at badger latrines when the sward length in the rest of the pasture was reduced (Benham, 1993; Hutchings & Harris, 1997). Furthermore, cattle do not readily detect badger urine at latrines (Benham, 1993; Hutchings & Harris, 1997), which may be particularly risky due to the relatively high numbers of bacilli that may be present (Gallagher & Clifton-Hadley, 2000).

Badgers and cattle appear to tolerate each other on pasture, and close contact may occur occasionally (Benham & Broom, 1989; Benham, 1993; Scantlebury et al., 2004; Bohm et al., 2009). Cattle have been seen to investigate dead or moribund badgers (Benham & Broom, 1989; Sleeman & Mulcahy, 1993), which may also create opportunities for disease transmission. There is also some evidence that badgers with advanced disease may exhibit aberrant behaviour, which brings them into direct contact with cattle (Cheeseman & Mallinson, 1981; Garnett et al., 2002).

Badgers have been found to regularly visit farm buildings in search of food and bedding material (Kruuk and Parish, 1985; Shepherdson et al., 1990; Brown 1993), and come into close direct contact with cattle (Tolhurst, 2006). Hence farm buildings may be a particular arena of risk for TB transmission (Corner 2006). They have been observed to visit a substantial proportion of monitored farms (Ward et al., 2008), with regularity (Garnett et al., 2002), and to defecate and urinate directly onto cattle feed whilst exploiting a range of resources.

1.5 Ecology & host population monitoring

Ecology

Badger ecology and social dynamics are of profound importance when considering management action to reduce the risk of disease transmission to cattle. Throughout much of the UK, badgers live in social groups and maintain a shared group territory (Kruuk 1989; Neal and Cheeseman 1996). In the areas of the UK where badgers reach relatively high densities, this territorial arrangement can be strongly defined, and groups can be significantly larger than those observed elsewhere in Europe (Cheeseman et al., 1981). In such areas, the territorial structure of badger populations appears to be relatively stable over time, with the location of boundaries changing little (Delahay et al., 2000). Badgers mark their territories with a range of scent marks, including faeces, urine and scent gland secretions (Roper et al., 1986; Brown et al., 1993) which may be concentrated at latrines, particularly in high density populations (Delahay et al., 2007b). There is evidence to suggest that this stable social arrangement may restrict rates of contact between badgers in different social groups, and hence limit the rate of spread of bTB in the population (Delahay et al., 2000). Culling has been shown to disrupt this stable social pattern (see Carter et al., 2007). Hence, some of the benefits of badger culling in terms of reduced bTB transmission to cattle may potentially be offset by the consequences of increased ranging behaviour among residual and immigrant badgers (Woodroffe 2005a), at least temporarily (Jenkins et al., 2008) (see section 1.6). At lower densities, badger social structure may be less strongly defined, and under such conditions the negative consequences of culling may be less marked (Corner, 2006).

Badgers are opportunistic foragers with a wide diet. In the UK they spend much of their active time foraging for earthworms and other invertebrates on pasture (Kruuk 1978a; Neal & Cheeseman 1996), but also exploit other resources, including stored feed and other items associated with farmyards and buildings (Garnett et al., 2002; Tolhurst 2006), and cattle troughs (Garnett et al., 2003). There is some evidence that badgers are more likely to visit farm buildings in search of such foods when their preferred earthworm prey are unavailable because of dry weather conditions (Garnett et al., 2002).

Host population monitoring

The Eurasian badger occurs in all states of Europe (Griffiths & Thomas 1993). Within this area it is absent only from arctic zones, high altitudes and some islands. In most European countries, national data on badger population size and trends are poor. Reviews of badger abundance in Europe based on published literature and questionnaires (Griffiths & Thomas 1993; Johnson et al., 2002) concluded that badger densities in the majority of countries were in the range 0.1 – 1.0 km⁻². Some countries had particularly sparse populations (e.g. Albania, the Netherlands, Estonia and Belgium), while others had notably higher densities (e.g. the UK, Rol and Sweden), and in several countries, no estimates of badger density were available. At the time of their review, Griffiths and Thomas (1993) concluded that badger populations were either stable or increasing in most European countries.

Studies in several European countries have generated estimates of badger density and / or distribution at a local scale (e.g. Wiertz & Vinck 1986; Aaris-Sorensen 1987; Schley et al., 2003; van Apeldoorn et al., 2005). However, systematic national monitoring programs are rare. Baseline surveys of the UK (Scotland, England and Wales, but not Northern Ireland) were carried out in the mid-1980s (Cresswell et al., 1990), and in the Rol (Smal, 1995) and Northern Ireland in the early 1990s (Feore et al., 1993). These investigations involved surveys of badger setts in random, representative samples of 1km squares across the respective countries. Badger 'main' setts were used as a proxy for the presence of badger

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social groups. Subsequent follow-up surveys were carried out in mainland UK and Northern Ireland with the aim of identifying any changes in badger population size. The badger population of the UK (excluding northern Ireland) was estimated to have increased by about 70% in the decade between the two surveys (Wilson et al., 1997), while in Northern Ireland no change was observed (Reid et al., 2008).

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1.6 Prevention and control

Culling

Culling assumes that reducing the population size of the target species results in a concomitant decrease in the prevalence and abundance of infectious individuals. However, this assumption may not hold, owing to the confounding influence of ecological phenomena such as social organisation, compensatory reproduction or immigration (Carter et al., 2007).

Since 1973, badger culling has been employed in the UK and RoI to supplement cattle-based controls to reduce the incidence of bTB in cattle. Gassing of badgers in their setts was carried out from 1975 to 1982, and was associated with declining bTB incidence in cattle. However, there was a national decline in bTB incidence in cattle over the same period, therefore the true impact of badger culling on cattle bTB remained unclear (Dunnet et al., 1986). Concerns over the humaneness of gassing led to it being replaced by cage trapping and shooting badgers. Over the subsequent period several different strategies of cage trapping and dispatch were employed, but the incidence of bTB in cattle increased significantly from its low point in 1979.

Several studies have been published on the effects of badger culling on the incidence of bTB in cattle (e.g. Clifton-Hadley et al., 1995a; Ó Máirtín et al., 1998; Eves, 1999). In general, these studies were associated with declines in cattle bTB incidence, although they lacked the experimental rigour required to establish cause and effect. However, more compelling evidence has since emerged from experimental field studies in the RoI (the Four Counties Trial, see Griffin et al., 2005; Corner et al., 2008) and the UK (the RBCT, see Donnelly et al., 2003; Donnelly et al., 2006; Jenkins et al., 2008a). The positive and negative effects of culling observed during the RBCT (described in section 1.4) and the changes in these with time after culling provide robust evidence of the complex outcomes of badger culling for TB dynamics in badgers and cattle. In the RoI the Four Counties study provided less equivocal evidence for the positive effects of culling. The differences in the outcomes of the experimental studies in the UK and RoI are of considerable interest, and merit further investigation.

Biosecurity

Possible options for reducing cattle contact with badger excretory products at pasture include keeping cattle from known latrine areas or setts using conventional or electric fencing and where possible, reducing the time spent by a herd in any one area of pasture. Another approach is to minimise cattle grazing at field edges where badger latrines are often concentrated (see Delahay et al., 2007b), by using fencing and/or employing strip-grazing regimes.

The physical exclusion of badgers from farm buildings is the primary option for minimising the risk of contact between badgers and cattle in buildings (Phillips et al., 2003; Garnett et al., 2002; Roper et al., 2003). Methods of excluding badgers include ensuring that farm building doors and gates are kept closed at night, bottom-sheeting gates and doors to prevent badger access, installing badger-proof doors on feed stores, and storing feed in closed bins or secure buildings (Defra 2007). Cattle feed delivered in raised troughs rather than placed on the ground may reduce the risk of contamination by badgers, and further work on secure trough design would be valuable (Garnett et al., 2003). Specifically designed electric fencing can be used to exclude badgers from particular locations (Tolhurst et al., 2008) but requires careful installation and maintenance, and may not be practical in all farmyard situations. Such measures will have cost and time implications but their effectiveness in reducing herd breakdowns has not yet been quantified.

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Vaccination

Vaccination has been most successfully employed in wildlife for the control of rabies in foxes in Europe (Pastoret & Brochier, 1999), which has laid the foundations for its practical consideration as an option to manage other infectious diseases in wild mammals (see Blancou et al., 2009). It has been demonstrated that vaccination with BCG can provide captive badgers with some protection against experimental infection with bTB (Stuart et al., 1988; Corner et al., 2008b; Lesellier et al., 2009a&b) and research is currently underway in the UK and RoI to develop an effective means for its delivery to badgers.

The UK government is currently funding a range of research to support the development, licensing and implementation of a BCG vaccine delivery system for badgers. The most economically viable and practically achievable approach to delivering vaccine to badgers over relatively large areas, is likely to involve an oral bait (Bourne et al., 2007; Delahay et al., 2003). Badger setts are easily recognisable structures at which to deliver oral bait, and studies using baits containing biomarkers placed around badger setts have shown uptake by up to 96% of animals subsequently captured (de Leeuw et al., 2006). In the UK and RoI, badger vaccine research is focused on the development of a palatable oral bait, within which BCG will remain viable.

In the absence of a licensed and effective oral vaccine bait, the UK government has announced plans to trap badgers and inject them with BCG from 2010 onwards, in some areas of England where bTB is a problem in cattle. Advantages of the trap and inject approach are that each animal will receive a known dose, non-target species will not be exposed to the vaccine and if a system of temporary marking is employed then it should be possible to avoid repeatedly dosing the same individual badgers. However, trapping badgers is labour intensive and expensive, and it would only be possible to deploy vaccine over a much smaller area for the same resources than if delivered using oral baits.

Simulation models have shown that a vaccination efficiency of 40-50% could result in a high probability of eradicating endemic disease (White & Harris, 1995; Smith & Cheeseman, 2002; Wilkinson et al., 2004).

Other approaches

Fertility control is based on the same principle as lethal control, which is to reduce the host population size below a threshold where infection can be maintained (Swinton et al., 1997). In this approach, animals are given an immunocontraceptive vaccine that renders them infertile, which in turn reduces population growth rates. This has the theoretical potential to be applied to badgers to reduce population size, without the social disruption associated with culling. However, considerable research into development of such a vaccine, delivery methods and the demographic and epidemiological consequences are required before widespread application of fertility control could be considered.

Combined management approaches, directed at both badgers and cattle, may offer opportunities to reduce the incidence of herd breakdowns. Possibilities include culling at the site of a disease outbreak, in association with ring vaccination of susceptible individuals in the surrounding area. This may help to minimise the negative effects of culling observed at the periphery of culled areas during the RBCT. However, without field evidence on the effectiveness of vaccination, the likely success of this approach is unknown. Selective culling of badgers by trapping and testing for bTB infection would theoretically allow the removal of infected animals only, minimising the number of animals culled and potentially reducing the magnitude of any disturbance to social structure. However, the effectiveness of such an approach relies on the availability of a diagnostic test that can be rapidly and easily

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employed in the field and is sufficiently sensitive and specific. The potential added benefits of combining such an approach with vaccination are not fully understood. In general, further work on modelling the outcomes of combined control strategies would be valuable, under different ecological and disease conditions.

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1.7 Knowledge gaps

Transmission routes

The precise routes of transmission between badgers and cattle remain largely unknown. This is because of the practical challenges of gathering information on transmission in the field.

Pathology

The factors that may predispose certain individuals to rapid disease progression and reactivation of latent infection are not yet understood, and in particular the potential role of stress. Another area where knowledge is poor is the relationship between disease progression and bacterial excretion. It is also of interest to investigate the strain types that infect wildlife and whether wildlife presents a special ecotype for *M. bovis*.

Diagnostics

Further work is required to develop more sensitive and rapid *in vitro* diagnostics that can be used to test live animals, as such tests may be valuable tools in the management of infection in badger populations. Information is also required on the relationship between exposure to environmental mycobacteria and host responses to diagnostic tests.

Epidemiology and ecology

Further work is required to determine the extent to which badgers with advanced disease may exhibit behavioural traits that increase the likelihood of onward transmission to other badgers and cattle. Also, results from the UK RBCT and the Four Counties Trial in the RoI raise the possibility that underlying differences in the prevailing density and social structure of badger populations may have influenced the outcome of culling operations in the two countries. Comparative ecological studies could help identify factors that relate to the likely success of badger culling. The majority of information on the epidemiology of bTB in badgers has originated from the UK and RoI, but little is known about their potential role in other parts of Europe where badgers are relatively abundant.

Host population monitoring

More accurate and precise methods for estimating badger abundance would be valuable for monitoring the demographic consequences of management interventions (e.g. culling, vaccination, fertility control). Several existing methods could be further refined to this end.

Control

In the UK and the RoI, badger vaccine safety and efficacy studies, and the research required to develop a licensed product and a strategy for its deployment are underway. This work seeks to address the major knowledge gaps associated with vaccination of badgers.

Several questions remain regarding the epidemiological consequences of culling-induced perturbation of badger populations, including the factors that may influence the magnitude of such effects, and methods of mitigation. The potential benefits of using vaccination to offset culling-induced perturbation, or in combination with selective culling, are not yet understood.

Further information is required on farming practices that may increase risks of transmission between badgers and cattle at pasture and in farm buildings, in order to identify high risk situations where control measures could be targeted. Research is currently underway in the UK to investigate practical measures for preventing badgers from entering farm buildings, but their effectiveness in relation to reducing herd breakdown risks is unknown.

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2. Wild Boar

In most areas where the Eurasian wild boar (*Sus scrofa*) has been introduced, hybridization with free-roaming domestic pigs has led to crossbreeding; producing what are often referred to as wild pig, feral swine or feral hog. Crossbreeding has also taken place in parts of the natural range of wild boar, such as in Southern Spain and on several Mediterranean islands (e.g. Di Marco et al., 2008). Data described here refers to Eurasian wild boar unless otherwise stated.

2.1 Prevalence & distribution

Prevalence of infection with *M. bovis* (and *M. caprae*) is best estimated by culture from pooled tonsil and mandibular lymph node samples, and PCR identification of the mycobacteria (Section 3.3). However, several reports have used pathological observations to estimate the prevalence of TB-compatible lesions in large-scale surveys. In this case, aetiology is confirmed at a metapopulation level by culture and PCR (e.g. Vicente et al., 2006).

Isolation of *M. bovis* from wild boar is commonplace in Europe (Table 1). This agent is frequently the only MTBC member found in wild boar (e.g. Aranaz et al., 1996; Gortázar et al., 2008). In Europe, cases of *M. bovis* have been reported in wild boar from Croatia (Pavlik et al., 2002), France (Table 1), Germany (Schulz et al., 1992), Hungary (Machackova et al., 2003), Italy (Biolatti et al., 1992, Serraino et al., 1999 and references in Table 1), Portugal (Duarte et al., 2008, Santos et al., in press), Russia (Starodinova 1974, in Bollo et al. 2000), Slovakia (Kalenski 1992, Machackova et al., 2003), Spain (Table 1), and in farmed wild boar from the UK (Delahay et al., 2002). Unfortunately, prevalence data on wild boar from Russia and Hungary are absent from the scientific literature. MTBC compatible lesions have been observed but not confirmed by culture in one wild boar from Bosnia and Herzegovina (Ivetic and Sudaric 1987 in Machackova et al., 2003). No mycobacteria of the MTBC were detected among 304 wild boar from the Czech Republic (Machackova et al., 2003).

The highest recorded bTB prevalence amongst wild boar originates from the Iberian Peninsula. In central and southern Spain, MTBC infected wild boar were confirmed by culture and PCR in 84% of mixed populations (n=57) of red deer and wild boar, and in 75% of populations of wild boar alone (n=8). In wild boar populations, the prevalence of individuals with bTB compatible lesions ranged up to 100% (mean prevalence = 42.5%; Vicente et al., 2006). A recent survey in Doñana National Park (southern Spain) confirmed *M. bovis* infection in 52% of 124 randomly sampled wild boar (Gortázar et al., 2008). Recent data from Portugal suggest that wild ungulates, including the Eurasian wild boar, may act as *M. bovis* maintenance hosts (Santos et al., in press). Outside Iberian Peninsula, for instance in some Italian regions, bTB has been considered as enzootic in wild boar (Biolatti 1992, Mignone 1996). However, other Italian studies in which lesions were limited to lymph nodes of the head, suggested that boar were end-hosts for *M. bovis* (Serraino et al., 1999).

An increasing trend of bTB compatible lesions was reported among wild boar carcasses inspected visually between 1992 and 2004 in Extremadura, Spain (Hermoso de Mendoza et al., 2006, Parra et al., 2006). In Doñana (Southern Spain), *M. bovis* infection prevalence in wild boar almost doubled from 1998-2003 to 2006-2007 (see Table 1). In the Brotonne forest, Northwest France, *M. bovis* infection prevalence remained stable between 2002 and 2007 despite intensive red deer and wild boar culling (Maeder et al., 2009). No other reports on bTB prevalence trends are currently available in the scientific literature.

Mycobacterium caprae has often been identified in Spain (Table 1). For example, 34% of 58 MTBC positive wild boar from Southern Spain were infected with *M. caprae* (Gortázar et al.,

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2005a). This agent has also been reported in one wild boar from Portugal (Duarte et al., 2008) and in seven animals from Hungary (Erler et al., 2004).

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Table 1. Prevalence of *M. tuberculosis* complex (MTBC) infection or tuberculosis-compatible lesions among Eurasian wild boar, reported in the scientific literature (C culture; C-C confirmed by culture; PCR-C confirmed by PCR; VI visual inspection).

Country	Region	Prev.	Sample	Method	Agent	Reference
Croatia		1.45%	69	C	<i>M. bovis</i>	Machackova et al., 2003
France	Northwest	37.5%	240	C	<i>M. bovis</i>	Zanella et al., 2008a
France	Northwest	31%	255	C	<i>M. bovis</i>	Maeder et al., 2009
Germany	Mecklenburg-Vorpommern	1.37%	7419	VI (C-C)	<i>M. bovis</i>	Schulz et al., 1992
Italy	Northwest	11.4%	2488	VI (PCR-C)	MTB complex	Bollo et al., 2000
Italy	Sardinia	1.11%	90	PCR	<i>M. bovis</i>	Zanetti et al., 2008
Italy	Sicily (wild pigs)	9.4%	149	PCR	MTB complex	Di Marco et al., 2008
Portugal		11.1%	162	C	<i>M. bovis</i>	Santos et al., (in press)
Slovakia	West and East	19.6%	46	C	<i>M. bovis</i>	Machackova et al., 2003
Spain	Doñana N.P.	52.4%	124	C	<i>M. bovis</i>	Gortázar et al., 2008 (2006-2007)
Spain	Doñana N.P.	28%	214	C	<i>M. bovis</i>	Romero et al., 2008 (1998-2003)
Spain	Extremadura	3.92%	8478	VI (C-C)	<i>M. bovis</i>	Hermoso de Mendoza et al., 2006
Spain	Madrid-CLM-Extrem.	46.1%	52	C	<i>M. bovis</i>	Aranaz et al., 2004
Spain	South-Central	42.5%	1060	VI (C-C)	<i>M. bovis</i> and <i>M. caprae</i>	Vicente et al., 2006

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2.2 The disease in wild boar

Pathogenesis and routes of infection

Little is known about the route of natural infection of boar with *M. bovis*. The frequent location of lesions in the mandibular lymph nodes may arise from entry via either the respiratory or ingestive routes, as these nodes receive drainage from the nasal, oral and tonsillar regions. Some wild boar develop lesions in thoracic lymph nodes only, while others do so exclusively in mesenteric nodes, indicating that either the ingestive or the respiratory route of infection may occur (Vicente et al., 2006, Martín-Hernando et al., 2007). Five milliliters of mycobacterial suspension were administered by the oropharyngeal route by emptying a needle-less syringe onto the back of the tongue (Ballesteros et al., 2009c). Food and/or air-borne mycobacterial infection via the tonsils is followed by a generalization of the disease to other organs including firstly the mandibular lymph nodes (IREC-NEIKER-VISAVET, unpublished data; see also section 2.4.6).

Clinical signs

Most infected wild boar do not show signs of disease. Only exceptionally, severely diseased animals may exhibit lethargy, abnormal behaviour, and eventually arthritis. No other clinical signs have been reported, even in experimentally *M. bovis* infected wild boar (Ballesteros et al., submitted), although tuberculosis appears to cause mortality among juvenile wild boar (Martín-Hernando et al., 2007; Gortázar et al., 2008).

Distribution of lesions

Typically (i.e. in 92-100% of cases) visible lesions are most often located in the mandibular lymph nodes (Gortazar et al., 2004, Parra et al., 2006, Martín-Hernando et al., 2007). Lesions are also described, but at lower frequencies, in other lymph nodes of the head, the medial retropharyngeal and parotid lymph nodes, and in the tonsils (Bollo et al., 2000, Martín-Hernando et al., 2007). Approximately 19 to 83% of infected wild boars have lesions in the lung or in the thoracic lymph nodes, mainly in the left bronchial (Martín-Hernando et al., 2007, Di Marco et al., 2008, Maeder et al., 2008). One to 49% have abdominal lesions, mainly as small necrotic foci in the mesenteric nodes (Parra et al., 2006, Martín-Hernando et al., 2007, Maeder et al., 2009). Other locations of lesions include spleen, liver and joints (Biolatti et al., 1992, Martín-Hernando et al., 2007).

In Spain, about 60% of infected wild boar of all age and sex classes had generalized TB, with lesions in more than one anatomical region. Large lesions in more than one anatomical region were more frequent among juveniles (Vicente et al., 2006, Martín-Hernando et al., 2007). In France, generalized bTB was found in 25-29% of infected wild boar (Zanella et al., 2008a, Maeder et al., 2009). In black wild pigs from Sicily (Italy) this figure increased to about 50% (Di Marco et al., 2008).

In general, the distribution and gross and histological appearance of tuberculous lesions in the Eurasian wild boar are similar to those in feral pigs (Nugent et al., 2002).

Gross pathology

Wild boar develop characteristically necro-calcified granulomas within thick fibrotic capsules. The granulomas grow with time, to develop larger lesions with more necrosis, mineralization and peripheral fibrosis. The spectrum of gross lesions includes a range from small (1 mm) white foci, to larger white-yellow nodules with necrosis and often calcification. Lesions of up to 15 cm are described (Martín-Hernando et al., 2007) but small lesions (< 1 cm) are frequent, being observed in about 40% of infected wild boar (Martín-Hernando et al., 2007).

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Some studies have ranked granulomas according to their size, and time-course of development (Bollo et al., 2000, Martín-Hernando et al., 2007). The largest lesions are often located in the lymph nodes of the head (Gortazar et al., 2004). Multiple nodules of 1-3 cm are often present in the same lymph nodes (Biolatti et al., 1992). Infection may be associated with non-visible gross lesions (NVL), as shown in 17% of MTBC culture positive Eurasian wild boar from Spain. In 50% of these NVL cases, tuberculous lesions were revealed histologically (Martín-Hernando et al., 2007).

Histopathology

The typical tuberculous granuloma in wild boar is formed by epithelioid macrophages and giant cells, sometimes of Langhans type, surrounded by lymphocytes, plasma cells and macrophages. More advanced lesions have central necrosis, with various degrees of calcification and peripheral fibroplasia. Small granulomas (up to 1 cm) are more cellular while larger ones have more extensive areas of necrosis, calcification and thicker fibrosis (Bollo et al., 2000, Gortazar et al., 2004, Martín-Hernando et al., 2007).

Acid-fast bacilli can be difficult to find but in one study were observed in 70% of sections taken, and most frequently in the lungs (Martín-Hernando et al., 2007). The mycobacteria identified by immunohistochemistry were shown mostly in the periphery of the granulomas (Biolatti, et al., 1992). Apparently there are fewer mycobacteria, at least when demonstrated by amplification of *M. tuberculosis* direct test (MTD) in the more chronic, fibronecrotic lesions (Bollo et al., 2000).

The lesions caused by *M. bovis* are histologically indistinguishable from those caused by other mycobacteria, such as *M. avium* or Group III mycobacteria (Ray et al., 1972).

Routes of bacterial shedding

TB lesions are frequently seen in thoracic lymph nodes and lungs, suggesting that respiratory excretion may occur (Machackova et al., 2003, Martín-Hernando et al., 2007). Even apparently local and well-contained mandibular lymph node lesions may be accompanied by lesions in the adjacent salivary glands, with shedding in saliva (Gortazar et al., 2004). Also, tonsillar lesions and those in the ileocaecal region can eventually contribute to the shedding of mycobacteria. In contrast, excretion of mycobacteria in urine appears to be less important in wild boar, since tuberculous lesions are not described in the kidneys. The observation of bTB lesions in the mammary glands of some sows suggests that pseudo-vertical transmission to piglets through infected milk may be possible (Martín-Hernando et al., 2007). Juvenile wild boar have a higher infection and lesion prevalence (Gortazar et al., 2008), and more extensive bTB lesions (Vicente et al., 2006, Martín-Hernando et al., 2007) than other age classes. These animals probably represent the main source of excreted mycobacteria in boar populations.

2.3 Diagnostics

Diagnostic tests parallel to those described for bovines do exist, but few scientific reports of their use in wild boar are available. Tests that detect cell-mediated immunity include skin tests and the IFN γ assay tests, whilst those that detect antibodies are based on ELISA.

Cellular immunology

The intradermal tuberculin (IDTB) test has been used for routine field detection of infected animals for almost a century (reviewed in Monaghan et al., 1994, de la Rua-Domenech et al., 2006), and is the official test for diagnosis in cattle in most countries. A review of the literature of pathogenesis and diagnosis of bovine tuberculosis in cattle estimated the sensitivity of the tuberculin test (at standard interpretation) to be 90% (Morrison et al., 2000), whereas others have reported a range between 52% and 100% with a median value of 80% (Vordermeier et al., 2006).

Skin tests using mycobacterial antigens (bovine and avian purified protein derivative (PPD)) and comparison to a mitogen (a plant derived phytohaemagglutinin) as a positive control have been evaluated recently in wild and farmed wild boar (Jaroso et al., submitted). According to authors, skin testing had a variable sensitivity (100% if only the four culture positive controls were considered (the small sample size is acknowledged), 57% if BCG-injected controls were included) and 78-87% specificity. However, the need to handle each animal twice, which is impractical for testing wild animals on a large scale and limits its use to experimental studies or farmed animals.

The IFN γ assay is an in-vitro alternative described by Wood and co-workers in 1990 (Wood et al., 1990) to overcome the problems associated with the IDTB test. Since then, it has been extensively applied to cattle and goats (e.g. Liebana et al., 1998; Gormley et al., 2006; Álvarez et al., 2008). The test is more sensitive than IDTB tests (Wood et al., 1992; Gonzalez Llamazares et al., 1999) and can detect infected animals at an earlier stage (Gormley et al., 2006).

Swine or wild boar IFN γ can be detected using an enzyme immunoassay (EIA) and an ELISA kit is commercially available. The conditions for the optimal use of this technique in wild boar must be determined, as must the optimal cut-off (Rothel et al., 1990). As an initial approach, the optimal conditions used for cattle and goats could be applied. Animals can be considered positive if the mean optical density (OD) of a sample stimulated with bovine PPD minus the mean OD of nil antigen is greater than 0.05 and greater than the same value of the sample stimulated with avian PPD (interpretation prescribed in the Spanish bovine tuberculosis eradication program).

There is only limited information on the use of the IFN γ as a diagnostic test in swine. The IFN γ test has been successfully evaluated in a small sample from naturally *M. bovis*-infected, open-air raised domestic pigs from Spain (Bezoz et al., 2007). In this study, heparinised blood samples were collected, and three aliquots from each were incubated with PBS, avian PPD and bovine PPD at 37°C for 18-24 hours. Detection of IFN γ in supernatant was performed in duplicate with the Pierce Endogen® porcine IFN γ ELISA kit, according to the manufacturer's instructions. Preliminary results of the IFN γ assay for the evaluation of the immune response after experimental infection with *M. avium* subsp. *avium* and *M. bovis* in two groups of wild boar, showed that the response was species-specific and may be related to the route of infection (IREC-NEIKER-UCM, unpublished data).

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The practicality of the assay is hampered by the need to stimulate blood samples in the laboratory within 8 hours of collection, to avoid impairing test performance (Gormley et al., 2004).

Serology

Studies of tuberculosis have suggested increased humoral and decreased cell mediated immune responses as disease progresses. Serology techniques can also be affected by cross detection of different mycobacteria. Although several tests are available for domestic and wild animals, their use is less generalized than skin testing. However, serological tests have been employed for the detection of bTB in a wide variety of wildlife species. The wild boar constitutes one exception to the generally low sensitivity of such tests (Chambers 2009).

The MultiAntigen Print ImmunoAssay (MAPIA) using a panel of 12 mycobacterial antigens (Lyashchenko et al., 2000) showed heterogeneous antigen recognition patterns, though MPB83 was the serodominant antigen target in wild boar.

Recently, two studies have shown promising levels of sensitivity and specificity with ELISA tests based either on bovine PPD (Aurtenetxe et al., 2008) or on a combination of natural and recombinant antigens (lateral-flow rapid test, Lyashchenko et al., 2008). The rapid test (developed by Chembio Diagnostic Systems Inc) is an animal-side disposable kit that can be performed in 20 minutes to minimize physical restraint of animals. Both studies coincide in reporting sensitivity of 73-77% and specificity of 96-97%. A close association between a strong antibody response and the presence of gross lesions in individuals infected with *M. bovis* has been observed in wild boar (Aurtenetxe et al., 2008; Lyashchenko et al., 2008), as in other species.

The use of ELISA tests may contribute to the diagnosis of bTB in wild boar and probably also in pigs, with an acceptable sensitivity and specificity and without the need to handle the animals twice as in the skin test. Thus, ELISA testing may become a useful tool in large-scale monitoring of bTB in wild boar (Aurtenetxe et al. 2008).

2.4 Epidemiology

Knowledge on feral pigs from Australia and New Zealand

Mycobacterium bovis infection in wild swine was first reported in Australia. In the Northern Territory, Asian water buffaloes that died at the end of the dry season provided infected carcasses for scavengers. However, the low prevalence of generalized bTB in feral pigs, the absence of pulmonary lesions, the lack of other obvious routes of excretion from infected pigs, and the lack of contact between feral pigs and other species, particularly water buffalo and cattle, led to the conclusion that feral pigs were dead-end hosts and not a source of bTB infection (Corner et al., 1981; McInerney et al., 1995). This hypothesis was subsequently validated when, 20 years later and after bTB was essentially eradicated from the bovid population, a survey showed that infection had almost disappeared from the feral pig population (Corner, 2006).

However, one study in New Zealand found that 33% of infected feral pigs had either lung or bronchial lesions, consistent with aerosol transmission of *M. bovis* (Wakelin and Churchman, 1991). In contrast, when Nugent et al. (2003) reviewed the role of pigs as bTB hosts (for the New Zealand Animal Health Board) they concluded that (1) pigs are highly susceptible to *M. bovis* infection; (2) the location of lesions suggests that pigs become infected by scavenging infectious carcasses of animals such as possums or deer; (3) despite the high prevalence of disease, feral pig populations cannot maintain the infection by themselves (i.e. they appear to be spill-over end hosts); (4) however, they may help maintain bTB in a region when infectious pig carcasses (or parts thereof discarded by hunters) are available for scavengers; (5) owing to their susceptibility to bTB, feral pigs can be useful as sentinels to detect the presence of infection in wildlife in an area (Nugent et al., 2002).

Evidence for the role of wild boar as a reservoir for MTBC mycobacteria in Europe

In the last decade, research groups in Spain have provided evidence that support the role of Eurasian wild boar as a reservoir host for *M. bovis*. This contradicts the experience from Oceania, and has important implications for the control of the disease in the country (reviewed in Naranjo et al., 2008a) (reviewed in Naranjo et al., 2008a). There seem to be differences across its distribution range and between closely related hosts (feral pigs). Evidence suggesting that wild boar may act as a reservoir of infection in Mediterranean areas include:

- i. Presence of common MTBC genotypes in wild boar, domestic and wild animals and humans.
- ii. High prevalence of *M. bovis* among wild boar in estates fenced for decades in complete absence of contact with domestic livestock and other wild ungulates.
- iii. TB lesions are frequently seen in thoracic lymph nodes and lungs, suggesting that respiratory infection and excretion may occur.
- iv. Extensive tuberculous lesions in more than one anatomical region occur in a high proportion of juvenile wild boar, which probably represent the main source of mycobacteria.

However, there is still a need to clarify infection and excretion routes, and to establish the minimum infective dose by each infection route.

Evidence for transmission within wild boar, between wild boar and deer, and between wild boar and livestock

In Spain, associations have been observed between the density of wild boar populations and cattle bTB incidents (unpublished reports to Castilla – La Mancha Government). In addition,

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Eurasian wild boar share MTBC genotypes of bovine and caprine origin with cattle, goats, domestic swine, deer and humans (Aranaz et al., 2004, Gortazar et al., 2005b, Parra et al., 2006).

In summary, the available information suggests that infection in Eurasian wild boar has important implications for the control of bTB in the Iberian Peninsula, and that control of the infection in wild boar populations will be necessary if complete eradication of the disease is to be achieved. Changes in wildlife management (towards more intensive models) and in livestock production (towards more extensive models) further complicate bTB epidemiology and control.

Risk factors for infection in wild boar

Individual risk factors include age but not sex (Vicente et al., 2006), genetics (Acevedo-Whitehouse et al., 2005), and behaviour (IREC, unpublished data). Wild boar display strong sociability at the maternal group, with presumably high contact rates during foraging, wallowing, and scavenging on carcasses.

Environmental risk factors include Mediterranean conditions (climate with marked dry seasons) in concomitance with the effects of management (e.g. food and surface water distribution). This is observed even in areas characterized by an absence of intensive management (e.g. Doñana National Park; Gortázar et al., 2008). Important phenomena include (1) the overabundance of Eurasian wild boar and other ungulates (Southern Spain has high densities of wild ungulate species due to intensive management of wild boar and red deer) maintained by artificial feeding and watering, containment of populations behind high wire fences and translocation (Gortázar et al., 2006; Vicente et al., 2007b); (2) the high availability of bTB infected carrion and viscera discarded by hunters; (3) the introduction of bTB through movements of wild or domestic ungulates; (4) the effects of management on population genetics, such as inbreeding (Acevedo-Whitehouse et al., 2005).

Molecular epidemiology

Understanding the epidemiology of tuberculosis is usually based on a combination of traditional disease tracing investigation and molecular typing. Some genetic elements of *M. bovis* can be exploited as strain-specific markers. The traditional standardised method of IS6110-RFLP has been replaced to a large extent by fast, cost-effective, PCR-based techniques that allow working with a large number of isolates. Currently, the most frequently used typing techniques are Direct Variable Repeat-spacer oligonucleotide typing (DVR-spoligotyping) and Mycobacterial Interspersed Repetitive Units - Variable Number Tandem Repeats (MIRU-VNTR). Several studies involving *M. bovis* strains from different countries suggest that the most appropriate typing method may be dependent on the geographical region and animal origin of the isolates that are being studied. The better discriminatory power is provided by a combination of methods.

Spacer oligonucleotide typing (spoligotyping) (Kamerbeek et al., 1997) is a PCR-based method that reveals the polymorphism of the DR region by detecting the presence or absence of specific spacer sequences of the region. The spoligotyping method has advantages in terms of simplicity, speed and cost. It has been chosen as an appropriate method to type *M. bovis* isolates cultured from wild animals, and has also been applied to isolates from farmed animals. MIRU-VNTR has been proposed as an alternative (Skuce et al., 2005, Allix et al., 2006).

Spoligotyping and MIRU-VNTR results indicate that *M. bovis* isolates from wild boar share the same profiles as those from other wild animals (red deer, fallow deer, Iberian lynx and

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red fox) in the same geographical area. The same patterns are also shared with domestic animals, such as cattle and goats (Aranaz et al., 2004, Gortázar et al., 2005b, Romero et al., 2008). In some areas of western Spain, the possibility of cross infection between wild boar and outdoor bred Iberian domestic pigs has been described (Parra et al., 2003). This reveals an epidemiological connection, either because of infection transfer between the two host populations or from a common source. Importantly, fingerprinting *per-se* cannot determine the direction of transmission between host populations. Molecular evidence of inter-species transmission of *M. bovis* has also been described in Portugal (Duarte et al., 2008), France (Haddad et al., 2001, Zanella et al., 2008a) and Italy (Goria et al., 2006).

Mycobacterium caprae (Aranaz et al., 2003) has also been isolated from wild boar with bTB compatible lesions in Spain (Aranaz et al., 2004, Gortázar et al., 2005a), Portugal (Duarte et al., 2008) and central Europe (Erler et al., 2004, Prodingler et al., 2005). In most cases, they were geographically linked with isolates with the same typing patterns from local cattle or goats. In Hungary, a correlation between the geographical distribution of infected wildlife and cattle has been described (Janosi et al., 2009).

Host-pathogen interaction

Differential gene expression has been characterized in Eurasian wild boar naturally infected with *M. bovis* (Naranjo et al., 2006a, 2006b, 2007a, 2007b, Pérez de la Lastra et al., 2009, Galindo et al., 2009). These studies suggest that while some mechanisms have been conserved in the host response to mycobacterial infection between different species, others may be species- and tissue-specific (Naranjo et al., 2007b). New mechanisms were identified by which mycobacteria modify the gene expression profile in this species (Pérez de la Lastra et al., 2009, Galindo et al., 2009). These studies highlight the importance of conducting genomics and proteomics studies of host-pathogen interactions in natural populations and question the extrapolation of results obtained in animal models and in vitro systems without confirmation in the mycobacteria reservoir species.

Tissue-specific differences were characterized in the wild boar response to mycobacterial infection (Naranjo et al., 2007b). Stress and inflammatory responses were characterized in mandibular lymph nodes and tonsils of Eurasian wild boar naturally infected with *M. bovis* (Naranjo et al., 2006a, 2006b, 2007a). The heat shock response was increased in tonsils, while the acute phase protein (APP) response was a characteristic of mandibular lymph nodes in infected animals. These results are in agreement with histopathological findings and may reflect differences in the role of mandibular lymph nodes and tonsils during mycobacterial infection in wild boar. The heat shock response may protect wild boar from environmental pathogens and is therefore induced in the tonsils that may be a relevant entrance site for mycobacteria in wild boar. Once mycobacterial infection has been established in wild boar, tuberculous lesions occur in mandibular lymph nodes, where APP response may reflect tissue injury in the granulomas or in organs affected by a generalization of the infection. These results support at the molecular level the suggested food and/or airborne mycobacterial infection route via the tonsils, followed by a generalization of the disease to other organs including mandibular lymph nodes in wild boar.

Analysis of differentially expressed genes contributes to the understanding of the cellular response to natural mycobacterial infection in Eurasian wild boar, and may impact on the identification of biomarkers of resistance and susceptibility to bTB in these species (Naranjo et al., 2006b, 2007b, 2008a, 2008b) and on the development of vaccination strategies for the control of bTB (Pérez de la Lastra et al., 2009).

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2.5 Ecology & host population monitoring

The Eurasian wild boar has a wide natural distribution including vast areas of Europe and North Africa, extending to Sri Lanka, Indonesia, Japan, Taiwan and Korea. As a result of introductions, Eurasian wild boar are also found in areas far from their original distribution (Lever 1994). The species is now free-living in several parts of the UK (Delahay et al., 2002) and in most of continental Europe except northern Scandinavia and most islands.

Ecology

Wild boar are present in a wide variety of environments throughout their distribution, although they exhibit a preference for forest habitat. Tooth eruption patterns allow wild boar to be categorised into four age classes (<6, 6-12, 12-24 and >24 months; see also EFSA 2009). They are gregarious animals, living in groups of variable size. Under natural conditions most groups comprise females and their offspring. Adult males are seen to form groups in autumn and winter, although males usually display solitary behaviour. Males may act as inter-group spreaders of disease and are also more prone to disperse greater distances. Wild boar tend to aggregate spatially due to social behaviour and irregular food availability, especially in the autumn months. Spatial aggregation and inter-group contact, and hence disease transmission risks, are enhanced if feeding or baiting (e.g. for hunting) takes place (Acevedo et al., 2007, Vicente et al., 2007b). Wild boar density figures in Europe usually range from 1 to 10 per km² (see references in Ruiz-Fons et al., 2008 and EFSA 2009). High densities (up to 90 individuals per km²) and the scarcity of water in Mediterranean countries during the summer also contribute to wild boar aggregation (Acevedo et al., 2007). As social behaviour of wild hosts may play an important role in the epidemiology of disease (see Cross et al., 2009), social structure should be taken into account in implementing disease control programs (Ruiz-Fons et al., 2008).

The wild boar is mostly a sedentary species with a short native-dispersal distance (<10 km). Matriarchal social groups are known to inhabit diurnal home-ranges varying from 150 to more than 2000 ha (mean = ~500ha); adult males roam between matriarchal groups and often inhabit larger areas (mean = 1000-2000ha). Fenced motorways constitute barriers that may be sporadically crossed by wild boar, especially over bridges or during drive hunts (see EFSA 2009).

Population dynamics

The European wild boar is a polygynous species with an autumnal breeding season influenced by environmental conditions. The breeding season occurs in autumn and farrowing takes place after 120 days, although some sows farrow in late summer. The peak of births occurs from February to April but may expand if food is available in large quantities. Most reproductively active wild boar sows are more than one year old, piglets do not breed and 30% to more than 60% of juvenile females may reproduce depending on food availability. Wild boar sows produce 3 to 7 piglets per year depending on age, body mass and food availability. Improvement of food supply is a significant factor in wild boar population growth. Across all age classes natural survival rates are approximately 0.7-0.8/year, but turnover may be much higher in hunted populations (Ruiz-Fons et al., 2006, see EFSA 2009).

bTB is an infection with slow progression of disease. Effects of chronic infectious diseases are often difficult to measure. There is observational evidence that juvenile wild boar with generalized bTB are under-represented in the population, suggesting enhanced mortality (Naranjo et al., 2008a, Martín-Hernando et al., 2007, Gortazar et al., 2008). However, the possibility that some infected wild boar resolve infection cannot be excluded. Broader

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ecological implications to consider are the probable exacerbation of disease due to climatic factors (e.g. the dry summer in Mediterranean areas).

Systems with multiple host species

Eurasian wild boar interact with the environment, wildlife, livestock and humans. Literature suggests that the multi-host situation in Mediterranean areas involves wild boar and deer species (Gortazar et al., 2008). In northern areas of the Iberian Peninsula, other reservoirs such as badgers may be involved (Sobrino et al., 2008). Studies in Spain suggest that wild boar are more susceptible to infection than red deer (Vicente et al., 2006, Gortazar et al., 2008). In southern Europe, bTB often exists in a multi-host situations, of which wild boar is an important part. The disease affects livestock and can also affect species of conservation concern (Woolhouse 2002, Phillips et al., 2003), but these situations are also relevant for conservation. For example, several Iberian lynxes (*Lynx pardinus*) have died due to bTB in their last two strongholds in southern Spain (e.g. Perez et al., 2001).

Although inter-species transmission probably occurs (see above concerning scavenging), most transmission may occur at the intra-species level due to ecological, behavioural and epidemiological factors (Gortazar et al., 2008). The significance of wild boar as bTB hosts relates to the high levels of infection observed and its ecological characteristics such as its scavenging behaviour and potential for dissemination of disease and interaction with domestic livestock (due to its ability to cross fences).

Host population monitoring

The geographical range and population densities of Eurasian wild boar are currently increasing in the Iberian Peninsula (Acevedo et al., 2006) and elsewhere in Europe (Saez-Royuela and Tellería 1986, EFSA 2009), reaching previously unrecorded levels (Geisser and Reyer 2004). This has contributed to the spread of many diseases, including bovine tuberculosis (Gortazar et al., 2006, Naranjo et al., 2008a). Thus, adequate methods for monitoring wild boar abundance and for identifying high density hotspots are required.

The most common means to estimate wild boar abundance is by using data derived from hunting, usually expressed as wild boar catch per surface area unit or as wild boar catch per hunting activity. Hunting derived data on wild boar abundance are most useful at large geographical scales (Acevedo et al., 2006).

A recently developed simple method of wild boar abundance estimation, based on the frequency of faecal droppings found on transects (FBII), also allows calculation of a spatial aggregation index. The FBII and the aggregation index were correlated with bTB prevalence in wild boar, and with the hunting index. Furthermore, the FBII and the aggregation index were more strongly correlated with disease prevalence than was the hunting index. Hence, at least in habitats with high wild boar densities, the FBII combined with the aggregation index constitutes a cheap and reliable alternative means of estimating wild boar abundance, which can be used for epidemiological risk assessment, even outside the hunting season and in areas with no available data on hunting activities (Acevedo et al., 2007). At a very local scale, precise calculations on wild boar density may be obtained by capture-recapture methods (e.g. Hebeisen, 2007).

2.6 Prevention and control

Preventing disease introduction

Wild boar are able to maintain bTB circulation even in the absence of frequent contacts with other ungulates (Vicente et al., 2006). Hence, it is beneficial to avoid the introduction of bTB into naïve populations. Pre-movement control of cattle by skin-testing is compulsory in many countries. However, this is often not the case for other domestic animals that may carry TB, including farmed deer, goats and pigs, and for wildlife species, notably the wild boar, though methods for bTB testing live wild boar are still poorly developed (see above). Hence, a careful pre-movement survey of the donor population, based on either on *post mortem* inspection and culture of a moderate sample of hunter-harvested wild boar (e.g. n = 30), or on a larger sample of ELISA-tested wild boar sera (e.g. n = 60) could help avoid translocating of infected individuals.

Culling

Host eradication may be an option in regions or islands where the wild boar is not native, or where populations comprise hybrids. However in this case, before aiming at eradicating hybrid wild boar it is valuable to control bTB among the local domestic pig breeds. Wild boar eradication is not a realistic option over the large areas and often inaccessible habitats in which they live.

The identification and management of over-abundant populations are key actions in the control of many infectious diseases (Gortázar et al., 2006). While wildlife culling is rarely an effective means of eradicating a wildlife-related disease, population reduction is a goal in many disease control efforts. This is a temporary measure, except if habitat modification is used to reduce host density more permanently or to alter host distribution or exposure to disease agents (Wobeser 2002). Observational data from central Spain have shown a relationship between wild boar abundance, wild boar aggregation, and bTB prevalence (Acevedo et al., 2007). A correlation between wild boar and red deer bTB prevalence was also shown in the same region (Vicente et al., 2006). However, experimental studies testing the effect of wild boar population control on bTB prevalence in boar and other hosts are lacking.

Biosecurity

Setting up barriers to prevent wildlife contact with domestic livestock is often impossible. Wildlife disease management aimed at minimizing spill-over to domestic animals will depend on the development of secure livestock husbandry practices (Conner et al., 2008). However, fencing or other means of limiting contact between infected wildlife and livestock, such as the use of dogs to reduce direct or indirect contact (Vercauteren et al., 2008) could be an option under certain conditions. Studies are required to assess different means of avoiding or reducing contact between wild boar and cattle.

Hygiene measures, such as the correct disposal of carcasses and hunting remains (gut piles) should become compulsory in wildlife areas with a history of TB. However, research is required to assess how to reconcile this with the conservation of carrion consuming endangered bird species (Gortázar et al., 2008).

It is clear that spatial aggregation, even more than density, is a major risk factor for bTB in wildlife. In fact, banning wildlife feeding has been proposed as a means to limit direct or indirect *M. bovis* transmission at feeding sites. However, the extremely high *M. bovis* infection prevalence found in Doñana National Park occurred in the absence of artificial feeding of wildlife, suggesting that a feeding ban alone would only have a limited effect on *M.*

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bovis prevalence in wildlife in Mediterranean habitats (Gortazar et al., 2008). In warm climates with marked dry seasons, waterholes may constitute a similar risk for disease spread as feeding sites. This is supported by several surveys on wildlife bTB in south-central Spain (e.g. Vicente et al., 2007b). Again, experiments testing the hypothesis that changes in food or water distribution may affect bTB epidemiology in wild boar are lacking.

Vaccination

Vaccination of wildlife is being considered as an option for bTB control in Ireland and the UK (badger), in New Zealand (possum) and in the USA (white-tailed deer). As already mentioned, delivery, safety and efficacy issues need to be addressed in order to prepare for licensing a vaccine. In the wild boar, ongoing research is producing valuable knowledge on these issues.

Specific baits for the oral delivery of vaccine preparations to 2–4 month-old wild boar piglets were developed and evaluated. Physical stability studies demonstrated that baits were stable for at least three days at temperatures as high as 42 °C. Bacterial viability in the baits and the antibody response in orally immunized wild boar were studied. Bait acceptance studies using artificial feeders in the field showed that baits were accepted by 2–3 month-old animals, the preferred age for vaccination (Ballesteros et al., 2009b).

Piglet feeders were shown to be highly selective for young wild boar. Baits disappeared faster in summer than in spring (i.e. ~70% consumption after the first day in selective feeders in summer, and 40% in spring). Therefore, a combination of a summer season and selective feeders was found to be a potentially reliable bait-deployment strategy for wild boar piglets under Mediterranean conditions. This delivery technique based on selective piglet feeders also has potential for other uses in the Eurasian wild boar and wild pigs under different management conditions (Ballesteros et al., 2009a).

Preliminary experiments in small numbers of captive wild boar showed no isolation of *M. bovis* BCG from faeces and from the environment, even after oral delivery of very high doses, and no *M. bovis* BCG isolation from tissues collected at necropsy (IREC-NEIKER-VISAVET, unpublished data).

Preliminary vaccination and challenge experiments suggest that a single BCG vaccination by the oral route may protect wild boar from infection by a virulent *M. bovis* field strain. These results also showed at the molecular level that wild boar responded to oral BCG immunization in a similar way to parental BCG vaccination (Pérez de la Lastra et al., 2009) and provided additional evidence that expression of selected genes correlates with protection to *M. bovis* infection in this species (IREC-NEIKER-VISAVET, unpublished data).

Assessing the effects of wildlife disease control

The success of any such management action must be assessed critically, including an analysis of the costs, the ecological consequences and the benefits to animal and human health and welfare. The wild boar is not an endangered species; they can be hunted, and their populations may even be repopulated with quarantined individuals from other areas, if that were deemed necessary.

Consideration of socio-economic and political issues is fundamental to successful management of wildlife diseases. Feeding bans are often politically charged because of long traditions of supplemental feeding and local economies that are built around this practice. In hot climates, any regulation of wildlife watering would raise strong opposition. Risk modelling may help identify successful strategies that balance the biological risks of disease

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transmission and the political risks of alienating hunters who assist with wildlife management (Conner et al., 2008).

More information on wild boar population control and disease management can be found in the EFSA report on classical swine fever (EFSA 2009).

2.7 Knowledge gaps and prospectus

Prevalence and distribution

Data on the prevalence and distribution of infection among wild boar are largely absent for much of Europe, except for Spain. Moreover, only a few reports on trends in infection are currently available.

Pathology

Research on the routes and intensity of bacterial excretion is still needed. Little is known about the molecular biology of the host-pathogen interface, particularly for wildlife host species. The application of genomics and proteomics to the characterization of host-pathogen interactions is essential to advance our knowledge of bacterial pathogenesis, infection and transmission and to identify potential diagnostic and vaccine candidate antigens.

Diagnosis

A much larger sample (several thousand) of confirmed positive wild boar would be needed to fulfil the requirements for accurate evaluation of the recently developed ELISA tests.

Epidemiology

There is a lack of knowledge regarding risks associated with specific *M. bovis* strains. Also there is no information on intra-specific and inter-specific transmission rates and routes of infection amongst wildlife (which may differ according to the species involved and the direction). This is crucial since assemblages of particular species may allow bTB persistence in complex multi-host situations (e. g. Mediterranean Spain). Local intensive studies suggest that continuing Eurasian wild boar monitoring, including different geographic areas (such as cattle free areas) will help further our understanding of disease dynamics and risks.

Ecology & host population monitoring

Mortality among juvenile wild boar has yet to be studied in any detail and quantified. There is also a need to study dispersion patterns, habitat use, aggregation behaviour and social interactions amongst wild boar, and between boar and other species. Data on these phenomena can be gathered through the use of new technologies like GPS-collars and proximity tags in field studies. Even more importantly, few data exist on the effects of increased hunting pressure (as a strategy to reduce wild boar numbers and tuberculosis) on social and spatial perturbation and compensatory reproduction in wild boar populations.

Prevention and control

Vaccination and challenge trials are currently being carried out with larger numbers of experimental subjects. In parallel, safety issues are being addressed and delivery experiments with biomarkers are finishing. If these experiments progress as expected, work towards licensing the use of oral BCG in free living wild boar will be the goal.

Other than vaccination, research on bTB control in wild boar is needed in the following fields:

- Barrier types and efficacy
- Ecology of carrion and gut pile consumption and management
- Epidemiological consequences of wild boar population reduction (on boar and other hosts)
- Quantification of contact and transmission rates at the livestock-wild boar interface, and identification of means to reduce these
- Experiments testing the hypothesis that changes in food or water distribution may affect transmission

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3. Deer

3.1 Prevalence & distribution

Various methods have been used to estimate prevalence in deer including passive surveillance (Delahay et al., 2002), more systematic deliberate trapping and *post mortem* examination (Delahay et al., 2007; Gortazar et al., 2008), and assessment of gross pathology alone (Vicente et al., 2006). Infection is easier to detect in farmed deer, as a greater range of diagnostic tests is available for use and frequent clinical inspection is possible (Griffin and Cross, 1987; Clifton-Hadley and Wilesmith, 1991; Griffin et al., 1998a; EFSA, 2008).

Infection has been detected in wild deer and prevalence estimates are available for a number of European countries (Table 2; Clifton-Hadley and Wilesmith, 1991, 2005; Bode, 1995; Delahay et al. 2002, 2007; Parra et al., 2006). The OIE recorded bovine tuberculosis in 2007 in red deer in Austria (3), France (43), Portugal (14), a single case of *M. bovis* from a roe deer in France, 11 infected fallow deer in Ireland and cases in all three species in the UK (OIE, 2007).

Fallow deer (*Dama dama*)

The first recorded case of bovine tuberculosis (bTB) in a feral deer in the British Isles was in a fallow deer with generalised bTB in Co Wicklow, Ireland in 1974 (Wilson and Harrington, 1976). Infection in fallow deer has been recorded in Denmark (Jorgensen et al., 1988), Ireland (Wilson and Harrington, 1976; O'Reilly and Daborn, 1995), Spain (Gortazar et al., 2008; Romero et al., 2008) and in GB (Delahay et al., 2002, 2007b; Paterson, 2008; Symmons, 2008).

An outbreak in farmed deer in Sweden occurred in the early 1990s and was traced back to deer imported from GB (Bölske et al., 1995) though the original infection was imported from Eastern Europe (Stuart et al., 1988).

Prevalence estimates are below 5% for much of GB (Delahay et al., 2007; Symmons, 2008; Paterson, 2008) but can vary regionally. In the Cotswolds region of western England prevalence estimates as high as 21% have been recorded (Paterson, 2008). In southern England and Wales *M. bovis* was isolated from 18.5% (n = 65) of fallow deer although it is likely that not all of these were genuinely wild deer (Delahay et al., 2002). Simpson (2000) reviewed all MAFF-held records of found dead fallow deer from 1976 to 1999 and estimated infection rates for *M. bovis* of about 1.2% in these wild deer.

In the Doñana National Park (DNP) in southern Spain prevalence estimates of 13–19% have been recorded in fallow deer (Gortazar et al., 2008; Romero et al., 2008). A survey between 1982 and 1984 subsequently identified *M. bovis* infection in 3% (n = 335) of fallow deer from a wildlife centre in the West of Ireland and in 15% (n = 40) of fallow deer in West Waterford, Ireland (O'Reilly and Daborn, 1995). Witte (1940) reported a prevalence of 20.9% in fallow deer in Germany but attributed these unusually high figures to the origins of the animals from a game park and to diagnostic error.

Roe deer (*Capreolus capreolus*)

Roe deer are susceptible to *M. bovis* infection, gross lesions are common. In some cases clinical disease is evident (Proud and Davis, 1998; Delahay et al., 2002). Infected roe deer have been recorded throughout southern England (Gunning, 1985; Rose, 1987; Proud and Davis, 1998; Delahay et al., 2002, 2007b; Paterson 2008) and isolated cases have been recorded in Germany (Schmidt, 1941), Switzerland (Bouvier, 1960), France (Zanella et al., 2008a), Italy (Balseiro et al., 2009) and Poland (Pavlik et al., 2005). Tuberculosis was also

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identified in a roe deer in Spain (Balsiero et al., 2009). Mycobacterial culture was unsuccessful but PCR identified MTBC mycobacteria and species other than *M. bovis* are only rarely identified in ruminants in Spain (Parra et al., 2006).

Infection in roe deer tends to be reported as isolated cases (Pavlik et al., 2005; Balsiero et al., 2009). In GB, prevalence estimates range between 1–3%, with higher prevalence in the Cotswolds region (Rose, 1987; Proud and Davis, 1998; Simpson 2000; Delahay et al., 2002, 2007b; Paterson 2008).

Red deer (*Cervus elaphus*)

Infection of red deer with *M. bovis* has been reported from Denmark (Clausen and Korsholm, 1991), Czech Republic (Pavlik et al., 1998), Ireland (Dodd, 1984; Quigley et al., 1997), Switzerland (Bouvier, 1963), Germany (Witte, 1940), Hungary (Pavlik et al., 2005), France (Zanella et al., 2008a), Portugal (Duarte et al., 2008), Spain (Parra et al., 2006; Hermoso de Mendoza et al., 2006; Vicente et al., 2006; Gortazar et al., 2008; Romero et al., 2008) and England (Delahay et al., 2002, 2007b; Paterson 2008).

In GB the prevalence of *M. bovis* infection in red deer is low. No positive animals were found from surveys of 189 red deer in southern England and Wales from 1971 to 1996 (Delahay et al., 2002). A subsequent survey that targeted bTB “hotspots” in southwest England found infection in 1.02% of red deer (Delahay et al., 2007b). In continental Europe the prevalence of infection in red deer can be high but varies regionally. The prevalence of *M. bovis* among wild red deer in Brotonne, France increased from 13% in 2001/02 to 24% in 2005/06, even after the implementation of control measures (Zanella et al., 2008a).

In Spain, infection is widespread in wild populations of Iberian red deer (*C. e. hispanicus*) (Parra et al., 2006; Vicente et al. 2006; Fernandez-de-Mera et al., 2009). Between 1997 and 2002 the prevalence of tuberculosis in red deer inspected visually in the Extremadura region of western Spain increased from 0.83% to 1.74% (Parra et al., 2006). Infected deer were identified in 14 of 17 sites where deer were sampled in central and southern Spain, with a concentration of infection in a core area around Castilla-La Mancha, Extremadura and Andalucía (Vicente et al., 2006). Prevalence estimates ranged from 1.1% with gross lesions in the Extremadura region (Hermoso de Mendoza et al., 2006) to 27% in red deer in the Doñana National Park (Gortazar et al., 2008).

In Austria *M. caprae* has been isolated from gross lesions from seven free living red deer (Glawischnig et al., 2003). One red deer infected with *M. caprae* was identified in Germany in 2005 and another in 2007 (CVO questionnaire; H.J. Bätza, 2009). *M. caprae* has also been identified in red deer in Spain (Gortazar et al., 2005).

Other species

M. bovis infection in sika deer (*Cervus nippon*) has been recorded in Ireland and the U.K. (Dodd 1984; Rose 1987; Delahay et al., 2002). In England, MAFF investigations collated by Delahay (2002) found infection in 2.1% of sika deer. Previous surveys in GB had identified infection in four of 81 (5%) sika deer (Rose, 1987).

Delahay et al. (2001a) reported *M. bovis* infection in a single wild Reeves’ muntjac (*Muntiacus reevesi*) shot in Gloucestershire, England. A subsequent survey of wild mammals concentrated in areas where bTB was common in cattle and badgers, identified 5.2% of muntjac infected with *M. bovis* (Delahay et al., 2007a).

M. bovis has also been recovered from semi-wild reindeer (*Rangifer tarandus*) in the UK (Lovell, 1930) and from elk (*Alces alces*) in Sweden (Hulphers and Lilleengen, 1947). An

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outbreak of *M. bovis* in a herd of axis deer (*Axis axis*) in a wildlife park in England occurred in 1964 (Jones et al., 1976).

A survey of 1,000 wild and captive cervids in Germany between 2002-2005 found no *M. bovis* in a mixed population of wild red, roe and fallow deer but detected *M. bovis* infection in some animals from a game reserve (Moser et al., 2007).

Table 2. Prevalence and identification of *M. bovis* or *M. caprae* in wild deer in Europe (adapted and updated from Clifton-Hadley and Wilesmith, 1991).

Species	Country	Region	Prevalence	Sample	Comments	Reference
Red deer	Austria		n/a			OIE 2002-2007
	Austria	Northern Alps		7 cases	1999-2001 <i>M. caprae</i> identified	Glawischnig et al., 2003
	Czech Republic			1 case	Free-ranging red deer in 1991	Pavlik et al., 1998
	Denmark	Jutland	0.6%	1/170	1983-1989	Clausen and Korsholm, 1991
	France	Normandy	13%	9/72	2001/02	Zanella et al., 2008a
			24%	33/138	2005/06 Submissions by hunters	
	Germany		0.09%	66/77035	Based on gross pathology	Reported in Witte, 1940 (CVO questionnaire; H.J. Bätza, 2009).
	Germany			2	<i>M. caprae</i> was identified 2005 and in 2007	
	GB	SW	<1%	1/136	Hunters submissions. Culture.	Paterson, 2008
	GB	SW	1.02%	2/196	Culture and post mortem	Delahay et al., 2007b.
	Hungary				6 red deer identified between 2000-2004; passive surveillance, submissions of suspect carcasses	Pavlik et al., 2005
	Ireland		2.6%	9/340	National park	Quigley et al., 1997
	Ireland		n/a			Dodd, 1984
	Italy		n/a			OIE 2002-2007
	Portugal		n/a			OIE 2002-2007
	Spain	Donana	15.5%	26/168	Bacteriology; random hunting for health surveillance 1998–2003	Romero et al., 2008
	Spain	Donana	27.4%	26/95	<i>M. bovis</i> isolation (2006-2007)	Gortazar et al., 2008
Spain	South & Central	0-50%		Prevalence of tuberculous-like lesions	Vicente et al., 2006	

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Species	Country	Region	Prevalence	Sample	Comments	Reference	
Roe deer	Spain	Extremadura	0.83%		1997	Parra et al., 2006	
			1.74%		2002		
		Spain	Extremadura	1.1%		Meat inspection of hunted carcasses hunted showed suspect lesions	Hermoso de Mendoza et al., 2006
		Switzerland		n/a			
		France	Normandy	0.02%	1/53		Bouvier, 1963
		GB	SW	0%	0/239		Zanella et al., 2008a
		GB	Cotswolds	3%	2/69	local incidences from 0% to 7%	Paterson, 2008
		GB		1.02%	9/885	Area endemic for cattle TB	Delahay et al., 2007b.
		GB		0.03%	1/36		Rose, 1987
		GB		0.9%		1971-1996; includes parkland deer.	Delahay et al., 2002
		GB		0.8%		1976-1999; 1013 wild roe deer, some overlap with Delahay et al., 2002	Simpson, 2000
		GB	SW	1.3%	3/236	all of which showed gross lesions and one of which showed clinical disease shot or found dead roe deer grazing near reactor cattle in an estate in (1993-1996)	Proud and Davis, 1998
		GB	Wiltshire			1980 and 1984	Rose, 1987
		GB	Wiltshire		1 case	Ailing buck, shot and submitted for post mortem and culture	Gunning, 1985
		Italy	Aosta Valley		1	RTA	Balseiro et al., 2009
	Italy					OIE 2002-2007	
	Poland				2 animals identified between 2000-2004	Pavlik et al., 2005	
	Spain	Valdés, Asturias		1	No mycobacteria cultured but PCR identified M. tuberculosis complex	Balseiro et al., 2009	
	Switzerland		1.2%	11/892	Associated with infected cattle	Bouvier, 1960	

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Species	Country	Region	Prevalence	Sample	Comments	Reference
Fallow deer	Denmark					Jorgensen 1988
	Germany		20.9%	3762/18042	Animals from large game reserves	Reported In Witte 1940
	GB	Cotswolds	20.5%	27/132	incidences from 0% to 26%	Paterson, 2008
	GB	SW	4.1%	2/121	local incidences from 1% to 13%	Paterson, 2008
	GB	Wye Valley	3.1%	4/128		Symmons, 2008
	GB		18.5%		1971-1996; not all wild deer	Delahay et al., 2002
	GB		1.2%		332 wild fallow deer; some overlap with Delahay et al., 2002.	Simpson 2000
	GB		4.4%	22/504	Area endemic for cattle TB	Delahay et al., 2007b.
	Ireland					OIE 2002-2007
	Ireland	South	15%			O'Reilly & Daborn 1995
	Ireland	West	3%			O'Reilly & Daborn 1995
	Ireland	Co. Wicklow		1 case	One found-dead female deer	Wilson and Harrington, 1976
	Spain	Donana	12.7%	17/134	Bacteriology; random hunting 1998–2003	Romero et al., 2008
Spain	Donana	18.5%	18/97	<i>M. bovis</i> isolation (2006-2007)	Gortazar et al., 2008	
Reeves' muntjac	GB		5.2%	3/58	Area endemic for cattle TB	Delahay et al., 2007b.
	GB		0%	0/5	1971-1996;	Delahay et al., 2002
Sika	Ireland					OIE 2002-2008
	GB		2.1%		1971-1996;	Delahay et al., 2002
	GB		5%	4/81		Rose 1987
Sika or sika x red	ROI		3.8%	5/130	<i>M. bovis</i> isolation. Survey of deer killed by licensed hunters in cattle breakdown areas 4 sika, 1 red x sika	Dodd 1984

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Species	Country	Region	Prevalence	Sample	Comments	Reference
Unspecified	GB	SW England SE England	1.1%	8/734 1/2000	Associated with cattle breakdowns and infected badgers	Philip 1989

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3.2 The disease in deer

Pathogenesis and routes of infection

The distribution of lesions indicates that inhalation and ingestion are the most common routes of infection in cervids. Frequent tonsillar involvement supports oral and/or respiratory exposure (Lugton et al., 1998). The time course of infection is generally slowly progressive and fulminant (acute) infections are less frequently observed.

Information about the time-course of lesion development is derived from experimental studies. In intra-tonsillar infected white-tailed deer (*Odocoileus virginianus*) the first gross lesions were observed in the lungs at day 42 post infection (pi) and consisted of necrosis and mineralization. At day 56 pi the lesions had a thin capsule of peripheral fibrosis. At day 328 pi there was liquefaction of the necrotic centres, giving the gross appearance of an abscess. There was a tendency for establishment of lesions in the caudal lobes of the lungs (Palmer et al., 2002).

Clinical signs

Most *M. bovis* infected cervids do not show any clinical signs. In advanced disease, emaciation is the most typical sign coupled with extensive lesions of severe chronic tuberculosis. Lethargy may occur. Enlargement of superficial lymph nodes may be observed (Schmidbauer et al., 2007), often accompanied by draining tracts in red deer (Lugton et al., 1998).

Distribution of lesions and pathological findings

Deer apparently have a high propensity to develop severe tuberculous disease. A range of observations from absence of gross lesions (no visible lesions) to single or multiple lesions of variable size, to severe generalized disease have been reported (Gavier-Widén et al., 2009; Zanella et al., 2008b; Martin-Hernando, in press).

There is a preponderance of lesions in medial retropharyngeal lymph nodes. Lesions also frequently occur in the lungs, in lymph nodes draining the lungs and airways (tracheobronchial and mediastinal), and in mesenteric lymph nodes. The tonsils are frequent sites of tuberculous infection, but do not always show gross lesions (Rohonczy et al., 1996; Lugton et al., 1998; Palmer et al., 2002). Purulent tonsillitis is observed in some cases. Tuberculous lesions may also occur in any organ or tissue, such as spleen, liver, bones, and others (Balseiro, et al., 2009; Gavier-Widen et al., 2009).

The reported frequency of involvement of the different body regions (head, abdomen and thorax), in natural tuberculosis in free ranging deer is highly variable. In a report in Spain, lesions were more frequent in the thorax in fallow deer than in red deer (Martin Hernando, in press), while tuberculosis affecting more than one region (head, abdomen, and/or thorax) was found to occur with a similar frequency in both red and fallow deer.

In wild red deer in Europe, granulomas, seen as caseous-abscesses, are of variable size, up to large (more than 30 cm in diameter) fibrous thinly encapsulated abscesses. Abscesses are often located in lymph nodes, such as mesenteric lymph nodes and adhering to adjacent tissues (Zanella et al., 2008b; Martin Hernando, in press; Rhyan et al., 1992). Calcification may not be observed grossly (Zanella et al., 2008b).

Wild red deer in France exhibited a high prevalence of macroscopic lesions (24.1%). The mesenteric lymph nodes were most commonly affected, either as a single site or in combination with lesions in retropharyngeal and pulmonary lymph nodes. There was a predominance of severe lesions (abscesses), and frequent generalized infection (18% of

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cases) (Zanella et al., 2008b). This description agrees with findings from red deer in other parts of Europe, such as Austria (Glawischnig et al., 2003). In Spain, lesions were most frequent in retropharyngeal lymph nodes, followed by involvement of the abdominal cavity, with lesions in the mesenteric lymph nodes and the ileocaecal valve, the latter having lesions in 60% of cases (Martin Hernando, in press). In Europe, it has been suggested that mesenteric lymph nodes are the main site of *M. bovis* replication in wild red deer (Zanella et al., 2008b).

In wild fallow deer in Spain (Martin Hernando, in press) and Ireland (Wilson and Harrington, 1976), the thoracic region was most frequently involved. Lesions consisted of enlargement of lymph nodes in the thoracic and abdominal cavities, with caseous-necrotic tubercles with some calcification. Similar lesions were observed in the lungs (Wilson and Harrington, 1976).

Cases in roe deer in England, Spain and Italy, were described as caseating tubercles, up to 6 cm, in the tonsils, lungs, mediastinal and bronchial lymph nodes, and an isolated case in the spleen (Gunning, 1985). *M. bovis* was cultured from one roe deer with no macroscopic lesions (Zanella et al., 2008a). Presence of AFB varied from none to abundant (Balseiro, et al., 2009; Gunning, 1985).

Tuberculous lesions have been described in the lungs, and retropharyngeal, submaxillary and mediastinal lymph nodes of sika deer (Dodd, 1984).

Descriptions of tuberculosis in white-tailed deer in North America indicate the medial retropharyngeal lymph nodes as the most commonly affected site followed by the lungs (Palmer et al., 2000). To our knowledge, tuberculosis has not been detected in introduced white-tailed deer populations in parts of Europe.

Gross and histopathology

The main difference between lesions in cervids and cattle is that in the former, fibrosis at the periphery of granulomas is thinner and necrosis in advanced lesions may liquefy, forming abscesses (Palmer et al., 2002). The gross tuberculosis lesion (granuloma) in cervids varies from:

- a. small (1 mm) white foci.
- b. round thinly-encapsulated tubercles with central caseous necrosis with or without calcification, varying from a few millimetres up to more than 10 cm in diameter. The area of necrosis expands as granulomas increase in size. Granulomas in lymph nodes may markedly enlarge the nodes and completely replace the lymphoid tissue.
- c. abscess-like lesions containing yellow-white purulent exudates. Abscesses or diffuse purulent inflammation, for example purulent pneumonia, appear to be a peculiar feature of tuberculosis in cervids and are often described in naturally infected cervids of all species (Palmer et al., 2000; Whiting and Tessaro, 1994). Abscesses often contain large numbers of mycobacteria and are considered to be important in the dissemination of the infection, especially if they open into an airway.
- d. diffuse tuberculous consolidation in the lungs

Microscopically, deer often have classic granulomas with partially mineralized, multifocal to coalescing caseous necrosis surrounded by a mantle of epithelioid macrophages, Langhans type multinucleated giant cells, lymphocytes, plasma cells, variable neutrophils and a variably thick fibrous capsule. Red deer may form suppurative abscesses or pyogranulomas and sika deer can have abundant, bizarre, large, irregular giant cells (Rhyan and Saari, 1995).

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3.3 Diagnostics

Diagnosis in non-bovines, including deer, has been the subject of several reviews (Livingstone, 2001; de Lisle et al., 2002; Cousins and Florisson, 2005; Chambers, 2009). The full range of tests including intra-dermal skin tests, PCR, culture, serology, IFN γ available for farmed deer has recently been reviewed and estimates of sensitivity and specificity derived from a meta-analysis were reported for each test (EFSA, 2008). Bacterial culture remains the diagnostic gold standard for the identification of *M. bovis* in wild deer (Aranaz, et al., 2004; Delahay et al., 2002, 2007b). Immunological methods for the detection of bTB in wildlife are important not only for diagnosis *per se* but in surveillance programmes that require live sampling then release of animals, and research activities.

Post mortem, culture and PCR

In wild deer, tuberculosis is often detected *post mortem*. Gross pathology has been used extensively as a preliminary diagnostic tool for deer, particularly in hunted deer and is often confirmed subsequently by bacterial culture (Aranaz et al., 2004; Zanella et al., 2008a; Paterson, 2008). In some cases gross pathology is used as a definitive method of diagnosis. For instance, prevalence estimates have been based on tuberculous-like lesions (Vicente et al., 2006). Estimates of sensitivity and specificity of necropsy or meat inspection obtained by a recent meta-analysis ranged from 63 to 81% and 72 to 94% respectively, with necropsy being more sensitive and specific (EFSA, 2008). Necropsy is often performed where tuberculosis is already suspected. In a deer survey in GB where deer stalkers were encouraged to submit samples and state where they identified lesions, only half of deer where hunters identified lesions by visual inspection were determined to be infected with *M. bovis* following culture (Paterson 2008). Conversely 14 of the 29 animals where *M. bovis* was isolated by culture had no visible lesions.

Histopathology is a highly sensitive and rapid tool for the detection of tuberculosis. The technique can be used to identify suspect cases of tuberculosis and can also enhance specificity by excluding tissue changes that are caused by helminth parasites, other bacterial or fungal infections and some neoplasms (de Lisle et al., 2002). However histopathology is limited in that it cannot differentiate between *M. bovis* infection and tissue changes resulting from infection with other mycobacteria (de Lisle et al., 2002). Sensitivity and specificity of histopathological analysis in naturally infected white-tailed deer in Michigan has been estimated at 98% and 87% respectively (Fitzgerald et al., 2000). The use of acid-fast staining improved specificity (from 87% to 97%) but reduced the positive predictive value (Fitzgerald et al., 2000).

Bacterial culture is the gold standard for establishing a diagnosis of tuberculosis in deer but is an expensive, lengthy process. It can take up to 12 weeks to ensure a sample is negative (Pritchard, 1988; Reid, 1997). The use of a genetic probe in white-tailed deer reduced this time considerably to 1–8 weeks. The probe had excellent sensitivity and specificity for the detection of tuberculosis, although it was specific to the MTBC group and could not be used to identify mycobacterium species (Fitzgerald et al., 2000). A species-specific PCR has been used to identify *M. bovis* infection in elk in less than 24 hours (Shringi et al., 2007). PCR is a faster, sensitive method of identification of tuberculosis infection in live animals, although despite widespread use, a standardised procedure for PCR does not yet exist (EFSA, 2008). Nasal swabbing from live animals has not been successful (de Lisle et al., 1984). Sensitivity and specificity estimates for culture (Sensitivity 74% (67-79%) and Specificity 97% (47-100%)) and PCR (Sensitivity 87% (90-92%) and Specificity 99.5% (94-100%)) have been recently reviewed (EFSA 2008).

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Gross pathology, histopathology and culture do not always recognize the same animals as being infected (Rohonczy et al., 1996). Studies in elk and red deer in Michigan found the sensitivity of gross pathology was 93% and was greater than histopathology (88% to 89% depending on the method used), but that gross pathology was less specific. Maximising sensitivity could be achieved by interpreting culture, *post mortem* observations and histopathology in parallel. It is not always possible to culture from visible lesions but conversely, *M. bovis* can be isolated from tissue where there are no visible lesions (Hunter, 1984; Clifton-Hadley and Wilesmith, 1991; Rohonczy et al., 1996).

Cellular immunology

The principal immunological response of the host to infection with pathogenic mycobacteria is the acquired cellular immune response, exemplified by the proliferation of lymphocytes and the production of cytokines such as IFN γ by T-cells. With relatively few exceptions (Eurasian badger and wild boar), the majority of bTB diagnostic tests for European wildlife based on cellular immunity have been developed for red deer (Table 3).

The mainstay of diagnosis of bTB in cattle is the intradermal delayed-type hypersensitivity reaction caused by injection of PPD-B (the tuberculin skin-test). This test appears to work well in deer (Corrin et al., 1993), but is impractical for free-ranging deer because of the need to measure the cutaneous reaction some 24-72 hours after the injection of tuberculin. A recent study applied the comparative cervical skin-test to wild red deer captured by the National Parks Services in Spain and obtained an apparent prevalence figure of 19% for 111 deer (Fernandez-de-Mera et al., 2009).

Table 3. Summary of CMI (cell-mediated immunity) assays employed in *Cervus elaphus* infected with *M. bovis*

Experimentally (E) or Naturally (N) infected	Method employed ¹	Stimulatory antigen(s)	Reference(s)
N	LPA	PPD-B, MPB70	Griffin et al., 1991
N	TST, LPA	PPD-B, PPD-A	Griffin et al., 1994
N	TST	PPD-B, PPD-A	Gaborick et al., 1996
E	qRT-PCR (for various cytokines)	PPD-B	Harrington et al., 2006, 2007
E	LPA, IFN γ EIA (commercial test)	PPD-B	Harrington et al., 2007

LPA = lymphocyte proliferation assay; TST = tuberculin skin test; qRT-PCR = quantitative real-time polymerase chain reaction; EIA = enzyme immunosorbent assay.

Increasingly, efforts have been made to develop *in vitro* assays of cell-mediated immunity (CMI) based on the measurement of lymphocyte proliferation or the detection of cytokines such as IFN γ produced following the stimulation of blood mononuclear cells in culture with mycobacterial antigens such as PPD, purified antigens or even peptides, such as the commercially available assay for the detection of IFN γ in deer (Cervigam, Prionics Ag, Switzerland) (Waters et al., 2008). In the US, the Cervigam test has been evaluated for use in *M. bovis*-infected white-tailed deer and reindeer with variable results (Palmer et al., 2004c; Waters et al., 2004). Results were more consistent for reindeer with specificity ranging from 83-94% depending on antigen used and the cut off value (Waters et al., 2008). Relatively

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little effort has been focused on the detection of cytokines other than IFN γ in deer, as the latter appears to be the cytokine most associated with mycobacterial infection across species. However, a quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) method was successfully applied to red deer, where levels of mRNA were determined for IL-2, IL-4, IL-10, IL-12p40, IFN γ , TNF α , and enzyme-inducible nitric oxide synthase (iNOS) using qRT-PCR on peripheral blood PBMC from *M. bovis* experimentally infected animals (Harrington et al., 2006). Different durations of stimulation with PPD-B were required for optimal measurement of different cytokines. Subsequently, success with the IFN γ qRT-PCR has been reported using whole-blood cultures from red deer (Harrington et al., 2007), thereby increasing the utility of the method. IFN γ mRNA levels correlated to the assay of IFN γ protein, and the qRT-PCR assay was found to be more accurate than either lymphocyte proliferation or the commercial IFN γ protein ELISA (Cervigam).

Serology

Whilst frequently less sensitive for the diagnosis of bTB in wildlife than assays of CMI, serological methods remain popular because of their relatively low cost, simplicity, speed, and ability to perform the test on stored rather than fresh samples. The most common format for serodiagnosis is the enzyme-linked immunosorbent assay (ELISA), in which a variety of target antigens have been used for deer: PPD-B, PPD-A, MPB70, *M. bovis* culture filtrate, and lipoarabinomannan (Griffin et al., 1991, 1994; Gaborick et al., 1996). With knowledge of the principal antigens recognized by infected animals, a commercial lateral-flow immunochromatographic test (ICT) (Stat-Pak, Chembio Diagnostic Systems, Inc.) was developed for the diagnosis of bTB in a variety of wildlife species, including white-tailed deer (Lyashchenko et al., 2008). The performance (the number of bTB positive and negative cases correctly identified by the test) of the Stat-Pak for the detection of bTB varied between species; being highest in white-tailed deer (97%).

Improving the sensitivity of immunodiagnosis

Antibody responses in naturally infected red deer were boosted by intradermal injection of PPD for skin testing (Griffin et al., 1994). The boosting effect of the skin test significantly increased the sensitivity of an ELISA performed 10 days later from 45.7% to 85.3%. Importantly, the 'boosted ELISA' revealed 11 heavily infected deer that had been negative in the skin test, revealing the value of performing additional tests, especially as adjuncts to any tests that are routinely performed or required by national legislation. Frequently, the sensitivity of serodetection has been improved by the inclusion of more than one antigen target. Sometimes specific purified antigens have been combined, and in other cases cruder antigen preparations have been used, such as PPD-B or *M. bovis* culture filtrate (Griffin et al., 1991, 1994). In a few cases in deer, non-proteinaceous, mycobacterial lipoarabinomannan (LAM) purified from the bacterial cell was used (Gaborick et al., 1996; Waters et al., 2004). Combining antigens was demonstrated, or believed, to result in improved sensitivity. An association between increased test sensitivity and more severe tuberculosis has been observed in red deer (Griffin et al., 1991). A few studies reported the greatest sensitivity of bTB detection when a combination of tests was used; such as the combination of a serological and a CMI-based test in red deer (Griffin et al., 1994). Whether the use of more than one test is practical or cost-effective is doubtful in some situations, and is an approach likely to result in an increased number of false positive results, although this was not the case for red deer (Griffin et al., 1994).

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Influence of other mycobacteria on test specificity

Many wildlife species will be variably exposed to environmental mycobacterial species (Corner et al., 1981; Stainsby et al., 1989; Bercovier and Vincent, 2001; Mikota et al., 2001). In such situations, common or cross-reacting antigens between the environmental mycobacteria and *M. bovis* may reduce test specificity. Use of individual antigens should improve the specificity of such tests but this cannot be taken for granted. For example, whilst MPB70 is a common antigen of choice, as it appears specific to *M. tuberculosis* complex mycobacteria, there is a cross-reacting antigen in *Nocardia asteroides* (Harboe and Nagai, 1984). Serotypes of *N. asteroides* have been isolated from soil samples and occasionally from non-bovine animals (Pier and Fichtner, 1981). Another common seroreactive antigen in *M. bovis* infection, MPB83 (Lyashchenko et al., 2008), is also expressed by the environmental mycobacterium, *M. kansasii* (Vosloo et al., 1997). Occasional cases of *M. kansasii* infection of deer do occur (Delahay et al., 2007b).

3.4 Epidemiology

Evidence for transmission amongst deer, between deer and other wildlife, and from cattle to deer

Genotyping has been used to investigate transmission pathways between deer, other wildlife species and domestic livestock (Duarte et al., 2008). Spatial associations between common strains of *M. bovis* do not indicate the direction of transmission, but do indicate flow of infection between species or infection from a common source. In Ireland, direct repeat restriction fragment length polymorphism (DR-RFLP) analysis has shown isolates of the same strain in feral deer that are common in cattle (Skuce et al., 1996). Spoligotyping and RFLP analysis has been used to show spatial associations between common strain types in cattle, badgers and deer in Ireland, suggesting that transmission is occurring between these species (Costello et al., 1999). Restriction endonuclease analysis (REA) was used in an epidemiological investigation of an outbreak in farmed fallow deer in Sweden and demonstrated transmission within deer populations (Bölske et al., 1995). All affected deer holdings had imported, or been in contact with, infected deer that had been imported into Sweden from Britain (Bölske 1995). This was subsequently confirmed with RFLP analysis using the insertion sequence IS6110 (Szewzyk et al., 1995).

Spoligotypes from Spanish wild deer show the same pattern as some domestic bovine isolates, suggesting transmission is occurring between these animals (Aranaz et al., 1996). Infected red deer, fallow deer and a range of wild and domestic animals from similar geographical areas were infected with the same *M. bovis* strains. Spoligotyping and mycobacterial interspersed repetitive units-variable number tandem repeats (MIRU-VNTR) was used by Romero et al. (2008) to establish association between and within infection in wild animal populations and cattle. In France, all wildlife (red deer, roe deer, wild boar, fox) found to be infected with *M. bovis* in an area in Normandy under study had the same spoligotype and VNTR pattern, suggesting transmission was occurring between species and this pattern was also identical to the strain that had been circulating in nearby cattle herds since at least 1995 (Zanella et al., 2008a). The finding of the same spoligotype pattern over several years in deer and wild boar, and infection in deer and boar in areas where there are no domestic cattle, suggests that infection is being maintained within the wildlife population in Spain (Aranaz et al., 2004), although it is not known if deer can maintain infection in the absence of another wildlife reservoir such as wild boar.

Risk factors for infection in wild deer

The differentiation of a spill-over host from a maintenance host is based on general pathology, such as the location of lesions, and ecological factors such as population density, behavioural characteristics and opportunities for interaction in the same habitat (Aranaz et al., 2004). According to these criteria some deer species and populations may be maintenance hosts in Spain (Aranaz et al., 2004) and in GB (Delahay et al., 2007b). High infection rates in adult red deer in the Brotonne Forest in France and the presence of infection in juveniles suggests that transmission is occurring within the red deer population, although the wild boar population is also affected (Zanella et al., 2008a).

Risk factors for the infection of wildlife from other wild species and from infected domestic animals include high population density, ecological and behavioural characteristics that enhance transmission opportunities, food shortages or other events that cause clustering of animals and events that force wildlife to extend their range or usual food source (Hunter, 1996; Delahay et al., 2001b, 2007b; Gortazar et al., 2007). Contact with farmed deer, the

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quality of fence lines between wild and farmed deer and ingress or egress by free ranging deer all serve to increase transmission opportunities (Hunter, 1996).

Parra et al. (2005) investigated risk factors associated with tuberculosis transmission to wild ungulates. Sampling was carried out on private estates where wild Iberian red deer and boar were kept for hunting purposes, and cattle and other livestock were also present. They found that the level of clustering, or transmission between species increased over time with the prevalence of disease and was associated with the period immediately after the deer reproductive season, which may facilitate active transmission.

It is common for infection in deer to be associated with infection in domestically farmed cattle, deer or other wild maintenance hosts (Aranaz et al., 2004; O'Brien et al., 2006). Early cases of infection in roe and sika deer were identified in Wiltshire and Dorset, in southern England, both areas of high infection in cattle (Rose, 1987). An investigation of farms with persistent breakdowns in Dorset found 4.3% of the local deer population was infected (Rose, 1987) compared with only 1 in 2000 deer where cattle breakdowns were uncommon (Phillip, 1989). In the Doñana National Park, where bTB is widespread in species such as red deer and wild boar, it has been suggested that infection came from cattle, as no cases of bTB in wildlife had been reported before an uncontrolled increase in the local cattle population (Aranaz et al., 2004). The highest prevalence estimates for wildlife were recorded in an area of Doñana where cattle had been excluded since 2005, suggesting that *M. bovis* can circulate within wildlife without the need for amplification in a cattle host.

In Spain high prevalence of tuberculous lesions in red deer in south and central Spain is associated with high prevalence of infection in wild boar (Parra et al., 2006; Vicente et al., 2006, 2007a). Known risk factors for infection in populations of red deer managed for game in Spain include increasing age and prevalence in wild boar (Vicente et al., 2006), sex and wild boar spatial aggregation at feeding and watering places (Vicente et al., 2007). Infected deer in England have been found in areas known to have infected badgers (Rose 1987; Delahay et al., 2002) but surveys have been concentrated in these areas and so results are somewhat skewed.

Host behaviour can enhance or limit the spread of disease within and amongst species. Fallow deer are expected to have a higher prevalence due to their feeding patterns (grazing rather than browsing), more gregarious nature and larger feeding ranges as they do not establish territories outside of the rutting season (Clifton-Hadley and Wilesmith, 1991). The clinical response to infection in deer can be diverse and unpredictable, from no appreciable disease, to a sub-acute response followed by sudden death (Clifton-Hadley 1991; Reid, 1997). Animals with low-grade disease can still shed bacteria over relatively large areas and these individuals remain a risk of further infection.

Transmission

The pattern of pathology in deer suggests that a respiratory route of infection such as nose to nose or fence-line contact is important (Delahay et al., 2002). Congregation around shared sources of food, especially in times of food scarcity provides opportunity for close contact and direct transmission. Fence line and nose to nose contact is considered a risk factor for transmission of infection between wild deer and either farmed deer or cattle, but animal activated cameras documented little interaction between wild and farmed white-tailed deer in Michigan, probably due to the social group structure of the deer populations (Vercauteren et al., 2007).

Contaminated feed is a source of infection for deer and for transmission between deer and cattle. *M. bovis* can survive on hay for 7 days and could still be isolated from samples of

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apples, corn and potatoes at 112 days after contamination (Palmer and Whipple, 2006). Transmission of *M. bovis* occurred in all white tailed deer given feed previously contaminated by experimentally infected deer, suggesting that this is an effective indirect source of transmission (Palmer et al., 2004b) The distribution of lesions in the indirectly infected deer was concentrated in the lungs, tracheobronchial and mediastinal lymph nodes, suggesting an aerosol route of infection, although the exposed deer were housed together and direct transmission between the deer could not be ruled out.

Under appropriate conditions in New Zealand, *M. bovis* can survive in the environment for up to 28 days (Jackson et al., 1995). Laboratory experiments demonstrated the survival of *M. bovis* for up to 4 weeks in soil held in 80% shade (Duffield and Young, 1985). *M. bovis* has been isolated from water on farm yards (Little et al., 1982b) and this could be an added source of contamination.

Human activities

The spread of urban populations and increasing agricultural requirement for productive land may force increased interaction between livestock and free ranging animals, thus increasing transmission opportunities (Hunter 1996).

In white tailed deer transmission of infection was thought to have been encouraged by the long-established public practice of feeding large volumes of supplementary feed to prevent migration and keep deer numbers high for hunting purposes (Miller and Kaneene, 2006). This situation has also been observed in red deer in Exmoor, Devon, southern England where management regimes and supplementary feeding resulting in overstocking has been suggested to be responsible for the spread of bTB in the deer population (Green, 2004). Similarly in Spain, increases in bTB in red deer and wild boar populations in the Extremadura Mediterranean region in Spain have been observed since a change in game management practices to increase the economic return from hunting. Practices such as more effective game fencing around the perimeter of estates, shrub removal and feed supplementation in times of food shortages have increased animal density and provided more opportunity for disease transmission (Hermoso de Mendoza et al., 2006; Parra et al., 2006).

Risk to cattle, domestic animals and humans from deer

Experimental studies show that *M. bovis* can successfully be transmitted from deer to cattle. All nine cattle that were transferred between pens that had housed experimentally inoculated deer became infected (Palmer et al., 2004a, b). Wild deer have been implicated in the transmission of infection to cattle (Wilson and Harrington, 1976) although a case control study in Northern Ireland found the presence of wild deer was not a risk factor for cattle bTB infection (Denny and Wilesmith, 1999).

Free ranging and captive deer have been implicated in the spread of tuberculosis in cattle in the USA, Canada and New Zealand (Essey and Koller, 1994; de Lisle et al., 2001; O'Brien et al., 2006), to humans (Fanning and Edwards, 1991; Wilkins et al., 2003) and to other species of deer and wildlife (O'Brien et al., 2006). In Michigan, a case control study of risk factors that influenced *M. bovis* infections in cattle found the prevalence of bTB in wild white tailed deer around the farm increased the risk of cattle breakdowns, and farms where measures had been taken to exclude wild deer or that bordered on land where wild deer were less common had a lower risk of cattle breakdowns (Kaneene et al., 2002). Wild deer are also likely to present a potentially significant risk of transmission to farmed deer (Gunning, 1985).

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Sharing feed with deer may provide a potential route for infection of cattle. The provision of unfinished feed from experimentally inoculated deer to cattle resulted in the infection of 4 out of 9 calves (Palmer et al., 2004a). In Switzerland roe deer were suspected of infecting cattle by contaminating pasture (Bischofberger and Nabholz, 1964).

Delahay et al. (2007b) carried out a semi-quantitative risk assessment to estimate the degree of risk to cattle (relative to that presented by the badger) from each species in which *M. bovis* had been isolated in their survey. Their assessment incorporated the range of prevalence estimates from each species, extent of potential bacterial excretion, likelihood of contact with cattle and approximate biomass. The results suggested that the risk to cattle from deer, particularly fallow and red deer, was potentially substantial, relative to that presented by the badger. However, given the variation in deer densities throughout GB, any risk is likely to be localised.

3.5 Ecology and host population monitoring

Ecology

The most widely distributed and abundant species of deer in Europe are red deer, roe deer and fallow deer. In some northern latitudes, European elk (moose) are found and reindeer persist in Scandinavia (usually semi-domesticated). Non-native species are also established in some European countries, such as Reeves' muntjac (Britain, Netherlands), Japanese sika (Austria, Denmark, Germany, Britain, France, Ireland) and Chinese water deer (*Hydropotes inermis*; Britain, France).

The feeding habits and habitat preferences of deer will have a significant impact on their exposure to *M. bovis* in the environment, and the likelihood of direct and indirect contact with domestic cattle. Deer can be classified according to their feeding strategies (Hofmann, 1985). Roe deer are selective browsers of herbs, shrubs and trees, red deer are both grazers of grasses and browsers of shoots and herbs, whereas fallow deer are mainly grazers of large volumes of grasses. Clearly grazers are more likely to consume contaminants from grass than browsers.

Red deer favour woodland, but have adapted to open moorland. Individuals in woodland populations tend to be largely solitary, or occur as mother-calf groups. On open ground, they may form large, single-sex groups, only mixing during the breeding season. Roe deer typically inhabit woodland, but can also be found in scrub. At low to medium density they are typically solitary, or exist in mother-kid-yearling groups. At high densities however, they may form mixed-sex herds, and can be observed occupying fields. The breeding season involves aggressive interactions between territorial males, whereas females occupy over-lapping home ranges. Fallow deer favour woodland but also extensively use arable and pasture. In large woodlands, most of the year is spent in single-sex herds, which come together during the breeding season. Populations inhabiting more open environments may persist in large, mixed-sex herds all year.

Herding behaviour results in high intra-specific contact rates, thus elevating the potential for disease transmission, maintenance and spread within the population. Such behaviour may occur seasonally and may differ between the sexes (see above), but may also be influenced by management practices. For example, supplementary feeding of deer results in high local densities, high contact rates, and has been linked to a high incidence of bTB among white-tailed deer in northern USA (Miller et al., 2003). Supplementary feeding of wild red deer (primarily to promote stocks for hunting) continues to be practised in parts of Europe (Putman and Staines, 2004).

As a general rule, most deer avoid direct contact with livestock. However, herding species (red and fallow) have been observed to be less wary of livestock in some localities (A. Ward & R. Delahay, pers. obs.). Deer may frequent grasslands that are used by cattle and may share feed put out for livestock at pasture, potentially leading to indirect inter-specific transmission (Palmer et al., 2004b). Deer may also investigate carcasses, sometimes by 'mouthing' them. In New Zealand investigation of dead and moribund *M. bovis*-infected possums (*Trichosurus vulpecula*) has been linked to transmission of bTB to red deer (Sauter and Morris, 1995; Lugton et al., 1998).

Population monitoring

Methods for estimating the abundance of deer have been extensively reviewed (Buckland, 1992; Mayle and Staines, 1998; Mayle et al., 1999). However, most of the techniques

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considered were used within relatively small areas, and typically aimed at producing 'snapshot' estimates of abundance, rather than detecting trends.

Of the many abundance estimation methods currently available, different approaches may be best suited to particular environmental circumstances (Mayle *et al.* 1999), and to addressing different questions. Across Europe, many countries collect cull returns through statutory or non-statutory agencies (www.face-europe.org). Many also collect information on hunter numbers, and in this way can use cull returns to gauge relative changes in population size and structure at the national scale and within regions (Davey and Aebischer, 2006).

For deer in open habitats, direct census counts of individual populations have been widely used, but their reliability and accuracy has been called into question (Clutton-Brock and Albon, 1989; Trenkel *et al.*, 1998), largely due to the inability of this method to estimate the undetected proportion of the population. However, as an indicator of presence and gross trends, direct counts may be useful. In woodland, deer abundance has most often been estimated using indirect methods, such as dung counts. While this approach can be used to produce reasonably accurate estimates of deer density, considerable effort is required to produce sufficiently precise estimates to detect even large changes over time (Smart *et al.*, 2004). More recently, distance sampling procedures have been applied to deer dung counts, and this has improved estimate precision considerably (Marques *et al.*, 2001). Nevertheless, due to uncertainty associated with species-specific defecation rates and dung persistence periods, low precision of density estimates is likely to remain a problem with dung counting methods, which may be more suited to use as indices of abundance or relative change. Another recently developed approach is to combine information on deer sightings and field signs to derive scores that relate to deer abundance (Cooke, 2006). Recent developments in distance sampling analytical procedures (Buckland *et al.*, 2004) and technology (e.g. forward looking infra red or thermal imaging) has meant that population surveys can be undertaken quickly over fairly large areas of land (e.g. tens of km²) and yet yield precise and accurate density and abundance estimates (Gill *et al.*, 1997; Smart *et al.* 2004; Guenzal, 1997).

3.6 Prevention and control

TB control in farmed deer relies on a test and slaughter policy based on the SICCT test and the application of movement restrictions (Anon, 1992; Bode, 1995; Reid, 1997; More et al., 2009) but there are few systems in place for the detection and control of infection in wild deer. With the exception of Finland and Sweden, bTB in wildlife is not a notifiable disease in EU Member States (de la Rua-Domenech, 2006). In GB The Tuberculosis Order (2007) designated bTB a notifiable disease in mammals (except man), where *M. bovis* is isolated in laboratory samples. bTB is notifiable in wild deer in GB under the Tuberculosis (Deer) Order 1989 (as amended) where suspicion of bTB in any deer (or carcase) must be notified to the Divisional Veterinary Manager (DVM) of Animal Health.

Several methods have been proposed for the control of disease in wildlife. These include the implementation of barriers between wildlife and livestock, the sanitary disposal of carcasses, culling, habitat management and feeding bans to achieve population control and treatment and vaccination of infected or susceptible animals (Gortazar et al., 2007). In many cases transmission to wild deer is associated with infected domestic livestock (Romero et al., 2008). Disease control in a true spill-over host should be possible through directing management efforts at the maintenance host (Corner, 2006). However feral red deer in France and Spain appear to be able to maintain infection levels in the absence of cattle infection (Romero et al., 2008).

Culling and hunting

The surveillance of wild host populations and the identification of over abundance is a first step in control (Gortazar et al., 2007), but overabundance is a difficult concept to define (Gortazar et al., 2005b). Population control in wildlife remains a contentious issue, particularly in infected but endangered or protected wildlife (Green, 2004). The success of culling wildlife as a method of controlling disease has been variable and has been the subject of intense debate (Gortazar et al., 2007). Situations where culling may be most appropriate include host populations that are geographically isolated or comprise non-native introduced species where legal and social constraints are minimal (Gortazar et al., 2007). Extensive depopulation has been employed to control tuberculosis in wildlife in isolated situations. An outbreak in Axis deer in a wildlife park in England between 1964 and 1974 was eventually contained by the removal and hand rearing of newborn calves and the complete destruction of the adult herd (Jones et al., 1976).

Less extreme population reduction and habitat modification can be used to alter host density, distribution and exposure to the pathogen (Gortazar et al., 2007). In late 2002, the increasing prevalence of *M. bovis* among a wild population of red deer in Normandy, France triggered the implementation of control measures, which included the reduction of red deer numbers in conjunction with a ban on supplementary feeding, the proper disposal of hunted viscera and the extension of fencing to reduce contact between wildlife and cattle. Passive surveillance continued to identify increasing infection, rising from 13% in 2001/02 to 24% in 2005/06. However the higher prevalence in adult animals (36%) by 2005/06 compared with juveniles (14%) suggested population control measures may have had some impact (Zanella et al., 2008a). Work to cull the entire red deer population in the area is ongoing (OIE, 2007).

Where culling large numbers of wild, particularly native animals, is socially unpalatable and culturally unacceptable other measures of correcting population overabundance have been utilised. The reduction of the white tailed deer population through increased hunting has been used with some success in Michigan (de Lisle et al., 2002; O'Brien et al., 2006). Despite bTB prevalence figures in the region of 5% in the white tailed deer population in a number of

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counties, widespread deer culling was not considered an option (O'Brien et al., 2006). In this region, where hunting was an integral part of the local economy and the white tailed deer was revered to the point where it had been declared the state mammal, bTB control took a two-fold approach. The first was to decrease the deer population by promoting increased hunting. More hunting permits were issued, including the unlimited issue of permits designed to allow the hunting of deer without antlers. This targeted younger deer and females, an unpopular hunted group, with the aim of reducing the reproductive capacity of the local white tailed deer population. This, in combination with a ban on supplementary feeding and baiting, halved the deer population in the counties where bTB was endemic and bTB prevalence reduced from 5% in 1995 to 1.7% in 2004. The prevalence in yearling animals, a proxy for the rate of new infection as these animals could only have been infected within the past year, decreased from 1.9% to 0% (O'Brien et al., 2006).

Biosecurity

Where test and slaughter, population control or vaccination campaigns are not possible another option that may be more culturally acceptable is containment. This can be achieved by the use of control zones, game-proof fences, cordons and movement control (Bengis et al., 2002). In most cases, other species where bTB is less commonly reported represent spill-over hosts and the best option for control in these wild life populations is control of disease in the maintenance host, by reducing disease incidence and also preventing contact between domestic and wild animals (Bengis et al., 2002). Farmed domestic species have been implicated in the transmission of *M. bovis* to wild deer in GB (Rose 1987; Phillip 1989), France (Zanella et al., 2008a), Spain (Aranaz et al., 2004; Romero et al., 2008) and the USA (O'Brien et al., 2006). Reducing contact between cattle and wildlife will reduce transmission between these hosts. In general, factors that reduced cattle access to wild white-tailed deer or discouraged contact between cattle and deer, such as the use of electric fencing and secure feed storage, reduced the risk of cattle infection in a case control study in Michigan (Kaneene et al., 2002).

Livestock protection dogs have been used for centuries to protect domestic animals from predation and can be used to prevent contact between wild deer and domestic species. The use of livestock protection dogs that had been bred to coexist with cattle reduced the rate of contact between cattle and white-tailed deer in Michigan. The dogs were particularly effective at protecting cattle feed (Vercauteren et al., 2008b).

The compulsory proper disposal of hunting carcasses has been proposed as a measure to reduce the availability of infected carcasses and prevent spread to scavengers (Gortazar et al., 2007). Correct disposal of viscera and the implementation of fencing to reduce contact between wildlife and cattle has played an integral part in the effort to control *M. bovis* in red deer and wild boar in northern France (Zanella et al., 2008a).

Modification of both human and animal behaviour may also reduce the incidence of disease. A ban on supplementary feeding was integral to the control of bTB in white-tailed deer in Michigan (Miller and Kaneene, 2006) with baiting and feeding of deer banned since 1998 in counties in Michigan where bTB has been reported in deer (de Lisle et al., 2002). Bans on supplementary feeding have also been implemented in the Brotonne Forest in France (Zanella et al., 2008a).

Vaccination

Vaccination has been proposed as a viable strategy for controlling disease in wildlife (Buddle et al., 2000; Gormley and Collins, 2000) but further evaluation and modification is needed for it to be a realistic option for use in wild deer. The use of BCG has been evaluated in deer

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(Griffin and Buchan, 1993; Griffin et al., 1998) and experimental studies have shown that BCG can provide protection against disease in red deer (Griffin et al., 1998) and white tailed deer (Palmer et al., 2009).

A deer model of experimental infection has been established and used to evaluate vaccination protocols in deer (Griffin et al., 1998; Griffin, 2000). A series of experimental studies have been carried out to identify variables that influence protective efficacy of vaccination using BCG (Pasteur 1173P2), including vaccine dose, viability and administration. Doses of between 10^4 and 10^7 provided significant levels of protection against infection and disease. Interestingly, higher doses protected against disease only. Single dose vaccines successfully protected against disease but booster vaccination within 6-8 weeks from the primary vaccination was necessary to protect against infection (Griffin et al., 1998). This is an obvious difficulty in the deployment to a wild animal disease reservoir. An adequate candidate vaccine for wild deer should aim to break transmission between wildlife and cattle and within wildlife, and does not have to prevent infection altogether (Buddle et al., 2006). Of more utility in wild animals is an oral route of vaccination and these studies showed that a single dose of live BCG administered via the tonsillar crypt gave similar levels of protection to a single dose administered subcutaneously (Griffin et al., 1998).

3.7 Knowledge gaps and prospectus

When dealing with an increasing disease that has become endemic in free-ranging wildlife it is considered vital to:

1. determine the spatial distribution of disease
2. identify major maintenance hosts
3. determine the prevalence rates in maintenance hosts
4. identify spill-over hosts
5. identify transmission models
6. identify the original source of infection
7. identify human activities that may increase transmission rate, e.g. supplementary feeding
8. evaluate ante-mortem tests in wildlife
9. vaccinate
10. identify physical barriers of movement for hosts (Bengis et al., 2002).

Many of these factors have yet to be determined with regard to infection in wild deer.

Prevalence estimates

True prevalence estimates of infection with *M. bovis* or *M. caprae* in wild deer are rare. The extent of infection in wildlife in most counties and transmission pathways from cattle or known maintenance hosts to other wildlife species are not well understood. Most investigations have a significant amount of bias. Several prevalence surveys in deer have examined carcasses obtained from hunters (Aranaz et al., 2004, Parra et al., 2006 ; Zanella et al., 2008a; Paterson 2008). Hunted animals are a biased sample, weighted heavily towards male animals and so do not necessarily provide an accurate reflection of prevalence (Aranaz et al., 2004). Other surveys have been conducted in response to persistent cattle breakdowns (Rose 1987), or in regions known to have on-going, extensive infection in cattle (Delahay et al., 2007b) or wildlife (Parra et al., 2006). There may be other reservoirs of bTB in animals or regions that have not yet been tested.

Large numbers of carcasses are necessary for the detection of low prevalence. In Spain prevalences of less than 1% were estimated using more than 50,000 deer carcasses (Parra et al., 2006). To obtain reliable estimates of prevalence, and also of the degree of risk of infection within this wildlife species to domestic animals, there needs to be an assessment of the size of the local population (Delahay et al., 2002). Few studies have attempted to assess the size of the wildlife population, exceptions include Delahay et al. (2007b), Vicente et al., (2007b) and Zanella et al. (2008a). Regional variation and degrees of spatial aggregation of *M. bovis* infection in deer have been recorded and this can substantially affect measures of prevalence (Delahay et al., 2007b). Clearly, further investigations and surveys in wild deer are required to fully evaluate and understand the extent of *M. bovis* infection in free-living deer.

The sensitivity of the diagnostic test used to establish infection influences prevalence estimates. In general prevalence estimates are derived from the gold standard of isolation of *M. bovis* from tissue culture. Necropsy examination for gross lesions is faster and less expensive than culture methods, and has been used in Spain to estimate the prevalence of tuberculosis in deer populations (Parra et al., 2005 ;Vicente et al., 2006). In many cases only animals with visible lesions are examined using mycobacterial culture (Delahay et al., 2002 and others). However, *M. bovis* is frequently isolated from deer where visible lesions have not been identified (Delahay et al., 2007b; Zanella et al., 2008a). Passive surveillance may

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overestimate disease prevalence if only animals with suspicious lesions undergo mycobacterial culture.

Transmission appears to be occurring within the red deer population in France and Spain, often in the absence of cattle infection (Romero et al., 2008; Zanella et al., 2008a). In both countries wild boar are an important reservoir host. In GB however, even in the presence of high levels of infection in both the badger and cattle populations, the prevalence of infection in red deer is relatively low (Delahay et al., 2002; 2007b).

Transmission

In general, with some notable exceptions (Zanella et al., 2008a) surveys for infection in deer have been confined to countries where bTB is endemic in cattle or other wildlife reservoirs such as GB (Delahay et al., 2002, 2007) and Spain (Romero et al., 2008). Nonetheless, infection in wild deer has been reported in a number of countries where bTB is considered to be eliminated. Countries with OTF status (year OTF status attained in brackets) in which infection has been identified in deer include Austria (1999), Czech Republic (2004), France (2000) and several other eastern European countries (de la Rua-Domenech, 2006). The mechanisms of transmission in the relative absence of cattle infection are not known. In addition, wild boar and deer populations in France (Zanella et al., 2008a) and Spain (Aranaz et al., 2004; Parra et al., 2005) can be heavily infected with *M. bovis* but the ability of deer to maintain infection and transmission in the absence of another wildlife reservoir is not clear.

Control

Factors that have been proposed to account for the variable success of BCG vaccination in mammals include environmental mycobacteria (Fine 1995) and infection with intestinal helminths (Elias et al., 2001). Both of these factors are potentially important in wild animal populations and their effect on BCG efficacy in deer is unknown.

4. Other species

4.1 Prevalence & distribution

M. bovis has a broad host range and has been identified in many species, particularly in countries where bTB in cattle is widespread (Delahay et al., 2002, 2007b; Martin-Atance et al., 2006). However, infection in wildlife has been also reported in countries where cattle are considered free from bTB (Anon, 2009), such as the Czech Republic (Pavlik et al., 2002), Hungary, Poland (Pavlik and Trcka, 2006), France (Zanella et al., 2008a) and Austria (Anon, 2009).

Various methods have been used to estimate prevalence in wild hosts including collation and analysis of existing data (Delahay et al., 2002), systematic trapping and *post mortem* examination (Delahay et al., 2007b), or live-sampling (Mathews et al., 2006) of animals for culture of *M. bovis*, or the estimation of serological prevalence (Martin-Atance et al., 2006). European records of *M. bovis* infection in wildlife, and estimates of prevalence (where available) are listed in Table 4.

The use of passive surveillance to estimate disease prevalence in wildlife populations is fraught with difficulties and there have been few systematic surveys of wildlife for bTB. In contrast with deer and wild boar where some countries conduct passive surveillance on hunted carcasses (Gortazar et al., 2008; Paterson, 2008) few other species are hunted, and some are the focus of conservation efforts (e.g. Iberian Lynx, European Bison). Delahay et al. (2002) reviewed annual reports compiled by the British Ministry of Agriculture (MAFF) between 1976 and 1997 for non-bovine species infected with *M. bovis*. Infection was confirmed by bacteriological culture of tissues obtained at *post mortem* examination in red fox, mink, ferret, mole and brown rat. In common with many of the available surveys, the MAFF investigations were likely to have been based on a biased sample of animals, and the true origin of many carcasses was uncertain. In many cases samples were submitted for culture where there was already a suspicion of infection and *post mortem* examination and collection protocols changed throughout the sampling period (Delahay et al., 2002).

A more systematic survey in south-west England, a part of GB where bovine tuberculosis in cattle is commonplace, found the prevalence of infection in wild hosts other than deer and badgers to be 3.2% (red fox *Vulpes vulpes*), 3.9% (stoat *Mustela erminea*), 4.2% (polecat *Mustela putorius*), 2.4% (common shrew *Sorex araneus*), 2.8% (yellow-necked mouse *Apodemus flavicollis*), 0.6% (wood mouse *Apodemus sylvaticus*), 1.5% (field vole *Microtus agrestis*) and 0.4% (grey squirrel *Sciurus carolinensis*) (Delahay et al., 2007b). During this study carcasses obtained from pest and game managers, road traffic accidents, systematic trapping and from vets and wildlife hospitals, were subjected to systematic *post mortem* examination and microbiological culture of tissues. A study using live-sampling to obtain clinical samples (e.g. faeces, urine, sputum) for culture, estimated the prevalence of *M. bovis* in farmland wildlife (excluding badgers and deer) to be less than 2% in GB, following the confirmation of infection in only one vole (Mathews et al., 2006). Live sampling is however, likely to be substantially less sensitive than *post mortem* examination. In Spain, serological surveys have identified antibodies against *M. bovis* in 3% of lynx and 4% of red foxes sampled, although infection was not confirmed by other methods (Martin-Atance et al., 2006).

Insectivores

Hedgehogs (*Erinaceus europaeus*) are susceptible to infection with *M. bovis* under laboratory conditions (Thorns et al., 1982). Griffith (1939) reported infection in a free-living hedgehog caught in Regent's Park, London. *M. bovis* was isolated from 4 out of 79

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hedgehogs in a region endemic for tuberculosis in New Zealand (Lugton et al., 1995). In GB *M. bovis* was isolated from two out of 162 moles (*Talpa europaea*) sampled between 1976 and 1997, although neither exhibited macroscopic lesions (Delahay et al., 2002). Although tuberculosis is thought to be rare in bats, very few have been subjected to examination. *M. bovis* was not isolated from the single Pipistrelle bat that was collected during MAFF investigations (1976-97) in GB (Delahay et al., 2002).

Lagomorphs

Rabbits (*Oryctolagus cuniculus*) are known to be highly susceptible to experimental infection with mycobacteria, particularly with *M. bovis*, which can be lethal (Wilson and Harrington, 1976). As a result, rabbits have been at times considered an inappropriate model for investigations of disease (Griffin, 2000). Nevertheless, there are very few reported cases of *M. bovis* infection in wild rabbits or hares. However, Griffith (1939) isolated *M. bovis* from rabbits on a UK fur farm, and mycobacteria with characteristics of *M. bovis* were isolated from a rabbit in Co. Cork, Ireland (P. Sleeman and F. Quigley personal communication in Delahay et al., 2002). In GB, various surveys of 347 (Delahay et al., 2007b), 146 (Delahay et al., 2002), 57 (Little et al., 1982b), and 21 rabbits (Gallagher, 1980) failed to identify a single infected case, despite being conducted following cattle breakdowns or in areas where bTB was endemic in cattle or other wildlife. Similarly, in New Zealand where infection is endemic in cattle and a number of wildlife reservoirs, a survey of 1,000 wild rabbits found no signs of infection (de Lisle et al., 1995). The only recent confirmed case of infection in a rabbit is from Central Otago, New Zealand where *M. bovis* was isolated from an animal found dead (Anon., 1980; Gill and Jackson, 1993).

M. bovis was detected in a free-living brown hare (*Lepus europaeus*) that was trapped during a possum control operation in an area of New Zealand with an exceptionally high prevalence of bTB in possums (Cooke et al., 1993).

Rodents

Despite their ubiquity, *M. bovis* is rarely found in rodents. Little et al. (1982b) isolated *M. bovis* from two out of 90 brown rats (*Rattus norvegicus*) trapped on a tuberculosis-affected cattle farm in Dorset. Infection was also isolated from two out of 167 rats collected from a further 19 cattle farms in England during a survey for *Brucella abortus* infection (Bosworth, 1940). However, *M. bovis* was not isolated from substantial numbers of mice, voles and squirrels collected following cattle herd breakdowns (Gallagher, 1980; Little et al., 1982b). A review of historical records indicated that *M. bovis* had been isolated from only five out of 412 brown rats over an 11 year period in GB (Delahay et al., 2002). As a result of subsequent investigations in England and Wales the prevalence of infection was estimated to be less than 3% in samples of common shrew, yellow necked mouse, wood mouse, vole and grey squirrel (Delahay et al., 2007).

Many rodents are susceptible to *M. bovis* infection, and mice have been used extensively as experimental models (Clark et al., 2008). The American meadow vole (*Microtus pennsylvanicus*) and house mouse (*Mus musculus*) are highly susceptible to infection with *M. bovis* via both oral and intranasal inoculation, resulting in gross lesions. In addition, meadow voles were capable of disseminating *M. bovis* in faeces although the viability of excreted bacilli was not evaluated (Clarke et al., 2007). The brown rat was the most difficult to infect with *M. bovis*, gross lesions did not develop and tissue cultures from only one of the 24 inoculated rats yielded *M. bovis*. The authors hypothesised that the rat may possess innate resistance to oral infection (Clarke et al. 2007). None of the 317 rats collected in a survey in GB showed evidence of *M. bovis* infection (Delahay et al., 2007b).

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Carnivores

In Europe *M. bovis* infection is perhaps most commonly reported in the fox after badgers, wild boar and deer. Infection in the fox is relatively common and has been recorded from GB (Gallagher, 1980; Little et al., 1982b, c; Delahay et al., 2002, 2007b), France (Zanella et al., 2008a) and Spain (Martin-Atance et al., 2005; Romero et al., 2008). In most surveys prevalence is less than 4% and cases have generally been associated with higher levels of infection in other wildlife species (Little et al., 1982b; Gallagher, 1980; Delahay et al., 2002 and 2007b; Martin-Atance et al., 2006; Zanella et al., 2008a).

In GB, Little et al. (1982b) identified infection in one out of seven foxes from a farm in Dorset where wildlife were investigated following a cattle herd breakdown. Gallagher (1980) isolated *M. bovis* from four foxes out of 103 collected in the Cotswolds area of England. Infection was confirmed in 1.2% of foxes collected during MAFF investigations (1976-97) (Delahay et al., 2002) and a subsequent survey in south west England found 3.2% of foxes were infected (Delahay et al., 2007b).

Elsewhere in Europe, *M. bovis* was detected in one fox out of seven collected from a national park in Spain (Martin-Atance, 2005) and a subsequent serological survey found 4% of foxes in the Doñana National Park (DNP) had evidence of infection (Martin-Atance et al., 2006). An infected fox was also found in the Bretonne region of France, where an outbreak in red deer and wild boar had occurred (Zanella et al., 2008a), and infected foxes have also been recorded in Switzerland (Bouvier, 1963). Furthermore, our questionnaire survey identified records of infection with *M. caprae* in a fox in Austria (Questionnaire, W. Glaswischnig, 2009) and *M. bovis* in a fox in Sicily, Italy (Questionnaire, R. Orusa, 2009).

Although the badger is the most well-known mustelid reservoir of *M. bovis*, ferrets are also highly susceptible to infection (Lugton et al., 1997). In New Zealand, wild ferrets are highly susceptible to *M. bovis*, with an overall prevalence of 32% in areas where bTB is endemic in cattle and other wildlife (Lugton et al., 1997). In areas where ferrets exist in high densities they may be considered a maintenance host for *M. bovis* (Caley and Hone, 2005). In 2005 *M. bovis* spoligotype SB0273 was isolated from a group of 12 ferrets at a rescue centre in East Sussex, England. This is the predominant strain found in cattle in this region (Anon, 2005; Lee et al., 2009) but it is not known whether the infection was acquired in the wild or in the rescue centre.

M. bovis was isolated from an otter found dead in Northern Ireland in 2008 (Anon., 2008). Again, the spoligotype and VNTR pattern was identical to that circulating in the local cattle population. Although there are previous reports of pulmonary tuberculosis in otters in Cornwall, England (Stevens 1957 in Delahay et al., 2002) and in a captive otter in France (Urbain and Nouvel, 1946), bacteriological confirmation was not performed in these instances.

Out of the 271 mustelid samples, collected during the MAFF investigations (1976-97) in GB, only one mink (*Neovison vison*; N=184; 0.5%) from south Wales and one ferret (*Mustela furo*; N= 26; 3.9%) were found to be infected (Delahay et al., 2002). In a subsequent systematic survey of wild mammals in south west England, 4.0% of stoats and 4.2% of polecats (*Mustela putorius*) were found to be infected with *M. bovis* but infection was not found in either feral ferrets or mink (Delahay et al., 2007b). However, *M. bovis* was isolated from one mink out of 10 collected from Co. Tipperary, Ireland (E. Costello pers comm. In Delahay et al., 2002), although examination of a further 15 mink from Co. Wicklow, Ireland failed to find a positive case (O'Crowley and Wilson, 1991).

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The Iberian lynx is a globally Critically Endangered species. The first case of bovine tuberculosis in a free-living Iberian lynx was reported in 1998 (Briones et al., 2000). *M. bovis* infection was detected by immunohistochemistry and PCR in 3 out of 17 animals found dead in conservation areas in Spain (Pena et al., 2006). In Doñana National Park, *M. bovis* was identified in 4 out of 10 found-dead lynx (Romero et al., 2008) and antibodies were detected in one (3%) out of 39 lynx (Martin-Atance et al., 2006).

Feral cats (*Felis catus*) are considered both a spill over host and a transmission risk to cattle in New Zealand (Ragg et al., 1995). In 2005 *M. bovis* was the most common mycobacterium isolated from domestic cats in the UK (Monies et al., 2006a). Prior to comprehensive controls of tuberculosis in cattle, including milk pasteurisation, *M. bovis* infection was common in cats in Europe and was associated with bTB in cattle (O'Reilly and Daborn, 1995). In 1949 the prevalence of infection in cats in north west England was estimated to be 13% (Jennings, 1949). A survey of cattle farms with a history of bTB in Pennsylvania, USA found *M. bovis* infected cats on 5 out of 12 farms (Snider et al., 1971), but a more recent survey in an endemic area in Michigan failed to find any infected cats on similar farms (Wilkins et al., 2008). In Britain the overwhelming majority of reports of infected cats were family pets, probably because these cases are more likely to be brought to the attention of a veterinarian than those amongst feral cats (Monies et al., 2000; Monies et al., 2006a)

Wild ruminants

In the Czech Republic tuberculosis assumed to be a result of *M. bovis* was reported in one wild goat (*Capra hircus*) from a game park in 1991, but only histological examination was performed (Pavlik et al., 2002). Infection was detected in one of 384 (0.26%) chamois (*Rupicapra rupicapra*) from a region in Switzerland where roe deer were also infected and suspected of re-infecting cattle (Bouvier, 1960).

In Poland infection with *M. bovis* was reported in a population of wild European bison (*Bison bonasus*) in a protected area in the Bieszczady mountains (Pavlik et al., 2002). The first case was diagnosed in 1997 and further cases were rapidly uncovered in subsequent years. It was suggested that the probable source of infection was *M. bovis* infected cattle that grazed in close proximity to the bison herd (Zorawski and Lipiec, 1997). Infection was so widespread that the entire bison population was culled (Pavlik, 2006).

Birds

Experimental studies have demonstrated that pigeons (*Columba* sp.) are susceptible to infection with *M. bovis* and starlings (*Sturnus* sp.) and crows (*Corvus* sp.) are moderately susceptible to experimental inoculation (Butler et al., 2001). However, the degree of susceptibility and exposure of these species to natural infection outside the laboratory is unknown, and confirmed natural infections have not yet been recorded in the EU. A response to our questionnaire from Italy reported infection in lanner falcon (*Falco biarmicus*), peregrine falcon (*Falco peregrinus*), Eurasian kestrel (*Falco tinnunculus*) and yellow-legged Gull (*Larus michahelis*) (Questionnaire, R. Orusa, 2009). All these species may scavenge on carcasses, which may have provided the opportunity for infection via the alimentary route.

Table 4: Occurrence and prevalence estimates for wild hosts (other than badgers, deer and boar) identified as being infected with *M. bovis* in Europe

Species	Country	Prevalence	Sample	Comments	Reference
Insectivores	GB	1.24%	2/162	between 1971 -1996	Delahay et al., (2002)
Mole					
Hedgehog	GB		1		Griffith (1939)
Rodents					
Common shrew	GB	2.4%	1/41		Delahay et al., (2007b)
Yellow necked mouse	GB	2.8%	1/36		Delahay et al., (2007b)
Wood mouse	GB	0.6%	2/337		Delahay et al., (2007b)
Field vole	GB	1.5%	1/67		Delahay et al., (2007b)
Bank vole	GB		1/1,307	Live sampling and culture of aspirates	Matthews et al., (2006)
Grey squirrel	GB	0.4%	2/450		Delahay et al., (2007b)
Brown rat	GB	1.21%	5/412	between 1971 -1996	Delahay et al., (2002)
	GB		2/90		Little et al., (1982b)
	GB		2/167		Bosworth (1940)
Crested porcupine (<i>Hystrix cristata</i>)	Italy			<i>M. bovis</i> isolated	Orusa, R., (pers. comm.)
Carnivores	Spain		4/10	Bacteriology; found dead 1998–2003	Romero et al., (2008)
Iberian lynx	Spain	3%	1/39	Serology on live animals, no bacterial confirmation	Martin-Atance et al. (2006)
	Spain	17.7%	3/17		Pena (2006)
Domestic cat	GB	16.7%	6/36	All six infected cats were found on the same premises.	Delahay et al., (2002)
	GB		12/98	Submissions to VLA in 2005 – not a survey.	Monies et al., (2006a)
Red fox	Austria		1	<i>M. caprae</i> (PCR ; spoligotype)	Glaswischnig, W. (pers. comm.)

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Species	Country	Prevalence	Sample	Comments	Reference
Red fox	France	2%	1/49		Zanella et al., (2008a)
	GB	3.2%	24/732,		Delahay et al., (2007b)
	GB	1.2%	12/993		Delahay et al., (2002)
	GB		1/7		Little et al., (1982)
	GB	3.9%	4/103		Gallagher (1980)
	Italy				OIE (2002-2007) Questionnaire, (R. Orusa, 2009)
	Spain	4%	5/118 1/7 2/5	DNP ; Serology confirmed by bacteriology DNP and south western Spain Bacteriology; found dead 1998–2003	Martin-Atance et al. (2005, 2006) Romero et al. (2008)
	Switzerland			From a region where tuberculous roe deer had also been found dead,	Bouvier (1963)
Stoat	GB	3.9%	3/78		Delahay et al., (2007b)
Polecat	GB	4.2%	1/24		Delahay et al., (2007b)
American mink	GB	0.54%	1/184	Submitted between 1971 -1996	Delahay et al., (2002)
	Ireland		1/10		Delahay et al., (2002)
Feral ferret	GB	3.9%	1/26	between 1971 -1996	Delahay et al., (2002)
Otter	France		1	Captive	Urbain and Nouvel (1946)
	Ireland		1	Wild; found dead	Anon. (2008); Lee et al., (2009)
Large herbivores European bison	Poland		12	1997-1999	Pavlik et al., (2002)
			14	2000-2004 Post mortem	Pavlik et al., (2005)
Wild goat	Czech Republic		1	Histological examination	Pavlik et al., (1998)
Chamois	Switzerland	0.26%	1/384		Bouvier (1963)

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Species	Country	Prevalence	Sample	Comments	Reference
Birds	Italy		1	No mycobacterial culture	OIE (2002-2007)
Lanner falcon					
Peregrine falcon	Italy		1	No mycobacterial culture	OIE (2002-2007)
Eurasian kestrel	Italy		1	<i>M. bovis</i> isolated	Orusa, R.
Yellow-legged Gull	Italy		1	<i>M. bovis</i> isolated	Orusa, R.

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4.2 The disease in other species

Although *M. bovis* affects a wide range of animal hosts, particularly mammals, there is wide variation in levels of natural susceptibility and propensity to develop severe disease between species (Francis, 1958; Une and Mori, 2007). For example, cervids are considered to be generally more susceptible than carnivores. The descriptions below refer to the most frequently observed presentation of bovine tuberculosis in the various species. However, all species, including those that usually develop mild forms of disease, have the capacity to develop generalized or advanced tuberculosis. Such progressive disease may typically be associated with emaciation, old age, concomitant infections and other factors that may negatively impact on the immune system of the host. The morphology of tuberculous lesions varies between species. For example many carnivores seldom develop overt disease, but typically exhibit discrete lesions with little necrosis and calcification. Histological examinations reveal that badgers, ferrets and other carnivores do not develop giant cells, and the main components of granulomas are epithelioid cells (fusiform macrophages).

Predators and scavengers may acquire infection through the consumption of infected prey or carcasses, and may consequently develop tuberculous lesions in the gastrointestinal tract and associated lymph nodes (often mesenteric lymph nodes). *M. bovis* may subsequently disseminate to other tissues, with the lungs and bronchomediastinal lymph nodes being targeted in particular. Retropharyngeal lymph nodes, draining the oral and nasal mucosae, are also frequent sites of lesions in all species.

Carnivores

It has been suggested that domestic (or feral) cats (*Felis catus*) are most frequently exposed to *M. bovis* through the consumption of contaminated milk, and subsequently develop lesions in mesenteric lymph nodes, the lungs and associated lymph nodes (Jennings, 1949). However, infection in cats has also been described in which lesions were absent from the gastrointestinal tract and mesenteric lymph nodes, but present in the lungs, lymph nodes of the head and kidneys, suggesting transmission via a route other than the consumption of infected material followed by disseminated disease (Monies et al., 2000). Tuberculous skin lesions can develop from bite and scratch wounds inflicted by tuberculous cats (Francis, 1958; Jennings, 1949).

A clinical case of bovine tuberculosis infection has been described in the Iberian lynx in Doñana National Park in Spain. The animal had a tuberculous arthritis with fistulisation in an elbow, but unfortunately the inner organs were not available for examination (Briones et al., 2000).

The majority of infected red foxes develop a NVL form of tuberculosis. Transmission in foxes is likely to arise as a result of scavenging on infected carcasses. In the UK, 23 of 24 infected foxes had NVL tuberculosis and one had lesions in the mesenteric lymph nodes (Delahay et al., 2007b). In Spain one confirmed case had NVL tuberculosis (Martin-Atance et al., 2005), and one other exhibited disseminated tuberculosis. The latter animal had gross calcified lesions in the mesenteric and submandibular lymph nodes, tuberculous lung abscesses and multiple small lung granulomas with acid fast bacilli (AFB) (Millán et al., 2008).

There are two recorded cases of the isolation of *M. bovis* from feral mink (from UK and Ireland) with no gross lesions reported (Delahay et al., 2002). One case of bovine tuberculosis was detected in a feral ferret in the UK, with no gross lesions observed (Delahay et al., 2002). NVL tuberculosis is a frequent presentation in feral ferrets in endemic areas in

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New Zealand, occurring in 27.8% of infected individuals (Lugton et al., 1997). Ferrets are most likely to become infected through scavenging carcasses. When gross lesions are present, they typically occur in the alimentary tract, liver, mesenteric and retropharyngeal lymph nodes. Ferrets with gross tuberculous lesions have higher numbers of *M. bovis* bacilli than those with only microscopic lesions (de Lisle et al., 2005). Between 50 and 90% of ferrets with no gross lesions showed microscopic granulomas in the liver (Lugton et al., 1997).

A UK survey found no evidence of lesions in one infected polecat, but in one of three infected stoats lesions were present in the mesenteric lymph nodes (Delahay et al., 2007b). One older report from the UK describes a case of an otter with advanced pulmonary tuberculous lesions and emaciation (Stephens, 1957).

Rodents and other small mammals

Wild rodents apparently develop mild disease or no gross lesions following natural *M. bovis* infection, and NVL tuberculosis may be more frequent than has been recognised (Gavier-Widen et al., 2009). Consequently, surveys for tuberculosis in wild rodents based on identification of gross lesions, would most likely only detect a small proportion of cases. Experimental infections of laboratory mice have confirmed their susceptibility to *M. bovis* and have shown that severe lesions may develop, although the severity of the disease is related not only to the dose of mycobacteria but also to the route of infection, with the respiratory route being more virulent than the intravenous (Logan et al., 2008). Experimental studies on a variety of rodents in the USA, showed that infection established in all meadow voles inoculated with *M. bovis*, with higher susceptibility to the intranasal than the oral route. The studies also showed, 14 of 24 house mice and only 1 of 24 brown rats became infected by the oral route (Clarke et al., 2007).

M. bovis has been isolated (by culture or the inoculation of guinea pigs) from tissues of wild brown rats that had no gross lesions (see Delahay et al., 2002). In a survey in the UK, five infected brown rats showed no gross lesions (Delahay et al., 2002). Likewise, experimental oral infection of a rat did not result in the development of gross lesions, but histological examination showed a focus of multinucleated giant cells with a few AFB in a tracheobronchial lymph node (Clarke et al., 2007). Occasionally, lesions have been observed in the digestive tract, indicative of oral infection (Rankin and McDiarmid, 1969).

No lesions were observed in two naturally infected wood mice and one yellow-necked mouse (Delahay et al., 2007b). House mice (*Mus musculus*) orally inoculated with a high dose of *M. bovis* developed multiple 1-5 mm, pale, tan, soft to gritty foci in the lungs, enlarged lymph nodes and spleen. Histological examination of the lesions showed they consisted of infiltrates of macrophages, epithelioid cells, few multinucleated giant cells, coagulative and variable numbers of AFB (Clarke et al., 2007).

Of the two field voles in which *M. bovis* infection has been confirmed in the UK, in one case no information was available on pathology (Delahay et al., 2002) and the other had NVL tuberculosis (Delahay et al., 2007b). NVL tuberculosis has been reported in one infected common shrew, two infected grey squirrels and two moles in the UK (Delahay et al., 2007b).

4.3 Diagnostics

Diagnosis in non-bovines has been the subject of a number of reviews (Livingstone, 2001; de Lisle et al., 2002; Cousins and Florisson, 2005; Chambers, 2009). In animals where bTB is more rarely identified, diagnosis is most frequently obtained at *post mortem* examination and confirmed by bacterial culture. The isolation of *M. bovis* from tissues obtained at *post mortem* examination remains the diagnostic gold standard for infection in wildlife, and this has been the method used in most wildlife surveys (Delahay et al., 2002, 2007b; Aranaz et al., 2004; Martin-Atance et al., 2005). However, there is great value in the development of immunological methods for the detection of bTB in wildlife not just for diagnosis but also for use in surveillance programs that require the live sampling then release of animals, and in research activities directed at the study of bTB and vaccination.

Post mortem examination, culture and PCR

In the majority of wild animals, infection with *M. bovis* is detected by finding gross lesions at *post mortem* examination and then confirmed by culture, or detected by culture alone (Aranaz et al., 2004; Delahay et al., 2007b; Zanella et al., 2008a). Live sampling to obtain faeces, urine and tracheal aspirates for culture was used in a survey to detect *M. bovis* infection in wildlife on UK farms with and without a history of recent bTB (Mathews et al., 2006). A total of 4,180 animals from 16 species, predominately rodents, rabbits, mustelids and foxes were examined but *M. bovis* was only isolated from a single bank vole and three badgers. Prevalence estimates for all the species examined were lower than those recorded in other surveys of British wildlife (Delahay et al., 2002, 2007b). The marked difference between the two approaches almost certainly relates to the lower sensitivity of live sampling for culture in relation to culture of tissues obtained during *post mortem* examination. Nasal swabbing from live animals has also been unsuccessful (de Lisle et al., 1984). *M. bovis* has been isolated from 16% (10/63) of faecal samples from live ferrets, although bacterial shedding via faeces was thought most likely to be associated with advanced disease which brings into question the diagnostic value of such an approach (Lugton et al., 1997).

Frequently only animals in which visible lesions are identified are subsequently submitted for mycobacterial culture (see Delahay et al., 2002). It is not always possible to culture from visible lesions, but conversely, *M. bovis* can be isolated from tissue where no lesions are visible (Little et al., 1982; Delahay et al., 2007b; Zanella et al., 2008a).

Cellular immunology

A feline IFN γ ELISPOT test has been adapted from a commercially available feline ELISPOT test as a diagnostic test for *M. bovis* (Rhodes et al., 2008). The assay shows good predictability for the detection of *M. bovis* and *M. microti* (Rhodes et al., 2008). Monoclonal antibodies to bovine IFN γ cross react with ovine and caprine IFN γ enabling the Bovigam test to be used as a diagnostic test for sheep and goats (Rothel et al., 1990).

In vitro tests based on CMI responses to *M. bovis* infection are invariably more sensitive than serological tests in the same species but are usually harder to develop and deploy, require longer to obtain a result, necessitate fresh samples of blood (that may need to be tested soon after taking), and, in the case of cytokine assays, may have limited or no cross-reactivity to other species. However, their improved sensitivity and relevance to anti-mycobacterial immunity often makes the investment of effort worthwhile. Reports of the successful application of qRT-PCR to measure cytokines (e.g. Harrington et al., 2006) and the diagnostic potential of an NO assay (Waters et al., 2002), provide hope that assays of CMI might be applied across a variety of different wildlife species.

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Serology

The use of crude antigen preparations and detection reagents based on protein A or G (Thoen et al., 1980a; Cousins, 1987) means that it has been relatively straightforward to develop an ELISA test for any given species without prior information of the repertoire of antigens recognized or the need for species-specific reagents. For this reason, it has proved possible to develop tests that can function across a variety of wildlife species (Thoen et al., 1980; Table 5).

Where it has been desirable to know the predominant seroreactivity of different species, sera have been screened using western blotting (Goodger et al., 1994) or more recently, multi-antigen print immunoassay (MAPIA) (Lyashchenko et al., 2000). This is a relatively straightforward blotting method whereby sera are screened for reactivity against a panel of putative antigens bound to nitrocellulose at known concentration.

One advantage of serological tests is that retrospective studies are very easy to conduct where serum samples have been kept frozen. By way of example, an indirect competitive ELISA using *M. bovis* MPB70 (a highly homologous antigen to MPB83) was used to perform a retrospective serological survey for bTB amongst wild carnivores from the Doñana National Park in Spain (Martin-Atance et al., 2006). Serum samples were tested from 118 red foxes, 39 Iberian lynx, 31 Eurasian badgers, 5 Egyptian mongoose (*Herpestes ichneumon*), 4 European genet (*Genetta genetta*), and 1 Eurasian otter. Antibodies to MPB70 were detected in 7 badgers, 5 foxes, and 1 Iberian lynx. No confirmation of *M. bovis* infection was sought in any of the animals tested. However, the study demonstrated the utility of serological assays, despite their generally low sensitivity, in determining the possible range and relative frequency of wildlife species infected with *M. bovis*. Although *M. bovis* infection was not confirmed in the single seropositive lynx, the presence of *M. bovis* infection in this population of Iberian lynx had already been confirmed by culture from an animal that had died previously of generalized bTB (Perez et al., 2001). Although not validated for this species, use of the ELISA demonstrated that it might be feasible to detect bTB infection in this critically endangered species without the need to resort to *post-mortem* examination.

In many cases, a simple method to obtain a blood sample from a captured wild animal without the use of chemical restraint would be a significant advantage, especially in the case of ICTs where the volume of blood needed is likely to be small (e.g. <0.03 ml). At present, the need to anaesthetize some species in order to obtain a blood sample limits the potential for rapid ICTs to be used 'animal-side' in the field. The advantages of serological tests are that they are relatively easy to develop, frequently work across species (although this needs to be demonstrated rather than assumed), are quick and relatively easy to perform in themselves, and can be deployed on archived material such as serum. As such, serological wildlife surveys are relatively inexpensive to perform and can allow those with responsibility for wildlife and disease management to focus their resources where most effective. However, an inability to detect antibodies to *M. bovis* in the samples tested does not necessarily imply the infection is absent from that species, as it may be regionally clustered or at a prevalence below the threshold of detection with the number of samples available, and/or the test may have low sensitivity for certain species.

Table 5. Summary of ELISA tests employed in non-bovine wildlife of the EU infected with *M. bovis*.

Species	Experimentally (E) or Naturally infected (N)	Target antigen(s)	References
Red fox	N	MPB70	Martin-Atance et al., (2006)
Llama (<i>Lama glama</i>)	N E	HKMB, HKMA, PPD-B, MPB70, PPD-B, PPD-A, LAM, PTB, SK	Thoen et al., (1980) Stevens et al., (1998)
Iberian lynx	N	MPB70	Martin-Atance et al., (2006)
Seal (Phocidae)	N	PPD-B, PPD-A	Cousins (1987)

MBCF = *M. bovis* culture filtrate; LAM = lipoarabinomannan; HKMB = heat-killed *M. bovis*; HKMA = heat-killed *M. avium*; PTB = crude carbohydrate antigen from *M. avium paratuberculosis*; SK = sodium lauroyl sarcosinate extract of *M. bovis* (no further details given).

4.4 Epidemiology

Molecular epidemiology

Spoligotypes from Iberian lynx in Spain show the same pattern as some domestic cattle and wild ungulates living in the same area, consistent with transmission occurring between these populations (Aranaz et al., 1996). Spoligotyping and mycobacterial interspersed repetitive units-variable number tandem repeats (MIRU-VNTR) was used by Romero et al. (2008) to demonstrate that the strains found in wildlife (artiodactyla, carnivores) in the DNP were those that were most prevalent in cattle. In France, the infected fox detected in the Brotonne region was infected with *M. bovis* with an identical spoligotype and VNTR pattern as the wild deer (red and roe) and wild boar reservoir, suggesting circulation within wildlife (Zanella et al., 2008a). This pattern was also identical to the strain that had been circulating in nearby cattle herds since at least 1995 (Zanella et al., 2008a).

Histopathological differences between infection in cattle and goats suggested that a strain existed that was better adapted to goats than classical *M. bovis* (Cousins, 2001). This was confirmed genetically and the strain was first renamed *Mycobacterium bovis* subsp. *Caprae*, and after significant taxonomic investigation was elevated to species status, namely *Mycobacterium caprae* (Aranaz et al., 2003). *M. caprae* is not restricted to goats in Spain and has been reported in cattle, wild boar and pigs in France, Austria and Germany (Aranaz et al., 2003). *M. caprae* has since been identified as the causative organism in a outbreak in a zoo that caused tuberculosis in a dromedary camel (*Camelus dromedarius*) and two bison (Pate et al., 2006) and has been reported in cattle, humans and red deer in Austria (Prodingier et al., 2002; Glawischnig et al., 2003) and in Croatia (Cvetnic et al., 2007). Neither the extent to which this organism is present in wildlife populations, nor its risk to cattle have been established yet. Although it is zoonotic, the actual risk of human infection is as yet undefined. It was estimated that up to a third of human infections with *M. bovis* in Germany were in reality a result of infection with *M. caprae* (Kubica et al., 2003).

Transmission

The persistence of *M. bovis* bacilli in infected animals after death acts as a source of infection for scavengers and facilitates transmission to predators such as foxes and lynx. In Switzerland, badgers and foxes were thought to have become infected by scavenging on roe deer carcasses (Bouvier, 1963). Lesions along the digestive tract suggested that a fox found with generalized tuberculosis in Spain had become infected from scavenging infected wild ungulate carcasses (Millan et al., 2008). Where infection in avian species has been reported it has been in birds of prey or scavengers, and it is likely that transmission occurred by the alimentary route.

In Michigan, where bTB was prevalent in white tailed deer, surveillance was extended to other deer species, scavengers, carnivores and rodents from 1996 to 2003 (O'Brien et al., 2006). *M. bovis* was detected in a range of opportunistic scavengers such as coyotes and foxes and the prevalence in scavengers as a whole was estimated to be 2.8%. Infection was thought to have occurred as a result of exposure to infected deer carcasses. However, the lack of visible lesions and disseminated disease in these spill-over hosts suggested few routes for shedding bacteria.

Where *M. bovis* has been isolated from carnivores it has generally been the same strain as that circulating in the cattle, badgers or artiodactyls (Aranaz et al., 1996; Aranaz et al., 2004; Delahay et al., 2007b; Romero et al., 2008; Zanella et al., 2008a). Although rabbits are the favoured prey of the lynx, their diet may also include fallow deer (Delibes, 1980). Deer may be a likely source of infection for lynx as *M. bovis* has been detected in both fallow and red

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deer in Spain (Table 2). Hedgehogs may also scavenge carrion, and suggested routes of transmission include the consumption of contaminated carcasses (Lugton et al., 1995) and milk (Griffith, 1939).

Prior to extensive disease control in cattle, *M. bovis* was frequently isolated from domestic cats and was associated with the consumption of contaminated milk from tuberculous cows (Jennings, 1949). More recently, clinical signs such as persistent abscesses or skin lesions that are unresponsive to antibiotic therapy, weight loss and submandibular lymphadenopathy have been reported in cats in New Zealand and the UK (de Lisle et al., 1990; Gunn-Moore et al., 1996; Monies et al., 2006b). The epidemiology of *M. bovis* infection in cats is still unclear but it is possible that recent changes in the clinical signs of infection in cats have resulted from a combination of the removal of infected cattle from farms and direct or indirect contact with infected wildlife (Monies et al., 2000).

Wildlife surveys that have taken place following cattle breakdowns have identified infection in foxes and rodents, but the source was unknown (Gallagher, 1980; Little et al., 1982b). *M. bovis* can survive for extended periods of time, dependant on suitable weather conditions (Duffield and Young, 1985; Jackson et al., 1995; Palmer and Whipple, 2006b). Estimates of *M. bovis* survival in the environment in New Zealand range from 14 to 28 days (Jackson et al., 1995) and studies in the UK were in broad agreement, depending on the time of year and the type of sample. For example, survival in faeces ranged from 14 days in summer to 28 days in winter.

Laboratory experiments demonstrate the survival of *M. bovis* for up to 4 weeks in 80% shade (Duffield and Young, 1985). *M. bovis* has been isolated from water in farm yards (Little et al., 1982a) and from feed (Palmer and Whipple, 2006). Viable *M. bovis* was recovered from hay at 7 days after inoculation and could still be isolated from samples of apples, corn and potatoes at 112 days after contamination (Palmer and Whipple, 2006).

Foxes and badgers are known to interact and share feeding sites (Macdonald et al., 2004). It has been suggested that infection in the fox could result from using empty badger setts (Gallagher, 1980). A number of other species may also use setts, usually after badgers have abandoned them but sometimes occupying part of the burrow system whilst badgers use another. Examples include rabbits, rodents (especially rats), polecats, weasel and otter (Neal & Cheeseman, 1996; Sleeman 1999).

The spread of urban populations and an increasing agricultural requirement for productive land may force increased interaction between livestock and free ranging animals, so increasing transmission opportunities (Hunter 1996). In most cases where *M. bovis* has been isolated from domestic cats, they have been from rural or suburban areas where bTB was endemic in cattle and/or badgers and the spoligotype patterns were consistent with those found in cattle in the region (Monies et al., 2000; Monies et al., 2006a)

In the Doñana National Park in Spain, where infection is widespread in red deer, wild boar, foxes and the Iberian lynx, it has been suggested that the original source was cattle, as no cases of tuberculosis in wildlife had been reported before an uncontrolled increase in the local cattle population (Aranaz et al., 2004; Romero et al., 2008). However, this is often difficult to determine as surveys for *M. bovis* in wildlife tend to be conducted in regions where infection is endemic in cattle (Gallagher, 1980; Little et al., 1982b; Delahay et al., 2002; Delahay et al., 2007b) and populations outside endemic areas are rarely systematically investigated. It has also been suggested that the initial source of an outbreak of bTB among European bison in Poland was an infected cattle herd that grazed on common land (Zorawski

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and Lipiec, 1997; Pavlik et al., 2002; Pavlik et al., 2005; Welz et al., 2005). Strain information was not reported but there had been no prior record of infection within this population.

Risks to cattle, conservation and public health

Delahay et al. (2007b) carried out a semi-quantitative risk assessment to estimate the degree of risk to cattle posed by several mammal species from which *M. bovis* had been isolated in the UK. Their assessment incorporated the range of prevalence estimates for each species, extent of bacterial excretion, likelihood of contact with cattle and approximate biomass, relative to the badger. Based on the evidence available, the risk from species other than deer and badgers was not considered likely to be epidemiologically relevant. However, for some species there was a high level of uncertainty associated with this conclusion because of small sample sizes, and insufficient information on pathology and the potential for onward transmission. Nevertheless, for some such as polecats, small sample sizes reflected low abundance and these species were therefore deemed unlikely to represent a high risk to cattle.

The survival of the Iberian lynx and the European bison are threatened by *M. bovis* infection in Europe. In Doñana National Park in Spain, antibodies to *M. bovis* were detected in one (3%) out of 39 lynx (Martin-Atance et al., 2006). The animal concerned was captured three times over a three year period and tested positive on each occasion. In Poland infection with *M. bovis* was reported in a population of wild European bison grazing in a protected area in the Bieszczady mountains (Pavlik et al., 2002). Infection was disseminated widely within the bison herd and was thought to have originated from nearby cattle (Zorawski and Lipiec, 1997).

Human exposure to infected wildlife represents a potential public health hazard for hunters, veterinarians and the general public (Fanning and Edwards, 1991; Wilkins et al., 2003). In New Zealand, up to 89% of cats infected with *M. bovis* were shown to come from areas where bTB was endemic in cattle and wildlife (de Lisle et al., 1990). However, these were, in all but one case, domestic pets and not feral cats. Similarly in the UK few infected feral cats have been detected and the overwhelming majority of cases in cats have been in family pets (Monies et al., 2000; Monies et al., 2006a; Monies et al., 2006b). The close relationship between companion animals and their owners suggests that infection in cats may constitute a credible public health hazard.

4.5 Ecology and host population monitoring

Ecology

Ecological and behavioural factors may have profound effects on the dynamics of *M. bovis* infection in wild hosts, they may be principal determinants of the likelihood of onward transmission to domestic animals, and will influence the choice of potential control measures (Delahay et al., 2009). Clearly the precise nature of such effects varies widely amongst different species, and detailed information may be unavailable in many cases. However, we can identify some key aspects of mammal ecology and behaviour which are likely to play an important role in disease maintenance, transmission and management.

Wild mammal populations exhibit social structure on several different levels, influenced by sex, age, relatedness, reproductive status, social dominance and environmental factors. The spatial organisation of a population will determine rates of contact amongst its members, which may be social, reproductive or aggressive in nature, and hence influence disease transmission. Individuals may be solitary or live in groups of varying size. Many carnivores (e.g. smaller mustelids) and terrestrial insectivores (e.g. hedgehogs) for example are relatively solitary, only coming together for the purposes of breeding and rearing young, but maintaining individual territories for the rest of the time. In such cases reproductive activity and aggressive encounters with con-specifics are likely to present opportunities for disease transmission. A common system amongst carnivores is for a reproductively mature male to defend a territory which encompasses those of several females (e.g. stoats, martens). In some carnivores family groups include a breeding pair and offspring (e.g. red fox) and in others, individuals of varying degrees of relatedness may live together in larger social groups (e.g. Eurasian badgers). The prevailing social system is likely to be related to the distribution of resources in the environment. Social systems not only vary amongst different species but may also differ across the geographic range of a single species. Individual and group ranges may exhibit differing degrees of territorial exclusivity, which will determine rates of contact with neighbours and hence the likelihood that infection can be effectively maintained within the population. For group-living species including some carnivores (e.g. badgers), deer (see above) and other ungulates (e.g. wild boar) inter- and intra-group (or herd) transmission rates may differ considerably. Interestingly, although much has been made of the importance of host density in determining transmission rates in wildlife populations, social structures may confound simplistic interpretations of this relationship.

The diet and feeding habits of different mammals will influence the likely levels of exposure to pathogens such as *M. bovis*. Infected prey species and carrion will be sources of infection for carnivores and scavengers. Those species that prey on known hosts of *M. bovis* may therefore be at particular risk. Herbivores on the other hand may be most likely to encounter bacilli whilst grazing on vegetation contaminated with the excretions of infected hosts. One potential route of infection for rodents (e.g. mice and squirrels) in the UK may be the incidental consumption of bacilli whilst foraging on undigested food items in faeces at badger latrines.

The distribution of food resources is an important determinant of habitat preferences, although other factors such as predator avoidance and sociality will also play a role. Nevertheless, population density is likely to be highest in food-rich habitats, with consequences for social behaviour (see above), disease transmission and maintenance. Habitat preferences will also determine the extent to which wild mammal hosts may make contact (direct or indirect) with cattle. Some species such as hedgehogs in the UK, may spend much time foraging on grazed pasture, providing opportunities for contact with cattle. In contrast, other species such as otters and mink are less likely to come into close contact

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with cattle owing to their preference for riverine habitats. Any assessment of the potential risk of transmission from wildlife to cattle should include consideration of the ecology and behaviour of the wild hosts (see Delahay et al., 2009).

Population monitoring

A wide range of approaches are available for monitoring the abundance of wild mammals (see Wilson and Delahay (2001) for a review of methods for terrestrial carnivores). Some approaches are of generic value whilst others are only appropriate for certain groups or species. A range of techniques could potentially be used to estimate the occurrence and abundance of some of the wild mammal species in which *M. bovis* infection has been identified (Table 5; Young et al., 2008). Estimation of occurrence can be carried out at a large scale, while estimation of abundance is usually only feasible at local scales. Some methods are speculative based on an assumption of further technological advances, such as the development of a mammal DNA library for use with remote DNA recovery.

Table 5. Methods recommended by Young et al. (2008) for monitoring the occurrence and abundance of selected wild mammals. Methods listed for estimating occurrence are those that could be used in a multi-species survey over a large area. Abundance methods are those that could potentially be used to estimate density at local scales.

	Occurrence	Abundance
Carnivores		
Fox	Direct observation, field signs	Distance sampling; hair / faecal DNA recovery; camera traps
Otter	Field signs	Hair / faecal DNA recovery; camera traps
Mink	Indirect detection stations, Field signs	Hair / faecal DNA recovery; camera traps
Polecat	Effective population size	Live trapping; DNA recovery from hair
Stoat	Direct observation; indirect detection stations; effective population size	Hair traps for DNA recovery
Feral cat	-	Directed local searches for colonies
Feral ferret	-	Live trapping; hair traps for DNA recovery
Lagomorphs		
Brown hare	Direct observation	Distance sampling
Rabbit	Direct observation	Distance sampling
Insectivores		
Hedgehog	Direct observation; droppings	Standardised spotlight searches; live trapping
Mole	Direct observation; field signs	Live trapping with capture-recapture
Shrews	Live trapping; indirect detection stations; field signs for some species	Live trapping with capture-recapture (in grids or webs for some species); hair DNA recovery; effective population size for some species

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<i>Rodents</i>		
Grey squirrel	Direct observation, field signs	Live trapping with catch recapture; hair DNA recovery
Brown rat	Direct observation	-
Wood & Yellow-necked mouse	Live traps; indirect detection stations	Live trapping with capture-recapture in grids or webs; hair DNA recovery
Field & Bank vole	Live-trapping; indirect detection stations	Live trapping with capture-recapture in grids or webs

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4.6. Prevention and control

Culling and Biosecurity

It is likely that most wild species in which bTB is less commonly reported, represent spill-over hosts and the best means of reducing infection in these populations is to control disease in the reservoir. Where spill-over occurs from domestic cattle, reducing disease incidence in herds and preventing contact between domestic and wild animals are likely to be beneficial (Bengis et al., 2002). However, once infection becomes established and self-maintaining within a wild host population, then additional action, directed at the wild maintenance host is likely to be required.

Effective surveillance is considered crucial for the control of diseases in wildlife (Gortazar et al., 2007). The extension of surveillance to other deer species and to scavengers, carnivores and rodents from 1996 -2003 in Michigan, where bTB was prevalent in white tailed deer, identified infection in a number of different scavengers (O'Brien et al., 2006). Submissions from hunters, road traffic accidents and trapped animals were examined for lesions and cultured. The identification of infection in these other species triggered control using different methods appropriate for each species. In foxes, coyotes, racoons and bob cats, population density was reduced using trapping and hunting. In other ruminants the practice of supplemental feeding was discouraged, and then outlawed, while densities were kept low and contact with other infected wildlife was restricted (O'Brien et al., 2006).

In most cases transmission to wildlife populations has most likely occurred from infected domestic livestock (Romero et al., 2008; Zanella et al., 2008a), and in a true spill-over host, successful control of infection should be possible by focussing management action on the maintenance host (Corner, 2006). In Northern Australia, where bTB was a significant problem in cattle and wildlife, control measures were restricted to the feral water buffalo which was considered the main reservoir of infection in wildlife. The pattern of pathology in feral pigs however indicated that they were a spill-over and end stage host, so efforts to control disease were not directed at pigs. In the presence of control of infection in feral buffalo but with no control targeted at feral pigs, the prevalence of bTB in the latter declined from 40% in the 1970's to 0.25% in the early 1990s (Corner, 2006).

Several methods have been proposed for the control of disease in wildlife, including culling, the implementation of barriers between wildlife and livestock, the sanitary disposal of potentially infected carcasses, habitat management, feeding bans, treatment and vaccination (Gortazar et al., 2007; Delahay et al., 2009). Population control in wildlife remains a contentious issue, particularly in infected but endangered or protected wildlife. Nevertheless, in isolated situations depopulation has been employed to control tuberculosis in some wild hosts. Depopulation was the eventual measure employed during an outbreak of *M. bovis* in protected, wild European bison in the Bieszczady mountains in Poland (Pavlik et al., 2002). In New Zealand, a widespread possum culling strategy which also includes ferrets is an integral component of the bTB control strategy in cattle, and has resulted in the elimination of *M. bovis* from six small areas of New Zealand (de Lisle et al., 2002). However, culling can have unpredictable effects on the demography and behaviour of wild mammal populations (see Carter et al., 2009).

The compulsory sanitary disposal of hunting carcasses has been proposed as a measure to reduce the availability of infected carcasses and prevent spread to scavengers (Gortazar et al., 2007). Correct disposal of viscera has been implemented in France in an attempt to reduce the incidence of *M. bovis* in red deer and wild boar, although infection has already spread to scavengers in the affected area (Zanella et al., 2008a).

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Vaccination

Much of the work in this area has focused on badgers (Lesellier et al., 2006, 2009a&b; Corner et al., 2008a&b), deer (Griffin et al., 1998; Griffin, 2000; Palmer et al., 2009) and possums (Ramsey et al., 2008; Tompkins et al., 2009). Vaccination is also a particularly attractive option for control of disease in endangered species such as the Iberian Lynx. However, further development and evaluation is required before vaccination can be considered as a realistic option for other wild host species. The development of an effective vaccination strategy for wild hosts presents technical challenges in methods of vaccine delivery and in assessing the proportion of the target population that has been vaccinated (Buddle et al., 2006). Practical limitations in the deployment of vaccines in wild animals may dictate that the optimal vaccination strategy would be a single administration of an oral bait (Buddle et al., 2006).

Research in New Zealand has shown that BCG vaccination can induce protection against disease in ferrets (Qureshi et al., 1999; Cross et al., 2000). Oral vaccination of ferrets with BCG (Pasteur 1173P2) resulted in less severe pathology after challenge with *M. bovis* than that observed in unvaccinated controls (Qureshi et al., 1999). Further work showed that subcutaneous inoculation of BCG also reduced the severity of infection with *M. bovis* after challenge. However, intra-duodenal inoculation, which was designed to mimic oral vaccine deployment, was not effective in reducing the degree or severity of infection in ferrets (Cross et al., 2000). When the BCG was protected and delivered orally in a lipid matrix to possums in a field trial, very high levels of efficacy were obtained (Tompkins et al., 2009).

While vaccination with the aim of interrupting transmission of *M. bovis* is the subject of ongoing research, recently the use of vaccination for the protection of endangered or valuable species has also received attention (de Lisle et al., 2002; Breed et al., 2009). Protocols already exist for the vaccination of wildlife for rabies control in Europe (Linhart et al., 1997; Rosatte et al., 2001), and oral baits have been successfully employed in various parts of the world to deploy vaccine to foxes, jackals (Linhart et al., 1997), racoons (Rosatte et al., 2001) and other species.

Other potential methods of control

Surveillance is considered pivotal to any disease control program. As the prevalence in each animal reservoir decreases the probability of detecting infection decreases and hence larger sample sizes are required. The use of sentinel animals has been suggested as a cost-effective way to infer prevalence in host populations when direct estimation is difficult. Pigs for example, have been proposed as sentinels for *M. bovis* infection in possums in New Zealand because of their susceptibility to infection and their scavenging habits (Nugent et al., 2002). Hedgehogs are scavengers, have restricted home ranges and are relatively easy to capture, so have also been suggested as potential sentinels for infection in other wildlife (Lugton et al., 1995).

Integrated control programs have had success in the control of diseases in wildlife. Control of a rabies outbreak in racoons in Ontario, Canada, was achieved using a point infection control (PIC) strategy that combined the radical reduction of host population density immediately around the epicentre, a trap-vaccinate-release strategy on the periphery of the outbreak, and the aerial deployment of an oral bait in the surrounding areas (Rosatte et al., 2001). The response was rapid and the vaccination strategy was extended to include other susceptible species.

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In the case of endangered species, where culling is not appropriate, the treatment of infected animals may be a realistic option. There are no reports of treatment in the Iberian lynx, but domestic cats have been treated with extensive and lengthy antibiotic therapy. The outcomes of such interventions have however been variable (Monies et al., 2000; Monies et al., 2006).

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4.7. Knowledge gaps and prospectus

The sporadic nature of reports of tuberculosis in wildlife species apart from badgers, wild boar, and deer, at least in part reflects the sporadic nature of investigations of bTB in these species. Most cases only come to light because they involve clinical disease (Millan et al., 2008). There has been little surveillance for infection in many wild hosts and what work has been done has focused on areas where there is a known wildlife reservoir of infection or infection is endemic in cattle (Delahay et al., 2002; Martin-Atance et al., 2006; Delahay et al., 2007b; Zanella et al., 2008a).

Prevalence

Estimates of the prevalence of *M. bovis* or *M. caprae* infection in wild animals are rare, and where they do exist they are likely to be from biased samples. Prevalence estimates alone cannot indicate the likely level of risk of transmission from a wild host population to cattle or other species. In order to assess these risks, prevalence estimates need to be interpreted in the context of other information, including host abundance. However, few studies have considered host density in assessments of *M. bovis* transmission risk (cf. Delahay et al. 2007b not known if some species display inherent resistance to *M. bovis* infection, and on the basis of field observations it is difficult to distinguish resistance from absence of exposure. Differences in the behaviour of hosts could profoundly influence the likelihood of contact with infected hosts (either domestic or wild) and potentially contaminated environments (e.g. cattle grazing). Rabbits should theoretically be at similar risk of exposure to *M. bovis* bacilli on pasture as cattle or deer, and yet infection in wild rabbits is extremely rare (Gill and Jackson, 1993), and unconfirmed to date in Europe. Interestingly rabbits are implicated in the transmission of *Mycobacterium avium* subspecies *paratuberculosis* (Daniels et al., 2003; Judge et al., 2006). Although often exposed to similar pasture and wildlife as cattle, the allelomimetic behaviour of sheep may reduce their opportunities for infection. Even when exposed to overwhelming infection pressure from infected cattle, few sheep relative to cattle were infected (Malone et al., 2003). Felids, particularly domestic cats, appear to be more susceptible to *M. bovis* than dogs. In New Zealand, *M. bovis* was isolated from 76 cats between 1974 and 1993 but only from two dogs (de Lisle, 1993). Cats are however also likely to roam more widely than dogs, and hence have more opportunity for contact with infected cattle or wildlife.

Transmission

Transmission pathways from cattle or known maintenance hosts to other wildlife species are still not well understood. For example, in scavengers such as foxes and the Iberian lynx, infection appears to be related to the ingestion of infected carrion (Aranaz et al., 2004; Millan et al., 2008) but whether transmission can occur within these species has yet to be determined.

Glossary

AFB: acid fast bacilli, mycobacteria visualized microscopically by Ziehl-Neelsen staining.

Bovine TB (bTB). Infection caused by *Mycobacterium bovis* and/or *Mycobacterium caprae*. Although strictly speaking only infection of cattle might be termed bovine tuberculosis, to avoid confusion with other *Mycobacterium* infections of wildlife, such as *Mycobacterium avium paratuberculosis*, we consistently use the terms bovine TB and bTB for infection of all wildlife hosts with *M. bovis*.

Culture: Culture is the isolation of the bacteria in the laboratory from the clinical (tissue, sputum, milk, etc.) or environmental sample into culture media. This is considered to be the gold standard for diagnosis. The main drawback is the required time as they can take 4-12 weeks to produce visible colonies.

Culture protocol: Before culture, samples should be ground and decontaminated to eliminate undesired rapidly growing contaminants that may over-grow mycobacteria. There are several protocols for decontamination but all are toxic to some extent for mycobacteria. *M. tuberculosis* complex organisms need to be cultured onto special solid (agar-based, egg-based) or liquid media.

Eradication campaign: Programmes directed to control or to eliminate infection in domestic animals (cattle). They are organised by governments or other official bodies and are usually based on a test-and-slaughter policy.

ELISA test: Serology detects the presence of antibodies (humoral immunity) in the serum or secretions of an animal (i.e. milk). There are several formats that can be used, such as the traditional ELISA assays, or the new rapid systems.

Genetic fingerprinting: Also called typing, it is the use of nucleic acid-based technique that provides specific identification of a microorganism. There are several techniques to detect DNA polymorphisms. Their usefulness and discriminatory power depend on the microorganism species and the geographical origin.

Generalized tuberculosis: lesions in at least two different anatomical regions (abdomen, thorax, head)

Interferon- γ test: The interferon-gamma (IFN γ) test is an in vitro method for measuring the cell mediated immune (CMI) response. Whole blood cultures are stimulated with antigens (tuberculin or other specific antigens). IFN γ is then detected in the blood culture supernatant using an ELISA assay. These ELISA assays are specific to family; some are commercially available (bovidae, suidae, primates).

IDTB: The intradermal tuberculin test (or tuberculin test) measures in vivo the cell-mediated immune response by injecting an antigen into the skin of an animal. The antigen is called tuberculin. The response, based on the swelling of the skin and/or presence of clinical signs, is read after 72 hours (delayed hypersensitivity). There are several protocols regarding site of injection, antigen, and measurements depending on the animal species.

Maintenance hosts: Maintenance hosts are those that can maintain infection in a population in the absence of cross-transmission from other species of domestic or wild animals, and may act as a source of infection to other species. The identification and control of disease in maintenance hosts is pivotal to a disease control programme.

Molecular epidemiology: Molecular epidemiology is the implementation of genetic fingerprinting techniques to a panel of isolates in order to obtain information about the

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dynamics of the epidemics. A combination of traditional disease tracing investigation and molecular typing is needed to understand the epidemiology of tuberculosis and provides a valuable insight into the importance of different hosts in the maintenance and spread of the infection.

M. bovis: *Mycobacterium bovis* is the causative agent of bovine tuberculosis and infects a wide range of domestic and wild animals. It has been reported worldwide (with only few exceptions) although prevalence (the number of animals that are infected) varies largely.

M. caprae: *Mycobacterium caprae* was initially described as causative agent of tuberculosis in goats. Subsequently, it has been found also in other domestic and wild animals but usually with lower prevalence than *M. bovis*. So far it has only been reported in Europe.

M. tuberculosis complex (MTBC): The *Mycobacterium tuberculosis* complex is a group of closely related mycobacteria which are human and animal pathogens. It receives the name from *M. tuberculosis (sensu stricto)* because this pathogen was the first one to be identified. The members of the complex show a degree of host specificity that does not preclude the possibility of infection of other species.

MIRU-VNTR typing: Acronym for mycobacterial interspersed repetitive unit–variable number tandem repeats. It detects the number of repeats of a target sequence in a defined locus (such mini-satellite loci) using PCR. The size of product can be detected by electrophoresis or sequencing. Several loci have been described, and the method involves analysis of a group of them.

NVL TB: Acronym for “no visible lesion tuberculosis” confirmed *M. bovis* infection, usually by culture, in animals which show no detectable macroscopic lesions at post-mortem examination

PCR: The Polymerase Chain Reaction is an in vitro amplification reaction that increase the amount of a specific target (nucleic acid) sequence to a detectable level (by agarose gel electrophoresis, hybridization or real-time detection). PCRs targeting various sequences are currently used for culture identification because they can detect low number of organisms and accurately distinguish between the species of mycobacteria. PCR can be used for direct detection of the organisms in clinical material but with a lower sensitivity.

RFLP: Restriction fragment length polymorphism studies the DNA polymorphism by cutting DNA with endonucleases, blotting onto membrane, and hybridization with repetitive genetic elements. Several genetic elements have been described (IS6110, IS1081, DR, PGRS). This technique is time-consuming and requires a considerable amount of DNA.

Spill-over host: Spill-over hosts need to continually acquire infection from other species in order for it to persist in the population. A spill-over host may be a dead-end host if it plays no significant role in the onward transmission of infection, or it may be an amplifying host which can increase the prevalence of infection in domestic animals or the number of species infected.

Spoligotyping: Spacer oligonucleotide typing (spoligotyping) is a PCR-based method that reveals the polymorphism of the Direct Repeat region by detecting the presence or absence of specific spacer sequences. The amplified product is detected by hybridization onto a spoligotyping membrane.

Tuberculosis: Chronic infection characterised by granulomatous lesions that are mainly located in the lung and associated lymph nodes but can also appear in any body location, depending on the route of infection and dissemination.

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Questionnaire acknowledgements

Respondents to questionnaires sent to CVOs

Country	Name	Affiliation
Austria	Dr. Renate Kraßnig	Federal Ministry of Healthx
Cyprus	Savvas Savva	Cyprus Veterinary Services, Ministry of Agriculture, Natural Resources and Environment
Czech Republic	Petr Kucinsky	State Veterinary Administration of the Czech Republic
Finland	Katri Levonen, DVM, PhD	Ministry of Agriculture and Forestry
GB	Josie Willett on behalf of Nigel Gibbens	Defra, Veterinary Science Team, Area 5B, Nobel House
Germany	Dr.Hans-Joachim Bätza	Federal Ministry for Food, Agriculture and Consumer Protection
Italy	Riccardo Orusa DVM	Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta
Italy	Donatella Capuano	Sanita
Latvia	Tatjana Ribakova	Food and Veterinary Service of Republic
Luxembourg	Dr. Albert Huberty	Administration des Services Vétérinaires
Poland	Emilia Łoś	Animal Health and Welfare Office General Veterinary Inspectorate
Portugal	Patrícia Tavares Santos	Divisão de Epidemiologia, Direcção de Serviços de Saúde e Protecção Animal, Direcção Geral de Veterinária, Largo da Academia Nacional de Belas Artes
Republic of Ireland	Martin Blake	Department of Agriculture, Fisheries & Food
Slovenia	Aleksandra Hari	
Spain	Jose Luis Saez LLorente	Ministry of Environment and Rural and Marine Affairs, Department of Animal Health
Sweden	Susanna Sternberg Lewerin	Department of Disease Control, National Veterinary Institute

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Respondents to questionnaires sent to wildlife researchers

Country	Name	Affiliation
Albania	Kastriot Korro	Faculty of Veterinary Medicine, Agricultural University of Tirana
Austria	Walter Glawischnig	GES Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH
Bosnia and Herzegovina	Nasir Sinanović	Veterinary Faculty, University of Sarajevo
France	Jean Hars	Unité sanitaire de la faune. Office national de la chasse et de la faune sauvage
France	Maria Laura Boschioli:	National & OIE/FAO Bovine tuberculosis and paratuberculosis Reference Lab. Unité Zoonoses Bactériennes - Bacterial Zoonoses Unit Agence Française de Sécurité Sanitaire des Aliments (AFSSA) - French Food Safety Agency
Italy	Alessandra Gaffuri	Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna
Italy	Lorenzo Battistacci	Istituto Zooprofilattico Sperimentale Umbria e Marche, Perugia
Italy	Stefano Gavaudan	Laboratorio Diagnostica Integrata Istituto Zooprofilattico Sperimentale Umbria e Marche Sezione di Pesaro Via dei Canonici 140
Italy	Dr. Pacciarini	Italian National Reference Centre for Bovine tuberculosis
Italy	Dr. Boniotti	Italian National Reference Centre for Bovine tuberculosis
Lithuania	Raimundas Lelesius,	Veterinary Institute of Lithuanian Veterinary Academy,
Norway	Kjell Handeland	National Veterinary Insititute, Norway
Poland	Justyna Bien	Witold Stefanski Institute of Parasitology, PAS
Portugal	Nuno Santos	Pygargus Lda Portugal, SAPO
Serbia	Mr Sara Savic	Naucni Institut za veterinarstvo "Novi Sad"
Switzerland	Marie-Pierre Ryser	Institute of Animal Pathology, Bern
	Stefano Gavaudan	Laboratorio Diagnostica Integrata, Istituto Zooprofilattico Sperimentale Umbria e Marche
Slovakia	Ladislav Molnar	University of Veterinary Medicine, Kosice
UK	Anthony Sainsbury	Institute of Zoology

Wildlife researchers who contributed to make contact with the wildlife TB specialist in their countries: Christa Mosler-Berger, Switzerland; Theodora Steineck, Austria; Martin Janovsky, Austria; Vincent Dedet, France; Mark Chambers, UK; Marc Artois, France; Vittorio Guberti, Italy.

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Respondents to questionnaires sent to National Reference Laboratories and Partner Institutions from the VENoMYC network.

Country	Name	Affiliation
Austria	Erwin Hofer Sandra Revilla-Fernández	Institute for Veterinary Disease Control, Austrian Agency for Health and Food Safety (AGES)
Belgium	A. Linden	Veterinary Faculty, University of Liege
	Marc Govaerts	Department of Bacteriology and Immunology Veterinary and Agrochemical Research Centre
Czech Republic	Ilona Parmova	State Veterinary Institute
	Ivo Pavlik	Veterinary Research Institute O.I.E. Reference Laboratories for Paratuberculosis and Avian Tuberculosis Department of Food and Feed Safety
Denmark	Steen B. Giese	National Veterinary Institute Technical University of Denmark
Finland	Jaana Seppanen	Finnish Food Safety Authority EVIRA
France	María Laura Boschiroli	National & OIE/FAO Bovine tuberculosis and paratuberculosis Reference Lab. Unité Zoonoses Bactériennes Agence Française de Sécurité Sanitaire des Aliments (AFSSA)
	Jean Hars	Unité sanitaire de la faune - Wildlife sanitary Unit Office national de la chasse et de la faune sauvage - French Game and wildlife Agency
Germany	Irmgard Moser	Friedrich-Loeffler-Institut Federal Research Institute for Animal Health
Hungary	Szilard Janosi	Central Agricultural Office, Veterinary Diagnostic Directorate
Italy	Simona Zoppi	S.S. Patologia Animale e Stabulario S.C. Diagnostica Generale Istituto Zooprofilattico Sperimentale Piemonte Liguria e Valle d'Aosta
Lithuania	Asta Pereckiene	Bacteriology Department, National Food and Veterinary Risk Assessment Institute
Latvia	Andra Utinane	National Diagnostic Centre of FVS
Luxembourg	Joseph Schon	Laboratoire de Médecine Vétérinaire
Republic of Ireland	Eamonn Costello	Central Veterinary Research Laboratory
Romania	Ion Sandu	Bacteriology Department, NRL tuberculosis Institute for Diagnosis and Animal Health
Sweden	Göran Bolske	Statens Veterinärmedicinska Anstalt
Slovakia	Zuzana Dindová	State Veterinary and Food Institute Bratislava- DSL Nitra

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