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Detection of *Borrelia burgdorferi* in dogs using line immunoblot assay

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ABSTRACT
Lyme borreliosis is a vector-borne disease caused by spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex and transmitted by ticks belonging to the *Ixodes* genus. The importance of dogs as sentinel species had been previously highlighted because the dogs are the host for vector ticks sharing habitats and being in close physical contact with humans. The purpose of the present study was to investigate the exposure of dogs to *B. burgdorferi* using immunological tests. Our study included a total of 49 dogs aged between 0.8-16 years. The serum of dogs was tested for the presence of antibodies against *B. burgdorferi*s.l. using immunoblot line assay. The positive results obtained from the screening of all 49 dogs were confirmed using species-specific immunoblot assays. Overall, 20.4% (10/49) of the samples were positive for *B. burgdorferi*s.l. expressing proteins like p41, OmpC, OmpA, p100. Immunoblot analysis confirmed the presence of antibody against *B. afzeli* in 5 cases (10.2%), *B. burgdorferi* sensu stricto in one case (2%), and the remaining were more likely infected with other *Borrelia* species. Our results show that investigation of exposure of dogs to *B. burgdorferi* can provide valuable information on the potential risk of infection with this pathogen in humans.
Keywords: *Lyme disease, Borrelia burgdorferi, line blot assay, dogs.*

1. INTRODUCTION

Lyme borreliosis is a vector-borne disease caused by spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex and transmitted by ticks belonging to the *Ixodes* genus. *Ixodes ricinus* is the main vector of *Borrelia burgdorferi* in Europe (Farkas et al., 2014). It affects a wide range of wild and domestic animals but only a small part show clinical symptoms (Ionita et al., 2013). The importance of dogs as sentinel species had been previously highlighted because the dogs are the host for vector ticks sharing habitats and being in close physical contact with humans (Ebani et al., 2014; Barth et al., 2014). The incidence of Lyme disease reaches from 2 to 40/100 000 in Germany and to 300/100 000 in Austria (Peltomaa et al., 2004). The estimated number of unrecognized cases is presumably higher, because a tick bite is not always recognized and the typical erythema chronicummigrans only develops in about 50% of *Borrelia* infections (Lawrenz, et al, 1999). The transmission of spirochetes takes place after the first 24 hours of ticks feeding, and the predominant species that causes Lyme disease are: *B. gariniic*ausing mainly neurological disease, *B. afzelii*associated with degenerative skin symptoms and less commonly *B. burgdorferi* s.s. (Arnaboldi et al., 2013) Relatively a small number of infected dogs demonstrate clinical signs, in Romania few data have been available concerning the occurrence of Lyme borreliosis in dogs. Therefore the aim of this study was to assess the seroprevalence of *B. burgdorferi*s.l. in dogs.

2. EXPERIMENTAL SECTION

Our study included a total of 49 dogs (32 males and 17 females) aged between 0.8-16 years. The blood was acquired from dogs examined at Veterinary Clinique ofthe Faculty of Veterinary Medicine, Bucharest, and serum was obtained by centrifugation at 4000 rpm, 10 minutes. The serum of dogs was tested for the presence of antibodies against *B. burgdorferi* sensu lato using immunoblot assay (Anti-Borrlia IgG Line Immunoassay, DRG, Germany). The positive results obtained from the screening of all 49 dogs were confirmed using species-specific immunoblot assays (Blot *Borrelia afzelii* IgG and Blot *Borreliaburgdorferi* sensu stricto IgG, TestLine, Czech Republic).Because regionally differing occurrence of subtypes (genospecies) and the known variability of the cell surface proteins of *Borrelia burgdorferi*, antigen mixtures are often preferred. Confirmatory tests like western blot, dot blot, line blot are essential and the serological test results have to be interpreted together with clinical picture (Hauser et al., 1997).

3. RESULTS SECTION

Our result showed that from 49 serum samples, 20.4% (10/49) were positive for *B. burgdorferi*s.l. expressing antibodies against p41 (flagellin), OspC, OspA, p100, VlsE antigens(Table 1), (Fig. 1). Immunoblot analysis confirmed the presence of antibodies against *B. afzeli* in 5 cases (10.2%), *B. burgdorferi* sensu stricto in one case (2%), and the remaining were more likely infected with other *Borrelia* species (Table 2), (Fig. 2). Dog's positive for *Borrelia* infection had aged between 0.8-13 years,

suggesting that the infection is not dependent on age. Principal vectors of *B. burgdorferi* s.l. in Europe, including urban and suburban ecosystems, are two tick species: *I. ricinus* and *I. persulcatus*, the latter only occurring in eastern and north-eastern Europe (Rizzoli et al., 2014). In many European countries the *Borrelia burgdorferi*s.l. have only been reported in the vector, the seroprevalence of canine borreliosis is not well documented to date (Barth et al., 2014).Other study from Denmark and Swedish found a prevalence in dogs of 16.1% and 20.7% respectively (Kramer et al., 2014). Barth et al., showed that the seroprevalences of B. burgdorferi in the dogs in Germany was 4.9% and 28.6% for Czech Republic. In a study complete by Mircean et al., in 2012 on dogs from Romania showed that the seroprevalence for the *B. burgdorferi* was 0.5%, 6 from 1146 serum samples were tested positive using SNAP(®) 4Dx(®). An associated risk factor was the type of dog: stray dogs were at risk being positive for *Dirofilaria immitis*, shelter dogs for *Erlichiacanis*, and hunting dogs for *B. burgdorferi* (Mircean et al., 2012). In another study from Romania, 276 serum samples from dogs were analyzed by ELISA and immunofluorescencetechniques. Kramer et al., (2013) analyzed more than 12.000 *Ixodes ricinus* ticks from all country of Romania and found that 1.4% are infected with *Borrelia burgdorferi*s.l. Furthermore, by reverse line blot hybridization and RFLP they identified three Borrelia genospecies: *B. afzelii*, followed by *B. garinii* and *B. burgdorferi* sensu s.s (10).The overall prevalence of anti-Borrelia antibodies was 6.52% (18/276) in dogs, with a significantly higher positivity recorded in a mid-country region. Seroreactivity was correlated with occupation, with working dogs being more exposed (Kiss et al., 2011).The reference methods for detection and identification of Borrelia species involves serological tests based on specific IgG and IgM antibody detection such as ELISA and Western blot or by molecular biology methods such as PCR and PCR-RFLP (Hauser et al., 1997).

Table 1. Screening of *Borrelia spp*. in serum of 49 dogs

Positive samples	Antigens detected
D1	p41, OspC
D3	p41, p100
D4	p100, p41, OspC
D7	p41, VlsE, OspA, OspC
D17	p100, p41
D19	p41, OspC
D24	p41, OspC
D31	p100, p41
D48	OspC
D49	p41

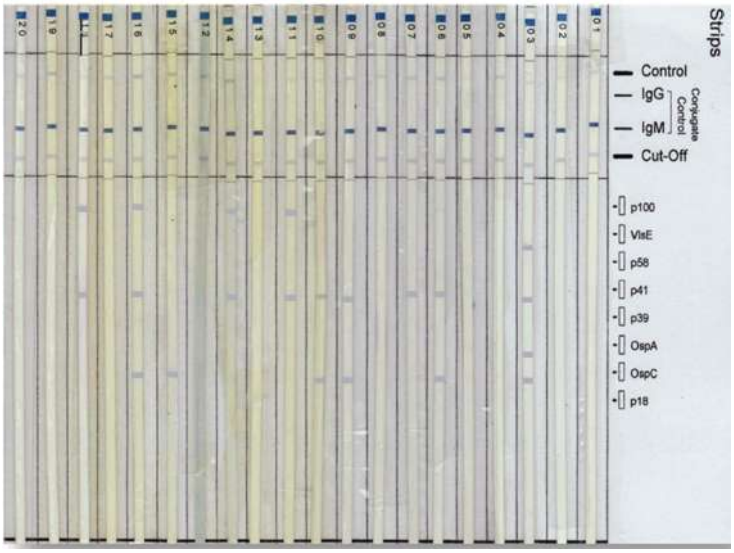

Figure 1. Screening of Anti-*Borrelia* IgG

Table 2. Identification of *Borrelia* species in positive cases

Positive samples	Antigens detected
Borrelia afzelii	
D17	OspA
D19	OspA, OspC
D24	OspA, BmpA
D31	OspA
D48	OspC
***Borrelia burgdorferi* sensu stricto**	
D49	OspC

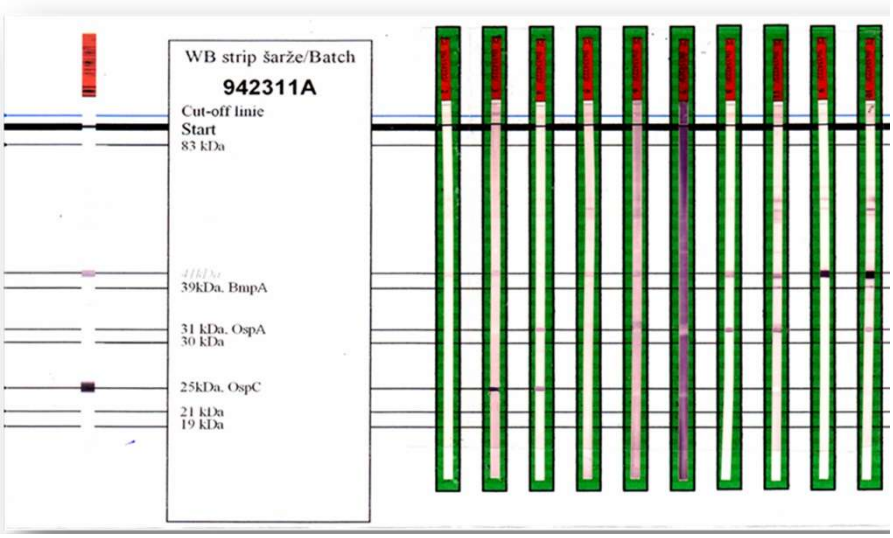

Figure 2. Identification of *Borrelia afzelii* IgG

4. CONCLUSIONS

Dogs and cats are heavily infested with ticks and might act as hosts (probably not reservoirs) or sentinels for Lyme borreliosis. The risk of exposure of dogs to numerous vector-borne pathogens has increased, and close relationship with humans in urban areas poses new concerns for human public health (Rizzoli et al., 2014). The occurrence of this important canine, but also zoonotic parasites should be more investigated for a better evaluation of dog's role as reservoir in boreliosis disease. Our results shows that exposure to *B. burgdorferi* infection in dogs can provide valuable information on the infectious potential for humans. The prevalent species identified in dogs was *B. afzelii* followed by *B. burgdorferi* s.s. and the infection does not influenced by the age.

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