CLINICAL REPORT

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Pneumonia infection by *Morganella morganii* in a male alpaca

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Abstract

After sudden death with a history of about two weeks ruminal tympany, a 3-year-old, male alpaca from Huantaiqi Zoo, Chongqing, China was presented to the Animal Diseases Rapid Diagnosis Center, Southwest University, Chongqing, China for diagnosis of the death causes. At necropsy, the primary pathological lesions were found in the lung. A pronounced hemorrhage with topical congestion and lobular pneumonia was identified. Sero-fibrinogenous pleural effusion was also detected in the thoracic cavity. After necropsy, the lung sample was processed for histological examination, while lung, hydropericardium, and heart-blood samples were processed for bacteriological examination. From the lung tissue, abundant fluid exudate was found in the pulmonary alveoli. Meanwhile, a mild to moderate hemorrhage and inflammatory cells infiltrations were also observed in the lung sections. Pure isolates on the 5.00% defibrinated sheep blood agar were submitted for identification by morphological and molecular methods. Sequencing results indicated that the Gram-negative sporadic bacilli were all belonged to *Morganella morganii*. To the best of our knowledge, this is the first record of *M. morganii* induced pneumonia in an alpaca.

Introduction

Morganella morganii, Gram-negative anaerobic rod bacterium, belongs to tribe Proteeae of family Entero-bacteriaceae and has been recognized as an increasingly important pathogen.[1] The disease spectrum associated with *M. morganii* infections is broad and the mortality of such infections remains high in reported cases.[2]

The alpaca (*Vicugna pacos*) is a domesticated South American camelid with the largest population in Peru. The breeding of this animal for wool and meat is an important agricultural commodity in the economy of Peruvian peasants.[3] The number of alpacas bred in China increases continuously as these animals become more and more popular. Alpacas are used for wool production, as companion or ornamental animals and for therapeutic treatment of humans. They are kept as pets in smaller groups or as livestock in larger herds.[4]

In this report, we described the case of male alpaca in Huantaiqi Zoo of Chongqing, China conferred for a necropsy to the Animal Diseases Rapid Diagnosis Center, Southwest University, Chongqing, China after sudden death.

Case Description

A 3-year-old male alpaca had been kept two years with other two alpacas in Huantaiqi Zoo of Chongqing, China originating from a larger alpaca breeding farm without records of *M. morganii* infection. The breeder reported a ruminal tympany persisting for about two weeks treated with sodium bicarbonate. The nutritional condition of the individual was low to normal and it was found dead by the breeder in the zoo. At necropsy (within 5 hr after death), a high-grade of sero-fibrinogenous pleural effusion was observed in the thoracic cavity. The lung showed a pronounced hemorrhage with topical congestion and lobular pneumonia was identified (Fig. 1A). Furthermore, limpid hydropericardium was detected and plenty of foam was presented in the trachea (Fig. 1B). No other pathological lesions were observed in any other organs. Selected samples from lung tissue were routinely processed for histopathological examination. Moreover, bacteriological examinations from the lung, heart-blood and hydropericardium were performed by routine laboratory tests using blood agar (containing 5.00%

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defibrinated sheep blood) at 37.00 ± 1.00 ˚C in aerobiosis for 24 hr. Samples of lung tissue were firstly collected, fixed in 10% neutral buffered formalin, then processed, paraffin-embedded, stained with Hematoxylin and Eosin and examined by standard light microscopy for histological studies. The air spaces of the pulmonary alveoli were obliterated with fluid exudate (Fig. 2A). A mild to moderate hemorrhage and inflammatory cells infiltration were also detected in the alveolar spaces (Fig. 2B).

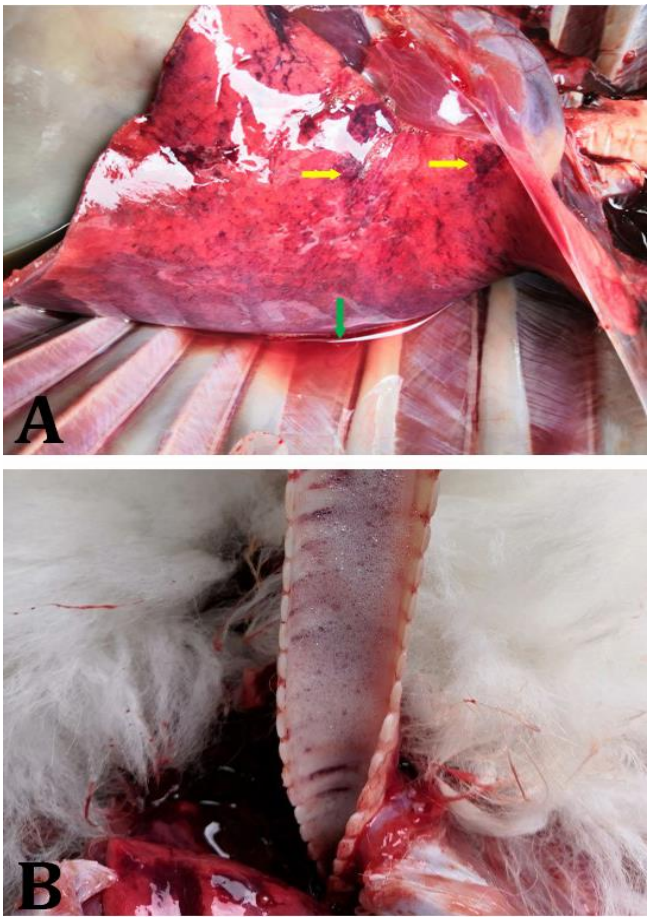

Fig. 1. A) Typical pathological changes in the alpaca. Noticeable hemorrhage and topically congestion in the lung (yellow arrows). Sero-fibrinogenous pleural effusion was notable in the thoracic cavity; the lesion areas were located with green arrow. **B)** Plenty of foam was presented in the trachea.

After incubation, a pure culture of translucid grey colonies with hemolysis on the blood agar was submitted for identification. Gram staining revealed Gram-negative sporadic bacilli. To confirm the identification of isolated strains obtained from the lung and hydropericardium, five clones (two isolated from the lung and three isolated from the hydropericardium) were undergone 16S rDNA sequencing. The chromosomal DNA from the isolates was extracted using the lysis buffer (Takara Biotech, Beijing, China) in accordance with the manufacturer's instructions. The supernatant was collected and stored at – 20 ˚C until used as polymerase chain reaction (PCR) templates. Primers based on 16S rDNA (F-5'-AGAGTTTGATCCTGGCTCAG-3' and R-5'-AAGGAGGTGATCCAGCCGCA-3') were designed as described previously.[5] For PCR amplification, each reaction was performed in a final volume of 50 µL containing 25.00 µL of premix Taq (Takara Biotech), 2.00 µL (10 µM) of each forward and reverse primers, 6.00 µL DNA template and 15 µL ddH_2O. The PCR cycling protocol was as follows: the first denaturation at 94 ˚C for 10 min followed by 30 cycles of denaturation at 94 ˚C for 30 sec, annealing at 55 ˚C for 30 sec and extension at 72 ˚C for 90 sec. After a final extension at 72 ˚C for 5 min, the tubes were cooled to 4 ˚C. Sequencing data of the partial 16S rDNA were analyzed for the homology using MegAlign5 software. Based on the sequencing results of 16S rDNA, all the five isolates shared 99.50% similarity with *M. morganii* strains registered in Genbank. According to the above-mentioned identification results, the alpaca was infected with *M. morganii* resulting in fatal pneumonia.

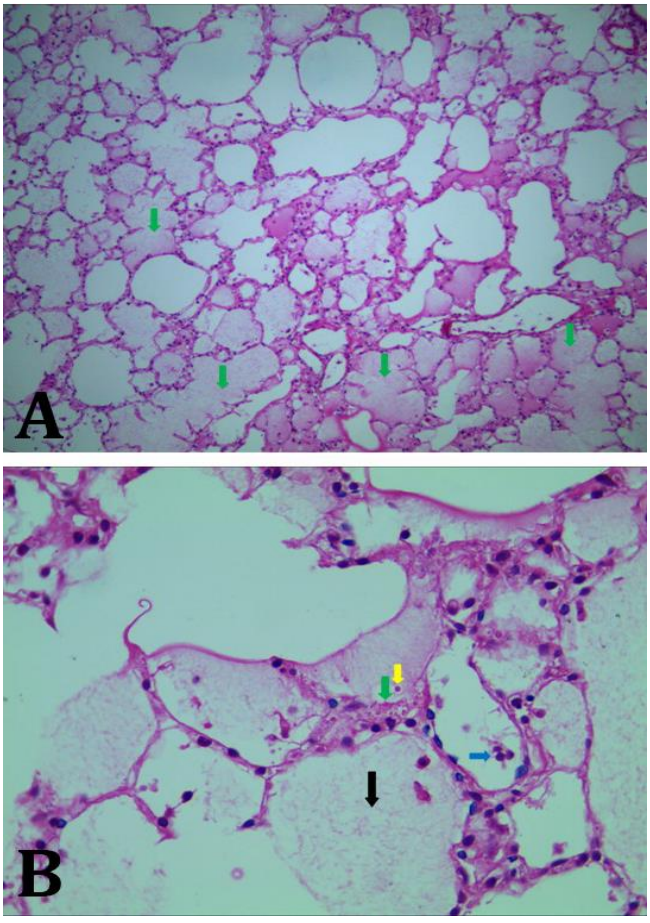

Fig. 2. Histopathological evaluation of alpaca lung tissue. **A)** The air spaces of the pulmonary alveoli were obliterated with fluid exudate and located with green arrows (H&E; 100×). **B)** A few red blood cells (yellow arrow), desquamated or necrotic epithelial cells (green arrows), inflammatory cells (blue arrow) and fluid exudate (black arrow) were detected in the alveolar spaces (H&E; 400×).

Discussion

The *M. morganii* is widely distributed in nature and commonly found in the environment and intestinal tracts of humans, mammals and reptiles as a part of the normal flora. Previously, *M. morganii* was considered to be a very unimportant pathogen and not much attention was given to it due to rarity and low potential for nosocomial epidemics.[2] However, the increased frequency of *M. morganii* infection and a considerable acceleration of the resistant genes spread in this bacterium caused wide-spread concern and it could not be neglected.[2] In this study, *M. morganii* was isolated from an adult alpaca. To the best of our knowledge, this is the first *M. morganii* isolation report from this animal. In this case, the carcass was promptly transported to the laboratory in several hr after death besides the low temperature in the winter (ranged 4 to 8 ˚C outside). Thus, we could cautiously conclude that the samples used for bacteria isolation were not contaminated by saprophyte bacteria. However, only five isolates were identified and the defect of small quantity could not roll out the possibility of co-infection with other common pathogens like *E. coli* and *Diplococcus pneumoniae*. In addition, bacteria were successfully isolated from lung and hydropericardium, while blood was negative. These findings indicated that bacteremia and septicemia could be rolled out in this study.

Hypersensitivity reaction is an unavoidable risk factor for the sudden death. Various causes can lead to allergic reactions, while medications, food or other substances might be more common among patients.[6] In the present report, the alpaca has only received sodium bicarbonate, without any other medicines or vaccines during the therapy of ruminal tympany. Food, drinking water and the feeding environment were consistent with the past, as the other two healthy alpacas. No clinical manifestations of hypersensitivity like dermatitis and asthma were found in this case. Thus, it was believed that hypersensitivity reaction should not be the key cause of death. However, in-depth research on this issue was not performed in this study.

As previously reported, *M. morganii* mainly causes sepsis, abscess, urinary tract infection, bacteremia, purple urine bag syndrome, chorioamnionitis, cellulitis, and wound infection.[2] But in this case, pneumonia was the primary type of infection resulting in the death of alpaca. A similar result had been found in a captive Jaguar in the Republic of Korea.[7] Furthermore, humans have also displayed pneumonia during morganelliasis, not with-standing in a lower ratio.[2,8] These findings indicated that the disease spectrum of *M. morganii* is diversified. Although *M. morganii* as a pneumonic pathogen in animals needs to be further elucidated, this case revealed that the organism has potential pathogenicity and should always be considered during the diagnosis of bacterial pneumonia in domestic and wild animals.

The breeding of alpacas in China has grown considerably in the last 10 years and these animals have gained great fame especially regarding wool or meat production or as hobby animals in zoos leading to a close contact to the humans pointing out the possible risk of transmission to humans. However, no information was found about this organism in the alpaca. Therefore, further investigation is needed to ascertain the pathogenic mechanism of *M. morganii* and to block the development of *Morganella* infections.

Acknowledgements

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Conflict of interest

The authors declare that there was not any conflict of interest.

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