



EPIZOOTIOLOGICAL STUDY OF THE OCCURRENCE OF CANINE BABESIOSIS IN SOUTHWESTERN SLOVAKIA

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ABSTRACT

This epizootiological study was carried out to investigate the occurrence of canine babesiosis in southwestern Slovakia. The study focused on the proportion of the species of ticks serving as babesia vectors of babesiosis in the ticks collected from selected locations close to Komárno, in southwestern Slovakia. Additionally, observations were made on the health and overall clinical signs in dogs suspected of having babesiosis. In 2014 we collected ticks from vegetation by the drag cloth (flagging) method and also directly from dogs. A totally of 622 ticks were collected: 491 *Dermacentor reticulatus* and 131 *Ixodes ricinus*. Representative samples of ticks (n=103) were examined by the molecular method and the presence of *Babesia* spp. DNA was identified in 12.5 % of the *Ixodes ricinus* ticks collected by flagging and in 9.5 % of the *Ixodes ricinus* ticks collected from the dogs. *Babesia canis* (KU681325) with 90 bp sequence, 100 % identical with *Babesia canis* isolates from dogs for example from: Turkey (KF499115), Rumania (HQ662634), Croatia (FJ209025), Poland (EU622792) and Russia (AY962186),

was confirmed after sequencing in one *Ixodes ricinus* female obtained from a dog. This was the first confirmation of the occurrence of *B. canis* in *Ixodes ricinus* ticks in Slovakia. In 2.2 % of the *Dermacentor reticulatus* ticks obtained from vegetation by flagging, we were able to diagnose the DNA of *Babesia canis*. In 4.8 % of the *Dermacentor reticulatus* ticks collected from dogs, the presence of *Babesia* spp. was confirmed. Thirty three dogs with suspicion of babesiosis were observed in an ambulance by their health and clinical signs. The loss of appetite was observed in 22 patients (66.7 %), apathy in 19 cases (57.6 %), and fever in 19 cases. Closer specification indicating babesiosis was finding engorged ticks on the dog bodies which occurred in 21 cases (63.6 %), haematuria in 8 cases (24.2 %), anaemia in 4 cases (12.1 %), and tremor in 6 cases (18.2 %). Other non-specific signs, such as diarrhoea, vomitus, slowed-down movement, ataxia and lacrimation were also observed in less than 18 % of the examined dogs. On the basis of the specific clinical signs, blood was withdrawn from 33 dogs for preparation of blood smears and in 19 of them (57.6 %) babesia in erythrocytes were confirmed microscopically.

Key words: *Babesia*; *Dermacentor marginatus*; dog; epizootiology; *Ixodes ricinus*; PCR; prevalence

INTRODUCTION

During the past two decades, the number of tick-borne pathogens has increased and currently ticks raise serious health problems in many European regions. Due to climate changes which affect the biology of these vectors, they are spreading tick-borne diseases. Babesiosis is a protozoan disease with a worldwide distribution which affects particularly the blood and reticuloendothelial system of mammals. Babesiosis occurs more frequently and is becoming an increasing veterinary problem because changing global conditions effects the spread of ticks which serve as the vector. In Slovakia, the highest prevalence of babesiosis has been recorded in the southwestern and southeastern regions.

The aim of this study was to investigate the occurrence of babesiosis in dogs in the southwestern region of Slovakia.

MATERIALS AND METHODS

The collection of ticks from canine patients, sampling of blood and preparation of blood smears was carried out at a private ambulance in Komárno, located in southwestern Slovakia. The drag cloth (flagging) method was used to col-

lect ticks from vegetation in this region. Blood smears were stained by the Diff Quick procedure [6] and evaluated microscopically at $\times 100$ magnification using oil immersion. In addition, we analysed the sequence of partial 18S rRNA of babesia from ticks by means of the PCR amplification of approximately 450 bp long fragment of 18S rRNA gene using primers BJ1 (5'GTCTTGTAATTGGAATGATGG3') and BN2 (5'TAGTTTATGGTTAGGACTACG3') [1].

Positive samples were purified employing a commercial kit ISOLATE II PCR, supplied by Bioline, and subsequently subjected to sequencing.

RESULTS

We collected a total of 622 ticks: 464 by flagging (59 *Ixodes ricinus* and 405 *Dermacentor reticulatus*) and 158 directly from the dogs (86 *Dermacentor reticulatus* and 72 *Ixodes ricinus*). Thirty three patients with suspected babesiosis were observed in a private ambulance for the clinical signs of the disease. A loss of appetite was observed in 22 dogs (66.7%), apathy in 19 (57.6%) and 19 patients had fevers. Additional clinical signs included: the presence of engorged ticks on dog bodies in 21 cases (63.6%), haematuria in 8 cases (24.2%), anaemia in 4 cases (12.1%), and tremor in 6 cases (18.2%). Other non-specific signs, such as diarrhoea, vomiting, slowed down movement, ataxia and lacrimation were observed in less than 18% of the patients. In order to confirm babesiosis, complete examination of

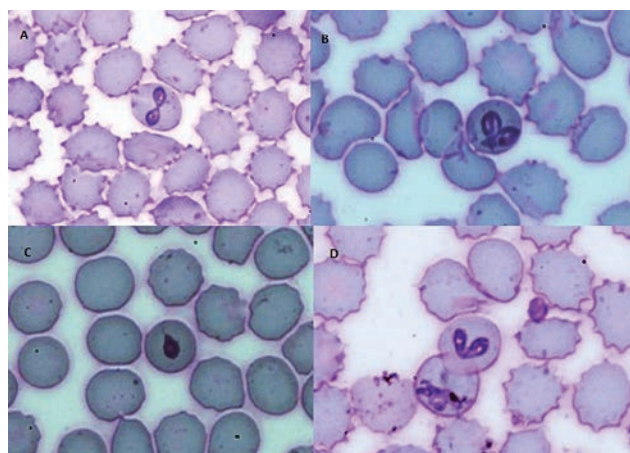


Fig. 1. *Babesia* in erythrocytes: A, D – Howell-Jolly bodies; A, B, D – pairs of pear-shaped *Babesia* in erythrocytes; C – tear-shaped *Babesia*; D – Maltese Cross-tetrahedral forms of *Babesia* (original pictures). Magn. $\times 100$

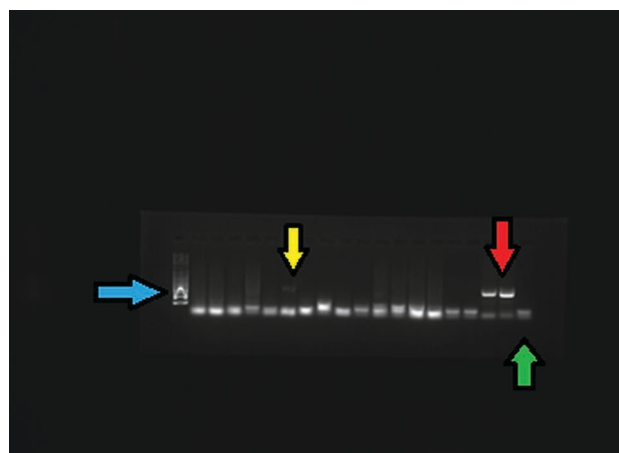


Fig. 2. Result of electrophoretic examination. Blue arrow points to DNA ladder, yellow arrow points to positive sample. Red and green arrows indicate positive and negative controls, respectively

the blood was carried out. The total number of dogs with suspicion of babesiosis was 33 and the disease was confirmed in all of them.

Blood smears from 33 patients were examined microscopically in the laboratory of the Parasitological Institute of the University of Veterinary Medicine and Pharmacy (UVMP) in Košice. The examination of blood smears showed the presence of typical forms of the parasite, such as tear-shaped forms (Fig. 1 C) and pairs of pear-shaped forms (Fig. 1 A, B, D), and also the so-called Maltese Cross-tetrahedral forms (Fig. 1 D) with light cytoplasm. The typical manifestation of haemolytic anaemia was the changed shape of erythrocytes which occurred in the form of echinocytes; the so-called Howell-Jolly bodies were also present (Fig. 1, upper left).

From the 622 collected ticks, we formed representative samples ($n = 103$), which were examined by the PCR for the presence of the DNA of babesia. The DNA of *Babesia* spp. was detected using this molecular method, in 2/16 ticks (prevalence 12.5 %) of the species of *Ixodes ricinus* which were collected by flagging (Fig. 2). In 1/45 ticks (prevalence 2.2 %) of *Dermacentor reticulatus* we diagnosed *Babesia canis canis*. The PCR examination of ticks collected directly from dogs presented the DNA of *Babesia* spp. in 2/21 (prevalence 9.5 %) of the *Ixodes ricinus*. The sequencing showed that one of these ticks was infected with *Babesia canis canis* (KU681325), with 490 bp sequence, which was 100 % identical with isolates of *Babesia canis* from dogs, for example from: Turkey (KF499115), Rumania (HQ662634), Croatia (FJ209025), Poland (EU622792), Russia (AY962186) and others. In 1/21 ticks (prevalence 4.8 %) of the *Dermacentor reticulatus*, we diagnosed the presence of *Babesia* spp. This was the first confirmed occurrence of DNA of *B. canis* in *Ixodes ricinus* ticks in Slovakia.

DISCUSSION

The dynamics of the spread of babesiosis in Europe has changed considerably in recent years. These changes have been ascribed frequently to the evidence of the extension of geographic territory and vertical distribution of ticks. Canine babesiosis has spread also to Slovakia, with the first clinical cases recorded in the eastern part of the country around Michalovce. Many authors in different studies have

discussed the issue of the most suitable and reliable method for the diagnosis of babesia. The diagnostic methods used for the detection of *Babesia* spp. infection differ in their successfulness. *Babesia* spp. are commonly detected by microscopic examination of blood smears but this technique has some limitations with regard to its low sensitivity with low parasitaemia and difficulties with distinguishing morphologically similar species. [3]. The method based on the proof of antigens of immunofluorescent antibodies is also used to assess exposure of dogs to *Babesia* spp. infection, but the specificity of this method is low due to antigenic cross reactivity [5, 6]. This method also fails to determine whether an ongoing infection is involved. The alternative techniques, such as polymerase chain reaction (PCR), were indicated by several authors as the most suitable way of detection and identification of *Babesia* spp. [7]. Despite the low recovery of parasites at low parasitaemia, the microscopic examination of blood smears is still one of the most frequently used methods owing to its simplicity and availability [5].

The first occurrence of canine babesiosis in Slovakia was reported by Chandoga et al. in 2001 [2]. Today this disease is a common seasonal health problem in the respective regions presented. This was confirmed by the study of Kubelová et al. [4] which focused on the prevalence of *Babesia canis canis* in *D. reticulatus* ticks in southeastern, southwestern and west Slovakia. The results presented by these authors confirmed that the highest prevalence of *B. canis canis* (14.7 %) was in the area around Michalovce. A significantly lower prevalence (2.3 %) was detected in the Gabčíkovo region (southwestern Slovakia). Our results showed that in the Komárno region (southwestern) the prevalence of *Babesia* spp. in *Ixodes ricinus* ticks reached 12.5 % and that of a *Babesia canis canis* in *Dermacentor reticulatus* reached 2.2 %.

CONCLUSIONS

This study provided the first proof of the occurrence of the DNA of *B. canis canis* in ticks of the species *Ixodes ricinus*. Up to this date, no study carried out in Slovakia reported *Ixodes ricinus* as a babesiosis vector.

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