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9	Corresponding Author	Organization Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM)
10		Division
11		Address Ronda de Toledo s/n, Ciudad Real 13071, Spain
12		e-mail mariana.boadella@uclm.es
13		Family Name Gortazar
14		Particle
15		Given Name Christian
16		Suffix
17	Author	Organization Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM)
18		Division
19		Address Ronda de Toledo s/n, Ciudad Real 13071, Spain
20		e-mail
21		Family Name Acevedo
22		Particle
23		Given Name Pelayo
24		Suffix
25	Author	Organization University of Malaga
26		Division Biogeography, Diversity, and Conservation Research Team, Animal Biology, Department of Sciences
27		Address Málaga 29071, Spain

28		e-mail	
29		Family Name	Carta
30		Particle	
31		Given Name	Tania
32		Suffix	
33	Author	Organization	Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM)
34		Division	
35		Address	Ronda de Toledo s/n, Ciudad Real 13071, Spain
36		e-mail	
37		Family Name	Martín-Hernando
38		Particle	
39		Given Name	María Paz
40		Suffix	
41	Author	Organization	Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM)
42		Division	
43		Address	Ronda de Toledo s/n, Ciudad Real 13071, Spain
44		e-mail	
45		Family Name	Fuente
46		Particle	de la
47		Given Name	José
48		Suffix	
49		Organization	Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM)
50	Author	Division	
51		Address	Ronda de Toledo s/n, Ciudad Real 13071, Spain
52		Organization	Oklahoma State University
53		Division	Department of Veterinary Pathobiology, Center for Veterinary Health Sciences
54		Address	Stillwater 74078, OK, USA
55		e-mail	
56		Family Name	Vicente
57		Particle	
58		Given Name	Joaquín
59	Author	Suffix	
60		Organization	Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM)
61		Division	

62	Address	Ronda de Toledo s/n, Ciudad Real 13071, Spain
63	e-mail	
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67	Abstract	<p>Monitoring is needed to identify changes in disease occurrence and to measure the impact of intervention. Using mycobacterial diseases as an example, we discuss herein the pros and cons of the current Spanish Wildlife Disease Surveillance Scheme providing suggestions for monitoring relevant diseases shared with wildlife in other regions facing similar challenges. Six points should be considered. This includes: (1) making sure the disease is properly monitored in the relevant domestic animals or even in humans; (2) also making sure that background information on wildlife population ecology is available to maximize the benefits of the monitoring effort; (3) selecting the appropriate wildlife hosts for monitoring, while being flexible enough to incorporate new ones if research suggests their participation; (4) selecting the appropriate methods for diagnosis and for time and space trend analysis; (5) deciding which parameters to target for monitoring; and finally (6) establishing a reasonable sampling effort and a suitable sampling stratification to ensure detecting changes over time and changes in response to management actions. Wildlife disease monitoring produces knowledge that benefits at least three different agencies, namely, animal health, public health and conservation, and these should combine efforts and resources. Setting up stable, comprehensive and accurate schemes at different spatial scales should become a priority. Resources are always a limiting factor, but experience shows that combined, cross-collaborative efforts allow establishing acceptable schemes with a low enough cost to be sustainable over time. These six steps for monitoring relevant shared diseases can be adapted to many other geographical settings and different disease situations.</p>
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Six recommendations for improving monitoring of diseases shared with wildlife: examples regarding mycobacterial infections in Spain

Mariana Boadella · Christian Gortazar · Pelayo Acevedo · Tania Carta ·
María Paz Martín-Hernando · José de la Fuente · Joaquín Vicente

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Abstract Monitoring is needed to identify changes in disease occurrence and to measure the impact of intervention. Using mycobacterial diseases as an example, we discuss herein the pros and cons of the current Spanish Wildlife Disease Surveillance Scheme providing suggestions for monitoring relevant diseases shared with wildlife in other regions facing similar challenges. Six points should be considered. This includes: (1) making sure the disease is properly monitored in the relevant domestic animals or even in humans; (2) also making sure that background information on wildlife population ecology is available to maximize the benefits of the monitoring effort; (3) selecting the appropriate wildlife hosts for monitoring, while being flexible enough to incorporate new ones if research suggests their participation; (4) selecting the appropriate methods for diagnosis and for time and space trend analysis; (5) deciding which parameters to target for monitoring; and finally (6) establishing a reasonable sampling effort and a suitable sampling stratification to

ensure detecting changes over time and changes in response to management actions. Wildlife disease monitoring produces knowledge that benefits at least three different agencies, namely, animal health, public health and conservation, and these should combine efforts and resources. Setting up stable, comprehensive and accurate schemes at different spatial scales should become a priority. Resources are always a limiting factor, but experience shows that combined, cross-collaborative efforts allow establishing acceptable schemes with a low enough cost to be sustainable over time. These six steps for monitoring relevant shared diseases can be adapted to many other geographical settings and different disease situations.

Keywords Disease monitoring · Paratuberculosis · Time trends · Tuberculosis · Wildlife diseases · Zoonoses

Introduction

The history of wildlife disease surveillance in Europe possibly started with the first passive surveillance schemes set up in Scandinavian countries in the 1930s (Mörner et al. 2002). Surveillance of rabies (King et al. 2004) and trichinellosis (Blancou 2001) started afterwards. However, the first scientific meetings did not occur until the early 1990s (Symposium on the health and management of free-ranging mammals held in Nancy, France, in 1991; First conference of the European section of the Wildlife Disease Association EWDA, in Paris, France, in 1994). These meetings prompted a more widespread interest in wildlife disease surveillance. In the last decades, classical swine fever in Eurasian wild boar (*Sus scrofa*; Rossi et al. 2005) and highly pathogenic avian influenza (Chen et al. 2005) further contributed to a growing interest on diseases shared

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M. Boadella (✉) · C. Gortazar · T. Carta ·
M. P. Martín-Hernando · J. de la Fuente · J. Vicente
Instituto de Investigación en Recursos Cinegéticos,
IREC (CSIC-UCLM-JCCM),
Ronda de Toledo s/n,
13071, Ciudad Real, Spain
e-mail: mariana.boadella@uclm.es

P. Acevedo
Biogeography, Diversity, and Conservation Research Team,
Animal Biology, Department of Sciences, University of Malaga,
29071, Málaga, Spain

J. de la Fuente
Department of Veterinary Pathobiology, Center for Veterinary
Health Sciences, Oklahoma State University,
Stillwater, OK 74078, USA

62 with wildlife such as zoonotic diseases and diseases that
63 have potential risk for domestic species (Gortazar et al.
64 2007). Detection of these relevant diseases in wildlife was
65 identified as a determinant of the structure and function of
66 European surveillance schemes (Leighton 1995). At a
67 worldwide scale, the World Organization for Animal Health
68 or OIE working group on wildlife diseases was also
69 established in 1994. It is now recognized that those
70 countries which conduct disease surveillance of their wild
71 animal populations are more likely to detect the presence of
72 infectious and zoonotic diseases and to swiftly adopt
73 counter measures (Mörner et al. 2002).

74 In Spain, the interest in wildlife diseases started in the
75 1980s and was boosted in 1989 with the emergence of
76 rabbit hemorrhagic disease in European wild rabbits
77 (*Oryctolagus cuniculus*; Villafuerte et al. 1994). In the last
78 decade, however, resources for studying wildlife diseases
79 increased after the identification of wildlife species as
80 actors in the epidemiology of important livestock diseases,
81 such as Aujeszky's disease (Müller et al. 1998), bluetongue
82 (Ruiz-Fons et al. 2008) and bovine tuberculosis (bTB;
83 Naranjo et al. 2008), and more recently after realizing the
84 importance of diseases in Iberian lynx (*Lynx pardinus*)
85 conservation (Millán et al. 2009). Risk factors for the
86 appearance of wildlife reservoirs are commonly the
87 spillover from domestic livestock in combination with
88 anthropogenic activities such as translocation of wildlife,
89 supplemental feeding of wildlife and wildlife populations
90 reaching densities beyond normal habitat carrying capaci-
91 ties (Gortazar et al. 2006; Palmer 2007). This, along with
92 the size of the Spanish livestock industry and the significant
93 proportion of free range breeding systems, prompted
94 specific calls for wildlife disease research in the national
95 grant scheme in 2006 and 2008 (INIA-FAU, [http://sp.inia.
96 es/ucc/contenidos/memo1.pdf](http://sp.inia.es/ucc/contenidos/memo1.pdf)).

97 Using mycobacterial diseases as an example, we discuss
98 herein the pros and cons of the current Spanish Wildlife
99 Disease Surveillance Scheme ([http://rasve.mapa.es/Publica/
100 Programas/NORMATIVA%20Y%20PROGRAMAS%
101 5CPROGRAMAS%5CFAUNA%20SILVESTRE%
102 5CPLAN%20NACIONAL%20DE%20VIGILANCIA%
103 20SANITARIA%20EN%20FAUNA%20SILVESTRE_2011.
104 PDF](http://rasve.mapa.es/Publica/Programas/NORMATIVA%20Y%20PROGRAMAS%5CPROGRAMAS%5CFAUNA%20SILVESTRE%5CPLAN%20NACIONAL%20DE%20VIGILANCIA%20SANITARIA%20EN%20FAUNA%20SILVESTRE_2011.PDF)), providing suggestions for wildlife disease monitoring
105 in other regions facing similar challenges.

106 Mycobacterial diseases in European wildlife

107 TB in Eurasian badgers (*Meles meles*) was first diagnosed
108 in Switzerland (Bouvier 1963), a country where no further
109 reports on wildlife TB exist in the scientific literature
110 (Wyss et al. 2000). Later, *Mycobacterium bovis* was
111 isolated from badgers in southwest England in 1971 and

Ireland in 1973. Since then, the infection in badgers has
112 been found throughout dense badger populations of
113 southwestern England and parts of Wales (Krebs 1997)
114 and throughout Ireland (Dolan 1993). By contrast, there
115 was no published TB case in badgers from the continent
116 since the first description in Switzerland in the 1960s, until
117 a recent case report from Spain (Sobrino et al. 2008). This
118 is surprising, since many countries in continental Europe
119 have both TB and badgers. Lower badger densities as
120 compared to Britain and Ireland may partly explain this
121 absence. However, a lack of targeted surveillance could
122 also contribute (Artois et al. 2009). 123

124 More recently, a growing body of evidence suggests that
125 other wildlife hosts do also act as *M. bovis* reservoirs in
126 different parts of Europe (Gortazar et al. [accepted for
127 publication](#)), including the Eurasian wild boar in Spain
128 (Naranjo et al. 2008) and Portugal (Santos et al. 2009) and
129 several cervids in different countries (e.g., Gortazar et al.
130 2008). As many countries attempt to eradicate bTB from
131 domestic livestock, success is impeded by spillback from
132 wildlife reservoirs. It will not be possible to eradicate *M.*
133 *bovis* from livestock until transmission between wildlife
134 and domestic animals is halted. Such an endeavor will
135 require a collaborative effort between agricultural, wildlife,
136 environmental and political interests (Palmer 2007). Now-
137 adays, TB is among the wildlife diseases receiving more
138 attention by scientists and government agencies. 138

139 Paratuberculosis in wildlife, by contrast, is receiving far
140 less attention in wildlife than TB. This disease, caused by
141 *Mycobacterium avium paratuberculosis* (MAP), has been
142 considered as a major disease of ruminants for more than a
143 century and has significant economic and welfare effects on
144 livestock in all continents. Recently, this bacterium has
145 received an increasing interest because of scientific evi-
146 dence that suggest that human infection with this microor-
147 ganism may be causing some, and possibly all, cases of
148 Crohn's disease (Naser et al. 2004; Uzoigwe et al. 2007).
149 The incidence of paratuberculosis is high in animals kept
150 intensively under environmental and husbandry conditions
151 which are conducive to the spread of the infection
152 (Chiodini et al. 1984). Cervids and other wild ruminants
153 have frequently been identified as MAP hosts, and high
154 prevalence along with clinical disease was reported in
155 some cases (Balseiro et al. 2008), but not in others (Carta
156 et al. [in press](#)). In Scotland, wild rabbits have been
157 identified as true wildlife MAP reservoirs too (Beard et
158 al. 2001), and a similar status may locally apply in Spain
159 (Maio et al. [in press](#)). 159

160 However, regular surveillance, other than the annual
161 reporting of TB cases and far more sporadic reporting of
162 wildlife paratuberculosis to the OIE, is not done at the
163 (European) country level, or at least not recorded in the
164 scientific literature. 164

165 **Wildlife disease monitoring**

166 Wildlife disease monitoring can be defined as the systematic
 167 recording of epidemiological data, with the specific purpose
 168 of detecting spatial and temporal trends as well as the
 169 presence/absence of the disease. Data and samples gathered
 170 can be used for detecting emerging diseases (Rhyan and
 171 Spraker 2010) and in retrospective studies (Oleaga et al.
 172 2008; Ruiz-Fons et al. 2008). Ideally, monitoring informa-
 173 tion should integrate data on the risk factors determining the
 174 pathogen epidemiology, such as host abundance and distri-
 175 bution, as they can inform us on potential disease spread in a
 176 given spatial or temporal frame. The concept is similar to
 177 surveillance, which is done in order to meet the objectives of
 178 controlling the disease (Artois et al. 2009). In contrast to
 179 disease surveillance, which may be passive based on clinical
 180 cases or active based on random sampling, monitoring is
 181 more often active.

182 Disease control at the human–livestock–wildlife inter-
 183 face should be based on a thorough knowledge of the
 184 “natural history” (ecology) of the disease agent and its
 185 human, domestic and wild hosts (Woodford 2009). Disease
 186 and population monitoring is a fundamental part of disease
 187 ecology. Figure 1 presents a diagram of how new diseases
 188 usually lead first to descriptive epidemiology and eventu-
 189 ally to risk factor analyses and control actions. If humans or
 190 domestic animals are affected, disease monitoring will start
 191 early in time. The decision to monitor this disease in

192 wildlife will depend on the relevance of wildlife hosts as
 193 disease reservoirs for humans or domestic animals or on the
 194 effects of the disease on wildlife population dynamics. Only
 195 if at least one of these options is suspected will monitoring
 196 of the disease among wildlife hosts be considered. As a
 197 consequence, wildlife disease monitoring usually starts
 198 much later in time. However, while this is the case for
 199 most regions in developed countries, in areas where wildlife
 200 species provide greater economic returns than livestock, the
 201 opposite might be the case. This has driven wildlife
 202 research and monitoring schemes in less developed
 203 countries where livestock and human health care are poor
 204 or nonexistent (Kock et al. 2002).

205 Disease monitoring in wildlife is promoted in order to
 206 obtain information to compare with the distribution and
 207 prevalence trends in livestock, as a basis for decision
 208 making regarding wildlife disease control, and as a means
 209 for assessing the effects of any disease management action.
 210 Monitoring, by definition, has no limited time frame.
 211 Monitoring wildlife disease trends requires adequate diag-
 212 nostic methods and differential diagnoses; a large-scale and
 213 long-term sampling network; the logistics linked to the
 214 preparation, distribution and conservation of valuable
 215 wildlife samples; and expertise for data management and
 216 analysis. In addition, a vital need exists to gather data from
 217 the ecology and wildlife management field in order to
 218 combine them with disease information regarding both
 219 wildlife and livestock (Delahay et al. 2009).

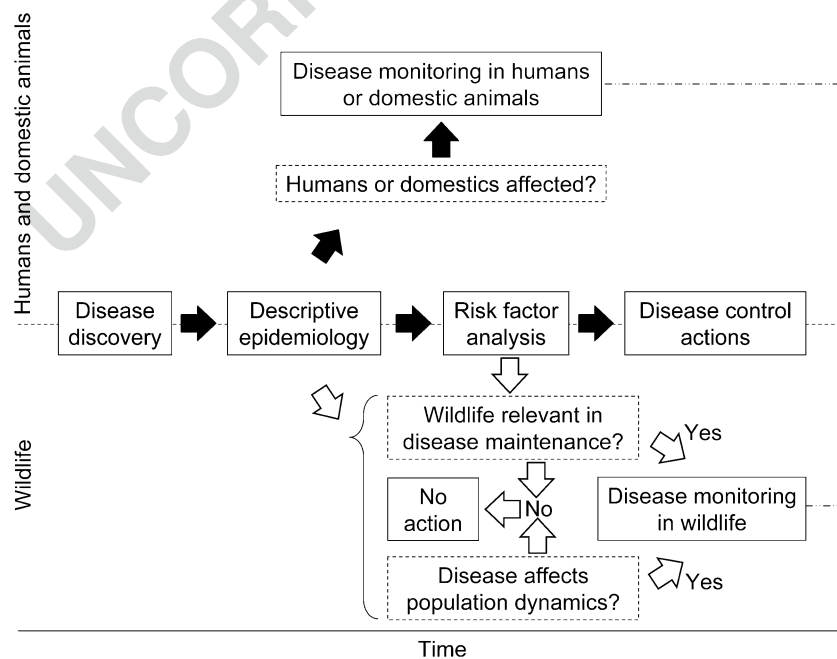


Fig. 1 Schematic representation of disease management in humans and domestic animals (*upper part*) and wildlife (*lower part*). *Dotted boxes* indicate decisions and the *arrow at the bottom* suggests time. Wildlife disease monitoring will mainly occur if wildlife species are

identified as significant reservoirs for humans or domestic animals, or if the disease has a significant impact on wildlife populations. This will probably happen later in time than monitoring in humans or domestics

220 **Recommendations for monitoring diseases in wildlife**

221 To properly monitor a wildlife disease, several points must
222 be considered. This includes: (1) making sure the disease, if
223 shared, is properly monitored in the relevant domestic
224 animals or even in humans; (2) also making sure that
225 background information on wildlife population ecology is
226 available to maximize the benefits of the monitoring effort;
227 (3) selecting the appropriate wildlife hosts for monitoring,
228 while being flexible enough to incorporate new ones if
229 research suggests their participation; (4) selecting the
230 appropriate methods for diagnosis and for time/space trend
231 analysis; (5) deciding which parameters to target for
232 monitoring: one or more disease agents, or lesions or
233 contact as revealed by serum antibodies; and finally (6)
234 establishing a reasonable sampling effort and a suitable
235 sampling stratification that can be prolonged over time.

236 First, if the disease is shared with humans or domestic
237 animals, do appropriate monitoring programs that allow, for
238 instance, trend comparisons between these and wildlife
239 exist? Regarding bTB, good information on the prevalence
240 and incidence in bovine livestock will be available in most
241 European situations. But, at the same time, information may
242 be lacking for other relevant — or potentially relevant —
243 domestic species such as goats and free-range pigs.

244 Second, wildlife disease monitoring will only make sense if
245 population monitoring is carried out at the same time, allowing
246 to link changes in abundance or management with changes in
247 disease indicators (Acevedo et al. 2007). This should include
248 not only the target wildlife hosts but also other relevant
249 competitor or prey species (Sobrino et al. 2009).

250 Third, wildlife disease monitoring should select for the
251 most appropriate wildlife hosts, considering not only
252 distribution, abundance, degree of protection, prevalence
253 and disease susceptibility but also ease of sample collection
254 and diagnostic sensitivity and specificity. For instance, in
255 Spain, TB has mainly been recorded in wild boar, red deer
256 (*Cervus elaphus*) and fallow deer (*Dama dama*; e.g.,
257 Gortazar et al. 2008), and as previously stated, sporadically
258 in badgers (Sobrino et al. 2008). TB has also occasionally
259 been described in red fox (*Vulpes vulpes*; Martín-Atance et
260 al. 2005) and Iberian lynx (*L. pardinus*; Peña et al. 2006).
261 However, wild boar are considered the best TB surveillance
262 target because of their wider distribution, higher abundance
263 and high availability as a game species and because of their
264 lesion distribution (Martín-Hernando et al. 2007). The
265 recent design of a specific and sensitive enough enzyme-
266 linked immunosorbent assay (ELISA) test (Aurtenetxe et al.
267 2008; Boadella et al. 2011) makes sample harvesting and
268 laboratory analyses relatively easy even if only head lymph
269 nodes and blood samples are available. By contrast, the
270 detection of TB-compatible lesions in cervids requires the
271 inspection of the head and neck, thorax and abdomen

(Vicente et al. 2006; Martín-Hernando et al. 2010). 272
Moreover, wild ruminants are often infected with other 273
mycobacteria, such as MAP, further compromising diag- 274
nostic specificity of some tests, particularly those based on 275
serum antibodies (Reyes-García et al. 2008; Carta et al. in 276
press). In turn, badgers have a more limited distribution in 277
Spain and are protected by law, making sampling difficult. 278
However, monitoring schemes should be flexible enough to 279
allow incorporating new species if research suggests their 280
participation in disease epidemiology (Delahay et al. 2001). 281

282 Fourth, the diagnostic and statistic methods should be 282
defined in a way that assures repeatability and data quality. 283
Diagnostic methods selected for wildlife disease monitoring 284
will depend on factors such as the selected host species and 285
expected sample size, the cost of each test, and its 286
specificity and sensitivity. Tests suitable for their use in 287
wildlife are not always available, and the difficulties 288
imposed by field sampling contribute to reduce test 289
sensitivity (Donnelly and Hone 2010). Statistical methods 290
will depend on factors such as the expected prevalence, the 291
geographic scale, the length of the time series and the 292
degree of change in time of the measured variable, being it 293
prevalence or lesion intensity (Joly et al. 2009). It is often 294
of use to study the age-specific prevalence rates, particu- 295
larly using juvenile prevalence as a proxy for incidence 296
(Wobeser 1994). Epidemiological data are peculiar from a 297
statistical perspective. Data with aggregated distributions 298
are usual in the epidemiological databases, so parametric 299
statistics, which are requiring normal distribution of the 300
data, cannot be generally used (e.g., Jewell 2009). So in 301
risk factor and disease trend assessment, generalized 302
models — with Poisson, negative binomial, zero-inflated 303
or binomial distributions — are needed. Information is 304
often generated at different spatial scales — from individual 305
to population or even to region — and so it is required to 306
use mixed models in which, by means of random variables, 307
pseudoreplication can be avoided (Zuur et al. 2009). 308
Another essential peculiarity is that the epidemiological 309
data of different host species is rarely available at the same 310
spatial resolution and at a high enough resolution to allow 311
meaningful inferences to be made. In general terms, data 312
analyzed should be referred to the same territorial units 313
(municipalities or provinces, for example), and the lowest 314
resolution will determine the spatial resolution of the 315
analysis (see Pfeiffer et al. 2008). 316

317 Fifth, once the host species are defined, it must be 317
decided what to target for monitoring. This means defining 318
the agent or agents: *M. bovis* only, or members of the *M.* 319
tuberculosis complex (MTBC), or MTBC and MAP, for 320
instance; and also defining what data will be needed, be it 321
the antigen by culture or PCR, specific antibodies or even 322
characteristic lesions (Vicente et al. 2006; Aurtenetxe et al. 323
2008; Santos et al. 2010). It is important to choose 324

325 parameters for which detection tools of known effective-
 326 nesses are available (Wobeser 1994). In addition, it is
 327 important to consider testing expenses and budget limita-
 328 tions. Thus, if funding is limited, it can be wise to combine
 329 more expensive techniques, such as culture, applied for
 330 confirmation to a subsample, with cheaper techniques such
 331 as gross pathology (e.g., Vicente et al. 2006). In most cases
 332 of mycobacterial disease monitoring, the target will be
 333 MTBC, but under certain circumstances, monitoring may
 334 need to include MAP because of the relevance of cross-
 335 reactivity to the tests used or because of the importance of
 336 MAP for the regional livestock industry (e.g., Balseiro et al.
 337 2008). Moreover, prevalence rates have a limited value for
 338 monitoring chronic diseases with a very protracted course
 339 (Wobeser 1994) such as mycobacterial infections.

340 Finally, it is of paramount importance to define an
 341 adequate and reasonable sample size as well as number and
 342 distribution of sampling localities according to statistical
 343 recommendations (Table 1). We must keep in mind the
 344 budget and the current and future logistic constraints such
 345 as the laboratory analysis throughput per day, the space
 346 available for short- and long-term sample storage and the
 347 design of proper databases and sample banking registers.
 348 Moreover, sampling must be adequately stratified by age
 349 and sex (Vicente et al. 2006), management (Vicente et al.
 350 2007) and study zone (Muñoz et al. 2010). Epidemiology
 351 software can help identify suitable sample sizes and can
 352 detect time trends when a known initial prevalence and an
 353 expected prevalence change are given (e.g., Win Episcopo,
 354 <http://www.clive.ed.ac.uk/cliveCatalogueItem.asp?id=B6BC9009-C10F-4393-A22D-48F436516AC4>;
 355 European Food Safety Authority 2010). For instance, sampling
 356 requirements will depend on the expected initial prevalence
 357 or the expected degree of change in these prevalences from
 358 time 1 to time 2 (Table 1). In order to spare costs, it may be
 359 advisable to pool samples for analysis (e.g., Tayce et al.
 360 2008) or to accumulate samples gathered during several
 361 years until the required sample size is achieved (see
 362 Table 2).
 363

Monitoring mycobacterial diseases in Spanish wildlife 364

365 Spain is a 504,782 km² country in southwestern Europe 365
 366 that includes two archipelagos, the Canary Islands off the 366
 367 West African coast, the Balearic Islands in the Mediterra- 367
 368 nean, and the autonomous towns of Ceuta and Melilla in 368
 369 the north of Africa. Based on habitat and climate features 369
 370 and wildlife population characteristics, Spain can roughly 370
 371 be divided into six bioregions (Muñoz et al. 2010; Fig. 2). 371
 372 The compulsory control of bTB in Spanish cattle has been 372
 373 successful, so that current individual cattle incidence is 373
 374 below 0.5%. However, the distribution of positive cattle 374
 375 herds is not uniform, with higher prevalence in Mediterra- 375
 376 nean habitats of the south and west of the Spanish 376
 377 mainland. Islands with no potential wildlife reservoirs are 377
 378 almost bTB-free (<http://rasve.mapa.es/Publica/Programas/NORMATIVA%20Y%20PROGRAMAS/PROGRAMAS/2010/TUBERCULOSIS/PROGRAMA%20NACIONAL%20DE%20ERRADICACION%20DE%20TUBERCULOSIS%20BOVINA.%20A%C3%91O%202010.PDF>). Of the sus- 381
 382 ceptible domestic hosts, bTB is only monitored in cattle 382
 383 and in goats living in close contact to cattle. Some 383
 384 regions have also implemented compulsory or voluntary 384
 385 bTB control programs in goats. In Spain, paratubercu- 385
 386 losis has been diagnosed for over 20 years in all three 386
 387 (cattle, sheep and goat) domestic ruminant species (Aller 387
 388 et al. 1973; Garrido and León-Vizcaíno 1979), but is not 388
 389 monitored. 389
 390

391 The current situation regarding tuberculosis in Spanish 391
 392 wildlife was recently reviewed (Gortazar et al. *in press*). 392
 393 Paratuberculosis, in turn, has been recorded in farmed red 393
 394 deer (Fernández-de-Mera et al. 2009), but preliminary data 394
 395 from nationwide surveys suggest that wildlife is only 395
 396 locally relevant in MAP epidemiology (Carta et al. *in* 396
 397 *press*). This is the case of fallow deer in an intensively 397
 398 grazed mountain area in northern Spain (Balseiro et al. 398
 399 2008) and possibly of European wild rabbits sharing 399
 400 pastures with infected domestic ruminants in southern 400
 401 Spain (Maio et al. *in press*). Sporadic records of MAP are 401
 402 also available for wild boar (Álvarez et al. 2005). 402

403 Therefore, wildlife TB prevalence is two orders of 403
 404 magnitude higher than in cattle, and it is most likely that 404
 405 certain wildlife reservoirs might locally interfere with 405
 406 the cattle bTB eradication efforts (Gortazar et al. 2008). 406
 407 In addition, TB has killed several endangered Iberian 407
 408 lynxes causing conservation concerns (Peña et al. 2006). 408
 409 These are clear reasons for targeting wild ungulates for 409
 410 TB monitoring and for taking into account the possible 410
 411 interference of MAP in certain diagnostic tools and host 411
 412 species (Boadella et al. 2011; Carta et al. *in press*). 412 **Q2**
 413 Table 3 presents an overview of the application of the six 413
 414 abovementioned recommendations to the current Spanish 414
 415 circumstances. 415

Q1t1.1 **Table 1** Sample effort needed for the detection of disease according to the expected prevalence (assuming a population size of >10,000) and for the detection of prevalence variations over 50% according to the initial prevalence (with a power of 90% and confidence level of 95%, Win Episcopo 2.0)

t1.2		Detection $P > 10,000$			
t1.3	Expected prevalence	0.1%	1%	5%	10%
t1.4	Required sample size	2,990	300	59	29
t1.5		Variation > 50%			
t1.6	Initial prevalence	1%	12%	30%	60%
t1.7	Required sample size	5,098	387	130	44

t2.1 **Table 2** Example regarding the Spanish wildlife disease surveillance scheme

t2.2		Birds	Carnivores	Hares	Rodents	Wild boar	Red deer	Roe deer	Wild bovinds
t2.3	BR 1	200	60	0	100	400	70	50	20
t2.4	BR 2	100	60	120	200	570	190	60	40
t2.5	BR 3	100	60	90	100	510	250	35	30
t2.6	BR 4	100	60	60	100	245	120	40	60
t2.7	BR 5	200	60	65	0	345	50	20	75
t2.8	BR 6	100			0				
t2.9	Total	800	300	335	500	2,070	680	205	225

Probability of detection: Annual samples by taxon and bioregion (BR 1–6). Shadings indicate that sampling is sufficient for the detection of prevalences of 10% (*light grey*), 5% (*medium grey*), and 1% (*dark grey*), with a power of 90% and confidence level of 95%; Win Episcope 2.0. *White boxes* represent situations where these levels are not achieved in only 1 year of sampling

416 **Discussion**

417 As our knowledge on wildlife diseases grows, disease
 418 control becomes more often an option. However, monitor-
 419 ing is needed to identify changes in disease occurrence and
 420 to measure the impact of interventions (McDonald et al.
 421 2008). Despite this fact, wildlife disease monitoring is
 422 largely in its infancy (Artois et al. 2009), and setting up
 423 stable, comprehensive and accurate schemes at different
 424 spatial scales (local, national and global) should become a
 425 priority for health authorities and wildlife managers.

In many countries including New Zealand, the United
 States and several ones in the European Union, wildlife
 vaccination as a means to contribute to bTB control in
 livestock is being seriously considered (e.g., Tompkins et
 al. 2009; Chambers et al. 2011; Corner et al. 2009;
 Ballesteros et al. 2009). In this context, the implementa-
 tion of wildlife TB-monitoring schemes is a real need.

One point to consider is who takes charge of the
 monitoring costs. Wildlife disease monitoring produces
 knowledge that benefits at least three different agencies,
 namely, animal health, public health and conservation. It

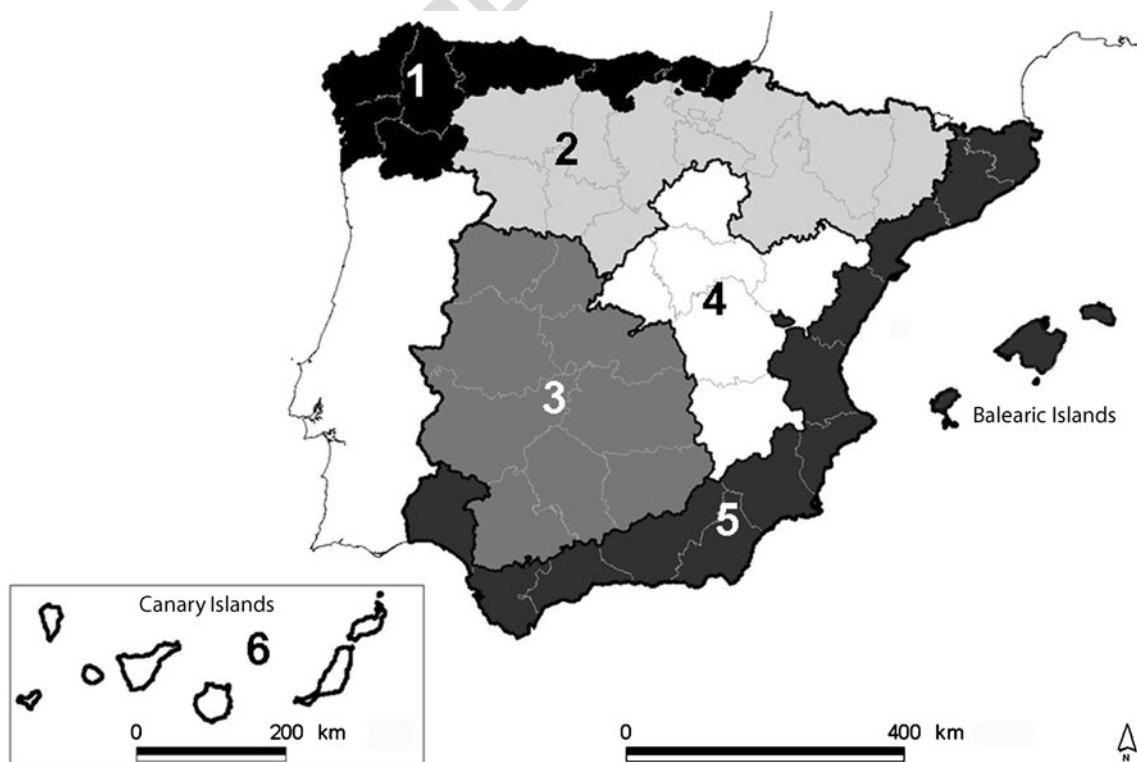


Fig. 2 Map of Spain, with a division into six large bioregions for sampling and wildlife disease monitoring, according to the Spanish Wildlife Disease Surveillance Scheme

t3.1 **Table 3** Main requisites, current circumstances, and recommendations for TB monitoring in Spanish wildlife

t3.2	Requisite	Current circumstances	Recommendations
t3.3	(1) Disease is properly monitored in the relevant domestic animals or even in humans.	Excellent monitoring in cattle.	Include most goat herds in monitoring.
t3.4		No nationwide compulsory monitoring in other domestic animals.	Improve information exchange with medics.
t3.5	(2) Background information on wildlife population ecology is available to maximize the benefits of the monitoring effort.	Human cases not always differentiated from <i>M. tuberculosis</i> .	
t3.6		Tools for estimating relative abundance and spatial aggregation are available for wild boar (Acevedo et al. 2007). No easy density estimation methods are available for wild boar.	Decide a tool (dung counts and/or hunting yields) and apply to all selected sampling sites.
t3.7		In deer, population density can be estimated (Acevedo et al. 2008, 2010).	Characterize other risk factors and monitor their changes through time.
t3.8		Management-related risk factors (feeding, waterholes, fencing) have been identified (Vicente et al. 2007) and are monitored.	
t3.9	(3) Select the appropriate wildlife hosts for monitoring, while being flexible enough to incorporate new ones.	Wild boar is an accessible and widespread game species and is more able to cross fences and likely to contact cattle than other ungulates; serosurveillance already exists for other infections.	Use wild boar as key indicator species.
t3.10		Deer are not as widespread.	Collect head lymph nodes and sera, along with data on sex and age.
t3.11		Badger distribution and abundance is limited.	Where available, use red deer, fallow deer and badger too.
t3.12	(4) Select appropriate methods for diagnosis and for time trend analysis.	Foxes are poor sentinels for mycobacterial diseases (Carta et al. in press).	
t3.13		Sensitive and highly specific ELISA available for wild boar (Aurtenetxe et al. 2008; Boadella et al. 2011), lesions easily detectable in wild boar heads (Martín-Hernando et al. 2007).	Use ELISA for calculating serum antibody prevalence, pathology for additional lesion scoring, and culture a subsample, for confirmation and molecular epidemiology.
t3.14		Cross-reactions and low sensitivity limit the use of ELISA in deer, and TB monitoring in deer requires inspecting whole carcass and using expensive and time-demanding pathology and culture (Martín-Hernando et al. 2010).	Expertise required for data management and statistical analysis.
t3.15	(5) Decide which parameters to target for monitoring: one or more disease agents, or lesions, or contact as revealed by serum antibodies?	Serum antibodies and TB-compatible lesions are time- and cost-effective in wild boar (Vicente et al. 2006; Aurtenetxe et al. 2008; Santos et al. 2010; Boadella et al. 2011).	Use wild boar serum antibody prevalence as main parameter, lesion scoring as additional tool.
t3.16			Pay attention to prevalence in juvenile age classes. Some proportion of culture confirmation is advisable for strain characterization and epidemiology.
t3.17	(6) Establish a reasonable sampling effort and distribution.	Wildlife sampling bioregions have been defined (Muñoz et al. 2010) and cattle bTB prevalence and distribution is well described.	Stratify sampling by bioregion and cattle bTB prevalence. Better sample from permanent sampling sites, which can be monitored for host abundance and management.
t3.18		Sampling effort depends on regional wild boar abundance and the collaboration of hunters and local authorities.	

437 would be wise to combine efforts and resources from all
 438 three compartments and to take advantage of the expertise
 439 of government agencies and academic institutions. Govern-
 440 ment attitudes toward wildlife disease research have changed
 441 during the last decades for reasons already listed in the
 442 “Introduction.” Now it is needed to convince other stakeholders

too such as the livestock industry, the hunting lobby or the
 conservationists, and even medics of the need to monitor
 wildlife diseases if we are thinking about their future control.
 Successful examples of collaboration between conservationists
 and vets [e.g., the detection and management of feline leukemia
 in the endangered Iberian lynx (López et al. 2009)], between

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AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES.

- Q1. Tables are renumbered here (original sequence: Table 2a, Table 2b, Table 1).
- Q2. The citation “Carta et al. 2011” (original) has been changed to “Carta et al. in press”. Please check if appropriate.

UNCORRECTED PROOF