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Association between the use of the acaricides, household type, tick bite and seropositivity against *Anaplasma phagocytophilum* and *Borrelia burgdorferi sensu lato* in clinically healthy dogs in Latvia

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Abstract

The aim of this study was to evaluate the association between seropositivity to *Anaplasma phagocytophilum* and *Borrelia burgdorferi sensu lato* and the following factors: (i) type of household (rural vs. urban), (ii) use of acaricides (type and seasonality/duration), and (iii) history of tick bite (season). Blood samples were collected from all the dogs in the household and dog owners were surveyed. Serological examination was performed with the SNAP 4Dx test kit (IDEXX). Of 400 clinically healthy dogs 44 (11%) were seropositive for *A. phagocytophilum* and 11 (2.75%) were seropositive for *B. burgdorferi sensu lato.* Seroprevalence against *A. phagocytophilum* was significantly higher in dogs from rural households. In a household with several dogs and with tick attachment in autumn the odds of being seropositive against *A. phagocytophilum* increased. The use of the acaricides (type and regularity of application) was not associated with significantly increased risk of being seropositive against *A. phagocytophilum* or *B. burgdorferi sensu lato*.

Key words: *Anaplasma phagocytophilum*, anaplasmosis, acaricide, *Borrelia burgdorferi*, borreliosis, dog, rural.
Abbreviations: CI, confidence interval; CGA, canine granulocytic anaplasmosis; OR, odds ratio; *s.l., sensu lato*; TBD, tick-borne diseases.

Introduction

In the last decade, interest in tick-borne diseases (TBD) in dogs has increased among veterinarians, dog owners and researchers. Based on research conducted on TBD in dogs in Scandinavian countries and information on human TBD in Latvia (Bormane et al. 2004; Carrade et al. 2009; Halperin, 2011), we suspected that two TBD, namely canine granulocytic anaplasmosis (CGA) and Lyme disease or borreliosis, may be present in dogs in Latvia. CGA is caused by a rickettsial microorganism *Anaplasma phagocytophilum* and borreliosis is caused by various spirochetes from the genogroup *Borrelia burgdorferi sensu lato* (*s.l.*) (Carrade et al. 2009; Halperin 2011). Two Ixodid ticks are prevalent in Latvia, *Ixodes ricinus* (Western and Central regions) and *Ixodes persulcatus* (Eastern region) (Bormane et al. 2004). Both tick species have been observed to carry *A. phagocytophilum* and the potentially pathogenic borrelia *Borrelia afzelii, Borrelia garinii, B. burgdorferi sensu stricto* and *Borrelia valaisiana* (Ranka et al. 2004; Agudelo et al. 2011).

Diagnosis of CGA and Lyme disease can be difficult, since dogs with CGA mostly present with nonspecific clinical signs – fever, lethargy, anorexia, joint problems (Egenvall et al. 2000; Jensen et al. 2007; Carrade et al. 2009), along with various changes in hematology/biochemistry values (Kohn et al. 2008; Pantchev 2010). Lyme disease can present with clinical signs similar to CGA or rarely, it may be associated with fatal glomerulonephritis with an incidence rate of 1.85% (Gerber et al. 2007; Dambach et al. 1997) or cardiac manifestation (Agudelo et al. 2011). Veterinarians should be aware that dogs can receive several tick-borne diseases from a single tick-bite and thus the clinical disease and treatment can be more complex (Beall et al. 2008; Carrade et al. 2011).

There are multiple factors that influence the occurrence and prevalence of TBD in dogs, e.g. the habitat, ectoparasite control, human behavior, removal technique of the attached ticks, and others (Foil et al. 2004; Randolph 2010). Tick control has been regarded as one of the main prophylactic measures to prevent tick-borne diseases in dogs. Additional activities, such as modification of the local habitat by trimming grass, cutting bushes and eradicating small rodents are helpful, but may not be applicable in all areas. It is known that early removal of the attached tick reduces the possibility of the infection, since migration of *A. phagocytophilum* from tick to the dog starts 24 to 48 h after tick bite, but for *B. burgdorferi s.l.* the time varies between 24 to 72 h (Carrade et al. 2009; Kohn et al. 2008; Straubinger 2000). Vaccination, another typical control measure against infectious diseases, is not available for *A. phagocytophilum*. Several vaccines targeted at various

outer membrane proteins of *B. burgdorferi s.l.* and having a borreliacidal effect inside or outside the tick have been developed (Straubinger 2000; Schuijt et al. 2011), but vaccine development still continues to be a challenging field of research. None of these vaccines is readily available in Latvia and dogs are not routinely vaccinated against borreliosis.

In order to provide Latvian veterinarians with scientific evidence regarding TBD and their prevention we believe that it is imperative to study these diseases locally. Veterinarians have a duty to inform people about zoonotic diseases. *A. phagocytophilum* is a causative organism of human granulocytic anaplasmosis; anaplasma transmission from dogs to humans has not been proven and is unlikely, but dogs serve as sentinels for human exposure (Carrade et al. 2009; Day 2011; Goossens et al. 2001).

We hypothesized that there are several factors that could have either positive or negative influence on the seropositivity against TBD in dogs in Latvia. The aim of this study was to evaluate if the following factors have an effect on the dog being seropositive against *A. phagocytophilum* and *B. burgdorferi s.l.*: habitat, acaricide use (type and seasonality), seropositive dog in a household, and known attachment of the tick (season and number of attached ticks).

Materials and methods

Serology

Peripheral blood samples from 400 clinically healthy dogs from various rural regions and urban areas in Latvia were collected. Selection of samples was representative of three distinct regions of tick habitat, comprising regions where *I. ricinus* or *I. persulcatus* ticks are predominant and a region where both of the above mentioned tick species are commonly found (Berzina et al. 2012). Plasma was used for serological examination with the SNAP 4Dx test kit (IDEXX Laboratories, Westbrook, Maine, USA), which detects antibodies against major surface protein p44/MSP2 of *A. phagocytophilum*, C6 peptide within membrane protein of *B. burgdorferi*, peptides p30 and p30-1 from the outer membrane of *Ehrlichia canis* and specific antigens of *Dirofilaria immitis* (Chandrashekar et al. 2010). The sensitivity and specificity of the test used in this study is described to be 99 and 100%, respectively for *A. phagocytophilum* and 98.8 and 100%, respectively for *B. burgdorferi s.l.* (Chandrashekar et al. 2010; Carrade et al. 2011). The test was performed on the day of sampling according to the manufacturer's instructions (Idexx Laboratories, http://www.idexx.com/pubwebresources/pdf/en_us/smallanimal/snap/4dx/snap-4dx-package-insert.pdf). Owner consent was received for obtaining blood samples and enrollment in the study. The study was approved by the Latvia State Food and Veterinary Service.

Survey

The survey of dog owners was carried out in collaboration with small animal veterinary clinics. Survey of information about the dog (age, sex, breed) included the following questions:

1. Is your dog living in arural or urban area? (Yes/No)
2. Are there several dogs in the household? (Yes/No)
3. Are you using any acaricides on your dog? (Yes/No)
   a. If yes, specify – what type (spot-on products, collars, other) and brand name.
   b. If yes, specify – how often (whole year; spring, summer, autumn; other).
4. Have you noticed any attached ticks on your dog? (Yes/No)
   a. If yes, specify – when it was most prominent (spring, autumn, summer, winter).
   b. If yes, specify – how many (less than 10, more than 10 at once).

Statistical analysis

Nonparametrical distribution of data was assumed. Statistical significance of the association between the seroprevalence and various parameters was assessed with the Fishers exact test (two tailed) or odds ratio with confidence intervals. Statistical significance was assessed among the following groups of dogs: seronegative vs. two groups of seropositive dogs (seropositive against *A. phagocytophilum* and against *B. burgdorferi s.l.*). Statistical software NCSS 2007 was used for statistical analysis (Kaysville, Utah, USA). Values of $p < 0.05$ were considered statistically significant.

Results

Seroprevalence

For the purpose of this study 400 paired surveys and blood samples were collected and analyzed. Seroprevalence against *A. phagocytophilum* was 11% (44/400, 95% CI 7.9 – 14.1%) (Berzina et al. 2012) and antibody level against *B. burgdorferi* 2.75% (11/400, 95% CI 1.2 – 4.4%). No dogs were seropositive for *E. canis*; no *D. immitis* antigens were found in any of the examined blood samples.

Among the analyzed groups of dogs, age, sex and breed were not associated with significantly higher seropositivity (all parameters, $p > 0.05$; Berzina et al. 2012).

Household type

Overall, more rural dogs (280/400) than urban dogs (120/400) were evaluated. The results on association between the presence of antibodies against *A. phagocytophilum*, habitat and number of seropositive dogs in the household are included in Table 1. *A. phagocytophilum* seroprevalence was significantly higher in dogs living in rural areas compared to those living in urban households ($p = 0.03$). Additionally, it was noted that risk of being seropositive

against *A. phagocytophilum* was significantly higher if there was more than 1 dog in a household [odds ratio (OR) 0.33, 95% CI 0.15 – 0.74]. Incidentally, all households with several dogs were in rural areas. Five dogs seropositive against *B. burgdorferi* were from urban, six from rural areas and there was no statistically significant difference in *B. burgdorferi* seroprevalence between the two types of households.

Use of acaricides

Acaricide use was reported by 71% (284/400, 95% CI 66.5 – 75.4%) of the dog owners. Of those, 37% (105/284, 95% CI 31.4 – 42.6%) reported that they consistently re-applied acaricides to their dogs during whole tick season (i.e., during spring, summer and autumn). Inconsistent acaricide application was reported by 63% (175/284, 95% CI 57.4 – 68.6%) of dog owners. Four respondents said they apply acaricides the whole year. We did not find a statistically significant difference between the application of acaricides and the seroprevalence or attachment of ticks ($p > 0.05$). Questions about the type of the product and its brand name were filled in only in 37% of cases (148/400, 95% CI 32.3 – 41.7%). Of all the products, spot-on products were most commonly used (76/148 respondents), 35/148 respondents indicated that they have used collars. Powder, shampoos and other products were used less frequently. Acaricide was used for three of the dogs seropositive to *B. burgdorferi*, the other eight dogs did not receive any anti-tick products.

Tick attachment

Forty dog owners reported that their dogs have not had an attached tick and none of these dogs had antibodies against *A. phagocytophilum*. Lack of tick attachment was associated with lower seroprevalence compared to dogs with reported tick attachment: for dogs with less than10 ticks attached, OR was 0.07 (95% CI 0.0048 – 1.32); for dogs with more than 10 ticks attached OR was 0.13 (95% CI 0.007 – 2.34). *A. phagocytophilum* seroprevalence was not significantly different between dogs that had less than 10 or more than 10 ticks attached ($p = 0.51$). Odds ratio showed positive correlation between seropositivity against *A. phagocytophilum* and tick attachment in the autumn compared to spring (OR 0.36, 95% CI 0.13 – 0.98) and summer (OR 0.36, 95% CI 0.17 – 0.76). All dogs with antibodies against *B. burgdorferi* previously had ticks attached – three of each with less than 10 ticks attached in spring and summer, two dogs with less than 10 ticks attached in autumn, and the remaining three dogs with more than 10 ticks attached in the autumn.

Discussion

Here we report that rural habitat, tick attachment in the autumn and household with several dogs were significantly associated with higher seroprevalence against *A. phagocytophilum*.

Higher seroprevalence in rural versus urban dogs has been described previously and suggests that exposure to tick habitat increases the number of seropositive dogs (Rand et al. 1991; Krupka et al. 2007; Carrade et al. 2009, Wu et al. 2009). Our data might be skewed since we sampled relatively more dogs from rural areas. It has been suggested that hunting might be an increased risk activity and might increase the number of seropositive dogs among this population (Krupka et al. 2007). However, our data did not support this hypothesis: seroprevalence in hunting dogs (12%) was only slightly higher than in healthy pet dogs (11%) and the difference was not significant (Berzina et al. 2012). One of the reasons for this disagreement between the rural versus urban and hunting versus pet dog seroprevalence in Latvia might be because our hunting dog group was too small (n = 41). Another reason for discrepancy between our results and other publications is that owners of hunting dogs in Latvia may recognize the risk and use acaricides more regularly than other owners. For example, in our study four dog owners that reported use of the acaricide during the whole year all had hunting dogs. Our finding that in a multiple dog household the risk of seropositivity against *A. phagocytophilum* increases, could be explained by the fact that infected ticks are residing in that particular area. This is supported by our finding of several higher *A. phagocitophilum* seroprevalence areas, namely, Tervete (rural area in Southern Central Latvia), Limbazi and Skrunda (small towns located in Northern and Western parts of Latvia, respectively) (Berzina et al. 2012). In addition, several dogs in the same household are more likely to be found in rural areas and dogs being social animals would be more active in a group. Transmission of granulocytic anaplasmosis from dog to dog via blood or saliva has not been proven (Carrade et al. 2009) and is not suspected to have happened in these households.

The finding that ectoparasite use had no protective effect against canine granulocytic anaplasmosis and borreliosis in dogs is a controversial observation. There are previously

Table 1. Parameters associated with increased seropositivity against A*naplasma phagocytophilum* in healthy dogs in Latvia

Parameter	Statistical significance	Odds ratio
Rural household	p = 0.03	–
Several dogs in the household	–	0.33, 95% CI 0.15 – 0.74
More than 10 ticks attached	–	0.13, 95% CI 0.007 – 2.34
Tick attachment in autumn	–	0.36, 95% CI 0.13 – 0.98

published studies that show protective effect (Blagburn et al. 2005) and studies that have failed to find protective effect (Wu et al. 2009; Kohn et al. 2011; Rand et al. 2011). In addition it is noteworthy that several tick species have become resistant to commonly used acaricides due to the target site mutations (Foil et al. 2004). However, the interpretation of our results is not unequivocal, since they are based on the owner survey and might be biased due to the fact that dog owners might not know whether the product they were using was an acaricide or was developed to repel and/or kill other ectoparasites. At least in some cases this was proved to be true, since the brand name mentioned by some owners was not an acaricide, but a flea repellent (data not shown). Most dog owners could not name the brand of the product they were using; this gives additional bias to the results since different products have been described to differ in their efficacy against ticks (Kidd et al. 2003). It could be questioned if dog owner surveys are an adequate way of data collection, but several researchers have used this method for data collection (Wu et al. 2009; Kohn et al. 2011). Kohn and colleagues (2011) had the same doubts as we expressed here, whether the acaricide products were applied correctly and were they acaricides at all. In our study, spot-on products were reported to be the most commonly used form of acaricide.

Wu and colleagues (2009) reported that dog owners in Korea reported tick attachment even after the use of the acaricide products on their dogs. No information on the exact use of the product was given in that case or is available in our study. The use of the acaricide for the whole tick season (spring till autumn) is recommended, since nymphs are more active in spring, but adult ticks have increased activity during autumn and both of these stages can transmit the bacteria (Carrade et al. 2009). Those dog owners that reported inconsistent use of acaricides often made remarks that they feel ticks are more common only in a particular season (spring, summer or autumn), or they often had forgotten to reapply the product as recommended or the use of acaricides was inconsistent due to financial constraints (data not shown). Acaricide use in pet animals should be encouraged by veterinarians, since pets living in close proximity to humans can bring ticks into homes.

Forty dog owners reported that their dog had never had a tick attached. This result most probably implies that the tick was not seen by the owner, since ticks can attach, feed and fall off without being noticed. The history of a tick bite or lack of one is not a predictor of the seropositivity both in humans and dogs (Leiby et al. 2002; Poitout et al. 2005). Jensen and colleagues (2007) reported that in dogs in Germany tick infestation was associated with significantly higher seropositivity against *A. phagocytophilum*. To decrease the risk of a successful transmission of bacteria via the tick bite, it is recommended to carefully remove all attached ticks as soon as possible. Attachment of the infected tick does not always result in the successful transmission of the parasite to the dog (Kidd, Breitschwerdt 2003). The transition from tick to the dog is different for *A. phagocytophilum* and *B. burgdorferi* and this might be one of the reasons that can explain why *B. burgdorferi* seroprevalence is considerably lower. After the bite, borrelias need activation within the tick and only then they start to migrate from the midgut to the salivary glands, as opposed to anaplasma that reside in the salivary glands and are transmitted sooner after the tick attachment. Other important factors that affect the seroprevalence include the numbers and viability of the agent within the tick, and species of the agent (Kidd, Breitschwerdt 2003).

None of the factors evaluated in this study had a significant association with seropositivity against *B. burgdorferi*. We explain this finding by the low number of seropositive dogs in our study.

To improve the shortcomings of this study prospective studies on TBD are warranted, but the results of the present study can aid veterinarians in small animal clinics in Latvia.

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