

The role of companion animals in the environmental circulation of tick-borne bacterial pathogens

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Abstract

Ticks are known as vectors of a wide range of pathogens of medical and veterinary importance; some of them of zoonotic concern constitute a hazard for the emergence of tick-borne diseases shared between humans and domestic animals and becoming a part of the 'One Health' concept. Canine and feline tick-borne diseases have emerged in recent years, performing an extensive geographic distribution and enlarged global prevalence. The present review focuses on the recent epidemiological studies on the emergence of tick-borne bacterial pathogens in dogs and cats, and the discussion whether pet ownership increases the risk of tick-borne diseases. A lot of data provide confirmation that dogs and cats themselves may substantially contribute to the circulation of the ticks and tick-borne bacterial pathogens in the environment. Molecular diagnostics of tick-borne pathogens infections generates a lot of problems like the choice of molecular methods and molecular markers for the detection of bacterial genomic DNA, but play an important role in the diagnosis of infections. The study provides some insight into molecular diagnostic techniques and new potentially recognized bacterial pathogens of this group. Protecting human and companion animal health from vector-borne infections requires controlling vector populations, containing development of novel, practicable strategies that will limit vectors and transmission of vector-borne disease pathogens.

Key words:

'One Health' concept, ticks, *Ixodes ricinus*, cats, dogs, bacterial tick-borne diseases, epidemiology, molecular tools, risk for pet owners

INTRODUCTION

In recent decades, the importance of vector-borne diseases in both human and veterinary medicine has spread dramatically. The spread of arthropod vectors to new areas, geographic wide-extent of pathogens, and recognition of new pathogens have all contributed to the heightened importance and attentiveness of vector-borne infections. In recent years, the frequency of some vector-borne diseases has been increasing partly due to climatic changes, especially global warming, which can also affect arthropod vector concentration, and has a direct influence on the abundance, geographical distribution and vectorial ability of arthropod vectors [1]. Additionally, other factors have been connected to the changing epidemiology of vector-borne diseases, like many ways of transport which have strongly increased, with intense animals production and also sport and companion animals. These movements provide very good conditions for the circulation of pathogens. The increasing mobility of the human populations and their companion animals due to the recreation model of spending time and travelling to distant places, support pathogen and vector exchanges [1, 2].

The vector-borne agents comprise a group of globally-distributed and spreading pathogens causing several zoonotic diseases transmitted by the bite of hematophagous arthropods, mainly ticks and mosquitoes. The most common

tick in northern Europe is *Ixodes ricinus*, which is known as a vector of a wide range of pathogens of medical and veterinary importance, such as viruses, bacteria and protozoa. A wide spectrum of tick-borne pathogens identified in many studies, constitute a hazard for the emergence of tick-borne diseases in domestic animals and in humans [3–6]. Dogs and cats may play a significant role in the transmission cycles of many agents of vector-borne diseases by serving as amplifying hosts [5, 6]. Many of these pet vector-borne pathogens can also affect humans due to their zoonotic potential. When molecular diagnostic techniques become available, more sensitive and specific differentiation of tick-borne agents will be possible, as tick-borne diseases are recognized as an emerging infectious threat not only to humans and pets, but also because of the public health importance of many of these infectious disease agents.

OBJECTIVE

The review focuses on the role of dogs and cats as companion animals in the environmental circulation of tick-borne bacterial pathogens and discusses whether pet ownership increases the risk of tick-borne diseases. Some insight is provided into molecular diagnostic techniques and new potentially recognized bacterial pathogens of this group.

'One Health' in companion animal vector-borne diseases. Canine vector-borne diseases shared between humans and dogs, point evidently to the obligation to join under the 'One

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Health' concept in all aspects, from epidemiology, clinic, diagnostic, therapy and prevention [7], and as described Mencke [8], are a perfect example of the importance of interdisciplinary cooperation.

The international importance of the 'One Health' concept of co-ordinated activity of those involved in human and animal health is a modern personification of a long tradition of comparative medicine, with an explosion of knowledge in the area of infectious disease research [9]. The modern 'One Health' pays the attention to zoonotic pathogens emerging from wildlife and production animal species, and is now considering the role of small companion animals, and especially for the vector-borne infectious diseases shared by man, dogs and cats. The most important of these are bacterial diseases: borreliosis, ehrlichiosis, rickettsiosis, anaplasmosis, bartonellosis, and protozoal leishmaniosis [10]. The impact of environmental changes (e.g. climate change, deforestation and urbanization) and lifestyle changes, including the increase in global human and animal movement, has also become a part of the 'One Health' concept [11]. Therefore, it seems that only a multidisciplinary 'One-Health' approach, integrating research outputs of experts from different disciplines, its veterinarians, molecular biologists, epidemiologists, physicians, and sociologists, etc., collectively with appropriate outreach can successfully make urban and peri-urban areas free from infection by tick-borne pathogens [8, 12]. The cited authors noticed that controlling canine vector-borne diseases obviously needs 'One Health' tactic and proper environmental control of arthropod vectored pathogens, based on ectoparasiticides with repellent properties to stop blood sucking with haematophagous, to block the interaction between the arthropod vector and the host which may conduct infections.

Tick-borne diseases in urban and peri-urban areas mean a rising risk for public and animal health in Europe. Certain tick-borne infections have recently strayed, emerging in new regions or re-emerging within endemic locations and creating increased alarm for public health, food security, and biodiversity protection [13]. Evidently, global warming changes the spread of tick-borne diseases, but not only climate conditions determine the geographical distribution of tick species, their population densities and dynamics, the possibility of their infection with pathogens for humans and animals, and finally the frequency of contacts of humans and domestic animals with infected ticks [12, 13]. Deforestation and reforestation are known as the factors which have a big influence on the transformation of biotopes, thus it affects tick host accumulations as well as tick infection rates [14]. *Ixodes ricinus* is mainly associated with mixed forests, but recently, abundant tick populations have been detected in European urban green areas, which are of public health importance due to the exposure of humans and companion animals to potentially infected ticks [15, 16]. In urban habitats, small and medium-sized mammals, birds, dogs and cats as companion animals, rarely larger mammals (roe deer and wild boar) play a role in vector ecology, in the maintenance of tick populations and as reservoirs of tick-borne pathogens. As it is known, urbanization, due to the restriction of natural areas, dramatically change the composition of wildlife species and affect the associated tick populations. In European cities, public parks, gardens, peri-urban areas, and cemeteries are principally the important

places where humans and companion animals can encounter potentially infected questing ticks [17]. A Polish study evaluating the prevalence of *A. phagocytophilum*, *Neorhlichia micurensis* and *Rickettsia* spp. in ticks collected from cats and dogs in an urban area (Wroclaw agglomeration, south-western Poland) demonstrated the high tick infestation rates and high prevalence of pathogens found in these ticks [18]. The authors concluded that dogs and cats themselves may significantly contribute to the circulation not only of the ticks but also pathogens in urban area.

Bacterial tick-borne diseases in dogs and cats. The major tick-borne infectious diseases are shared by man, dogs and cats. Among the bacterial diseases transmitted by *I. ricinus* the most significant are borreliosis, anaplasmosis, ehrlichiosis, and rickettsiosis.

Lyme borreliosis (LB) is known as one of the main emerging tick-borne disease in temperate regions in Europe and North America [19]. In Europe, the most recent cases of canine borreliosis have been noted in almost all countries [20–22], including Poland [23–27].

In recent years, reported cases of human borreliosis in Poland (according to the National Institute of Public Health) have increased more than 4 times, from 22.8 per 100,000 in 2012 to 55.2 per 100,000 in 2016; in 2015, there were 13,625 reported cases and 21,200 in 2016, but with considerable under-reporting, the number of cases was probably several times higher. The highest number of human cases of borreliosis in Poland in 2016 was registered in the Silesian Province, but the highest incidence rate was in Podlaskie Province with 134.9 per 100,000 people (Polish Institute of Hygiene).

Borrelia burgdorferi sensu lato (Bbsl) is a group of spirochete bacteria species, some of which cause LB in humans and dogs. To date, it has been supposed that in European dogs, the same as in humans, three pathogenic species of *Bbsl*: *B. afzelii*, *B. garinii* and *B. burgdorferi sensu stricto* are the most common European circulating genospecies, and are the etiological agents of LB [26, 28–31]. In The Netherlands, Belgium and Luxembourg, *B. valaisiana* has been also found [32–34], and in Polish and Belgian studies, *B. valaisiana*, *B. spielmanii* and *B. lusitanae* [4, 35] were detected. Thus, dogs as well as humans are hosts for many species of *Bbsl*, because the borrelicidal ability of serum of dogs and humans is evident only in certain genospecies of *Bbsl* [36]. Thus, both dogs and humans harbour more species than wild animal species, which generate phylogenetically older *Bbsl* species-host systems, and these animals may act even as non-competent reservoir hosts [36]. Apart from many genospecies of *Bbsl*, dogs harbour other tick-borne agents and dual or triple infections may occur.

***Anaplasma* spp.** (Rickettsiales, Anaplasmataceae) are intracellular bacteria and two species infect dogs: *Anaplasma phagocytophilum*, the causative agent of granulocytic anaplasmosis, and *A. platys*, causing thrombocytic anaplasmosis. The latter species has a worldwide distribution, with descriptions of infection from South and North America, Australia, Asia and Africa, and is associated with the *Rhipicephalus sanguineus* tick. *A. phagocytophilum* occurs in the northern hemisphere, and it is vectored by hard ticks of the *Ixodes ricinus*, among them, the sheep tick

I. ricinus in Europe. *A. phagocytophilum* has been detected across Europe, with varying prevalence in ticks, ranging from 1.7% in north-eastern Slovakia [37], 4.1% in Germany [38], 10.4% in central Italy [39] to 20.5% in Spain [40]. In Poland, the pathogen *A. phagocytophilum* is reported to occur in its vector *I. ricinus* in numerous studies and the prevalence in ticks has been ranging from 2.9% in the central region [41] to 4.5% in the north-west [42]. In south-western Poland, 2 species of ticks, *I. ricinus* and *I. hexagonus* collected from dogs and cats were infected in 14.4% [18]. In man (mainly forest workers as a particularly tick-exposed group within the population), antibodies against *A. phagocytophilum* have also been detected, e.g., in 17.7% in north-eastern Poland [43] and 19.8% in the eastern region [44].

In a few studies, blood samples from dogs have been screened with molecular methods with the results of 2/192 dogs being DNA positive for *A. phagocytophilum* [45], 0.5% of 408 dogs [27], and 14% of dogs suspected of having Lyme disease being positive for *A. phagocytophilum* [46], and 1/79 dogs being positive in a group of apparently healthy sled dogs [47]. Italian research showed *A. phagocytophilum* DNA in 7.5% of 50 dogs [48]. Polish research [26] showed the co-occurrence of *A. phagocytophilum* DNA in the blood of dog undergoing treatment for borreliosis. The same dog also proved positive for the presence of borrelia DNA. Co-occurrence of both pathogens did not disturb the clinical picture of borreliosis, and the administered treatment was also effective for the mixed infection.

Seroprevalence rates in dogs from European countries are generally high, i.e. 8.76% in central Italy [49], 10.6% of cats in Romania and Hungary [50], and 13.5% of cats in Portugal [51]. In a large study in Poland, 3,094 serum samples were taken from dogs throughout all 16 Polish provinces and 12.3% and 3.75% of the dogs were positive for *A. phagocytophilum* and *B. burgdorferi* s.l., respectively, and co-infections with both pathogens were recorded in 1.71% of all examined dogs [52].

In addition to the occurrence of the pathogen in dogs, it has also been reported in diverse forms of wildlife in Poland, e.g., roe deer [53] and wild cervids [54]. Even though this aspect is not examined very often within the canine or feline population, there is a clear risk of infection by frequently reported *A. phagocytophilum*-positive *I. ricinus* ticks in Poland.

Neoehrlichia mikurensis (NM, formerly *Candidatus Neoehrlichia micurensis*, CNM), another member of Anaplasmataceae (Rickettsiales), reported for ticks from several parts of Europe [38, 55]. Severe cases have been reported mainly in immunocompromised human patients [56, 57]. The causative agent, the intracellular bacterium NM, is considered an emerging tick-borne pathogen in Europe where it is transmitted by *I. ricinus* acting as a vector for numerous zoonotic tick-borne pathogens [58]. NM has been identified as one of the most prevalent microorganism in *I. ricinus*, indicating frequent exposure of animals to this potentially pathogenic species [59]. Dogs also appear to be infected by NM [60]. In a Croatian retrospective study with archival tissues from 19 dogs which died from haemolytic anaemia, NM was detected and confirmed by sequencing in a 3.5-months-old dog [61]. This finding is probably the first case in which NM may have been pathogenic for younger dogs. There is no evidence of pathogenicity in other domestic animal species. Recently, the presence of NM in samples

of dogs' blood was detected in Brandenburg, northern Germany; in 3 from 1,023 samples [62], but for now, the role of NM in disease development in companion animals remains unclear yet [56].

Cats also are hosts for *I. ricinus* ticks and feline vector-borne diseases have emerged in recent years, giving a broader geographic distribution and increased worldwide occurrence [5]. Domestic cats play a crucial role in the transmission cycles of some tick-borne agents by existence as reservoirs and amplifying hosts, the conditions that the 'One Health' concept requires [9]. Kaplan et al. [10] have recently notified that the detection of vector-borne pathogens is very challenging as some of them occur in healthy cats and the clinical signs are unspecific. PCR-based protocols applied to vector-borne pathogens are very effective for detecting and typify infecting pathogens, for monitoring treatment after chemotherapy, and for assessing the role that subclinically-infected cats can play in the spread of infections [10].

Generally, in the scientific literature there are only few reports on LB in cats. The explanation might be a different tick infestation in cats compared to dogs, a low exposure to tick-borne infections, or a low alertness of veterinarians for tick-borne diseases in feline patients [63]. The aim of the study by Pantchev et al. [63] was to determine the proportion of antibodies against *Bbbsl* in feline sera and to compare the significance of feline and canine borreliosis. Specific antibodies against the C6-peptide of *Bbbsl* in cats were detected by a test based on an enzyme immunoassay technique. The proportion of *Borrelia* antibody-positive cat sera was significantly lower than that determined for dogs during the same time period and residing in the same regions. All antibody positive cats came from countries endemic for LB (Germany, Sweden and Belgium), and all positive cats (except one) presented clinical signs. Obtained data revealed that diagnosis of 'feline LB' is rare in cats, but LB should be considered in cats with compatible clinical signs (leg lameness, and to a lesser extent, neurological signs) when other differential diagnoses have been ruled out.

The molecular detection and species identification of feline vector-borne bacteria and protozoa with veterinary and zoonotic importance, and evaluation of associated risk agents in cats, were the aims of the study of Maia et al. in Portugal [5]. 649 cats with clinical signs compatible with vector-borne diseases, from veterinary medical centres and animal shelters were examined. *Anaplasma* spp./*Ehrlichia* spp., *Babesia* spp., *Bartonella* spp., *B. burgdorferi* sensu lato, *Hepatozoon* spp. and *Leishmania* spp. infections were assessed by PCR in blood samples. 29.9% of the cats were PCR-positive for at least one of the tested genera, and 5.15% cats were positive to 2 – 3 of the genera; the most common were protozoa (9.9–5.4%) then *Anaplasma* spp./*Ehrlichia* spp. (5.4%), *Bartonella* spp. (2.9%) and *B. burgdorferi* s.l. complex (2.2%). In conclusion, the authors noted that the occurrence of feline tick-borne agents in southern Portugal, some of a zoonotic character, shows the need of attention of the veterinarians, owners and public health authorities for the risk of infection, and the control quantity should be implemented to prevent the infection of cats, other vertebrate hosts and people.

In the north-eastern part of the USA, where *I. scapularis* is a main tick-vector, the seroprevalence of antibodies against *B. burgdorferi* and *A. phagocytophilum* in cats was examined by Magnarelli et al. [64] to determine whether cats develop serum antibodies against antigens of *B. burgdorferi* and

A. phagocytophilum, and whether coinfection with the 2 organisms occurs. Serum samples from 84 healthy cats and 9 cats with lameness, fever, anorexia, or fatigue serum antibodies against 2 pathogens, were measured with an ELISA incorporating with Western blot analysis, or indirect fluorescent antibody (IFA) staining. ELISA results indicated that 47% sera contained antibodies against *B. burgdorferi* antigens, and seropositivity rates of 30% and 38% were detected for antibodies against *A. phagocytophilum* via IFA and ELISA testing, respectively. 16% of sera had antibodies against both pathogens. The authors concluded that cats living in areas infested by *I. scapularis* ticks are exposed to *B. burgdorferi* and *A. phagocytophilum* and, in some cases, may be coinfectd; nevertheless, most cats appeared to be healthy. In the opinion of Pennisi et al. [65], *A. phagocytophilum* is the most important feline pathogen among rickettsial organisms, and coinfections may occur. However, on the pathogenesis of these agents in cats, little information is available. Clinical signs are usually reported soon after tick infestation and are generally non-specific, involving fever, anorexia and lethargy and joint pain may occur. Because some rickettsial species are of zoonotic concern, direct contact with cat saliva should be avoided because of potential contamination by *Rickettsia felis*, for example. Infected cats are 'sentinels' of the occurrence of rickettsial pathogens in ticks and fleas in a given geographical zone, and the authors concluded that they signal a risk for people exposed to vectors [65, 66].

Molecular diagnostics. Unlike in the case of human infection, a typical case Lyme disease in dogs is not easy to document, mainly because many of the animals exposed to *B. burgdorferi* do not develop clinical abnormalities [67, 68]. Nevertheless, the diagnostics of *Bbsl* infections should be based mainly on clinical symptoms and an assessment of the risk of exposure to infected ticks, combined with diagnostic tests, including the assessment of *Borrelia* spp. antibodies of IgM and IgG class. The common diagnostic tests for *Borrelia* spp. are divided into 2 different groups. The first one includes indirect tests, which make it possible to assess the contact of an individual with the pathogen (serum antibodies), and the second group, called direct tests, are able to detect the pathogen in the clinical material (culture collection, microscopic methods, PCR and real-time PCR). However, lack of standardization of PCR for the identification of *Borrelia* spirochaetes makes it impossible to compare the reports on this subject. PCR detection of *Bbsl* uses various molecular markers, i.e. DNA sequences located in the bacterial chromosome and in plasmids. As many studies have shown, some markers, as well as PCR protocols, are useful for genospecies differentiation of *Bbsl* and phylogenetical analysis from the genetic material of ticks, others for clinical diagnostics. The spirochete expresses OspA, but not OspC, when is present in the midgut of unfed ticks, but during a tick's blood meal some spirochetes stop expressing OspA and express OspC instead [69]. Some genes of *Bbsl* are expressed only in a mammalian host or have significantly upregulated expression in that environment, for example, VlsE, DbpA, BBK32, Erp, and Mlp proteins. For these reasons the detection of bacteria genomic DNA may play an important role in the diagnosis of *Borrelia* infections [70–72].

The collection and analysis of questing ticks, and investigation of host-associated ticks for the occurrence

of pathogens is a valuable method for providing insight into the transmission patterns of tick-borne diseases [73, 74]. For tick samples, nested-PCR or PCR-RFLP targeting the flagellin gene (*flaB*), the intergenic spacer region (IGS) located between 5S and 23S rRNA, and the *glpQ* gene, as well as conventional PCR targeting the 16S rRNA, are often used for *Borrelia* DNA detection [75, 76]. Another study [77] revealed that the High-Resolution DNA Melting method with the *groEL* gene as marker is very useful for environmental samples. It enables identification of 8 *Borrelia* species, including *B. miyamotoi* from the relapsing fever borreliae group, and 7 from the *B. burgdorferi* s.l. complex (*B. garinii*, *B. afzelii*, *B. burgdorferi* s.s., *B. valaisiana*, *B. lusitanae*, *B. bissetii*, *B. spielmanii*) in questing *I. ricinus*. The study by Dunaj et al. [78] and Jahfari et al. [79] demonstrated that RLB (Reverse Line Blot Hybridization) might be easily used in *Borrelia* DNA detection with genospecies-identification occurring in *I. ricinus* ticks. The real-time PCR for blood samples would be more useful [72].

It is believed that tick-based investigation is essential for monitoring human and/or animal disease emergence. Dogs can be used to deliver blood samples for serological surveys; however, there is an alternative method when pets are used as deliverers of tick samples for PCR analysis for the detection of the pathogen. In the USA, there is the evaluation of dogs as sentinel species for emergence of Lyme disease in areas with ticks infestation. This is very useful because by removing ticks from dogs for identification and pathogen testing, veterinarians can play an important role in early detection in areas with increasing risk of Lyme disease [80]. In conventional PCR assay, the limited number of different targets is used, usually the pathogens target is known to be vectored by certain tick species collected at particular sites [81]. The study of Michelet et al. [59] demonstrates the utility of a fast tool that allows for the comprehensive testing of high numbers of tick-borne pathogens in ticks. In this study, a method using multiple primers/probe sets was employed, in order to achieve high-throughput detection of tick-borne pathogens, which permitted the simultaneous detection of 25 bacterial and 12 parasitic species. Additionally, it allowed for the discovery of rare pathogens, such as *Bartonella henselae*. The study was conducted on the DNA samples of *I. ricinus* nymphs collected from vegetation from 3 European countries.

The aim of Jahfari et al. [79] was to detect the nucleic acid of specific pathogens in human blood through amplification with PCR and real-time PCR, and in ticks, using the RLB technique to detect infection by most of tick-borne pathogens. In comparison to the DNA amplification with PCR, available serological tests generally have a low specificity and sensitivity, mainly during the early stage of the infection. In addition, the authors noticed, although culturing is considered the most reliable method in demonstrating the presence of bacteria, it is time consuming. The limitations of this methodology are well known; therefore, the interpretation of these results should be made with caution. Also, it should be noted that the absence of DNA of a pathogen cannot be interpreted as the absence of the infectious agent. Cerar et al. [82] and Elfving et al. [83] mentioned that besides the limits of PCR-based methods, the time of sample collection after a tick bite and beginning of an antibiotic treatment, or the tissue tropism of the pathogen, strongly affect the ability of pathogen detection. In a study by Jahfari et al. [79], only in one of the 291 patients

with an *erythema migrans* – caused by *B. burgdorferi* s.l. – the DNA of this pathogen was detected in blood, which confirms that the chance of detecting *B. burgdorferi* s.l. DNA in blood samples of confirmed Lyme borreliosis patients is very low. Neither *Rickettsia helvetica* nor *R. monacensis* was detected in 626 blood samples, whereas recent molecular evidence for their presence was found in the cerebrospinal fluid of neuroborreliosis patients, and in a skin sample of an EM patient [84, 85]. Bil-Lula et al. [72] have noted that the real-time PCR assay seems to be very useful in testing blood sampled patients, first of all in the early stage of the disease, before an antibody response develops, and in cases where the patient does not have classic symptoms.

Canine borreliosis occurs most often in the articular form, affecting joints of limbs, usually the wrist or instep [86, 87]. One or 2 joints become swollen and the inguinal and prescapular lymph nodes become enlarged. The associated symptoms are: ‘malaise’, expressed in elevated body temperature, loss of appetite, fatigue, and lameness in subsequent days. Dogs suffering from borreliosis rarely develop myocarditis, although the older ones develop the renal form of this disease [86, 87]. In addition, co-infections with 2 or more pathogens enhance diagnostic difficulties.

Molecular methods are useful for disentangling infections and accurately describing the prevalence and geographical distribution of tick-borne diseases, or in establishing the best strategies for treatment and control. Molecular diagnostics of tick-borne pathogens infections generate a lot of problems in the choice of molecular methods or molecular markers for the detection of bacterial genomic DNA.

For the detection of *Rickettsia* spp., a conventional PCR or a real-time PCR targeting the *gltA* genome region is used [18, 88, 89]. A real-time PCR targeting *msh2* gene fragment is performing to detect *A. phagocytophilum* [89, 90]. In order to detect *Neohelminthospora mikurensis*, a real-time PCR targeting the partial *groEL* gene (99 bp) is used [91, 92].

Has pet ownership increased the risk for tick encounters and tick-borne diseases? Dogs and cats live in the close vicinity of their owners and can act as direct sentinels for the infection of humans [80]. Pet dogs can be a very sensitive indication of infection risk and give a good signal of the exposure of their owners to infected ticks, because they largely share the same environment and visit the same outdoor areas [93, 94]. Many authors from many parts of the world have shown that the rate of *B. burgdorferi* infection in humans and dogs depends on the intensity of occurrence of ticks, associated with the geographic locality of their biotope, and indicates the point of risk for humans and dogs [95]. Dogs living in tick-infested areas, in which cases of human LB have also been noted, produce *B. burgdorferi* s.l. antibodies [26, 96–99]. The results of some studies prove that from an epidemiological point of view, canines should be considered as risk animals because they accompany people in risk-predisposed areas. Additionally, even removing ticks or cleaning infested animals after a walk risks exposure [9, 98]. There is a risk of human infection when ticks are crushed during removal from a pet and tick salivary gland material can be exposed to the hands of an owner [9]. However, the same author concluded that infected dogs and cats constitute a minimal threat to people, even though infected ticks can be carried into the domestic habitat [9]. Even though *Borrelia* can be transmitted from dogs to ticks, dogs are not

considered important reservoir hosts [19, 100], and some studies examining seropositivity in dogs, their owners and other local residents, found no correlation between dog ownership and infection risk. For example, Goossens et al. [101] did not observe any correlation between the frequency of *Borrelia* s.l. antibodies in a hunting dog and the serum of the dog’s owner. They concluded that direct transfer of ticks between hunters and hunting dogs is insignificant and one’s own dog is not an LB hazard.

Numerous studies prove that dogs and cats can be used as sentinels for LB. Serological studies in the USA displayed that exposure of dogs to *B. burgdorferi* reflects the geographical distribution of human LB reports [19], and the use of dog sera to detect and quantify the risk for human LB in a certain region is considered to be more sensitive than the use of incidence reports of human clinical cases [101]. Seroprevalence has been found to be greater in dogs than in humans, due to their greater habitat exposure and greater risk of *B. burgdorferi* infection in dogs than people [102]. The use of dog sera also has the advantage over human serology because the seroprevalence among dogs is more likely to reveal the real environmental risk of LB, due to the short half-life of canine antibodies against *B. burgdorferi* [101].

The most recent American research carried out by Jones et al. [94] shows that cat and dog pet owners are at increased risk of encountering ticks, and that pet owners are at an increased risk of developing tick-borne disease. They examined whether pet ownership increased the risk for tick encounters and tick-borne disease among residents of 3 Lyme disease-endemic States in the USA as a nested cohort within a randomized controlled test. Pet-owning households had 1.83 times the risk of finding ticks crawling on and 1.49 times the risk of finding ticks attached to household members, compared to people without pets. The authors concluded that pet owners should be attentive of this risk and be reminded to conduct regular tick checks of all household members, including pets.

Because several authors suggest that the epidemiological profile of borreliosis in dogs is an indicator of the potential risk to people in the area, for that reason the precise mapping of the risk of exposure to LB in frequently visited forests is an important method for targeting prevention and control measures [103]. Moreover, according to the results of a study carried out in the USA by the Center for Disease Control and Prevention (CDC), the more dogs with LB, the higher the frequency of this tick-borne illness in humans. On the basis of data from ‘Prevalence Maps’ presented by the Companion Animal Parasite Council (www.PetsAndParasites.org), it was established that the humans in areas with a higher than average number of dogs with borreliosis have a greater risk of contracting the disease. It is important to educate pet owners about the risk of tick-borne diseases as well as the need of prevention. Lyme disease research; prevalence maps are crucial implements in the education of humans and veterinary health care professionals and pet owners about this increasing problem. Protecting human and animal health from vector-borne infections requires continuing attention on controlling vector populations, containing the development of novel, practicable strategies that will limit transmission of vector-borne disease pathogens.

CONCLUSIONS

1. The 'One health' concept includes the role of small companion animals, specifically for the vector-borne infectious diseases that are shared by humans, dogs and cats.
2. Domestic dogs and cats play a crucial role in the transmission cycles of some tick-borne agents by existence as reservoirs and amplifying hosts – the conditions that the 'One Health' approach requires.
3. From the epidemiological point of view, in the case of humans and domestic animals, urban or peri-urban areas are important with vector ecology playing a very important role in the maintenance of tick populations.
4. *Neoehrlichia mikurensis* next to *Borrelia burgdorferi* sensu lato or *Anaplasma phagocytophilum*, indicating frequent exposure of animals to this potentially pathogenic species, is considered as an emerging tick-borne pathogen in Europe where it is transmitted by *I. ricinus*.
5. Molecular diagnostics of tick-borne pathogens infections generates a lot of problems, e.g. the choice of molecular methods and molecular markers for the detection of bacterial genomic DNA, but play an important role in the diagnosis of infections.
6. Dogs and cats living in the close vicinity of their owners can act as direct sentinels for the infection of humans. It is believed that the seroprevalence of *B. burgdorferi* s.l. indicates dogs to be perfect sentinels.
7. Precise mapping of the risk of exposure to Lyme borreliosis in frequently visited forests is an important method for targeting prevention and control measures.
8. Protecting humans and companion animals health from vector-borne infections needs controlling the vector populations, containing development of novel strategies that will limit the risk of transmission of vector-borne pathogens.

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