Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

J. Comp. Path. 2015, Vol. 152, 269–273

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

www.elsevier.com/locate/jcpa

DISEASE IN WILDLIFE OR EXOTIC SPECIES

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

B. Moreno[*,†], R. Bolea[*,†], M. Morales[†], I. Martín-Burriel[*,‡], Ch. González[§] and J. J. Badiola[*]

** Centro de Investigación en Encefalopatías y Enfermedades Transmisibles Emergentes, † Unidad de Microbiología e Inmunología, Departamento de Patología Animal, ‡ Laboratorio de Genética Bioquímica (LAGENBIO-i3A), Departamento de Anatomía, Embriología y Genética Animal, Facultad de Veterinaria, Universidad de Zaragoza, Miguel Servet 177 and § Centro de Recuperación de Fauna Silvestre de La Alfranca, Servicio de Biodiversidad, Dpto. de Agricultura, Ganadería y Medio Ambiente, Gobierno de Aragón, Finca La Alfranca s/n, Pastriz, Zaragoza, Spain*

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

Correspondence to: B. Moreno (e-mail: bmoreno@unizar.es).

0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2014.12.012

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.

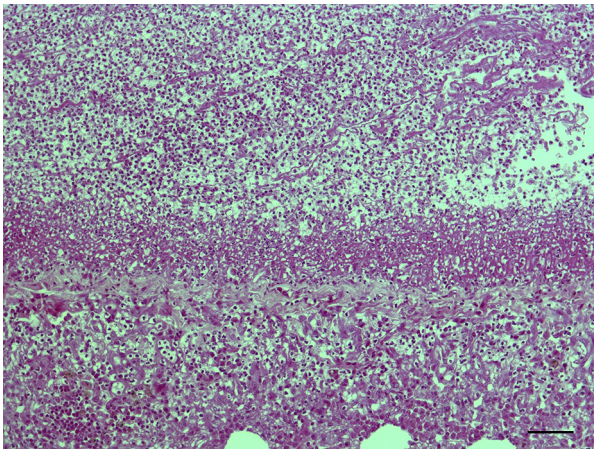

Fig. 1. Section of lung, showing severe pyogranulomatous pleuritis with abundant fibrin and numerous neutrophils and macrophages. Alveolar lumina are present at the base of the image. HE. Bar, 200 µm.

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

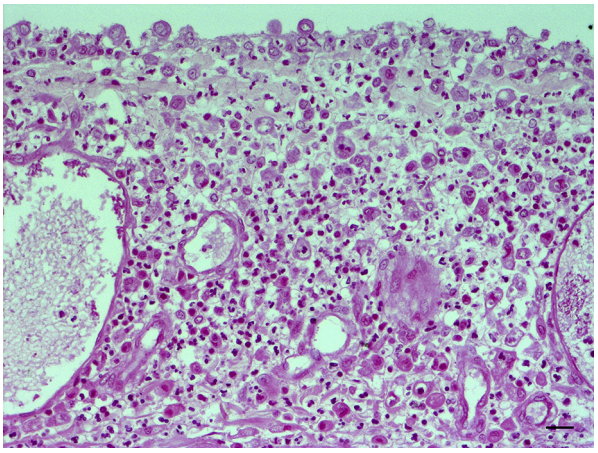

Fig. 2. Detail of pyogranulomatous pleuritis showing numerous neutrophils and macrophages. Metaplastic mesothelial cells are noted at the top of the image. HE. Bar, 20 µm.

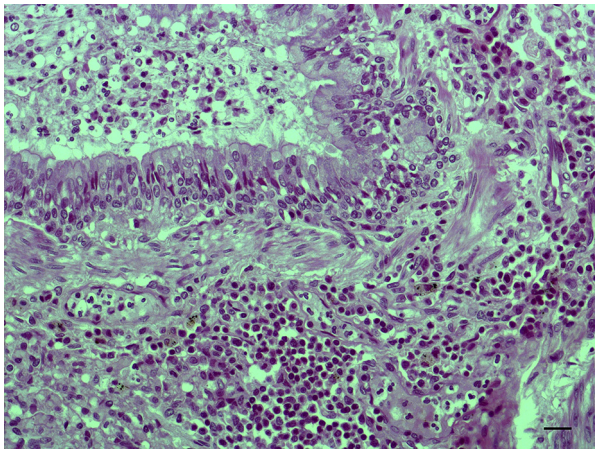

Fig. 3. Section of bronchiole showing catarrhal bronchiolitis and a surrounding infiltrate of mononuclear cells. HE. Bar, 20 μm.

compatible with *Streptococcus* spp. was the only significant microbiological finding. No canine distemper virus, coronavirus or other pathogens inducing granulomatous lesions were demonstrated. The *Streptococcus* sp. was tentatively classified as *Streptococcus acidominimus/pluranimalium* by the API 20 Strep system. A continuous sequence of 1,432 base pairs (bp) of the *16S rRNA* gene was obtained from the isolate (GenBank accession number KF021280). A BLAST comparison with the *16S rRNA* sequences in GenBank revealed a high similarity with *S. halichoeri* strain M512/02/1 (99.51% identity), being the next similar species *Streptococcus dysgalactiae* subsp. *equisimilis* strain CIP 105120 (97.23% identity). The isolate clustered with *S. halichoeri* in both ML and MP phylogenetic trees (Fig. 4), with 100% in both trees. Similarly, a 697 bp sequence was obtained for the *rpoB* gene (Genbank accession number: KF138590). This sequence

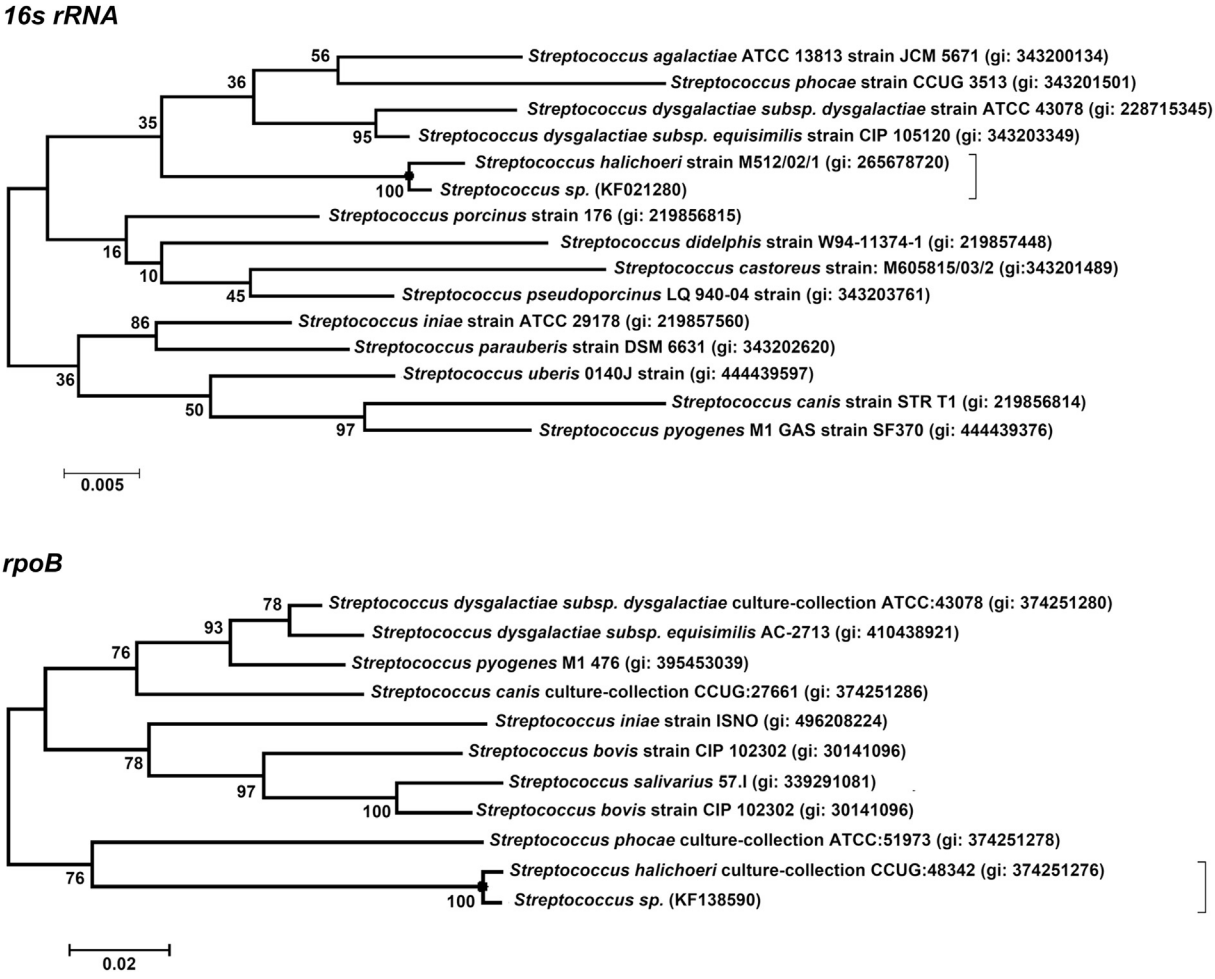

Fig. 4. Unrooted trees based on *16s rRNA* and *rpoB* gene sequences inferred by using the maximum likelihood method based on the Kimura 2-parameter model. Bootstrap values (expressed as percentages of 1,000 replications) are shown next to the branches. The trees are drawn to scale, with branch lengths measured as the number of substitutions per site (bars, 0.5% and 2% sequence divergence). Selected branches correspond with the groups of the new isolate. The same groups and bootstrap values were obtained in the maximum-parsimony trees (data not shown).

displayed 99.28% similarity with the corresponding gene of *S. halichoeri* culture-collection CCUG:48,342 (JN580236.1) and 99.22% with *S. halichoeri* strain CIP 108195 (DQ232471.1), with the third most similar sequence being that corresponding to *S. dysgalactiae* subsp. *equisimilis* AC-2713 (HE858529.1). As for the *16S rRNA* analysis, the new strain clustered together with *S. halichoeri*, displaying 100% bootstrap values for both ML and MP trees (Fig. 4).

In the present case, a gross diagnosis of tuberculosis was made initially, but TB was excluded following microscopical examinations including ZN staining and the absence of Langhans's type giant cells, which are not typically found in badgers. Other pathogens such as fungi, *Nocardia* spp., *Actinomyces* spp. or coronavirus that can induce similar granulomatous lesions were also ruled out. The only significant microbiological finding was a pure culture of *S. halichoeri* suggesting an aetiological role for this organism. The identification of *S. halichoeri* was only achieved after molecular studies. Sequencing of the *16S rRNA* gene has become the new 'gold standard' for defining species and genus (Clarridge, 2004). It has been suggested that a minimum of 99% sequence similarity should be present for species identification, with an ideal value >99.5%. Our results met this criterion; however, for some strains from the *Streptococcus* genus, the *rpoB* gene has been proposed as an alternative to *16S rRNA* (Vos *et al.*, 2012). The high similarity observed between the 697 bp *rpoB* gene fragment sequenced and this gene in two strains of *S. halichoeri*, strongly support the results obtained in the *16S rRNA* analysis.

The genus *Streptococcus* includes numerous gram-positive organisms with worldwide distribution (Köhler, 2007). Most species are commensals of the upper respiratory tract and lower urinary tract and may cause opportunistic infections, typically of a suppurative nature. In badgers, the only reference of *Streptococcus* spp. isolation comes from a study of bronchoalveolar lavage fluid in which a light growth of alpha and beta-haemolytic streptococci was detected (McCarthy *et al.*, 2009). *S. halichoeri* was first reported in UK grey seals (*Halichoerus grypus*) with skin wounds (Lawson *et al.*, 2004) and, very recently, from a man with empyema (Foo and Chan, 2014). The occurrence of *S. halichoeri* in the European badger and the grey seal, two species with a very different way of life, is an interesting finding. One possible explanation for this could be the lack of accurate information about bacterial species in wild carnivores. Additionally, the recent isolation in a human being raises the possibility of a more widespread distribution of *S. halichoeri* and that this *Streptococcus* sp. could be an emerging zoonotic infection (Foo and Chan, 2014). *S. halichoeri* infection might be underdiagnosed due to difficulties in identification by traditional techniques (Foo and Chan, 2014). In the present case sequencing of the *16S rRNA* gene permitted identification of this species, although routine techniques failed in the identification.

The origin of the infection in this case remains unclear. Pleuritis and pyothorax are often associated with pneumonic spread from bacteria aspirated from the oropharyngeal microflora. Other routes include haematogenous spread and, less often, bite wounds and perforation of the oesophagus or reticulum (Caswell and Williams, 2007). In the present case, no external injuries suggesting fighting or accidents during trapping were observed and the origin was suspected to be the aspiration of oropharyngeal flora. Further studies are necessary, however, to confirm if *S. halichoeri* is a normal inhabitant of the oral mucosa of badgers. On the other hand, respiratory bacterial infections, as often observed with streptococci, are favoured by stress or previous viral infection (Caswell and Williams, 2007). Canine distemper virus is an important respiratory pathogen in dogs and mustelids, often predisposing to secondary bacterial infections (Barron and Rosenthal, 2012). Although canine distemper was not identified, the possibility of co-infection with other viral pathogens cannot be excluded. In the present case, the stress of being trapped and handled may have precipitated the disease.

This report represents the third isolation of *S. halichoeri* since its first description, the second in a new animal species and demonstrates that it can be associated with respiratory pathology resembling tuberculosis in badgers. Interestingly, the isolation from a man with empyema suggests that this streptococcal species might be associated mainly with suppurative lesions of the respiratory system. It also emphasizes that molecular techniques may be needed for a final identification of some bacterial species and that more studies are needed in wildlife to demonstrate their potential role as reservoirs of both known and unknown pathogens.

Acknowledgments

The authors thank A. I. Sánchez and C. Cons for technical assistance.

Conflict of Interest Statement

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

References

Barron HW, Rosenthal KL (2012) Respiratory diseases. In: *Ferrets, Rabits and Rodents: Clinical Medicine and Surgery*, KE Quesenberry, JW Carpenter, Eds., Elsevier Saunders, St Louis, pp. 78–85.

Caswell JL, Williams KJ (2007) Respiratory system. In: *Jubb, Kennedy & Palmer's Pathology of Domestic Animals*, 5th Edit., Vol. 2, MG Maxie, Ed., Elsevier Saunders, St Louis, pp. 575–578.

Clarridge JE 3rd (2004) Impact of *16S rRNA* gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiology Reviews*, **17**, 840–862.

Corner LA, O'Meara D, Costello E, Lesellier S, Gormley E (2012) The distribution of *Mycobacterium bovis* infection in naturally infected badgers. *Veterinary Journal*, **194**, 166–172.

Drancourt M, Roux V, Fournier PE, Raoult D (2004) *rpoB* gene sequence-based identification of aerobic gram-positive cocci of the genera *Streptococcus*, *Enterococcus*, *Gemella*, *Abiotrophia* and *Granulicatella*. *Journal of Clinical Microbiology*, **42**, 497–504.

Foo RM, Chan D (2014) A fishy tale: a man with empyema caused by *Streptococcus halichoeri*. *Journal of Clinical Microbiology*, **52**, 681–682.

Gallagher J, Clifton-Hadley RS (2000) Tuberculosis in badgers; a review of the disease and its significance for other animals. *Research in Veterinary Science*, **3**, 203–217.

Graham E, Lamm C, Denk D, Stidworthy MF, Carrasco DC *et al.* (2012) Systemic coronavirus-associated disease resembling feline infectious peritonitis in ferrets in the UK. *Veterinary Record*, **171**, 200–201.

Köhler W (2007) The present state of species within the genera *Streptococcus* and *Enterococcus*. *International Journal of Medical Microbiology*, **297**, 133–150.

Lawson PA, Foster G, Falsen E, Davison N, Collins MD (2004) *Streptococcus halichoeri* sp. nov., isolated from grey seals (*Halichoerus grypus*). *International Journal of Systematic and Evolutionary Microbiology*, **54**, 1753–1756.

McCarthy G, Shiel R, O'Rourke L, Murphy D, Corner L *et al.* (2009) Bronchoalveolar lavage cytology from captive badgers. *Veterinary Clinical Pathology*, **38**, 381–387.

Millán J, Candela MG, López-Bao JV, Pereira M, Jiménez MA *et al.* (2009) Leptospirosis in wild and domestic carnivores in natural areas in Andalusia, Spain. *Vector Borne Zoonotic Diseases*, **9**, 549–554.

Origgi FC, Plattet P, Sattler U, Robert N, Casaubon J *et al.* (2012) Emergence of canine distemper virus strains with modified molecular signature and enhanced neuronal tropism leading to high mortality in wild carnivores. *Veterinary Pathology*, **49**, 913–929.

Quinn JH, Girard YA, Gilardi K, Hernandez Y, Poppenga R *et al.* (2012) Pathogen and rodenticide exposure in American badgers (*Taxidea taxus*) in California. *Journal of Wildlife Diseases*, **48**, 467–472.

Sobrino R, Dubey JP, Pabón M, Linarez N, Kwok OC *et al.* (2008) *Neospora caninum* antibodies in wild carnivores from Spain. *Veterinary Parasitology*, **155**, 190–197.

Vela AI, Pérez M, Zamora L, Palacios L, Domínguez L *et al.* (2010) *Streptococcus porci* sp. nov., isolated from swine sources. *International Journal of Systematic and Evolutionary Microbiology*, **60**, 104–108.

Vickerman MM, Brossard KA, Funk DB, Jesionowski AM, Gill SR (2007) Phylogenetic analysis of bacterial and archaeal species in symptomatic and asymptomatic endodontic infections. *Journal of Medical Microbiology*, **56**, 110–118.

Vos M, Quince C, Pijl AS, DE Hollander M, Kowalchuk GA (2012) A comparison of *rpoB* and *16S rRNA* as markers in pyrosequencing studies of bacterial diversity. *PloS One*, **7**, e30600.

[*Received, September 23rd, 2014*
Accepted, December 22nd, 2014]