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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Isolation and Phylogenetic Characterization of *Streptococcus halichoeri* from a European Badger (*Meles meles*) with Pyogranulomatous Pleuropneumonia

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Summary

Clinical and pathological studies in European badgers (*Meles meles*) are limited. Badgers play a significant role in the epidemiology of bovine tuberculosis (TB) in some countries and an accurate diagnosis is needed for this infection. However, the lesions of bovine TB are similar to those associated with other pathogens, making pathological diagnosis difficult. In the present study, *Streptococcus halichoeri* was isolated from a European badger with pyogranulomatous pleuropneumonia and suspected of having tuberculosis. TB and other pathogens able to induce similar lesions were ruled out. Comparative *16S rRNA* and *rpoB* gene sequencing studies showed an identity of 99.51% and 98.28%, respectively, with *S. halichoeri*. This report represents the third description of this bacterium and the first in an animal species other than the grey seal (*Halichoerus grypus*). It also shows that *S. halichoeri* can be associated with a pathological process characterized by granulomatous inflammation and resembling tuberculosis.

Keywords: badger; pleuropneumonia; 16S rRNA; *Streptococcus halichoeri*

Clinical and pathological studies in European badgers (*Meles meles*) are limited. Only canine distemper virus infection seems to be an important cause of death in this species (Origgi *et al*., 2012). The badger plays a role in the epidemiology of bovine tuberculosis (TB) in Great Britain, with a high percentage of badgers having TB lesions (Corner *et al*., 2012). Serological studies have shown that badgers have antibodies against several infectious agents typically associated with dogs, cats or man (Sobrino *et al*., 2008; Millán *et al*., 2009; Quinn *et al*., 2012) and a potential involvement in the epidemiology of several domestic animal and human diseases has been suggested.

Pyogranulomatous inflammation can be associated with several pathogens including *Mycobacterium* spp., *Nocardia* spp., *Actinomyces* spp., fungi or the pyogranulomatous form of coronavirus infection (Gallagher and Clifton-Hadley, 2000; Caswell and Williams, 2007; Graham *et al*., 2012). In the present study, we report the isolation and phylogenetic identification of *Streptococcus halichoeri* from a badger with pyogranulomatous pleuropneumonia initially diagnosed as TB.

A female European badger, approximately 1 year of age, was found trapped in a concrete pipe and sent to the Wildlife Rehabilitation Centre of La Alfranca, Zaragoza, Spain. Clinical examination revealed good body condition, with a body weight of

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6.605 kg. A superficial abrasion in the back and eroded claws were the only external lesions observed. Antiparasitic treatment was administered and the badger was kept at the Centre for 8 days, showing normal behaviour and an apparent recovery, but it died suddenly without any preceding clinical signs.

A complete necropsy examination was performed at the Rehabilitation Centre and samples of lung with pleura and pericardium, tracheobronchial and mediastinal lymph nodes and spleen were sent for histopathological studies. They were processed routinely and stained with haematoxylin and eosin (HE), Ziehl–Neelsen (ZN) and periodic acid–Schiff (PAS) stains. Pleural samples were sent for microbiological and molecular studies. These were cultured onto MacConkey and sheep blood agar plates (Oxoid, Madrid, Spain) and incubated for 24 h at 37°C, both aerobically and anaerobically. They were also cultured in Sabouraud's agar for fungal growth for 8 days. Standard biochemical tests and the API 20 Strep system (bioMèrieux, Lyon, France) were performed on the colonies.

Molecular studies were also performed on the isolate. For phylogenetic analysis, the *16S rRNA* gene and the *rpoB* gene were amplified as previously described (Drancourt *et al.*, 2004; Vickerman *et al.*, 2007). Sequences were aligned and compared with the known *16S* ribosomal RNA sequences from bacteria and Archaea and with the nucleotide collection published in the NCBI database using the BLAST tool (http://www.ncbi.nlm.nih.gov/). Public sequences with identity higher than 95% for *16S rRNA* and 85% for *rpoB* were selected for phylogenetic study. Sequence alignments, maximum likelihood (ML) and the maximum parsimony (MP) phylogenetic trees were constructed following standard procedures (Vela *et al.*, 2010). The reliability of the phylogenetic trees was confirmed using 1,000 bootstrap replications. Direct immunofluorescence for feline coronavirus was performed with a commercial kit (VMRD, Pullman, Washington, USA; CJ-F-FIP-10ML) and a nested PCR for canine distemper virus was performed with a commercial kit (Genekam Biotechnology AG, Duisburg, Germany; Ref. K. 031).

The most significant lesions were found in the thoracic cavity, where there was a very thickened and nodular left pleural surface with underlying purulent exudate and severe pericarditis. The lung was congested and collapsed and the heart was dilated. A congested and slightly enlarged liver and a mottled and mildly enlarged spleen with multifocal white pinpoint foci were also seen. Microscopically, there was severe pyogranulomatous pleuritis and pericarditis (Fig. 1). Numerous neutrophils, macrophages, some small giant cells, fibrin and, in some areas, neovascularization were observed (Fig. 2). Mesothelial cells were metaplastic showing a rounded morphology (Fig. 2). The lung showed atelectasis, congestion and haemorrhage, with alveoli occupied by numerous macrophages, some of them with foamy cytoplasm and neutrophils. Mild to moderate proliferative catarrhal bronchiolitis was seen (Fig. 3). Some airways and blood vessels were surrounded by a slight infiltration of mononuclear cells (Fig. 3). PAS and ZN stains were both negative. The only other significant lesions were found in the spleen, which showed moderate follicular hyperplasia, and in the lymph nodes, in which there was lymphoid depletion in the follicular areas.

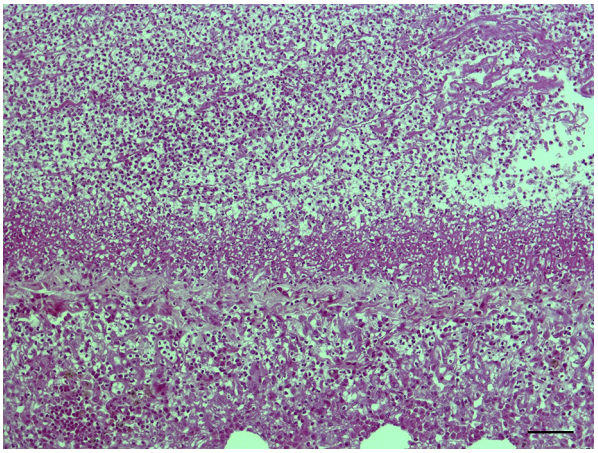

Fig. 1. Section of lung, showing severe pyogranulomatous pleuritis with abundant fibrin and numerous neutrophils and macrophages. Alveolar lumina are present at the base of the image. HE. Bar, 200 µm.

A pure growth of gram-positive, catalase and oxidase-negative, aerobic coccoid organisms,

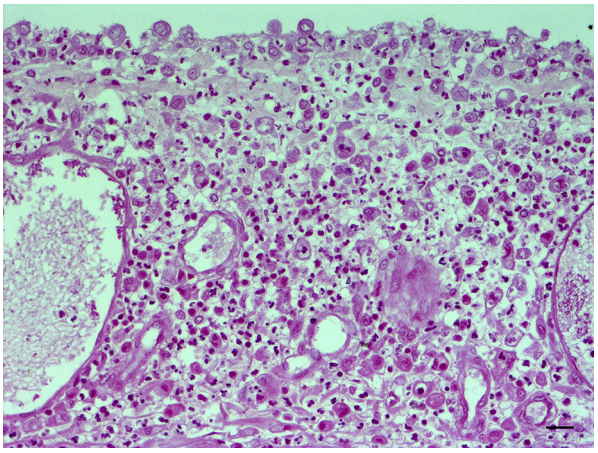

Fig. 2. Detail of pyogranulomatous pleuritis showing numerous neutrophils and macrophages. Metaplastic mesothelial cells are noted at the top of the image. HE. Bar, 20 µm.