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Short communication

Clinical outbreak of babesiosis caused by *Babesia capreoli* in captive reindeer (*Rangifer tarandus tarandus*) in the Netherlands

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ABSTRACT

From a herd of captive reindeer (*Rangifer tarandus tarandus*) consisting of two males and seven females with five calves, three calves were diagnosed on post mortem examination with a *Babesia capreoli* infection. The diagnosis was indicated by PCR and when the other reindeer were examined two adult females and a one-year-old male were *Babesia*-positive. Molecular characterization of the 18S rDNA of the parasite showed complete identity with known *B. capreoli* sequences. *Ixodes ricinus* has been demonstrated to be a competent vector for *B. capreoli* from infected roe deer (*Capreolus capreolus*), the natural host of *B. capreoli*.

The *B. capreoli* infection in these reindeer may have been transmitted by infected ticks (*Ixodes ricinus*) originating from roe deer living in the forest and meadows surrounding the enclosure.

1. Introduction

Babesiosis is a tick-borne disease caused by protozoal parasites of the genus *Babesia*. Tick-transmitted pathogens are becoming increasingly important, however, not all aspects of the transmission dynamics of tick-borne pathogens are known. (Dantas-Torres, 2015; Lindgren and Jaenson, 2006).

Acute *Babesia* infection can cause hemolysis resulting in hemolytic anemia with icterus and hemoglobinuria. Infected animals may show normocytic, normochromic anemia, a low packed cell volume and red blood cell count and, depending on the stage of the disease, variable counts of white blood cells (Bartlett et al., 2009). Clinical signs can include high fever, hemoglobinuria, jaundice, shaking, myalgia and abdominal pain but asymptomatic infections are also possible.

Babesia spp. multiply within the erythrocytes by asynchronous binary fission which produces gametocytes that can be ingested by blood-feeding arthropods such as castor bean ticks (*Ixodes ricinus*). Within the tick gut, after conjugation, the gametocytes undergo multiple rounds of fission and migrate to different tissues within the tick. Before transmission further development occurs in the tick's salivary gland (Gray et al., 2010).

Babesia spp. are widespread throughout Europe. In wild and domestic ruminants *B. divergens*, *B. venatorum* and *B. capreoli* are of major importance (Hoby et al., 2009; Rizzoli et al., 2014). *B. divergens* is found in cattle (Zintl et al., 2003). *B. venatorum* seems to be common in roe deer (*Capreolus capreolus*), typically occurring without clinical signs although these have occasionally been described in reindeer in Switzerland (Robert et al., 2008) and the Netherlands (Kik et al., 2011). Asymptomatic roe deer seem to be the mammalian maintenance host of *B. capreoli* in Europe (Michel et al., 2014; Hoby et al., 2009). In Germany and Switzerland clinical infections have been reported in captive reindeer, alpine chamois (*Rupicapra rupicapra*), and red deer (*Cervus elaphus*) (Hoby et al., 2009; Wiegmann et al., 2015). Based on case reports of clinical cases and on tick surveillance, *B. divergens* and *B. venatorum* are considered to be endemic in the Netherlands but *B. capreoli* has not been described in the Netherlands to date (Wielinga et al., 2009).

Here we describe the first clinical outbreak of babesiosis caused by *B. capreoli* in captive reindeer in the Netherlands.

2. Case description

In 2015 an outbreak of clinical babesiosis occurred in a reindeer population at Ouwehand Zoo in Rhenen, the Netherlands. The reindeer herd consisted of 14 animals: 2 adult males, 7 adult females and 5 calves, all of which had been born in May of that year.

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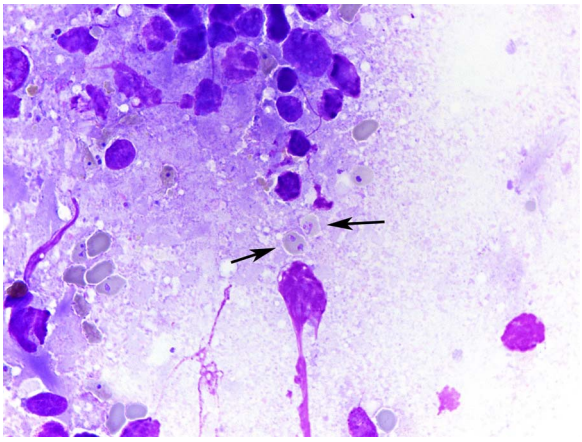

Fig. 1. Cytology of the lung of a reindeer infected with *Babesia*. The protozoal inclusions are indicated by arrows.

A one-month-old calf (8267) suddenly died without exhibiting clinical signs. At necropsy jaundice of the sclera, pleurae, serosa of the intestines and large vessels as well as hemoglobinuria were evident. The liver was slightly enlarged and yellow. The spleen was enlarged. Cytology was performed on the liver, spleen, lungs and large intestinal contents which were stained with Hemacolor® (Merck, Darmstadt, Germany). Cytological examination showed 2–3 µm large intra-erythrocytic inclusions consistent with *Babesia* spp. (Fig. 1). DNA was extracted from spleen material using the DNeasy blood & tissue kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). PCR was performed using primers (forward primer: 5′-GACACAGGGAGGTAGTGACAAG-3′ and reverse primer 5′-CTAAGAATTTCACCTCTGACAGT-3′) amplifying the V4 region of the 18S ribosomal RNA gene of *Babesia/Theileria* (Gubbels et al., 1999). The PCR produced a signal indicating the presence of *Babesia* and/or *Theileria* DNA. Two weeks later, another calf of the same age (1888) showed heavy breathing and fever (40.2 °C). Procaïnbenzylpenicillin (Dopharma Research B.V. Raamsdonkveer, the Netherlands; 20,000 IU/kg/day, subcutaneously for 5 days) was administered combined with a single intravenous injection of flunixin meglumine (1 mg/kg; Finadyne, Intervet Nederland B.V., Boxmeer, the Netherlands). The calf was diagnosed with laryngeal diphtheria and a tracheotomy was performed. A modified tracheal tube was placed under local anesthesia. One week after surgery the condition of the calf suddenly deteriorated and the calf was euthanized.

Necropsy confirmed the initial diagnosis of laryngeal diphtheria. Additional findings included hepatosplenomegaly, dark red kidneys (on cut surface) and light red urine. Intra-erythrocytic inclusions were present in cytology. Histology revealed acute necrosis in the liver and the tubular epithelium of the kidney and hemosiderin pigment in the spleen. PCR on whole blood of the calf produced a signal indicating the presence of *Babesia* and/or *Theileria* DNA. In light of these findings the remaining calves were treated with imidocarb dipropionate (Carbesia 8.5%, Pharmacy Department, Faculty of Veterinary Medicine, Utrecht, the Netherlands) 3 mg/kg subcutaneously on days 1, 2, 6, 9 and 21 (Bartlett et al., 2009).

One week later, a one-year-old male reindeer was euthanized due to acute respiratory distress; no post mortem examination was performed. However, in the cytology of a blood smear an estimated 10% of the erythrocytes contained inclusions consistent with *Babesia* spp. Subsequently, blood was taken from five adult females (all mothers of newborn calves), one adult male and the remaining calves (which had received three doses of imidocarb propionate at that time) for complete blood count (CBC), blood chemistry and *Babesia* testing with PCR.

There were no significant changes in CBC and blood chemistry values of any of the animals. Two adult females (3667 and 5192) tested positive for *Babesia/Theileria* by PCR (Table 1). Due to the absence of clinical signs or changes in CBC or blood chemistry it was assumed these animals were subclinically infected. Infected adult animals were not treated with imidocarb in order to ensure a good antibody response and thereby reduce herd susceptibility (Bartlett et al., 2009). PCR products from the four positive samples (1888, 3667, 5192 and 8267) were sequenced by dideoxy-dye termination sequencing of both strands and compared with sequences from isolates in GenBank (http://www.ncbi.nlm.nih. gov/). The sequences were aligned and analyzed using BioNumerics 6.6 (Applied Maths, Kortrijk, Belgium). The sequences obtained from the reindeer samples were all identical and 100% similar to the 18S rDNA *B. capreoli* sequences in Genbank (Accession number FJ944828 and FJ944827). The 18S rDNA sequence from the reindeer parasite exhibited the characteristic substitutions at positions 631 and 663 (from A to G and A to T, respectively) from *Babesia divergens* rDNA (Malandrin et al., 2010).

Table 1
Information, clinical signs and PCR results from all individual reindeer in the herd.

ID (sex)	year of birth	Babesia (PCR)	Clinical signs
9514 (♀)	2004	neg	None
9781 (♂)	2015	neg	Died without previous signs (30-6-2015)
3667 (♀)	2009	pos	None
1888 (♀)	2015	pos	Larynx diphtheria; euthanized (22-6-2015)
4729 (♀)	2015	neg	None
5192 (♀)	2013	pos	None
newborn	2015	nd	Died (30-5-2015)
8267 (♂)	2015	pos	Died without previous signs (12-6-2015)
3427 (♀)	2014	neg	None
2013 (♀)	2014	neg	None
2466 (♂)	2012	neg	None
male (♂)	2014	pos[a]	Died after acute respiratory distress (22-7-2015)

nd = not done; reindeer within one line are related (mother and calf).
[a]Positive blood smear instead of PCR.

Tick dragging throughout the enclosure and examination of the remaining animals yielded no ticks. An *I. ricinus* nymphal tick was recovered from the clothing of one of the veterinarians, but tested negative for *Babesia/Theileria* by PCR.

3. Discussion

To our knowledge this report describes the first fatal cases of *B. capreoli* infections in captive reindeer in the Netherlands. Apart from five cases in free-ranging chamois (*Rupicapra rupicapra*) in Switzerland, no fatal cases of babesiosis due to *B. capreoli* have been reported (Hoby et al., 2009).

There is a single report of a fatal case of *B. venatorum* (formerly called B*abesia* sp. EU1) infection in a captive reindeer in the Netherlands (Kik et al., 2011). Also, in another Dutch zoo in June 2015 two adult forest reindeer (*R. t. fennicus*) exhibiting icterus, acute liver necrosis and hemoglobinuria, with cytological intra-erythrocytic *Babesia* spp. inclusions, tested positive for *B. venatorum* in PCR tests (unpublished data). Based on case reports of clinical cases and on tick surveillance, *B. divergens* and *B. venatorum* are considered to be endemic in the Netherlands but no reports on the occurrence of *B. capreoli* have been published so far (Wielinga et al., 2009). However, *B. capreoli* was detected in a recent survey in *I. ricinus* ticks originating from a national park in the Netherlands (unpublished data).

Adult roe deer and reindeer are often subclinically infected with *Babesia* spp. (Michel et al., 2014; Penzhorn, 2006). Clinical disease has been described (Langton et al., 2003) but appears to occur rarely in adult animals.

In Germany subclinical infections with different *Babesia* species

seem to be common in captive reindeer and this might pose a risk for naïve animals (Wiegmann et al., 2015). Clinical signs are usually more severe in naïve and immunocompromised animals (Bartlett et al., 2009). Only the young calves in this population showed clinical signs and died due to a *B. capreoli* infection whereas some adult animals were subclinically infected. Of the four PCR-positive animals, two were young calves (8267 and 1888) and the other two were adult females (3667 and 5192), both mothers of the deceased calves. Positive diagnosis in the one-year-old male was based on the presence of intra-erythrocytic organisms in its blood smear; unfortunately no post mortem or PCR testing were performed.

The route and time of introduction of *B. capreoli* into the reindeer herd is unknown. Since 2011, no new reindeer were introduced into the herd. Despite the fact that no ticks were found on the animals in the herd it is possible that transmission occurred via *Babesia*-positive ticks. The enclosure of the reindeer herd is surrounded by forest and meadows where roe deer are frequently seen. Roe deer may themselves have acted as a vector for the spread of *Babesia*-positive ticks. However, as direct contact between roe deer and the captive reindeer can be ruled out in this zoo, it is possible that *Babesia*-positive ticks may have entered the enclosure on wild small mammals or birds or even from willow branches (*Salix* spp.) collected from the surrounding area. To prevent new outbreaks of babesiosis the focus should be on the prevention of tick infestation in captive reindeer by use of acaricidal products during the tick season.

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