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Short communication

Pathology and genetic findings in a rare case of *Mycobacterium caprae* infection in a sow

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ABSTRACT

Bovine tuberculosis, a reemerging zoonosis in diverse ecological scenarios, has been reported in the autochthonous Nebrodi black pig breed population used for meat production in Italy. During a routine abattoir inspection in 2013, 24 of 299 carcasses (8%) of Nebrodi black pigs presented tuberculosis-like lesions at pathologic examination. *Mycobacterium bovis* was isolated from 23 animals and *M. caprae* from a 3-year-old sow. The sow showed severe diffuse lesions involving the visceral organs, right coxofemoral joint, and mammary glands. Isolation of *M. caprae* from mammary glands is uncommon, with only one other case involving a sow reported so far; however, *Mycobacteria* infection of the mammary glands may be transmitted from lactating sows to piglets, contributing to the spread and maintenance of bovine tuberculosis in swine. Genotyping analysis showed *M. caprae* spoligotype SB0866 and profile 4,1,5,4,4,11,4,2,4,3,8,7 MIRU-VNTR (mycobacterial interspersed repetitive units-variable number of tandem repeats). The worldwide prevalence of this spoligotype is very low. The finding of severe, diffuse tuberculous lesions strongly suggests that Nebrodi black pigs are susceptible for *Mycobacterium* spp. and that they might act as a distributor for these microorganisms. Since natural ecosystems with multiple contacts among different livestock species and wild animals are very common in Mediterranean regions, current surveillance and eradication plans for bovine tuberculosis will need to be extended to other potential reservoir species in regions where extensive and traditional breeding systems are operated.

1. Introduction

Bovine tuberculosis (bTB) is a neglected, though reemerging zoonosis with broad implications for livestock production, agricultural trade, and public health (Menin et al., 2013). *Mycobacterium caprae*, a species within the *Mycobacterium tuberculosis* complex first identified in 2003 (Aranaz et al., 2003), has been isolated mainly in Europe and with increasing frequency in cattle in Spain (Rodríguez et al., 2011). A recent report on comparative genomics analysis suggests that *M. caprae* is one of the *M. bovis*-related mycobacteria that have evolved to infect not only goats and sheep but also other hosts such as wild boar, red deer, cattle, and humans, with high lesion scores (De La Fuente et al., 2015). Swine are receptive animals for infection by several species of *Mycobacterium* primarily through the oral route probably due to their omnivorous nature and habit of eating infected carrion (Gortazar et al., 2003; Nugent et al., 2015). Though generally considered as bTB spillover hosts, there is evidence that wild boars might act as bTB reservoir hosts in Spain (Martìn-Hernando et al., 2007; Vicente et al., 2006). Because domestic pigs and wild boars are known to be infected by *M. bovis* and

M. caprae and because both can infect a wide range of animal species, current eradication programs targeting only *M. bovis* in cattle have been expanded in many countries to address *M. caprae* infection in a manner similar to *M. bovis* (Rodriguez-Campos et al., 2014).

While the prevalence of bTB has gradually declined thanks to cattle health surveillance systems in many regions of Italy, controlling the disease remains a major problem in Sicily, particularly in natural areas like the Nebrodi National Park where there is extensive farming of mixed livestock and contact between domestic and wild animals is frequent. A recent outbreak of bTB has been reported in a fallow deer

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(*Dama dama*) herd (Amato et al., 2016) that shares the same geographical area as the Nebrodi black pig (*Sus scrofa*), an autochthonous breed reared in free or semi-free roaming conditions. The pigs roam wooded areas for most of their lives, foraging mainly on acorns roots, bulbs, and fruit. They are captured shortly before slaughtering and are fattened by feeding with small amounts of grain, bran, and barley. Nebrodi black pig has been recently identified as an epidemiological reservoir for bTB (Di Marco et al., 2012). Here we report a rare case of *M. caprae* infection involving the mammary glands and other tissues of an adult sow. The anatomo-pathological features and the genotyping characterization of the isolated strain are described.

2. Materials and methods

An abattoir survey was carried out in the first half of 2013 in the province of Messina (Italy) to examine pathological signs of bTB infection in Nebrodi black pigs and to further address their role as a potential reservoir host for tuberculosis infection. The lymph nodes of the head and internal organs (lungs, liver, spleen, stomach, intestines, and respective lymph nodes) of 299 slaughtered Nebrodi black pigs were examined for macroscopic lesions. Lesions were sampled for tuberculosis suspicion when caseous, calcified lesions were observed. For the purposes of this study, additional histopathology and bacteriological analyses were performed to confirm the diagnosis in the cases of abnormal pathology findings. For microbiological examination, tissue samples were processed and cultured in liquid and solid media (Middlebrook 7H9 broth and Löwenstein-Jensen medium) according to the official culture protocol (OIE Manual, 2010). For histological examination, samples were fixed in 10% neutral buffered formalin and routinely processed to obtain 4-μm sections stained with hematoxylin and eosin and Ziehl-Neelsen stain. Molecular characterization and genotyping of the isolates were performed by spoligotyping and mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR) analysis (ETRA-E, VNTR 2163a, 2163b, 4052, 3155, 1895, 3232, and MIRU-26), as described elsewhere (Boniotti et al., 2009).

3. Results

Twenty-four of the 299 carcasses (8%) presented bTB-like lesions at gross examination. Macroscopic examination revealed localized granulomatous lesions affecting almost exclusively the lymph nodes of the head, thorax, and abdomen (submandibular, retropharyngeal, tracheobronchial, gastric, intestinal lymph nodes). Of particular interest were the severe generalized lesions of the tonsils, mammary glands, and right coxofemoral joint noted in a 3-year-old sow. The lesions of the lymph nodes of the head, thorax, and abdomen usually appeared as caseous necrotic-calcified tubercles (< 1 cm in diameter) or larger lesions (> 1 cm in diameter) encased in a thick fibrotic capsule. The lungs, liver, and spleen showed features consistent with protracted and disseminated tuberculosis. Small, translucent gray nodules with a pale yellow core, detectable in both surface and section, were observed in the tonsils. Multiple granulomas of different size containing yellowish-green exudate were present in three mammary glands (Fig. 1). A voluminous mass (10 cm) was observed in the right coxofemoral joint (Fig. 2). Transversal bone sections showed thickening of the joint capsule with extensive areas of necrosis and inflammation. X-ray examination revealed bone remodeling of the trochanter and ischial osteolysis (Fig. 3). Histological examination confirmed that lesions appeared as classic granulomas containing a variable number of multinucleated giant cells, macrophages, and lymphocytes, with minimal or abundant calcifications at the center. Occasional neutrophils in and around the necrotic core were observed, together with numerous acid-fast bacilli (Fig. 4). Four isolates obtained from the coxofemoral joint, lungs, iliac lymph nodes, and mammary glands were identified as *M. caprae*. MIRU-VNTR typing showed spoligotype SB0866 and MIRU-VNTR profile 4,1,5,4,4,11,4,2,4,3,8,7.

Fig. 1. Multiple disseminated granulomas involving the mammary gland of a sow infected by *M. caprae*. A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM03976.

Fig. 2. Right coxofemoral joint of a sow infected by *M. caprae*. Granulomas with a caseous-necrotic center and severe fibroblastic reaction in the joint capsule. A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM03977.

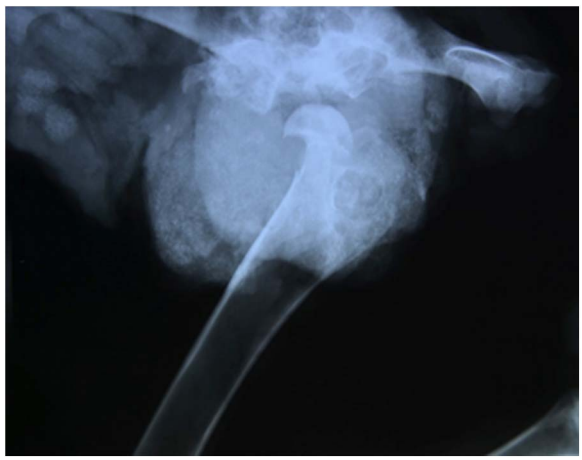

Fig. 3. X-ray showing bone remodeling of the trochanter and ischial osteolysis in the right coxofemoral joint of a sow infected by *M. caprae*.

4. Discussion

The bTB infection rate is about 4% in cattle (Italian Reference Centre for Bovine Tuberculosis) and 7% in pigs in Sicily (Di Marco et al., 2012). In the Nebrodi Park ecosystem, traditional farming practices with extensive mixed breeding (cattle, pigs, sheep and goats, poultry, horses) can facilitate contact between infected and uninfected

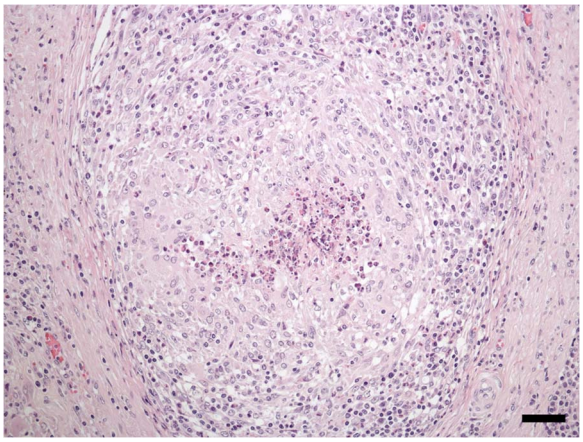

Fig. 4. Mammary gland of a sow infected by *M. caprae*. Large granuloma with a neutrophilic core surrounded by epithelioid cells, macrophages, and lymphocytes. H & E stain. A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM03978.

animals and interspecies transmission of the disease, as supported by evidence of clonal diffusion (*M. bovis* isolates with the same spoligotype and VNTR genotype) among different animal species (unpublished data). The national tuberculosis eradication program is based on skin testing and culling of animals that test positive or inconclusive. Given the occurrence of outbreaks of bTB in areas where the mixed breeding system is operated, this schedule needs to be extended to all susceptible species, including goats, sheep, and pigs. Moreover, and implemented through disease control plans should include use of the gamma-interferon assay (Pesciaroli et al., 2012). An active epidemiological surveillance plan at the abattoir should be implemented, as well as a wildlife monitoring plan for.

4.1. *M. caprae* infection and mammary gland involvement

M. caprae is the dominant species in cattle in central European countries (Erler et al., 2004). In German patients, *M. bovis* and *M. caprae* were involved in 69% and 31% of cases of bTB, respectively (Kubica et al., 2003). *M. caprae* has been detected in only about 9% of isolates from cattle and rarely reported in red deer in Italy (Chiari et al., 2014). In the present survey, *M. caprae* infection (genotype SB0866 and MIRU-VNTR profile 4,1,5,4,4,11,4,2,4,3,8,7) was identified for the first time in an adult sow. Spoligotype SB0866 has been reported in 1 goat, 1 cow, and 1 pig in Spain (Rodrìguez et al., 2011), while in mainland Italy it is mostly limited to Campania. Only 4 of 630 isolates were identified as spoligotype SB0866 in Sicily over a 10-year period, 2 of which with the same spoligotype and MIRU-VNTR profile from adjacent cattle herds, suggesting clonal interspecies transmission (Italian Reference Centre for Tuberculosis).

This is the first report of *M. caprae* isolated from the mammary glands in swine in Italy. To the best of our knowledge, one previous case of *M. caprae* isolated from the mammary glands in a sow was reported in Croatia during a bTB outbreak in a small family farm with infected cows; however, the tuberculosis lesions in the mammary glands were detected only in the positive sow (Cvetnic et al., 2006). In a previous study, 3 of a total of 127 European wild boars that tested positive for the *Mycobacterium tuberculosis* complex presented mammary gland lesions (Martìn-Hernando et al., 2007).

The presence of diffuse granulomas and the recovery of positive isolates from the mammary glands of lactating sows suggests that the infection could be transmitted to piglets through the milk. This could have a considerable impact on the epidemiology and clinical outcome of the infection, especially in natural ecosystems where young animals infected during lactation might easily succumb to the disease and other animals eating their carcasses would increase the spread of *Mycobacteria*. Also, the survivors would spread the bacteria and go on to develop calcified lesions. Indeed, a high percentage of calcified lesions has been observed among European wild boar weanlings and juveniles (Martìn-Hernando et al., 2007).

4.2. Comparative pathology between M.caprae and M. bovis infection

A recent study on comparative pathology in wild boars hunted in Spain showed that, because the lesions caused by *M. caprae* are more prone to excrete tubercle bacilli than *M. bovis*, animals infected by *M. caprae* can be considered as a high-risk source of infection for other animals (García-Jiménez et al., 2013). The same study reported that more than 50% of the wild boars carried *M. caprae*, indicating an increase in its prevalence in these animals in Spain. The *M. caprae* infection in the single sow in our study was similar in terms of pathological features to changes seen in several other pigs infected with *M. bovis* (data not shown); however, more anatomic pathology observations are needed, and *M. caprae* infection rates are currently lower in Italy (Italian Reference Centre for Bovine Tuberculosis). In addition, the host's genetic background should also be considered, since different animal species and/or breeds might be more or less susceptible to different species of *Mycobacteria*.

4.3. Alternative Tb control program

Further analysis is necessary to address these various aspects. What can be said is that differential regional management of bTB eradication and control needs to be implemented in natural ecosystems worldwide. Surveillance plans for bTB should be expanded beyond cattle health control and *M. bovis* detection, as has been done in some countries (Rodriguez-Campos et al., 2014). Regionalization of control plans adapted to different scenarios could provide a good strategy for the eradication and control of *Mycobacteria* infection in cattle (Livingstone et al., 2006). In different natural ecosystems, the role each animal species plays in spreading the disease depends on several factors (i.e., host abundance and host interactions), and it has been observed that spillover hosts can occasionally transmit bTB infection to true maintenance hosts if they reach a high density (Nugent, 2011). Throughout the Mediterranean there are many areas with a high density of mixed livestock species and wild animals with multiple continuous contacts among them. It has been reported that the probability of *Mycobacterium tuberculosis* complex excretion is lower in wild boars of the Atlantic than the Mediterranean areas of Spain (Muñoz-Mendoza et al., 2013).

5. Conclusion

In this unusual case of *M. caprae* infection by a rare spolygotype strain in a sow the anatomic pathological findings confirmed the greater invasiveness of *M. caprae* as compared to *M. bovis*, with the involvement of several different organs and tissues, including the mammary glands. The 7% prevalence of bTB in Nebrodi black pigs strongly suggests that they might act as a natural bTB reservoir in natural ecosystems where different livestock and wild animals share the same pastures.

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