



Original article

Ticks on dogs and cats: A pet owner-based survey in a rural town in northeastern Switzerland



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ARTICLE INFO

Article history:

Received 19 November 2014
 Received in revised form 23 January 2015
 Accepted 25 January 2015
 Available online 13 February 2015

Keywords:

Dog
 Cat
Ixodes ricinus
Ixodes trianguliceps
Ixodes hexagonus
Dermacentor reticulatus

ABSTRACT

Changes in the endemic foci of tick populations and invasions of tick species to new areas have become evident in Europe, leading to changes in the epidemiology of tick-transmitted diseases. However, data about tick infestations of pet animals are limited. Following the recent identification of a new focus of canine babesiosis in northeastern Switzerland, we investigated the occurrence of tick vectors in this region by using a pet owner-based sampling strategy. All dog owners in a rural town were sent postal requests to send ticks from their dogs and cats over two consecutive years, beginning in April 2012. In total 3003 ticks were submitted for identification from 249 dogs (approximately 20% of the resident dog population) and from 117 cats. *Ixodes ricinus* was the most abundant species identified in 96.8% ($n = 2124$) and 74.3% ($n = 601$) of the individual samples submitted from dogs and cats, respectively. Two other tick species, *I. hexagonus* and *Dermacentor reticulatus*, were recorded on both host species, with host infestation prevalences below 2%. On cats (but not on dogs), as many as 24.0% ($n = 194$) of the specimens were identified as a fourth tick species, *I. trianguliceps*. Overall, 93.5% of the ticks were adults (93.8% and 93.0% in dogs and cats), 4.4% nymphs (5.7% in dogs and 1% in cats) and 2% larvae (0.5% and 6.0% in dogs and cats), respectively. The highest infestation intensity was 49 *I. ricinus* ticks from an individual dog. However, 55.6% of the submissions from dogs and 24.8% from cats contained only one tick. This survey demonstrated that pet owners can contribute to a cost-effective tick surveillance and identified a new tick focus of *D. reticulatus*. The finding of *I. trianguliceps* exclusively on cats might be related to behavioural traits of the cats or to a more readily detection of these very small ticks during petting by their owners.

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Introduction

Hard ticks (Ixodidae), frequently found on dogs and cats, bear the vector competence for a broad spectrum of pathogens of medical and veterinary significance (ESCCAP, 2012; Gray et al., 2009; Hillyard, 1996). In recent years, there have been changes in the endemic foci of tick populations, and invasions of tick species in central and northern Europe have become evident, causing changes in the epidemiology of tick-transmitted diseases (Beugnet and Marie, 2009; Jongejan and Uilenberg, 2004; Leschnik et al., 2002; Medlock and Jameson, 2010). For example, canine babesiosis is a severe and often life-threatening disease in dogs, caused by the protozoan *Babesia canis* which is transmitted by *Dermacentor reticulatus* (Halos et al., 2014). In Switzerland, there is a stable endemic focus in the western part of Lake Geneva (Jacquier, 1974; Pfister et al., 1993; Porchet et al., 2007), and four focal outbreaks in dogs,

without a travel history, have recently been identified in eastern areas of the Swiss midplains (Sager et al., 2005; Schaarschmidt et al., 2013, 2006). We have identified a new focus in northeastern Switzerland, with 15 confirmed indigenous cases over the last 4 years (unpublished data). Therefore we aimed to investigate the occurrence of tick vectors in this region. Data on tick infestations of pet animals are rare, and it is presumed that different sampling strategies influence the composition of tick samples (Lavender and Oliver, 1996). Most published data on ticks of pet animals were obtained with specimens collected in veterinary practices (Claerebout et al., 2013; Földvári and Farkas, 2005; Nijhof et al., 2007; Ogden et al., 2000; Papazahariadou et al., 2003; Smith et al., 2011). Alternatively, ticks can be collected from the environment, by either experimentally walking dogs in the infested area or by flagging (Duscher et al., 2013; Jennett et al., 2013; Leschnik et al., 2012; Schorn et al., 2011).

Our study describes a systematic and cost-effective, pet owner-based sampling strategy to investigate tick occurrence on dogs and cats following an outbreak of canine babesiosis in a town surrounded by recreation areas in northeastern Switzerland.

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Materials and methods

Study site

The study was performed in the municipality of Rapperswil-Jona (population of approx. 26,000 inhabitants), which is located in the eastern part of Switzerland (47.233° N, 8.823° E) on the shores of the lake of Zurich. The altitude ranges between 407 m and 544 m above sea level. The vegetation around the town is predominantly characterised by deciduous woodland, bushes, agricultural areas and some rough grassland, rivers and marsh. The surrounding areas are popular recreation areas and attractive sites for dog walking.

Tick sampling strategy and identification

At the beginning of April 2012 and April 2013, letters were sent to all dog owners of the municipality (799 and 783 letters were sent in 2012 and 2013, respectively, to a total of 967 people). Their addresses were made available by the municipal administration, since all dogs (but not cats) in Switzerland are marked by a microchip and are registered (Swiss Federal Council directive No. 916.401). The dog owners were invited to collect ticks from their dogs and cats, store the ticks between adhesive tapes or in a suitable tube and send them to our laboratory. Furthermore, they were asked to provide data on the animals (dog/cat, age, sex, and breed) and on date and putative geographical site of tick exposure. The study lasted for 2 years and was terminated end of March 2014. Tick species, stage and gender were determined using standard keys (Estrada-Peña et al., 2004; Hillyard, 1996; Nosek and Sixl, 1972), and the ticks were stored individually in 70% ethanol at -20°C .

Tracking *D. reticulatus* in the environment

To verify the presence of *D. reticulatus* foci, the owners of dogs infested with this species were contacted by telephone and asked where exactly they usually walk their dogs. Questing ticks along the common walking paths in the three identified distinct areas were collected by flagging the vegetation on four occasions (twice each in June and September 2012). The cotton flagging blanket was inspected every 20 m for the presence of ticks.

DNA isolations and PCRs

DNA from individual *D. reticulatus* ticks was extracted according to Wenk et al. (2012) using a mixer mill (Retsch®, MM 300). PCRs were performed using two sets of primers, both amplifying part of the *Babesia* spp. 18S rRNA gene as described (Casati et al., 2006; Hilpertshäuser et al., 2006). To check whether amplifiable DNA was extracted, a tick-specific PCR targeting the mitochondrial 16S rRNA gene (Black and Piesman, 1994) was also performed. The latter PCR, combined with direct sequencing of the amplicons (Syngene GmbH, Schlieren, Switzerland) was also used to confirm tick species identification (Bown et al., 2006).

Case definition and statistical analysis

Every sample containing ticks was classified as an infestation, and co-infestations (when different tick species in the same sample were observed) were counted as different infestation events for each species. Thus, an individual animal could give rise to several infestation events, caused by infestations at different dates and/or by several tick species. The Pearson's χ^2 -test was used to analyse differences amongst infestation events in dogs and cats for each tick species, and for the number of ticks independent of species. Similarly, differences in life stages of the ticks received from dogs and cats were analysed with respect to the total number of ticks

per host animal. A p -value <0.05 was considered as statistically significant. Statistical analysis was performed in R version 2.14.2 (R Development Core Team, 2012).

Results

In total 967 dog owners were invited by letter twice (April 2012 and 2013) to send ticks from their dogs and cats. Data were collected for 2 years after the initiation of the study. Ticks were received from 18.6% and 16.5% of the dog owners in Year 1 and Year 2, respectively, and 20.7% and 19.1% of the dog population was covered (as an owner can have more than one dog). Ticks were submitted in every month of the 2 year study. A total of 3003 ticks (1719 and 1284 per study year) were submitted from 249 individual dogs and 117 individual cats. From 102 dogs and 11 cats, ticks were sent for both years of the investigation. Thus, samples were submitted from 184 and 167 dogs in Year 1 and Year 2 respectively, and from 64 cats in each of the 2 years. Overall, 521 and 360 infestation events for dogs and 124 and 86 for cats were recorded in 2 years, respectively.

Data about the ticks from dogs and cats are summarised in Table 1. *Ixodes ricinus* was the most abundant species representing 96.8% ($n=2124$) and 74.3% ($n=601$) of the ticks submitted from dogs and cats (statistically significant difference among the host species). Two other tick species, *I. hexagonus* and *D. reticulatus*, were recorded in both host species, with abundances below 2%. In cats, as many as 24.0% ($n=194$) of the specimens were identified as a fourth tick species, *I. trianguliceps*. The identity of two specimens of each developmental stages of this species was confirmed by PCR and sequencing. Of the ticks received, 93.8% and 93.0% were adult ticks isolated from dogs and cats, respectively. In contrast, the percentages of nymphs (total 4.4%; 5.7% in dogs and 1% in cats) and larvae (total 2%; 0.5% in dogs and 6.0% in cats) were significantly different between the two host species. Co-infestations were recorded on only 8 dogs with both *I. ricinus* and *D. reticulatus*. The number of ticks per infestation event is shown in Fig. 1. The highest intensity of infestation was 49 *I. ricinus* ticks from an individual dog, but 55.6% of the submissions from dogs contained only a single tick, whereas in cats this percentage was significantly lower (24.8%).

The presence of a local population of *D. reticulatus* was confirmed, by flagging along a 3 km long section, in one of the areas where infested dogs were regularly walked (Joner Allmeind, 47.226° N, 8.856° E). Thus, 5 female and 3 male *D. reticulatus* as well as 427 *I. ricinus* were collected. At least one *Derma-centor* specimen was obtained at any of the four collection dates (two each in June and September). All *Derma-centor* ticks received either from pet owners ($n=50$) or collected by flagging ($n=8$) were PCR-negative for *Babesia* spp.

Discussion

Our strategy to collect ticks in a newly identified focus of *Babesia* transmission consisted of systematically requesting residential dog owners to collect and send ticks from their pet dogs and cats. Thus, ticks from about 20% of the overall resident dog population were obtained. In the first 8 months after sending the request letters, ticks were obtained from 20.2 and 18.7% of the dog population. In a British study covering the 8 months between March and October, 22.9% (810/3534) of individual dogs thoroughly inspected at veterinary practices were infested by ticks (Smith et al., 2011). Thus, the analogous infestation rates found in this previous study and our study confirm that our approach is suitable to obtain reasonable estimates of canine tick infestation. The slight decline of tick infestations observed in the second year of our study might be caused by the fact that

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