Ticks and Tick-borne Diseases xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Short communication

Uneven seasonal distribution of *Babesia canis* and its two 18S rDNA genotypes in questing *Dermacentor reticulatus* ticks in urban habitats

Sándor Hornok[a,*], Kitti Kartali[a], Nóra Takács[a], Regina Hofmann-Lehmann[b]

[a] *Department of Parasitology and Zoology, Faculty of Veterinary Science, Szent István University, Budapest, Hungary*
[b] *Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland*

ARTICLE INFO

Article history:
Received 23 December 2015
Received in revised form 13 February 2016
Accepted 22 February 2016
Available online xxx

Keywords:
Dermacentor reticulatus
Canine babesiosis
Seasonality
Urban biotopes

ABSTRACT

It has been reported from cities in Central Europe that clinical cases of canine babesiosis are most frequent in spring time, despite the fact that the peak activity of *Dermacentor reticulatus* (the vector of *Babesia canis*) is during autumn. The present study was initiated to evaluate the seasonal distribution of *B. canis*-infected *D. reticulatus* ticks in this context.

In two habitats of Budapest 852 *D. reticulatus* adults were collected between August, 2014 and June, 2015. Among the molecularly analysed 413 ticks 8.2% were PCR positive for piroplasms. Both formerly reported 18S rDNA genotypes of *B. canis*: ("A" and "B") were identified. In habitat-1 *B. canis*-infected ticks were detected only in spring. Similarly, in habitat-2 *B. canis*-infected ticks occurred significantly more frequently during winter and spring than in the autumn (24.6% vs. 1.4%), and their monthly distribution showed significant negative correlation with tick size. The prevalence of infected ticks was the highest (43.5%) in late February. In addition, a month-dependent time-shift was noted in the appearance of the two *B. canis* 18S rDNA genotypes: the less pathogenic "A" predominating earlier, and the more pathogenic "B" later.

It is known from literature that *D. reticulatus* individuals that moult to adult in the spring are smaller in size. Thus, the above results suggest that in urban habitats the occurrence of *B. canis*-infected ticks (or their questing activity) is more likely, when there are freshly emerged adults in the population, i.e. early in the questing season. It was also observed that the temporal distribution of *D. reticulatus* ticks carrying different *B. canis* genotypes was not random.

1. Introduction

Hard ticks (Acari: Ixodidae) transmit a variety of pathogenic microorganisms greater than any other vector arthropods (Jongejan and Uilenberg, 2004). Highly relevant to the vector role of ticks, several aspects of the spatio-temporal variations of pathogen prevalence in ticks have been demonstrated, which will significantly influence the epidemiology of relevant tick-borne diseases. For instance, pathogen-carrier ticks may quest for a host higher on the vegetation (Romashchenko et al., 2012) or may concentrate in small foci in an endemic area (Goethert and Telford, 2009). Concerning seasonal changes in pathogen prevalence, the majority of studies have focused on *Ixodes ricinus*. In this tick species the prevalence of infection with *Anaplasma phagocytophilum* (Polin et al., 2004), with *Rickettsia helvetica* (Kantsø et al., 2010) and with *Borrelia burgdorferi* s.l. (Mysterud et al., 2013) was the highest in the spring.

However, to the best of our knowledge, no such phenomena were reported in the case of *Dermacentor* spp. *Dermacentor reticulatus* is the species of the genus with the most northern distribution in Europe, occurring in all countries of the continent except Ireland, Iceland and Scandinavia. In support of its increasing importance, the geographical range of *D. reticulatus* was observed to expand in Central, then towards both North-Western and North-Eastern Europe over the past decades (Karbowiak, 2014). Furthermore, *D. reticulatus* is frequently found in urban biotopes (Hornok et al., 2014a). It is a competent vector of *Babesia canis*, an important protozoan parasite of dogs. The life cycle of *D. reticulatus* (from egg laying in late spring, through the activity of larvae and nymphs in summer, until the emergence of adults in autumn) can be completed in one vegetation period, but can be extended to two years when adults hybernate twice before feeding (Nosek, 1972).

Studies (encompassing several years) in the regions of large cities in Central Europe attest that clinical cases of canine

* Corresponding author. Tel.: +36 1 478 4187; fax: +36 1 478 4193.
E-mail address: hornok.sandor@aotk.szie.hu (S. Hornok).

http://dx.doi.org/10.1016/j.ttbdis.2016.02.016
1877-959X/

Table 1
Monthly data of collected tick numbers, mean size of males and females (mm) with standard deviation, and results of molecular analyses. Red, bold numbers indicate sample groups with *B. canis* positivity. Blue background highlights periods with smaller mean tick size. The number of dots indicate the number of ticks containing the relevant genotype.

	months		August	September	October	November	December	January	February	March	April	May	June
HABITAT-1	**tick number**	male	2	41	45	46	15	21	33	38	26	9	0
		female	0	15	45	76	16	27	39	46	49	29	3
	mean size		3.6±0.1	3.6±0.5	3.7±0.1	3.7±0.4	3.7±0.4	3.7±0.3	3.7±0.3	3.7±0.3	3.6±0.3	3.5±0.2	3.3±0.3
	***B. canis* positive**	male	nd	0/11	0/22	0/11	0/11	0/11	0/11	0/11	**1/11**	0/9	nd
	/ all tested	female	nd	0/12	0/24	0/12	0/12	0/12	0/12	0/12	0/12	**4/14**	nd
	genotype 18S-A										•		
	genotype 18S-B											••••	
HABITAT-2	**tick number**	male	0	10	20	7	6	12	8	9	15	1	0
		female	0	13	28	16	20	10	18	22	15	1	0
	mean size		-	3.6±0.4	3.7±0.3	3.7±0.3	3.6±0.3	3.5±0.4	3.5±0.4	3.5±0.3	3.5±0.3	3.4±0.1	-
	***B. canis* positive**	male	-	0/10	0/11	0/7	0/6	0/12	**3/8**	**2/9**	**3/12**	nd	-
	/ all tested	female	-	**1/13**	0/12	0/16	**2/17**	**4/10**	**7/15**	**5/14**	**2/11**	nd	-
	genotype 18S-A			•			••	••••	•••••••••	••••••	•		
	genotype 18S-B								•	•	••••		

Abbreviation: nd - not done.

babesiosis are most frequent in spring time (Csikós et al., 2001; Pavlović et al., 2002), despite the fact that the peak activity of *D. reticulatus* is during the autumn (Széll et al., 2006; Hornok, 2009). Therefore, the present study was initiated to evaluate the seasonal distribution of *B. canis*-infected *D. reticulatus* ticks in urban biotopes of the region. For comparison with *B. canis* (which is a transovarially transmitted pathogen), the detection of *A. phagocytophilum* was also attempted, because the latter is transstadially maintained in *I. ricinus* and was reported to be highly prevalent in *D. reticulatus* in Eastern Europe (Karbowiak et al., 2014).

2. Materials and methods

2.1. Sample collection and preparation

D. reticulatus originated from two urban habitats of southern Budapest: (1) in the northern part (coordinates: 47°27′20.5″N, 19°4′21.3″E) of the large (257 km^2) Csepel-island on the Danube; and (2) in an urban park with neglected vegetation, Orbán-hegy (coordinates: 47°24′ 31.8″N, 19°7′33.5″E), enclosed by streets. Both habitats can be characterized by uncut meadow-grass, scattered bushes and few (mainly oak) trees. As for potential hosts of *D. reticulatus*, dogs, rodents, hedgehogs, hares and pheasants are known to be present. These two sites have been reported to be important habitats of *D. reticulatus* (Hornok et al., 2014a). Ticks were collected monthly (between August, 2014 and June, 2015, at the end of each month) in both habitats from the grass with the dragging-flagging method (drag cloth: 1 m × 1 m, examined every 30 s) along ten 100 m long transects (i.e. in ca. 1000 m^2). All specimens were immediately put into 70% ethanol and stored at room temperature until morphological identification by standard keys. The size of ticks was measured (from the antero-lateral apex of the scutum to the end of idiosome, with 0.1 mm precision, under stereo microscope) in order to evaluate the presence of freshly moulted adults in the population (which are smaller: Nosek, 1979).

A representative number, i.e. approximately twenty three ticks (randomly sampled 11 males and 12 females, if available) per month from both habitats were processed for molecular analyses (exception: 46 ticks in October from habitat-1; 22 ticks in January from habitat-2: Table 1). The DNA from these altogether 413 ticks was extracted by using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) as reported (Hornok et al., 2014b). The quantity/quality of DNA was assessed by a NanoDrop Thermo Scientific Multiscan Go spectrophotometer (Thermo Fisher Scientific Inc., Loughborough, UK).

2.2. Molecular analyses

The presence of *B. canis* was evaluated by a conventional PCR that amplifies an approx. 500 bp fragment of the 18S rDNA gene of piroplasms (original method in Casati et al., 2006; modification described in Hornok et al., 2015), followed by gel electrophoresis (1.5% agarose) and sequencing of all PCR positive samples at Biomi Inc. (Gödöllő, Hungary). Obtained sequences were not submitted to GenBank, because all were 100% identical to those of formerly reported *B. canis* isolates in Hungary (KP834449 for genotype "A", KP834450 for genotype "B").

In addition, the detection of *A. phagocytophilum* was attempted with a TaqMan real-time PCR that amplifies part of the major surface protein 2 (msp2) gene (original method in Courtney et al., 2004; modification described in Hornok et al., 2014b).

2.3. Statistical analyses

Confidence intervals (CI) for the prevalence rates were calculated at the 95% level according to Sterne's method (Reiczigel, 2003). Seasonal prevalence data were analysed with *Chi*-square test. The relationship of monthly distributions was assessed by Spearman's rank correlation. Differences were considered significant when $P < 0.05$.

Please cite this article in press as: Hornok, S., et al., Uneven seasonal distribution of *Babesia canis* and its two 18S rDNA genotypes in questing *Dermacentor reticulatus* ticks in urban habitats. Ticks Tick-borne Dis. (2016), http://dx.doi.org/10.1016/j.ttbdis.2016.02.016