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Granulocytic Ehrlichiosis in a Roe Deer Calf in Norway

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ABSTRACT: A case of granulocytic ehrlichiosis is described in a roe deer (*Capreolus capreolus*) calf from Norway. The calf was heavily infested with *Ixodes ricinus* and died from *Escherichia coli* septicemia. Granulocytic *Ehrlichia* sp. was detected by polymerase chain reaction (PCR) from several organs and sequence determination identified a variant of human granulocytic ehrlichiosis (HGE) agent. This is the first report of a possible clinical granulocytic *Ehrlichia* sp. infection in a roe deer.

Key words: *Capreolus capreolus*, case report, *Ehrlichia* sp. granulocytic ehrlichiosis, HGE, roe deer.

Tick-borne fever (TBF), caused by *Ehrlichia phagocytophila*, has been considered for decades as a common disease in domestic ruminants along the coast of southern Norway (Øverås, 1972; Stuen, 1998). In the present paper, a case of possible clinical granulocytic ehrlichae infection in a roe deer (*Capreolus capreolus*) calf is described.

The calf and its twin were born on the island Ådnøy, on the southwest coast of Norway. The sheep tick *Ixodes ricinus*, is abundant on this island. Roe deer calves are normally born in late May to early June in Norway (Østbye and Bjørnsen, 1990). The calf had been observed for several weeks together with its sibling and mother. On July 18 the calf had lost its normal fright and flight response and was observed from a close distance. Many ticks were seen on its head and the calf was lame in one leg. The calf was found dead 3 days later and examined post mortem. The estimated age of the calf was 7 to 8 wk.

A routine necropsy including aerobic bacterial cultivation was conducted. Tissue samples were fixed in 10% neutral buffered formalin and processed for histopathology. Five to 10 g of spleen, lung, brain, kidney, and liver tissue were frozen at −20 C for polymerase chain reaction (PCR) analysis. The PCR analysis for detection of DNA from granulocytic ehrlichae was performed according to Olsson Engvall et al. (1996), with some modifications (Stuen and Olsson Engvall, 1999).

The calf weighed 4.3 kg, and showed signs of putrefaction. More than 200 *I. ricinus*, mainly nymphs and larvae, were found especially on the head and neck. The calf was in normal body condition, but showed signs of dehydration. Petechial hemorrhages were found in the lungs. There were no signs of diarrhea. The spleen was dark and swollen with subcapsular petechiae. Histopathology showed petechiae in various organs. Pulmonary lesions indicated a subacute to chronic interstitial pneumonia, with septal edema and cellular infiltration in the interalveolar septa by both mononuclear and neutrophil inflammatory cells. In some areas the alveolar lumen was collapsed. Alveolar macrophages were abundant. Pleomorphic intracellular structures similar to *E. phagocytophila* inclusions were seen in alveolar epithelial cells and also in alveolar macrophages. However, due to putrefaction, it was difficult to characterize these structures further, especially since immunohistochemistry was not available. The pulmonary findings were similiar to those observed in lambs experimentally infected with *E. phagocytophila* (Munroe et al., 1982; Campbell et al., 1994).

All tissues examined were positive by PCR. Positive PCR analyses performed on samples from different internal organs indicated a systemic infection with granulocytic ehrlichae (Olsson Engvall et al., 1996; Stuen and Olsson Engvall, 1999). In

order to determine the *Ehrlichia* sp. present, reverse line hybridization and sequencing of a portion of the 16S rRNA gene (bp. 28-455) were performed (Schouls et al., 1999). Both tests identified the presence of a 16S rRNA sequence identical to that of a variant of the human granulocytic ehrlichiosis (HGE) agent (GenBank accession number AJ242783). The HGE agent (accession number U02521) and its variant both have 16S rRNA gene sequences very similar to that of *E. phagocytophila* (Schouls et al., 1999). A strain of *Escherichia coli* in pure culture was cultivated from the heart, lungs, kidney, brain, and spleen. No further characterization of this strain was performed.

These results show that roe deer in Norway can be infected with granulocytic *Ehrlichia* sp. Healthy roe deer in UK has previously been found infected with granulocytic *Ehrlichia* sp. (McDiarmid, 1965), and a recent PCR investigation from the UK indicates that roe deer could be natural hosts for *E. phagocytophila* (Alberdi et al., 2000). Clinical ehrlichiosis in reindeer (*Rangifer tarandus tarandus*) has been demonstrated following experimental infection with *E. phagocytophila* (Stuen, 1996a). However, to the authors' knowledge, the present case may be the first clinical case of this infection described in a roe deer.

Escherichia coli septicemia was diagnosed in the calf, which has previously been reported as a systematic bacterial infection in roe deer in Sweden (Aguirre et al., 1999). However, in the present study, the calf was infected with both *E. coli* and granulocytic ehrlichae. A similar double infection has earlier been observed in Norway in a moose calf, which died of a *Klebsiella pneumonia* septicemia together with an *E. phagocytophila* infection (Jenkins et al., 2001). Both *E. coli* and *K. pneumonia* are opportunistic pathogens (Krieg and Holt, 1984), and the question arises whether infection with these bacterial species should be regarded as secondary due to immunosuppression caused by a primary granulocytic ehrlichae infection. The main consequence of an *E. phagocytophila* infection in sheep is the ensuing immunosuppression (Larsen et al., 1994) that leads to secondary infections, such as *Staphylococcus aureus* pyemia (Brodie et al., 1986) and *Pasteurella haemolytica* (*trehalosi*) septicemia (Stuen, 1996b). Opportunistic infections in HGE patients indicate that this infection also may cause host defense impairments in humans (Dumler et al., 1999). However, further investigations are needed to clarify whether granulocytic ehrlichae may cause serious immunosuppression and accordingly represent a health problem in wild ruminants.

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