# Six recommendations for improving monitoring of diseases shared with wildlife: examples regarding mycobacterial infections in Spain

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REVIEW

# Six recommendations for improving monitoring of diseases shared with wildlife: examples regarding mycobacterial infections in Spain

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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

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## 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

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

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

Using mycobacterial diseases as an example, we discuss herein the pros and cons of the current Spanish Wildlife Disease Surveillance Scheme (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 in other regions facing similar challenges.

## Mycobacterial diseases in European wildlife

TB in Eurasian badgers (*Meles meles*) was first diagnosed in Switzerland (Bouvier 1963), a country where no further reports on wildlife TB exist in the scientific literature (Wyss et al. 2000). Later, *Mycobacterium bovis* was isolated from badgers in southwest England in 1971 and Ireland in 1973. Since then, the infection in badgers has been found throughout dense badger populations of southwestern England and parts of Wales (Krebs 1997) and throughout Ireland (Dolan 1993). By contrast, there was no published TB case in badgers from the continent since the first description in Switzerland in the 1960s, until a recent case report from Spain (Sobrino et al. 2008). This is surprising, since many countries in continental Europe have both TB and badgers. Lower badger densities as compared to Britain and Ireland may partly explain this absence. However, a lack of targeted surveillance could also contribute (Artois et al. 2009).

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

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

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

## Wildlife disease monitoring

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

Disease control at the human–livestock–wildlife interface should be based on a thorough knowledge of the "natural history" (ecology) of the disease agent and its human, domestic and wild hosts (Woodford 2009). Disease and population monitoring is a fundamental part of disease ecology. Figure 1 presents a diagram of how new diseases usually lead first to descriptive epidemiology and eventually to risk factor analyses and control actions. If humans or domestic animals are affected, disease monitoring will start early in time. The decision to monitor this disease in wildlife will depend on the relevance of wildlife hosts as disease reservoirs for humans or domestic animals or on the effects of the disease on wildlife population dynamics. Only if at least one of these options is suspected will monitoring of the disease among wildlife hosts be considered. As a consequence, wildlife disease monitoring usually starts much later in time. However, while this is the case for most regions in developed countries, in areas where wildlife species provide greater economic returns than livestock, the opposite might be the case. This has driven wildlife research and monitoring schemes in less developed countries where livestock and human health care are poor or nonexistent (Kock et al. 2002).

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

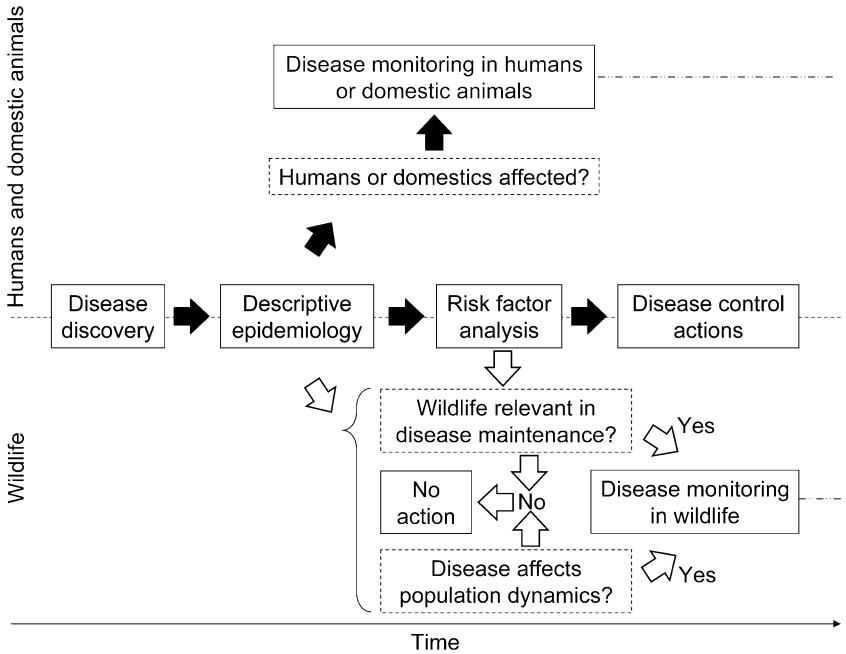


**Fig. 1** Schematic representation of disease management in humans and domestic animals (*upper part*) and wildlife (*lower part*). *Dotted boxes* indicate decisions and the *arrow at the bottom* suggests time. Wildlife disease monitoring will mainly occur if wildlife species are identified as significant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

## Recommendations for monitoring diseases in wildlife

To properly monitor a wildlife disease, several points must be considered. This includes: (1) making sure the disease, if shared, 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/space trend analysis; (5) deciding which parameters to target for monitoring: one or more disease agents, or lesions or contact as revealed by serum antibodies; and finally (6) establishing a reasonable sampling effort and a suitable sampling stratification that can be prolonged over time.

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

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

Third, wildlife disease monitoring should select for the most appropriate wildlife hosts, considering not only distribution, abundance, degree of protection, prevalence and disease susceptibility but also ease of sample collection and diagnostic sensitivity and specificity. For instance, in Spain, TB has mainly been recorded in wild boar, red deer (*Cervus elaphus*) and fallow deer (*Dama dama*; e.g., Gortazar et al. 2008), and as previously stated, sporadically in badgers (Sobrino et al. 2008). TB has also occasionally been described in red fox (*Vulpes vulpes*; Martín-Atance et al. 2005) and Iberian lynx (*L. pardinus*; Peña et al. 2006). However, wild boar are considered the best TB surveillance target because of their wider distribution, higher abundance and high availability as a game species and because of their lesion distribution (Martín-Hernando et al. 2007). The recent design of a specific and sensitive enough enzyme-linked immunosorbent assay (ELISA) test (Aurtenetxe et al. 2008; Boadella et al. 2011) makes sample harvesting and laboratory analyses relatively easy even if only head lymph nodes and blood samples are available. By contrast, the detection of TB-compatible lesions in cervids requires the inspection of the head and neck, thorax and abdomen (Vicente et al. 2006; Martín-Hernando et al. 2010). Moreover, wild ruminants are often infected with other mycobacteria, such as MAP, further compromising diagnostic specificity of some tests, particularly those based on serum antibodies (Reyes-García et al. 2008; Carta et al. in press). In turn, badgers have a more limited distribution in Spain and are protected by law, making sampling difficult. However, monitoring schemes should be flexible enough to allow incorporating new species if research suggests their participation in disease epidemiology (Delahay et al. 2001).

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

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

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

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

**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 Episcope 2.0)

| | Detection $P$>10,000 | | | |
|---|---|---|---|---|
| Expected prevalence | 0.1% | 1% | 5% | 10% |
| Required sample size | 2,990 | 300 | 59 | 29 |
| | Variation>50% | | | |
| Initial prevalence | 1% | 12% | 30% | 60% |
| Required sample size | 5,098 | 387 | 130 | 44 |

## Monitoring mycobacterial diseases in Spanish wildlife

Spain is a 504,782 km$^2$ country in southwestern Europe that includes two archipelagos, the Canary Islands off the West African coast, the Balearic Islands in the Mediterranean, and the autonomous towns of Ceuta and Melilla in the north of Africa. Based on habitat and climate features and wildlife population characteristics, Spain can roughly be divided into six bioregions (Muñoz et al. 2010; Fig. 2). The compulsory control of bTB in Spanish cattle has been successful, so that current individual cattle incidence is below 0.5%. However, the distribution of positive cattle herds is not uniform, with higher prevalence in Mediterranean habitats of the south and west of the Spanish mainland. Islands with no potential wildlife reservoirs are 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 susceptible domestic hosts, bTB is only monitored in cattle and in goats living in close contact to cattle. Some regions have also implemented compulsory or voluntary bTB control programs in goats. In Spain, paratuberculosis has been diagnosed for over 20 years in all three (cattle, sheep and goat) domestic ruminant species (Aller et al. 1973; Garrido and León-Vizcaíno 1979), but is not monitored.

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

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

**Table 2** Example regarding the Spanish Wildlife Disease Surveillance Scheme

| | Birds | Carnivores | Hares | Rodents | Wild boar | Red deer | Roe deer | Wild bovids |
|---|---|---|---|---|---|---|---|---|
| BR 1 | 200 | 60 | 0 | 100 | 400 | 70 | 50 | 20 |
| BR 2 | 100 | 60 | 120 | 200 | 570 | 190 | 60 | 40 |
| BR 3 | 100 | 60 | 90 | 100 | 510 | 250 | 35 | 30 |
| BR 4 | 100 | 60 | 60 | 100 | 245 | 120 | 40 | 60 |
| BR 5 | 200 | 60 | 65 | 0 | 345 | 50 | 20 | 75 |
| BR 6 | 100 | | | 0 | | | | |
| TOTAL | 800 | 300 | 335 | 500 | 2070 | 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

## Discussion

As our knowledge on wildlife diseases grows, disease control becomes more often an option. However, monitoring is needed to identify changes in disease occurrence and to measure the impact of interventions (McDonald et al. 2008). Despite this fact, wildlife disease monitoring is largely in its infancy (Artois et al. 2009), and setting up stable, comprehensive and accurate schemes at different spatial scales (local, national and global) should become a 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 implementation 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

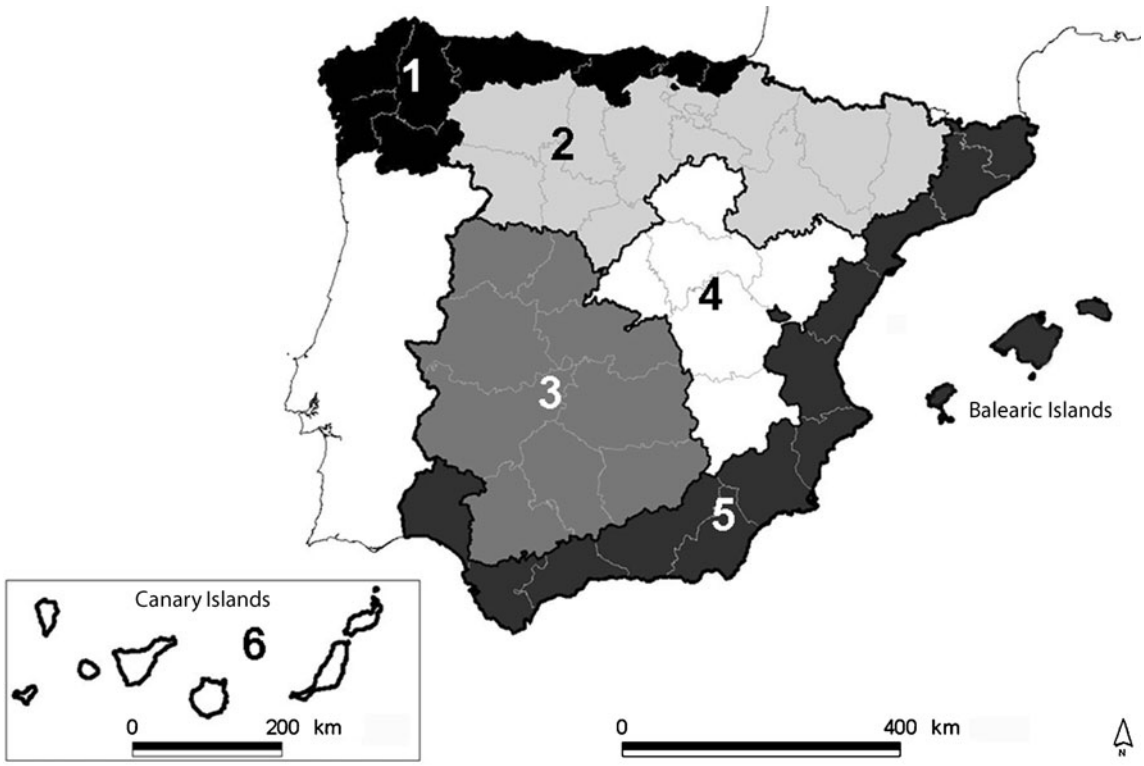


**Fig. 2** Map of Spain, with a division into six large bioregions for sampling and wildlife disease monitoring, according to the Spanish Wildlife Disease Surveillance Scheme

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

| Requisite | Current circumstances | Recommendations |
|---|---|---|
| (1) Disease is properly monitored in the relevant domestic animals or even in humans. | Excellent monitoring in cattle. | Include most goat herds in monitoring. |
| | No nationwide compulsory monitoring in other domestic animals. | Improve information exchange with medics. |
| | Human cases not always differentiated from *M. tuberculosis*. | |
| (2) Background information on wildlife population ecology is available to maximize the benefits of the monitoring effort. | 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. |
| | In deer, population density can be estimated (Acevedo et al. 2008, 2010). | Characterize other risk factors and monitor their changes through time. |
| | Management-related risk factors (feeding, waterholes, fencing) have been identified (Vicente et al. 2007) and are monitored. | |
| (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. |
| | Deer are not as widespread. | Collect head lymph nodes and sera, along with data on sex and age. |
| | Badger distribution and abundance is limited. | Where available, use red deer, fallow deer and badger too. |
| | Foxes are poor sentinels for mycobacterial diseases (Carta et al. 2011; Sobrino et al. 2011). | |
| (4) Select appropriate methods for diagnosis and for time trend analysis. | 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. |
| | 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. |
| (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. |
| | | Pay attention to prevalence in juvenile age classes. Some proportion of culture confirmation is advisable for strain characterization and epidemiology. |
| (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. |
| | Sampling effort depends on regional wild boar abundance and the collaboration of hunters and local authorities. | |

would be wise to combine efforts and resources from all three compartments and to take advantage of the expertise of government agencies and academic institutions. Government attitudes toward wildlife disease research have changed during the last decades for reasons already listed in the "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

vets and medics regarding many zoonoses such as trichinellosis (e.g., Wacker et al. 1999); and between conservationists, medics and vets [e.g., in zoonoses where wildlife are both reservoirs and victims, such as TB (Gortazar et al. 2005, 2008)] should serve as a trigger for future collaborations.

The six steps for surveillance of relevant shared diseases can be adapted to many other geographical settings and different disease situations. Regarding mycobacterial diseases, these are worldwide distributed and do frequently affect multihost systems at the domestic animal–wildlife interface as described for Spain. In any such situation, similar requisites to those outlined in Table 2 do apply. The same requisites are also valid for other disease systems, if they affect domestic animals or humans. For instance, deer might be better indicators of bluetongue virus circulation than vaccinated domestic sheep or cattle (Rodríguez-Sánchez et al. 2010).

Wildlife disease-monitoring programs that are integrated within national animal health surveillance infrastructures should have the capacity to respond promptly to the detection of unusual wildlife mortality and to institute epizootiological researches into new and emerging wildlife diseases (Mörner et al. 2002). Increased training and preparedness of human and animal health staff and government agencies, improved communication and continued research will enhance wildlife-monitoring efforts (Belant and Deese 2010). Resources are always a limiting factor, but the developments toward the monitoring of TB in Spanish wildlife show that combined efforts of local and national government agencies, along with the commitment of trans-disciplinary research, can allow setting up acceptable schemes with a low enough cost to be sustainable in time. Improvements, such as extending animal TB surveillance to goats and pigs and establishing improved links and data exchange with the human health system, are still needed. There exist opportunities for similar approaches elsewhere regarding other diseases, hosts, and geographic circumstances.

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