

TUBERCULOSIS IN WILD BOAR (SUS SCROFA) IN THE WESTERN LIGURIA REGION *

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ABSTRACT

Mycobacterium bovis, the causative agent of bovine tuberculosis, can infect a wide range of domestic and wild animals. The infection has been described in wild boars (*Sus scrofa*) as well. In the Western Liguria Region (Northern Italy) the first case of tuberculosis in a wild boar was observed in 1989. To understand the relationship between mycobacterial infections' existence in livestock vs wildlife, a surveillance activity, targeting hunted animals, has been carried out over the last 25 years. The aim of this work is to show the local evolution of the disease within the wild boar population based on 2002-2016 data.

During the study period, about 48,000 animals were submitted to anatomopathological examination. The majority of affected wild boars were caught during hunting seasons, in territories located nearby infected livestock or pastures where cattle from infected livestock used to graze.

The observed decreasing trend in wild boars is likely associated with the progressive fading out of tuberculosis in the local cattle (last outbreak in 2012) that led in 2014 to the recognition of official free status of the province. A different trend has been observed for infections associated to *M. microti*, a microorganism unrelated to cattle. Our data suggest that wild boar could be considered as a sentinel of *M. bovis* in an area without necessarily acting as a reservoir or a vector of infection.

Keywords: Wild boar, Tuberculosis.

RÉSUMÉ

Mycobacterium bovis, l'agent causal de la tuberculose bovine, peut infecter un large éventail d'animaux domestiques et sauvages. L'infection a également été décrite chez les sangliers (*Sus scrofa*). Dans la région de Ligurie occidentale, au nord de l'Italie, le premier cas de tuberculose chez un sanglier a été observé en 1989.

Pour comprendre la relation entre l'existence des infections mycobactériennes chez le bétail et la faune sauvage, une activité de surveillance, ciblant les animaux chassés, a été menée au cours des 25 dernières années. L'objectif de ce travail est de montrer l'évolution locale de la maladie au sein de la population de sangliers sur la base des données 2002-2016.

Au cours de la période, environ 48 000 animaux ont été soumis à un examen anatomopathologique. La majorité des sangliers touchés par la maladie ont été capturés pendant les saisons de chasse sur les territoires situés à proximité d'élevages infectés par *Mycobacterium bovis*, ou dans des pâturages où l'on faisait paître du bétail provenant d'élevages infectés.

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La tendance à la baisse observée pourrait être associée à la disparition progressive de la tuberculose chez les bovins de cette zone (dernier foyer en 2012) jusqu'à la reconnaissance du « statut officiellement indemne » (éradication de la maladie), obtenu par la province d'Imperia en 2014.

Une tendance différente a été observée pour les infections associées à *M. microti*, un microorganisme sans rapport avec le bétail. Nos données suggèrent que le sanglier pourrait être considéré comme un « animal sentinelle » de *M. bovis* dans un territoire donné, sans nécessairement agir comme réservoir ou vecteur d'infection.

Mots-clés : sangliers, tuberculose.



I - INTRODUCTION

The risk of spreading pathogens among wildlife, livestock and humans is increasing in recent years - [Jones et al., 2008]; many factors are related to this increased risk, such as animal husbandry (e.g., intensification, range farming), natural habitat (e.g., forest cover, connectivity), and climate change (e.g., mean temperature, rainfall), but also the changes in human society (e.g., mobility) [Gortázar et al., 2014].

The emergence of pathogens in wildlife, livestock and humans is dependent on interactions between the final host and reservoir hosts and their environment. The role of environment is relevant, influencing the relation between host and pathogen as well as disease transmission [Haydon et al., 2002].

Bovine tuberculosis (TB) is mainly a disease of domestic cattle, but can affect many other domestic and wild species, as well as humans. The disease is a worldwide important zoonosis that causes significant human mortality in developing countries. In Europe, human mortality caused by TB is very limited thanks to measures to control the disease in animal hosts (e.g. testing and culling of infected livestock) and to prevent public health threats.

Mycobacterium bovis, the causative agent of TB, and closely related other mycobacteria of the Mycobacterium tuberculosis complex (MTC) have an extensive host range including bovines, small ruminants such as goats and sheep, and a wide range of wildlife species [Gortazar et al., 2007; Neill et al., 2005].

*Domestic and non-domestic animals may be considered either as maintenance (or reservoir) hosts or non-maintenance (or spill-over) host for bovine tuberculosis. In reservoir host species, infection can persist through horizontal transfer in the absence of any other source of *M. bovis* and may as well be transmitted to other susceptible hosts. In contrast, spill-over hosts become infected with *M. bovis* but the infection only occurs sporadically or persists within these population if a true maintenance host is present in the ecosystem. If the source of infection is removed, the prevalence for this disease is reduced and it can only be maintained in the long term by another source [Haydon et al., 2002].*

*The disease in wildlife population has serious implication for several reasons: often infected animals show no clinical signs even though lesions are well developed, being therefore infectious to other species, especially gregarious species, such as wild boars (*Sus scrofa*); moreover, no practical treatment or preventive measure exists for free-ranging wildlife [Bollo et al., 2000].*

Since the 1930s, when tuberculosis was reported for first time in wild boar [Kindinger, 1934], infection has been described in this species in several European countries and can reach high prevalence, particularly in areas of the Iberian Peninsula, where boars are maintenance hosts. Boars also may become infected by scavenging infected carcasses [EFSA CFP/EFSA/AHAW/2008/3].

Wild boars are highly susceptible to TB and mycobacterial infections are usually diagnosed by post mortem detection of causative agents in tissues and cultural isolation from lesions (mainly in the lymph nodes of the head); by macroscopic examination, lesions show high degree of polymorphism with appearance being either exudative or proliferative [Mignone et al., 1991].

The potential of transmission to cattle population across Europe was evaluated by Hardstaff and coll., [2014], by determining the level of TB hazard for which a given wildlife species is responsible: wild boars were the species with the greatest ability to transmit the disease to cattle. Known risk factors for TB include temporal and spatial overlap at the interface between livestock and wild ungulates [Barasona et al., 2013]. However, the role of wild boars in the epidemiology of TB is still under discussion; in Spain wild boars are considered to be reservoir hosts [Naranjo et al., 2007], whereas in North-western Italy as spill-over hosts [Serraino et al., 1999; Dondo et al., 2007]. It is possible that their role varies depending on the local epidemiological context and wildlife management practices [Cousins and Florison, 2005]. According Machakowa and coll. [2003] most of their reported cases refers to tuberculosis caused by *M. bovis* in pasture areas shared with cattle herds. Serraino and coll. [1999] hypothesized that wild boars are the end host for *M. bovis* infection, because infected wild boars were detected only in areas where infected cattle were also present. Dondo and coll. [2007] pointed out that the incidence of *M. bovis* infection in wild boars is directly related to the local prevalence and incidence of the disease in cattle. This was shown in certain areas of North-western Italy where the prevalence of *M. bovis* in wild boars was related to the prevalence in cattle since they shared the same

habitat and pasture areas [Tittarelli, 2013]. Mignone and coll. [1997] suggested that the likely way of infection in wild boar was the oral one, through the exposure to wet territories contaminated by infected bovine faeces.

In the Western Liguria Region (North-western Italy) the first case of tuberculosis in a wild boar was observed in 1989 [Mignone et al., 1991]. The diagnosis was based on anatomopathological examination of hunted wild boars and confirmed by microbiological methods, biological bioassay and histopathological examination.

To understand the relationship between the existence of the disease in livestock vs. wildlife, a surveillance activity, targeting hunted and dead animals, has been carried out in the mentioned area over the last 25 years. This activity is carried out by the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZSPLV), in collaboration with the local Game Management Unit "Comprensorio Alpino" (CA), the Hunting Territorial Fields "Ambiti Territoriali di Caccia" (ATC), the Provincial Administration, and the National Forestry Service (CFS). The hunting area, divided into CA and ATC, covers about 33,600 hectares (ha) and 74,700 ha respectively out of an overall Imperia's area of 115,600 ha. Commingling of cattle and wild boars is common in CA area, in particular, where domestic and wild ruminants share resources during spring and summer.

Most of the animals underwent viscera examination, as well as organs and tissue sampling for chemical, bacteriological and virological analysis.

The aim of this work is to show the local evolution of the disease within the wild boar population.

II - MATERIAL AND METHODS

The current study covers the years 2002-2016 and is focused on an area, limited to Imperia province (Western Liguria Region, figure 1), where the wild boars hunting activity is managed into a CA, mountainous area, and an ATC, hilly or flat area. A dedicated database was set up based on both wild boars sampling data and results of the laboratory analysis performed at IZSPLV. Animal individual data such as sex, age, weight, site where the animal was shot down or found, were collected. The animals were divided into three categories using the

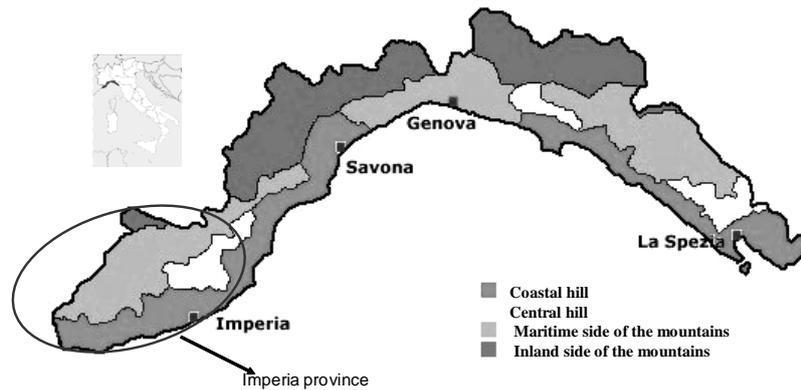
weight as a proxy for increasing age: piglet <15 Kg; subadult 15-40 Kg; adult >40 Kg. Laboratory analysis included an anatomopathological examination to detect macroscopic lesions compatible with tubercular lesions and subsequent confirmation by microbiological and histopathological methods, molecular identification and typing of strains. The impact of the investigated disease on the wild boar population was evaluated in term of prevalence (cases out of 100 wild boars were submitted to post-mortem inspection). The

occurrence of anatomopathological lesions was compared between ATC and CA. Moreover, the potential for association between disease occurrence and age and sex was assessed through logistic regression modelling.

The software used for the data analysis was Stata14.1 (StataCorp).

Figure 1

Map of Liguria Region



III - RESULTS AND DISCUSSION

Over the study period, about 49,000 wild boars were submitted to post-mortem inspection with an overall decreasing trend in the crude prevalence of anatomopathological lesions compatible with tubercular status (figure 2). Dondo and coll. [2007], in a GIS based study, carried out between 2000 and 2006, highlighted the simultaneous presence of homologous strains in wild boar and cattle populations in same areas and period. Tittarelli [2013] while reporting the eradication of TB from *M. bovis* in the bovine populations of the Imperia's area leading to the recognition of official free status in 2014, showed the overlapping location of the residual bovine outbreaks in the early 2000s with the detection of TB associated to *M. bovis* in wild boars.

Moreover, parallel to the mentioned epidemiological decreasing trend in cattle, we observed a steady decreasing trend in the occurrence of anatomopathological lesions compatible with the disease (figure 3), regardless

the different environment (ATC vs. CA). This observation may be consistent with an inter-species transmission that, however, is not enough to permit the persistence of the infection among the wild boars and leading to its fading out.

M. microti and *M. bovis* (both members of the *Mycobacterium tuberculosis* complex) and *M. TB* complex were the most common laboratory findings. Over the last few years, tuberculosis-like lesions due to *Mycobacterium microti*, recently described as causative agent, were particularly relevant.

The last case of tuberculosis from *M. bovis* in wild boars in Imperia province has been reported in 2012, while over the following years the presence of *M. microti* has been detected (figure 4).

The logistic regression model exploring the effect of age and sex (figure 5) indicated no evidence of an association with disease occurrence.

Figure 2

Anatomopathological lesions compatible with tubercular status: number of wild boars submitted to examination and crude prevalence (cases/100) by year

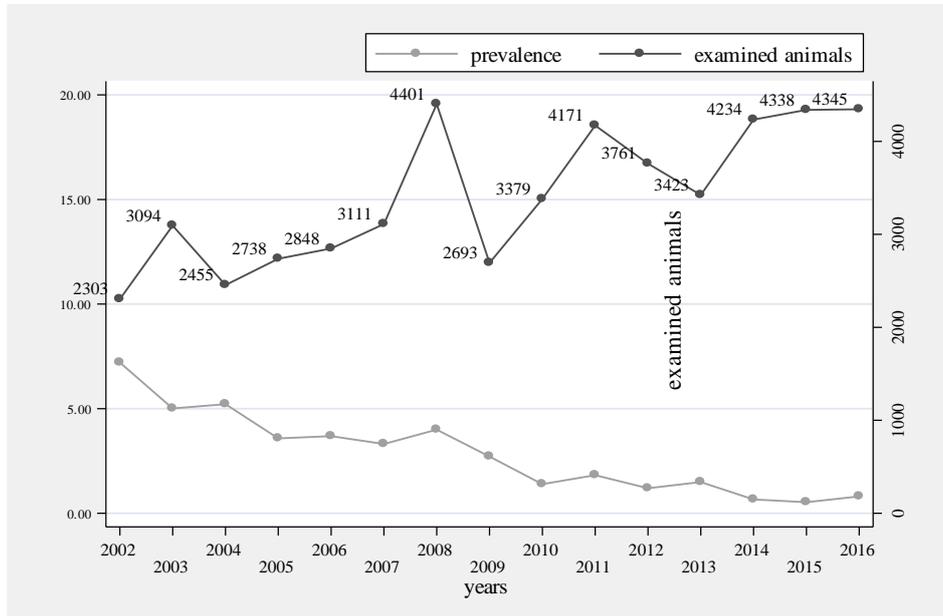


Figure 3

Trend of the prevalence of anatomopathological lesions compatible with tubercular status by environment (ATC vs. CA)

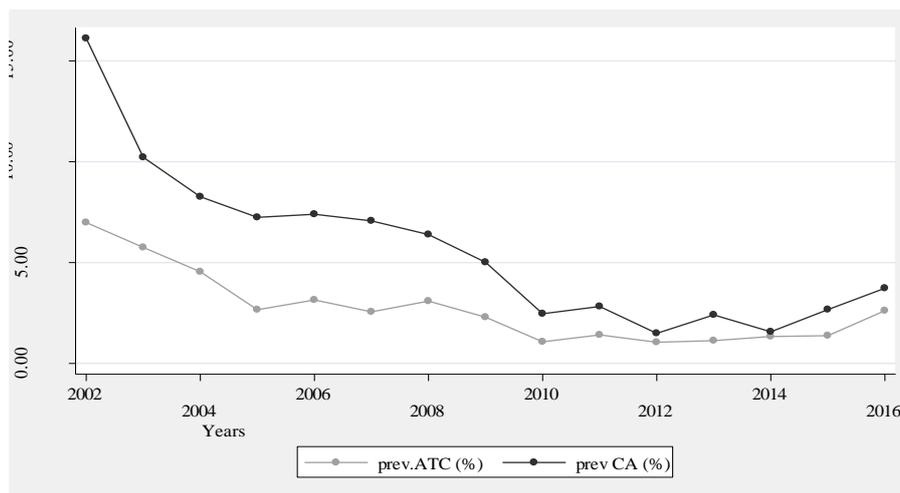


Figure 4

Findings based on cultural diagnosis: positive cases per year and agent

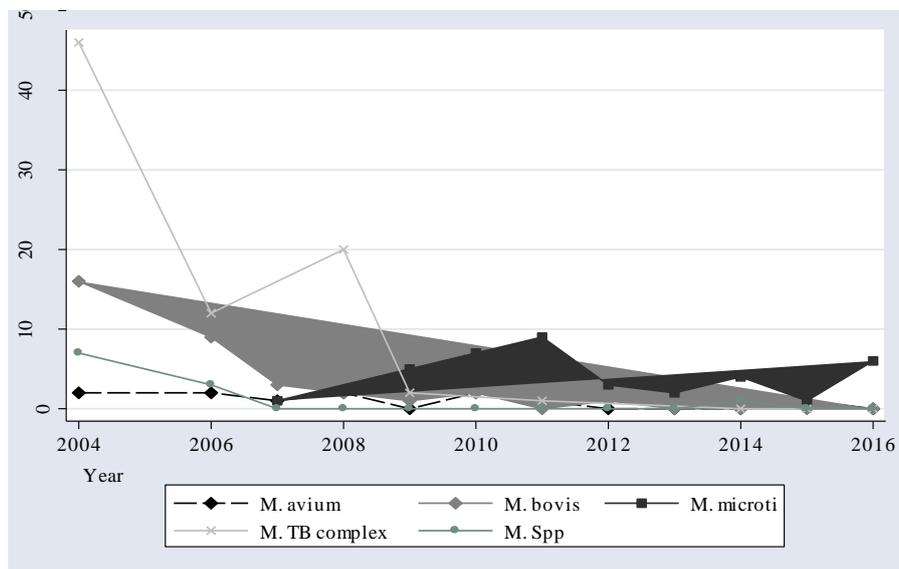
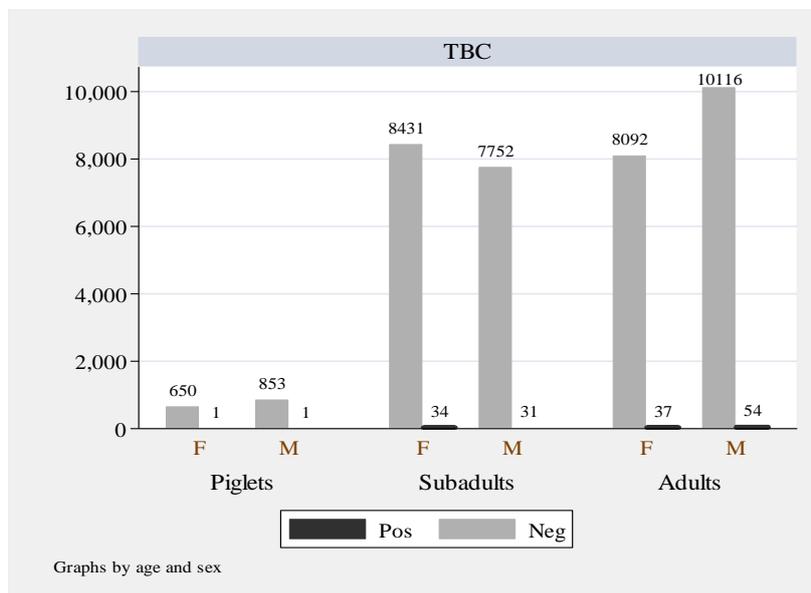


Figure 5

Tubercular status by category (age and sex)



IV - CONCLUSION

Our data are consistent with the hypothesis of wild boar being an environmental sentinel of *M. bovis* without necessarily acting as a reservoir or a vector of infection.

In the past, in the study area, cases of *M. bovis* infections in wild boar had been epidemiologically associated to cases raising in cattle herds due to the overlapping of their respective habitat and pasture areas. This was a consequence of the expansion of ecotourism, changes in land-use practices, and escalating competition for resources that were responsible for an increased contact between free-ranging wildlife, domestic animals and humans.

No fading out is associated with the mycobacterial infections of wild boars by *M. microti*: in this case the transmission is not associated with the coexistence of cattle but likely with the wild boar habits of rooting and eating dead small rodents.

The confirmation of mycobacterial infections in a wildlife population, which is not easily controllable as it is the case for wild boars, raises problems of both public health and wildlife management. It is therefore important to carry out effective surveillance activities targeting not only humans and livestock, but also wildlife species potentially involved in the epidemiological cycle.

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