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Systemic Bovine Tuberculosis in a Crested Porcupine (*Hystrix cristata*) in the Marche Region, Italy

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ABSTRACT: Tuberculosis is a worldwide zoonosis involving a wide range of hosts among domestic and wild animals. We describe tuberculosis caused by *Mycobacterium bovis* in a wild crested porcupine (*Hystrix cristata*) found dead in the district of Macerata, Marche Region, Italy in 2019.

Mycobacterium bovis, the causal agent of bovine tuberculosis, has been isolated from humans and a wide range of domestic and wild animals. *Mycobacterium bovis* infection is probably underestimated in humans worldwide (Olea-Popelka et al. 2017) and *M. bovis* has been detected in cattle, small ruminants, camelids, swine, horses, cats, and dogs (Rodriguez-Campos et al. 2014).

Within Europe, *M. bovis* has been isolated in free-living wildlife including wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), red fox (*Vulpes vulpes*), European badger (*Meles meles*), stoat (*Mustela erminea*), Iberian lynx (*Lynx pardinus*), rodents (Muridae and Sciuridae), and others (Gavier-Widén et al. 2012). It has not been detected previously in wild crested porcupine (*Hystrix cristata*), although one case of *Mycobacterium pinnipedi* isolated from a porcupine in a German zoo has been reported (Jurczynski et al. 2011).

In May 2019, a wild adult male porcupine in excellent conditions was found dead due to a vehicular collision, in the area of Matelica (Macerata, Italy) and was submitted for postmortem examination by local public veterinary services. The liver, kidneys, lungs, and heart were delivered to the local official laboratory (Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche), where histologic, bacteriologic, and molecular tests were performed.

During postmortem examination, sarcomatous nodular miliary lesions were detected in the liver, kidneys, pleura, pericardium, mediastinal, and mesenteric lymph nodes (Fig. 1). Additionally, the basal and apical lung lobes were affected by infiltrative diffusive lesions without any macroscopic evidence of necrosis or calcification.

Tissue samples for histology were fixed in buffered formalin, processed, embedded in paraffin wax, sectioned at 4 μm, and stained with H&E and Ziehl-Neelsen (Z-N) stains.

On histology, diffusive granulomatous foci were detected in mesenteric lymph nodes; some were confluent and subverted the architecture of the organ. They were characterized by a necrotic center with degenerated/apoptotic cells surrounded by epithelioid-histiocytic cells, giant multinucleated Langhans-type cells containing rod-shaped Z-N positive bacteria, plasma cells, and lymphocytes. Similar lesions were observed in the liver, lungs (Fig. 2), and kidneys.

Macroscopic and microscopic findings were consistent with generalized tuberculosis.

We extracted DNA from a pool of organs using a commercial kit, following the manufacturer's instructions (Genomic DNA isolation kit, Norgen Biotek Corp., Thorold, Ontario, Canada). A PCR reaction with primers Tb1-F/Tb1-R specific for bacteria of the *M. tuberculosis* complex was performed (Kulski et al. 1995) and positive specimens were used for mycobacterial culture and genotyping. A pool of lungs, lymph nodes, liver, and kidney were homogenized; decon-

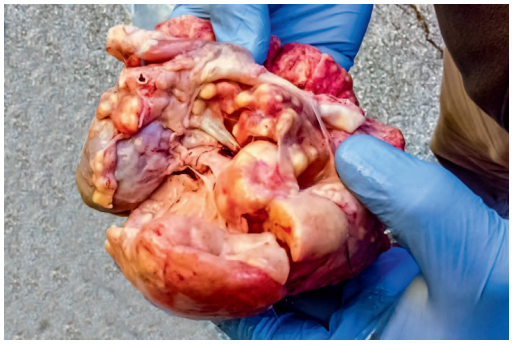

Figure 1. Lung and mediastinal lymph node of a crested porcupine (*Hystrix cristata*) found dead from a vehicular collision in the district of Macerata, Marche Region, Italy, in 2019. Pleuropneumonia and polyserositis with typical sarcomatous nodular lesions, necrosis, and associated mediastinal lymphadenitis are present.

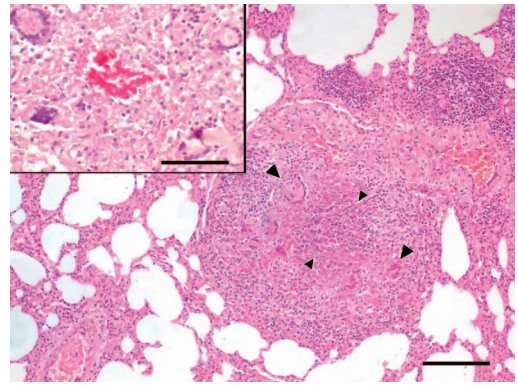

Figure 2. Lung of a crested porcupine (*Hystrix cristata*) found dead from a vehicular collision in the district of Macerata, Marche Region, Italy, in 2019, showing a typical granulomatous lesion. Note the well-defined granuloma inside the lung parenchyma, characterized by a necrotic central area in which foci of dystrophic calcification are beginning to develop (small arrows). Many large and multinucleated Langhans-type giant cells are present inside the granuloma, surrounding the area of necrosis (big arrows). H&E stain. Bar=250 μm. In the insert, the center of the granuloma shows strong staining with red for acid fuchsin, indicating a large presence of live mycobacteria and bacterial wall products. Multinucleated Langhans-type giant cells are evident, surrounding the central area of the granuloma, which is occupied by necrotic-caseous material. Ziehl-Neelsen stain. Bar=100 μm.

taminated with hexadecylpyridinium chloride and sodium hydroxide; centrifuged; and cultured on Lowestein Jensen and Stonebrink medium. Tubes were incubated at 37 C in capnophilic conditions (5–10% CO_2 atmosphere) for 10–15 d and at 37 C in aerobic conditions for up to a further 10 wk, being observed once a week for the presence of colonies (World Organization for Animal Health 2019).

A *Mycobacterium* sp. was isolated from the examined organs, and typical colonies were stained with Z-N followed by biochemical and molecular tests in order to identify the *Mycobacterium* species. A multiplex-PCR reaction was performed on DNA extracted from colonies, to distinguish among *M. avium*, *M. tuberculosis* complex, and *M. intracellulare* by amplifying regions in the MPB70 gene and in the rRNA subunit 16S gene (Kulski et al. 1995). Samples positive for *M. tuberculosis* complex were submitted to two amplifying protocols to discriminate between species: one based on a PCR amplifying a region on the locus GyrB, and an enzymatic digestion of the obtained fragments by the restriction endonuclease RSA I and SAC II (Kasai et al. 2000; Chimara et al. 2004); the second consisting of seven PCR reactions amplifying genomic regions on the loci 16S rRNA, Rv0577, IS1561, Rv1510, Rv1970, Rv3877/8, and Rv3120 (Huard et al. 2003). Spoligotyping (Kamerbeek et al. 1997) and multiple-locus, variable-number, tandem-repeat analysis (MLVA) with a group of 12 genes (Boniotti et al. 2009) were also performed. The bacterium was identified at species level as *Mycobacterium bovis* and at molecular typing level as spoligotype BCGlike 0120 and MLVA profile ETR-A 3, ETR-B 3, ETR-C 5, ETR-D 3, ETR-E 3, QUB11A 10, QUB11B 4, QUB26 4, QUB1895 4, QUB15 3, QUB3232 6, MIRU26 5.

In Italy since 1995, the application of eradication programs has greatly reduced the number of tuberculosis cases in cattle herds; most of the Italian Provinces have become free of the disease, including all the Provinces of Marche Region, with the exception of Macerata (European Commission 2018). In this Province, the bacterium is still circulating due to the transmission from domestic ani-

mals to wildlife and the occurrence of maintenance hosts (Gavaudan et al. 2019).

Mycobacterium bovis has been isolated from cattle and hunted wild boars in Macerata, with an increasing incidence in wild boars reported in the last 5 yr (Gavaudan et al. 2019). In wild boars, lesions are mainly localized in the mandibular and retropharyngeal lymph nodes, with occasional involvement of the lungs and mediastinal lymph nodes.

The strain identified in the porcupine shows the same spoligotype and MLVA profile as the strain circulating in cattle and wild boar in the examined area. This highlights the presence of a multihost system with a domestic-wildlife interface having the wild boar as a maintenance host, likely to spread the infection to other potential wild host species such as porcupine.

Although the porcupine is probably a spillover host, behaviors such as communal nesting, together with susceptibility to development of severe lesions, could promote transmission of *M. bovis*. Further epidemiological investigation is needed to understand any role of this species in the spread of bovine tuberculosis in this district.

LITERATURE CITED

Boniotti MB, Goria M, Loda D, Garrone A, Benedetto A, Mondo A, Tisato E, Zanoni M, Zoppi S, Dondo A, et al. 2009. Molecular typing of *Mycobacterium bovis* strains isolated in Italy from 2000 to 2006 and evaluation of variable number-tandem repeats for geographically optimized genotyping. *J Clin Microbiol* 47:636–644.

Chimara E, Ferrazoli L, Leão SC. 2004. *Mycobacterium tuberculosis* complex differentiation using gyrB-restriction fragment length polymorphism analysis. *Mem Inst Oswaldo Cruz* 99:745–748.

European Commission. 2018. *European Commission implementing decision (EU) 2018/1983 of 26 October 2018 amending Annexes I and II to Decision 2003/467/EC as regards the declaration of certain regions of Italy as officially tuberculosis-free and officially brucellosis-free in relation to bovine herds (notified under document C (2018) 6981)*. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018D1983&from=EN. Accessed June 2020.

Gavaudan S, Conquista M, Angeloni G, Perugini G, Maresca C, Scoccia E, Aconiti Mandolini N, Barchiesi F, Pesca C, Morandi F, et al. 2019. Tuberculosis in wild boar, epidemiology and control perspectives. In: *Proceedings of 11th international symposium on wild fauna,* Wild Animals Vigilance Euro-Mediterranean Society, Viterbo, Italy, 24–29 September, p. 134.

Gavier-Widén D, Chambers M, Gortázar C, Delahay R, Cromie R, Lindén A. 2012. Mycobacteria infections. In: *Infectious diseases of wild mammals and birds in Europe*, Gavier-Widen D, Duff JP, Meredith A, editors. Wiley-Blackwell, Oxford, UK, pp. 265–292.

Huard RC, de Oliveira Lazzarini C, Butler WR, Van Soolingen D, Ho JL. 2003. PCR-based method to differentiate the subspecies of the *Mycobacterium tuberculosis* complex on the basis of genomic deletions. *J Clin Microbiol* 41:1637–1650.

Jurczynski K, Lyashchenko KP, Gomis D, Moser J, Greenwald R, Moisson P. 2011. Pinniped *tuberculosis* in Malayan tapirs, (*Tapirus indicus*) and its transmission to other terrestrial mammals. *J Zoo Wildl Med* 42:222–227.

Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, Bunschoten A, Molhuizen H, Shaw R, Goyal M, et al. 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *J Clin Microbiol* 35:907–914.

Kasai H, Ezaki T, Harayama S. 2000. Differentiation of phylogenetically related slowly growing mycobacteria by their gyrB sequences. *J Clin Microbiol* 38:301–308.

Kulski JK, Khinsoe C, Price T, Christiansen K. 1995. Use of a multiplex PCR to detect and identify *Mycobacterium avium* and *M. intracellulare* in blood culture fluids of AIDS patients. *J Clin Microbiol* 33:668–674.

Olea-Popelka F, Muwonge A, Perera A, Dean AS, Mumford E, Erlacher-Vindel E, Forcella S, Silk BJ, Ditiu L, El Idrissi A, et al. 2017. Zoonotic tuberculosis in human beings caused by *Mycobacterium bovis*—A call for action. *Lancet Infect Dis* 17:e21–e25.

Rodriguez-Campos S, Smith NH, Boniotti MB, Aranaz A. 2014. Overview and phylogeny of *Mycobacterium tuberculosis* complex organisms: Implications for diagnostics and legislation of bovine tuberculosis. *Res Vet Sci* 97:S5–S19.

World Organization for Animal Health. 2019. *Manual of diagnostic tests and vaccines for terrestrial animals*. https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/. Accessed May 2021.

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