

The importance of dogs in eco-epidemiology of Lyme borreliosis: a review

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ABSTRACT: *Borrelia burgdorferi* sensu lato, is endemic in most of the regions in Europe. Pathogen circulates in nature involving ticks vector (mainly *Ixodes ricinus* in Europe) and wide spectrum of reservoir animals like rodents, game animals, birds as well as pets. Considering the close association of a dog and humans, and their similar activities in nature, it is necessary to evaluate the significance of a dog as an important animal in ecology and epidemiology of Lyme borreliosis. Antibody profile in Lyme disease is the most characteristic feature in dogs that helps to evaluate the changes in disease prevalence in particular area and helps to assess the risk factors for human. The article reviews overall eco-epidemiological importance of dogs in Lyme disease surveillance.

Keywords: *Borrelia burgdorferi*; dogs; ecology; epidemiology

Lyme disease is the most common arthropod-borne disease of humans in Europe and North America. In Europe, disease has been reported in variety of animal species closely associated with human (Kasbohrer and Schonberg, 1990; Parker and White, 1992; Blowey *et al.*, 1994; Ciceroni *et al.*, 1997; Magnarelli *et al.*, 1997; Stefancikova *et al.*, 2000). Approximately 40 species of mammals and birds have been recognized as a reservoir for *Borrelia burgdorferi* (Bb) (Gern *et al.*, 1998). Among the pet animals dog has been identified as the competent reservoir for *B. burgdorferi* sensu stricto (Mather *et al.*, 1994). Moreover, various researchers have proposed the dog as 'sentinel animal' for the detection of emerging risk areas of Lyme disease (Lindenmayer *et al.*, 1991; Falco *et al.*, 1993; Merino *et al.*, 2000; Bhide *et al.*, 2002). In Europe considerable study on canine Lyme borreliosis has been done with respect to symptoms and seroprevalence. The most com-

mon symptom in dog is migratory arthritis without divergent radiographic findings (Magnarelli *et al.*, 1987). Intermittent lameness can also be seen with several episodes. Other clinical signs consist of anorexia and general malaise. There are some reports of heart block (Levy and Dury, 1988), neurological sign like seizures (Azuma *et al.*, 1993), and fatal kidney failure (Dambach *et al.*, 1997). Although the various symptoms have been reported so far, the diagnosis of Lyme borreliosis in dogs is much more difficult. Recent serological techniques have made the diagnosis easier and more confirmative.

Antibody profile in dogs in Lyme disease is an interesting criterion that can be used for risk assessment of Lyme disease in humans. Purpose of this review is to focus on the possible use of dog as a marker for identification of new developing Lyme disease foci and to elaborate their eco-epidemiological importance.

Canine Lyme disease serodiagnosis and antibody profile

Till today sensitive serological techniques like ELISA, western blot, immuno fluorescent assay (IFA) etc. are developed to detect and confirm the *B. burgdorferi* infection in dogs. With the help of standard ELISA or IFA, antibody titers can be detected between 4–6 weeks after exposure to infected ticks. Serological tests are also developed to distinguish between early and late stages of *Borrelia* infection. Though the sensitive ELISAs are used widely in humans and pet animals, their specificity is still doubtful. False positive results can be obtained because of vaccination especially in the dogs. In efforts to improve the specificity of serological tests as well as to distinguish between antibodies after *Borrelia* infection and vaccination several strategies have been attempted, for example use of flagellin-enriched (Coleman and Benach, 1987) and purified (Hansen *et al.*, 1988) antigen preparations or specifically VIsE antigen (Liang *et al.*, 2000), and the use of recombinant antigens of *B. burgdorferi* expressed in *Escherichia coli* (Zumstein *et al.*, 1992). Recently, the more specific and confirmative borreliacidal antibody test has been used in dogs (Callister *et al.*, 2000). *B. burgdorferi* infection in humans and other animals results in production of killing (borreliacidal) antibodies. These antibodies are directed against several *B. burgdorferi* proteins including outer surface protein A (OspA), OspB, OspC, decorin binding protein A (DbpA) and outer membrane protein p66 (Scriba *et al.*, 1993; Probert and Lefebvre, 1994; Rousselle *et al.*, 1998; Exner *et al.*, 2000). Borreliacidal antibodies can be detected in dogs one week after attachment of infected tick (Callister *et al.*, 2000). These borreliacidal antibodies not only increase the specificity but also effectively distinguish between early and late Lyme disease.

In untreated infected animal, antibody level increases (IgG), reaching maximum at approximately 90–120 days after tick exposure, and then remain at its level up to one and a half year in the absence of re-exposure (Straubinger, 2000). On the other hand shorter span of anti-*Borrelia* antibodies has been reported in dogs. Moreover, Hovius *et al.* (1999) and Goossens *et al.* (2001) have reported obligatory yearly reinfection to maintain seropositivity in dogs. On the contrary the period of seropositivity in humans after an infection with *B. burgdorferi* is much longer.

Advantages of canine seroprevalence over serosurvey in other animals

Prediction of potential area for Lyme disease is a difficult task. An epidemiologist may experience more complexities in declaring any new geographical area as emerging risk zone for Lyme borreliosis. The complexity heightens particularly when human case prevalence is low. To identify the endemic area it is also very necessary to study vector-host relationship, vector population and anti-*Borrelia* antibody prevalence in the reservoir hosts. Some researchers have suggested a close association between population/distribution of *Ixodid* ticks and Lyme disease prevalence in humans and dogs (Lissman *et al.*, 1984; Magnarelli *et al.*, 1987). Canine anti-*Borrelia* serosurvey offers a promising tool for targeting areas presenting potential human risk (Rand *et al.*, 1991, 1996). Advantages of canine serosurvey over other animals are: simplicity in sample collection, effective follow up and/or feed back, known history of treatment and vaccination, and greater correlation for Lyme disease risk assessment to human being. On the other hand, tick vector distribution surveys, flagging, small mammal trapping or examination of deer and other wild free-living animals are laborious and time consuming (Eng *et al.*, 1988). Moreover, the seroprevalence in wild animals can not be applied directly to assess the Lyme disease risk to common people who have rare or no contact to forested areas. Measuring the tick density and prevalence of infected ticks in and around cities especially parks, playgrounds and recreational places near human habitat is one of the imperative approach to assess a Lyme risk for common people. As dogs have free and frequent access to such areas, combination of tick density and tick infectivity study with canine seroprevalence can be effective tool to judge the actual Lyme disease risk in the area under study.

Facts of canine seroprevalence

Till to date considerable work has been done in the field of canine seroprevalence. Anti-*Borrelia* antibodies in dogs have been reported in most of the major European countries (Table 1). Particularly in Slovakia, seropositivity in hunting dogs was 40% whereas; in service and pet dogs positivity observed was 11.80% and 29.40%, respectively (Stefancikova *et al.*, 1996). Difference in seropositivity according to

Table 1. Prevalence of anti-*Borrelia* antibodies in dogs from different parts of the world

Country	County	Method of detection	Prevalence (%)	No. of sample (n)	Reference
Bolivia	Cordillera	ELISA	0.0	43	Ciceroni <i>et al.</i> , 1997
Brazil	Cotia	ELISA	9.7	237	Joppert <i>et al.</i> , 2001
Croatia	Gorski Kotar	ELISA	40.0	10	Poljak <i>et al.</i> , 2000
Czech Republic	Prague	IHA	53.7	169	Sykora <i>et al.</i> , 1990
Germany	–	ELISA	7.2	665	Wittenbrink <i>et al.</i> , 1996
Germany	Berlin	ELISA	10.1	189	Kasbohrer and Schonberg, 1990
Germany	Berlin	IFA	5.8	189	Kasbohrer and Schonberg, 1990
Germany	Bavaria	IFA	35.5	130	Weber <i>et al.</i> , 1991
Israel	–	WB	10.0	40	Beneth <i>et al.</i> , 1998
Italy	Tyrrhenian coast	IFA	0.0	23	Mannelli <i>et al.</i> , 1999
Japan	Tokyo	ELISA	27.3	387	Arashima, 1991
Mexico	Monterrey	IFA	16.0	850	Salinas-Melendez <i>et al.</i> , 1999
Netherlands	(hunting dogs)	ELISA	18.0	448	Goossens <i>et al.</i> , 2001
	(Pet dogs)	ELISA	17.0	75	Goossens <i>et al.</i> , 2001
Slovakia	Kosice	ELISA	26.9	78	Stefancikova <i>et al.</i> , 1996
	Kosice (hunting dogs)	ELISA	45.3	75	Stefancikova <i>et al.</i> , 1998
	(service dogs)	ELISA	18.3	60	Stefancikova <i>et al.</i> , 1998
	(pet dogs)	ELISA	17.6	68	Stefancikova <i>et al.</i> , 1998
Spain	Castilla y Leon	IFA	21.0	308	Delgado and Carmenes, 1995
Spain	Soria	IFA	11.6	146	Merino <i>et al.</i> , 2000
Spain	Leon	IFA	2.10	95	Rojo Vazquez, 1997
Sweden	–	ELISA	3.9	588	Egenvall <i>et al.</i> , 2000
USA	Rhode Island	ELISA	52.0	227	Hinrichsen <i>et al.</i> , 2001
USA	Illinois	ELISA	56.9	1 077	Guerra <i>et al.</i> , 2000
USA	Fort Detrick	ELISA	20.0	440	Sheets <i>et al.</i> , 2000
USA	California	ELISA	2.3	917	Olson <i>et al.</i> , 2000
USA	Alabama	IFA	1.70	579	Wright <i>et al.</i> , 1997
USA	New York	ELISA	49.2	1 446	Falco <i>et al.</i> , 1993
USA	Oklahoma	ELISA	11.7	223	Mukolwe <i>et al.</i> , 1992
USA	Columbia	ELISA	24.3	37	Stockham <i>et al.</i> , 1992
USA	Maine	ELISA	4.34	828	Rand <i>et al.</i> , 1991
USA	Texas	IFA	5.5	2 409	Cohen <i>et al.</i> , 1990
USA	Oklahoma	IFA	18.0	259	Rodgers <i>et al.</i> , 1989
USA	Connecticut	IFA	66.5	155	Magnarelli <i>et al.</i> , 1987
USA	Hudson Valley	IFA	76.3	114	Magnarelli <i>et al.</i> , 1987
USA	New Jersey	IFA	34.7	423	Schulze <i>et al.</i> , 1987
USA	Wisconsin	IFA	53.0	380	Burgess, 1986

use and nature of dogs is also reported by Cohen *et al.* (1990), Stefancikova *et al.* (1998) and Merino *et al.* (2000) whereas, antibody prevalence was not associated with sex and season (Delgado and Carmenes, 1995). Outdoor activity is the prime factor, which

governs percent seropositivity against Lyme borreliosis in any given species of animal. In short, the difference in seroprevalence may due to differences in tasks performed by dogs and therefore the different tick exposition (Daniels *et al.*, 1993). Age de-

pendent variation in seroprevalence of Lyme disease in dogs is reported in Slovakia (Stefancikova *et al.*, 1996), Spain (Merino *et al.*, 2000) and North America (Cohen *et al.*, 1990; Lindenmayer *et al.*, 1991). Some researchers have tried to correlate the seropositivity and geno-phenotypic characteristics of dogs. In dogs with hard type of hairs greater seropositivity against *B. burgdorferi* was reported in comparison to others (Merino *et al.*, 2000). No correlation between size of dog and positivity was reported. Similarly gender is a factor, which does not affect the seropositivity (Magnarelli *et al.*, 1987; Delgado and Carmenes, 1995; Merino *et al.*, 2000). Apart from above explained factors, environment can also play an important role. Dogs living at higher altitude expressed minor seroprevalence in comparison with dogs living in lower region (Lindenmayer *et al.*, 1991). Study in Soria province in Spain by Merino *et al.* (2000) confirmed this hypothesis by comparing the seroprevalences in dogs from other altitudes. All environmental factors ultimately control the tick population in specific area. Tick population governs vector-host relationship as well as tick attachment rate and thus affect the seropositivity in dogs. In the canine surveillance system for Lyme borreliosis in Wisconsin and Illinois (Guerra *et al.*, 2001), seroprevalence pattern by county (0–40%) was significantly correlated with human incidence of Lyme disease and with abundance of tick vector, *Ixodes scapularis*. In the same study a geographic information system (GIS) was used to integrate environmental data with the location of the residences of the dogs to determine environmental risk factors. In Europe environmental risk factors for Lyme disease have been determined using satellite, climatological, and ecological data (Estrada-Pena, 1997; Daniel *et al.*, 1998; Randolph, 2000). Thus, seropositivity in dogs is positively associated with increased tick exposure, time spent outdoor, living in deciduous forested areas etc. Because of close similarity between Lyme disease risk factors of dogs and humans, canine surveillance system is useful method for assessing the risk as well as geographic distribution of Lyme disease.

Complement resistance of *Borrelia burgdorferi* and reservoir competence of dog

Complement-mediated killing of *B. burgdorferi* in hosts have ecological implications as it can determine the reservoir competence (Kurtenbach *et al.*,

1998b; Hovius *et al.*, 2000). The pattern of serum complement sensitivity of different *Borrelia* genospecies matches the known reservoir status of many vertebrate species (Kurtenbach *et al.*, 1998b). Studies indicate that *B. garinii* and *B. valaisiana* are mainly transmitted to ticks by avian hosts whereas, *B. afzelii* is transmitted to tick by rodents (Humair *et al.*, 1995; Kurtenbach, 1998a). *In vitro* canine complement sensitivity test (Hovius *et al.*, 2000) against three different *Borrelia* strains (B31, *B. burgdorferi* sensu stricto; pKo, *B. afzelii*; and A87S, *B. garinii*) showed B31 and pKo as resistant species to dog complement than A87S. It was observed that *Borrelia* isolates differ in their ability to activate complement and resist killing by serum bactericidal activity (Brade *et al.*, 1992). Though there is no extensive study available to compare species specific complement sensitivity of *Borrelia* and reservoir competence of dog, one can extrapolate the available complement sensitivity results to propose reservoir status of dog for particular *Borrelia* species (Hovius *et al.*, 2000). Such a correlation was made previously in rodents and squirrels by Kurtenbach *et al.* (1998b). Rodent complement resistance of *B. afzelii* parallels the prime transmission competence of rodent species (Humair *et al.*, 1995) and squirrels (Craine *et al.*, 1997). Furthermore, complement mediated lyses of *B. garinii* explains why the European rodents are insufficient reservoir for European *B. garinii* strains, while its resistance to pheasant complement makes clear the concept of reservoir competence ability of pheasant for the same *Borrelia* genospecies. In case of an incompetent reservoir sika deer active killing of *Borrelia* by complement takes place (Nelson *et al.*, 2000). Similarly lysis of *Borrelia* regardless of genospecies correlates the incompetent reservoir nature of deer explained by Jaenson and Talleklint (1992).

Lyme disease risk assessment for pet owners and hunters

Overall Lyme disease risk assessment data compiled in various reviews and reports (Flisiak and Zabicka, 1995; Arteaga and Garcia-Monco, 1999; Werner *et al.*, 2001), indicate the morbidity exceeds 100 cases per 100 000 inhabitants per year in central Europe. Comparatively higher prevalence observed in outdoor workers than indoor workers in southwest Sweden (Werner *et al.*, 2001) and Spain (Arteaga and Garcia-Monco, 1999) indicates posi-

tive correlation between human contact with tick vector and Lyme disease risk. Only measurement of human contact with a tick is not sufficient criteria to assess the risk of Lyme disease. Apart from this criterion, the population of reservoir competent domestic, wild as well as pet animals in particular area is necessary to study.

There are many controversies about zoonotic importance of pet animals as far as Lyme disease is concern. Even if some authors have had put forth hypothesis about greater risk of Lyme disease to pet owners (Mather *et al.*, 1994), there is no concrete evidence of direct infection from pet animals or dogs to human. Survey in the Netherlands by Goossens *et al.* (2001), showed no correlation between sole ownership of dogs and seropositivity against Lyme disease. However, recently a case of one and a half year old girl suffering from gonarthitis has been reported by Zajadacz and Juskiewicz (2002) with high antibody titre. The girl was never in the forest and had no contact with animals except a pet dog. Authors suggested the most possible transmission of Lyme disease from pets to the girl.

Hunting dogs usually carry infected ticks from the forest. Loosely attached ticks as well as infected females from dog drop near human habitat. Female ticks lay eggs in the spring, which hatches to larvae. These Ixodid larvae preferentially feed on small mammals and rodents. Presence of rodent population in and around human habitat facilitates feeding of larvae and nymphs and consequently helps in establishment of the tick population. Rodents are known competent reservoir from which Ixodid larvae acquire *Borrelia* infection. In the following spring larvae moult into nymphs, with an acquired infection from rodents. Ixodid nymphs have wide host range including dogs and humans. Nymphs moult to adult in fall and act as the most important source of infection for dogs. Transmission of *Borrelia* from Ixodid ticks to dogs, cats and human has been reported (Smith *et al.*, 1993). Higher seroprevalence (33%) in the domestic cats does not exclude the importance of this pet animal in Lyme disease epidemiology (Magnarelli *et al.*, 1990).

To conclude, screenings of dog for seropositivity is good indicator of actual and present risk of Lyme disease in particular area due to shorter span of anti-*Borrelia* IgG antibodies. Dogs stay seropositive for a much shorter period after an infection with *Borrelia*. On the other hand the seropositivity in other animals as well as in humans persists for several years. Similar seroprevalence in hunting

dogs and humans particularly in hunters (Goossens *et al.*, 2001) illuminates close relation and linked epidemiological aspects of Lyme disease. Evolution and establishment of Lyme disease focus may occur quickly due to favourable climatic conditions and geoeological suitability of central Europe for tick vectors. Hunting dogs can serve as seroindicators and/or sentinel for identifying new focuses as well as assessing the changes in endemicity of well known focuses of Lyme disease.

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