Mycobacterium bovis in Wildlife

Introduction

Tuberculosis (TBC) is a disease caused by microorganisms of the *Mycobacterium Tuberculosis complex* (Figure 1) including *Mycobacterium tuberculosis* and *Mycobacterium bovis*, etiological agent apiece of human and animal tuberculosis.

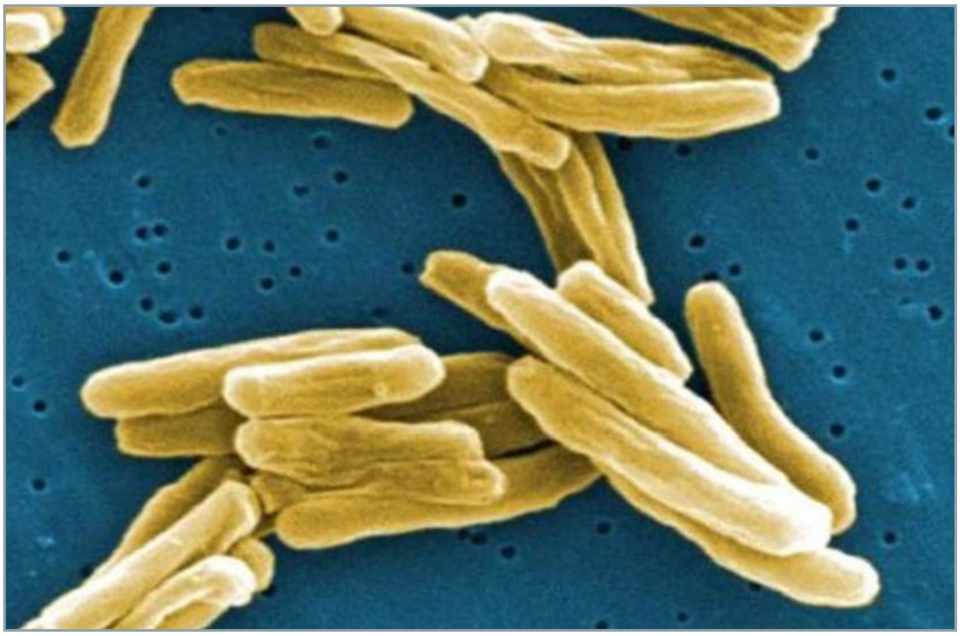


Figure 1.
M. tuberculosis

About 8,8 million of new human TBC cases are diagnosed each year worldwide, equal to 122 cases each 100.000 inhabitants, with 1,5 million of deaths in 2010 (WHO,2011). The 58% of TBC cases is reported in Asia, specifically in India and China where the highest number of diagnosis is performed, equal to 26% and 12% of total cases respectively.

Despite significant progresses have been made in the control and/or eradication of tuberculosis in industrialized countries, the disease remain one of the most important infectious disease in humans and livestock in different countries of the world (WHO 2010).

The Mycobacterium genus includes more than 190 species, some of which are pathogenic both to humans and animals. Pathogenic Mycobacteria which can infect humans and/or animals (domestic and wild) are: *M. tuberculosis*, *M. leprae* (responsible for human leprosy), *M. bovis*, *M africanum*, *M pinnipedii*, *M. bovis subsp caprae* e *M. microti*.

Tuberculosis caused by *M. bovis* is a zoonosis (Perez-Lago et al., 2014), humans can be infected through the inhalation of infected aerosols, the contact with infected animals, or through the ingestion of contaminated foods and drinks. Raw milk produced by infected animals represents an important source of infection for humans. At global level, an high number of TBC cases are caused by *M. bovis* and bovines represents the main reservoir of the infection (Müller *et al*., 2013)

In animals as well the main routes of infection are inhalation of infected aerosols and ingestion of contaminated tissues (Palmer, 2013). A specific example of oral route infection has been recorded in some areas of Spain where wild boars have been infected by feeding on infected deer carcasses (Gortazar *et al*., 2012). Mycobacterium bovis can be eliminated also through secretion and excretion, such as urine and feces (Barasona *et al*., 2015).

It is suspected that characteristics like soil type and ph might play a role in the persistence of *M. bovis* in the environment, but further studies are needed to clarify this relationship (Barbier *et al*., 2017). Experimental studies on different substrates have demonstrated that *M. bovis* can survive in the environment also for a long time: until 12 months in sterilized soils incubated in controlled laboratory conditions (Ghodbane *et al*., 2014). In Michigan (USA) it has been demonstrated the persistence of the bacteria until 88 days under meteorological natural conditions (Fine *et al*., 2011) at low temperatures (4 °C) (Barbier *et al*., 2017) protected from solar radiations (ultraviolet), as for example in feces (Tanner *et al*., 1999), corn, hay, water or in fresh and humid soil during winter and spring (Fine *et al*., 2011; Jackson *et al*., 1995; Barbier *et al*., 2017).

A wide range of domestic and wild animals can be infected by *M. bovis* (Biet *et al*., 2005), and even though livestock is considered the principal host, among wild animals some species are recognized as reservoir, including: European badger (Meles meles) in Great Britain and Ireland, red deer (*Cervus elaphus*) and brushtail possum (*Trichosurus vulpecula*) in New Zeland, African buffalo (*Syncerus caffer*) in South Africa, wild boar (*Sus scrofa*) in the Iberian Peninsula and white-tailed deer (*Odocoileus virginianus*) in Michigan, USA (Palmer, 2013; Naranjo *et al*., 2008).

The presence of a wildlife reservoir may reduce the efficacy of eradication programs in livestock, especially where the infection has been reduced or eradicated in domestic animals (Aranaz *et al*., 2004; Gortázar *et al*., 2007).

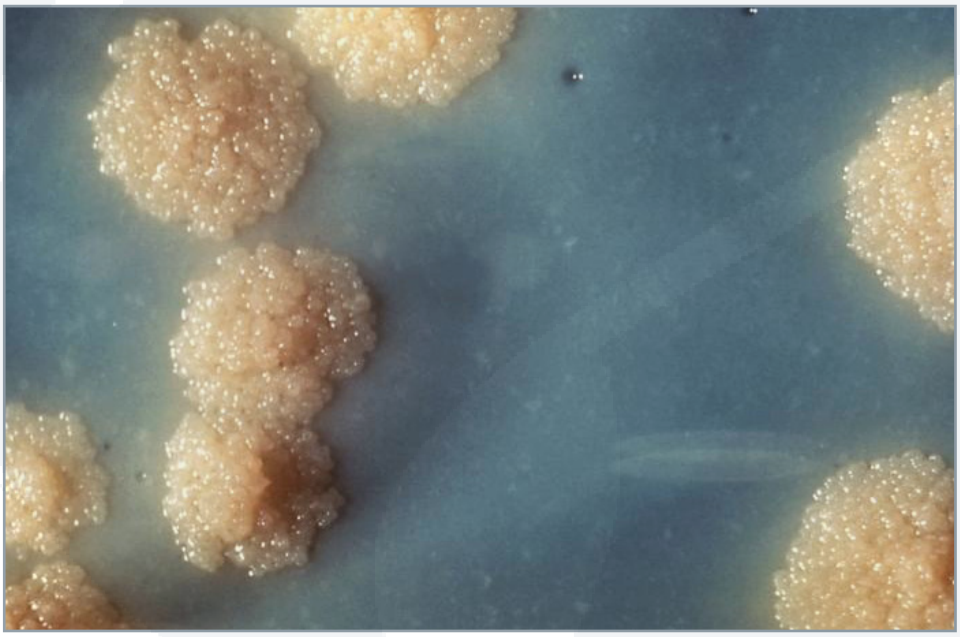
It must be taken into account that the endemization of the infection in wild populations is a complex phenomenon which requires the interaction between many factors such as density and social behaviors of wild animals, frequency of contact with domestic animals, availability of trophic resources and the alteration of habitats, for example through the use of fences or through the land abandonment.

The complexity of this mechanism is well described in some areas of Spain, where wild boars and red deer are responsible of the maintenance of the infection in the environment and act as spill-over to domestic cattle. (Gortázar *et al*., 2012).

Transmission between wild and domestic animals may occur at common foraging and watering areas (Kaneene *et al*., 2002). In the Iberian Peninsula, for example, the highest prevalence of tuberculosis in deer and wild boars has been reported in the south-western area, where protected natural areas are present.

Conditions which are believed to influence the transmission and persistence of TBC at local level are: (1) the high density of wild animals (2) the concentration of animals around foraging and watering points, and (3) a Mediterranean weather, with hot and dry summer, which improve the aggregation of animals around watering points (Palmer, 2013).

Figure 2.
Colture in vitro of *Mycobacterium tuberculosis* showing the morphology of the colony



It's important therefore to understand that tuberculosis must be monitored in all the animal species potentially involved in the epidemiological cycle, both domestic and wild, including in the monitoring process all the stakeholders: administrators, farmers, hunters, environmentalists and academics.

In Spain tuberculosis is present especially where there is an overlapping of pastures used by domestic (bovine and caprine, ovine and suidae also at a local level) and wild animals (wild boar, deer and fallow deer, badgers).

Badger is also strongly suspected to have an important role in transmission of TBC in the North of Spain (Gortázar *et al*., 2012). The infection has been reported in France in wild boars, where a possible association between the presence of badgers and bovine herds with TBC has been suspected (Payne *et al*., 2012).

Eurasiatic wild boar (*Sus scrofa*) with steady increase is considered to have an important role in the epidemiology of tuberculosis.

In Spain, the eurasiatic wild boar is reported as the main reservoir, responsible of the persistence of TBC into the wild. Prevalence rates of infection in this species are over the 50% (Vicente *et al*., 2013), with one third of the piglets at risk to get infected in the first 6 months of life (Che' Amat *et al*., 2015).

Monitoring tuberculosis in these species, therefore, is of fundamental importance to better understand possible mechanisms of persistence of the infection in certain territories.

In France, with the purpose of estimating the exposition of wild boar to the *M. tuberculosis* complex (MTC), 2.080 serum samples of hunted wild boars were collected in 58 France "departments".

The samples were tested using ELISA as first method of detection. From the results it was possible to deduce that the exposition of wild boars to the MTC was related with outbreaks of TBC in cattle: the average distance from infected wild boars to livestock outbreak was 13 Km, coherent with the home-range of a wild boar (a male can cover until 38 Km) (Richomme *et al*., 2013).

The diagnosis intra-vitam in wild animals is limited due to the difficulty of capturing and manipulating animals on a regular base. Surveillance, therefore, is based mainly on post mortem examination of hunted (Santos *et al*., 2010) or found death animals.

Cultural exam of M.bovis led to a definitive diagnosis of tuberculosis. It can be made on frozen tissues at -20 °C and on live animals from bronchial suction, urine or tampons (for example feces, abscess, wounds).

On death animals, the collection of 2 gr of tissue gathered from organs with lesions and from interested lymph nodes can be adequate for bacteriological culture and molecular characterization.

It is necessary underline, as already stated, that conditions and mechanisms responsible of infection maintenance in wild animals can be different, according to the geographic areas and species involved. Consequently, control measures should be adapted to local circumstances. Otherwise, in many European areas, wild boars and deer populations are growing rapidly both geographically than demographically.

One of the control methods widely used in the past was depopulation. Depopulation was realized through an intense hunting activities with the aim of reducing the density of wild animals up to a level which unable the maintenance of the transmission in the population.

This approach was used in the United Kingdom in badgers with contrasting results. If in some areas a decrease of incidence of TBC in cattle was reported, on the other hand in peripheral areas an increasing rate of infection was noticed.

This can be explained by the pressure that hunting has on the social structure of badgers with an enlargement of animals home range and therefore of the likelihood of contacts between infected and healthy individuals (Palmer, 2013).

Additional measures of control are based on prevention of contact between wild and domestic animals avoiding situations of promiscuity such as the sharing of watering and foraging points.

Nevertheless the most promising measure of control is the use of vaccines in wild population.

Studies on wild boar using oral vaccination through the distribution of baits two times per summer (Buddle *et al*., 2003), have reported a strong protective response against field strain of M. bovis (Gortazar *et al*. 2014). Other experimental studies using oral vaccination have been performed on badgers in which high levels of protection against infection were confirmed (Gormley *et al*., 2017).

An experiment completed in United Kingdom demonstrated the absence of interference with tubercoline test in the case of accidental ingestion by cattle of oral bait, containing inactivated vaccines, distributed for wild animals (Jones *et al*., 2016).

Appendix. Results of exams from 2010 at IZSAM laboratories

In Abruzzo, as in other Italian regions, in the last 10 years the density and geographical distribution of certain wild species have increased resulting in an enlargement of the overlapping with human activities.

In order to understand the potential involvement of wild ungulates in the transmission cycle of the bovine tuberculosis, in those provinces still not officially free of infection a monitoring protocol has been developed to be applied during necropsy procedures. For each sample a set of metadata is collected through a specific form.

From 2010 to August 2017 815 wild ungulates were tested for *Mycobacterium spp*., of which 413 were wild board, 253 roe deer, 111 deer and 20 Apennine chamois. Around 80% of examined animals came from the province of L'Aquila.

Isolated mycobacteria were identified by PCR and PCR-RFLP. Only two animals, both wild boars, tested positive, one of them to *Mycobacterium avium* (2017) and the other one to *Mycobacterium kumamotonense* (2015).

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