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Original papers

Dermacentor reticulatus (Fabricius, 1794) and *Babesia canis* (Piana et Galli-Valerio, 1895) as the parasites of companion animals (dogs and cats) in the Wrocław area, south-western Poland

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ABSTRACT. Tests performed in 2013 and 2014 revealed the occurrence of three tick species parasitizing pet cats and dogs in the Wrocław Agglomeration. In total, 1,455 tick specimens were removed from 931 hosts (760 dogs and 171 cats) in 18 veterinary clinics. The dominant tick species was *Ixodes ricinus* (n=1272; 87.4%), followed by *I. hexagonus* (n=137; 9.4%) and *Dermacentor reticulatus* (n=46; 3.2%). Females were the most often collected development stage among *I. ricinus* and *D. reticulatus,* and nymphs among *I. hexagonus*. Additionally, *D. reticulatus* ticks (n=337) were then collected from vegetation in the Wrocław area to detect *Babesia canis*; however, none was found positive. Only 9.0% of dog blood samples sent to VETLAB were positive for *Babesia* spp. Negative results for *B. canis* from ticks may result from the short period of the occurrence of *D. reticulatus* in the Wrocław area and therefore the vector-pathogen cycle may not have been fully established at the time of the study. Nevertheless, *D. reticulatus* is expanding its range, and the size of its population in the Wrocław Agglomeration is increasing. The presence of the pathogenic *Babesia* spp. combined with the occurrence of its main vector¸ *D. reticulatus*, suggests that the epizootiological situation in the area can change and may pose a new veterinary problem in the future.

Key words: *Dermacentor reticulatus*, *Babesia canis*, pets, Wrocław, Poland

Introduction

From the veterinary and medical points of view, two of the most dangerous of the 16 hard tick species (Ixodidae) constituting a permanent component of Polish fauna [1,2] are *Ixodes ricinus* (Linnaeus, 1758), recognized as the main vector of Lyme disease and posing the greatest risk for people, and *Dermacentor reticulatus* (Fabricius, 1794), the vector of the unicellular parasite,

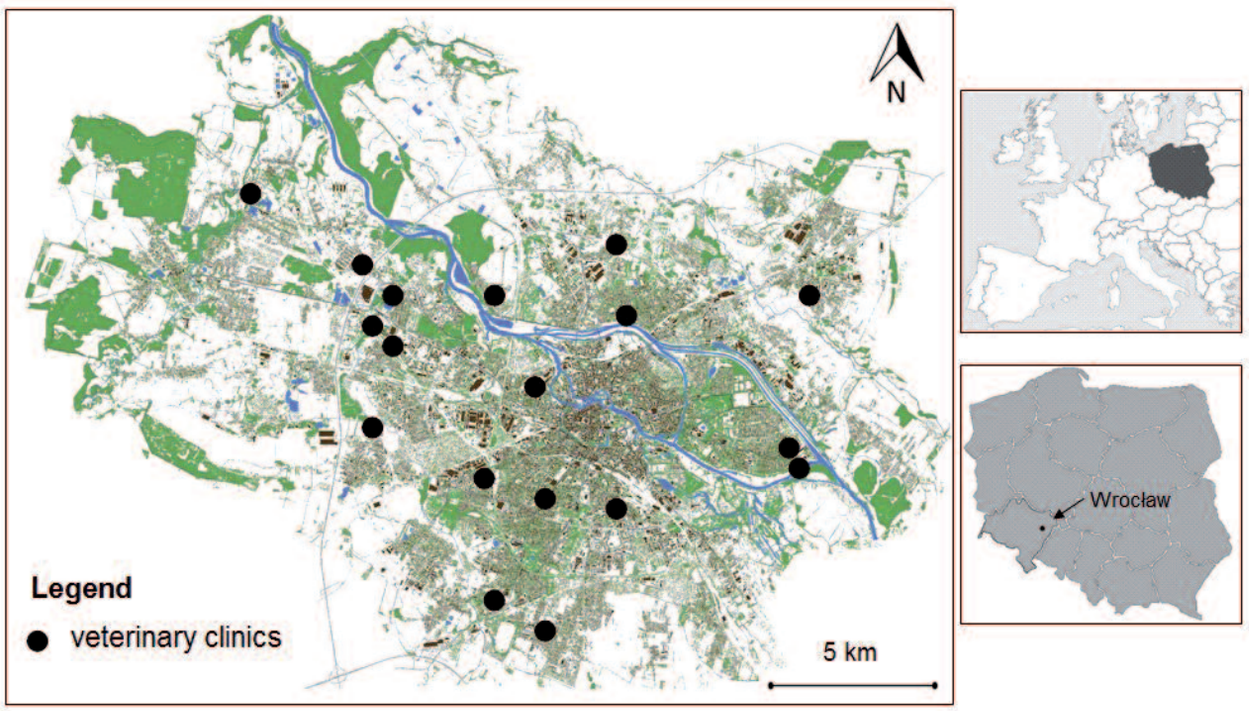

Fig. 1. The location of 18 veterinary clinics in the Wrocław Agglomeration (SW Poland), 2013–2014; (Wroclaw Spatial Information System)

piroplasma (*Babesia canis*), which represents a health problem mostly for dogs and wild canids, such as wolves or foxes [3,4]. The prevalence of *B. canis* in the wild canids is documented usually in clinical cases. It had also been reported that only red foxes were infected by *Babesia microti*-like small piroplasm [5–8].

Apart from these two tick species, three more in Poland can parasitize dogs and cats, but with lower transmitting importance, i.e., *I. hexagonus* Leach, 1815, *I. rugicollis* Schulze et Schlottke, 1929, and *I. crenellates* Koch, 1844. Additionally, *Rhipicephalus sanguineus* (Latreille, 1806) has been noted in Poland as an adventive species.

Canine babesiosis, one of the most common tick-borne diseases (TBD) among dogs, can be caused by different *Babesia* species depending on the geographical region [9,10]. *B. vogeli* is usually transmitted by *Rh. sanguineus* ticks and occurs in subtropical and tropical climatic zones; *B. rossi* is found mainly in Africa and is carried by ticks from the genus *Haemaphysalis* Koch, 1844; *B. gibsoni* mostly in Asia with the vector *H. longicornis* Neumann, 1901; *B. conradae* has been identified in the USA and has no known vectors. *B. canis* has been described in Europe, and is vectored by *D. reticulatus* and *Rh. sanguineus* ticks.

It is known that the risk of babesiosis, just as any other TBD, is strictly associated with the size of the tick population in the habitats, especially in urban areas, where the potential hosts (e.g., dogs, cats) are present, together with a suitable infection level of ticks. Although it is very important to estimate the risk of TBD among animals, the process is a difficult one. Tick infestation and infection with pathogens depend on many inter-related biotic and abiotic factors [11]. Complex studies are very helpful in such cases, combining field research, evaluating the presence and the abundance of ticks in the environment and their prevalence and intensity on hosts, with laboratory tests based on the molecular detection of pathogens transmitted by ticks.

The aim of the study was to evaluate the epizootic situation of babesiosis in the Wrocław Agglomeration, SW Poland, by determining pet infestation by ticks, especially *D. reticulatus,* and the prevalence of *Babesia* spp.

Materials and Methods

In the years 2013 and 2014, ticks were collected from dogs (n=760) and cats (n=171) in 18 veterinary clinics located in the Wrocław Agglomeration, in the south-western part of Poland (Fig. 1). In March and November 2014 and 2015, *D. reticulatus* ticks were also collected from

vegetation. Tick specimens were determined by life stage, sex and species [12].

To detect pathogen DNA, ticks from dogs and cats were kept in 70% ethanol, while questing *D. reticulatus* were stored alive at 4°C until DNA extraction was performed. Before the procedure, ticks were washed in distilled water. The ammonium hydroxide (NH_4OH) method was used to extract the DNA of unfed ticks [13]. In the case of engorged ticks, a Tissue Genomic Extraction GPB Mini Kit with proteinase K (Genoplast Biochemicals, Poland) was used according to the manufacturer's instructions. The obtained lysates were stored at −20°C for further analysis. To amplify *B. canis* DNA, PCR was performed for all *D. reticulatus* ticks obtained from cats and dogs (n=46), or from vegetation (n=337) with the BAB1 forward primer and BAB3 reverse primer [14].

Additionally, to determine the extent of *B. canis* infection in pet dogs, blood samples were obtained from the dogs by veterinary physicians at their clinics between January 2013 and August 2015. The blood was drawn into EDTA tubes and sent to VETLAB Veterinary Laboratory in Wrocław for testing. It is important to note that these dogs were not the hosts of the ticks tested for *B. canis* infection. The blood smears were stained with May-Grünwald-Giemsa (1000×) in order to detect blood parasites (*Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., *Mikrofilaria* spp., *Mycoplasma* spp.).

Results

In total, 1455 tick specimens were removed from 931 pets (760 dogs and 171 cats) in 18 veterinary clinics, and 337 *D. reticulatus* ticks were collected from vegetation. The dominant tick species removed from cats and dogs was *I. ricinus* (n=1272; 87.4%), followed by *I. hexagonus* (n=137; 9.4%) and *D. reticulatus* (n=137; 3.2%). Females comprised the most frequently collected development stage for *I. ricinus* (91.2%) and *D. reticulatus* (67.4%), while the nymph was the most common form of *I. hexagonus* (71.5%) (Table 1).

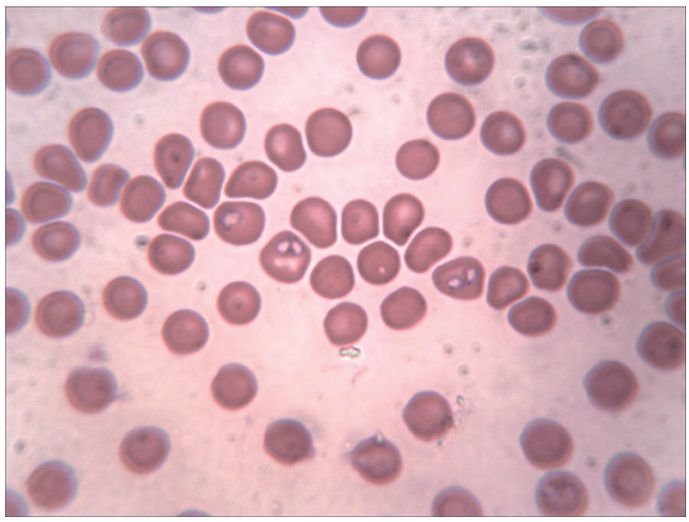

Fig. 2. Positive *Babesia* spp. (merozoite) blood smear from the dog (orig.)

D. reticulatus ticks (n=46) were removed mainly from dogs (n=34) and only two cats. While all *D. reticulatus* females (n=31) were attached to hosts and were engorged, this wasn't true for any of the male ticks. Both ticks specimens collected from cats were females.

PCR tests revealed that none of *D. reticulatus* adult ticks collected from vegetation (n=337) nor from cats and dogs (n=46) was positive for *B. canis*. However, blood smears confirmed the presence of *Babesia* spp. (Fig. 2) in 9.0% (n=36) of dog blood samples (n=401).

Table 1. Structure of tick population collected from pets (dogs and cats) in the Wrocław Agglomeration (SW Poland), 2013–2014

Species	number (%) of tick stages				
	females	**males**	**nymphs**	**larvae**	**Total**
Dermacentor reticulatus	31 (67.4%)	15 (32.6%)	0	0	**46 (100%)**
Ixodes hexagonus	32 (23.4%)	0	98 (71.5%)	7 (5.1%)	**137 (100%)**
Ixodes ricinus	1160 (91.2%)	103 (8.1%)	9 (0.7%)	0	**1272 (100%)**
Total	1223 (84.1%)	118 (8.1%)	107 (7.4%)	7 (0.5%)	**1455 (100%)**

Discussion

Our results confirm that *I. ricinus* and *D. reticulatus* are the most common species of ticks collected from pets in Poland [15–18]. *D. reticulatus* appears to be expanding its distribution range in western Poland [19–24] but *I. ricinus* remains the dominant tick species in that area. In southern European countries, pets are attacked by a wider range of tick species than in Poland. For example, pets in Hungary are attacked by six species: *D. reticulatus* (46.8%), *I. ricinus* (43.2%), *I. canisuga* (5.6%), *Haemaphysalis concinna* (2%) and only one specimen of *D. marginatus* and *I. hexagonus* [32]. In Belgium, the most commonly removed tick species from pets is *I. ricinus* (76.4%), followed by *I. hexagonus* (22.6%); *Rhipicephalus sanguineus* (0.3%) and *D. reticulatus* (0.8%) are in the minority [28].

All the *D. reticulatus* specimens collected as part of the present study, either from vegetation (n=337) or from dogs (n=44) and cats (n=2), were found to be negative for *Babesia canis* infection. This result is in keeping with previous studies conducted by Mierzejewska et al. [25] who confirmed the presence of *Babesia* spp. only in ticks collected from the Eastern population of *D. reticulatus* ticks in Poland, east of the Vistula River. The prevalence of *Babesia* spp. in *D. reticulatus* ticks varies across Eastern and Central Poland. In Warsaw, 11% of *D. reticulatus* removed from dogs were found to be infected [26], while the prevalence in ticks collected from vegetation ranges from 0 to 14.8% [25]. Also, most cases of canine babesiosis in Poland are recorded in the eastern part of the country [27]. In Western Europe, depending on the study, the level of *Babesia* spp. infection in *D. reticulatus* has been found to be zero in Belgium [28] or low, 1.6% in Belgium and the Netherlands [29]. In Slovakia [30] studies conducted on questing *D. reticulatus* ticks revealed the prevalence of *B. canis* only in females (1%). However, a few years later Kubelová et al. [31] detected *B. canis* DNA in both females (3.5%) and males (1.9%). In Hungary [32] only female *D. reticulatus* ticks were examined for *B. canis* and the prevalence was very high (29.9%).

However, the detection of *Babesia* spp. in 9.0% (n=36) dog blood samples highlights the risk of canine babesiosis around the area of Wrocław, the capital of Lower Silesia, in south-western Poland. These results emphasise the need for constant monitoring of ticks and their infections to control the epizootiological situation in the area. It has been shown that tick intensity is the same in both high-impact and low-impact anthropogenic areas [33]. The lack of *Babesia canis* infection in the *D. reticulatus* ticks in the Wrocław area could be attributed to the short period of occurrence of these ticks in the area, the possibility that the infection level is very low or the number of tested ticks was not enough to detect infection.

Conclusions

Dermacentor reticulatus, the main vector of *Babesia canis*, the aetiological agent of canine babesiosis, known to be endemic in north-eastern (Warmińsko-Mazurskie Voivodeship) and central Poland (Masovia Voivodeship), was found on dogs and cats, as well as on vegetation in Wrocław, south-western Poland. Pets were also parasitized by two others tick species, *Ixodes ricinus* which was dominating one and *I. hexagonus*.

The lack of detection of *B. canis* in tested samples of the collected *D. reticulatus* ticks may be attributed to two causes. Firstly, the presence of *D. reticulatus* in Wrocław, an area historically free of this tick species, is short and hence, the host-parasite relationship may not have been fully established. Secondly, the infection level of piroplasms in the ticks is low.

Nevertheless, this appearance of *Babesia* spp. infections in dogs may be the first sign of canine babesiosis in the Wrocław area. The presence of *D. reticulatus* ticks combined with that of canine piroplasmosis can pose a problem for veterinary services. Therefore, constant environmental and laboratory monitoring of ticks should be provided.

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