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BABESIA CAPREOLI INFECTIONS IN ALPINE CHAMOIS (*RUPICAPRA R. RUPICAPRA*), ROE DEER (*CAPREOLUS C. CAPREOLUS*) AND RED DEER (*CERVUS ELAPHUS*) FROM SWITZERLAND

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ABSTRACT: Five cases of fatal babesiosis in free-ranging chamois (*Rupicapra r. rupicapra*) attributed to infections with *Babesia capreoli* were recently recorded in two regions of the Swiss Alps. To investigate the ecologic factors that possibly lead to those fatal *B. capreoli* infections in chamois, blood, ticks, and demographic data of 46 roe deer (*Capreolus c. capreolus*), 48 chamois, and nine red deer (*Cervus elaphus*) were collected in 2006 and 2007 in both affected regions. Whereas no parasitic inclusions were found by microscopical examination of blood smears, *B. capreoli* was identified by polymerase chain reaction/sequencing in blood of 12 roe deer (26%, 95% confidence interval [CI]: 14.3–41.1), one chamois (2%, CI: 0–6.1), and one red deer (11%, CI: 0.3–48.2). Prevalence of *B. capreoli* was significantly higher in roe deer compared with chamois ($P<0.001$). All 214 ticks were identified as *Ixodes ricinus*, and significantly more roe deer (63%, CI: 47.5–76.8) were infested compared with chamois (21%, CI: 10.5–35.0, $P<0.001$). Overall, prevalences of both tick infestation and *Babesia* infection increased significantly ($P<0.001$) with decreasing altitude, and *Babesia*-positive samples were detected significantly more often from animals with tick infestation compared with animals without ticks ($P=0.040$). Our results indicate that roe deer may play an important reservoir role for *B. capreoli*. It is hypothesized that the expansion of the presumed vector *I. ricinus* to higher elevations and its increased abundance in overlapping habitats of roe deer and chamois may favor the spillover of *B. capreoli* from roe deer to chamois.

Key words: *Babesia capreoli*, babesiosis, chamois, elevation, epidemiology, red deer, roe deer, Switzerland.

INTRODUCTION

Babesiosis, caused by intraerythrocytic protozoan parasites of the genus *Babesia*, is a globally important tick-transmitted disease. It has been reported in several domestic and free-ranging mammal species and is gaining importance as an emerging zoonosis in humans (Taylor et al., 2001; Herwaldt et al., 2003). Disease ranges from silent infections to hemolytic anemia, cardiovascular shock, and multiple organ failure, depending on factors such as age, immunocompetence, and coinfections of hosts with other pathogens (Homer et al., 2000).

Until recently, reports of fatal babesiosis in free-ranging bovids from Europe were restricted to a chamois (*Rupicapra r. rupicapra*) from Switzerland (Bouvier, 1965) and an ibex (*Capra pyrenaica*) from Spain (Marco et al., 2000). However, in both cases diagnosis was presumptive, and no molecular characterization of the parasites was performed. In 2005 and 2006, fatal hemolytic anemia due to acute babesiosis was diagnosed in five adult chamois found dead in two distinct areas of the Swiss Alps (Hoby et al., 2007). Initial molecular genetic analyses of part of the 18S rRNA gene revealed 99–100% identity with GenBank entries attributed to *Babesia divergens* of cattle origin and *Babesia capreoli* of wild ruminant origin. Further sequence analyses of the rDNA internal transcribed spacers 1 and 2 (ITS1, ITS2) allowed the researchers to distinguish the cattle from the chamois isolates.

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Additionally, *Babesia* isolates of apparently healthy roe deer (*Capreolus c. capreolus*) were indistinguishable by these analyses from the isolates of chamois. Hence, *B. capreoli* was finally considered as the causative agent of the fatal hemolytic anemia in chamois (Schmid et al., 2008).

Babesiosis caused by *B. divergens* sporadically occurs in cattle in Switzerland (Schmid et al., 2008). In contrast, it is not considered as a common disease of chamois, and it has not been observed in free-ranging chamois from Switzerland during at least the past 10 yr of passive surveillance at the Centre for Fish and Wildlife Health (University of Bern, Bern, Switzerland). The occurrence of five fatal cases in the Swiss Alps raised the question of whether babesiosis in chamois should be considered as an emerging disease or whether it has been present in the affected areas for years but remained undiscovered. Studies from Slovenia (Duh et al., 2005), Poland (Sawczuk et al., 2005), and France (Bonnet et al., 2007) have shown that free-ranging cervids may act as a reservoir for *Babesia* spp. that have high sequence identities at the 18S rRNA gene with *B. capreoli* and *B. divergens*. We therefore hypothesized that 1) free-ranging cervids such as roe deer and red deer (*Cervus elaphus*) may act as carriers for *B. capreoli* and thus play a role as reservoir hosts of this piroplasm in Switzerland; and 2) Alpine chamois are less frequently exposed to *Ixodes ricinus* ticks, the likely vector of *B. capreoli*, and thus are highly susceptible to infection and develop fatal babesiosis. To test our hypothesis, we investigated the prevalence of *B. capreoli* infection and tick infestation in free-ranging cervids and chamois from the affected regions.

MATERIALS AND METHODS

The study areas included the Tössstock region (47°19′N, 8°90′E, approximately 100 km^2) at 600 to 1,300 m above sea level (a.s.l.) in the northeastern Swiss Alps and the Simmental/Gantrisch region (46°40′N, 7°2E, approximately 140 km^2) at 650 to 2,100 m a.s.l. in the northwestern Swiss Alps. In both areas, the vegetation is characterized by deciduous, mixed, and coniferous forest and prealpine to alpine grassland. Chamois and roe deer are present in large numbers, whereas red deer are less common (Hausser, 1995; federal hunting statistics 2006 and cantonal population estimations).

Sample kits, including a questionnaire for the collection of demographic and geographic (coordinates, altitude) data, were distributed to local hunters and game wardens before the regular hunting seasons (summer and autumn) of 2006 and 2007. Participants were asked to obtain EDTA blood samples from the thoracic and/or heart cavities of chamois, roe deer, and red deer, to inspect the carcasses for ticks, and to send the samples to the laboratory immediately after collection. In total, 48 chamois, 46 roe deer, and nine red deer samples were collected. Ninety samples originated from hunted animals and 13 from carcasses that did not have pathologic changes associated with babesiosis. Both sexes and all age classes were represented (Table 1).

Blood smears from all animals were prepared immediately, stained with Giemsa and examined under light microscopy (magnification, 1,000×). The rest of the blood samples as well as the ticks were stored at −20 C until analysis. DNA was extracted from blood using a commercially available kit (DNA blood mini kit, QIAGEN, Hombrechtikon, Switzerland) following the manufacturer's instructions. Polymerase chain reaction (PCR) with primers detecting a broad range of *Babesia* spp. was performed, and samples yielding weak positive results were retested in a second PCR specific for *B. capreoli/B. divergens* (Hilpertshauser et al., 2006). Amplicons were directly sequenced by a private company (Microsynth, Balgach, Switzerland). Three of the roe deer samples were used in a parallel study for detailed molecular genetic identification of the isolates (Schmid et al., 2008), confirming the presence of *B. capreoli*. The species, stage, and gender of each tick were determined with a stereomicroscope according to Cotty (1985).

The two-tailed Fisher's exact test (FET) was used to compare prevalences. Differences in elevation distribution of the species were studied with the Mann-Whitney *U*-test (*U*). To assess differences in tick prevalence between the species in relation to elevation, five categories were defined (≤900 m a.s.l., 901–1,200 m a.s.l.; 1,201–1,500 m a.s.l.; 1,501–1,800 m a.s.l.; ≥1,801 m a.s.l.), and the FET was reapplied. The Kruskal-Wallis one–way analysis of variance (ANOVA) was used to reveal differences of *Babesia* and tick

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TABLE 1. Demographic data, geographic origin, prevalences of tick infestation and *Babesia* infections in samples of chamois (*Rupicapra r. rupicapra*), roe deer (*Capreolus c. capreolus*), and red deer (*Cervus elaphus*) collected in 2006 and 2007 in two regions of Switzerland.

	Chamois (n=48)	Roe deer (n=46)	Red deer (n=9)
Demographic data			
Male	29	25	8
Female	19	21	1
0–1 yr	6	9	1
≥1–2 yr	12	18	1
≥2 yr	30	19	7
Origin			
Tössstock	18	38	8
Simmental/Gantrisch	30	8	1
Altitude	1,347 (405)[a]	857 (163)	950 (55)
	610–2,060[b]	570–1,500	800–1,200
Prevalence			
Ixodes ricinus ticks	10/48 (20.8)[c]	29/46 (63.0)	6/9 (66.7)
	10.5–35.0[d]	47.5–76.8	22.9–92.5
Babesia capreoli	1[f]/48 (2.1)[e]	12/46 (26.1)	1/9 (11.1)
	0–6.1[d]	14.3–41.1	0.3–48.2

[a] Mean elevation in meters above sea level (standard deviation).

[b] Altitude range in meters above sea level.

[c] Number of animals infested with *I. ricinus*/total number of animals tested (percentage).

[d] 95% confidence interval.

[e] Number of blood samples positive for *B. capreoli* by polymerase chain reaction and sequencing of part of the 18S rRNA gene/total number of animals tested (percentage).

[f] Apparently healthy 2-yr-old male.

prevalence in relation to elevation. Statistical procedures were performed with the NCSS 2007 software (Number Cruncher Statistical Systems, Kaysville, UT, USA) and Excel (Microsoft, Redmond, Washington, USA). Values of $P<0.05$ were considered significant.

RESULTS

A compilation of demographic data, origin and elevation of samples, prevalence of tick infestation, and prevalence of *B. capreoli* infection as detected by PCR is summarized in Table 1. No parasitic inclusions could be visualized in any of the blood smears. Sequence analysis (385–439 base pairs [bp] of the 18S rRNA gene) of 12 amplicons revealed 99.5–100% identity with GenBank entries attributed to *B. capreoli* of wild ruminant origin and two retested samples reacted positive in the second specific PCR. The prevalence of *B. capreoli* was significantly higher in roe deer compared with chamois (FET, $P<0.001$), whereas no significant differences were noted between roe deer and red deer (FET, $P=0.433$) or chamois and red deer (FET, $P=0.289$). Prevalence of *B. capreoli* infection in roe deer did not differ between sexes and age classes.

In total, 214 ticks were obtained from the three ruminant species; all were identified as *I. ricinus* (169 adult females and 45 males). Chamois were sampled at significantly higher elevations than roe deer (U, $P<0.001$) and red deer (U, $P=0.003$). Ticks were more often collected from roe deer (FET, $P<0.001$) and red deer (FET, $P=0.011$) than chamois. However, prevalence of tick infestation did not differ between species within the same elevation category (FET, $P=0.078$–1.0). Overall, both tick (ANOVA, $P<0.001$) and *Babesia* (ANOVA, $P<0.001$) prevalence

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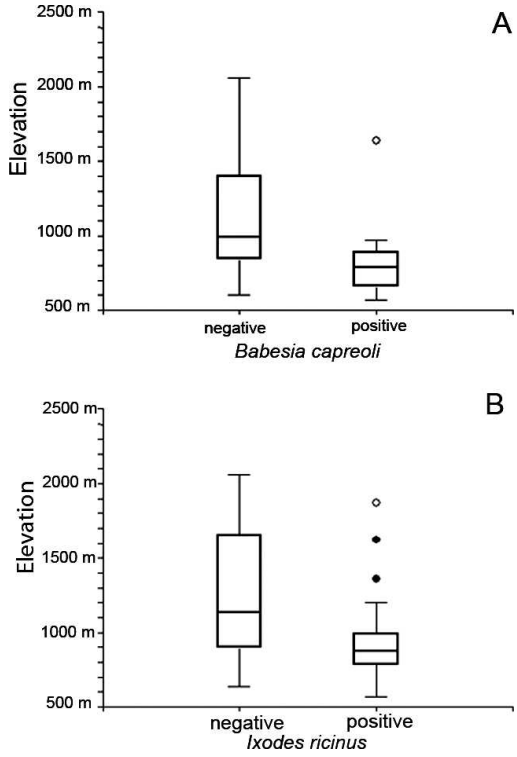


FIGURE 1. Elevation range of sampled wild ungulates (48 chamois, 46 roe deer, and nine red deer) from Switzerland in function of their status of infection/infestation with *Babesia capreoli* (A) and *Ixodes ricinus* (B). Results between *B. capreoli* negatives (n=89; median=1,000 m) and positives (n=14; median=795 m) and between *I. ricinus* negatives (n=58; median=1,140 m) and positives (n=45; median=880 m) were highly significant (one-way analysis of variance, $P<0.001$).

increased significantly with decreasing elevation (Fig. 1), and *Babesia*-positive samples originated significantly more often from animals with tick infestation compared with animals without ticks (FET, P=0.040).

DISCUSSION

DNA of *B. capreoli* was detected in numerous roe deer from both regions where fatal babesiosis has been recorded in chamois. In contrast, only a single positive sample each originated from chamois and red deer. Piroplasms were not detected in the blood smears of any PCR-positive animals examined in this study, whereas parasitic inclusions were abundant in chamois with clinical babesiosis (Hoby et al., 2007). This indicates that blood smear examination may be an important tool to diagnose acute cases of babesiosis but is not appropriate to detect low level parasitemia of *B. capreoli*.

The prevalence of the parasite was significantly higher in roe deer compared with chamois, and no fatal cases have been recorded in roe deer. This supports our hypothesis that roe deer play a role as reservoir host of *B. capreoli*. These results further suggest that *B. capreoli* is endemic in roe deer in our study areas and cannot be considered as an emerging infection in local roe deer populations. Recent investigations in sympatric cattle from the Tössstock region revealed no evidence for babesial infections (Schmid et al., 2008), supporting the hypothesis of a sylvatic cycle of this piroplasm. The epidemiologic role of red deer is unclear. Only nine red deer samples could be examined, of which only one was positive. Presumably, red deer can cope with the infection similarly to roe deer. Due to the minor occurrence of red deer compared with roe deer in the two affected regions, its potential impact as a reservoir there is less important but might be considerable in areas where the species is abundant. The very low prevalence of infection in healthy chamois suggests that chamois are less commonly exposed to infection and/or are highly susceptible to babesiosis. However, to address the significance of the single positive sample from an apparently healthy chamois and better define the susceptibility of chamois to *B. capreoli* infection, further investigations and experimental infections are needed.

Babesia capreoli is most probably transmitted by *I. ricinus*. This is the most abundant tick species in Switzerland (Hilpertshauser et al., 2006), and infected *I. ricinus* have been detected in one of the regions where babesiosis has been seen in chamois (Schmid et al., 2008). In the

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present study, the prevalence of *I. ricinus* was significantly lower in chamois compared with roe deer, which are one of the most important hosts for *I. ricinus* (Carpi et al., 2007). Because no difference was found in prevalence of tick infestation between species within the same elevation category, infestation seems to be more dependent on the elevation than host species. Indeed, it has been demonstrated that *I. ricinus* infestation on roe deer decreases above 1,125 m (Chemini et al., 1997). In contrast to roe deer, chamois use habitats mostly around timberline (approximately 1,800 m a.s.l.), up to 3,000 m (Hausser, 1995). Thus, they are naturally less exposed to both tick infestation and *B. capreoli* infection than roe deer and might be less adapted to this tick-transmitted hemoparasite than roe deer.

There is evidence that the elevation distribution limit of *I. ricinus* shifted considerably toward higher altitudes in central Europe in the past years (Materna et al., 2005; Cadenas et al., 2007). Increasing vector density in natural chamois habitat may put this species at an increased risk of infection with tick-transmitted pathogens (Daniel et al., 2004) and may explain the apparently emerging character of babesiosis in this species; however, the impact of babesiosis on chamois populations in the affected regions remains unclear. If climate change leads to the expansion of tick and possibly roe deer distribution to higher altitudes (Böhm et al., 2001; Leonard et al., 2002; Walther et al., 2002), further cases of fatal babesiosis in chamois may be expected.

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