Ticks and Tick-borne Diseases 5 (2014) 785–789

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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

Short communication

Occurrence of ticks and prevalence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. in three types of urban biotopes: Forests, parks and cemeteries

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ARTICLE INFO

Article history:
Received 27 January 2014
Received in revised form 18 March 2014
Accepted 28 May 2014
Available online 22 July 2014

Keywords:
City
Ixodes
Dermacentor
Haemaphysalis
Zoonosis
Anaplasma
Borrelia

ABSTRACT

The aim of the present study was to compare different urban biotopes for the occurrence of ixodid tick species, for the population density of *Ixodes ricinus* and for the prevalence rates of two emerging, zoonotic pathogens. Altogether 2455 ticks were collected from the vegetation on 30 places (forests, parks, cemeteries) of Budapest, Hungary. *I. ricinus* and *Haemaphysalis concinna* were collected in all three biotope types, but *Dermacentor reticulatus* only in parks and forests, and *D. marginatus* only in a forest. Highest population density of *I. ricinus* was observed in neglected parts of cemeteries. In females of this tick species the prevalence rates of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. were significantly lower in cemeteries, than in parks or forests. In conclusion, risks associated with the presence of ticks and tick-borne pathogens may be high in a city, but this depends on biotope types, due to habitat-related differences in the vegetation, as well as in the availability of tick hosts and pathogen reservoirs.

Introduction

Hard ticks (Acari: Ixodidae) are regarded as the most important transmitters (vectors) of animal and human pathogens in the temperate zone (Jongejan and Uilenberg, 2004). In such epidemiological scenarios cities may represent a unique interface where ticks can regularly find both human and animal hosts, in favour of the transmission of zoonotic tick-borne agents.

However, urban existence may not necessarily represent an opportunity for ticks, as in environments heavily populated by people they have to establish and survive under the influence of human activities. In large cities the state of vegetation and availability of hosts (particularly of adult ticks) may be the most significant limiting factors for perpetuating the tick life cycle (Dautel and Kahl, 1999; Uspensky, 2014).

Studies assessing urban tick activity and risks associated with tick-borne pathogens frequently focus either on one tick species (e.g. Overzier et al., 2013) or on one kind of habitat (e.g. Di Luca et al., 2013). However, ecologically the urban environment can be characterized by a mosaic-like pattern of heterogeneous biotopes (Dautel and Kahl, 1999). Information is scarce in the literature on the comparison of such urban biotopes that provide different conditions for the tick life cycle according to vegetation type and the availability of hosts.

Thus the aim of the present survey was to investigate three types of urban biotopes (forests, parks and cemeteries) for the presence of questing ticks and for the prevalence of tick-borne pathogens. In particular, the study focused on the habitat-related occurrence of ixodid species, on the population density of the most important antropophilic tick species in Europe, *I. ricinus*, and on the prevalence rates of two emerging, zoonotic pathogens, *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato (s.l.). For molecular analysis females of *I. ricinus* were chosen, because both *A. phagocytophilum* and *B. burgdorferi* s.l. have transstadial transmission (Ogden et al., 1998; Stanek et al., 1986), and therefore differences in the infection prevalence of these two pathogens will most likely manifest in the adult ticks (as a consequence of different hosts of immature stages in different biotopes). Higher prevalence of *A. phagocytophilum* and *B. burgdorferi* s.l. in adult *I. ricinus*, than in nymphs collected in the same place, is confirmed by literature data (Overzier et al., 2013).

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http://dx.doi.org/10.1016/j.ttbdis.2014.05.010
1877-959X/

Materials and methods

Sample collection

Altogether 30 study sites (representing three biotopes) were selected in Budapest, Hungary (Fig. 1) and sampled during the main spring tick season (March–June) in 2013.

Cemeteries ($n = 5$) were represented by their "neglected parts", i.e. either out of use or where the vegetation was not well maintained (uncut). All cemeteries included in the study are enclosed by approx. 2 m high, mostly concrete fence, and no dogs are allowed to enter. In public **parks** ($n = 12$) the tree/bush covering was sparse and the grass was periodically lawn. In deciduous **forests** ($n = 13$), the tree covering was continuous, the vegetation intact. These three biotopes were different in the availability of potential tick hosts.

Nineteen sites were visited only once or twice in March, April or May, to obtain data on the occurrence of tick species, taking into account their seasonality (Hornok, 2009). On 11 places representing the three biotopes ($n = 2, 5, 4$, respectively) tick collections were performed at monthly interval, under similar (dry) weather conditions and at the same time of the day. These places were selected randomly, but taking into account that a coherent area of at least 300×100 m should have been available for sampling along transects.

Ticks were collected from the vegetation by the dragging-flagging method according to the protocol of the EDENext program. In brief, a white towel, measuring 1 m × 1 m, was drawn over the vegetation along three 100 m long transects per suspected tick habitat (i.e. 300 m^2). Ticks attached to and removed from the collecting device were immediately put into and stored in 70% ethanol. Species were identified by using standard morphological keys (Hillyard, 1996).

Molecular analyses

DNA was extracted from 240 *I. ricinus* females (80 randomly selected ticks collected at two sites per each biotope type) individually, using extraction controls, as described (Hornok et al., 2010).

For the detection of *A. phagocytophilum* a TaqMan real-time PCR, that amplifies part of the gene encoding a major surface protein (*msp2*), was performed (Courtney et al., 2004). The probe was modified as 6-FAM-TGG TGC CAG GGT TGA GCT TGA GAT TG-TAMRA (5′–3′). The assay consisted of 40 cycles, and the results were regarded as positive if the threshold cycle (Ct) value was below 39. The detection limit of this PCR is 0.125 ratio (one-eights) of an *A. phagocytophilum* infected cell (Courtney et al., 2004).

Evaluation of the presence of *B. burgdorferi* s.l. was done using a TaqMan real-time PCR targeting a portion of the flagellin gene (Leutenegger et al., 1999). Primers were: B.398f (5′-GGG AAG CAG ATT TGT TTG ACA-3′) and B.484r (5′-ATA GAG CAA CTT ACA GAC GAA ATT AAT AGA-3′), and the probe B.421p (5′–3′: 6-FAM-ATG TGC ATT TGG TTA TAT TGA GCT TGA TCA GCA A-TAMRA). The test consisted of 45 cycles, and the results were interpreted as positive when the Ct value was below 45. The analytical sensitivity of the test is 10 copies of a cloned PCR product (Leutenegger et al., 1999).

The cut-off Ct values of both TaqMan real-time PCRs were defined according to the relative diagnostic sensitivities and specificities (Leutenegger et al., 1999; Courtney et al., 2004). All PCR tests were run while including appropriate positive and negative controls.

Statistical analyses

Population density of *I. ricinus* was expressed as the number of adults and nymphs per 300 m^2 of each habitat (Overzier et al., 2013), where monthly collections were performed (i.e. 11 places). Exact confidence intervals (CI) for prevalence rates at the 95% level were calculated according to Sterne's method (Reiczigel, 2003). Mean values were compared with Mann–Whitney *U*-test, and sample prevalence data by using Fisher's exact test. Differences were regarded significant when $P < 0.05$.

Results

Occurrence of tick species according to biotope types

In the study period 2455 ticks, belonging to four species, were collected. Out of the 30 study sites there was only one public park (with cut grass and trimmed vegetation), where no ticks could be found (Fig. 1).

The most common tick species in urban habitats was *I. ricinus*, with an abundance of 78.6% (706 males, 621 females, 494 nymphs, 108 larvae: 1929 of 2455 ticks, CI: 76.9–80.2%). It was collected in 27 of 29 tick habitats (Fig. 1 and Table 1). Taken together all four available species, habitats of *I. ricinus* were situated the closest to the city centre (even within 1 km). On 16 sites only *I. ricinus* was found, and in cemeteries exclusively this species was collected on four out of five places (Table 1).

The abundance of *H. concinna* was 17.8% (nine males, seven females, 23 nymphs, 398 larvae: 437 of 2455 ticks, CI: 16.3–19.3%). It was found in six out of 29 tick habitats (Fig. 1, Table 1). Similarly to *I. ricinus*, the presence of *H. concinna* was also observed in all three biotope types.

The third most common species, *D. reticulatus* had an abundance of 3.6% (33 males, 55 females: 88 of 2455 ticks, CI: 2.9–4.4%). Despite being restricted to two biotope types (parks and forests: Table 1), it appeared to be more widespread than *H. concinna*, with nine habitats. The fourth identified species, *D. marginatus* was represented by a single female specimen, from a forest with cattle and horses kept nearby (Fig. 1 and Table 1).

Most frequently *I. ricinus* and *D. reticulatus* occurred together (on seven premises), followed by the simultaneous presence of *I. ricinus* and *H. concinna* on six sites. Cohabitation of these three species was also demonstrated in two parks.

Population density of Ixodes ricinus according to biotope types

From April to June the mean population density of *I. ricinus* was equilibrated between parks and forests. However, it was significantly higher in cemeteries, than in parks in April ($P = 0.047$), and also in comparison with both parks ($P = 0.026$) and forests ($P = 0.032$) in June (Table 1).

Prevalence of Anaplasma phagocytophilum and Borrelia burgdorferi s. l. in Ixodes ricinus females according to biotope types

The cumulative prevalence of *A. phagocytophilum* was 8.8% (21 samples PCR positive out of 240, CI: 5.5–13.1%). The number of PCR positive samples was significantly lower in cemeteries, than in parks ($P = 0.03$), or parks and forests taken together ($P = 0.015$) (Table 1).

Altogether 40.8% of ticks were infected with *B. burgdorferi* s.l. (98 samples PCR positive out of 240, CI: 34.6–47.3%). Significantly fewer *I. ricinus* females harboured this pathogen in cemeteries, than in forests ($P = 0.024$) (Table 1).

Discussion

In the present study four tick species (*I. ricinus*, *H. concinna*, *D. reticulatus*, *D. marginatus*) could be collected from the vegetation in