# Progress in the Control of Bovine Tuberculosis in Spanish Wildlife

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**Abstract**

Despite the compulsory test and slaughter campaigns in cattle, bovine tuberculosis (bTB) is still present in Spain, and the role of wildlife reservoirs is increasingly recognized. We provide an update on recent progress made in bTB control in Spanish wildlife, including aspects of epidemiology, surveillance, host-pathogen interaction and wildlife vaccination.

At the high densities and in the particular circumstances of Mediterranean environments, wild ungulates, mainly Eurasian wild boar and red deer, are able to maintain *M. bovis* circulation even in absence of domestic livestock. Infection is widespread among wild ungulates in the south of the country, local infection prevalence being as high as 52% in wild boar and 27% in red deer. Risk factors identified include host genetic susceptibility, abundance, spatial aggregation at feeders and waterholes, scavenging, and social behaviour. An increasing trend of bTB compatible lesions was reported among wild boar and red deer inspected between 1992 and 2004 in Southwestern Spain. Sporadic cases of badger TB have been detected, further complicating the picture.

Gene expression profiles were characterized in European wild boar and Iberian red deer naturally infected with *M. bovis*. The comparative analysis of gene expression profiles in wildlife hosts in response to infection advanced our understanding of the molecular mechanisms of infection and pathogenesis, revealed common and distinctive host responses to infection and identified candidate genes associated with resistance to bTB and for the characterization of host response to infection and vaccination.

Ongoing research is producing valuable knowledge on vaccine delivery, safety and efficacy issues. Baits for the oral delivery of BCG vaccine preparations to wild boar piglets were developed and evaluated. The use of selective feeders during the summer was found to be a potentially reliable bait-deployment strategy. Safety experiments yielded no isolation of *M.*

*bovis* BCG from faeces, internal organs at necropsy and the environment, even after oral delivery of very high doses. Finally, preliminary vaccination and challenge experiments suggested that a single oral BCG vaccination may protect wild boar from infection by a virulent *M. bovis* field strain.

Keywords: *Cervus elaphus*; Disease control; *Meles meles*; *Sus scrofa*; Wildlife epidemiology

## 1. Introduction

Bovine tuberculosis (bTB), a chronic infectious disease shared between livestock and wildlife, has a complex epidemiology, often with climate and habitat-mediated peculiarities. Four potential wildlife bTB reservoirs (as reviewed in EFSA 2009) exist in mainland Spain: Eurasian wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and Eurasian badger (*Meles meles*). Eurasian badgers are scarce in Mediterranean habitats and more abundant in Atlantic ones (Revilla and Palomares, 2002), and wild ungulates are continuously expanding in range and in numbers throughout the whole Peninsula (e.g. Gortazar et al. 2000, Delibes-Mateos et al. 2009), but are largely absent from the Islands.

Spain is a major livestock producer within the European Union. There are about 6,250,000 cattle in 143,000 herds. Since test and slaughter campaigns became compulsory, bTB in cattle declined significantly from 12% herd prevalence in 1987 to 1.68% in 2008. However, cattle bTB distribution is not uniform in the country. Island regions are almost bTB free, and most of the northern Spanish mainland (Atlantic climate region) is also almost bTB free. In contrast, several regions of central and southern Spain still have high bTB prevalence (Figure 1). In infected regions, bTB is consistently more prevalent among beef cattle and bullfighting cattle, which often share wildlife habitats, than among the generally fenced dairy cattle (http://rasve.mapa.es//Publica/Sanidad/documentos/INFORME%20FINAL%20TÉCNICO%20TB%202008.pdf).

Moreover, bTB is also present among goat and pig livestock, although information on prevalence is lacking. About 2,500,000 pigs are raised in open air systems. Most belong to the Iberian pig breed, which is raised in open evergreen oak woodland habitats of the south western Iberian Peninsula. Contact with domestic ruminants and with wildlife occurs in this ecosystem, and cases of *M. bovis* infection have been recorded (Gómez-Laguna et al. 2010). Molecular typing suggests that *M. bovis* strains of pigs may be shared with livestock and wildlife species (Parra et al. 2005). Regarding goats, current numbers in Spain are around 3,000,000 (http://www.mapa.es/estadistica/pags/anuario/2008/). Not only *M. caprae*, but also *M. bovis* strains are identified in goats (Gutiérrez and García-Marín, 1999).

The role of wildlife reservoirs in bTB epidemiology is increasingly recognized, worldwide (EFSA 2009). However, the definition of a wildlife reservoir is somewhat controversial. In a broad sense, a reservoir is defined as one or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the defined target population (Haydon et al. 2002). For being a competent bTB reservoir, any host species must be susceptible, able to transmit the disease, and abundant enough (Corner 2006). In Spain, the wildlife component of this reservoir is composed of one or more sympatric host species, with marked regional differences. Essentially, wild ungulates are responsible for bTB maintenance in Mediterranean regions of continental Spain, and the badger could have some relevance in the more humid Atlantic regions (see below).

Herein, we provide an update on recent progress made in bTB control in Spanish wildlife, including aspects of epidemiology, surveillance, host-pathogen interaction and wildlife vaccination.

## 2. The wildlife factor in bTB epidemiology in Spain

The Iberian Peninsula in the southwestern end of Europe is largely dominated by Mediterranean climate, with mild to cold, dry winters, hot and dry summers, and limited rainfall (usually less than 600 mm per year), which is concentrated in spring and autumn. The northern strip of the Peninsula, from Portugal to the Pyrenees, is characterized by an Atlantic climate, with up to 2000 mm rainfall per year.

### 2.1. Island regions

Both Spanish island regions (the Balearic Islands in the Mediterranean and the Canary Islands in the Atlantic) are almost bTB free. This is interesting from a wildlife perspective, since these regions are lacking all four potential wildlife reservoirs. For comparison, bTB was diagnosed among feral black pigs from the Italian Mediterranean island Sicily and the disease still constitutes a problem for livestock in this region (Di Marco et al. 2008).

### 2.2. Atlantic Spain

It is not known if wildlife represents a significant bTB reservoir in northern Spain. Eurasian badgers, a well known bTB reservoir in Ireland and the UK, are more common and abundant in Atlantic than in Mediterranean habitats in Spain (Revilla and Palomares, 2002), and sporadic cases of *M. bovis* infection have been detected in different Spanish regions, even in the north (Sobrino et al. 2008, A. Balseiro pers. comm.). However, prevalence figures based on large enough sample sizes, which are difficult to obtain in this protected species, are currently lacking. Moreover, recent monitoring data suggests that badger densities are increasing (Sobrino et al. 2009).

Very few cases of *M. bovis* infection have been reported among wild ungulates from Atlantic habitats in Spain, despite locally intense sampling. However, the bTB outbreak among red deer and wild boar from the Brotonne forest in France shows that stable situations with a high infection prevalence may well occur in Atlantic habitats (Zanella et al. 2008). One spillover case has been reported in a roe deer (*Capreolus capreolus*, Balseiro et al. 2009).

Considering together both the continued reduction of cattle bTB prevalence up to levels close to official eradication, and the nearly absence of wildlife bTB cases detected in Atlantic Spain, the current role of wildlife in bTB epidemiology seems of little relevance in this region, and eradication among cattle will hopefully be achieved soon. However, sporadic cases diagnosed in Eurasian badgers, along with increasing demographic trends in several potential bTB hosts (Gortazar et al. 2000, Acevedo et al. 2006, Sobrino et al. 2009), indicate that maintaining a targeted surveillance is advisable (see below).

### 2.3. Mediterranean Spain

In semiarid Mediterranean habitats of central and southern Spain, several wild ungulates contribute to bTB maintenance in a multi-host system (Gortazar et al. 2008). The epidemiologically most relevant wildlife hosts include the Eurasian wild boar (Naranjo et al. 2008a), and two cervids of the subfamily cervinae: the red deer and the fallow deer. Spillover hosts include the red fox (*Vulpes vulpes*; Millán et al. 2008) and the endangered Iberian lynx (*Lynx pardinus*; Peña et al. 2006), among others. Habitat constraints determine that badgers exist only at very low densities in Mediterranean Spain (Revilla and Palomares, 2002).

Bovine TB is widespread among wild ungulates in central and southern Spain (Vicente et al. 2006), local prevalence of culture confirmed infection being as high as 52% in wild boar and 27% in red deer (Gortazar et al. 2008). At the high densities and in the particular circumstances of Mediterranean environments, Eurasian wild boar and red deer are able to maintain *Mycobacterium tuberculosis* complex (MTBC) circulation in the absence of domestic livestock, for instance in fenced estates (Figure 2, panel a) and protected natural areas with no domestic ruminants (Gortazar et al. 2005). Red deer alone and wild boar alone maintain bTB circulation in the absence of other wildlife hosts (Vicente et al. 2006). The situation is worst among farm-like hunting enclosures called “cercones”, where virtually all wild boar become infected (Acevedo et al. 2007). The reservoir role of fallow deer has been less studied, but is possibly relevant at a local scale (Aranaz et al. 2004, Gortazar et al. 2008, Jaroso et al. unpublished data). Table 1 displays relevant facts about these three host species in Mediterranean habitats.

Individual risk factors for *M. bovis* infection in wild ungulates include the host species, wild boar consistently showing higher *M. bovis* infection prevalence than deer (Vicente et al. 2006, 2007b, Gortazar et al. 2008), and red deer showing higher prevalence than fallow deer (Gortazar et al. 2008); sex in red and fallow deer (males more prevalent, Vicente et al. 2006, Gortazar et al. 2008); age in deer and in wild boar (age-increasing trends; Vicente et al. 2006, Gortazar et al. 2008); and host genetic susceptibility (e.g. Acevedo-Whitehouse et al. 2005 and Naranjo et al. 2008a in wild boar, Fernández-de-Mera et al. 2009b, in red deer).

In multi-host systems such as those occurring in Mediterranean Spain, the infection levels of sympatric host species do also contribute to the risk factors for a given one (e.g. wild boar on red deer and vice versa; Vicente et al. 2007b). Belonging to an *M. bovis*-infected social group is a significant risk factor for infection in red deer and wild boar, but not for fallow deer (Gortazar et al. submitted).

Environmental risk factors are often, but not always, linked to artificial management of wildlife habitats and wildlife populations in high-wire fenced estates (Vicente et al. 2007a, 2007b). Fencing (Figure 2, panel a) can affect bTB epidemiology through a reduced host genetic variability. Using microsatellite markers, Acevedo-Whitehouse et al. (2005) found that genetic variability, fencing and wild boar abundance had significant effects on bTB infection. The strongest effects were observed for genetic heterozygosity, with relatively less heterozygous wild boar being more likely to be infected. Fencing may increase the chances of mating amongst close relatives, and contribute to a reduced genetic variability and reduced disease resistance. This was also suggested in red deer, where Fernández-de-Mera et al. (2009a, 2009b) showed significant loss in variability of the drb2 MHC-II locus over only 16 years in a fenced red deer population.

Moreover, fencing is commonly associated with other well known bTB risk factors such as feeding and translocating (Figure 2, panel c), and in general with wildlife overabundance (Gortazar et al. 2006). Artificial feeding and watering causes spatial aggregation and allows maintaining ungulate densities above the carrying capacity of a given habitat. Disease prevalence has been linked to spatial aggregation and high densities (Acevedo et al. 2007, Vicente et al. 2007a, 2007b). However, high *M. bovis* prevalences are found even in protected areas where no feeding and no translocations of wild ungulates take place, such as the Doñana National Park (DNP, Gortazar et al. 2008), possibly because of the high densities and habitat mediated spatial aggregation occurring in DNP. Aggregation and *M. bovis* transmission may occur more often in certain habitats. For instance hardwood *Quercus* spp. forest availability was associated with increased bTB risk in red deer and wild boar in central Spain (Vicente et al. 2007b). Miller et al. (2003) suggested that woodland areas provide shady, moist conditions under which *M. bovis* might survive longer in the environment.

Finally scavenging, including hunting gut-pile consumption (Figure 2, panel d), is most probably a significant risk factor for wild boar and for carnivores. Infection through consumption of contaminated materials may increase the probability of contacting *M. bovis* (Gortazar et al. 2008). Ongoing research is evidencing that the amount of carrion scavenged by wild boar in Mediterranean Spain compares to that by specialized scavengers (vultures), and even locally this suid becomes the top consumer (unpublished data).  Figure 2 suggests that gut-piles may occasionally attract herbivores such as red deer.

The main purpose of controlling bTB in Spanish wildlife is to prevent the infection of the domestic stock, and also humans. In this sense, ongoing research is addressing wildlife-domestic livestock interaction to elucidate shared epidemiology and to identify control measures such as those related to safe husbandry practices. Also, large scale studies on spatiotemporal incidence patterns of bTB in livestock herds are including wildlife related features, which will provide a first approximation to the relative contribution of wild animals, and a fine biogeographical picture of the problem.

## 3. Wildlife disease surveillance

### 3.1. Defining the concepts

Two important concepts in wildlife disease epidemiology need to be defined: surveillance and management. Surveillance is the ongoing recording of diseases in wildlife populations with a view to disease management. Surveillance data are used to identify the areas to be targeted for control, and to anticipate spatial and temporal trends so that pre-emptive management interventions can be used to reduce disease risks (Artois et al. 2009). Wildlife disease management begins with surveillance; knowing if diseases are present, their past and current distribution, and the trends in their prevalence. Possibilities for disease management, always in addition to surveillance, include disease control through different means, disease eradication, which is usually not realistic, or taking no action, if the cost-benefit analysis suggests that this is the most convenient option (Wobeser, 1994).

### 3.2. Methods in bTB surveillance

The development of effective schemes for the surveillance of disease in wildlife populations is becoming increasingly important (Artois et al. 2009). Tools available for bTB surveillance include visual inspection for macroscopic bTB-compatible lesions or combinations of visual inspection with culture (e.g. Vicente et al. 2006); systematic culture of selected tissues (e.g. Gortazar et al. 2008); and even serology or combinations of serology and culture (e.g. Aurtenetxe et al. 2008, Vicente et al. 2007b). In Spain, surveillance is based on large postmortem samples obtained from hunter-harvested wildlife. However, alternative techniques need to be used in protected wildlife such as badgers, or in protected areas where hunting is banned.

Bacteriological culture is the gold standard test for determining bTB infection prevalence. In DNP red and fallow deer, taking culture samples from both the tonsil and retropharyngeal lymph node increased the rate of isolation of *M. bovis* by 22% over culture of the retropharyngeal lymph node alone (Martín-Hernando et al. 2010). Similarly, 19% of DNP wild boar yielded *M. bovis* isolates from the tonsil but not from the mandibular LN samples (unpublished data). Hence, pools of both tissues are now used for surveillance purposes.

Investigation of wild deer for bTB compatible lesions should ideally include examination of the medial retropharyngeal, left tracheobronchial, mediastinal, mesenteric and ileocaecal lymph nodes (LNs) (Martín-Hernando et al. 2010). Otherwise, the inspected organs should be clearly stated to allow comparisons. In the wild boar visible lesions are most often (i.e. in 92-100% of cases) located in the mandibular lymph nodes (Gortazar et al. 2003, Parra et al. 2006, Martín-Hernando et al. 2007). This makes targeted surveillance in this host easy. However, for epidemiological purposes it is interesting to sample at least also the left tracheobronchial LN of wild boar, to estimate the number of individuals with thoracic extended lesions (Martín-Hernando et al. 2007). Ideally, lesion identification should be carried out by trained staff in a systematic manner, and the presence of infection at the local level should later be confirmed by culture. Such information is considered to be valuable for exploring the magnitude and general distribution of bTB in wildlife, provided a large enough sample size is obtained from an extensive area (Vicente et al. 2006). Further, molecular characterization of the isolates from different species (including livestock) would provide additional information to clarify both the local epidemiology of bTB and large spatiotemporal patterns (Gortazar et al. 2005).

The recent development of ELISA tests for the detection of antibodies against *M. bovis* will facilitate studies on large areas and long time series. Two recent studies in wild boar coincide in reporting sensitivity of 73-77% and specificity of 96-97%. A close association between 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). More data on the utility of these techniques for other hosts such as red and fallow deer are needed.

3.3. Prevalence trends among Spanish wildlife

An increasing trend of bTB compatible lesions was reported among wild boar and red deer inspected between 1992 and 2004 in Extremadura, south-western Spain (Parra et al. 2006). In Doñana NP (Southern Spain), *M. bovis* infection prevalence increased from 1998-2003 to 2006-2007 by 100% in wild boar and by 50% in red deer (Romero et al. 2008, Gortazar et al. 2008). Among 14 wildlife populations included in a 10 year survey in south central Spain, 11 presented increasing levels of bTB compatible lesions and of incidence among juvenile wild boar, in contrast to only 3 sites with decreasing bTB (unpublished data; Figure 3). However, no nationwide figures on bTB trends are available for wildlife.

Field methods for estimating wild ungulate abundance and spatial aggregation (e.g. Acevedo et al. 2007, 2008) have been implemented, allowing matching population monitoring with disease surveillance. At a national scale, a Wildlife Disease Surveillance Program is being set up. This scheme is expected to provide quality information on disease trends in Spanish wildlife.

## 4. Host-pathogen interactions

Substantial evidence suggests that genetic and environmental factors contribute to the pathogenesis and differences in susceptibility of humans and mice to *M. tuberculosis* (Fernando and Britton, 2006). In Eurasian wild boar, genetic factors have been associated with resistance to bTB at the population level (Acevedo-Whitehouse et al. 2005). In this study, wild boar microsatellite marker variability was correlated with bTB providing evidence of both general and single-locus associative effects on bTB, and several loci revealed high homology to regions of the genome with known immune function (Acevedo-Whitehouse et al. 2005).

Recent studies demonstrated by suppression-subtractive hybridization and proteome analysis differential gene expression in tonsils and mandibular lymph nodes of tuberculous and non-tuberculous wild boar exposed to natural *M. bovis* infection (Naranjo et al. 2006a; 2007a). Microarray gene expression profiling also showed differential gene expression in Iberian red deer lymph nodes and wild boar peripheral blood mononuclear cells in response to natural *M. bovis* infection (Fernández de Mera et al. 2008, Galindo et al. 2009). These studies showed tissue-specific expression profiles that suggested differences in the role that tonsils and mandibular lymph nodes play in response to *M. bovis* infection in wild boar (Naranjo et al. 2007b). Furthermore, these studies suggested candidate gene markers associated with bTB resistance in wild boar and characterized genes that could be used to monitor host response to pathogen infection and vaccination (Naranjo et al. 2006b; 2008b; Pérez de la Lastra et al. 2009). One of these genes, methylmalonyl-CoA mutase (MUT) was upregulated in *M. bovis*-exposed uninfected animals and specific alleles were associated with resistance to bTB in wild boar (Naranjo et al. 2006b; 2008b; Pérez de la Lastra et al. 2009). Other genes such as complement component 3 (C3), interferon gamma (IFN-gamma), interleukin 4 (IL-4) and Regulated on Activation, Normal T Expressed and Secreted cytokine, also known as CCL5 (RANTES) were downregulated in infected wild boar and upregulated in parentally and orally BCG-immunized animals when compared to non-immunized controls (Pérez de la Lastra et al. 2009; Ballesteros et al. 2009c). These results also provided additional evidence that expression of selected genes correlates with protection to *M. bovis* infection after oral BCG vaccination in wild boar (Ballesteros et al. 2009c).

These studies identified new mechanisms by which wildlife hosts respond to *M. bovis* infection and how the pathogen circumvents host immune responses to establish infection. Furthermore, gene expression profile in vaccinated animals showed BCG-specific responses that are different from those observed in naturally *M. bovis*-infected wild boar which may be used to monitor BCG vaccination during experimental vaccine studies in this species. Gene expression studies in naturally-infected wildlife bTB reservoirs are important for functional genomics and vaccine studies to aid in disease control.

**5. Wild boar oral vaccination with BCG**

Whether wildlife vaccination will become a realistic option in the frame of bTB control in Spain will depend on factors such as the long term success of cattle bTB control in wildlife habitats, and the results of ongoing vaccination trials in other countries, such as Ireland and UK in badgers and New Zealand in possums (*Trichosurus vulpecula*). Current vaccination research in Spain is focused on wild boar. Delivery, safety and efficacy issues need to be addressed in order to be prepared for eventually licensing a vaccine. Recently, baits for the oral delivery of vaccine preparations to 2–4 month-old wild boar piglets, the preferred age for vaccination, were developed and evaluated. The use of selective feeders during the summer was found to be a potentially reliable bait-deployment strategy (Ballesteros et al. 2009a; 2009b). Safety experiments yielded no isolation of *M. bovis* BCG from faeces, internal organs at necropsy and the environment, even after oral delivery of very high doses. Finally, preliminary vaccination and challenge experiments suggest that a single oral BCG vaccination may protect wild boar from infection by a virulent *M. bovis* field strain (Ballesteros et al. 2009c).

**6. Discussion**

The ecology of *M. bovis* in Spain represents a multi-host system, with a relevant role for wildlife, but also for domestic reservoirs and for the environment. There are huge differences between regions, with almost no bTB and no wildlife reservoirs in the islands; almost no bTB and few wildlife and habitat risk factors in the Atlantic mainland; and still high bTB prevalence in the Mediterranean habitats of the mainland, partly explained by wildlife and habitat risk factors. As bTB prevalence has dropped in livestock, the relative importance of a potential wildlife reservoir may increase. Thus, wildlife aspects need to be considered in the strategy to control bTB in Spain (http://ec.europa.eu/food/animal/diseases/eradication/reportsanco-10584-2007btbsubgroupsevillarev110-1-08.pdf ). Moreover, wildlife numbers and wildlife distribution are continuously changing, with potential impacts on epidemiology.

Regarding wildlife, steps have been taken towards disease control. Firstly, new regulations of wild animal translocations (Royal Decree 1082/2009; http://www.boe.es/boe/dias/2009/07/23/pdfs/BOE-A-2009-12206.pdf) and on disposal of hunting carcass remains (both at the EU and the regional level), if properly enforced, will contribute to limit bTB spread. Secondly, new diagnostic tools and setting up a Wildlife Disease Surveillance scheme will allow documenting the spatial and temporal trends of bTB in wildlife. In Spain, the variety of surveillance systems is broad, and a need exists to find effective ways to share and exchange data among regions and coordinate on a global scale.

This will hopefully improve our ability to identify new health risks in wildlife populations and enhance our capability to manage them when necessary (Artois et al. 2009).

Wildlife fencing, feeding, and keeping overabundant populations for hunting or other recreational purposes, is still a problem of paramount importance in disease epidemiology, not only regarding bTB (Gortazar et al. 2006). To contribute to solve this problem, census methods have been developed (Acevedo et al. 2007) or adapted to Mediterranean habitats (Acevedo et al. 2008). This allows getting accurate estimations of the wildlife densities before and after a given management. Now it is the responsibility of the environment authorities to achieve a reduction of the current wildlife densities to more sustainable levels, and to contribute also to reduce the spatial aggregation of wildlife at feeders or waterholes (Figure 2, panel b). However, the success of any such management action must be assessed critically, including an analysis of the costs, of the ecological consequences and of the animal and human health, welfare, and conservation benefits.

This, in turn, urgently requires applied research regarding waterhole ecology, wild boar and deer space use and movements, carrion and gutpile consumption by birds and mammals, and particularly on the effect of any attempt to control bTB in wildlife, for instance through population control or testing the hypothesis that changes in food or water distribution may affect transmission rates. Ongoing research is also addressing wildlife-domestic livestock interaction in order to provide safer husbandry practices.

Despite the initial characterization of gene expression profiles in wildlife in response to *M. bovis* infection and BCG vaccination, further experiments are required to identify specific genes associated with protective response in these animals to monitor immune response after vaccination and to establish gene markers for genetic studies and assisted breeding in farmed game.

New vaccination and challenge trials are currently being conducted with larger numbers of experimental subjects. In parallel, safety issues are being addressed and, in the field, bait delivery experiments with biomarkers are being analyzed to evaluate the coverage of our vaccination strategy and model the possible impact on bTB control in wildlife under the conditions found in Spain. If these experiments progress as expected, work towards licensing the use of oral BCG in free living wild boar will be the goal. However, vaccination will not be a golden bullet but just one of several tools available for bTB control in wildlife in Spain.

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**Conflict of Interest Statement**

The authors have no conflict of interest.

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**Table 1.-** Current situation in Spain, status regarding bovine tuberculosis (bTB), and selected bTB risk factors among three wild ungulates.

| | **Host species** | | | |
|---|---|---|---|---|
| **Fact** | Eurasian wild boar | Red deer | Fallow deer | References |
| Situation in Spain | | | | |
| - Current distribution (1) | Widespread | Patchy | Local | Palomo and Gisbert, 2002 |
| - Population trend | Increasing | Increasing | Stable to increasing | Gortazar et al. 2000, Acevedo et al. 2006 |
| - Abundance | Range 1 to $90/km^2$, even higher in farm-like enclosures | Mean 21, locally up to $69/km^2$ | Usually lower than red deer, but locally up to $50/km^2$ | Acevedo et al. 2007, 2008 |
| Status regarding bTB | | | | |
| - Prevalence | 42.5% (max 100%) | 13.7% (max 50%) | ? (max 18.5%) | Vicente et al. 2006, Gortazar et al. 2008 |
| - % of generalized bTB | 57.8-61.2% | 57.1-70% | 73.3% | Vicente et al. 2006, Martín-Hernando et al. 2007, 2010 |
| - % of lung lesions | 38.1% | 30% | 80% | Martín-Hernando et al. 2007, 2010 |
| Known bTB risk factors | | | | |
| - Individual | Age, Genetics, | Age, Sex, Genetics | Age, Sex | Acevedo-Whitehouse et al. 2005, Vicente et al. 2006, Gortazar et al. 2008, Fernández-de-Mera et al. 2009 |
| - Intra and inter-specific | Social group, Red deer bTB prevalence | Social group, Wild boar bTB prevalence | Not known | Vicente et al. 2007, Gortazar et al. (Submitted) |
| - Environmental | Density, Fencing, Hardwood *Quercus* spp. forest availability, Spatial aggregation of wild boar at artificial watering sites | Density, Hardwood *Quercus* spp. forest availability, Spatial aggregation of wild boar at artificial watering and feeding sites | Not known | Acevedo-Whitehouse et al. 2005, Vicente et al. 2007a, 2007b |
| - Ability to cross fences | High | Low | Low | Unpublished data from GPS-tagged wild boar |
| - Use of carrion | Frequent | Occasional | No | Unpublished data from camera trap surveys |

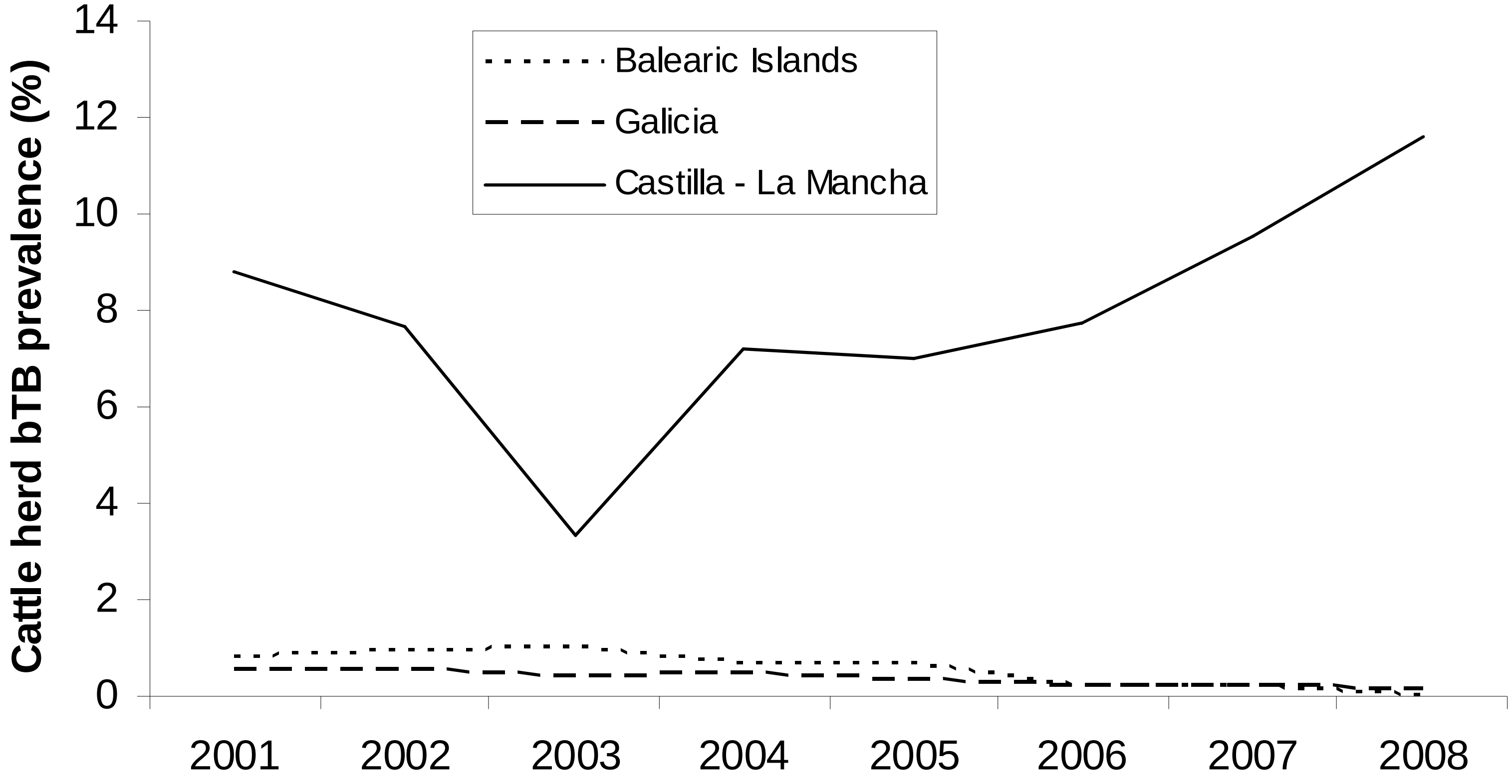
(1) All three are absent from the island regions.

## Figure legends

**Figure 1.-** Recent trends in cattle herd bovine tuberculosis (bTB) prevalence (in %) in three ecologically contrasting Spanish regions, the Balearic Islands (Mediterranean climate, no potential wildlife reservoirs), Galicia (Atlantic climate, potential wildlife reservoirs abundant), and Castilla – La Mancha (Mediterranean climate, potential wildlife reservoirs abundant and frequent high wire fencing & feeding). Prevalence data from MARM (http://www.mapa.es).

**Figure 2.-** Risk factors for bovine tuberculosis in Spanish wildlife. (a) High wire fencing nowadays occupies over 50% of suitable wildlife habitat in some provinces. While most fences are not wild boar proof, they reduce the genetic variability and disease resistance of red deer and wild boar. (b) Feeding and artificial watering causes aggregation of wild ungulates, in the picture wild boar. (c) Wildlife translocations imply the risk of spreading diseases. (d) Carrion consumption. Red deer stag consuming material from a hunting gut pile. The picture was taken by a movement triggered camera set up after a driven hunt to record carrion consumption.

**Figure 3.-** Ten year trends in the prevalence of bovine tuberculosis compatible lesions in wild boar from south central Spain. In panel (a), total lesion prevalence for each of the 14 populations in 2001-2004 and 2007-2009 is shown. Panel (b) shows the prevalence by age class for a population with declining trend (NV) and a population with an increasing trend (CB). These differences are most evident among yearlings.


14
12
10
8
6
4
2
0
Cattle herd bTB prevalence (%)
Balearic Islands
Galicia
Castilla - La Mancha
2001
2002
2003
2004
2005
2006
2007
2008


(a)
(b)
(c)
(d)
2008/01/31
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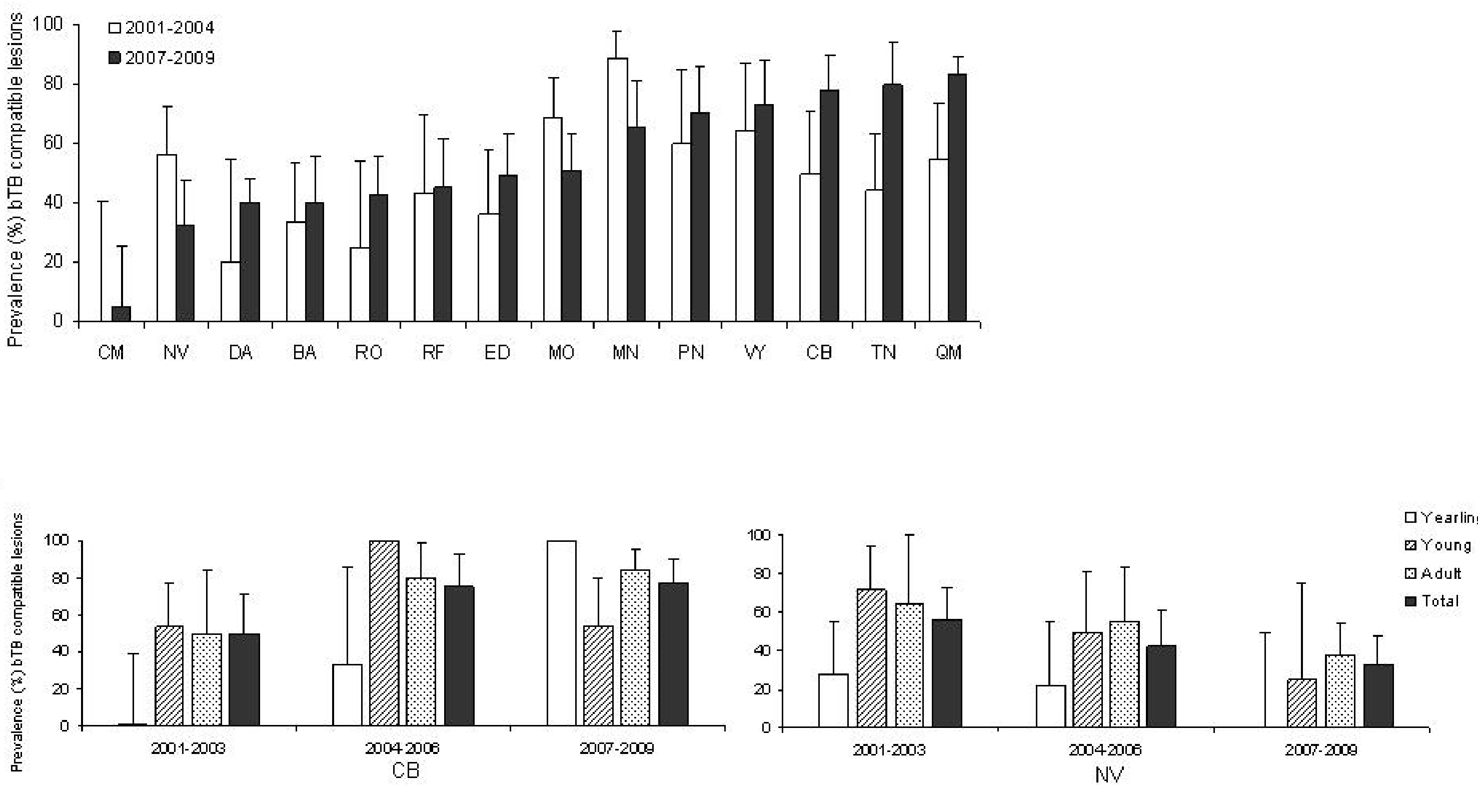
A
B



Prevalence (%) bTB compatible lesions
2001-2004
2007-2009
CM
NV
DA
BA
RO
RF
ED
MO
MN
PN
VY
CB
TN
QM
Yearling
Young
Adult
Total
2001-2003
2004-2006
2007-2009
CB
NV