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ORIGINAL PAPER

Seroprevalence of canine babesiosis in Hungary suggesting breed predisposition

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Abstract Six hundred fifty-one blood samples were collected from urban and rural dogs in various parts of Hungary to measure antibody levels to *Babesia canis* with indirect fluorescent antibody test. Thirty-seven (5.7%) of the sera showed positivity with titers between 1:80 and 1:10,240. Seroconverted dogs were found in 13 locations of the country. It is concluded that canine babesiosis is becoming more prevalent in Eastern Hungary. Seropositivity increased then declined with age, reaching a maximum in case of 3.1- to 5-year-old dogs. Prevalence of antibodies to *B. canis* was significantly higher among german shepherds and komondors. This suggests a genetic predisposition of german shepherd dogs to chronic babesiosis (carrier status) with long-term maintenance of their seropositivity. On the other hand, heavy-coated komondors are phenotypically more suitable for repeated exposure to ticks, potentially infected with *B. canis*. This is the first report on the seroprevalence of canine babesiosis in Hungary.

Introduction

Babesia canis, the large piroplasm of dogs, is a unicellular parasite infecting red blood cells and transmitted by hard ticks. According to its proposed nomenclature, three subspecies are distinguished: *Babesia canis canis* transmitted by *Dermacentor reticulatus* in the temperate zone, *Babesia canis vogeli* with *Rhipicephalus sanguineus*, and *Babesia canis rossi* with *Haemaphysalis leachi* as their vectors in warmer climates (Uilenberg et al. 1989; Carret et al. 1999). The geographical distribution of the causative agent and thus the occurrence of babesiosis are largely dependent on the habitat of relevant hard tick species, therefore being regarded as endemic to certain regions (Martinod et al. 1986).

In Europe, most autochthonous infections with *B. canis* were detected in southern countries (for review, see Trotz-Williams and Trees 2002). However, with increasing activity of travel, this piroplasm was more and more frequently brought to the north (Gothe and Schmid 1995; Losson et al. 1999), implying a chance for becoming established (Uilenberg et al. 1985; Zahler and Gothe 1997; Sager et al. 2005). Because babesiosis has been indigenous in Hungary for long (Wetzl 1905), it was reported that it could provide the source of several imported cases to Western Europe, particularly to Austria and Germany (Reuss 1993; Gothe and Schmid 1995; Edelhofer 1995; Zahler and Gothe 1997).

The aim of the present study was to undertake an extensive survey to estimate the seroprevalence of canine babesiosis in Hungary due to the lack of data on its current status.

Materials and methods

Blood samples were collected from 651 dogs by saphenous venopuncture between June and August in 2005. Places of origin were the two largest cities in Hungary (Budapest and

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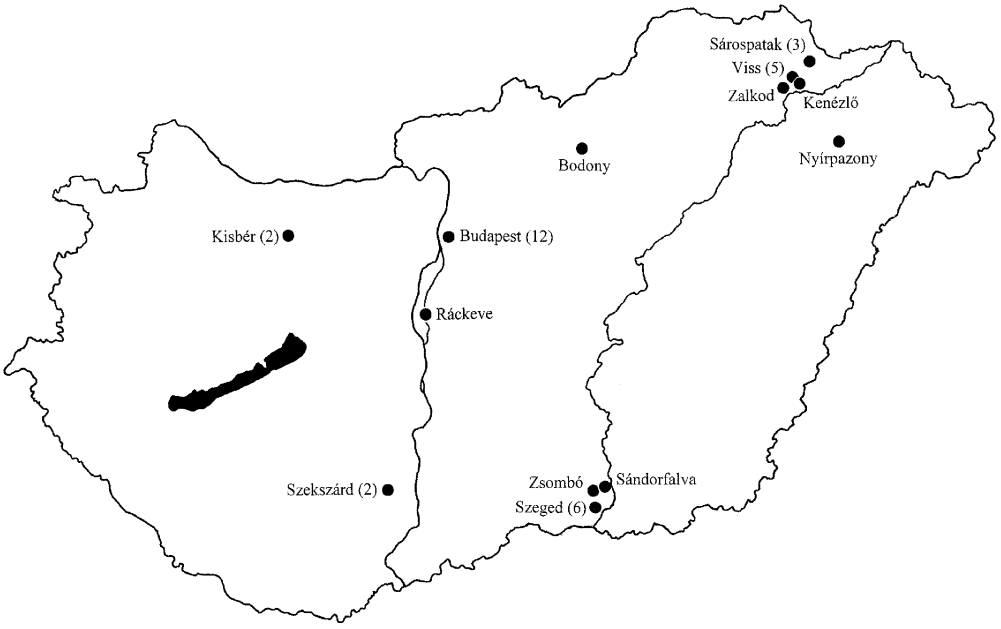

Fig. 1 Geographical distribution of places in Hungary where seropositivity of dogs was detected (if in more than one animal, the appropriate *number* is shown next to the *dot*)

Debrecen) with 338 samples, and 111 other areas in the countryside, including altogether 586 household, 41 farm, and 24 stray dogs in this survey. Data of animals regarding their age, sex, breed, keeping place, and owner status were carefully recorded. Serum was obtained by centrifugation in no later than 2 days then samples were stored at −20°C until evaluation.

Sera were tested for the presence and level of IgG antibodies against *B. canis* by indirect fluorescent antibody test (IFAT). In brief, the anticoagulated whole blood of a naturally infected dog (Hungarian strain) was washed three times in phosphate-buffered saline (PBS, pH 7.2) with centrifugation at 1,500×*g* for 10 min. The pellet was resuspended in PBS containing 1% bovine serum albumin and served as antigen. All sera were screened at dilutions of 1:20 and 1:40 in PBS. Fluorescein isothiocyanate-labeled sheep anti-dog IgG (heavy and light chains) immunoglobulins (The Binding Site, Birmingham, UK) were used as conjugate at a dilution of 1:30 in PBS. Serum samples showing fluorescence at the dilution of 1:40 were further titrated using twofold serial dilutions, of which 1:80 was taken as the positive threshold titer (cut-off value). Sera from dogs naturally infected with *B. canis* were used as positive controls.

Exact confidence intervals for the prevalence rates were calculated according to Sterne's method. Data were

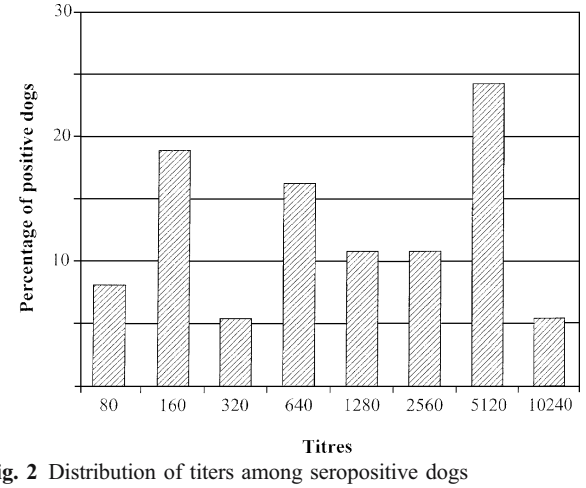

Fig. 2 Distribution of titers among seropositive dogs

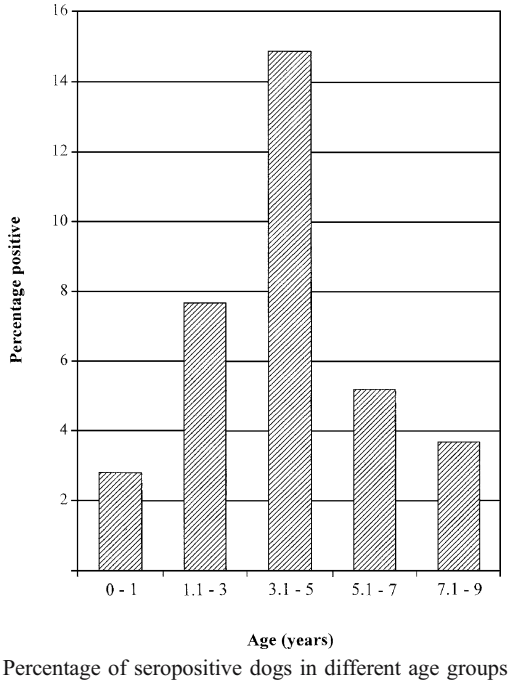

Fig. 3 Percentage of seropositive dogs in different age groups

compared by using Fisher's exact test and differences were regarded significant when $P \leq 0.05$.

Results

Positive samples were detected in 13 places of the country, mostly in East Hungary (Fig. 1). *B. canis* specific antibodies were present in 37 (5.7%; 95% confidence interval: 4.0–7.8%) of the 651 sampled dogs with titers between 1:80 and 1:10,240 in the IFAT (Fig. 2). Four further animals had titers (1:40) below the cut-off value. There was no correlation between titers and age, sex, breed purity, or keeping place.

The mean age of all sampled dogs was 5.4 years and 4 years for animals with seroconversion. Seropositivity increased then declined with age (Fig. 3), reaching a maximum in case of 3.1- to 5-year-old dogs with a significantly higher rate of infection when compared to other age groups (P=0.00013). Seropositivity was not detected above 9 years of age.

No significant differences were demonstrated between the prevalence rates of canine babesiosis among male and female, pure and mixed breed, or urban and rural dogs. Three of the 41 farm dogs and 2 of the 24 stray dogs had seroconversion, which also means a nonsignificant association when compared to household dogs.

However, prevalence of antibodies to *B. canis* was significantly higher among german shepherd (9 out of 53 or 17%, P=0.00166) and komondor dogs (3 out of 4 or 75%, P=0.00065) than in the case of other breeds. The keeping place (five urban and four rural from various regions) and mean age (3.4 years) of german shepherds with seroconversion was not significantly different from that of other breeds showing seropositivity. All three infected komondors lived in different locations of the same county and had high titers (1:2,560 to 1:5,120) in the IFAT.

Discussion

This is the first report on the seroprevalence of canine babesiosis in Hungary. Although infections with *B. canis* are most common in certain regions of Southern Europe, they were documented in several countries on the continent north of the Mediterranean basin, i.e., in Austria, Germany, Belgium, the Czech Republic, and Slovakia (Gothe and Wegerdt 1991; Kucera 1992; Edelhofer 1995; Losson et al. 1999; Chandoga et al. 2002). Autochthonous cases, sometimes suggestive of establishment, were also reported in The Netherlands and in Switzerland, implying a spread towards northern countries (Uilenberg et al. 1985; Pfister et al. 1993; Sager et al. 2005). Most of these infections were imported from surrounding or southern countries (Gothe and Schmid 1995; Zahler and Gothe 1997), underlining the importance to monitor the occurrence of canine babesiosis in endemic areas.

Regarding such countries, there is a relative lack of data on prevalence rates in the literature, in part, because most studies focus on clinical cases. In the present survey, 5.7% of the dogs were found seropositive, which is lower than the 14.1% reported in France (Cabannes et al. 2002). Other studies, for instance, carried out in Italy, showed prevalence rates between 0.8 and 17% (Traldi et al. 1988; Trotz-Williams and Trees 2002). Previous data based on clinical and hematological findings indicate that *B. canis* is endemic in some parts of Western Hungary and Budapest (Horváth and Papp 1996). However, according to the present results, this geographical range was expanded and canine babesiosis is becoming more prevalent in Eastern Hungary. This may be due to a gradual shift in the density of tick species in relevant habitats with a more widespread occurrence of *D. reticulatus*, the biological vector of *B. canis canis* (Földvári and Farkas 2005b; Sréter et al. 2005).

The well-documented seasonality of clinical babesiosis, also observed in Hungary with peaks around March–April and October–November (Martinod and Gilot 1991; Csikós et al. 2001), has no particular influence on serological surveys carried out at other times of the year or during a shorter period because elevated antibody levels may persist for months or even years (Schindler et al. 1966). Predominantly higher titers observed in the present study than those detected after experimental or natural infections (Schindler et al. 1966; Weiland and Kratzer 1979; Cabannes et al. 2002) may reflect repeated exposure and/or the previously documented high immunogenicity of the Hungarian isolate of *B. canis* when compared to others (Hauschild et al. 1995). Antibodies were most prevalent in the age group of 3.1 to 5 years, which is in contrast with the findings of Cabannes et al. (2002) who demonstrated a peak rate of infection between 6 and 10 years of age. However, both results are in accordance with the observations that dogs 3 years of age or older may have a significantly higher risk of being seropositive to *B. canis* than dogs below 1 year of age (Yamane et al. 1994). Older dogs, on the other hand, are usually less active and in this way, less subjected to environmental pressure for infestation with ticks (lowering the chance for the transmission of *B. canis*), which may explain the apparent absence of seroconversion in dogs above 9 years of age in the present study.

The lack of significant differences between urban and rural dogs in their seropositivity may indicate a similar level of exposure to potentially infected vectors because urban dogs are frequently taken on excursions. No association was found between the status of seroconversion and dog keeping on farms, as supported by the contradic-

tion in the literature, some reporting a lower (Vidor et al. 1989), while others a higher rate of infection in farm dogs (Wlosniewski et al. 1997). Similarly, low prevalence rates among stray dogs are consistent with the findings of others (Deplazes et al. 1995).

Predisposition of german shepherd and komondor breeds to babesiosis was hitherto not documented, although Bizzeti et al. (1997) described high prevalence rate (61.3%) among 31 asymptomatic german shepherds, and Tarello (2001) found an association between babesiosis and the immunity-dependent manifestation of fistulas in this breed. Because a previous study (Martinod et al. 1986) based on clinical and hematological examination did not find german shepherd dogs more susceptible to *B. canis* infection than others, the present results may indicate genetic predisposition to chronic (subclinical) babesiosis with a long-term carrier status and maintenance of seropositivity in this breed. Parasite clearance is effected by T cells during babesiosis (Homer et al. 2000). German shepherds were also demonstrated to have such deficiency in cell-mediated immunity to *Ehrlichia canis* that does not simultaneously alter the level of their specific humoral response, but makes them prone to develop chronic ehrlichiosis (Nyindo et al. 1980).

On the other hand, more frequent infections of komondor dogs may be explained by their phenotype. The heavy hair coat is essentially an easy-to-catch target for hard ticks, which then can remain unnoticed by the owner for long, consequently with a higher chance for the transmission of or repeated exposure to *B. canis*. This is further substantiated by the high titers all three seropositive komondors had. Increased susceptibility or resistance of other breeds to babesiosis reported elsewhere (Martinod et al. 1986) was not observed in the present study.

The subspecies of large babesia identified in Hungary is *B. canis canis* (Földvári and Farkas 2005a) as its vector, *D. reticulatus* is widespread (Földvári and Farkas 2005b). However, the geographical region of another subspecies seems to expand towards the north as the formerly African ssp. *B. canis vogeli* was reported from France, Spain, and Slovenia (Zahler et al. 1998; Caccio et al. 2002; Duh et al. 2004). Recently, autochthonous infestation with its hard tick vector, *R. sanguineus*, was recognized (Hornok and Farkas 2005) and unidentified small babesiae were also detected in Hungary (Farkas et al. 2004). These findings confirm the need for further studies on the etiology and epidemiology of canine babesiosis in Hungary.

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