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Short communication

Babesiosis in free-ranging chamois (Rupicapra r. rupicapra) from Switzerland

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Abstract

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1. Introduction

Babesiosis is a globally important tick-transmitted disease relevant to veterinary and public health. It is caused by intraerythrocytic protozoan parasites of the genus Babesia, and has been reported in a number of mammal species including humans. Disease spectrum ranges from silent infection to haemolytic anaemia, cardiovascular shock and multiorgan failure (Homer et al., 2000).

Four Babesia spp. have been described as cause of naturally acquired fatal babesiosis in wild ruminants in Europe. Babesia bovis was reported in a chamois (Rupicapra r. rupicapra) from Switzerland (Bouvier, 1965) and B. ovis in a Spanish ibex (Capra pyrenaica) (Marco et al., 2000). However, in both cases identification of the parasites was merely presumptive as it was based on morphological characteristics of the intraerythrocytic inclusions, and supported by an indirect fluorescent antibody test of unproven specificity in the ibex case. Babesia capreoli has been described microscopically in acute fatal cases of babesiosis in captive and free-ranging roe deer (Capreolus c. capreolus) from different countries of central and northern Europe (Enigk and Friedhoff, 1962; Hinaidy, 1987; Dorrestein et al., 1996). Babesia divergens, which is the cause of a
widespread cattle disease and the agent of rare human babesiosis in Europe (Zintl et al., 2003), has been identified by molecular means in a farmed reindeer (*Rangifer t. tarandus*) in Scotland (Langton et al., 2003). However, to date, *B. capreoli* is not distinguishable by molecular or serological methods from *B. divergens* (Garcia-Sanmartin et al., 2007). In this report, we describe five cases of fatal babesiosis in chamois, with partial molecular characterisation of the infectious agent.

### 2. Materials and methods

In June 2005, two adult female chamois (F1 and F2) were found dead in the eastern Swiss Alps (Tössstock region, 47°19’N, 8°90’E); a third adult female (F3) was found dead in May 2006 (Table 1). In June and July 2006, carcasses of an adult female (F4) and an adult male (M1) were found dead in the north-western Swiss Alps (Simmental and Gantrisch region, 46°40’N, 7°23’E). F1 and F4 were both followed by apparently healthy kids (K1 and K4), which were shot by local game wardens after discovery of the dead dams due to their poor chance of survival. F3 was lactating, but no kid could be spotted. All five adults and both kids were submitted for detailed pathological investigation. Age of the animals was determined by counting the annual growth rings on the horns. Complete necropsy of the carcasses was carried out. They were well-preserved or only slightly autolytic. Blood was collected from the thoracic and heart cavities and stored at −20 °C. Samples of the spleen of all animals were stored at −20 °C. Tissue specimens of the inner organs (lung, liver, spleen, kidney, rumen, abomasum, small and large intestine, lymph nodes, adrenals, urinary bladder) of all animals, the reproductive organs of the adult animals and the brain of 5/7 animals were collected. They were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm, stained with haematoxylin and eosin, and examined microscopically. Selected slides were also stained with Giemsa, Prussian blue and Okajima stains. Giemsa-stained blood smears were prepared for examination under the light microscope (magnification 1000×). Parasitological examinations of faeces from all adult animals included sedimentation, flotation and the Baermann–Wetzel method (Eckert, 2000). Bacteriological cultures of liver, kidney and spleen were performed in 4/5 adult animals. Blood or spleen samples were analysed by PCR. DNA was extracted using commercially available kits (DNA blood mini kit and DNeasy tissue kit, respectively, Qiagen, Hombrechtikon, Switzerland) following the manufacturer’s instructions. Amplification of part of the 18S rRNA gene specific for *Babesia* spp. was done by PCR according to Hilpertshauser et al. (2006), and amplicons were directly sequenced by a private company (Microsynth, Balgach, Switzerland). DNA samples were analysed by conventional PCR for *Anaplasma* spp. (Goodman et al., 1996), *Anaplasma marginale* (MSP5 and MSP4) (Torioni de Echaide et al., 1998; de la Fuente et al., 2002) and *Theileria* spp. (Hofmann-Lehmann et al., 2004), and by real-time qPCR for *Anaplasma phagocytopilum* (Pusterla et al., 1999).

### 3. Results

A compilation of data on the chamois including age, sex, weight, body condition as well as selected results of the examinations are summarised in Table 1.

#### 3.1. Necropsy and histopathology

Predominant macroscopic findings in all five adult chamois consisted of pale mucous membranes and...
musculature, swollen spleen with soft pulpy consistency (Fig. 1), and haemoglobinuria. Jaundiced serosal membranes were noticed in three cases (F2, F3 and M1). Various degrees of interstitial and alveolar pulmonary oedema were seen in 4/5 adult animals. No significant macroscopical lesions were observed in the kid of F1 (K1), whereas K4 showed a slightly swollen spleen. In the adult chamois, histological examination revealed haemosiderosis in the red pulp of the spleen, which was engorged with erythrocytes and macrophages and surrounded by various degrees of lymphoplasmacytic infiltration of the marginal zone. In the liver, moderate hypoxic lesions characterised by centrilobular vacuolar degeneration and focal hepatocellular necrosis were present. Kidney lesions were seen in only two cases (F1 and F2) and were characterised by mild, multifocal, tubular epithelial degeneration and multifocal, granular or globular haemoglobin casts in slightly dilated, cortical tubuli. Additional findings consisted of mild, chronic verminous bronchopneumonia and non-reactive sarcosporidiosis in the heart in all animals. No significant lesions were seen in sections of the other organs including brain. In the kids, histological alterations were restricted to K4, revealing similar pathological lesions in the spleen as in the adult animals and a minimal, focal, non-suppurative polioencephalitis.

3.2. Blood smears, coproscopy and bacteriology

In all the adult chamois, blood smear examination revealed slight anisocytosis, hypochromasia and small, round to pyriform, basophilic inclusions (Fig. 2) located at the periphery of the erythrocytes. There were up to three distinct inclusions within a single erythrocyte, sometimes linked in pairs lying at an angle of 160–180°.

The mean size of the inclusions was 1.47 μm in length (range 1.09–2.01 μm) and 0.84 μm in width (range 0.55–1.05 μm). The size and morphology of the parasites were consistent with merozoites of small Babesia spp., Theileria spp. or Anaplasma spp., supporting a tentative diagnosis of haemolytic anaemia due to an acute infection. Degree of parasitaemia in the blood smears varied of approximately 1.5–15%, depending on the individual. A few extracellular parasites were present singly, presumably released from ruptured erythrocytes. No inclusions could be detected in the blood smears of the kids. Gastrointestinal nematode eggs and Eimeria spp. oocysts were present in all animals, and Muellerius spp. larvae were detected in three faecal samples. All bacteriological cultures of liver, kidney and spleen were negative.

3.3. PCR/sequencing

All animals except kid K1 were positive for Babesia spp., and sequence analyses (385–396 bp of the 18S rRNA gene) revealed 99–100% identities with GenBank entries attributed to B. divergens or B. capreoli of domestic or wild ungulate origin. Sequences determined in this study were deposited in GenBank (accession numbers EF545557 (F1), EF545558 (F2), EF545559 (F3), EF545560 (F4), EF545561 (M1), EF545562 (K4)). A. phagocytophilum PCR was positive for K1, but not for the other animals. All samples tested negative for A. marginale and Theileria spp.

4. Discussion

To the authors’ knowledge, this is the first report of babesiosis in chamois confirmed by partial molecular characterisation of the etiological agent, and the first
report of a *B. divergens/B. capreoli*-like infection in chamois. Gross and microscopic lesions in all fatal cases were characteristic of an acute piroplasm infection (Valli, 2007), and consistent with descriptions of acute, fatal *B. divergens* infections in cattle (Gründer, 2002), *B. capreoli* infections in roe deer (Dorrestein et al., 1996) and presumptive *B. bovis* infection in chamois (Bouvier, 1965). Body condition varied considerably between the affected chamois. We suggest that emaciation in some animals was due to harsh climatic conditions and food limitations during winter and early spring (Crampe et al., 2002), but was not related to the *Babesia* infection. Indeed, pathological findings were consistent with an acute course of the disease.

With the exception of one chamois found in July (M1), the other four fatal cases (F1–F4) occurred between May and June. This time of the year coincides with a stressful period for females, including birth and lactation. It has been shown that fatal babesiosis may be triggered by stressful events (Penzhorn, 2006). Three of the affected females (F1, F3 and F4) were lactating; one female (F2) was not lactating but emaciated. However, the male (M1) was in a good body condition, indicating that the disease not only affects stressed or weakened individuals. *Babesia* spp. are transmitted by ticks, and May–June corresponds to the main peak of nymphal *Ixodes ricinus* tick activity (Perret et al., 2000). Thus, this apparently seasonal occurrence of babesiosis could also be linked to tick activity.

Morphological characteristics of the intraerythrocytic inclusions in the blood smears were compatible with a small *Babesia* spp. infection, however, infection with *Theileria* spp. or *Anaplasma* spp. could not be excluded. Furthermore, morphological differentiation of *Babesia* and *Theileria* spp. is more difficult or even impossible due to degenerative processes of the merozoites, and molecular diagnosis is indispensable. PCR analysis revealed an organism very closely related to *B. divergens/B. capreoli*, which is considered the causal agent. However, this result is based on sequencing of a relatively short segment of the 18S rRNA gene. Currently, morphological, serological and molecular data do not allow to differentiate between *B. divergens* and *B. capreoli* (Garcia-Sammartin et al., 2007). Thus, further studies including multilocus analyses are necessary to more precisely characterise these parasites and to investigate the relatedness of the isolates from our study.

Infections with *Anaplasma* spp. have been detected in serological studies in chamois from Switzerland, although not in relation to clinical manifestation (Liz et al., 2002; Dreher et al., 2005), and co-infections have been shown to occur in Swiss cattle (Hofmann-Lehmann et al., 2004). In the present study, PCR analysis for *A. phagocytophilum* was only positive in the kid without pathological findings (K1), supporting the diagnosis of fatal babesiosis in the diseased animals. Interestingly, *B. divergens/B. capreoli*-like sp. DNA was amplified by PCR from the blood in the kid with a slightly swollen spleen (K4), although no parasite could be detected in the blood smears of this animal. Splenomegaly might be interpreted as an early disease sign before parasites can be detected in the blood smear. Alternatively, the kid might have been through a transient subclinical babesiosis. Indeed, low-level parasitaemia and reduced susceptibility to disease due to innate resistance is known to occur in calves (Zintl et al., 2005).

Fatal babesiosis in chamois has been reported in a single animal from Switzerland more than 30 years ago (Bouvier, 1965). Babesiosis has not been observed in free-ranging chamois from Switzerland during at least the past 10 years of our passive surveillance, and the potential impact of this apparently emerging disease on the affected chamois population remains unclear. In case of a newly introduced *Babesia* sp., many fatal cases might be expected (Penzhorn, 2006). An increased mortality was suspected in the chamois population in the Tössstock region in spring 2005 (P. Spörri, personal communication). In contrast, neither increased mortality nor population decrease have been reported in the Simmental and Gantrisch region.

The origin of the *B. divergens/B. capreoli*-like parasites affecting chamois is unknown. Infection through ticks feeding on sympatric cattle and free-ranging cervids has to be considered. Indeed, *Babesia* spp. revealing very high sequence identities with *B. divergens* have recently been isolated in apparently healthy, free-ranging roe and red deer (*Cervus elaphus*) in Slovenia and Poland (Duh et al., 2005; Sawczuk et al., 2005). In order to investigate the susceptibility of chamois to *B. divergens/B. capreoli*-like infection and the possible reservoir role of domestic and/or free-ranging wild ruminants, the prevalence of *Babesia* spp. in these species in the affected areas has to be determined.

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References


