



CASE REPORT

***Streptococcus pluranimalium* Isolated from a Canine Respiratory Case: Identification and Experimental Infection in Mice**

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ABSTRACT

A 4-month-old Tactic dog was presented for evaluation of respiratory syndrome. A strain of *Streptococcus pluranimalium* was isolated and confirmed by biochemical characteristics and 16S rRNA sequence analysis. To determine the virulence of this bacterium, BALB/c mice were challenged by intraperitoneal injection with 10^8 CFU *S. pluranimalium*. Lung, liver, heart, kidney, spleen and brain were harvested at intervals for analysis. Lung tissue showed the earliest bacterial detection at 2 days and all the collected tissues demonstrated greater colony counts at 96h following exposure to the bacterium. Histopathological examinations showed that lung cells became widened with thickening of the bronchiolar lumen and narrowing of alveolar septa. Exudate in macrophages significantly increased lung congestion and severe consolidation. The main lesions in the brain were characterized by edema of nerve cells and non-supportive encephalitis, indicating that *S. pluranimalium* is capable of inducing brain damage by crossing the blood-brain barrier (BBB). It is concluded that *S. pluranimalium* may be one of causative agents for canine infectious respiratory disease.

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INTRODUCTION

Canine infectious respiratory disease (CIRD) is a constant challenge in dogs. There are multiple causative agents, including bacterial pathogens such as *Bordetella bronchiseptica* and viral infections such as canine parainfluenza or canine influenza virus. Bacterial infections caused by various species of *Streptococcus* are much more common in puppies and older dogs with weakened immune systems. The genus *Streptococcus* comprises a large number of Gram-positive cocci, non-motile and chain-forming bacteria, including both harmless commensals and formidable pathogens (Facklamm, 2002; Krzyściak *et al.*, 2013). *Streptococcus zooepidemicus* has been reported to cause severe systemic disease in dogs (Chalker *et al.*, 2003), and this bacterial disease has become increasingly implicated in fatal outbreaks in dogs over the past five years. However, there have not been reports describing CIRD associated with *S. pluranimalium*. This report described the bacteriological and pathological findings in a dog suffering from respiratory disease associated with *S. pluranimalium*.

Case history: A 4-month-old Tactic dog was presented for evaluation of respiratory syndrome in Animal Clinics of Nanjing Agricultural University, China. The syndrome included depression, anorexia, coughing, sneezing and copious nasal discharge. The hematological examination showed the elevated count of white blood cells and neutrophil granulocytes.

Bacterial isolation and identification: The bacterial isolates were obtained from nasopharyngeal swabs and identified as most likely to be *Streptococcus* species by phenotypic characterization. Isolates were cultured on TSB (tryptic soy broth) containing 5% defibrinated sheep blood and incubated at 37°C. The colonies were α -hemolytic and 1-2 mm in diameter. Typical colonies were Gram-stained and observed for Gram-positive cells in chains or pairs. Single colonies were harvested for biochemical characterization, and the biochemical profiles were as follows: positive reaction in ribose, sucrose and hydrolysis of esculin while negative for Voges-Proskauer (acetoin production), arginine dihydrolase, catalase, urease, mannitol, inulin, starch, glycogen, arabinose,

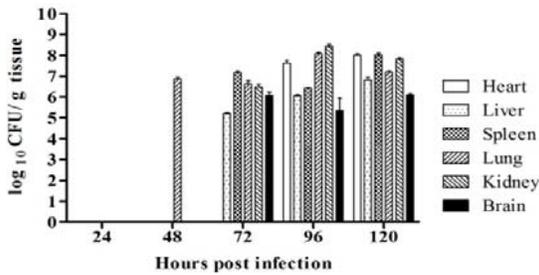


Fig 1: Bacterial load in heart, liver, spleen, lung, kidney and brain of mice after inoculation of *S. pluranimalium*. Bacteria are expressed as log₁₀ number of CFU per gram of heart, liver, spleen, lung, kidney and brain.

sorbitol, maltose and lactose. Further PCR amplification of 16S rRNA gene was performed for the confirmation of the bacteria using a pair of oligonucleotide primer (5'AGAGTTTGATCMTGGCTCA/TACGGYTACCTGTTACGACTT-3'). BLAST analysis against the GenBank database identified a closest match to *S. pluranimalium* (>98% sequence identity).

Bacterial load and lesions: BALB/c mice (5-6 weeks, 18-20g) were purchased from Animal Experiment Center, Yangzhou University. All the mice were housed in individual compartments in stainless steel wire cages. Prior to this study, the approval for conducting experiments was obtained from Animal Ethics Committee of Nanjing Agricultural University. Infectious agent was diluted in phosphate buffer saline (PBS) and injected 50 μ L (10^8 CFU) into 15 mice by intraperitoneal route. Another 15 mice were inoculated with PBS only and designated as control group. Mice were sacrificed by cervical dislocation. Tissues from three mice, including

heart, liver, lung, kidney, spleen and brain, were collected on each time point of 24, 48, 72, 96 and 120 hours post inoculation (hpi). Bacterial load was determined from heart, liver, spleen, lung, kidney and brain. For histopathological examination, tissue samples collected from 96 hpi were fixed in 10% neutral buffered formalin. After fixation, the tissues were embedded in paraffin. Tissue sections of 4 μ m were stained with hematoxylin and eosin, and then examined under microscope.

Bacterial load was determined from heart, liver, spleen, lung, kidney and brain (Fig. 1). At 24 hpi, this bacterium could not be isolated from all tissues, while at 48 hpi, it was isolated from lung only. At 72 hpi, no bacteria were isolated from heart, while the bacterial number in all tissues continued to increase with time over the course of the infection. At 96 hpi, bacterial loads in lung and kidney exceeded 10^8 cfu/g, 10^6 cfu/g in liver, 10^5 cfu/g in brain and 10^6 cfu/g in spleen and heart. At 120 hpi, bacterial loads in heart and spleen were 10^8 cfu/g, 10^6 cfu/g in liver and brain, and 10^7 cfu/g in kidney and lung.

Histopathological examination of tissue samples revealed that lesions appeared in the lungs and brain of the infected mice (Fig. 2), while no lesions were found in the kidney, liver, spleen and heart. The histopathological examination for the lung showed inflammation as well as destruction of epithelial cells (Fig. 2a). Airway involvement was characterized by inflammatory cells in the lumen. Lungs interstitial became widened, bronchial lumen became narrow and alveolar septum was thickened. There was increased infiltration of the inflammatory cells including macrophages. Interstitial pneumonia was also noticeable with occurrence of severe lung congestion (Fig. 2b). Inflammation with interstitial, airway necrosis and diffused consolidation was also observed (Fig. 2c).

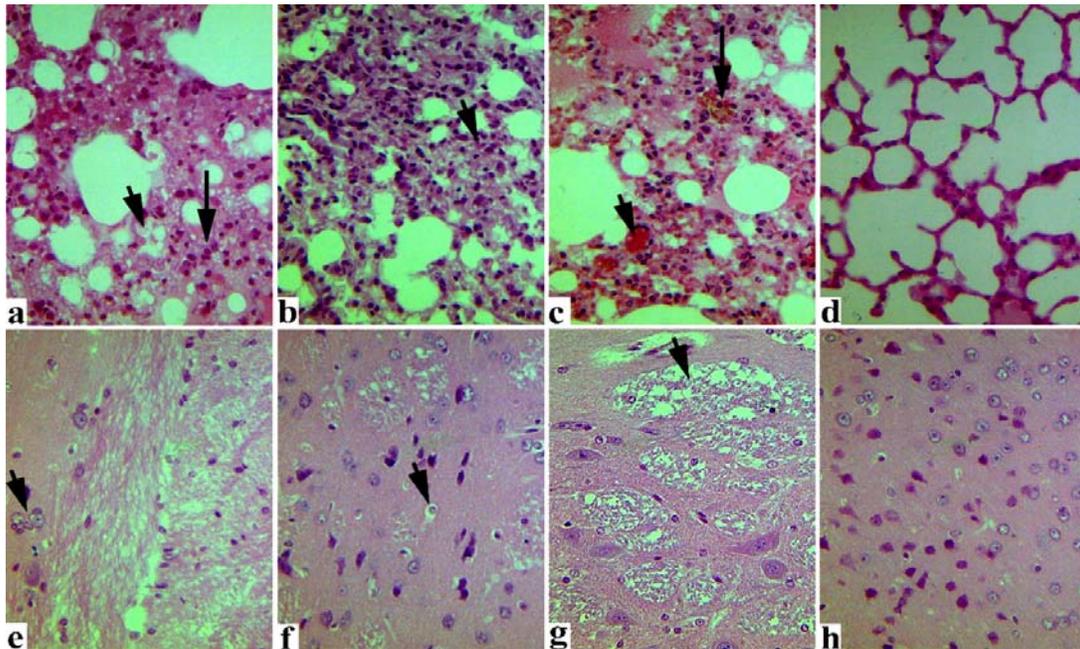


Fig 2: Histopathological changes in the lungs (a-d) and brains (e-h) of the mice at 96 h. p. i. after H&E staining at 400 \times . a) Lung showing infiltration in bronchiolar lumen (short arrow) and alveolar cavity widened with interstitial cells (long arrow), b) interstitial pneumonia with alveolar septum thickened by a number of inflammatory cells (arrow), c) airway necrosis, infiltration of inflammation cells (long arrow) and consolidation (short arrow) and d) no histopathological lesions in PBS control group. e) Brain showing glial nodules (arrow), f) neuronal satellitosis (arrow), g) dissolution of the nerve fibers and neuronal necrosis (arrow) and h) no histopathological lesions in PBS control group.

Swelling and edema of the nerve cells in the brain following *S. pluranimalium* infection were observed. Microglial nodules were detected (Fig. 2e) and glial cells were increased significantly, with some proliferated glial cells gathering around nerve cells to form satellite phenomenon (Fig. 2f). However, non-suppurative inflammation was induced after challenge with the bacterium. Nerve fibers were dissolved and neurons showed spongy necrosis (Fig. 2g). No histopathological lesions were found in the lung (Fig. 2d) and the brain (Fig. 2h) from PBS control group.

DISCUSSION

S. pluranimalium is a documented pathogenic agent in a wide range of species. This bacterium was first proposed as a new species by Devriese *et al.* (1999). In contrast to most streptococcal species, *S. pluranimalium* has been isolated from very different animal host species and variable body locations of the species: mastitic milk, vagina and cervix of cattle, crop, lungs and lesions of canary, tonsil of goat and cat (Devriese *et al.*, 1999), humans (Dhotre *et al.*, 2014), and brain and cerebrospinal fluid of neonatal calf (Seimiya *et al.*, 2007). In the present study, the biochemical results of new *S. pluranimalium* isolates were similar to what was reported by other authors (Devriese *et al.*, 1999; Hedegaard *et al.*, 2009; Seimiya *et al.*, 2007; Twomey *et al.*, 2012). Diseases associated with *S. pluranimalium* have been reported to be septicemia, endocarditis and salpingitis in broilers (Hedegaard *et al.*, 2009) and reproductive disorders in cattle and sheep (Foster *et al.*, 2008; Foster *et al.*, 2010). To our knowledge, this is the first report to identify *S. pluranimalium* in canine respiratory disease.

S. pluranimalium is an emerging pathogen, and the mechanisms of its pathogenesis are largely unknown. Our report showed that the mouse model could be used to assess the virulence of the bacterium. The pathological findings in the brain showed that *S. pluranimalium* is capable of inducing brain damage by migrating into the brain by crossing the blood-brain barrier (BBB). However, it is unclear how circulating bacteria cross BBB. Complete understanding of the pathogenesis of *S.*

pluranimalium should lead to the development of novel strategies to prevent the infection of this bacterium.

Conclusion: *S. pluranimalium* may be one of the causal agents of canine respiratory disease. This organism may act alone to cause disease or it may intensify the effects of other known pathogens, resulting in more significant symptoms. The veterinarian needs to be aware of the pathogen at clinics.

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