

< Case Report >

Multiple liver infection by *Sphingomonas* sp. and *Bacillus* sp. in a wild rodent (*Apodemus agrarius*): a case report

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Abstract

The first case of liver infection caused by *Sphingomonas* sp. and *Bacillus* sp. in a wild rodent is reported. A captured wild rodent, *Apodemus agrarius* (*A. agrarius*), presented with multiple liver abscess-like nodules (diameter 0.7~2.4 mm) in which Gram-positive and Gram-negative bacilli were detected simultaneously. These were grown in aerobic and anaerobic cultures, respectively, and were identified as *Sphingomonas* sp. and *Bacillus* sp., respectively, according to 16S rRNA sequencing.

Key words : Liver infection, *Sphingomonas*, *Bacillus*, Wild rodent

INTRODUCTION

Liver infection are caused by various organisms including bacteria, fungi, and parasites, are the most common type (Mavilia et al, 2016). The liver infection is classified by etiology: pyogenic, amoebic, and fungal liver abscess, those were caused by bacteria, *Entamoeba histolytica*, and fungus, respectively.

The most common bacterial pathogen in primary liver infection is *Staphylococcus aureus*, followed by *Streptococcus agalactiae*, *Escherichia coli* (*E. coli*), viridans streptococcus, *S. epidermidis*, and *Salmonella* species, whereas *E. coli* is the leading organism in secondary liver infection, followed by *S. aureus*, *Klebsiella pneumoniae*, viridans streptococcus, and *Candida albicans* (Mavilia et al, 2016). *Sphingomonas* is a Gram-negative, aerobic, rod-shaped bacterium that is ubiquitous in the natural environment and rarely causes disease in humans or other animals (An et al, 2013). *Bacillus* is Gram-pos-

itive and rod-shaped, consisting of anaerobes or facultative anaerobes, and is found in nature, particularly in soil. A couple of liver infection cases caused by *Bacillus* species have been reported (Harris, 1905; Latsios et al, 2003).

Here, we present an unusual case of multiple liver infection caused by *Sphingomonas* sp. and *Bacillus* sp. in a wild rodent, *Apodemus agrarius* (*A. agrarius*), which were identified via 16S rRNA sequencing.

CASE HISTORY

During a population survey on wild rodents in Gangwon-do Province in the Republic of Korea from February 2016 to June 2016, a total of 171 wild rodents were captured in the area of Hwacheon-gun using Sherman traps (H.B. Sherman, Tallahassee, FL, USA). The captured rodents included *A. agrarius* (n=162, 94.7%), *A. peninsulae* (n=2, 1.2%), *Micromys minutus* (n=2, 1.2%), and *Myodes rufocanus* (n=5, 2.9%). Animals were eu-

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thanized according to the protocols of the Institutional Animal Care and Use Committee at Yonsei University Wonju Campus. One of them, *A. agrarius*, had abscess-like nodules in the liver, which were subjected to histological and microbiological analysis.

RESULTS

There were numerous abscess-like nodules on the surface of liver of the wild rodents, and the size of nodules ranged from 0.7 to 2.4 mm in diameter (Fig. 1A). In the histological examination, there were demarcated granulomas in the liver parenchyma (Fig. 1B), which was surrounded by lymphocytes at the margin and filled with fibroblast or epithelioid cells in the content of granuloma (Fig. 1C). Nodule specimens were subjected

to Gram staining and bacterial culture using blood agar plates under both aerobic and anaerobic conditions. Interestingly, Gram-positive and Gram-negative bacilli were detected simultaneously (Fig. 2A). Culture results from nodule specimens demonstrated Gram-negative bacilli in the aerobic culture and Gram-positive bacilli in the anaerobic culture (Fig. 2B and 2C). Amplification and sequencing of the 16S rRNA gene were performed on each bacillus according to a previous report (Suzuki et al, 1996); the bacilli were identified as *Shingomonas* sp. (100% identity; GenBank accession number LN832009) and *Bacillus* sp. (99% identity; GenBank accession number LC049103), respectively.

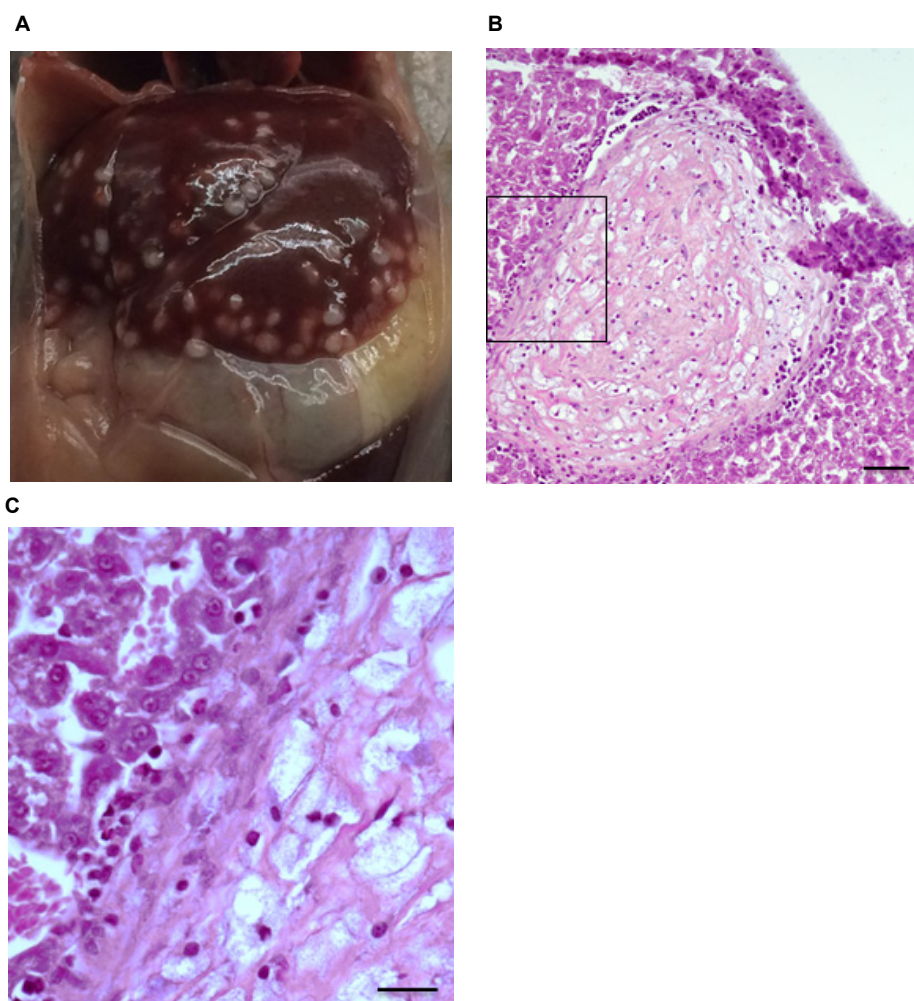


Fig. 1. Gross and histological lesions of the liver of a wild rodent showing multiple abscesses. (A) Gross appearance of the liver showing numerous abscesses on the surface. (B) Histological lesions of the liver sections showing the demarcated granuloma in the liver parenchyma (Hematoxylin and eosin staining, Bar 30 μ m). (C) The higher magnification of the marked area in panel (B) (Hematoxylin and eosin staining, Bar 10 μ m).

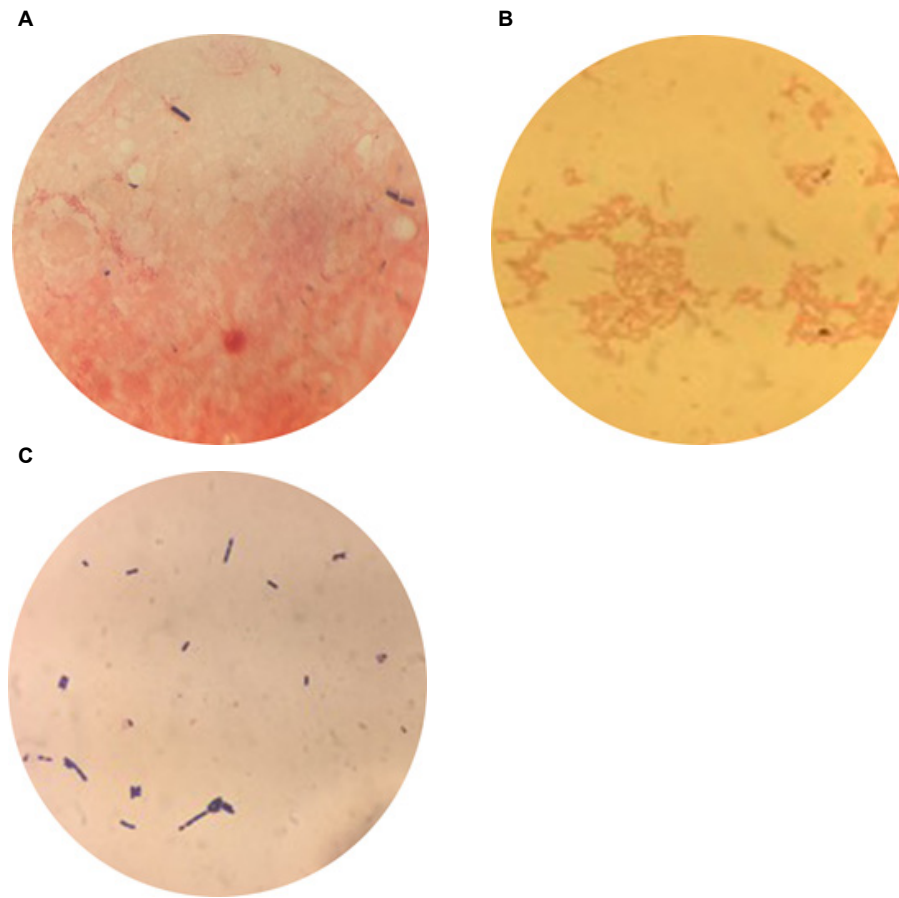


Fig. 2. Gram staining on liver nodule specimens and bacterial cultures from liver nodules. Gram staining of specimens from liver nodules showing Gram-negative and Gram-positive bacilli (A). Gram staining of bacteria grown under aerobic conditions showing Gram-negative bacilli (B) and bacteria grown under anaerobic conditions showing Gram-positive bacilli (C) on blood agar plates. Magnification $\times 1,000$.

DISCUSSION

In the present study, we report the first case of liver infection in a wild rodent caused by *Sphingomonas* and *Bacillus*. *Sphingomonas* is an aerobic, non-spore-forming, non-fermentative, Gram-negative bacillus with catalase and oxidase activity (An et al, 2013). The habitat of *Sphingomonas* has not been fully defined but it can be found in the natural environment, particularly in water and soil. The first case of a *Sphingomonas* species, *Sphingomonas paucimobilis* infection in a human, was described by Holmes et al. in 1977 (Holmes et al, 1977). *Sphingomonas* has been reported to cause a variety of human diseases (Hahler et al, 1997). However, to date, no hepatic infection by *Sphingomonas* species has been reported. This is the first natural case of liver infection caused by *Sphingomonas* in an animal or a human.

Bacillus is a genus of Gram-positive and rod-shaped bacteria that can be an obligate anaerobe or a facultative anaerobe. *Bacillus* species are ubiquitous in nature, par-

ticularly in soil, and include both free-living and parasitic pathogenic species. The first case of liver infection caused by *Bacillus* was reported by Harris in 1905, in which *Bacillus mortiferus* caused abscesses in the liver, lungs, and spleen of a patient (Harris et al, 1995). There was also a case report on *Bacillus cereus*, a food-borne pathogen, causing liver infection in an old man (Latsios et al, 2003). The *Bacillus* strain isolated in the present study did not cause severe lesions in comparison with *Bacillus cereus*, which caused fulminant liver failure (Mahler et al, 1997) and multiple abscesses in the liver as well as in brain (Kotiranta et al, 2000).

The sources of liver infection caused by *Sphingomonas* and *Bacillus* are not clear, but they may be peritoneal sources rather than hematogenous sources. In the review of Mavilia et al. a mixed flora including aerobic and anaerobic bacteria commonly caused liver infection via a peritoneal source, while a single organism was determined to be the cause of liver infection via the hematogenous spread of infection (Mavilia et al, 2016).

Bacterial liver infection is usually associated with enteric Gram-negative bacilli and anaerobic bacteria. The liver infection in the present study was caused not by enteric Gram-negative bacilli but other Gram-negative bacilli. In addition, *Bacillus* was also associated with liver infection. These results might provide a valuable information on the possible causative agents of liver infection, which are important for the effective treatment.

CONCLUSION

In conclusion, this is the first natural case of liver infection caused by *Sphingomonas* and *Bacillus* species in a wild rodent, *A. agrarius*.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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