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2 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 Q1 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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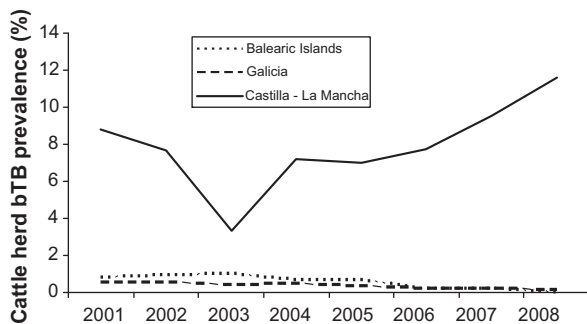


Fig. 1. Recent trends in cattle herd bovine tuberculosis (bTB) prevalence (in %) in three ecologically contrasting Spanish regions, the Balearic Islands (Mediterranean climate, no potential wildlife reservoirs), Galicia (Atlantic climate, potential wildlife reservoirs abundant), and Castilla - La Mancha (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%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 *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/anuario/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 ([Sobrinho 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 ([Sobrinho 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



Fig. 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.

111 forest in France shows that stable situations with a high
112 infection prevalence may well occur in Atlantic habitats
113 (Zanella et al., 2008). One spillover case has been reported
114 in a roe deer (*Capreolus capreolus*; Balseiro et al., 2009).

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

126 2.3. Mediterranean Spain

127 In semiarid Mediterranean habitats of central and
128 southern Spain, several wild ungulates contribute to bTB
129 maintenance in a multi-host system (Gortazar et al., 2008).
130 The epidemiologically most relevant wildlife hosts include
131 the Eurasian wild boar (Naranjo et al., 2008a), and two
132 cervids of the subfamily cervinae: the red deer and the
133 fallow deer. Spillover hosts include the red fox (*Vulpes*
134 *vulpes*; Millan et al., 2008) and the endangered Iberian lynx
135 (*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 (Fig. 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

Table 1
Q4 Current situation in Spain, status regarding bovine tuberculosis (bTB), and selected bTB risk factors among three wild ungulates.

Fact	Host species			References
	Eurasian wild boar	Red deer	Fallow deer	
Situation in Spain				
Current distribution ^a	Widespread	Patchy	Local	Palomo and Gisbert (2002)
Population trend	Increasing	Increasing	Stable to increasing	Gortazar et al. (2000), Acevedo et al. (2006)
Abundance	Range 1–90/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), Martin-Hernando et al. (2007, 2010)
% of lung lesions	38.1%	30%	80%	Martin-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. (2009a,b)
Intra and inter-specific	Social group, Red deer bTB prevalence	Social group, Wild boar bTB prevalence	Not known	Vicente et al. (2007a,b,c), Gortazar et al. (submitted)
Environmental	Density, fencing, hardwood <i>Quercus</i> spp. forest availability, spatial aggregation of wild boar at artificial watering sites	Density, hardwood <i>Quercus</i> 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,b)
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

^a All three are absent from the island regions.

163 deer showing higher prevalence than fallow deer (Gortazar
 164 et al., 2008); sex in red and fallow deer (males more
 165 prevalent, Vicente et al., 2006; Gortazar et al., 2008); age in
 166 deer and in wild boar (age-increasing trends, Vicente et al.,
 167 2006; Gortazar et al., 2008); and host genetic susceptibility
 168 (e.g. Acevedo-Whitehouse et al., 2005; Naranjo et al.,
 169 2008a, in wild boar; Fernández-de-Mera et al., 2009b, in
 170 red deer).

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

179 Environmental risk factors are often, but not always,
 180 linked to artificial management of wildlife habitats and
 181 wildlife populations in high-wire fenced estates (Vicente
 182 et al., 2007a,b). Fencing (Fig. 2, panel a) can affect bTB
 183 epidemiology through a reduced host genetic variability.
 184 Using microsatellite markers, Acevedo-Whitehouse et al.
 185 (2005) found that genetic variability, fencing and wild boar
 186 abundance had significant effects on bTB infection. The
 187 strongest effects were observed for genetic heterozygosity,
 188 with relatively less heterozygous wild boar being more
 189 likely to be infected. Fencing may increase the chances of
 190 mating among 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,b) 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 (Fig. 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 capa-
 city of a given habitat. Disease prevalence has been linked
 to spatial aggregation and high densities (Acevedo et al.,
 2007; Vicente et al., 2007a,b). 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. Aggrega-
 tion 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 con-
 sumption (Fig. 2, panel d), is most probably a significant

219 risk factor for wild boar and for carnivores. Infection
220 through consumption of contaminated materials may
221 increase the probability of contacting *M. bovis* (Gortazar
222 et al., 2008). Ongoing research is evidencing that the
223 amount of carrion scavenged by wild boar in Mediterra-
224 nean Spain compares to that by specialized scavengers
225 (vultures), and even locally this suid becomes the top
226 consumer (unpublished data). Fig. 2 suggests that gut-piles
227 may occasionally attract herbivores such as red deer.

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

239 3. Wildlife disease surveillance

240 3.1. Defining the concepts

241 Two important concepts in wildlife disease epidemiol-
242 ogy need to be defined: surveillance and management.
243 Surveillance is the ongoing recording of diseases in wildlife
244 populations with a view to disease management. Surveil-
245 lance data are used to identify the areas to be targeted for
246 control, and to anticipate spatial and temporal trends so
247 that pre-emptive management interventions can be used
248 to reduce disease risks (Artois et al., 2009). Wildlife disease
249 management begins with surveillance; knowing if diseases
250 are present, their past and current distribution, and the
251 trends in their prevalence. Possibilities for disease
252 management, always in addition to surveillance, include
253 disease control through different means, disease eradica-
254 tion, which is usually not realistic, or taking no action, if the
255 cost-benefit analysis suggests that this is the most
256 convenient option (Wobeser, 1994).

257 3.2. Methods in bTB surveillance

258 The development of effective schemes for the surveil-
259 lance of disease in wildlife populations is becoming
260 increasingly important (Artois et al., 2009). Tools available
261 for bTB surveillance include visual inspection for macro-
262 scopic bTB-compatible lesions or combinations of visual
263 inspection with culture (e.g. Vicente et al., 2006);
264 systematic culture of selected tissues (e.g. Gortazar
265 et al., 2008); and even serology or combinations of
266 serology and culture (e.g. Aurtenetxe et al., 2008; Vicente
267 et al., 2007b). In Spain, surveillance is based on large post-
268 mortem samples obtained from hunter-harvested wildlife.
269 However, alternative techniques need to be used in
270 protected wildlife such as badgers, or in protected areas
271 where hunting is banned.

272 Bacteriological culture is the gold standard test for
273 determining bTB infection prevalence. In DNP red and
274 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 retro-
pharyngeal lymph node alone (Martin-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 retro-
pharyngeal, left tracheobronchial, mediastinal, mesenteric
and ileocaecal lymph nodes (LNs) (Martin-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; Martin-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 (Martin-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.

263 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; Fig. 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

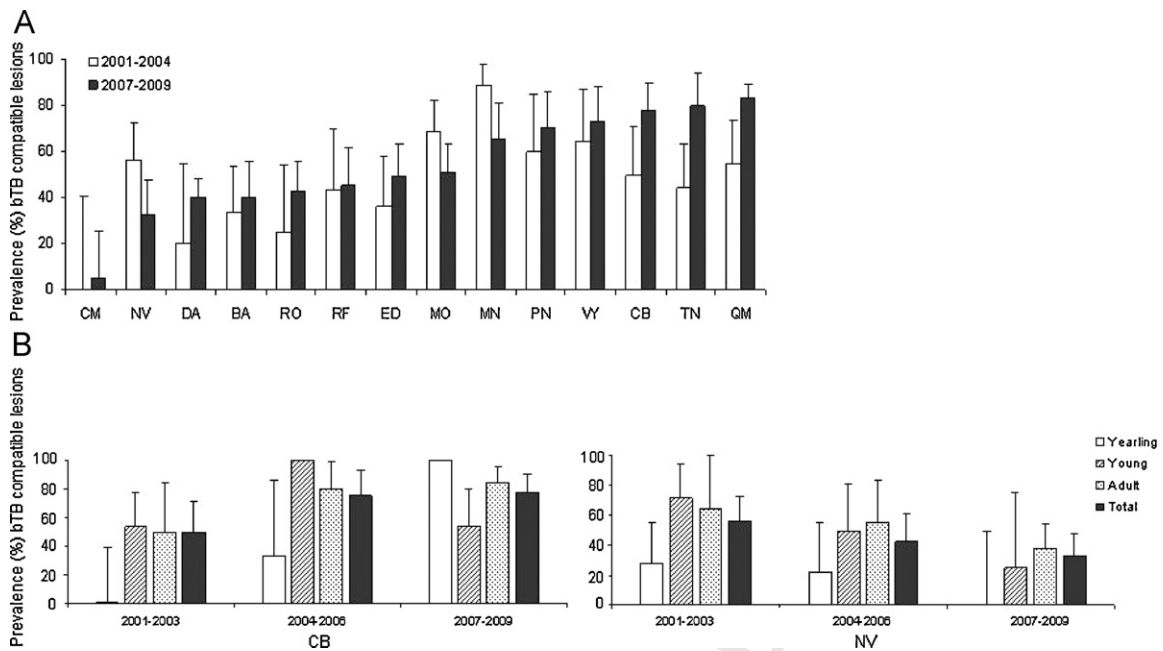


Fig. 3. Ten year trends in the prevalence of bovine tuberculosis compatible lesions in wild boar from south central Spain. In panel (a), total lesion prevalence for each of the 14 populations in 2001–2004 and 2007–2009 is shown. Panel (b) shows the prevalence by age class for a population with declining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

334 monitoring with disease surveillance. At a national scale, a
 335 Wildlife Disease Surveillance Program is being set up. This
 336 scheme is expected to provide quality information on
 337 disease trends in Spanish wildlife.

338 4. Host–pathogen interactions

339 Substantial evidence suggests that genetic and environ-
 340 mental factors contribute to the pathogenesis and
 341 differences in susceptibility of humans and mice to *M.*
 342 *tuberculosis* (Fernando and Britton, 2006). In Eurasian wild
 343 boar, genetic factors have been associated with resistance
 344 to bTB at the population level (Acevedo-Whitehouse et al.,
 345 2005). In this study, wild boar microsatellite marker
 346 variability was correlated with bTB providing evidence of
 347 both general and single-locus associative effects on bTB,
 348 and several loci revealed high homology to regions of the
 349 genome with known immune function (Acevedo-White-
 350 house et al., 2005).

351 Recent studies demonstrated by suppression-subtrac-
 352 tive hybridization and proteome analysis differential gene
 353 expression in tonsils and mandibular lymph nodes of
 354 tuberculous and non-tuberculous wild boar exposed to
 355 natural *M. bovis* infection (Naranjo et al., 2006a, 2007a).
 356 Microarray gene expression profiling also showed differ-
 357 ential gene expression in Iberian red deer lymph nodes and
 358 wild boar peripheral blood mononuclear cells in response
 359 to natural *M. bovis* infection (Fernández-de-Mera et al.,
 360 2008; Galindo et al., 2009). These studies showed tissue-
 361 specific expression profiles that suggested differences in
 362 the role that tonsils and mandibular lymph nodes play in
 363 response to *M. bovis* infection in wild boar (Naranjo et al.,
 364 2007b). Furthermore, these studies suggested candidate
 365 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 vac-
 cinated 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

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

417 6. Discussion

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

434 Regarding wildlife, steps have been taken towards
435 disease control. Firstly, new regulations of wild animal
436 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
439 (both at the EU and the regional level), if properly enforced,
440 will contribute to limit bTB spread. Secondly, new
441 diagnostic tools and setting up a Wildlife Disease
442 Surveillance scheme will allow documenting the spatial
443 and temporal trends of bTB in wildlife. In Spain, the variety
444 of surveillance systems is broad, and a need exists to find
445 effective ways to share and exchange data among regions
446 and coordinate on a global scale. This will hopefully
447 improve our ability to identify new health risks in wildlife
448 populations and enhance our capability to manage them
449 when necessary (Artois et al., 2009).

450 Wildlife fencing, feeding, and keeping overabundant
451 populations for hunting or other recreational purposes, is
452 still a problem of paramount importance in disease
453 epidemiology, not only regarding bTB (Gortazar et al.,
454 2006). To contribute to solve this problem, census methods
455 have been developed (Acevedo et al., 2007) or adapted to
456 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
(Fig. 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.

Conflict of interest statement

The authors have no conflict of interest.

Uncited reference

Delahay et al. (2002).

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