

Questing *Dermacentor reticulatus* harbouring *Babesia canis* DNA associated with outbreaks of canine babesiosis in the Swiss Midlands

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ABSTRACT

In 2011 and 2012, outbreaks of clinical canine babesiosis were observed in 2 areas of the Swiss Midlands that had no history of this disease so far. In one area, cases of canine babesiosis occurred over 2 consecutive tick seasons. The outbreaks involved 29 dogs, 4 of which died. All dogs were infected with large *Babesia* sp. as diagnosed in Giemsa-stained blood smears and/or PCR. These were identified as *B. canis* (formerly known as *B. canis canis*) by subsequent partial sequencing of the 18S rRNA gene of *Babesia* sp. Interestingly, the sequence indicated either a genotype with heterogeneity in the ssrRNA gene copies or double infection with different *B. canis* isolates. None of the dogs had a recent travel history, but one had frequently travelled to Hungary and had suffered twice from clinical babesiosis 18 and 24 months prior to the outbreak in autumn 2011. Retrospective sequencing of a stored blood DNA sample of this dog revealed *B. canis*, with an identical sequence to the *Babesia* involved in the outbreaks.

For the first time in Switzerland, the partial 18S rRNA gene of *B. canis* could be amplified from DNA isolated from 19 out of 23 adult *Dermacentor reticulatus* ticks flagged in the same area. The sequence was identical to that found in the dogs. Furthermore, one affected dog carried a female *D. reticulatus* tick harbouring *B. canis* DNA. Our findings illustrate that, under favourable biogeographic and climatic conditions, the life-cycle of *B. canis* can relatively rapidly establish itself in previously non-endemic areas. Canine babesiosis should therefore always be a differential diagnosis when dogs with typical clinical signs are presented, regardless of known endemic areas.

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Introduction

Canine babesiosis caused by large *Babesia* spp. occurs in many tropical and subtropical areas worldwide. Formerly, all described large *Babesia* of dogs have been considered as subspecies of *B. canis* (Cacciò et al., 2002), but the different vector tick specificity as well as unambiguous differences in the DNA have led to their reclassification into 3 individual species, namely *B. canis*, *B. rossi*, and *B. vogeli* (Schnittger et al., 2012). *B. canis*, transmitted by the hard tick *Dermacentor reticulatus*, is the most important species in Europe and can cause severe clinical disease in affected animals. The main clinical signs are fever, thrombocytopenia, anaemia, haemoglobinuria, and splenomegaly (Tenter and Deplazes, 2006).

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Even though most of Europe has a climate that could support *D. reticulatus* activity (Beugnet et al., 2009), the distribution of this species is highly focal (Gray et al., 2009). In the past decades, a spread of *D. reticulatus* and also of *B. canis* has been observed, and canine babesiosis is now considered as an emerging infectious disease in multiple European areas (Matijatko et al., 2012). In Germany for instance, new foci of *D. reticulatus* have been described (Dautel et al., 2006). Countries as far north as Belgium (Cochez et al., 2012), the Netherlands (Matjila et al., 2005), and Norway (Øines et al., 2010) have reported the occurrence of *D. reticulatus* or autochthonous infections with *B. canis*. The causes of the geographical extension are not completely understood, but most probably climate changes, habitat suitability, dynamics in host populations, and also anthropogenic factors all contributed (Gray et al., 2009; Léger et al., 2012).

In Switzerland, all reported cases of autochthonous canine babesiosis occurred in the biogeographic region 'Swiss Midlands' (Gonseth et al., 2001; Fig. 1). In this region, however, only Geneva represents a stable endemic focus (Jacquier, 1974; Pfister et al.,

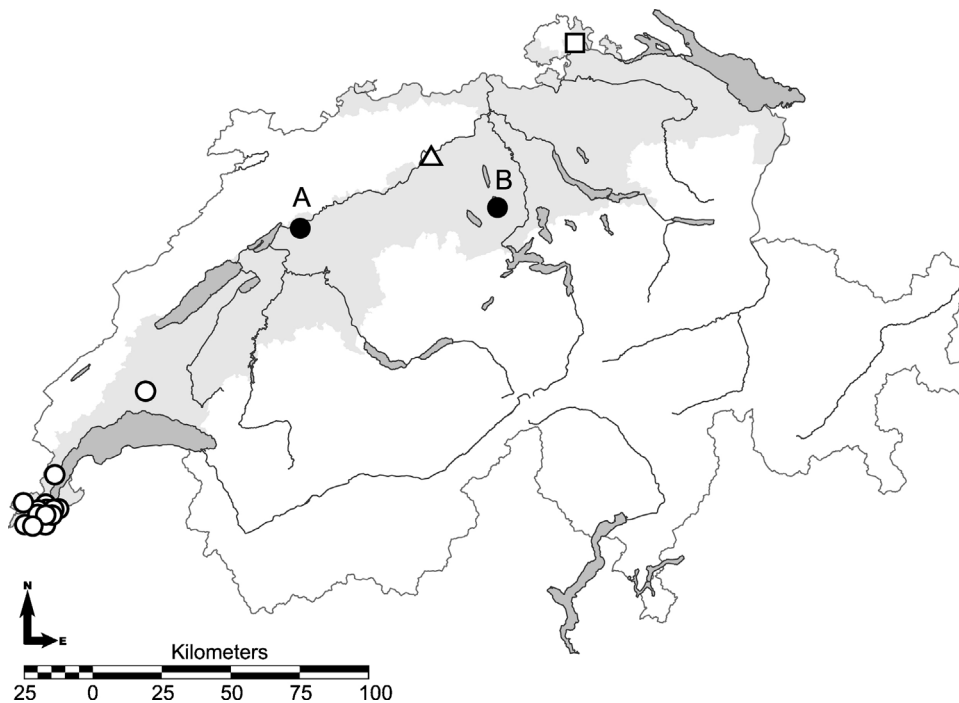


Fig. 1. Map of Switzerland showing locations of different canine babesiosis outbreaks in dogs without travel history. Solid circles show the location of the 2 described outbreaks in Dotzigen (A) and in Baldegg (B). Open circles: Porchet et al. (2007); open triangle: Sager et al. (2005); open square: Schaarschmidt et al. (2006). All reported outbreaks originated from the biogeographic region 'Swiss Midlands' (light-shaded area; Gonseth et al., 2001). Questing adult *D. reticulatus* ticks harbouring *B. canis* DNA were found in area B (geodata: Swiss Federal Office for Environment, CH 3003 Bern, Switzerland).

1993), and further dissemination along Lake Geneva was observed (Porchet et al., 2007; Fig. 1). *D. reticulatus* has only been collected in Geneva, but *B. canis* DNA was not demonstrated in those ticks (Pfister et al., 1993). Central, northern, and eastern Switzerland were historically considered free from canine babesiosis, but some years ago, 2 clustered cases of this disease in dogs with no travel history occurred (Sager et al., 2005; Schaarschmidt et al., 2006). These 2 outbreaks were restricted to one tick season, involved only 6 dogs, and the respective transmitting ticks had not been found. Very little is known regarding molecular characterisation of *B. canis* from Switzerland, with only 2 sequences originating from infected dogs deposited in GenBank (Casati et al., 2006).

Here, we report on 2 outbreaks of canine babesiosis in 2011/2012 in seemingly non-endemic areas of the Swiss Midlands and provide the molecular characterisation of the causative agents involved. In one area, *D. reticulatus* harbouring *B. canis* DNA could be collected for the first time in Switzerland. Anamnestic investigations as well as analysis of climate data during the outbreaks provided some insight into the possible mechanisms of the establishment of new foci for *D. reticulatus* and *B. canis*.

Materials and methods

Areas

The recreational areas of Dotzigen (47.186° N, 7.340° E), area A, and Baldegg (47.186° N, 8.277° E), area B, belong to the biogeographic region 'Swiss Midlands' (Gonseth et al., 2001; Fig. 1). Biogeographic regions are defined by the statistical evaluation of the local flora and fauna (Gonseth et al., 2001). The Swiss Midlands are characterized by a moderate relief with an altitude ranging between 260 m and 960 m above sea level (a.s.l.) (Gonseth et al., 2001) and by a fragmented landscape of deciduous and mixed woodland, agricultural areas, and rivers and lakes (Federal Office

for Spatial Development, 2012). The climate is continental with an Atlantic influence (mean annual rainfall 1203 mm and mean annual temperature 9.1 °C at the Station Biel; Federal Office of Meteorology and Climatology, 2012). The areas A and B are both characterized by the presence of water, a river in the former and a lake in the latter instance, bordered by environmentally valuable shrubs and woods and surrounded by agricultural land such as prairies and fields. Area A is situated about 430 m and area B about 460 m a.s.l. Both areas are very popular for dog walks.

Climate data

In 2011, Switzerland experienced the highest mean temperature since the beginning of the climate recordings in 1864, with 1.8–2.4 °C above the long-term mean temperatures from 1961 to 1990 (Federal Office of Meteorology and Climatology, 2012). More specifically, March to May and August to September were too warm, with >4 °C and >2 °C above the long-term mean, respectively. Rainfall reached only 70% of the long-term mean in area A and 90% in area B (Federal Office of Meteorology and Climatology, 2012). In contrast, February 2012 was characterized by unusually cold temperatures below 0 °C for more than 2 weeks (Federal Office of Meteorology and Climatology, 2012). Spring 2012 was also a dry and outstandingly warm period (2–2.5 °C above the long-term mean), with March reaching temperatures 3–4.5 °C above the long-term mean temperatures (Federal Office of Meteorology and Climatology, 2012).

Dogs

Nine diseased dogs of area A (spring 2011) and 19 diseased dogs of area B (autumn 2011 and spring 2012) could be included in the study. Clinical assessments of the dogs and haematological analyses were carried out. Indirect immunofluorescence antibody

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