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Review

Evidence of the role of European wild boar as a reservoir of *Mycobacterium tuberculosis* complex

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Abstract

Bovine tuberculosis (bTB) is caused by *Mycobacterium bovis* and closely related mycobacteria of the *Mycobacterium tuberculosis* complex. They have an extensive host range and may cause zoonotic TB. A major obstacle to bTB eradication in livestock is the implication of wildlife in the natural cycle of the pathogen. The identification of wildlife reservoir hosts is crucial for the implementation of effective control measures. The European wild boar (*Sus scrofa*) is frequently considered a spillover or dead end host rather than a true reservoir, and scientific evidence is conflicting outside Mediterranean Spain. The aim of this review is to update current scientific evidence of the wild boar as a TB reservoir and to underline those aspects that need further research. Evidences supporting that wild boar is a TB reservoir host include: (i) presence of common *M. tuberculosis* complex genotypes in wild boar, domestic and wild animals and humans, (ii) high prevalence of *M. bovis* among wild boar in estates fenced for decades in complete absence of contact with domestic livestock, and other wild ungulates (iii) TB lesions are frequently seen in thoracic lymph nodes and lungs, suggesting that respiratory infection and excretion may occur, and (iv) extensive tuberculous lesions in more than one anatomical region occur in a high proportion of juvenile wild boar that probably represents the main source of mycobacterial excretion. Hence, epidemiological, pathological and microbiological evidence strongly suggests that, at least in Spanish Mediterranean ecosystems, wild boar are able to maintain TB infection in the wild and are most probably able to transmit the disease to other species, acting as a true wildlife reservoir. These results expand the list of wildlife species that act as natural reservoirs of TB in different parts of the world and suggest the need to control the infection in wild boar populations for the complete eradication of the disease in Spain.

Keywords: Bovine tuberculosis; *Mycobacterium tuberculosis* complex; Wildlife; Reservoir host; European wild boar

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Contents

1. Introduction

Bovine tuberculosis (bTB) is caused by *Mycobacterium bovis* and closely related mycobacteria of the *Mycobacterium tuberculosis* complex. They have an extensive host range and may cause zoonotic TB (Gortazar et al., 2005; Neill et al., 2005). Livestock species of economic significance such as cattle, goats and pigs are susceptible to infection with *M. bovis* and this has important implications for veterinary and public health (Amanfu, 2006). In addition, losses due to bTB are also relevant when endangered wildlife species are involved (Briones et al., 2000; Pérez et al., 2001; Amanfu, 2006).

Despite the efficacy of the skin test in identifying tuberculosis cattle (Pollock et al., 2006; Macdonald et al., 2006), the disease has not been eradicated from many countries (Amanfu, 2006; Pavlik, 2006), because of the reservoir of *M. bovis* in wildlife (Morris et al., 1994; Cousins, 2001; Corner, 2006; Collins, 2006; Thoen et al., 2006; Amanfu, 2006). Therefore, the identification of wildlife reservoir hosts is crucial for the implementation of effective control measures (Gortazar et al., 2007). If wildlife species are identified as reservoirs of *M. bovis* infection, effective tools for diagnosis of *M. bovis*-infected animals and measures, such as field management to avoid inter-specific contact or vaccines are needed for the control of bTB (Corner, 2006; Nishi et al., 2006; Michel et al., 2006; Pollock et al., 2006; Ballesteros et al., 2007).Tuberculosis is a major concern in Spain because prior to 1995 this country had among the highest bTB cattle infection rates in the European Union (Caffrey, 1994; Liébana et al., 1995; Pavlik, 2006). Test and slaughter strategies have proven successful and reduced bTB prevalence in Spanish cattle from 1.4% in 1996 to 0.3% in 2005 (http://rasve.mapa.es/Publica/Programas/NORMATIVA%20Y%20PROGRAMAS/PROGRAMAS/2007/TUBERCULOSIS/PROGRAMA%20NACIONAL%20DE%20ERRADICACIÓN%20DE%20LA%20-TUBERCULOSIS%20BOVINA,%20AÑO%202007.PDF; Ballesteros et al., 2007). However, the disease has not been eradicated and regional differences in campaign effectiveness have become evident. In the regions of southern Spain with highest cattle bTB prevalence, wildlife species such as European wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) show high prevalence of bTB, which suggests that the disease is shared between domestic and wild hosts (Gortazar et al., 2005; Vicente et al., 2006). Southern Spain has high densities of wild ungulate species due to intensive management of wild boar and red deer that includes artificial feeding and watering, containment of populations behind high wire fences and translocations. This contrasts with northern Spain, where bTB prevalence is lower and game-management of wildlife is less common (Vicente et al., 2006, 2007). In addition, environmental factors such as summer draughts and limited water availability promote aggregation of wildlife at waterholes in the Mediterranean habitats of southern Spain (Acevedo et al., 2007; Vicente et al., 2007).

It is known that wild and domestic animals share common *M. tuberculosis* complex genotypes thus suggesting transmission between these animal species (Aranaz et al., 1996, 2004; Gortazar et al., 2005; Hermoso de Mendoza et al., 2006). Therefore, the control of TB in wildlife species and the avoidance of contact between wildlife and livestock, particularly in the case of the wild boar, is considered a priority for bTB control in Spain (Parra et al., 2003, 2005; Aranaz et al., 2004; Gortazar et al., 2005; Vicente et al., 2006, 2007). However, wild boar and its relative the feral pig are frequently considered spillover or dead end hosts rather than true reservoirs, and scientific evidence is conflicting outside Mediterranean Spain (e.g. Serraino et al., 1999; Machackova et al., 2003; Corner, 2006).

The aim of this article is to review the scientific evidence supporting the case for the European wild boar as a TB reservoir in Mediterranean Spain and to underline aspects that need further research.

2. Factors affecting pathogen reservoir hosts and transmission of *M. bovis*

Wildlife species can play an important role in the epidemiology of bTB. For this reason, it is important to distinguish between maintenance and spillover hosts. Maintenance hosts are those that can maintain infection in an area in the absence of cross-transmission from other species of domestic or wild animals. Spillover hosts need a continuing acquisition of infection from other species to maintain the infection (Morris et al., 1994). Both, maintenance and spillover hosts may act as a disease vector (Corner, 2006). However, a true bTB reservoir with epidemiological implications for disease control is the maintenance host with the possibility to transmit the pathogen to other species.

The transmission of *M. bovis* is dependent on a number of factors that include (i) the number of infected animals, (ii) the number of susceptible animals, (iii) the routes of infection, (iv) the anatomical location of infection and lesions, (v) the structure of tuberculous lesions, (vi) the routes and levels of pathogen excretion, and (vii) the minimum infective dose by each infection route (Corner, 2006). Direct or indirect contact between bTB wild hosts and livestock is required and occurs at shared pastures, water holes and feeders (Garnett et al., 2002). Generally, the most effective route of infection between wildlife species and from wildlife to domestic animals is the airborne route (Corner, 2006). However, other possible routes through sharing of water holes and feeding sites and the consumption of infected carcases have been considered (Corner, 2006; Hermoso de Mendoza et al., 2006; Coleman and Cooke, 2001; Delahay et al., 2002; Vicente et al., 2007).

3. Examples of wildlife reservoirs for *M. bovis*

Wildlife species have been demonstrated as reservoir hosts for *M. bovis* in different regions of the world. Wildlife ruminants such as the African buffalo (*Syncerus caffer*) and the Canadian bison (*Bison bison*) have been implicated in the epidemiology of bTB in the Kruger National Park, South Africa and Canada, respectively (De Vos et al., 2001; Nishi et al., 2006).

The European badger (*Meles meles*) has been implicated in the transmission of *M. bovis* to cattle and is the most important wildlife reservoir of bTB in Ireland and the United Kingdom (Phillips et al., 2003; Delahay et al., 2002). The behavior of infected terminally-ill badgers changes, which results in the animals loosing fear to cattle and coming in close contact with them, thus excreting aerosol that constitute a risk for transmission of bTB to cattle (Gavier-Widen et al., 2001; Corner, 2006).

The Brushtail possum (*Trichosurus vulpecula*) is the major wildlife reservoir of bTB in New Zealand and terminally-ill tuberculous possums are highly infectious for *M. bovis* (Coleman and Cooke, 2001). As with badgers, changes in the behavior of terminally-ill tuberculous possums increase the risk of contact and TB transmission to cattle (Coleman and Cooke, 2001).

The white-tailed deer (*Odocoileus virginianus*) is considered a wildlife reservoir of bTB in the United States. White-tailed deer populations have increased due to game-management and aerosol transmission is facilitated by high animal densities (Corner, 2006). In the last years, a significant decrease in bTB prevalence in white-tailed deer in Michigan has been achieved using control strategies such as restrictions on supplemental feeding and baiting of deer (O'Brien et al., 2006). Elk (*Cervus elaphus*) have been proposed as a wildlife reservoir in Canada (Nishi et al., 2006).

In summary, several species belonging to different taxonomic groups are able to act as bTB reservoirs depending on the particular ecosystem. In those situations where true wildlife reservoirs have been identified, bTB control has been attempted by culling (e.g. badgers; Donnelly et al., 2007), limiting contacts at the wildlife–livestock interface (e.g. banning artificial feeding; Brown and Cooper, 2006), and more recently with experiments for wildlife vaccination with BCG (Wedlock et al., 2005; Buddle et al., 2006; Lesellier et al., 2006) and the first delivery of a badger vaccine in the wild (Richard Delahay, personal communication).

4. Evidence of the role of European wild boar as a reservoir for *M. tuberculosis* complex in Spain

In the last decade, research groups in Spain have provided evidence that support the role of European wild boar as a reservoir host for *M. bovis* with important implications for the control of the disease in the country. The evidences discussed below may be relevant for the identification of TB reservoirs in other European countries where wild boar populations may be maintenance host for *M. bovis* infection (Pavlik, 2006).

4.1. Number of infected and susceptible animals

Wild boar and feral pigs are considered important disease reservoirs affecting the efforts to control infectious diseases (Ruiz-Fons et al., in press). The geographical range and population densities of the European wild boar are currently increasing in the Iberian Peninsula (Acevedo et al., 2006) and elsewhere in Europe (Saez-Royuela and Tellería, 1986; Acevedo et al., 2006). Artificially high wild boar densities that have negative ecological consequences are common in Mediterranean Spain and contribute to the problems associated with disease control (Gortazar et al., 2006).

Wild boar share *M. tuberculosis* complex genotypes of bovine and caprine origin with cattle, goats, domestic swine, deer and humans (Gortazar et al., 2005; Parra et al., 2006; Aranaz et al., 2004). In Spain, *M. bovis* has been circulating among wild ungulates in estates fenced for over 20 years in complete absence of contact with domestic livestock (Gortazar et al., 2005). The mean prevalence of TB-compatible lesions in wild boar from southern Spain is 44%, with some local populations showing up to 100% apparent prevalence (Vicente et al., 2006). Prevalence estimates based on *M. bovis* isolation from tonsils and mandibular lymph nodes reach up to 60% (unpublished results). In southern Spain, spatial aggregation due to game-management practices such as fencing, feeding and watering correlate with high bTB prevalence in wild boar (Acevedo et al., 2007; Vicente et al., 2007).

Thus, both the susceptibility to the disease and the number of infected animals suggest a possible reservoir role of the European wild boar in southern Spain.

4.2. Anatomical location of infection and localization and structure of tuberculous lesions

In a recent work, Martín-Hernando et al. (2007) described the extent and distribution of lesions in 127 culture positive European wild boars. Macroscopic TB-compatible lesions were found in 82.7% and an additional 8.7% had microscopic lesions only. In the study, 42.2% of wild boar had localized TB lesions while 57.8% of the animals had generalized TB. Mandibular lymph nodes were affected in 92.2% of the animals, in agreement with previous findings that identified these lymph nodes as an important site for TB inspection and surveillance in wild boar (Gortazar et al., 2003). Moreover, pathological studies evidenced TB lesions in 51% of the lungs or thoracic lymph nodes (Martín-Hernando et al., 2007). In cases with localized TB, granulomas were characterized by a mixed inflammatory cell population whereas strongly necrotic-calcified granulomas were more prevalent in cases with generalized TB infection. Significantly, tuberculosis lesions in more than one anatomical region were more frequent among juvenile wild boar, and mycobacteria were most frequently observed in the lung.

4.3. Pathogen infection and possible excretion routes

The finding of wild boar with thoracic tuberculous lesions only (usually bronchial lymph nodes), along with wild boar with abdominal lesions only (usually mesenteric lymph nodes), suggests that both respiratory and food-borne contamination may occur (Martín-Hernando et al., 2007). However, the data available does not allow elucidating whether the respiratory of digestive route is more relevant for TB infection in wild boar. Although, the infection routes in wild boar are unknown, we hypothesize that mycobacteria enter through oral mucosa and tonsils, from where infection of mandibular lymph nodes and other organs may occur. This hypothesis is consistent with food/water-borne and direct oro-nasal infections. Food/water-borne infections could occur at shared water and feeding sites that are common in game-managed states in Spain. Direct oro-nasal contact between animals is frequent in wild boar behaviour (Ruiz-Fons et al., 2007). Consumption of carrion and discarded offal from hunter-killed animals by wild boar is probably an important risk factor for TB infection in Spain (unpublished results).

In our studies, extensive tuberculous lesions in more than one anatomical region have been found in a high proportion of juvenile wild boar that probably represent the main source of mycobacterial excretion (Martín-Hernando et al., 2007). Juveniles are the dispersing age group in wild boar, (Truve and Lemel, 2003) and may therefore contribute to the geographical spread of TB. Weaners and juveniles (preferably females) associate in matriarchal groups where intimate contact between individuals during social and foraging activities are frequent and could facilitate pathogen transmission directly and/or indirectly.

The wild boar studied in south-central Spain present a high proportion of lung lesions, sometimes with large numbers of mycobacteria (Martín-Hernando et al., 2007; Parra et al., 2003, 2006). These observations suggest that the lung has a major role in TB transmission from infected wild boar to susceptible animals (Palmer et al., 2002). Mycobacteria can be excreted either as aerosols through the mouth and nose, or be swallowed and excreted with the faeces. This second route could be important because of the potential to contaminate food, pastures and water, but needs to be confirmed in wild boar. Another possibility considering the high proportion of tuberculous lesions in mandibular lymph nodes and tonsils of wild boar is the excretion of mycobateria through saliva. Mycobacteria have been observed in the excretory ducts of wild boar mandibular salivary glands (Gortazar et al., 2003). In contrast, the urinary excretion of mycobacteria seems unimportant in wild boar, since tuberculous lesions and mycobacteria were not found in the kidneys. The finding of a few sows with TB lesions in the mammary glands suggests that transmission to the piglets through infected milk is possible (Martín-Hernando et al., 2007).

In summary, the pathological characteristics of the tuberculous lesions and the associated tissue damage in various organs in wild boar indicate that at least those animals with large lesions and generalized infections have the potential to excrete *M. bovis* by several routes, which reinforces the potential for this species to be a true TB reservoir (Parra et al., 2003; Vicente et al., 2006, 2007; Martín-Hernando et al., 2007).

4.4. Minimum infective dose by each infection route

Presently, the minimum *M. bovis* infective dose for wild boar is unknown. However, the finding of tuberculous lesions with large number of mycobacteria in the lungs suggests that the minimum infective dose may be reached at least through the respiratory transmission route. For example, the minimum infection dose in cattle is five bacilli or less if delivered by aerosol to the lungs (Corner, 2006).

5. Impact of TB on wild boar populations

There is evidence that juvenile wild boar with generalized TB are under-represented in the population and are being lost from the population faster than other age groups. These evidence include (i) similar representation across ages of the different stages of granuloma development with a high percentage of calcified lesions found among weaners and juveniles, (ii) the proportion of generalized TB was constant across ages, (iii) juveniles presented the highest proportion of large TB lesions, and (iv) lung lesions were less frequent in adults (Martín-Hernando et al., 2007). Limited survival of juveniles with large TB lesions could be expected since healing of large lesions is unlikely. However, Bollo et al. (2000) described that lesions in wild boar could result in nodules of fibrous tissue with reduced number of bacilli. In a non hunted wild boar population with high TB prevalence, prevalence was lower in adults than in juveniles, again suggesting differential mortality of infected juvenile wild boar (unpublished results). However, a continued exposure to infection may occur and adults probably have a greater ability to control infection and recover from disease (Corner et al., 1981).

Recently, Naranjo et al. (2006, 2007) characterized by proteomic and transcriptomic analyses the differential stress/inflammatory responses in mandibular lymph nodes and oropharyngeal tonsils of European wild boar naturally infected with *M. bovis*. The results suggested that the up-regulation of serum amiloid A expression and other stress/inflammatory responses in mandibular lymph nodes and tonsils of infected wild boar may contribute to organ damage and death in *M. bovis*-infected juvenile wild boar (Segalés et al., 2005).

6. Comparative TB pathology and epidemiology in wild boar and feral pigs

While some previous studies found that most wild suids had lesions exclusively in mandibular lymph nodes (e.g. 62% in Australian feral pigs; Corner et al., 1981), findings in wild boar showed a high proportion of animals with generalized lesions affecting more than one anatomical region (Martín-Hernando et al., 2007).

Feral pigs are considered spillover (or dead end) hosts for bTB in Australia (Corner et al., 1981; Corner, 2006). There, feral pig densities are almost 10 times lower than wild boar densities in Spain (Acevedo et al., 2006; Hone, 1990), and several other differences in habitat (Acevedo et al., 2006), management (Vicente et al., 2007), and even genetic factors (Acevedo-Whitehouse et al., 2005) may contribute to explain these apparently contradictory results.

In Australia, the low prevalence of generalized bTB disease in feral pigs, the absence of pulmonary lesions, the lack of other obvious routes of excretion from infected pigs, and the lack of contact between feral pigs and other species, particularly water buffalo and cattle, lead to the conclusion that feral pigs were an end host and not a source of bTB infection (Corner et al., 1981). This hypothesis was subsequently validated when, 20 years after the above mentioned study and after bTB was essentially eradicated from the bovid population, a survey showed that bTB had almost disappeared from the feral pig population (Corner, 2006).

In Mediterranean Spain the situation is entirely different. Available field and molecular epidemiology and histopathological evidence (Gortazar et al., 2005; Parra et al., 2006; Aranaz et al., 2004; Vicente et al., 2006; Martín-Hernando et al., 2007), document a high proportion of generalized TB disease and a frequent involvement of the lungs in wild boar. Thus, at least in the particular ecosystem of Mediterranean habitats and management systems for wild boar, the European wild boar seems capable of acting as a true TB reservoir (Table 1).

The finding of 17% of infected wild boar without visible macroscopic tuberculous lesions (Martín-Hernando et al., 2007) further supports the role of

Table 1
Comparative data on feral pig and wild boar ecology and TB epidemiology

	Australia	Spain	References
Animals/km^2	<11	up to 90	Corner et al. (1981); Acevedo et al. (2007)
TB prevalence	0–40%	18–100%	Corner et al. (1981); Wakelin and Churchman (1991); Knowles (1994); Lugton (1997); Vicente et al. (2006, 2007)
Prevalence trend	Decreasing	Increasing	Parra et al. (2006); Corner (2006)
Lung lesions	Not found	38–52%	Corner et al. (1981); Gortazar et al. (2003); Martín-Hernando et al. (2007)
Percent generalized TB	25%	58%	Corner et al. (1981); Martín-Hernando et al. (2007)
Host status	Spillover	Reservoir	Corner (2006); Vicente et al. (2006, 2007)

this species as TB reservoir. Most of the previous studies were based on wild boar or feral pigs presenting macroscopic TB compatible lesions (Vicente et al., 2006; Corner et al., 1981; Bollo et al., 2000; Gortazar et al., 2003). However, the percentage of infected wild boar without visible macroscopic lesions is similar to that reported for other bTB wildlife reservoir hosts such as badgers, (Gallagher et al., 1998) and lower than that reported for possums (de Lisle et al., 2005) and white-tailed deer (Kaneene et al., 2002).

7. Future research needs

Despite the evidences discussed above to support the role of European wild boar as a TB wildlife reservoir host, several questions need further investigations. Presently, the minimum infective dose by each infection route is unknown and controlled experiments are needed to clarify this issue. The results discussed here were obtained from naturally infected wild boar populations. However, controlled experimental infections are needed for a better understanding of the pathobiology and immune response of wild boar to mycobacterial infection. Most of the information analyzed in these studies was obtained post-mortem. The development of rapid test for the diagnosis of TB in live animals would improve current epidemiological studies and control programs. Effective vaccines, preferably an oral vaccine, are also necessary for the control of TB in wild boar and other wildlife reservoir species. However, vaccination strategies could be combined with other control measures aimed at reducing risk factors of disease transmission identified in epidemiological studies.

8. Conclusions

The results discussed herein support the role of the European wild boar as a TB reservoir in Mediterranean Spain. Epidemiological, pathological and microbiological evidence strongly suggests that, at least in Spanish Mediterranean ecosystems, wild boar are able to maintain TB infection in the wild and are most probably able to transmit the disease to other species, thus acting as a true wildlife reservoir. The finding of European wild boar as a TB reservoir host expands the list of wildlife species that act as natural reservoirs of the infection in different parts of the world. These results have important implications for the control of TB in Spain and suggest the need to control the infection in wild boar populations if complete eradication of the disease is to be achieved.

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