



## 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 *Mycobacterium 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.

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### 1. Introduction

Bovine tuberculosis (bTB), a chronic infectious disease shared between livestock and wildlife, has a complex epidemiology, often with climate and habitat-mediated

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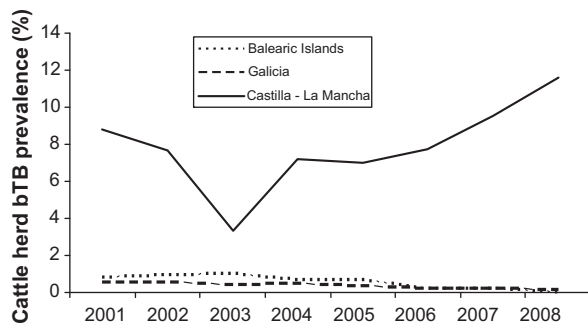


Fig. 1. Recent trends in cattle herd bovine tuberculosis (bTB) prevalence (in %) in three ecologically contrasting Spanish regions, the Balearic Islands (Mediterranean climate, no potential wildlife reservoirs), Galicia (Atlantic climate, potential wildlife reservoirs abundant), and Castilla – La Mancha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

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 (Fig. 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%20TECNICO%20bTB%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 *Mycobacterium 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/analisis/2008/>). Not only *Mycobacterium 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). How-

ever, 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). 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

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