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A Paretic Condition in an *Anaplasma phagocytophilum* Infected Roe Deer Calf

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ABSTRACT: This paper describes a case of *Anaplasma phagocytophilum* infection in a roe deer (*Capreolus capreolus*) calf in Norway. The calf was found deserted, paretic, and heavily infested with *Ixodes ricinus* ticks. It was euthanized and investigated postmortem. *Anaplasma phagocytophilum* was detected in several tissues by polymerase chain reaction (PCR) and 16S rRNA sequence analyses. Analyses for *Borrelia burgdorferi* sensu lato and tick-borne encephalitis (TBE) virus infections were negative. This is the first report of a possible paretic condition in *A. phagocytophilum* infected roe deer.

Key words: *Anaplasma phagocytophilum*, *Capreolus capreolus*, case report, granulocytic ehrlichiosis, paresis, roe deer.

Tick-borne fever (TBF), caused by *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophila*), has for decades been considered a common disease in domestic ruminants along the coast of southern Norway (Øverås, 1972; Stuen, 1998). Several other mammalian species, including wild cervids and humans, have also been found infected with *A. phagocytophilum* (Bakken et al., 1994; Jenkins et al., 2001). In the present paper a paretic condition in an *A. phagocytophilum* infected roe deer (*Capreolus capreolus*) calf is described.

The calf was found alive, but unable to stand, on August 2004 in Vest-Agder County, southwestern Norway. The calf held its head in an upright position, but it was unable to move its limbs. The sheep tick *Ixodes ricinus* is abundant in the area and many ticks were seen on the head and body of the calf. It was euthanized for animal welfare reasons and the whole body was frozen at −20 C for later examination. The estimated age of the calf was about 3 mo (Østbye and Bjørnsen, 1990).

A routine necropsy including aerobic bacterial cultivation from brain, heart, kidney, liver, lungs, and spleen was conducted. Tissue samples were not processed for histopathology because of putrefaction. However, 5- to 10-g tissue samples from brain, heart, blood, kidney, liver, lungs, and spleen tissues were frozen at −20 C for polymerase chain reaction (PCR) analysis and DNA sequencing. All sampled tissues were tested for DNA from *A. phagocytophilum* and *Borrelia burgdorferi* sensu lato, while only brain tissue was tested for RNA from tick-borne encephalitis (TBE) virus. Serum was tested for neutralizing antibodies to TBE virus (Vene et al., 1998). The concentrations of cobalt, copper and selenium in the liver were determined on a wet weight (ww) basis by atomic absorption spectroscopy (Bernhoft et al., 2002).

Total genomic DNA was isolated from tissue and blood samples using a commercially available kit (DNeasy Tissue kit; QIAGEN, Valencia, California, USA) and the DNA content was measured spectrophotometrically. For *A. phagocytophilum* analysis, the samples were subjected to a seminested PCR strategy, using primers 16S-F5 (5′-AGTTTGATCATGGTTCAGA-3′) and ANA-R4B (5′-CGAACAACGCTTGC-3′) for initial amplification of a 507 bp fragment of the 16S rRNA gene in *A. phagocytophilum*. The subsequent seminested reaction with primers 16S-F5 and ANA-R5 (5′-TCCTCTCAGACCAGCTATA-3′) produced a 282 bp fragment. The amplified products of the initial PCR were diluted at 1:100 in distilled water and 2 μl was used

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as a template in the second reaction. The PCR was performed in 25 μl reaction volumes containing 2.5 mM $MgCl_2$, 0.2 mM dNTP, 0.5 μM of each primer, 0.7 U AmpliTaq Gold enzyme (Applied Biosystems, Foster City, California, USA), and approximately 100 ng of DNA. Cycling parameters were 95 C for 5 min, followed by three cycles of 94 C, 55–52 C (touchdown of 1.0 C per cycle), and 72 C for 30 sec each, another 35 cycles (25 cycles for the seminested reaction) of 94 C, 52 C, and 72 C for 30 sec each, and finally a 5 min incubation at 72 C.

Anaplasma phagocytophilum variants were detected by direct DNA sequence determination of PCR products. The PCR products were sequenced in both directions using Big Dye terminator cycle sequencing chemistry and capillary electrophoresis (ABI 310; Applied Biosystems). Sequences were visually inspected from chromatograms.

Tissue samples were also tested for *Borrelia* spp. infection using the SL primers of Demaerschalck et al. (1995). These primers were designed to target the *ospA* gene of all of the *Borrelia burgdorferi* sensu lato genospecies involved in Lyme disease. Cultured isolates of *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* were kindly supplied by National Health Institute, Oslo, Norway (I. S. Aaberge) and they also provided known positive controls for the amplification reaction. PCR was performed on 150 ng genomic DNA in 25 μl reaction volumes with AmpliTaq Gold polymerase and the reaction buffer recommended by the supplier (Applied Biosystems). Cycling parameters were 95 C for 7 min, followed by 40 cycles of 94 C, 53 C, and 72 C for 30 sec each, and a final 5 min incubation at 72 C. To identify TBE virus in the brain tissue, a reverse transcription PCR (RT-PCR) was performed according to Schrader and Süss (1999).

The calf weighed only 6.5 kg and had signs of dehydration. More than 300 *I. ricinus* were found, mainly nymphs and larvae concentrated on the head. Body fat was not observed around the heart, intestines, and kidneys. Gross pathologic changes were not detected in the brain, spinal cord, vertebrae, or extremities. Petechial subendothelial hemorrhages were found in the heart. The spleen was enlarged and swollen with subcapsular petechiae. Little content was seen in the rumen, and there was no sign of diarrhea.

Bacterial cultivation was negative. However, all tissues examined by PCR were positive for *A. phagocytophilum*. Sequence analysis of the 16S rRNA gene revealed a genetic variant of *A. phagocytophilum* previously not identified in clinical cases of wildlife (GenBank accession number AJ242784; Table 1). The PCR and RT-PCR analyses targeting *B. burgdorferi* s.l. and TBE virus, respectively, proved negative. Neutralizing antibodies to TBE virus were not detected. The concentrations of cobalt, copper, and selenium in the liver tissue were 0.11, 43.0, and 0.05 μg/g ww, respectively.

The positive results of PCR analyses performed on tissue samples from several internal organs indicated systemic infection with *A. phagocytophilum* (Stuen and Olsson Engvall, 1999). Also, splenomegaly with subcapsular hemorrhages as seen in this deer is the most typical pathologic finding in animals that have died of *A. phagocytophilum* infection (Stuen and Olsson Engvall, 1999; Stuen et al., 2001a). A clinical case in a roe deer calf has been described where septicemic infections with both *A. phagocytophilum* and *Escherichia coli* were found and it was questioned whether infection with *E. coli* should be regarded as secondary due to immunosuppression caused by a primary *A. phagocytophilum* infection (Stuen et al., 2001a). In the present case, no other bacterial infections were detected.

Recently, several heavily tick-infested roe deer have been found dead or severely ill in the same geographical area. A similar paretic condition has also earlier been observed in five roe deer calves heavily infested with ticks. Unfortunately, none of

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TABLE 1. The 5′ end of the 16S rRNA gene sequences of 86 *A. phagocytophilum* isolates in diseased or dead mammals in Norway identified and compared with similar sequences from GenBank.

Mammalian species (number of isolates)	Sequence (nucleotide position)				GenBank accession no.
	80	92	93	100	
Sheep (6), cattle (1), human (2)[a]	A	A	A	G	U02521 (prototype)
Sheep (35), cattle (1), horse (1), moose (1)[b]	A	A	A	A	M72220
Sheep (28)	G	A	A	A	AF336220
Sheep (7)	A	A	G	G	AY035312
Roe deer (1)	A	G	A	G	AJ242783
Cattle (1), roe deer (1)[c]	A	G	A	A	AJ242784

[a] Bjöersdorff et al., 1999.

[b] Jenkins et al., 2001.

[c] Present case.

these calves were further examined (R. Moseid, pers. comm.).

A paretic condition may be caused by several factors, such as brain nematodes (e.g., *Elaphostrongylus* spp; Handeland et al., 2000), copper deficiency, exhaustion, spinal abscesses, infections, tick paralysis, and trauma. In the present case, brain nematodes, spinal abscesses, and trauma can be excluded. However, tick paralysis cannot be excluded, although toxins from *I. ricinus* are seldom mentioned in this context (Goethe and Neitz, 1991).

The knowledge of normal liver concentrations of cobalt, copper, and selenium in young roe deer is limited. The observed concentrations of cobalt and copper in this deer were within the normal ranges found in domestic and wild ruminants in Norway (Frøslie et al., 1987; A. Bernhoft, pers. inform.). Related to the knowledge of hepatic selenium in domestic ruminants, the measured selenium concentration in this roe deer must be regarded as deficient (Van Metre and Callan, 2001). The implication of low selenium status is unknown, but selenium deficiency may cause myodegeneration and impaired immunity. Unfortunately, histopathology was not performed. Because the present calf was cachectic, however, the paretic condition was most probably caused by exhaustion due to starvation and infestation/infection.

Blood loss due to heavy tick infestation may cause anemia and general weakness. A connection between tick infestation and starvation has already been mentioned as a cause of death in roe deer in Sweden (Alonso Aguirre et al., 1999). However, *A. phagocytophilum* infection may also cause severe illness, as observed in experimentally infected reindeer *Rangifer tarandus tarandus* (Stuen, 1996). In young lambs, the bacterium may cause lameness and pyemia (Brodie et al., 1986).

Experimental infection with *A. phagocytophilum* in red deer and reindeer showed that red deer were only subclinically infected while reindeer had a severe clinical reaction (Stuen, 1996; Stuen et al., 2001b). Thus, the agent may be more pathogenic in both roe deer and reindeer than in red deer. One possible reason for this could be an acquired resistance of red deer caused by a long-term exposure to *I. ricinus* and *A. phagocytophilum* (Wickel, 1996; Dumler and Brouqui, 1997). In Norway, red deer normally live in coastal lowland areas where *I. ricinus* is common, whereas the roe deer has expanded into tick-infested areas only during the last 40-50 yr (Østbye and Bjørnsen, 1990).

Six 16S rRNA gene variants of *A. phagocytophilum* have been identified in Norway. So far, two of these variants have been found in seriously infected roe deer (Stuen et al., in press). Although the variants can be distinguished on the basis

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of one or two nucleotide differences only, studies indicate that there may be biologic, ecologic, and pathologic differences between them (Massung et al., 2002; Stuen et al., 2003). However, whether all *A. phagocytophilum* variants cause clinical manifestations in cervids is unknown. In the present case, the same 16S rRNA gene variant has been found in diseased cattle (Table 1).

Hunting statistics for the past decade indicate a reduced production of roe deer calves in the present area, while tick infestation has increased in the same period (R. Moseid, pers. comm.). Further investigations are needed to clarify whether ticks and *A. phagocytophilum* represent a health problem in roe deer populations.

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