Case report

Fatal acute babesiosis associated with *Babesia venatorum* infection (*Babesia* sp. EU1) in a captive reindeer calf in Switzerland

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ABSTRACT

Babesia venatorum was isolated from a captive reindeer calf in Switzerland. The clinical signs consistent with acute babesiosis included hemolytic anemia and hemoglobinuria. The diagnosis was made based on visualization of intraerythrocytic parasites in the stained blood smears and confirmed by PCR analysis of the 18S rRNA gene, with subsequent species identification within *Babesia* confirmed by sequencing. The reindeer calf was initially treated with supportive care and an antiprotozoal drug (imidocarb dipropionate) but died a few days after hospitalization. *Babesia venatorum* is also known as *Babesia* sp. EU1 and can infect different mammalian species, including humans. The current case report aims to increase awareness among veterinarians and reindeer owners about the presence and the associated risk of this zoonotic pathogen. Considering the high morbidity and possible mortality associated with acute babesiosis, captive reindeer should receive tick prevention measures and be tested for subclinical infections in endemic area.

1. Introduction

Babesiosis is a tick-borne disease caused by globally distributed protozoal parasites of the genus *Babesia*. *Babesia* infections are reported in a wide range of domestic and wild mammalian hosts. These parasites are primarily of veterinary interest; however, the increased number of human cases worldwide has raised the question of whether *Babesia* members may also be emerging human pathogens (Suarez et al., 2019).

Babesia venatorum is also known as *Babesia* sp. EU1. The definitive taxonomic name is still under debate since a full formal species description is not yet available (Jiang et al., 2015). *Babesia venatorum* (*B. venatorum*) was first isolated from two splenectomized human patients in Austria and Italy (Herwaldt et al., 2003). Another human case was subsequently documented in Germany (Haselbarth et al., 2007). More recently, several human cases were reported in China (Jiang et al., 2015). *B. venatorum* was also isolated from ticks collected from healthy pets in Belgium (Lempereur et al., 2011) and from rodents in Switzerland (Burri et al., 2011). *B. venatorum* is closely related to, but clearly distinct from, *Babesia divergens* and *Babesia odocoilei*. *B. venatorum* was subsequently isolated from roe deer (*Capreolus capreolus*) in different European countries (Bonnet et al., 2007; Michel et al., 2014). The

frequent isolation in roe deer suggested that these animals play an important role as a reservoir of the parasite and that the infection is not restricted to only one geographic area in Europe.

Three *Babesia* species have been identified in reindeer: *B. venatorum*, *Babesia odocoilei* and one unidentified species closely related to *Babesia divergens* (Wiegmann et al., 2015). These *Babesia* species are all known to be transmitted by the vector tick *Ixodes ricinus* (Bonnet et al., 2007). *B. venatorum* normally leads to asymptomatic infections in reindeer. However, few cases of acute babesiosis due to *Babesia venatorum* have been reported in captive reindeer in Germany (Wiegmann et al., 2015) and in the Netherlands (Kik et al., 2011). A single case in Switzerland had been reported previously in a conference abstract (Robert et al., 2008).

2. Case description

A three-month-old male reindeer (*Rangifer tarandus*) calf presented with severe apathy, dyspnea and weight loss. The reindeer calf was a member of a small herd in Switzerland (Canton of Thurgau) composed of seven adults and two juvenile animals. All animals were kept outside in a large enclosure. All reindeers of the herd were annually tested for

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Table 1
Hematological values were given by the Sysmex XT2000iV (Sysmex Corporation, Kobe, Japan) and manual packed cell volume (PCV). The reference range used, mean and standard deviation for reindeer were previously reported (Miller et al., 2013).

Parameters	Values[a]	Mean ± SD[b]	Range
Manual PCV (%)	**10**	47 ± 0.01	39–57
HTC (%)	**10.8**	47 ± 0.01	39–57
RBC (×10⁶/μL)	**2.59**	10.7 ± 0.17	9.24–11.96
Hb (g/dL)	**5.2**	17.3 ± 2.58	14.5–21.1
MCV (fL)	41.7	44.4 ± 0.3	40.3–48
MCH (pg)	20.1	16.3 ± 0.1	15–17.5
MCHC (g/dL)	**48.1**	36.6 ± 2	35.1–38.4
PLT (×10³/μL)	184	253 ± 24	74–603
WBC (×10³/μL)	**12.4**	2.96 ± 0.19	0.96–5.19
Neutrophils (×10³/μL)	**8.58**	1.91 ± 0.18	0.56–4.52
Eosinophils (×10³/μL)	**0.19**	0	0–0.01
Monocytes (×10³/μL)	**1.05**	0.01 ± 0.01	0–0.03
Lymphocytes (×10³/μL)	**2.16**	0.90 ± 0.06	0.37–1.69

[a] Values outside the reference range are listed in bold letters.
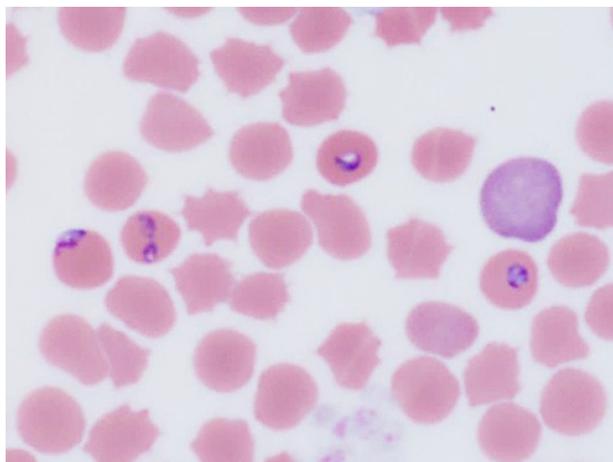[b] SD = standard deviation of the mean.

Fig. 1. Wright-Giemsa-stained EDTA blood smear showing intraerythrocytic inclusions of variable shapes (signet ring and piriform) morphologically compatible with *Babesia/Theileria* organisms; a polychromatophilic erythrocyte, an erythrocyte with basophilic stippling and a small platelet aggregate are also seen (1000× magnification).

fecal parasites and treated with antiparasitics in case of positive results. No history of tick infestation has been reported. At clinical presentation, the calf was very thin, dehydrated and hardly capable of standing. Tachycardia (110 bpm) and dyspnea were present. Mucous membranes were icteric and the temperature was 39.7 °C. Urine was dark-brown. The animal immediately received intravenous fluids (Ringer lactate with the first bolus of 1 L over 45 min and then 2 mL/kg per hour). No blood was available for transfusion. After blood examination and suspected *Babesia* infection, Imidocarb dipropionate treatment (Carbesia 3 mg/kg) was initiated. Complete hematology and biochemistry were requested for further investigation. Blood smears stained with Wright-Giemsa were also evaluated microscopically (Table 1). Based on previously reported biochemical and hematological reference values (Miller et al., 2013), severe anemia with mild to moderate leukocytosis due to mild neutrophilia and lymphocytosis and moderate monocytosis was present (Table 1). On blood smear examination, some of the erythrocytes contained small (1–2 μm in length) pleomorphic piroplasms (signet ring or pear-shaped) with an eccentric magenta-stained nucleus and basophilic cytoplasm (Fig. 1), resembling *Babesia* or *Theileria* organisms. In biochemical profiling, interference was recognized due to the marked hemolysis of the sample resulting in uninterpretable results. Hemoglobinuria was confirmed with urinalysis. Total nucleic acids

(TNA) were purified from 100 μL of EDTA-anticoagulated blood using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostic). TNA was stored at −80 °C until use. During nucleic acid extraction, a negative control of 100 μL of PBS was concurrently prepared to monitor cross-contamination. The PCR for the detection of the 18S rRNA gene of *Babesia* sp. from the blood samples was performed using 5 μL of TNA, 0.5 μM each primer (primers according to (Casati et al., 2006) BJ1: 5′-GTC TTG TAA TTG GAA TGA TGG-3′; BN2: 5′-TAG TTT ATG GTT AGG ACT ACG-3′), 1 unit of Taq polymerase (Qiagen AG, Basel, Switzerland) in a total volume of 50 μL (buffer provided by the manufacturer). The reaction mixtures were subjected to an initial denaturation step of 10 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and elongation at 72 °C for 2 min. Amplification was completed by a further 5 min step at 72 °C. For the PCR, positive (plasmid standard) and negative controls (nuclease-free water) were included. Primers used in this case were designed according to the sequence of the 18S rRNA gene and were able to detect many different *Babesia* species (Casati et al., 2006). The amplicon was sequenced from both directions (Synergene Biotech GmbH, Schlieren, Switzerland), and the consensus sequence was used for further analyses. PCR analysis confirmed the nature of the suspected blood parasites, and sequencing showed a homology of 100% (447/447 bp), with corresponding sequences of *B. venatorum,* i.e., GenBank KU351818 (roe deer from Germany) and JQ711228 (*Ixodes ricinus* from Belarus). The GenBank ID of the present case is MK584708, and the specific evolutionary relationships of the taxa are given in Fig. 2. The animal died 36 h after hospitalization.

3. Discussion

Fatal acute babesiosis due to *B. venatorum* have been rarely reported in captive reindeers (Kik et al., 2011; Robert et al., 2008; Wiegmann et al., 2015). The current case report aims to increase the awareness of veterinarians and reindeer owners about the presence and the associated risk of this zoonotic pathogen.

Hematological findings during clinical babesiosis are normally characterized by a decrease in hemoglobin concentration, PCV and red blood cell counts during high parasitemia, while total leukocyte count may increase. Thrombocytopenia is a common hematological feature in canine babesiosis (Kettner et al., 2003). Inconsistent findings on platelet count have been reported for *B. venatorum.* Only a few clinical cases have been reported in reindeer with only one case reporting thrombocytopenia (Robert et al., 2008). In humans, *B. venatorum* is associated with thrombocytopenia in 13% of the infected cases (Jiang et al., 2015). Thrombocytopenia has been also reported in two splenectomized human patients infected by *B. venatorum*; one of the patients was however under chemotherapy (Herwaldt et al., 2003). In the present case, the reindeer calf showed normal platelet count, suggesting that *B. venatorum* is not causing consistent thrombocytopenia in acutely infected reindeer. Interestingly, the morphological features of the intraerythrocytic parasites seen in the blood smear of the present case resemble the morphology of the parasites seen in the reported human case infected with *B. venatorum* in Germany (Haselbarth et al., 2007) and in China (Jiang et al., 2015). The signet ring forms of the parasite were most commonly seen (Fig. 1) in the infected erythrocytes of the present reindeer, followed by paired piriforms. Tetrads were rarely recognized in the present case and were also infrequently seen in the blood smears of the infected humans in China (Jiang et al., 2015).

B. venatorum is transmitted by the hard tick *Ixodes ricinus* (*I. ricinus*). *B. venatorum* has been isolated from *I. ricinus* in Switzerland and other European countries (Casati et al., 2006; Hilpertshauser et al., 2006). The vector capacity of *I. ricinus* has been demonstrated under natural and experimental conditions (Bonnet et al., 2007). *I. ricinus* is also the most prevalent and widely distributed tick in Europe and frequently bites humans. For this reason, *B. venatorum* has received increased attention as a potential agent of emerging tickborne disease in humans.
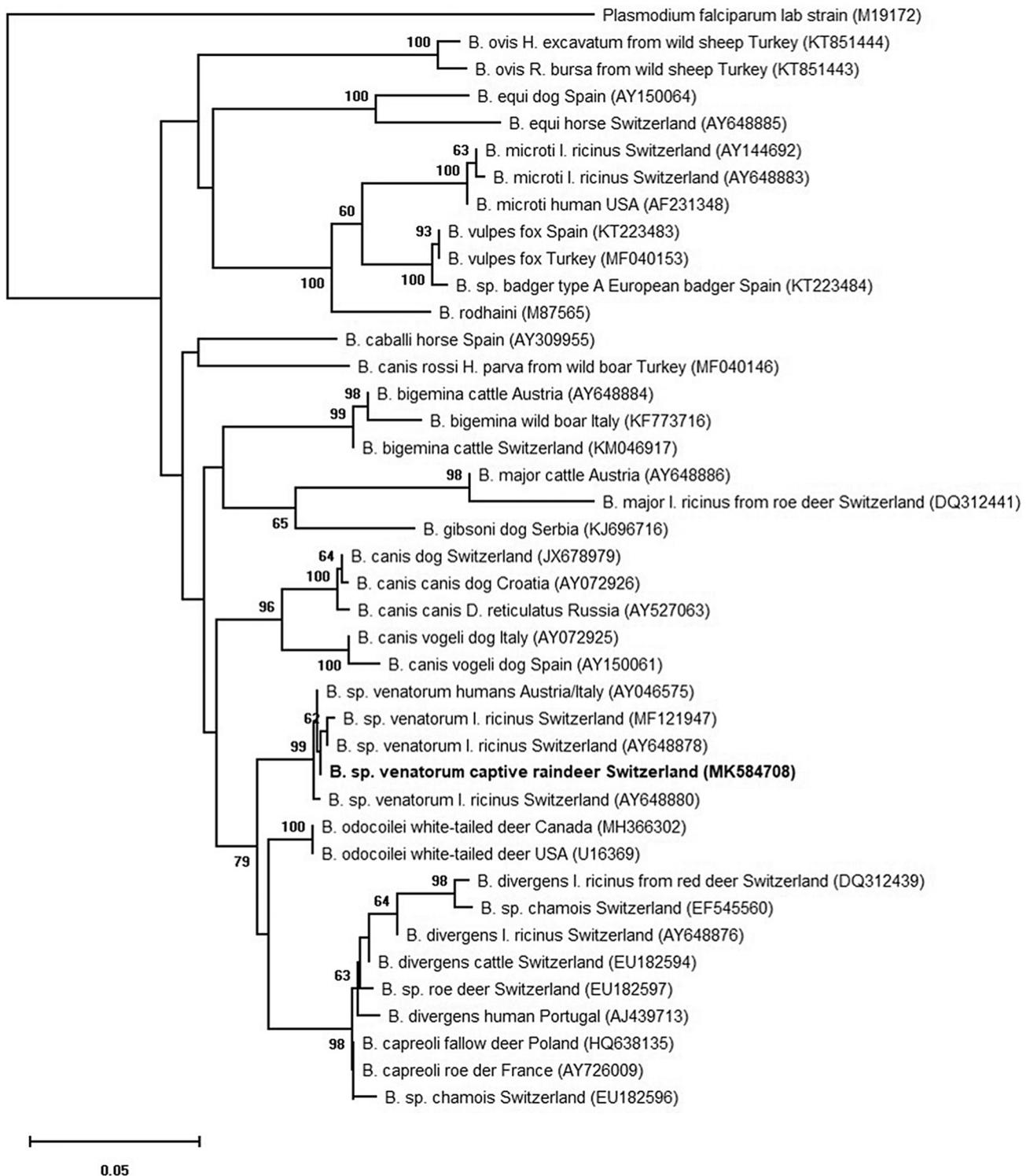
Fig. 2. Phylogenetic tree of partial 18S rRNA gene sequences

The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 1.08550841 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). Only values greater than 60 are displayed. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in units of the number of base substitutions per site. The analysis involved 41 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 552 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018). Reindeer sequence in bold, *Plasmodium falciparum* is used as outgroup. Accession numbers of all sequences are given in parenthesis.

Interestingly, *Ixodes persulcatus* was identified as vector for transmission of *B. venatorum* in China (Jiang et al., 2015).

B. venatorum is the most prevalent *Babesia* species detected in *I.*

ricinus collected in Switzerland (Gigandet et al., 2011; Lommano et al., 2012). *B. venatorum* has also been detected in the south (Casati et al., 2006) as well as in the western region of Switzerland (Lommano et al.,

2012). Interestingly, *B. venatorum* has also been identified in ticks collected in the suburban forest close to Neuchâtel in Switzerland (Gigandet et al., 2011). These findings clearly identify a potential risk of exposure to infected ticks for humans from this area. The prevalence of *Babesia* species differs among European countries but appears to be lower in urban and suburban regions than in rural areas. These differences are likely connected to the presence of competent reservoir hosts. However, the prevalence of *B. venatorum* in Swiss reindeer herds is currently not known and should be further investigated.

In conclusion, *B. venatorum* can cause fatal infections in reindeer. The overall prevalence in this animal species is currently not known. Given the presence and zoonotic potential of this organism, *B. venatorum* requires increasing attention from Veterinary Public Health services. It is also advisable to further evaluate the prevalence of *B. venatorum* in reservoir host animals.

Ethic statement

The animal was treated humanely and received a high standard of veterinary care at all times.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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