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1 **Progress in the Control of Bovine Tuberculosis in Spanish Wildlife**

2

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15

16

16 **Abstract**

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

21 At the high densities and in the particular circumstances of Mediterranean environments, wild
22 ungulates, mainly Eurasian wild boar and red deer, are able to maintain *M. bovis* circulation
23 even in absence of domestic livestock. Infection is widespread among wild ungulates in the
24 south of the country, local infection prevalence being as high as 52% in wild boar and 27% in
25 red deer. Risk factors identified include host genetic susceptibility, abundance, spatial
26 aggregation at feeders and waterholes, scavenging, and social behaviour. An increasing trend
27 of bTB compatible lesions was reported among wild boar and red deer inspected between
28 1992 and 2004 in Southwestern Spain. Sporadic cases of badger TB have been detected,
29 further complicating the picture.

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

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

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

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

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45 1. Introduction

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

54 Spain is a major livestock producer within the European Union. There are about 6,250,000
55 cattle in 143,000 herds. Since test and slaughter campaigns became compulsory, bTB in cattle
56 declined significantly from 12% herd prevalence in 1987 to 1.68% in 2008. However, cattle
57 bTB distribution is not uniform in the country. Island regions are almost bTB free, and most
58 of the northern Spanish mainland (Atlantic climate region) is also almost bTB free. In
59 contrast, several regions of central and southern Spain still have high bTB prevalence (Figure
60 1). In infected regions, bTB is consistently more prevalent among beef cattle and bullfighting
61 cattle, which often share wildlife habitats, than among the generally fenced dairy cattle
62 (<http://rasve.mapa.es//Publica/Sanidad/documentos/INFORME%20FINAL%20TÉCNICO%20OTB%202008.pdf>).
63

64 Moreover, bTB is also present among goat and pig livestock, although information on
65 prevalence is lacking. About 2,500,000 pigs are raised in open air systems. Most belong to
66 the Iberian pig breed, which is raised in open evergreen oak woodland habitats of the south
67 western Iberian Peninsula. Contact with domestic ruminants and with wildlife occurs in this
68 ecosystem, and cases of *M. bovis* infection have been recorded (Gómez-Laguna et al. 2010).
69 Molecular typing suggests that *M. bovis* strains of pigs may be shared with livestock and

70 wildlife species (Parra et al. 2005). Regarding goats, current numbers in Spain are around
71 3,000,000 (<http://www.mapa.es/estadistica/pags/anuario/2008/>). Not only *M. caprae*, but also
72 *M. bovis* strains are identified in goats (Gutiérrez and García-Marín, 1999).

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

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

87

88 **2. The wildlife factor in bTB epidemiology in Spain**

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

94 2.1. Island regions

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

100

101 2.2. Atlantic Spain

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

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

115 Considering together both the continued reduction of cattle bTB prevalence up to levels close
116 to official eradication, and the nearly absence of wildlife bTB cases detected in Atlantic
117 Spain, the current role of wildlife in bTB epidemiology seems of little relevance in this

118 region, and eradication among cattle will hopefully be achieved soon. However, sporadic
119 cases diagnosed in Eurasian badgers, along with increasing demographic trends in several
120 potential bTB hosts (Gortazar et al. 2000, Acevedo et al. 2006, Sobrino et al. 2009), indicate
121 that maintaining a targeted surveillance is advisable (see below).

122

123 2.3. Mediterranean Spain

124 In semiarid Mediterranean habitats of central and southern Spain, several wild ungulates
125 contribute to bTB maintenance in a multi-host system (Gortazar et al. 2008). The
126 epidemiologically most relevant wildlife hosts include the Eurasian wild boar (Naranjo et al.
127 2008a), and two cervids of the subfamily cervinae: the red deer and the fallow deer. Spillover
128 hosts include the red fox (*Vulpes vulpes*; Millán et al. 2008) and the endangered Iberian lynx
129 (*Lynx pardinus*; Peña et al. 2006), among others. Habitat constraints determine that badgers
130 exist only at very low densities in Mediterranean Spain (Revilla and Palomares, 2002).

131 Bovine TB is widespread among wild ungulates in central and southern Spain (Vicente et al.
132 2006), local prevalence of culture confirmed infection being as high as 52% in wild boar and
133 27% in red deer (Gortazar et al. 2008). At the high densities and in the particular
134 circumstances of Mediterranean environments, Eurasian wild boar and red deer are able to
135 maintain *Mycobacterium tuberculosis* complex (MTBC) circulation in the absence of
136 domestic livestock, for instance in fenced estates (Figure 2, panel a) and protected natural
137 areas with no domestic ruminants (Gortazar et al. 2005). Red deer alone and wild boar alone
138 maintain bTB circulation in the absence of other wildlife hosts (Vicente et al. 2006). The
139 situation is worst among farm-like hunting enclosures called “cercones”, where virtually all
140 wild boar become infected (Acevedo et al. 2007). The reservoir role of fallow deer has been
141 less studied, but is possibly relevant at a local scale (Aranaz et al. 2004, Gortazar et al. 2008,

142 Jaroso et al. unpublished data). Table 1 displays relevant facts about these three host species
143 in Mediterranean habitats.

144 Individual risk factors for *M. bovis* infection in wild ungulates include the host species, wild
145 boar consistently showing higher *M. bovis* infection prevalence than deer (Vicente et al.
146 2006, 2007b, Gortazar et al. 2008), and red deer showing higher prevalence than fallow deer
147 (Gortazar et al. 2008); sex in red and fallow deer (males more prevalent, Vicente et al. 2006,
148 Gortazar et al. 2008); age in deer and in wild boar (age-increasing trends; Vicente et al. 2006,
149 Gortazar et al. 2008); and host genetic susceptibility (e.g. Acevedo-Whitehouse et al. 2005
150 and Naranjo et al. 2008a in wild boar, Fernández-de-Mera et al. 2009b, in red deer).

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

156 Environmental risk factors are often, but not always, linked to artificial management of
157 wildlife habitats and wildlife populations in high-wire fenced estates (Vicente et al. 2007a,
158 2007b). Fencing (Figure 2, panel a) can affect bTB epidemiology through a reduced host
159 genetic variability. Using microsatellite markers, Acevedo-Whitehouse et al. (2005) found
160 that genetic variability, fencing and wild boar abundance had significant effects on bTB
161 infection. The strongest effects were observed for genetic heterozygosity, with relatively less
162 heterozygous wild boar being more likely to be infected. Fencing may increase the chances of
163 mating amongst close relatives, and contribute to a reduced genetic variability and reduced
164 disease resistance. This was also suggested in red deer, where Fernández-de-Mera et al.
165 (2009a, 2009b) showed significant loss in variability of the drb2 MHC-II locus over only 16
166 years in a fenced red deer population.

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

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

187 The main purpose of controlling bTB in Spanish wildlife is to prevent the infection of the
188 domestic stock, and also humans. In this sense, ongoing research is addressing wildlife-
189 domestic livestock interaction to elucidate shared epidemiology and to identify control
190 measures such as those related to safe husbandry practices. Also, large scale studies on
191 spatiotemporal incidence patterns of bTB in livestock herds are including wildlife related

192 features, which will provide a first approximation to the relative contribution of wild animals,
193 and a fine biogeographical picture of the problem.

194

195 **3. Wildlife disease surveillance**

196

197 3.1. Defining the concepts

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

208

209 3.2. Methods in bTB surveillance

210 The development of effective schemes for the surveillance of disease in wildlife populations
211 is becoming increasingly important (Artois et al. 2009). Tools available for bTB surveillance
212 include visual inspection for macroscopic bTB-compatible lesions or combinations of visual
213 inspection with culture (e.g. Vicente et al. 2006); systematic culture of selected tissues (e.g.
214 Gortazar et al. 2008); and even serology or combinations of serology and culture (e.g.
215 Aurtenetxe et al. 2008, Vicente et al. 2007b). In Spain, surveillance is based on large post-

216 mortem samples obtained from hunter-harvested wildlife. However, alternative techniques
217 need to be used in protected wildlife such as badgers, or in protected areas where hunting is
218 banned.

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

225 Investigation of wild deer for bTB compatible lesions should ideally include examination of
226 the medial retropharyngeal, left tracheobronchial, mediastinal, mesenteric and ileocaecal
227 lymph nodes (LNs) (Martín-Hernando et al. 2010). Otherwise, the inspected organs should be
228 clearly stated to allow comparisons. In the wild boar visible lesions are most often (i.e. in 92-
229 100% of cases) located in the mandibular lymph nodes (Gortazar et al. 2003, Parra et al.
230 2006, Martín-Hernando et al. 2007). This makes targeted surveillance in this host easy.
231 However, for epidemiological purposes it is interesting to sample at least also the left
232 tracheobronchial LN of wild boar, to estimate the number of individuals with thoracic
233 extended lesions (Martín-Hernando et al. 2007). Ideally, lesion identification should be
234 carried out by trained staff in a systematic manner, and the presence of infection at the local
235 level should later be confirmed by culture. Such information is considered to be valuable for
236 exploring the magnitude and general distribution of bTB in wildlife, provided a large enough
237 sample size is obtained from an extensive area (Vicente et al. 2006). Further, molecular
238 characterization of the isolates from different species (including livestock) would provide
239 additional information to clarify both the local epidemiology of bTB and large spatiotemporal
240 patterns (Gortazar et al. 2005).

241 The recent development of ELISA tests for the detection of antibodies against *M. bovis* will
242 facilitate studies on large areas and long time series. Two recent studies in wild boar coincide
243 in reporting sensitivity of 73-77% and specificity of 96-97%. A close association between
244 strong antibody response and the presence of gross lesions in individuals infected with *M.*
245 *bovis* has been observed in wild boar (Aurtenetxe et al. 2008; Lyashchenko et al. 2008). More
246 data on the utility of these techniques for other hosts such as red and fallow deer are needed.

247

248 3.3. Prevalence trends among Spanish wildlife

249 An increasing trend of bTB compatible lesions was reported among wild boar and red deer
250 inspected between 1992 and 2004 in Extremadura, south-western Spain (Parra et al. 2006). In
251 Doñana NP (Southern Spain), *M. bovis* infection prevalence increased from 1998-2003 to
252 2006-2007 by 100% in wild boar and by 50% in red deer (Romero et al. 2008, Gortazar et al.
253 2008). Among 14 wildlife populations included in a 10 year survey in south central Spain, 11
254 presented increasing levels of bTB compatible lesions and of incidence among juvenile wild
255 boar, in contrast to only 3 sites with decreasing bTB (unpublished data; Figure 3). However,
256 no nationwide figures on bTB trends are available for wildlife.

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

262

263 **4. Host-pathogen interactions**

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

272 Recent studies demonstrated by suppression-subtractive hybridization and proteome analysis
273 differential gene expression in tonsils and mandibular lymph nodes of tuberculous and non-
274 tuberculous wild boar exposed to natural *M. bovis* infection (Naranjo et al. 2006a; 2007a).
275 Microarray gene expression profiling also showed differential gene expression in Iberian red
276 deer lymph nodes and wild boar peripheral blood mononuclear cells in response to natural *M.*
277 *bovis* infection (Fernández de Mera et al. 2008, Galindo et al. 2009). These studies showed
278 tissue-specific expression profiles that suggested differences in the role that tonsils and
279 mandibular lymph nodes play in response to *M. bovis* infection in wild boar (Naranjo et al.
280 2007b). Furthermore, these studies suggested candidate gene markers associated with bTB
281 resistance in wild boar and characterized genes that could be used to monitor host response to
282 pathogen infection and vaccination (Naranjo et al. 2006b; 2008b; Pérez de la Lastra et al.
283 2009). One of these genes, methylmalonyl-CoA mutase (MUT) was upregulated in *M. bovis*-
284 exposed uninfected animals and specific alleles were associated with resistance to bTB in
285 wild boar (Naranjo et al. 2006b; 2008b; Pérez de la Lastra et al. 2009). Other genes such as
286 complement component 3 (C3), interferon gamma (IFN-gamma), interleukin 4 (IL-4) and
287 Regulated on Activation, Normal T Expressed and Secreted cytokine, also known as CCL5
288 (RANTES) were downregulated in infected wild boar and upregulated in parentally and

289 orally BCG-immunized animals when compared to non-immunized controls (Pérez de la
290 Lastra et al. 2009; Ballesteros et al. 2009c). These results also provided additional evidence
291 that expression of selected genes correlates with protection to *M. bovis* infection after oral
292 BCG vaccination in wild boar (Ballesteros et al. 2009c).

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

300

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

302 Whether wildlife vaccination will become a realistic option in the frame of bTB control in
303 Spain will depend on factors such as the long term success of cattle bTB control in wildlife
304 habitats, and the results of ongoing vaccination trials in other countries, such as Ireland and
305 UK in badgers and New Zealand in possums (*Trichosurus vulpecula*). Current vaccination
306 research in Spain is focused on wild boar. Delivery, safety and efficacy issues need to be
307 addressed in order to be prepared for eventually licensing a vaccine. Recently, baits for the
308 oral delivery of vaccine preparations to 2–4 month-old wild boar piglets, the preferred age for
309 vaccination, were developed and evaluated. The use of selective feeders during the summer
310 was found to be a potentially reliable bait-deployment strategy (Ballesteros et al. 2009a;
311 2009b). Safety experiments yielded no isolation of *M. bovis* BCG from faeces, internal
312 organs at necropsy and the environment, even after oral delivery of very high doses. Finally,

313 preliminary vaccination and challenge experiments suggest that a single oral BCG
314 vaccination may protect wild boar from infection by a virulent *M. bovis* field strain
315 (Ballesteros et al. 2009c).

316

317 **6. Discussion**

318 The ecology of *M. bovis* in Spain represents a multi-host system, with a relevant role for
319 wildlife, but also for domestic reservoirs and for the environment. There are huge differences
320 between regions, with almost no bTB and no wildlife reservoirs in the islands; almost no bTB
321 and few wildlife and habitat risk factors in the Atlantic mainland; and still high bTB
322 prevalence in the Mediterranean habitats of the mainland, partly explained by wildlife and
323 habitat risk factors. As bTB prevalence has dropped in livestock, the relative importance of a
324 potential wildlife reservoir may increase. Thus, wildlife aspects need to be considered in the
325 strategy to control bTB in Spain

326 ([http://ec.europa.eu/food/animal/diseases/eradication/reportsanco-10584-](http://ec.europa.eu/food/animal/diseases/eradication/reportsanco-10584-2007btbsubgroupsevillarev110-1-08.pdf)
327 [2007btbsubgroupsevillarev110-1-08.pdf](http://ec.europa.eu/food/animal/diseases/eradication/reportsanco-10584-2007btbsubgroupsevillarev110-1-08.pdf)). Moreover, wildlife numbers and wildlife
328 distribution are continuously changing, with potential impacts on epidemiology.

329 Regarding wildlife, steps have been taken towards disease control. Firstly, new regulations of
330 wild animal translocations (Royal Decree 1082/2009;

331 <http://www.boe.es/boe/dias/2009/07/23/pdfs/BOE-A-2009-12206.pdf>) and on disposal of

332 hunting carcass remains (both at the EU and the regional level), if properly enforced, will

333 contribute to limit bTB spread. Secondly, new diagnostic tools and setting up a Wildlife

334 Disease Surveillance scheme will allow documenting the spatial and temporal trends of bTB

335 in wildlife. In Spain, the variety of surveillance systems is broad, and a need exists to find

336 effective ways to share and exchange data among regions and coordinate on a global scale.

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

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

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

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

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

368

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373

374 **Conflict of Interest Statement**

375 The authors have no conflict of interest.

376

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

546

Fact	Host species			References
	Eurasian wild boar	Red deer	Fallow deer	
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 <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, 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

547

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

549

549 **Figure legends**

550

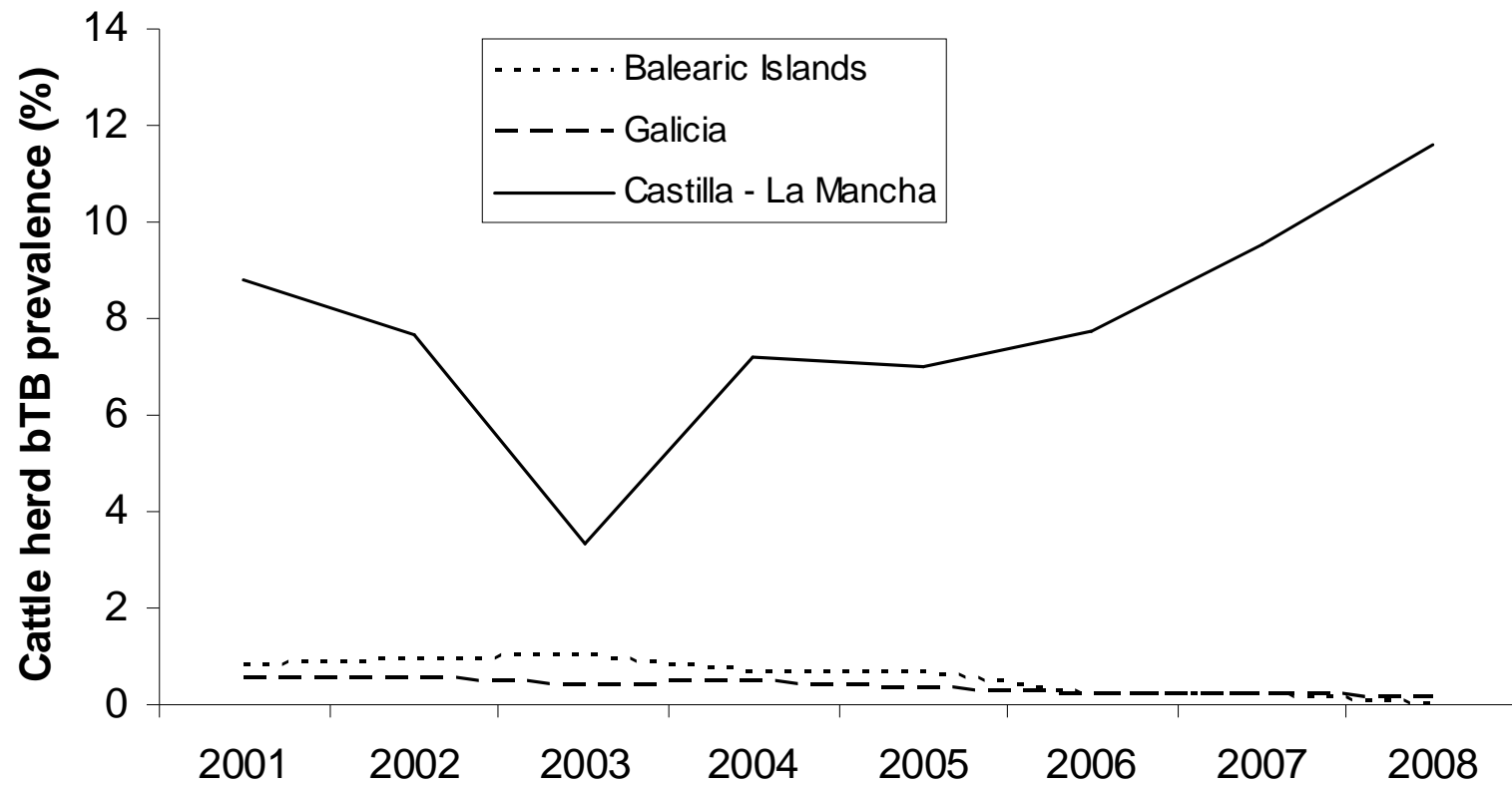
551 **Figure 1.-** Recent trends in cattle herd bovine tuberculosis (bTB) prevalence (in %) in three
552 ecologically contrasting Spanish regions, the Balearic Islands (Mediterranean climate, no
553 potential wildlife reservoirs), Galicia (Atlantic climate, potential wildlife reservoirs
554 abundant),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

557

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

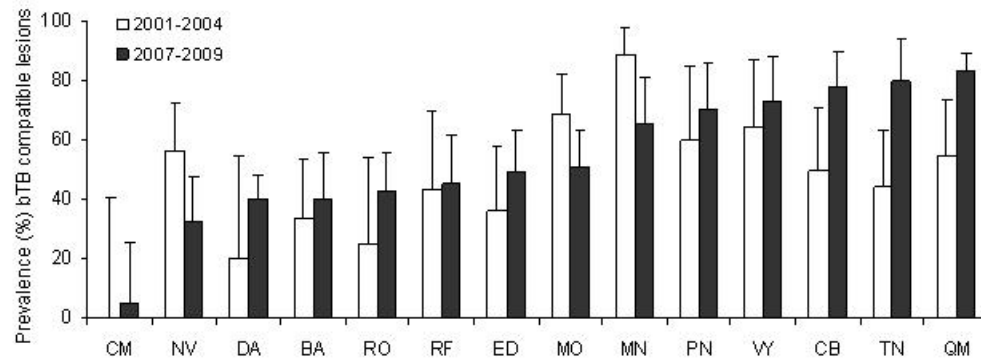
566

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





A



B

