



NOTE

Wildlife Science

Identification of *Moraxella lacunata* from pulmonary abscesses in three zoo herbivores

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ABSTRACT. Although *Moraxella lacunata* causes conjunctivitis, keratitis, endocarditis, and otolaryngitis in humans, its infection is rare in animals. We report three cases of asymptomatic pulmonary abscesses caused by *M. lacunata* in zoo herbivores, including two elks (*Cervus canadensis*) and a common eland (*Taurotragus oryx*). In all cases, macroscopic findings included coalescence of lung lobes and severe pulmonary abscesses filled with cheese-like materials in cysts. Microscopic findings included pneumonia characterized by marked fibrin exudates in alveolar spaces and infiltration of inflammatory cells. *M. lacunata* was identified in bacterial cultures from pulmonary abscesses using biochemical API 20NE system. *M. lacunata* is rarely isolated from zoo animals; however, herein, we describe the first report of pulmonary abscesses caused by *M. lacunata* infection.

KEY WORDS: antimicrobial susceptibility, asymptomatic pulmonary abscess, *Moraxella lacunata*, pathology, zoo animal

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The genus *Moraxella* is a gram-negative, aerobic coccobacillus or rod-shaped bacterium and usually has low virulence [11, 22]. Fourteen different species of *Moraxella* have been isolated from various animals and humans, and several species exist as natural normal flora [16, 22]. Of these species, *Moraxella catarrhalis* is the most important human pathogen associated with eye infections, respiratory tract infections, and chronic obstructive pulmonary disease [17]. *Moraxella bovis* causes infectious bovine keratoconjunctivitis, known as pinkeye, in cattle and has been found in the nasal passages of cattle [6, 22]. *Moraxella lacunata* can cause conjunctivitis, keratitis, endocarditis, and otolaryngitis in humans [5, 9, 13, 23], but is rarely found in animal cases [7, 24].

Here, we report three cases of pulmonary abscesses caused by *M. lacunata* infection in three zoo herbivores: two elks (*Cervus canadensis*) and one common eland (*Taurotragus oryx*). The two elks and the common eland died with no preliminary clinical signs but pulmonary abscesses were found during the postmortem examinations. The animals were housed in enclosed facilities at the Daejeon O-World Theme Park located in central Korea (36°17'19.00" N, 127°23'52.04" E) and were fed a diet of alfalfa, timothy hay, and commercial pelleted feeds.

A necropsy was performed according to a standard protocol. Lung tissue samples were collected and fixed in 10% neutral buffered formalin for 2 weeks, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin for evaluation using light microscopy. The samples were cultured on blood agar (Asan Pharmacy, Seoul, Korea) at 37°C for 12–18 hr. A pure culture was isolated on the agar following cold storage in a refrigerator for diagnosis. The colonies were confirmed using Giemsa staining and light microscopy. Classical phenotypic tests were performed using a biochemical API 20NE identification kit (bioMérieux, Marcy l'Etoile, France).

The susceptibility of the etiologic isolates to antibiotics was determined by the disc diffusion method [12]. The isolates were cultured in Mueller-Hinton (MH; Difco, Detroit, MI, U.S.A.) broth, and the turbidity of the suspension was adjusted to 0.5 McFarland standard (1.5×10^8 /ml). The bacterial cultures were subsequently inoculated into MH agar plates. The following antibiotics were applied to the discs (BBL; Becton Dickinson, Sparks, MD, U.S.A.): ampicillin (10 μg), amikacin (30 μg), bacitracin (10 μg), cephalothin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), cefazolin (30 μg), colistin (10 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), novobiocin (30 μg), enrofloxacin (5 μg), norfloxacin (10 μg), penicillin (10 μg), streptomycin (10 μg), trimethoprim-sulfamethoxazole (25 μg), oxytetracycline (30 μg), and vancomycin (30 μg). Resistance breakpoints were defined according to the National Committee for Clinical Laboratory Standards for gram-negative bacteria [12].

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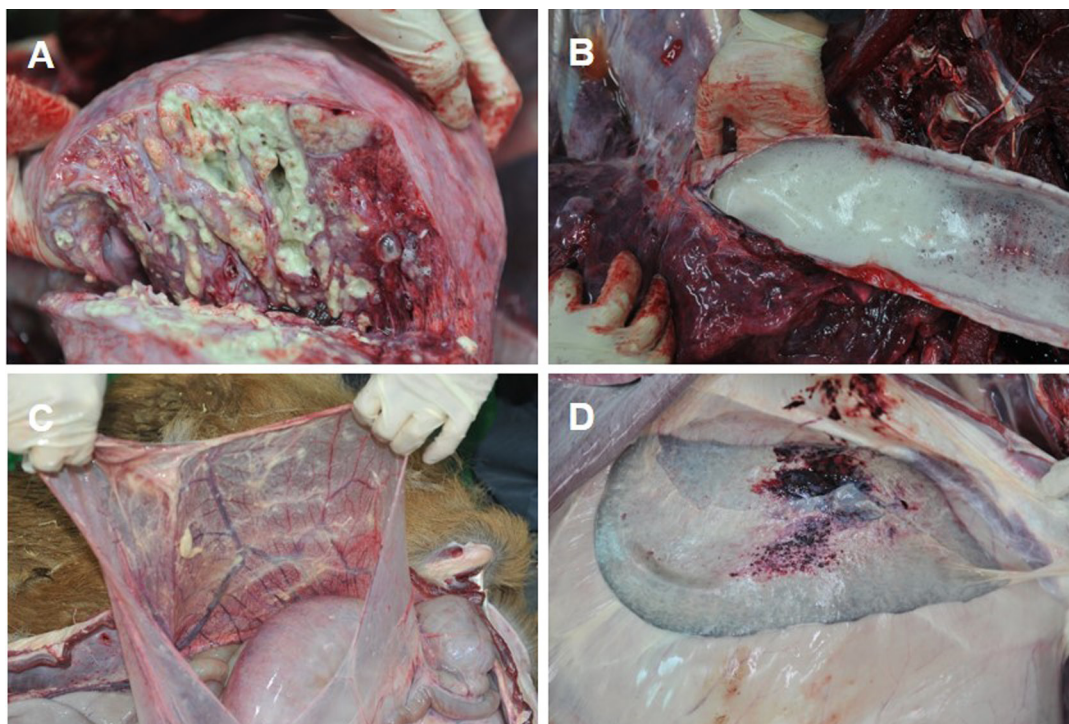


Fig. 1. Gross findings of the lung and abdominal lesions. A) Cut surface of the lung showing abscesses of variable size containing yellowish curd-like material. B) Foamy exudates in trachea. C) White fibrinous material attached to the peritoneum (greater omentum). D) Hemorrhages on the surface of spleen.

Case 1: In December 2013, an 18-year-old male elk had anorexia with slowed down behavior. He could not stand and showed clinical signs only one day before death. The animal was treated with dexamethasone (0.25 mg/kg, Dexorone; Handong Pharmacy, Seoul, Korea) and 5% dextrose (5% dextrose injection; Samyang Anipharm, Seoul, Korea) delivered at 100–150 ml/hr via intramuscular and intravenous injections, respectively. The next day, however, the animal died despite supportive fluid therapy. At necropsy, severe pulmonary abscesses were found bilaterally in the lung lobes together with congestion which was more severe in the caudal lobes. The abscesses showed 5–10 cm multiple cystic shapes containing yellowish curd-like materials variable in size at the cut surface (Fig. 1A). In addition, there was pneumonic congestion generally which was more severe in the caudal than cranial lobes. Especially, the caudal lobes were covered with inflammatory milky colored membranes and bilateral coalescence of cranial and caudal pulmonary lobes. The trachea had foamy exudates (Fig. 1B) and hydrothorax and ascites were also observed. The greater omentum presented inflammation and hemorrhagic signs (Fig. 1C). Microscopically, alveolar spaces were filled with fibrin exudates and hemorrhage, accompanied by macrophage and neutrophil infiltration (Fig. 2).

Case 2: In March 2015, an 8-year-old male elk had anorexia with occasional depression for 2 weeks before death. However, the animal died suddenly. At necropsy, severe pulmonary abscesses were found bilaterally in the lung lobes together with congestion which was more severe in the caudal lobes. Similar to case 1, the caudal lobes were covered with inflammatory milky colored membranes. Trachea was filled with foamy exudates (Fig. 1B) and hemorrhages were observed on the surface of spleen (Fig. 1D). Microscopically, alveolar spaces were filled with fibrin exudates and hemorrhage, accompanied by macrophage and neutrophil infiltration.

Case 3: In September 2015, a 14-year-old male common eland had anorexia for three days before death. However, the animal died suddenly. At necropsy, severe pulmonary abscesses were found bilaterally in the lung lobes together with congestion which was more severe in the caudal lobes. The caudal lobes were covered with inflammatory pseudo-membranes and bilateral coalescence of cranial and caudal pulmonary lobes. The cut surfaces had various sizes of cysts (Fig. 1A). The trachea was filled with foamy exudates (Fig. 1B). Hemorrhages were observed on the surface of spleen (Fig. 1D). Other symptoms, including hydrothorax and ascites, were also detected. Microscopically, fibrosis and infiltration of neutrophils were observed in the lung.

Since bacteremia were suspected in all three cases, the lung tissue, pleural effusion fluids, and ascites were smeared directly and the cocobacillic or rod-shaped bacteria with tinted violet were detected by Giemsa staining (Fig. 3). When the lung tissue and hemorrhagic pleural effusion fluids were pure cultured, the bacteria were identified as *M. lacunata* using a biochemical API 20NE identification kit. For differential diagnosis, the lung tissues were smeared and tested for tuberculosis using acid-fast staining and Lowenstein-Jensen medium (BBL) cultivation [8], but the results were negative. In an antimicrobial susceptibility test, *M. lacunata* was resistant to bacitracin, novobiocin, and vancomycin but sensitive to the others that were tested.

In human, *M. lacunata* is normally found in the oral cavity, cranial respiratory tract, and conjunctiva [9, 11, 14]. It usually causes

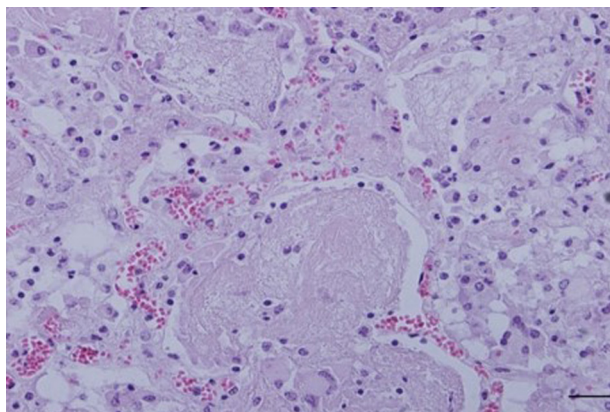


Fig. 2. Histopathological finding of pulmonary abscess. Infiltration of neutrophils and macrophages with hemorrhage and fibrin exudates in alveolar spaces. Magnification at $\times 400$. Hematoxylin and eosin staining.

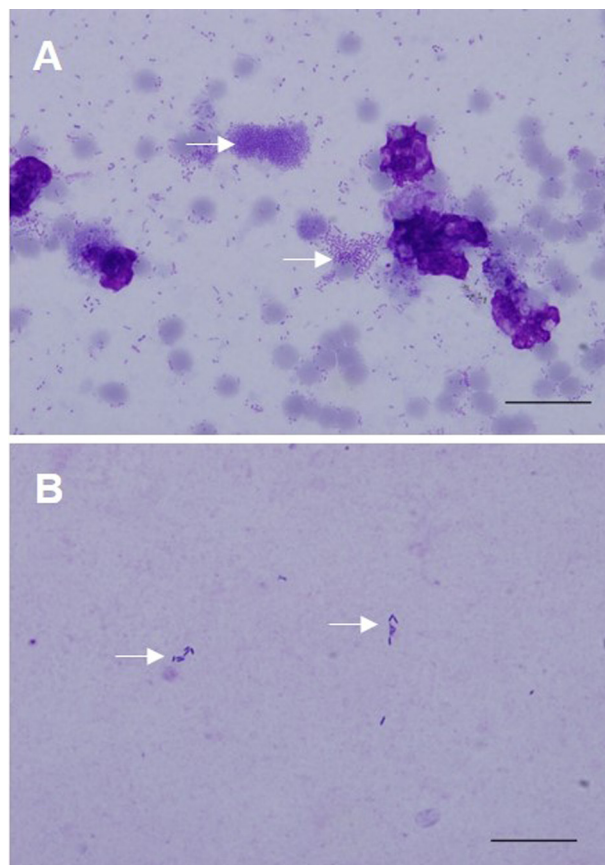


Fig. 3. Direct smears of (A) lung lesion and (B) hemorrhagic pleural effusion fluid. A) Coccobacilli (white arrows) on a smear sample of the lung lesion. B) Rod-shaped organisms (white arrows) on a smear sample of pleural fluid. Magnification at $\times 1,000$. Giemsa staining.

otitis, sinusitis, conjunctivitis, and pharyngitis in children and has been reported to cause keratitis, meningoencephalitis, skin and soft tissue infection, and systemic infections including endocarditis, septicemia, and septic arthritis in humans [3, 11, 13, 20, 23]. Furthermore, *Moraxella* spp. are considered one of the three major genera of bacteria causing pneumonia in children, in addition to *Haemophilus* and *Streptococcus*. Regarding animals, including giant pandas, macropods, and marine mammals, *Moraxella* spp. are associated with normal flora in dental plaque and ocular lesions [1, 10, 21]. In addition, *Moraxella* spp. have been isolated from dog and cat bite lesions [19]. However, there are several reports of *Moraxella* spp. infections resulting in mucohemorrhagic rhinitis, caseous lymphadenitis, keratoconjunctivitis, and septic arthritis [6, 15, 22].

M. lacunata causing pulmonary abscesses has not previously been reported in animals. Moreover, these cases are the first reported in herbivores such as elks and the common eland. Generally, pulmonary abscesses are caused by etiologic bacteria such as *Brucella* spp., *Mycoplasma*, *Pasteurella multocida*, and parasites [2, 4, 18]. *M. lacunata* is part of the normal flora in humans and animals but is rarely isolated from clinical cases [3, 11]. Initially, *M. lacunata* was considered innocuous and non-pathogenic owing to its low virulence in healthy humans and animals [6, 17, 24]. However, there are known predisposing factors such as an immunodeficient or immunocompromised condition, immunosuppression therapy, respiratory impairment, or a severely stressed environment [17, 20, 23]. *Moraxella* spp. infection has been described in animals with infections in which the predisposing factors seemed to be a suppressed immune system, immunodeficiency drugs, and stressed conditions [1, 10, 21].

Based on the antimicrobial susceptibility test, *M. lacunata* was sensitive to most of the tested drugs such as penicillin, cephalosporin, aminoglycosides, quinolones, and tetracycline and resistant to only three drugs: bacitracin, novobiocin, and vancomycin. This result was similar to previous reports [13, 20].

In conclusion, this study describes for the first report of pulmonary abscesses due to *M. lacunata* infection, which is rare in herbivores. As seen in these cases, *M. lacunata* can cause an opportunistic infection that may result in pulmonary abscesses and asymptomatic death.

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