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Ehrlichiosis in a Moose Calf in Norway

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ABSTRACT: A case of granulocytic ehrlichiosis in a moose calf (*Alces alces*) in Norway is described. The animal was heavily infested with ticks (*Ixodes ricinus*), and died from a *Klebsiella pneumoniae* septicemia. Examination of blood smears from the calf revealed cytoplasmic inclusions (morulae) typical of infection with *Ehrlichia phagocytophila* in the granulocytes. *Ehrlichia* sp. was detected by polymerase chain reaction (PCR) in blood from the calf, and in the ticks. Sequence determination identified it as *E. phagocytophila*. This is the first report of ehrlichiosis in moose.

Key words: *Alces alces*, case report, *Ehrlichia phagocytophila*, ehrlichiosis, moose, tick-borne fever.

In July 1999 a sick female moose calf (*Alces alces*) was found in the area of Marka (9°48'E, 59°8'N; Porsgrunn municipality, southern Norway) by a local farmer. The animal displayed apathy and paralysis of the hind quarters which the farmer found reminiscent of tick-borne fever, as occurring in his sheep flock. This prompted him to bring it to the laboratory at A/S Telelab. On examination the calf was moribund, and heavily infested with ticks (*Ixodes ricinus*); estimated age was 6 to 8 wk; rectal temperature was 39.5 C. Ticks were collected and citrated blood was taken from the jugular vein of the calf. The animal died 2 hr after examination and was necropsied.

Blood smears from the calf were prepared and stained with May-Grünwald Giemsa stain (Dacie and Lewis, 1991) for microscopical examination. Blood from the calf, and from five engorged ticks, was examined by polymerase chain reaction (PCR), using generic *Ehrlichia* primers PER1 and PER2 (Goodman et al., 1996). Primers were from Cybergene (Huddinge, Sweden); Amplitaq Gold DNA polymerase

was from PE-Biosystems (Foster City, California, USA); and deoxynucleotide triphosphates were from Applied Biosystems (Epsom, UK). The same samples also were tested for generic *Borrelia*, using species-specific primers for *B. afzelii*, *B. garinii*, and *B. burgdorferi*. Primers were supplied by Cybergene. The PCR method was based on that described by Demaerschalck et al. (1995), with the following modifications: PCR, using primers GI, GII and GIII, was performed as a multiplex PCR reaction using an annealing temperature of 65 C; the number of amplification cycles used was extended to 40; the concentration of MgCl₂ was 2.0 mM. The reaction was performed as a hot start PCR using 1.5 units of Amplitaq Gold per reaction, and the reaction was first incubated at 93 C for 10 min to activate the enzyme. Species determination was performed using a reverse line probe assay and sequencing of a portion of the 16S rRNA gene (bp. 28–455) which differentiates all known variants of the *E. phagocytophila* genogroup (Schouls et al., 1999). At necropsy, samples of the brain, spinal cord, skeletal muscles, lungs, heart, liver, kidneys, adrenal glands, spleen and urinary bladder were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin for histological examination (Culling et al., 1985). Samples of the lungs, liver and bladder urine were seeded on calf blood agar plates (Atlas, 1993) for bacteriological examination.

Examination of the blood smears revealed the presence of cytoplasmic inclusions (morulae) typical of *Ehrlichia phagocytophila*, in 3 to 4% of the neutrophilic granulocytes (data not shown). Polymerase

chain reaction revealed the presence of *Ehrlichia* sp. in the calf's blood, and in the blood from the ticks. The presence of a 16S rRNA sequence identical to that of the *E. phagocytophila* prototype sequence could be demonstrated both by line probe assay and sequencing techniques (Schouls et al., 1999). *Borrelia afzelii* was found in the ticks, but the calf's blood was negative for *Borrelia* sp.

Gross examination revealed a pale, emaciated carcass weighing 36.5 kg. The animal showed a bilateral, incomplete cataract, and lung edema. Numerous hemorrhages were present in the dura mater of the brain, on the epicardium and adrenal glands, and in the walls of the ureters. The urine was turbid and reddish. The pericardial sack contained yellowish, serous fluid mixed with sheets of fibrin, and the liver, spleen, and kidneys were swollen. Histologically, there were focal hemorrhages and necroses and aggregates of rod-shaped bacteria in the organs. Bacteriological examination revealed *Klebsiella pneumoniae* in pure culture from the lungs and liver, whereas both *K. pneumoniae* and *Staphylococcus aureus* were isolated from the urine.

The present study demonstrates *E. phagocytophila* in a moose calf that died from *K. pneumoniae* septicemia. *Ehrlichia phagocytophila* (tick-borne fever) is endemic in sheep and cattle in southern Norway where the vector (*Ixodes ricinus*) is abundant, and heavy losses may occur in lambs (Stuen, 1996a). In sheep, tick-borne fever produces profound effects on the immunological defence system, which increases susceptibility to disease and mortality from intercurrent infections (Larsen et al., 1994). Such secondary infections may cause pyemia or septicemia in lambs (Brodie et al., 1986; Stuen, 1996a). *Klebsiella* sp. are regarded as opportunistic pathogens in animals (Henton, 1994). We conclude that this moose calf died from a *K. pneumoniae* septicemia that may have been secondary to, and facilitated by, a primary infection with *E. phagocytophila*.

To our knowledge, this is the first reported case of *Ehrlichia* sp. in moose. Granulocytic *Ehrlichia* sp. have been reported in mule deer (*Odocoileus hemionus hemionus*), black-tailed deer (*Odocoileus hemionus columbianus*), elk (*Cervus elaphus nannodes*) and white-tailed deer (*Odocoileus virginianus*) in the USA (Foley et al., 1998; Belongia et al., 1997). Tick-borne fever has been induced in sheep by inoculation with blood from roe deer (*Capreolus capreolus thottii*), fallow deer (*Dama dama*) and red deer (*Cervus elaphus scoticus*) in England (Foggie, 1962; McDiarmid, 1965). However, there is but one report of clinical ehrlichiosis in a cervid (Stuen, 1996b). In this study, three reindeer (*Rangifer tarandus tarandus*) experimentally infected with *E. phagocytophila* developed parasitemia, fever, and neutropenia; one animal died during the experiment. To what extent wild ruminants may function as a reservoir for *E. phagocytophila* in domestic animals remains unanswered.

This work would not have been possible without the initiative and determination of the farmer B. Jacobsen who carried the sick animal 2 km through rough country on a hot summer day to bring it to the laboratory for study.

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