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Wildlife Tuberculosis: A Systematic Review of the Epidemiology in Iberian Peninsula

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1. Introduction

Mycobacterium bovis is the main etiological agent of bovine tuberculosis, infecting many species of wild and domestic mammals and also man. Bovine tuberculosis is a chronic and contagious infectious disease that has been reported to infect wild ungulates, carnivores, marsupials and primates (de Lisle *et al.*, 2002). Bovine tuberculosis (bTB) also occurs worldwide in livestock (Humblet *et al.*, 2009), causing annual economic losses estimated at 3 billion USD in 1995 (Steele, 1995). It remains a serious risk for animal health, and a threat for human health in many developing countries (Etter *et al.*, 2006). Several countries successfully eradicated bovine tuberculosis in livestock through test-and-slaughter and/or abattoir surveillance programs. Yet other countries, using similar strategies, did not achieve eradication and some even face the re-emergence of the disease (Schiller *et al.*, 2010). In Europe for instance, the prevalence of bTB in cattle is increasing in several countries (Gordejo & Vermeersch, 2006). Moreover current eradication and control programs in livestock in Europe are facing a range of challenges as stamping out is becoming a less attractive option for economic and environmental reasons and due to animal welfare concerns (Whiting, 2003).

Some of the abovementioned difficulties in eradicating bTB in cattle may relate with the occurrence of the disease in wildlife (Schiller *et al.*, 2010). In fact it has been demonstrated that the complete elimination of bTB can be extremely complicated by persistent infection of wild hosts, such as badgers in the United Kingdom, white tailed deer in the United States and brushtail possum in New Zealand (Corner, 2006). The single successful example of bTB eradication in a wildlife host is the Australian case, where it was accomplished through stamping out, which eliminated introduced water buffalo *Bubalus arnee*, the only maintenance host in that ecosystem, (Corner, 2006). This is not an option when autochthonous, protected or economic and socially valuable species are involved (Artois *et al.*, 2001). In most cases, an integrated control program is needed (Horan *et al.*, 2008), but this is often hampered by the lack of epidemiological data (Artois *et al*., 2001; Corner, 2006).

Bovine tuberculosis control programs in cattle are in place for several decades in Iberian Peninsula and consequently incidence has been decreasing (Allepuz *et al*., 2011; Cunha *et al*., 2011). However in the last few years incidence has stabilized, or even slightly increased in both Portugal and Spain (Allepuz *et al*., 2011; Cunha *et al*., 2011). The role of wildlife hosts in this scenario remains speculative; nevertheless the existence of wildlife reservoirs may compromise the goal of eradication in cattle. Besides livestock, attention should be given to spill-over from wildlife to other domestic animals (e.g. goats and free-ranging pigs) and even to humans, namely hunters and others that handle wild ungulate carcasses (Gortazar *et al*., 2011b, in press). Wildlife-to-human transmission of *M. bovis* is hard to prove and no single case has been documented in Iberian Peninsula, but it is known to occur elsewhere (e.g. USA – Wilkins *et al*., 2008). Bovine tuberculosis is also one of the main infectious diseases affecting the critically endangered Iberian lynx *Lynx pardinus*, with several free-ranging and captive lynx killed by this infection (Millán *et al*., 2009). Iberian lynx is subject to an intensive multinational conservation program in Iberian Peninsula, which includes releasing captive-bred animals to former range. The persistence of *M. bovis* on the environment and in prey species poses a threat to this conservation action (Millán *et al*., 2009).

Iberian Peninsula ecosystems display a high degree of human intervention and have experienced some profound changes in the last decades. The most important alterations were a shift from domestic ungulate to wild ungulate production for hunting purposes (Miguel *et al*. 1999) and an increasing intensification of the later (Vargas *et al*. 1995). This management of wild ungulate populations aims to increase profits by increasing harvest, translating into increased densities of hunted species. This has been accomplished through introduction/restocking, provision of food and water (mostly during the summer shortage), fencing and sometimes even medication (Miguel *et al*. 1999, Gortázar *et al*., 2006). All these changes have potential implications on bTB epidemiology (Gortázar *et al*., 2006).

In the Iberian Peninsula, ungulates such as the wild boar *Sus scrofa* and the red deer *Cervus elaphus* have been recognized as the most important maintenance hosts for wildlife tuberculosis (Gortázar *et al*., 2011b). Nevertheless other species have also been identified as locally non-negligible hosts, such as the fallow deer *Dama dama* and the badger *Meles meles* (Gortázar *et al*., 2011b; Balseiro *et al*., 2011). Several other species of ungulates and carnivores were also found infected (Rodriguez *et al*., 2010). This situation fits the definition of a multi-host pathogen within a multi-species ecosystem (Renwick *et al*., 2007; Gortázar *et al*., in press), in which pathogen persistence and spread is dependent on the density of each maintenance host species and also on the effective interspecies contact rate (dependent on the ecology of each species).

Research on host-pathogen interaction usually deals with single-host single-pathogen systems, where disease persistence depends solely on the intra-species transmission rate (Tompkins *et al*., 2001). If transmission is density-dependent, then population thresholds for disease invasion and persistence are expected and have been described (Swinton *et al*., 2001). By contrast, in multi-host pathogens systems, disease persistence is dependent on both intra and inter-species transmission rates and densities of several host species (Renwick *et al*., 2007). Moreover, these rates depend on pathological, epidemiological, ecological and behavioural factors (Corner, 2006).

In such a complex epidemiological setting, it is imperative to determine the precise role of each host species in pathogen maintenance before comprehensive control measures are undertaken. Much has been investigated in the last decade regarding wildlife tuberculosis epidemiology in Iberian Peninsula. In order to contribute to understanding the mechanisms underlying wildlife tuberculosis persistence in the multi-host ecosystems of this region, under widely different ecological and management pressures, we report a systematic bibliographic review on this subject. The aim of this review was to survey the peer-reviewed literature for evidence of the: *i*) epidemiological status of each host species; *ii*) determinants of wildlife tuberculosis occurrence; *iii*) geographical structuring of wildlife tuberculosis in the Iberian Peninsula; *iv*) time trends in wildlife tuberculosis occurrence.

2. Methods

We conducted a systematic bibliographic review for epidemiological studies on tuberculosis in wildlife in Iberian Peninsula by searching MEDLINE/PubMed, up to the 31st of August 2011, using MeSH and keywords: "*Mycobacterium bovis*", "*Mycobacterium caprae*", "wild boar", "deer", "epidemiology", "Iberian Peninsula", "Portugal" and "Spain". Combinations used were: ("Portugal" OR "Spain") AND ("*Mycobacterium bovis*" OR "*Mycobacterium caprae*"), ("*Mycobacterium bovis*" OR "*Mycobacterium caprae*") AND "wild boar" AND "epidemiology" and ("*Mycobacterium bovis*" OR "*Mycobacterium caprae*") AND "deer" AND "epidemiology". Abstracts were selected according to their relevancy and excluded if dealing exclusively with laboratory or pathology investigations, domestic species or humans or other geographical regions. Articles were reviewed in full text.

For each article, information about the type of epidemiological study and study design, sample size and sampling methodology, screening and diagnostic tests used, prevalence rate, time frame of the study, study areas, characteristics of the populations studied, risk factors identified and host epidemiological status was summarized and presented in table format for easy comparison. Due to their idiosyncrasies, molecular epidemiology articles were characterized differently according to the number of isolates studied, genotyping technique, mycobacterial species reported, number of genotypes found, host and geographical clustering of genotypes and study areas. Due to differing methodologies and sometimes incomplete reporting of results, meta-analysis was not applicable except for a small number of studies.

For the purpose of this review, wildlife tuberculosis was defined according to the OIE definition of bovine tuberculosis, but *Mycobacterium caprae* was also considered etiological agent, besides *M. bovis*.

3. Results

The bibliographic search yielded 286 articles. Initially, title and abstracts were reviewed and 247 articles excluded because they deal only with laboratory/pathology investigations (n=74), domestic animals (n=41), humans (n=50), other geographical regions (n=79), or were review/model articles (n=3). Full text papers were then reviewed and further 6 papers were excluded because they focused exclusively on laboratory/pathology investigations. Therefore 33 articles were selected as of interest to the present review.

Reference	Type	Sampling strategy	Sample n	Screening test	Diagnostic test	Time frame & tendency	Prevalence (rate)	Fencing	Study areas
Aranaz *et al.* (2004)	SU	Targeted (hunted)	96		BC		51 (53,1%)	MX	7 area SW Spain
Acevedo-Whitehouse *et al.* (2005)	CS	Targeted (hunted)	175		BC	2000-2003	82 (47%)	MX	7 areas SW Spain
Parra *et al.* (2005)	CS	Scanning (hunted)	112	MI	BC		112	FE	1 region W Spain
de Mendoza *et al.* (2006)	CS	Scanning (hunted)	8.478	MI	BC	1992-2004 increasing	333 (3,92%)	MX	1 area W Spain
Parra *et al.* (2006)	CS	Scanning (hunted)	34.582	MI	BC	1997-2002 increasing	625 (1,81%)	MX	1 region W Spain
Vicente *et al.* (2006a)	CS	Targeted (hunted)	1.060		GP BC (not all)	1999-2004	(42,51%, mean estate rate)	MX	57 areas SW Spain
Vicente *et al.* (2006b)	CS	Targeted (hunted)	412		GP BC (not all)	1999-2004	(18,2%-100%)	FE	19 area SW Spain
Gortázar *et al.* (2008)	CS	Targeted (culled)	124		BC	2006-2007	65 (52,4%)	FR	1 area SW Spain
Romero *et al.* (2008)	SU	Targeted (culled)	214		BC	1998-2003	60 (28,0%)	FR	1 area SW Spain
Santos *et al.* (2009)	CS	Targeted (hunted)	162		BC	2005-2007	18 (11,1%)	FR	8 areas South-central Portugal
Cunha *et al.* (2011)	SU	Scanning (hunted)	343	MI	BC	2002-2010	(63%)	MX	Several areas across Portugal
Gortázar *et al.* (2011a)	CS	Targeted (culled)	124		BC	2006-2007	62 (50%)	FR	1 area SW Spain
Pinto *et al.* (2011)	CS	Targeted (hunted)	132	GP	BC	2008-2009	21 (15,9%)	MX	1 area Central Portugal

Table 1. Studies dealing with wild boar included in the analysis. Classification: SU – survey; CS - cross sectional study; CC – case-control study; Screening/diagnostic test: MI – official meat inspection scheme; GP – gross pathology; BC – bacteriological culture; SE – serology; Fencing: FR – free-ranging populations; FE – fenced populations; MX – mixed free-ranging and fenced populations.

3.1 Characterization of published articles

Investigation of bTB epidemiology in wild boar and red deer (most often studied hosts) are mostly cross-sectional (11/14), the rest being surveys (Tables 1-2). Most studies opt for targeted surveillance on hunted (6/14) or culled (3/14) animals, the rest relying on scanning surveillance in routine meat inspection schemes for detection of macroscopic lesions-like lesions (Table 1-2). The mean number of animals studied in targeted-design studies is 278 for wild boar (n=9, range 96-1.060) and 401 for red deer (n=6, range 95-1.368). Thirteen out of fourteen studies use bacteriological culture as the diagnostic test. Nevertheless most of them (9/14) also include a previous screening test (usually gross pathology or routine meat inspection schemes), followed by bacteriological culture when macroscopic lesions were observed (Table 1-2).

As regards studies on other host species (ungulates and carnivores), 5/14 are case reports, 6/14 surveys while 3/14 are cross sectional studies (Table 3). Five out of twelve studies rely on passive surveillance of haphazardly found carcasses and 3/12 on targeted surveillance of purposefully trapped animals. Most of these studies deal with carnivore species. As expected regarding novel host species, 3/12 studies are case reports (Table 3). Mean number of animals studied in survey studies is 105 for fallow deer (n=4, range 89-134), 63 for badger (n=3, range 2-157) and 15 for Iberian lynx (n=5, range 1-39). Most other species (Table 5) are dealt in single studies, usually as case reports. Serologic tests were used in 3/9 studies investigating other host species, such as Barbary sheep and carnivores (Table 3).

3.2 Prevalence rates

For the wild boar populations surveyed by targeted-design studies using bacteriological culture as diagnostic test on all animals (n=6), prevalence rates ranged 0,11-0,53, with a meta prevalence rate of 0,36 (Table 5). Including all studies, regardless of design, prevalence rates ranged 0,18-1 (Table 1). For the red deer populations surveyed by targeted-design studies using bacteriological culture as diagnostic test on all animals samples (n=3), prevalence rates ranged 0,02-0,27, with a meta prevalence rate of 0,21 (Table 5). Including all studies, regardless of design, prevalence rates ranged 0,01-0,44 (Table 2). For the fallow deer populations surveyed by targeted-design studies using bacteriological culture as diagnostic test on all animals samples (n=4), prevalence rates ranged 0,13-0,67, with a meta prevalence rate of 0,28 (Table 5). For other host species, the sample size and/or the study design do not allow meta analysis.

3.3 Trends

Few studies address or allow addressing the time trend of bTB prevalence rates. In Doñana, bTB was not detected in targeted wildlife health surveillance until 1990's, when the population of cattle greatly increased, while in 2000's high prevalence rates were found in all ungulate species (Gortázar *et al.*, 2008). In fact, prevalence rates in this area increased from 1998-2003 to 2006-2007 by 100% in wild boar and 50% in red deer (Gortázar *et al.*, 2011b). In Extremadura region, West-central Spain, prevalence rates detected in routine meat inspection schemes steadily raised from 1994-2004, while not detected in 1992-1993 (de Mendonza *et al.*, 2006). One study area in South-eastern Portugal showed an increase in *M. bovis* infection rates in wild boar from 0,46 in 2005/06 (Santos *et al.*, 2009) to 0,78 in 2009/11 (Santos *et al.*, unpublished data).

Reference	Type	Sampling strategy	Sample n	Screening test	Diagnostic test	Time frame & tendency	Prevalence (rate)	Fencing	Study areas
Aranaz *et al*. (2004)	SU	Targeted (hunted)	108		BC		26 (24,1%)	MX	5 areas SW Spain
Parra *et al*. (2005)	CS	Scanning (hunted)	59	MI	BC		59	FE	1 region W Spain
de Mendoza *et al*. (2006)	CS	Scanning (hunted)	36.144	MI	BC	1992-2004 increasing	394 (1,09%)	MX	1 area W Spain
Parra *et al*. (2006)	CS	Scanning (hunted)	50.009	MI	BC	1997-2002 increasing	591 (1,18%)	MX	1 region W Spain
Vicente *et al*. (2006a)	CS	Targeted (hunted)	1.368		GP BC (not all)	1999-2004	(13,71% mean rate)	MX	21 areas SW Spain
Vicente *et al*. (2006b)	CS	Targeted (hunted)	574		GP BC (not all)	1999-2004	(0-44,0%)	FE	19 areas SW Spain
Gortázar *et al*. (2008)	CS	Targeted (culled)	95		BC	2006-2007	26 (27,4%)	FR	1 area SW Spain
Romero *et al*. (2008)	SU	Targeted (culled)	168		BC	1998-2003	26 (15,5%)	FR	1 area SW Spain
Castillo *et al*. (2010)	CS	Scanning (hunted)	551	MI	BC	2007-2009	28 (5,1%)	MX	2 areas SW Spain
Cunha *et al*. (2011)	SU	Scanning (hunted)	544 samples with lesion	MI	BC	2002-2010	(51%)	MX	Several areas across Portugal
Gortázar *et al*. (2011a)	CS	Targeted (culled)	95		BC	2006-2007	24 (25,3%)	FR	1 study area SW Spain
Pinto *et al*. (2011)	CS	Targeted (hunted)	339	GP	BC	2008-2009	35 (10,3%)	MX	1 area Central Portugal

Table 2. Studies dealing with red deer included in the analysis. Classification: SU – survey; CS - cross sectional study; CC – case-control study; Screening/diagnostic test: MI – official meat inspection scheme; GP – gross pathology; BC – bacteriological culture; SE – serology; Fencing: FR – free-ranging populations; FE – fenced populations; MX – mixed free-ranging and fenced populations.

Reference	Type	Sampling strategy	Sample n	Diagnostic test	Time frame	Prevalence (rate)	Fencing	Study areas
Briones *et al*. (2000)	CR		1 Iberian lynx	BC		1	FR	1 - SW Spain
Pérez *et al*. (2001)	CR		1 Iberian lynx	BC		1	FR	1 - SW Spain
Aranaz *et al*. (2004)	SU	Targeted (hunted)	89 fallow deer	BC		60 fallow deer (67,4%)	MX	2 area SW Spain
		Scanning (carcasses)	4 Iberian lynx			3 Iberian lynx		
Atance *et al*. (2005)	SU	Scanning (carcasses)	7 red fox 2 mongoose 2 genets 1 Iberian lynx 4 mustelids	BC		1 red fox	FR	1 area SW Spain
Atance *et al*. (2006)	SU	Targeted (trapped)	118 red fox 5 mongoose 4 genets 39 Iberian lynx 32 mustelids	SE (ELISA MPB70)		5 red fox (4%) 1 Iberian lynx (3%) 7 badger (23%)	FR	1 area SW Spain
Gortázar *et al*. (2008)	CS	Targeted (culled)	97 fallow deer	BC	2006-2007	18 (18,5%)	FR	1 area SW Spain
Millán *et al*. (2008)	CR		1 red fox	BC		1	FR	1 area SW Spain
Romero *et al*. (2008)	SU	Targeted (culled)	134 fallow deer	BC	1998-2003	17 (12,7%)	FR	1 area SW Spain
		Scanning (carcasses)	10 Iberian lynx			4 (40%)		
			5 red fox			2 (40%)		
Sobrino *et al*. (2008)	CR		1 badger	BC		1	FR	1 area SW Spain
Candela *et al*. (2009)	CS	Targeted (hunted)	61 Barbary sheep	SE (icELISA MPB70)	1999	(50%)	FR	1 area SE Spain
Millán *et al*. (2009)	SU	Targeted (trapped) Scanning (carcasses)	26 Iberian lynx 33 red fox 24 mongoose 10 gennet 2 badger	BC PCR SE (cELISA MPB70)	2004-2006	SE: 1 red fox 1 mongoose 2 badger BC: 2 red fox 2 Iberian lynx	FR	2 area SW Spain
Balseiro *et al*. (2009)	CR		1 roe deer	PCR IHC		1	FR	1 area N Spain
Gortázar *et al*. (2011a)	CS	Targeted (culled)	100 fallow deer	BC	2006-2007	21 (21%)	FR	1 area SW Spain
Balseiro *et al*. (2011)	SU	Targeted (trapped) Passive (carcasses)	157 badger (121 found dead, 36 trapped)	BC	2006-2010	8 found dead (6,6%) 0 trapped	FR	Several areas across Spain

Table 3. Studies dealing with other host species included in the analysis. Classification: SU – survey; CS - cross sectional study; CC – case-control study; Screening/diagnostic test: MI – official meat inspection scheme; GP – gross pathology; BC – bacteriological culture; SE – serology; IHC – immunohistochemistry; ELISA - enzyme-linked immune serum assay; Fencing: FR – free-ranging populations; FE – fenced populations; MX – mixed free-ranging and fenced populations.

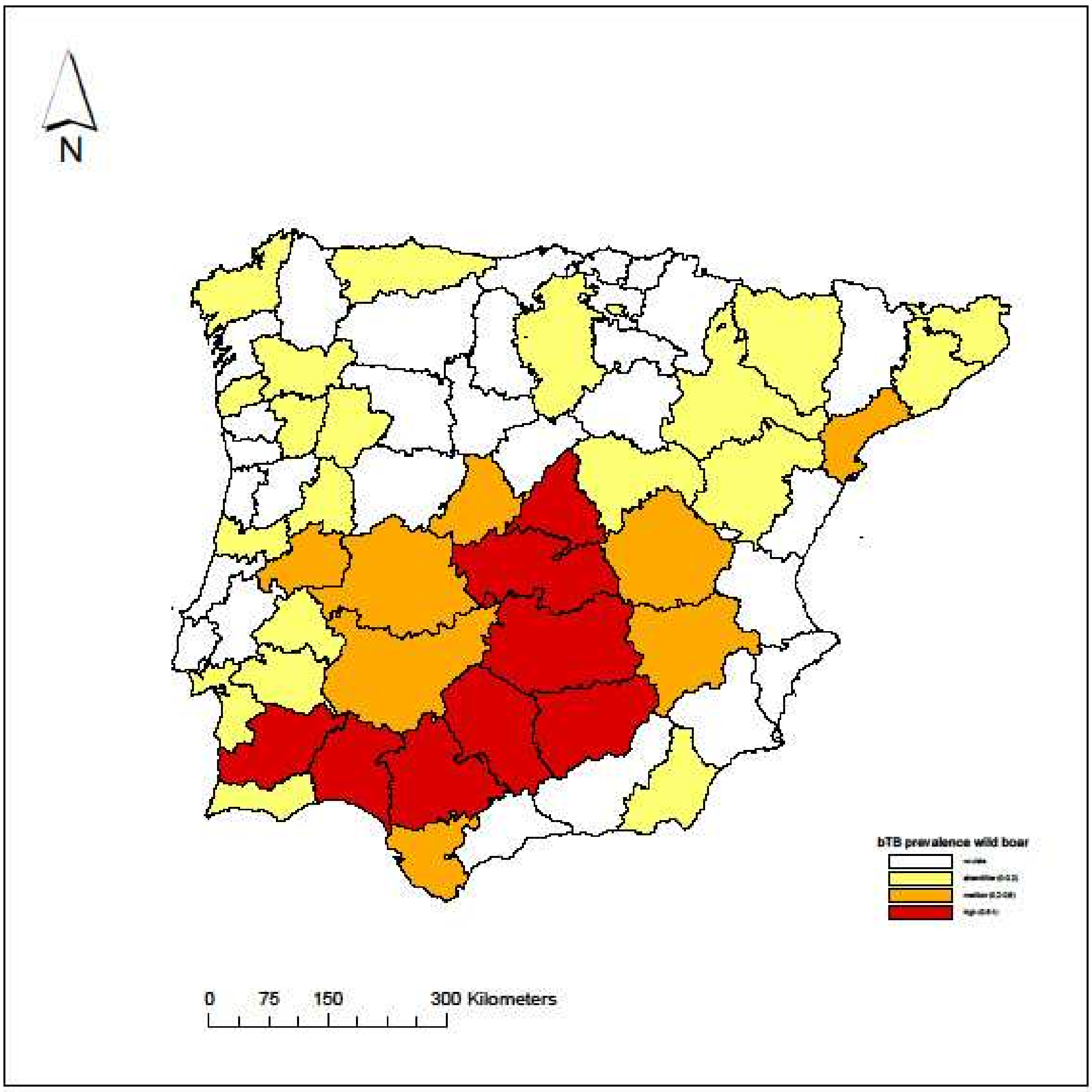

Fig. 1. Map displaying reported prevalence rates for bTB in the wild boar by administrative divisions of Iberian Peninsula (provinces in Spain, districts in Portugal). Bacteriological culture data (Aranaz *et al*., 2004; de Mendoza *et al*., 2006; Vicente *et al*., 2006a; Gortázar *et al*., 2008; Santos *et al*., 2009; Pinto *et al*., 2011) and serology data (Boadella *et al*., 2011; Santos *et al*., unpublished data) combined. The highest recorded prevalence for each administrative division is shown.

Again, few published articles address or allow addressing the geographical trend in bTB prevalence rates. In South-central Spain, an area roughly corresponding to Sierra Morena and Montes de Toledo was shown to have high prevalence rates, which declined towards the periphery of the area (Vicente *et al*., 2006a). In Doñana, wild boar and red deer show an increasing South-North gradient in prevalence rates (Gortázar *et al*., 2008). In Portugal, bTB was not detected in western regions, while present in the eastern portion of the country (Santos *et al*., 2009). Also in Eastern-central Portugal, wild boar and red deer populations show an increasing North-South gradient in prevalence rates (Pinto *et al*., 2011). In South-central Spain, lack of geographical autocorrelation in prevalence rates was reported (Vicente *et al*., 2006b).

3.4 Determinant factors of disease

Several risk and protective factors for bTB in both wild boar and red deer have been identified (Table 4). Most of the identified risk factors relate to host and other sympatric host's population factors, but also to environmental, management and historical factors. On the other hand, protective factors are mainly associated with environmental variables (Table 4). Notably, only one study has identified fencing, feeding and watering of wild ungulate populations as risk factors.

Determinants of disease		Wild boar	Red deer
	Type of risk factor		
Risk factors	Host population	Reproductive season Age Sex bTB prevalence rate in sympatric wild boar Wild boar abundance	Reproductive season Age Sex bTB prevalence rate in sympatric red deer
	Other hosts	Red deer presence Red deer abundance bTb prevalence rate in sympatric red deer	bTb prevalence rate in sympatric wild boar
	Environmental	Agro forestry land cover	
	Management	Aggregation at watering sites	Aggregation of wild boar at watering and feeding sites Fencing Supplementary feeding Presence water ponds Presence of livestock
	Historical	Past cattle density Distance to historical refuges	Past cattle density
Protective factors	All	Shrub land cover Distance to freshwater Sparse forestry land cover Genetic variability	Distance to freshwater

Table 4. Determinant factors of bTB occurrence identified in wild boar and red deer epidemiological studies in the Iberian Peninsula.

3.5 Host epidemiological status

Wild boar and red deer are usually considered maintenance hosts in Iberian Peninsula and epidemiological evidence has been gathered to support this view (Table 5) based on the characterization of populations maintaining high bTB prevalence rates despite long-term lack of contacts with cattle. Fallow deer, Barbary sheep and badger are also discussed as possible maintenance hosts, while all other reported hosts are considered spillover. Wild boar, red and fallow deer have been suggested as possible reservoirs of infection for livestock.

3.6 Molecular epidemiology

The most commonly identified causative agent of bTB in Iberian Peninsula has been *M. bovis*, although a small proportion (0,05, n=829) of *Mycobacterium caprae* was reported in 6/15 studies. *M. caprae* is much more frequent among isolates from wild boar (0,08, n=502) than from red deer (0,01, n=327). *Mycobacterium avium*-complex mycobacteria and other mycobacteria have also been isolated from wild hosts, but they fall out of the scope of the present review. Molecular epidemiology studies rely mostly on spoligotyping (14/15), usually coupled with MIRU-VNTR typing (9/15) (Table 6).

4. Discussion

4.1 Characterization of published articles

Most epidemiological studies on wild boar or red deer are cross-sectional, allowing for the estimation of prevalence rates and simultaneously the identification of risk or protective factors. A few of the earliest studies were surveys; also classified as such were some molecular epidemiology articles that allow calculating prevalence rates. As knowledge of bTB on other species is more recent, a larger proportion of these studies are case reports and surveys. A comparatively large number of studies address molecular epidemiology.

Notably absent from the literature are case-control studies, which could shed light on the importance of specific determinants of disease, such as fencing and provision of feed and water. The same should be mentioned for experimental studies, were exposure to a certain determinant of disease is manipulated and the effect on disease occurrence is then measured. This design could be of great help to ascertain the role of each species in the persistence of bTB, trough manipulation of host density. The same can be said for epidemiological modelling, which could provide a theoretical framework for understanding bTB persistence in Iberian Peninsula and test the effect of different control measures (Thrushfield *et al.*, 1995) and also to identify key data on host populations and wildlife tuberculosis that is missing or that is not feasible or up to date.

Most articles resort to targeted surveillance of hunted or culled animals, which allows prevalence estimation. Culling is expected to be less sex and age-biased than recreational hunting, which focuses on specific age (adults) and sex (males) classes. The hunting method used for harvesting the animals (drive hunts) is less selective than trophy hunting, allowing access also to females and juvenile/subadult animals (Fernández-Llario & Mateos-Quesada, 2003; Martínez *et al.*, 2005). Hunted animals are usually considered a representative sample of the population for health monitoring, at least for non neurological or debilitating diseases (Conner *et al.*, 2000). Nevertheless it should be kept in mind that some sampling biases can be present (Wilson *et al.*, 2001).

Taxonomic Order	Species	Diagnostic technique	Mycobacterial species	Meta prevalence	Epidemiological status	References
Artiodactyla	Wild boar *Sus scrofa*	Bacteriology culture other	*M. bovis* *M. caprae*	276/771 (35,8%)	Maintenance host Reservoir?	Aranaz *et al*. (2004) Acevedo-Whitehouse *et al*. (2005) Gortázar *et al*. (2008) Romero *et al*. (2008) Santos *et al*. (2009) Pinto *et al*. (2011) others
	Red deer *Cervus elaphus*	Bacteriology culture other	*M. bovis* *M. caprae*	78/371 (21,0%)	Maintenance host Reservoir?	Aranaz *et al*. (2004) Gortázar *et al*. (2008) Romero *et al*. (2008) Pinto *et al*. (2011) others
	Fallow deer *Dama dama*	Bacteriology culture other	*M. bovis*	116/420 (27,6%)	Maintenance host? Spillover host? Reservoir host?	Aranaz *et al*. (2004) Gortázar *et al*. (2008, 2011) Romero *et al*. (2008) others
	Chamois *Rupicapra pyrenaica*	Bacteriology culture	*M. bovis*		Spillover host	Rodríguez *et al*. (2010)
	Mouflon *Ovis orientalis*	Bacteriology culture	*M. bovis*		Spillover host	Rodríguez *et al*. (2010)
	Barbary sheep *Ammotragus lervia*	Bacteriology culture other	*M. bovis*		Spillover host? Maintenance host?	Candela *et al*. (2009) Rodríguez *et al*. (2010)
	Roe deer *Capreolus capreolus*	IHC PCR	*M. bovis*		Spillover host	Balseiro *et al*. (2009)
Carnivora	Iberian lynx *Lynx pardina*	Bacteriology culture other	*M. bovis*	9/40	Spillover host	Briones *et al*. (2000) Pérez *et al*. (2001) Aranaz *et al*. (2004) Atance *et al*. (2006) Romero *et al*. (2008) Millán *et al*. (2009) Rodríguez *et al*. (2010)
	Red fox *Vulpes vulpes*	Bacteriology culture other	*M. bovis* *M. caprae*	5/45	Spillover host	Atance *et al*. (2005, 2006) Millán *et al*. (2008, 2009) Romero *et al*. (2008) Rodríguez *et al*. (2010)
	Badger *Meles meles*	Bacteriology culture other	*M. bovis*	8/121	Maintenance host	Atance *et al*. (2006) Sobrino *et al*. (2008) Rodríguez *et al*. (2010) Balseiro *et al*. (2011)

Table 5. Bovine tuberculosis host species described in the Iberian Peninsula. For references and meta prevalence rate calculations for wild boar, red and fallow deer only targeted-design studies using bacteriological culture as diagnostic test on all animals samples are used. Meta prevalence in carnivores is exclusively based on passive-design studies.

Reference	Sample n (Isolates)	Technique	Time frame	Genotypes n	Host clustering	Study areas
Aranaz *et al*. (1996)	4 wild boar 2 red deer (129 cattle, 44 goat, 1 sheep, 2 cat)	SP		24 spoligotypes (2 clusters)	Sheep/goat isolates clustered apart from other species	
Parra *et al*. (2003)	37 wild boar (25 Iberian pig)	SP MV	1998-2001	8 spoligotypes 43 combined (14 clusters, 21 unique profiles)	4 Iberian pig-only clusters 7 wild boar-only clusters 2 common clusters (14 genotypes)	1 area W Spain
Aranaz *et al*. (2004)	33 red deer 62 fallow deer 58 wild boar 3 Iberian lynx (50 cattle)	SP	1996-2002	21 spoligotypes	17 genotypes in wild boar (4 exclusive) 8 genotypes red deer (none exclusive) 6 fallow deer (1 exclusive) 10 cattle (3 exclusive)	7 areas SW Spain
Gortázar *et al*. (2005)	58 wild boar 19 red deer	SP MV	1999-2002	11 spoligotypes 19 combined	10 spoligotypes wild boar (5 exclusive) 6 spoligotypes red deer (1 exclusive)	24 areas SW Spain
Parra *et al*. (2005)	112 wild boar 59 red deer (6 cattle, 28 Iberian pig, 2 goat)	SP MV	1998-2003	14 spoligotypes 131 combined (28 clusters, 76 unique profiles)	22 clusters wild boar (8 exclusive) 13 clusters red deer (3 exclusive) 7 clusters pig (2 exclusive) 3 clusters cattle (1 exclusive) 1 cluster goat	1 area W Spain
de Mendoza *et al*. (2006)	11 wild boar 8 red deer (5 cattle)	SP MV	1992-2004	(4 clusters, 10 unique profiles)		1 area W Spain
Duarte *et al*. (2008)	21 red deer 6 wild boar (258 cattle, 8 goat)	SP	2002-2007	29 spoligotypes	11 spoligotypes red deer (2 exclusive) 5 spoligotypes wild boar (none exclusive) 27 spoligotypes cattle (15 exclusive)	Portugal
Romero *et al*. (2008)	60 wild boar 26 red deer 17 fallow deer 4 Iberian lynx 2 red fox (54 cattle)	SP MV	1998-2003	9 spoligotypes	3 spoligotypes wild boar (none exclusive) 2 spoligotypes red & fallow deer & red fox (none exclusive) 2 spoligotypes Iberian lynx (1 exclusive) 11 spoligotypes cattle (8 exclusive)	1 area SW Spain
Duarte *et al*. (2009)	13 red deer 4 wild boar (157 cattle, 7 goat)	MV	2002-2007	87 genotypes	12 genotypes red deer (8 exclusive) 4 genotypes wild boar (1 exclusive) 78 genotypes cattle (71 exclusive)	Portugal
Santos *et al*. (2009)	14 wild boar	SP	2005-2006	4 spoligotypes		3 areas Portugal
Rodríguez *et al*. (2010)	204 wild boar 141 red deer 229 fallow deer 2 chamois 1 mouflon 6 Iberian lynx 2 red fox 1 badger	SP	1992-2007	252 spoligotypes	26 spoligotypes wild boar (6 exclusive) 22 spoligotypes red deer (2 exclusive) 13 spoligotypes fallow deer (1 exclusive) 1 spoligotype chamois (none exclusive) 1 spoligotype mouflon (1 exclusive) 3 spoligotypes lynx (none exclusive)	Spain

	(5585 cattle, 33 goat, 7 pig, 3 cat, 1 dog)				2 spoligotypes red fox (none exclusive) 1 spoligotype badger (none exclusive) 239 spoligotypes cattle (207 exclusive) 3 spoligotypes goat (1 exclusive) 2 spoligotypes pig (none exclusive) 3 spoligotypes cat (1 exclusive) 1 spoligotype dog (none exclusive)	
Cunha *et al*. (2012)	74 red deer 36 wild boar	SP MV	2008-2009	27 spoligotypes	21 spoligotypes red deer (11 exclusive) 15 spoligotypes wild boar (5 exclusive) 6 spoligotypes exclusive of wildlife vs 23 spoligotypes exclusive of domestic species	4 regions South-Central Portugal
Gortázar *et al*. (2011)	24 red deer 21 fallow deer 62 wild boar	SP MV	2006-2007	9 spoligotypes 13 genotypes combined	8 genotypes red deer (2 exclusive) 6 genotypes fallow deer (none exclusive) 5 genotypes wild boar (none exclusive)	1 area SW Spain
Pinto *et al*. (2011)	27 red deer 21 wild boar	SP	2008-2009	8 spoligotypes	8 spoligotypes red deer (4 exclusive) 4 spoligotypes wild boar (none exclusive)	1 area Central Portugal
Rodriguez *et al*. (2011)	14 wild boar 1 red deer 1 red fox (542 goat, 229 cattle, 2 sheep, 2 pig)	SP MV	1992-2009	15 spoligotypes	4 spoligotypes wild boar (none exclusive) 1 spoligotype red (none exclusive) 1 spoligotype red fox (none exclusive) 12 spoligotypes goat (6 exclusive) 9 spoligotypes cattle (2 exclusive) 2 spoligotypes sheep, pig (none)	Spain

Table 6. Molecular biology studies included in the analysis. SP: spoligotyping, MV: MIRU-VNTR mycobacterial interspersed repetitive units-variable number of tandem repeats.

On the other hand, studies of wild ungulates relying on routine meat inspection for detection of macroscopic tuberculosis-like lesions, do not allow for a reliable estimation of prevalence, which is underestimated in this situation (de Mendonza *et al.*, 2006). Nevertheless this type of design allows increasing sample size, which makes them suited for long-term surveillance rather than detailed epidemiological studies (de Mendonza *et al.*, 2006) and were mostly used in the first surveys and cross-sectional studies after bTB was detected in wildlife in Iberian Peninsula. The investigations on carnivore species, most of which are not hunted, tend to rely on passive surveillance schemes based on haphazardly found carcasses. This sampling design does not allow to estimate prevalence rates due to extensive sampling bias (e.g. Taylor *et al.*, 2002). Targeted sampling in these species has been attempted using serological tests but results should be interpreted with caution since these techniques have not yet been validated in these species.

The number of animals studied is usually adequate to determine prevalence rates with relatively small confidence intervals, at least in the easily available hunted species. The same cannot be said for most studies on protected carnivore species, where the collection of biological samples from a large number of animals is inherently difficult.

Bacteriological culture is the reference test for diagnosing bTB although it is expensive and time-consuming (de Lisle *et al.*, 2002). As the financial resources needed to perform bacteriological culture on a large number of samples are scarcely available, most surveys use other methods (usually gross pathology) as screening tests and only perform bacteriological culture for lesion-positive animals, sometimes as pooled samples. This introduces a bias and it was shown that the sensitivity of gross pathology was 72,2% of that obtained from bacteriology in the wild boar (Santos *et al.*, 2010). The same trend has been reported elsewhere for deer (Rohonczy *et al.*, 1996; O'Brien *et al.*, 2004).

4.2 Prevalence rates

Overall prevalence rates reported for bTB in wild boar, red deer and fallow deer in Iberian Peninsula are among the highest recorded for these species worldwide (Corner, 2006; Nishi *et al.*; 2006, Wilson *et al.*, 2008). Interestingly, prevalence rates in wild boar are invariably higher than in sympatric red or fallow deer (Gortázar *et al.*, in press).

Most studies report no sex differences in infection rates, but Santos *et al.* (2009) reported a significantly higher infection rate in female wild boar, presumably linked to more frequent social behaviour of females compared to males. Several studies report age differences in infection rates in wild boar, but data is conflicting since some authors reported increasing prevalence rates with age (e.g. Vicente *et al.*, 2006a,b), while others found higher prevalence rate in juveniles (e.g. Gortázar *et al.*, 2008; Santos *et al.*, 2009). Age and sex differences in prevalence rates were also reported in red deer (Vicente *et al.*, 2006a), which were higher for males and increased with age. This gender difference was already reported for cervids in North America (O'Brien *et al.*, 2006).

4.3 Trends

The few published data about the temporal dynamics of bTB prevalence rates are unanimous in showing an increasing trend across Iberian Peninsula in both wild boar and red deer (de Mendonza *et al.*, 2006; Gortázar *et al.*, 2008, 2011b, in press; Santos *et al.*, 2009, unpublished data). Gortázar *et al.*, (2011b) recently reported that 11/14 wild ungulate populations from central Spain show increasing bTB prevalence rates as assessed by gross pathology. This strongly supports previous interpretations that bTB is an emerging disease in wildlife in Iberian Peninsula.

The highest prevalence rates for bTB reported in wild ungulates in Iberian Peninsula lie in the central-south-western mountain chains of Montes de Toledo-Sierra Morena-Contenda (e.g. Vicente *et al.*, 2006a; Santos *et al.*, 2009) and Doñana (Gortázar *et al.*, 2008). Prevalence rates decline to the periphery of this region; the detected limits of this bTB core area are the provinces of Cáceres/Ávila to the north, eastern Portugal to the West, the Mediterranean coast to the South and Teruel to the East. bTB has not been detected or only sporadically in the northern, western and eastern periphery of Iberian Peninsula, despite locally intense surveillance (Gortázar *et al.*, 2011b). This pattern, coupled with the abovementioned increase in prevalence over time, strongly suggests that the disease is expanding from the central core area.

Interestingly, this core region of high bTB prevalence rates coincides with the main historical refuge of the wild boar in Spain (Tellería & Saez-Royuela, 1985) and, to some extent, in Portugal (Lopes & Borges, 2004). In the beginning of the XXth century, Iberian populations of wild ungulates were at their lowest level due to intense direct persecution and were largely restricted to a few mountain regions. Starting in 1960's, wild boar populations expanded from these refuges (Tellería & Saez-Royuela, 1985; Acevedo *et al.*, 2011) to a point they nowadays occupy almost all Iberian Peninsula (Rosell, 2001). Natural expansion of red deer also occurred but not to such a great extent as in the wild boar case and was much dependent upon translocations (Soriguer, 1998; Acevedo *et al*., 2011).

As suggested by Santos *et al*. (2009) for Portugal, wildlife bTB could be similarly expanding from the historical refuges with a lag comparative to its host's expansion. This lag could be explained by the threshold theory for disease persistence, as reported for other bTB hosts such as the possum *Trichosurus vulpecula* in New Zealand – Lloyd-Smith *et al*., 2005). As wild ungulate populations expanded, densities at the front of the expansion wave were too low (Holland *et al*., 2007) to allow for the persistence of bTB, even if presumably some infected hosts were involved in that expansion event. As a consequence, wildlife bTB initially remained confined to the historical refuges, despite dispersion of infected hosts. As ungulate distribution continued to expand, densities increased in a gradient centred at the historical refuges and eventually reached the threshold level. At that point, bTB, introduced by infected immigrants from the historical refuges, could persist and spread its distribution, a process seemingly still taking place.

This hypothesis could be tested by comprehensive geographical spatial analysis of the distribution of bTB in Iberian Peninsula, but the proposed natural expansion pattern has probably been much obscured by translocation and intensive management of ungulates for hunting purposes (Vargas *et al*. 1995; Miguel *et al*. 1999; Castillo *et al*., 2010). In fact, in South-central Spain lack of geographical autocorrelation in prevalence rates was suggested to be due to extensive fencing of intensively-managed big game hunting estates, which impair animal movements (Vicente *et al*., 2006b). On the other hand, wild ungulate translocations for hunting purposes occur frequently and may spread *M. bovis* to areas where it is absent today. Interestingly, *M. bovis* was isolated from wild boar in Portugal in two areas widely out of the known distribution of the disease (Santos *et al*., 2009; Cunha *et al*., 2012), one of which coincides with the release site of red deer originating from a population harbouring the same genotype of *M. bovis*. This provides circumstantial evidence for the role of translocations on bTB geographical spread.

More spatial data of bTb occurrence in Iberian Peninsula is urgently needed. The advent of sensitive, specific, reproducible and cheap serologic tests allows such large-scale research to be conducted, at least for wild boar (Boadella *et al*., 2011). This should improve the understanding of bTB occurrence across Iberian Peninsula.

4.4 Disease determinant factors

Most risk factors for bTB in wild boar and red deer identified in Iberian Peninsula are host population factors, most of them abundance-related. It is interesting to note that in the wild boar-red deer system, the abundance of each species influences bTB occurrence in the other species, further supporting the multi-host pathogen status of bTB in Iberian Peninsula ecosystems.

The number of risk factors related to management is greater for the red deer (n=5) than for the wild boar (n=1). This suggests that bTB occurrence in red deer populations is more dependent on management practices, while wild boar is competent to act as maintenance host under low-intensity management. This hypothesis could be tested by a case-control study of bTB occurrence in both species across a gradient of intensity of management.

Interestingly, among the protective risk factors described for bTB in Doñana, distance to freshwater sources is highlighted. Much remains to be known on the conditions necessary for the survival of mycobacteria in the environment, but humidity seems to favour it (Humblet *et al.*, 2009), particularly in the arid summer conditions of southern Iberian Peninsula. This suggests that environmental contamination with mycobacteria, particularly at watering sites, and indirect routes could play a role in disease transmission among wild ungulate species.

4.5 Host status

Wild boar and red deer are usually referred as maintenance hosts in Iberian Peninsula and evidence is available as populations maintaining high prevalence rates for several years, even decades, in the absence of domestic cattle which could theoretically serve as reservoirs for wildlife (e.g. Vicente *et al.*, 2006a; Gortázar *et al.*, 2008). It seems consensual that high-density sympatric populations of wild boar and red deer can maintain bTB at a high prevalence independent of the existence of other hosts (e.g. de Mendonza *et al.*, 2006; Vicente *et al.*, 2006a; Gortázar *et al.*, in press). This seems also to be independent of intensity of management for hunting purposes, favouring high density of animals through habitat management, feeding and watering (Miguel *et al.*, 1999), as even non-intensively managed but high-density populations of wild boar show high bTB prevalence rates (Santos *et al.*, 2009).

It should be noted that in most of Iberian Peninsula densities far above the natural carrying capacity of wild boar and red deer occur, even in the absence of intensive management, because natural predators of these species (essentially wolf *Canis lupus*) have been eliminated during the last 50 years (Rico & Torrente, 2000). Packer *et al.* (2003) have shown through modelling that removal of predators can lead to an increase on pathogens' prevalence. Furthermore, Barber-Meyer *et al.* (2007) have shown that wolf restoration in Yellowstone had significant impacts on the seroprevalence of several pathogens of deer, even though those populations were previously subject to predation by other species.

It could be hypothesized that the current bTB high prevalence rates in wildlife in Iberian Peninsula derives from severe changes on the ecosystems caused by intensive management for hunting purposes (Gortázar *et al.*, 2006) and eventually also predator eradication (Rico & Torrente, 2000). Experimental studies where host density is manipulated through large-scale culling are absent from the literature and could help to understand the role of artificialization of the ecosystems in the persistence and expansion of bTB. The picture is further complicated by the difficulty in separating the effect of each host species, as they usually occur in sympatry in the core area. Nevertheless, wild boar populations have been reported to show high bTB prevalence rates even in the absence of sympatric deer (Vicente *et al.*, 2006a).

Fallow deer and badger are most likely local maintenance hosts where they occur at high density, notably in scattered populations of fallow deer and in Atlantic Iberian Peninsula for the badger. On the other hand, other carnivore and ungulate species infected in Iberian Peninsula are most likely spillover hosts, with the possible exception of exotic Barbary sheep.

4.6 Molecular epidemiology

Studies reviewed are rather concordant in concluding that genotypes seem to be geographically clustered as each location has a few predominant genotypes, responsible for the majority of the infections. Concurrently, there is also a wide variety of locally rare genotypes. Local genotypes tend to be the same in different sympatric species, both domestic and wild, supporting the local interspecies transmission of *M. bovis*.

5. Conclusion

In summary, published evidence suggests that bTB is a natural pathogen of autochthonous wild ungulates in Iberian Peninsula, where wild boar and red deer act as maintenance hosts. Bovine tuberculosis is an emergent disease in these hosts, the expansion from the core high prevalence area in south-western Iberian Peninsula being fuelled by high densities of these species due to intensive management for hunting purposes. Several other species of ungulates and carnivores are affected by bTB, most probably as spillover hosts, but fallow deer and badger could serve as maintenance host in some locations. Although shown to be an important emerging infection, large gaps remain in the knowledge of the epidemiology of bTB in wildlife, such as intra and inter-species transmission routes, geographical distribution and effectiveness of control methods. Applying different epidemiological study designs, such as case-control and experimental studies, spatial analysis and modelling could shed light on this subject.

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