Journal of Zoo and Wildlife Medicine 48(1): 220–223, 2017

FATAL *ACINETOBACTER BAUMANNII* INFECTION IN THE CRITICALLY ENDANGERED EUROPEAN MINK (*MUSTELA LUTREOLA*)

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Abstract: The present study reports the first case of fatal *Acinetobacter baumannii* infection in the critically endangered European mink (*Mustela lutreola*). Gross examination revealed a severe, diffuse hemorrhagic pneumonia and generalized congestion as main features. Microscopically, the main lesions were an acute, severe fibrinous–hemorrhagic pneumonia associated with proliferation of coccobacilli and generalized acute–subacute congestion. Cultures yielded *A. baumannii*; the species was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and the strain presented a multidrug-resistant pattern. The results are not only of conservation concern but also of public health concern given *A. baumannii* is one of the most important pathogens implicated in nosocomial infections in humans.

Key words: *Acinetobacter baumannii*, European mink, *Mustela lutreola*, pneumonia, public health

BRIEF COMMUNICATION

In October 2013, a 7-yr-old, male captive-bred European mink (*Mustela lutreola*) was shipped to the Cordoba Municipal Zoo Park (CMZP) (southern Spain) for captive breeding within the European ex situ conservation program. In February 2014, the animal was admitted at the CMZP hospital with clinical signs of severe tachycardia (230–260 beats/min), tachypnea with increased breath sounds, depression, lethargy, and low body temperature (36.5–37.8°C). Radiographs demonstrated a widespread increase in radiodensity, mostly in the cranial and middle lobes of the right lung, and bronchoalveolar pattern of the apical regions (Fig. 1). Plasma biochemistry values revealed a marked leukopenia (white blood cell count 1.07×10^9/L).

The mink was treated with a combination of marbofloxacin (2 mg/kg body weight [BW]), amoxicillin–clavulanic acid (15 mg/kg BW), furosemide (4 mg/kg BW), and aminophylline (5 mg/kg BW). Intraosseous fluid therapy (lactated Ringer solution) and oxygen were also administered. Despite treatment the mink died 5 hr after admission. At necropsy, the animal was in good body condition and weighed 1.16 kg. Gross examination revealed a severe, bilateral, acute hemorrhagic pneumonia, slight reddish pleural effusion, acute–subacute congestion of major organs, and moderate splenomegaly. No other abnormalities were observed.

Tissue samples from lung, heart, liver, spleen, kidney, urinary bladder, esophagus, stomach, intestine, pancreas, and adrenal cortex were fixed in 10% neutral buffered formalin, routinely processed, and stained with hematoxylin and eosin, periodic acid–Schiff, Frasser-Lendrum, and Gram stain. Histopathologic examination of the lungs revealed severe generalized acute congestion, with the alveoli and bronchioles diffusely filled with edema and moderate to abundant cellular exudates composed of neutrophils, macrophages, erythrocytes, and detached pneumocytes. Numerous gram-negative coccobacilli were observed admixed with the edema, cell debris, and proteinaceous material. Macrophages frequently contained intracytoplasmic coccobacilli (Fig. 2a, b). In the spleen the red pulp showed acute congestion, few venous microthrombi in vessels, and occasional megakaryocytes. In the myocardium, acute congestion, interfibrillar edema, and extensive myofibril coagulation was observed. The liver presented centrilobular congestion and the kidneys showed congestion and multiple microhemorrhages in the corticomedullary junction. Acute congestion and microhemorrhages were

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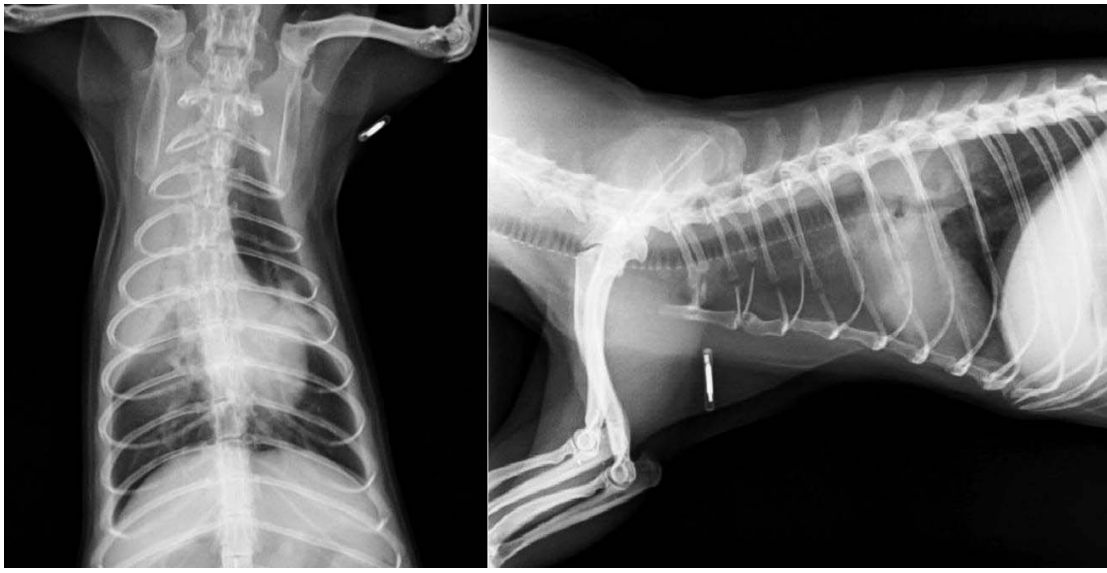

Figure 1. Ventrodorsal (left) and laterolateral (right) projections of the thoracic cavity by radiographic analysis.

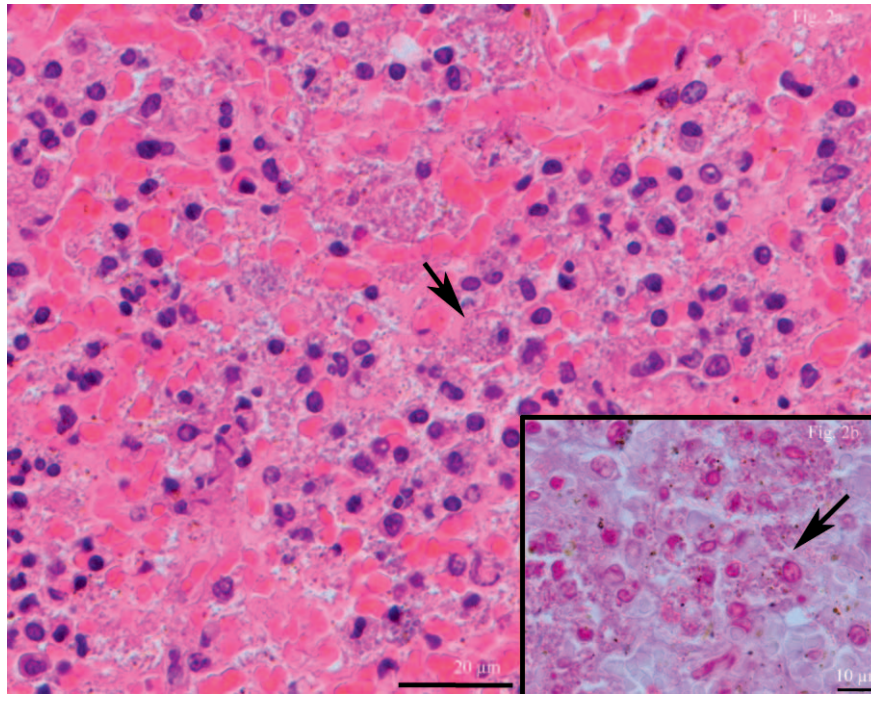

Figure 2. **a.** Lung. At high magnification the alveoli are filled with macrophages and neutrophils, as well as erythrocytes; numerous coccobacilli appear in the cytoplasm of large macrophages (arrow). The alveolar capillaries are congested. Hematoxylin and eosin, ×40. **b.** Lung. Numerous gram-negative coccobacilli are present in the cytoplasm of macrophages in the alveolar lumina (arrow). Gram stain, ×100.

found in the adrenal cortex. Congestion was also observed in the esophagus, stomach, intestine, and pancreas. A morphologic diagnosis of acute, severe fibrinous–hemorrhagic pneumonia with intralesional coccobacilli and acute septic shock was made.

The indirect immunofluorescent antibody test yielded negative results (titers $<1:20$) for canine distemper virus. Thus, the acute character of clinical signs and the normal values of gamma globulin (11.7% of total protein) using protein electrophoresis indicated absence of Aleutian disease infection.[2] Lung and kidney samples were subjected to bacteriology analyses and cultured individually by using standard procedures. Samples were directly cultured on blood agar base supplemented with 5% sterile defibrinated sheep blood (Oxoid ltd, RG24 8PW, Basingstoke, Hampshire, England). Pure colonies based on uniform colony morphology were obtained after 24–48 hr of aerobic incubation at 37°C from both organs. Gram staining revealed gram-negative coccobacillus. Isolates were tested using API 20NE (API CS; BioMerieux, 69280, Marcy l'Etoile, France) according to the manufacturer's recommendations. Bacterial isolates were also analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) using a Microflex LT bench top mass spectrometer (Bruker Daltonics, 28359, Bremen, Germany) as described.[11] The isolates were identified as *Acinetobacter baumannii* with a score ≥ 2.0, which indicate that the identification was reliable at the species level according to the manufacturer's criteria. The ability of MALDI-TOF to differentiate among *Acinetobacter* species has been previously demonstrated.[13] Besides, in this study, out of the 10 results provided by MALDI-TOF for every analyzed sample, *A. baumannii* was the only species detected.

Antimicrobial susceptibility of *A. baumannii* isolates was determined by the disc diffusion method[1] using a panel of 27 antimicrobial agents. The diameter of the bacterial growth inhibition was measured on the basis of clinical laboratory standards (Neo-Sensitabs potency according to CLSI 2006 and Veterinary Practise CLSI 2006). The bacterium was resistant to amoxicillin–clavulanic acid, ampicillin, cefazolin, cefotaxime, cefoxitin, ciprofloxacin, chloramphenicol, enrofloxacin, florfenicol, fosfomycin, lincomycin, metronidazole, neomycin, oxytetracycline, penicillin G, streptomycin, tetracycline, trimethoprim–sulfamethoxazole, and vancomycin. Sensitivity was found for amikacin, cefepime, ceftazidime, cotrimoxazole, gentamicin, imipenem, piperacillin–tazobactam, and tobramycin.

The results obtained in the present study indicate that the European mink died by an acute septic infection caused by *A. baumannii*. Even though fatal pneumonia associated with *A. baumannii* has been reported recently in American minks (*Neovison vison*),[10] to the authors' knowledge, this is the first report on acute mortality by *A. baumannii* infection in the critically endangered European mink. Histopathologic lesions observed in lung were in accordance with those previously reported in American minks infected with *A. baumannii*.[10]

European mink is a semiaquatic mustelid species native to Europe. Their populations have been reduced more than 90% in the last 50 yr, and currently, only isolated populations are present in Northern Spain, Western France, and Eastern Europe. The progressive disappearance of the European mink, which is considered as critically endangered,[7] has been mainly attributed to anthropogenic factors. Overexploitation, habitat destruction, watercourse quality loss, illegal hunting and trapping, introduction of the alien American mink, hybridization with European polecat (*Mustela putorius*), and diseases have been important factors in the decrease of their populations.[9] To save this species from extinction, ex situ and in situ conservation projects, which include habitat preservation, mink population monitoring, and captive breeding, have been initiated to maintain the genetic variability and produce individuals for future reintroduction efforts.

Acinetobacter baumannii is considered as one of the most important pathogens implicated in nosocomial infections in human hospitals.[5] In particular, this bacterium affects immunocompromised patients causing local or generalized septic processes. The main clinical symptoms associated with *A. baumannii* infection in humans include pneumonia and respiratory, urinary, and bloodstream infection. *Acinetobacter baumannii* infection has been also reported in animal species, mostly from intensive care units.[4–6,12] Moreover, *A. baumannii* has been also isolated in both human body and head lice, and the role of fleas as competent vectors for *A. baumannii* in American mink has been also suggested.[3,8,10] Because ectoparasites (including ticks, fleas, and lice) were not detected in the present case, this hypothesis could not be tested.

Acinetobacter baumannii is able to express a variety of mechanisms that make it frequently multidrug resistant.[14] The strain isolates in the

European mink presented a multidrug-resistant pattern being resistant to 19 out of the 27 antibiotics tested. The results are in accordance with those found in human and other species including the American mink.[10,14] Besides implications for the conservation of endangered species, *A. baumannii* is of public health concern because of its zoonotic potential. Additional studies are needed to determine the role of the European mink in the epidemiology of *A. baumannii* and its implication in the transmission to other species with which they share habitat.

Acknowledgments: The authors would like to thank the Municipal Council of Cordoba for providing technical support. The authors thank Sergio Sepúlveda Cano for reviewing the English of the manuscript.

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Received for publication 10 April 2016