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Mycoplasma ovipneumoniae Associated with Polymicrobial Pneumonia in a Free-ranging Yearling Barren Ground Caribou (*Rangifer tarandus granti*) from Alaska, USA

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ABSTRACT: *Mycoplasma ovipneumoniae* has been reported in association with respiratory disease in the wild only in members of the subfamily Caprinae of the family Bovidae. We identified *M. ovipneumoniae* in a cervid: a free-ranging barren ground caribou (*Rangifer tarandus granti*) yearling with polymicrobial bronchopneumonia.

A yearling, free-ranging, female barren ground caribou (*Rangifer tarandus granti*) from the Fortymile herd was found dead in eastern interior Alaska, US (64°30′N, 143°30′W) in May 2018. This animal, along with 49 other calves, had been helicopter-darted and radio-collared by Alaska Department of Fish and Game (Fairbanks, Alaska, USA) biologists in October 2017. The mortality signal was detected on 16 May 2018, approximately 145 km from the original capture location. The carcass was recovered and submitted on 18 May 2018 to Alaska Department of Fish and Game for necropsy.

At necropsy, the carcass weighed 34 kg, 9 kg less than expected based on its October mass of 47.6 kg and the typical 4.5 kg average over-winter loss of mass. It was mildly autolyzed and emaciated, with serous atrophy of epicardial adipose tissue. There were 26 live warble fly larvae (*Hypoderma* sp.) in the dorsal subcutis, and eight live nasal bot fly larvae (*Cephenemyia* sp.) in the oropharynx. A small amount of bloody fluid drained from the nares, and the trachea contained bloody foam. The tonsils bulged from the crypts and the retropharyngeal and mediastinal lymph nodes were enlarged and dark purple or mottled dark red and brown in coloration on capsular and cut surfaces. The lungs were diffusely edematous. The cranial and middle lung lobes were consolidated and dark red to purple; the remainder of the pulmonary parenchyma had multifocal to coalescent regions of similar discoloration (Fig. 1A). The abomasal serosa had paintbrush hemorrhages, multifocal hemorrhage was scattered throughout the pancreas, and hemorrhage surrounded the right trigeminal nerve.

Tissue samples were fixed in 10% neutral buffered formalin, routinely processed into paraffin, sectioned, stained with H&E at Wyoming State Veterinary Laboratory (Laramie, Wyoming, USA), and sent to the US Department of Agriculture, Agricultural Research Service, Animal Disease Research Unit (USDA-ARS-ADRU, Pullman, Washington, USA). Approximately 75% of examined lung tissue exhibited suppurative bronchopneumonia with intralesional coccobacilli colonies (Fig. 1B, C). A few scattered to regionally many (>1,000) thin-walled, embryonated nematode eggs and a few free larvae (*Protostrongylus* sp.) were within alveoli (Fig. 1D). Diffuse atrophy of adipose tissue was identified in the bone marrow, tongue, and heart. Moderate numbers of protozoal (*Sarcocystis* sp.) cysts were within muscle fibers of the tongue, heart, and esophagus, and adjacent to a salivary gland.

We placed lung tissue and a nasal mucosa swab into universal transport media and sent on ice to the USDA-ARS-ADRU. We extracted DNA from aliquots taken from both sample types immediately and following 1 d of 37 C culture in atmospheric oxygen, and PCR was performed using commercial kits (QIAamp DNA Mini Kit and QIAGEN Multiplex PCR Kit; QIAGEN, Germantown,

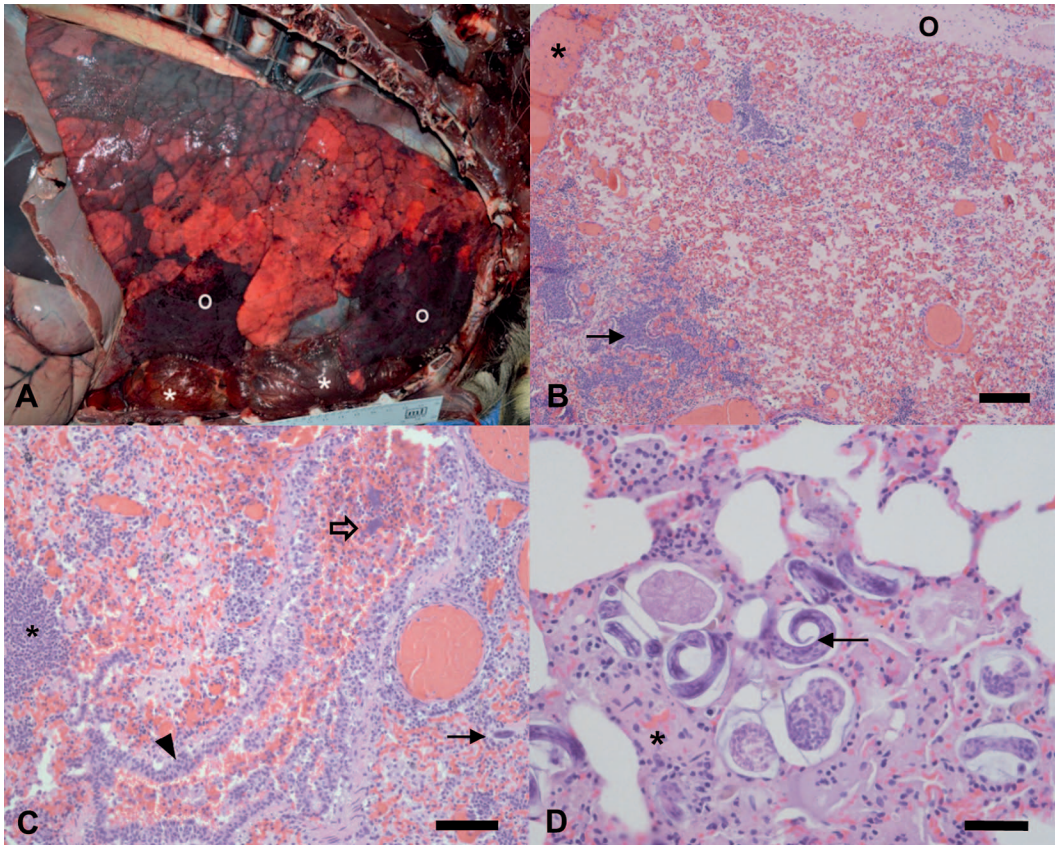

Figure 1. Gross and histopathologic findings from a yearling barren ground caribou *Rangifer tarandus granti*) found dead on 16 May 2018 in eastern interior Alaska, USA. (A) Gross image of the opened thorax illustrating consolidated and discolored dark red to purple cranial and middle lung lobes (o) and multifocal to coalescent regions of similar discoloration across the remainder of the pulmonary pleura. Pulmonary lobules are accentuated by septa that are expanded by edema and hemorrhage. Mediastinal lymph nodes are markedly enlarged and mottled dark red and brown (*). (B) The lungs are congested, with hemorrhage expanding the pleura (*), and edema expanding interlobular septa (o). Bronchioles are filled with numerous inflammatory cells (arrow) and alveoli are filled with edema and varying amounts of hemorrhage. H&E. Bar=200 μm. (C) Bronchioles are occasionally lined by mildly hyperplastic epithelium (arrowhead). Bronchioles and alveoli contain numerous degenerate neutrophils, fewer macrophages, edema, fibrin, hemorrhage, cellular debris, and small coccobacilli colonies (open arrow). Inflammation obliterates bronchioles (*) and necrosis and hemorrhage efface the parenchyma of the most severely affected areas. Free nematode larvae are occasionally noted within the most severely affected regions of the lungs (arrow). H&E. Bar=100 μm. (D) Alveoli contain many embryonated nematode eggs and free larvae having a pointed tail, consistent with *Protostrongylus* sp. (arrow). Alveolar walls surrounding the nematode eggs and larvae are thickened by fibrosis (*); acute inflammation was not centered on the nematodes. H&E. Bar=50 μm.

Maryland, USA). Partial *16S* ribosomal (r)RNA and *16S-23S* ribosomal RNA intergenic sequence (IGS) PCR assays and sequencing were performed to test for *Mycoplasma ovipneumoniae*. For the *16S* rRNA gene, published forward (LMF; McAuliffe et al. 2003) and reverse (MGSO; van Kuppeveld et al. 1992) primers were each used at 0.5 μM final concentration with the following cycler conditions: 15 min denaturation followed by 35 cycles of 95 C denaturation for 60 s, 58 C annealing for 90 s, and 72 C extension for 90 s, and a final 5 min extension followed by a 4 C hold. For the IGS region, a published PCR protocol (Besser et al. 2012) was used with modifications to the cycler conditions: 15 min

denaturation followed by 35 cycles of 95 C denaturation for 30 s, 55 C annealing for 30 s, and 72 C extension for 60 s, and a final 2 min extension followed by a 4 C hold. Amplicons of correct size were Sanger sequenced by Erofins Genomics (Louisville, Kentucky, USA). Forward and reverse sequences from all samples (nasal and lung, pre- and post-culture) were merged, manually inspected for errors, and trimmed using Sequencher® 5.2.2 software (Gene Codes, Ann Arbor, Michigan, USA). The *16S* rRNA gene sequence was trimmed to 919 base pairs (bp) corresponding to base range 75–993 of *M. ovipneumoniae* type strain Y98 (GenBank no. NR_025989.1), and the IGS sequence was trimmed to 457 base pairs. Each of the four merged and trimmed sequences were identical to one another at both genomic regions. A BLASTN (National Center for Biotechnology Information 2018) query identified the partial *16S* rRNA and IGS sequences to have the highest coverage and identity to *M. ovipneumoniae* Y98, with 100% coverage for both sequences, and 99% and 97% identity for *16S* rRNA and IGS sequences, respectively. Sequences were submitted 2 August 2018 to NCBI and assigned accession numbers MH707327 and MH707328.

Aerobic culture on fresh lung tissue, performed at Washington Animal Disease Diagnostic Laboratory (Pullman, Washington, USA), identified many *Pasteurella multocida* and *Mannheimia granulomatis*; leukotoxin A was not detected by PCR in lung tissue or *Pasteurellaceae* isolates. The presence of *M. ovipneumoniae* in fresh lung tissue was confirmed by Washington Animal Disease Diagnostic Laboratory by *Mycoplasma* spp. (universal) PCR and sequencing and *M. ovipneumoniae*-specific real-time PCR. Tonsillar tissue and tissue from an ear notch, sent to Wyoming State Veterinary Laboratory, were negative on PCR for cervid adenovirus and bovine viral diarrhea virus, respectively.

Although several reports exist of *M. ovipneumoniae* carriage and infection in species outside of the subfamily Caprinae, including cattle (*Bos taurus*), beira antelope (*Dorcatragus megalotis*), and multiple members of the subfamily Capreolinae including caribou (Wolfe et al. 2010; Gull et al. 2014; Highland et al. 2018), this is the first report of detection associated with pneumonia in a caribou or any wildlife species in Alaska. The role of *M. ovipneumoniae* in polymicrobial pneumonia described in this report is unclear, considering the poor nutritional condition, co-infections, and parasitic burden observed at necropsy. Although a few bronchioles had evidence of epithelial hyperplasia (Fig. 1C), distinct histologic changes typical of *M. ovipneumoniae* infection, including hyperplasia of bronchoalveolar lymphoid tissue and bronchiolar epithelium, were not obvious. Regardless, identification of *M. ovipneumoniae*-associated polymicrobial pneumonia was significant, because *M. ovipneumoniae* has been implicated as a primary causative agent of bighorn sheep (*Ovis canadensis*) epizootic polymicrobial pneumonia in western North America (Besser et al. 2013). The role of *M. ovipneumoniae* in caribou mortality and the impact of this pathogen on Alaskan caribou and other wildlife is unknown. Research is currently underway to understand the host range, distribution, and impact of *M. ovipneumoniae* in Alaska.

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