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BRIEF COMMUNICATION

Babesia odocoilei infection in a North American elk (*Cervus elaphus canadensis*)

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Abstract A 13-year-old, male, North American elk (*Cervus elaphus canadensis*) from a commercial herd was presented for a sudden onset of lethargy, anorexia, and hematuria to Palmer Veterinary Clinic, Plattsburgh, NY, USA. The elk, originated from Ohio, was from a herd of 60 animals with an age range of 2 months to 13 years. Clinically, over a 5–7-day period, the elk began to distance itself from the remainder of the herd, and became anorectic and eventually recumbent. Results of the admitting complete blood count indicated a normocytic, normochromic, nonregenerative anemia. The animal was serologically negative for elk wasting disease. Supportive treatment did not result in clinical improvement. The elk was euthanized because of the rapidly declining condition. At necropsy, the spleen was diffusely enlarged and the mesenteric and periaortic fats were yellow. Microscopically, the only finding of note was extensive splenic erythrophagocytosis. Microscopic examination of a peripheral blood film stained with Wright–Giemsa revealed that about 5% of erythrocytes contained protozoal parasites. The morphologic characteristics of these organisms were consistent with *Babesia* organism, most likely *Babesia odocoilei*. Using a nested polymerase chain reaction, the organism was identified as *B. odocoilei*. To the authors' knowledge, this is the first report of *B. odocoilei* infection in North American elk in New York State, USA. We recommend that infection with *B. odocoilei* be considered in any case of acute hemolytic anemia in elk.

Keywords *Babesia odocoilei* · Babesiosis · Elk

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Case presentation

A 13-year-old, male, North American elk (*Cervus elaphus canadensis*) from a commercial herd was presented for a sudden onset of lethargy and hematuria to Palmer Veterinary Clinic, Plattsburgh, NY, USA. The elk, originated from Ohio, was from a herd of 60 elks with an age range of 2 months to 13 years. Clinically, over a 5–7-day period, the elk began to distance itself from the remainder of the herd, and became anorectic and eventually recumbent. Results of the admitting complete blood count indicated a normocytic, normochromic, nonregenerative anemia (hematocrit 29%, reference interval 37–55%). The serum clinical chemistry data revealed hypoalbuminemia (2.1 g/dL, reference interval 2.5–3.5 g/dL), hypocalcemia (7.1 mg/dL, reference interval 8.0–12.0 mg/dL), hypophosphatemia (2.7 mg/dL, reference interval 4.0–8.6 mg/dL), hyperbilirubinemia (4.5 mg/dL, reference interval 0.0–0.7 mg/dL), and increased urea nitrogen (BUN) concentration (45 mg/dL, reference interval 10–25 mg/dL). The animal was serologically negative for elk wasting disease. Supportive treatment (fluids, electrolytes, and antibiotics) did not result in clinical improvement, and the elk was euthanized because of the rapidly declining condition.

At necropsy, the spleen was diffusely enlarged and the mesenteric and periaortic fat were yellow. Tissue samples collected during necropsy were fixed in buffered formalin and embedded in paraffin. Tissue sections were cut at 4 mm and stained with Harris hematoxylin and eosin Y (Fisher Scientific, Pittsburg, PA, USA). On histologic examination, the only finding of note was extensive splenic erythrophagocytosis.

Microscopic examination of a peripheral blood film stained with Wright–Giemsa was performed. Erythrocyte density appeared mildly decreased, suggestive of anemia. Erythrocyte features included moderate anisocytosis, and few echinocytes, schistocytes, and keratocytes. Occasional polychromasia, Howell–Jolly bodies, and basophilic stippling were noted. About 5% of erythrocytes contained protozoal parasites. The organism was pleomorphic and exhibited linear, amoeboid, paired pyriform, and signet ring form measuring 1–2× 3–4 μm (Fig. 1). Rare extracellular organisms were seen free within the background. The morphologic characteristics of these organisms were consistent with *Babesia* organism, most likely *Babesia odocoilei*. Microscopic examination of stained blood films from other animals in the same herd was negative for *Babesia* spp. and other hemoparasites.

Blood collected in EDTA-containing tubes were sent to the Department of Veterinary Pathobiology, Texas A&M University (College Station, TX) for molecular confirmation of the identity of the organism. Using a nested polymerase chain reaction (PCR, Ramos et al. 2010), the organism was identified as *B. odocoilei*.

Discussion

B. odocoilei (phylum Apicomplexa, order Piroplasmorida, family Babesiidae) is a tick-borne, intraerythrocytic parasite that is known to infect cervids. *B. odocoilei* causes acute, often fatal babesiosis in elk (*C. elaphus canadensis*), reindeer (*Rangifer tarandus tarandus*), and caribou (*R. tarandus caribou*) (Holman et al. 2003, 2000). *B. odocoilei* is mostly transmitted by *Ixodes* spp. especially *Ixodes scapularis* and *Ixodes dammini* (Armstrong et al. 1998; Keirans et al. 1996; Waldrup et al. 1990). The organism was first isolated from white-tailed deer (*Odocoileus virginianus*) in Texas (Emerson and Wright 1968). The first report of naturally acquired acute fatal babesiosis in a North American elk (*C. elaphus canadensis*) caused by an organism morphologically similar to *B. odocoilei*, which later genetically confirmed to be this species, was described in a farmed herd in Texas, USA (Holman et al. 2000, 1994). Outbreaks in farmed herds have been reported in several states in the USA including Indiana, Minnesota, Texas, and Wisconsin (Gallatin et al. 2003; Holman et al. 2003, 2000). Serologic prevalence rates of up to 100% have been reported in elk herds in New Hampshire, USA (Schoelkopf et al. 2005).

Because of the similarity in clinical signs, infection with *B. odocoilei* may be misdiagnosed as immune-mediated hemolytic anemia or other hemolytic anemias. Some infected animals may not show any clinical signs of disease. It has been suggested that subclinical babesiosis in elk may

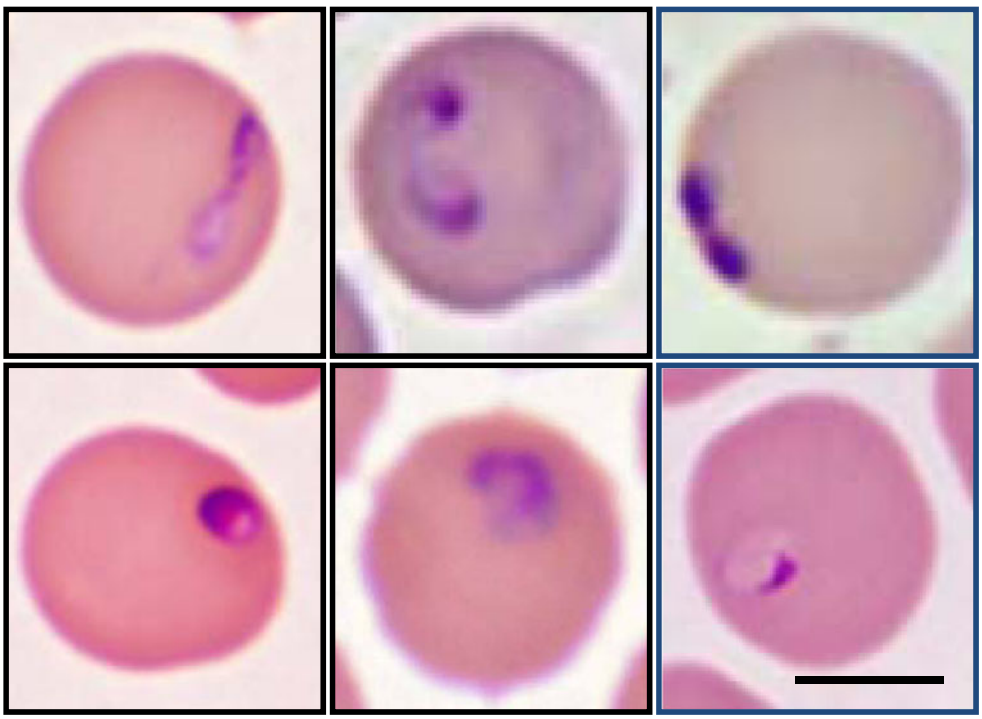

Fig. 1 Peripheral blood smear from a North American elk infected with *B. odocoilei*. Note the paired pyriform and signet ring form within erythrocytes; Wright–Giemsa stain, *scale bar* 5 μm

be due to variations in susceptibility of individual animal, tick burden, number of infecting organisms, and level of stress-induced immunosuppression (Gallatin et al. 2003).

Identification of the organisms in circulating erythrocytes is essential to avoid misdiagnosis and to obtain a definitive diagnosis of *B. odocoilei* infection. The diagnosis is usually confirmed by visualization of intraerythrocytic parasites in freshly prepared thin blood smears stained with Romanowsky-type stains such as Wright or Giemsa. In subclinical babesiosis, molecular biologic methods such as PCR assays and speciation of the organism by sequencing a hypervariable region of the 18S rRNA gene can be employed for detecting *B. odocoilei* (Schoelkopf et al. 2005; Holman et al. 2003, 2000). Immunodiagnostic assays such as immunofluorescent antibody test for detecting anti-*B. odocoilei* antibody are also useful tools (Schoelkopf et al. 2005; Holman et al. 2000; Goff et al. 1993; Waldrup et al. 1989). Electron microscopy has been also used to characterize this pathogen. *B. odocoilei* is characterized by its close proximity to the erythrocyte membrane, membranous structures resembling feeding organelles, and reproduction via a method resembling budding sensu stricto (Droleskey et al. 1993).

Although characteristic leukogram changes are not associated with natural or experimental infection with *B. odocoilei*, inflammation has been reported in some animals (Gallatin et al. 2003). There is no clinical chemistry changes characteristic of *B. odocoilei* infection. Hypoalbuminemia, hypocalcemia, and hypophosphatemia present in this case are probably due to decreased dietary intake. Hypocalcemia may have been also in part because of decreases in the protein-bound blood calcium fraction secondary to the low serum concentration of albumin. Increased BUN concentration may have been due to dehydration as no other findings were present to support renal damage. Hyperbilirubinemia in the absence of liver pathology was most likely due to extravascular hemolysis.

The elk in this report originated from Ohio; however, it is unknown whether the animal was infected in New York or in Ohio. Although *B. odocoilei* has been recently identified in captive reindeers at a New York zoological institution (Bartlett et al. 2009), to the authors' knowledge, *B. odocoilei* infection in North American elk in New York State, USA has not been previously reported.

B. odocoilei infection may result in hemolytic anemia and anorexia and may cause significant mortality among free-ranging animals. Therefore, captive cervids should receive tick prevention, be tested for subclinical infections in endemic areas, and receive aggressive treatment for acute infections when clinical babesiosis is suspected (Bartlett et al. 2009). Furthermore, effective animal management practices including appropriate tick control measures should be implemented to prevent transmission of this tick-borne pathogen to susceptible animals (Schoelkopf et al. 2005). Supportive care and antiprotozoal drugs such as imidocarb dipropionate (Imizol, Schering-Plough Animal Health, Union, New Jersey, USA) have been used to successfully treat *B. odocoilei* infection in elk (Gallatin et al. 2003) and reindeer (Bartlett et al. 2009). We recommend that infection with *B. odocoilei* should be considered in any case of acute hemolytic anemia in elk.

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