Original Article

Serological evidence of Borreliosis among companion dogs in Fars Province, South of Iran

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Summary

Lyme Borreliosis is a vector-transmitted zoonotic disease caused by the spirochetes from the *Borrelia burgdorferi* sensu lato complex. Dogs are the most susceptible domestic animals and serve as an appropriate animal model for detection of the endemic areas for Lyme borreliosis. The aim of the present study was to evaluate the occurrence and seroprevalence of *B. burgdorferi* infection among companion dogs population in Fars province, South of Iran, from December 2014 to September 2015. Blood samples were collected from 181 asymptomatic dogs, mostly referred to Veterinary Hospital of Shiraz University for regular vaccination. The antibody detection against *B. burgdorferi* was made by indirect Enzyme-linked Immunosorbent Assay (ELISA), employing *B. burgdorferi* VlsE antigen. A logistic regression model was developed to analyze multiple risk factors associated with seropositivity. Of 181 serum samples, 2 (1.1%) showed antibodies against *B. burgdorferi*, one was a 3-month old male watching dog with mix breed, and the other was a 2.5 year old female great din. Since no vaccination program was running in the studied population, positive samples were considered as naturally infected. The results of this study revealed the presence of *B. burgdorferi* infection among the companion dogs population in Fars province. In areas like Iran, where human cases of *B. burgdorferi* are not common or remain unreported, the public health implications of Lyme borreliosis seroprevalence in dogs are quite significant.

Key words: Lyme Borreliosis, *Borrelia burgdorferi*, Dogs, Fars.

Introduction

Lyme borreliosis is a vector-transmitted zoonotic disease caused by the spirochetes from the *Borrelia burgdorferi* sensu lato complex (Steere et al., 2004). Genus *Borrelia* includes a complex of species that can be functionally summarized as Lyme disease and relapsing fever *Borrelia* (Krupka and Straubinger, 2010). Lyme disease causative agents are mostly transmitted by ticks of the genus *Ixodes* and possibly by other arthropods such as fleas and blood sucker insects (Bowman et al., 2009; Hanifeh et al., 2012; Rostami et al., 2014). Several animal species including birds, rodents, dogs, cats and deer could serve as reservoirs of *Borrelia*

and some of them have an important role in introducing the tick and bacteria to human (Hanifeh., et al 2012; Goossens et al., 2001; Leonhard et al., 2010). Considering the importance of the disease in human beings, special attention is made on domestic animals and particularly on dogs. It seems that dogs are the most susceptible domestic animals, and serve as an appropriate animal model for detection of the endemic areas for Lyme borreliosis. Dogs can be assumed as an intermediary source of human infection and are used as "sentinel animals" for estimating the risk of Lyme disease in human (Hanifeh et al., 2012; Goossens et al., 2001; Merino et al., 2000; Mosallanejad et al., 2015).

The most common clinical manifestation of Lyme disease in dogs is migratory arthritis. The spirochetes disseminate into the joints and local inflammatory reactions can cause pain, swelling and lameness. Other but less common symptoms reported in dogs are fever, anorexia, lethargy, skin lesions, lymphadenopathy and abortion (Krupka and Straubinger, 2010; Bowman et al., 2009; Goossens et al., 2001; Greene et al., 1988). Nevertheless, many dogs fail to develop any clinical signs and high antibody titers can be developed in asymptomatic dogs. But at the same time, *Borrelia* has been isolated from the blood of some dogs with low antibody titers (Mosallanejad et al., 2015; Greene et al., 1988). These findings emphasize that definite diagnosis of canine borreliosis is almost difficult. However, evidences of tick exposure in endemic areas, presence of compatible clinical signs and detection of antibodies in blood serum and exclusion of other infectious diseases could be informative (Skotarczak, 2002; Speck et al., 2007).

Bacterial isolation and culture, as the most reliable detecting methods for *Borrelia* spp., are time consuming and are not always practical. No specific pathognomonic features exist in laboratory findings and biochemistry and hematology profiles are not usually diagnostic (Krupka and Straubinger, 2010; Rostami et al., 2014; Liang et al., 2000; Nielssen et al., 2002). Hence, serological tests have been widely used for indirect detection of *Borrelia* by detecting antibodies in the serum of infected animals (Hanifeh et al., 2012; Mosallanejad et al., 2015; Liang et al., 2000; Lindenmayer et al., 1990). In experimentally infected dogs, detectable IgG titers were produced 4 to 6 weeks after tick exposure and even after antibiotic treatment were persisted for years. While determining the exact time of exposure and the length of time that IgG level remains elevated is difficult in natural infections, it could be somewhat the same as the first one (Krupka and Straubinger, 2010; Greene et al., 1988; Lindenmayer et al., 1990).

Previous studies regarding the prevalence of *B. burgdorferi* in companion dogs have shown variable results in different provinces of Iran (Hanifeh et al., 2012; Mosallanejad et al., 2015). This diversity could be due to the different geographic area, endemicity of the disease and abundance of the vector. Therefore, the status of borreliosis should be defined separately for each area. The aim of the study described here was to evaluate the occurrence and seroprevalence of *B. burgdorferi* infection among companion dogs population in Fars province, South of Iran. This is the first study regarding

the prevalence of canine Lyme borreliosis in Fars province. Since dogs may serve as a potential source of infection for human beings, the public health implications of these results are quite significant.

Materials and Methods

The present study was conducted on 181 blood samples collected from companion dogs in Fars province, Shiraz district. Shiraz is located at an elevation of 1484 m above sea level and its climate is semi-arid, with mild winters and warm summers (Saboohi et al., 2012). The dogs subjected to the study were referred to the Veterinary Hospital of Shiraz University, from December 2014 to September 2015, mostly for regular vaccination. Amongst the specimens, 107 were male dogs and 74 were female. The dogs were classified into 3 groups according to their age (group 1, <1 year; group 2, 1–5 years; and group 3, >5 years). They were also classified as pure and mix-breed according to their breed. At the time of blood collection, 21 dogs showed respiratory or gastrointestinal signs, but all others were asymptomatic and revealed no clinical signs of Lyme disease, including fever, arthritis or lameness (Table 1). Each serum sample was accompanied by a questionnaire to be completed by the owner and included information related to the place where dog kept, exposure to ticks, type of food and relation to other dogs. None of the animals included in this study had been vaccinated against Lyme borreliosis and as no *Borrelia* vaccine is available in Iran.

Blood samples were collected from the cephalic veins and sera were separated and stored at -20°C until serological assays. Immunoglobulin G (IgG) class antibodies against *B. burgdorferi* were detected using indirect Enzyme-linked Immunosorbent Assay (ELISA), employing *B. burgdorferi* VlsE (Variable major protein-like sequence, expressed) antigen. VlsE is an outer membrane lipoprotein which is highly conserved among species of *B. burgdorferi* sensu lato complex and only expressed in mammalian hosts (Krupka and Straubinger, 2010; Liang et al., 2000). The antibodies against the VlsE antigen were detected using a commercial ELISA kit (NovaTec Immunodiagnostica GmbH, Germany).The diagnostic specificity and sensitivity of the ELISA test were >95 and 93.3%, respectively. According to the manufacturer's instructions, absorbance values higher than 10% over the cut-off were considered positive, while absorbance values lower than 10% below the cut-off were considered negative.

Relationships between the prevalence of positive cases and age, sex, breed, type of housing, type of food and exposure to other dogs were examined using chi-square analysis and Fisher's exact test by SPSS software, version 16 (SPSS, Inc. Chicago, IL, USA). Multiple logistic regression analysis was performed to evaluate the effects of different risk factors on the disease. P value less than 0.05 was considered statistically significant.

Results

IgG antibodies against *B. burgdorferi* were detected in 1.1% (n=2) of the companion dogs in Shiraz district. One of them was a 3-month old male watching dog with mix breed, while the other was a 2.5 year

old female great din. Both of dogs were kept in the garden and in contact with other dogs. No clinical symptoms of Lyme disease were observed in the studied dogs, though, positive samples showed respiratory and gastrointestinal signs (Table 1).

Table 1. Seroprevalence of *Borrelia burgdorferi* infection among companion dogs in Shiraz province, Iran.

Variable	Category	No. of dogs examined (%)	No. of positive dogs (%)
Age (year)	< 1	81 (44.8)	1 (1.23)
	1-5	89 (49.2)	1 (1.12)
	> 5	11 (6.6)	0
Sex	Male	107 (59.1)	1 (0.93)
	Female	74 (40.9)	1 (1.35)
Type of housing	Indoor	19 (10.5)	0
	Watchdog	146 (80.7)	2 (1.36)
	Stray	16 (8.8)	0
Breed	Pure	96 (53)	1 (1.04)
	Mix	85 (47)	1 (1.17)
Type of food	Raw food	76 (42)	1 (1.31)
	Cooked food	95 (52.5)	1 (1.05)
	Both	10 (5.5)	0
Exposure to other dogs	Yes	117 (64.6)	2 (1.7)
	No	64 (35.4)	0
Clinical signs	Lyme Borreliosis	0	0
	Respiratory	9 (4.9)	2 (22.22)
	Gastrointestinal	12 (6.52)	2 (16.66)
	No signs	160 (88.39)	0
Total		181 (100)	2 (1.1)

Since no vaccination program was running in the studied population, positive samples were considered as naturally infected. Because of the low prevalence of canine borreliosis in the studied population, no statistical analysis was possible.

Discussion

Results of the present study showed the evidence of exposure to *B. burgdorferi* infection in the companion dog population in South of Iran. The overall seroprevalence of 1.1% in this study was in great agreement with the earlier reports of 1.09% from France (Pantchev et al., 2009) and 1.2% from United States of America (Carrade et al., 2011) using SNAP® 4Dx® and ELISA techniques as screening assays. However, our seroprevalence was higher than the prevalence reported from east of Turkey (ELISA, 0.0%) (Icen et al., 2011), Bolivia (ELISA, 0.0 %) (Ciceroni et al., 1997), Italy (western blot, 0.0 %) (Mannelli et al., 1999) and Romania (SNAP® 4Dx®, 0.5 %) (Mircean et al., 2012), while it was lower than the seropositivity reported from Czech Republic (ELIZA, 6.5%) (Pejchalova 2006), Netherlands (ELISA, 17 %) (Goossens 2001), Spain (IFA, 21 %) (Amusategui et al 2008), West of Turkey (ELISA, 23.2%) (Bhide et al., 2008), Serbia (ELISA, 25.81 %) (Savić et al., 2010) and Germany (SNAP® 4Dx®, 35.5 %) (Barth et al., 2012).

Documented information regarding the prevalence of Lyme borreliosis among Iran dogs population is restricted to two studies from Northern and South-western provinces. A study conducted on 273 serum samples collected from three Caspian provinces of Golestan, Mazandaran and Guilan reported the overall seroprevalence of 8.1% using ELISA technique. The seroprevalence of *B. burgdorferi* in provinces of Golestan, Mazandaran, and Guilan were 20.91%, 2.91%, and 0.91%, respectively (Hanifeh et al 2012). Seroprevalence of *Borrelia*

infection among companion dogs in Ahvaz district (Southwest of Iran) was 9.52% identified by Immunochromatography assay (ICA) (Mosallanejad et al., 2015). It seems that prevalence of *Borrelia* infection is different not only between countries, but also between different regions within a country. These differences could be attributed to different geographical areas, environmental factors, exposure to the tick vectors and diagnostic laboratory methods (Merio et al., 2000; Nielssen et al., 2002).

The lower prevalence of *Borrelia* infection in Shiraz district could be due to the geographical conditions and activity of tick vectors. Based on the literature, *Ixodes ricinus* and *I. persulcatus* are the main vectors of *B. burgdorferi* in Europe and Eurasia, respectively (Hanifeh et al., 2012; Goossens et al., 2001; Pantchev et al., 2009). Low altitude and relative humidity of more than 80% are necessary for survival and activity of *Ixodes* genus (Merino et al., 2000; Guerra et al., 2001). A comprehensive tick survey carried out on different geographical areas of Iran (North, North-west/North-east, South and Central regions) showed that *Ixodes* ticks were only present in the Caspian region (Rahbari et al., 2007). Semi-arid climate of Shiraz district along with high temperature and low humidity during the spring and summer seasons are the main limiting factors which decrease the survival rate of carrier ticks and consequently the prevalence of infection in this area. Therefore, it seems that dogs in the Northern areas of the country are at a higher risk of *Borrelia* infection.

Variety in the seroprevalence of *Borrelia* infection among different areas could also be as a result of different diagnostic methods used for detection of antibodies. Serological assays such as ELISA, Immunofluorescent assay (IFA) and Immunochromatography assay (ICA) are widely used as screening tests for *Borrelia* infection, while Immunoblotting assays are more considered as confirmatory tests (Krupka and Straubinger, 2010; Hanifeh et al., 2012; Mosallanejad et al., 2015; Lindenmayer et al., 1990; Guerra et al., 2001). Among serological tests, ELISA presented greater sensitivity relative to IFA and ICA. Moreover, using recombinant antigens for ELISA coating, the specificity of the test has been significantly increased (Krupka and Straubinger, 2010; Liang et al., 2000; Lindenmayer et al., 1990). In the present study, IgG antibodies against *B. burgdorferi* were detected using ELISA method, employing *B. burgdorferi* VlsE antigen. Surface accessibility, flexibility, hydrophobicity and proximity to the site recognized by helper T cells are characteristics which positively determine the VlsE antigenicity (Liang et al., 2000a; Liang et al., 2000b). VlsE undergoes antigenic variation and allow the spirochete to escape efficiently from the host immune responses (Krupka and Straubinger, 2010; Liang et al., 2000a). VlsE antigen is highly conserved among genospecies of *B. burgdorferi* sensu lato complex, and therefore recombinant VlsE ELISA is expected to be highly sensitive and specific diagnostic technique for Lyme disease (Liang et al., 2000a; Liang et al., 2000b).

In conclusion, the results of this study revealed the presence of *B. burgdorferi* infection among the companion dogs

population in Fars province, South of Iran. However, a positive antibody test may not be sufficient to differentiate the Lyme borreliosis disease from prior exposure in a dog's history. Further studies including molecular detection of *B. burgdorferi* sensu lato from companion dog population and isolated ticks in suspected areas are highly recommended. In areas like Iran, where human cases of *B. burgdorferi* are not common or remain unreported, the public health implications of Lyme borreliosis seroprevalence in dogs are quite significant.

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Conflicts of Interest: None.

References

Amusategui I., Tesouro MA., Kakoma I. and Sainz Á. (2008). Serological reactivity to *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Neorickettsia risticii*, *Borrelia burgdorferi* and *Rickettsia conorii* in dogs from northwestern Spain. *Vector Borne and Zoonotic Diseases Journal*, 8(6), pp. 797-804.

Barth C., Straubinger RK., Sauter-Louis C. and Hartmann K. (2012). Prevalence of antibodies against Borrelia burgdorferi sensu lato and Anaplasma phagocytophilum and their clinical relevance in dogs in Munich, Germany. *Berliner und Münchener tierärztliche Wochenschrift*, 125 (7-8), pp. 337-44.

Bhide M., Yilmaz Z., Golcu E., Torun S. and Mikula I. (2008). Seroprevalence of anti-Borrelia burgdorferi antibodies in dogs and horses in Turkey. *Annals of Agricultural and Environmental Medicine*, 15(1), pp. 85-90.

Bowman D., Little SE., Lorentzen L., Shields J., Sullivan MP. and Carlin EP. (2009). Prevalence and geographic distribution of *Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia canis*, and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey. *Veterinary Parasitology*, 160(1), pp.138-48.

Carrade D., Foley J., Sullivan M., Foley CW. and Sykes JE. (2011). Spatial distribution of seroprevalence for *Anaplasma phagocytophilum, Borrelia burgdorferi, Ehrlichia canis*, and *Dirofilaria immitis* in dogs in Washington, Oregon, and California. *Veterinary Clinical Pathology*, 40(3), pp.293-302.

Ciceroni L., Bartoloni A., Ciarrocchi S., Pinto A., Guglielmetti P., Valdez Vasquez C., Gamboa Barahona H., Roselli M. and Paradisi F. (1997). Serologic survey for antibodies to *Borrelia burgdorferi* in sheep, goats and dogs in Cordillera Province, Bolivia. *Journal of Veterinary Medicine*, 44, pp.133-37.

Goossens H., Van Den Bogaard A. and Nohlmans M. (2001). Dogs as sentinels for human Lyme borreliosis in The Netherlands. *Journal of Clinical Microbiology*, 39(3), pp. 844-48.

Greene RT., Walker RL., Nicholson WL., Heidner HW., Levine JF., Burgess EC., Wyand M., Breitschwerdt EB. and Berkhoff HA. (1988). Immunoblot

analysis of immunoglobulin G response to the Lyme disease agent (*Borrelia burgdorferi*) in experimentally and naturally exposed dogs. *Journal of Clinical Microbiology*, 26(4), pp. 648-53.

Icen H., Sekin S., Simsek A., Kochan A., Celik O. and Altas M. (2011). Prevalence of Dirofilaria immitis, Ehrlichia canis, Borrelia burgdorferi infection in dogs from Diyarbakir in Turkey. *Asian Journal of Animal and Veterinary Advances*, 6(4), pp.371-8.

Krupka I. and Straubinger RK. (2010). Lyme borreliosis in dogs and cats: background, diagnosis, treatment and prevention of infections with Borrelia burgdorferi sensu stricto. *Veterinary Clinics of North America: Small Animal Practice*, 40(6), pp.1103-19.

Leonhard S., Jensen K., Salkeld DJ. and Lane RS. (2010). Distribution of the Lyme disease spirochete Borrelia burgdorferi in naturally and experimentally infected western gray squirrels (Sciurus griseus). *Vector Borne and Zoonotic Diseases journal*, 10(5), pp.441-6.

Liang FT., Aberer E., Cinco M., Gern L., Hu CM., Lobet YN., Ruscio M., Voet PE Jr., Weynants VE. and Philipp MT. (2000). Antigenic conservation of an immunodominant invariable region of the VlsE lipoprotein among European pathogenic genospecies of *Borrelia burgdorferi* SL. *Journal of Infectious Dis*eases, 182(5), pp.1455-62.

Liang FT., Jacobson RH., Straubinger RK., Grooters A. and Philipp MT. (2000). Characterization of a Borrelia burgdorferi VlsE invariable region useful in canine Lyme disease serodiagnosis by enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology*, 38(11), ppt.4160-6.

Lindenmayer J., Weber M., Bryant J., Marquez E. and Onderdonk A. (1990). Comparison of indirect immunofluorescent-antibody assay, enzyme-linked immunosorbent assay, and Western immunoblot for the diagnosis of Lyme disease in dogs. *Journal of Clinical Microbiology*, 28(1), pp.92-6.

Mannelli A., Cerri D., Buffrini L., Rossi S., Rosati S., Arata T., Innocenti M., Grignolo MC., Bianchi G., Iori A. and Tolari F. (1999). Low risk of Lyme borreliosis in a protected area on the Tyrrhenian coast, in central Italy. *European Journal of Epidemiology*, 15(4), pp. 369-75.

Merino F., Serrano J., Saz J., Nebreda T., Gegundez M. and Beltran M. (2000). Epidemiological characteristics of dogs with Lyme borreliosis in the province of Soria (Spain). *European Journal of Epidemiology*,16(2), pp. 97-100.

Mircean V., Dumitrache MO., Györke A., Pantchev N., Jodies R., Mihalca AD. and Cozma V. (2012). Seroprevalence and geographic distribution of *Dirofilaria immitis* and tick-borne infections (*Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato, and *Ehrlichia canis*) in dogs from Romania. *Vector Borne and Zoonotic Diseases Journal*, 12(7), pp. 595-604.

Mosallanejad B., Avizeh R., Jalali MHR. and Pourmahdi M. (2015). A serological survey on Borrelia burgdorferi infection among companion dogs in Ahvaz district, southwestern Iran. *Comparative Clinical Pathology*, 24(6), pp. 1559-63.

Nielssen A., Carr A. and Heseltine J (2002). Update on canine lvme disease. *Veterinary Medicine*, 97(8), pp.604-10.

Pantchev N., Schaper R., Limousin S., Norden N., Weise M. and Lorentzen L. (2009). Occurrence of Dirofilaria immitis and tick-borne infections caused by Anaplasma phagocytophilum, Borrelia burgdorferi sensu lato and Ehrlichia canis in domestic dogs in France: results of a countrywide serologic survey. *Parasitology Research*, 105(1), pp.101-14.

Pejchalová K., Žákovská A., Fučík K. and Schánilec P. (2006). Serological confirmation of Borrelia burgdorferi infection in dogs in the Czech Republic. *Veterinary Research Communications*, 30(3), pp.231-8.

Rahbari S., Nabian S. and Shayan P. (2007). Primary report on distribution of tick fauna in Iran. *Parasitology Research*, 101(2), pp. 175-7.

Rostami A., Zaeimi M., Piazhak N. and Mazaheri Nezhad Fard R. (2014). A case report of atypical borreliosis in a dog. *Iranian Journal Of Veterinary Science and Technology*, 3(2), pp. 49-56.

Saboohi R., Soltani S. and Khodagholi M. (2012). Trend analysis of temperature parameters in Iran. *Theoretical and Applied Climatology*, 109(3-4), pp. 529-47.

Savić S., Vidić B., Lazić S., Lako B., Potkonjak A. and Lepšanović Z. (2010). *Borrelia burgdorferi* in ticks and dogs in the province of Vojvodina, Serbia. *Parasite Journal* ,17(4), pp. 357-61.

Skotarczak B. (2002). Canine borreliosis-epidemiology and diagnostics. *Annals of Agricultural and Environmental Medicine*, 9(2), pp. 137-40.

Speck S., Reiner B., Streich W., Reusch C. and Wittenbrink M. (2007). Canine borreliosis: a laboratory diagnostic trial. *Veterinary Microbiology Journal*, 120(1), pp. 132-41.

Steere AC., Coburn J. and Glickstein L. (2004). The emergence of Lyme disease. *Journal of Clinical Investigation*, 113(8), pp. 1093-101.