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Serological Reactivity to *Borrelia burgdorferi Sensu Lato* in Dogs and Horses from Distinct Areas in Romania

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Abstract

Lyme disease is a perfect model of the complex relationship between host, vector, and the vector-borne bacteria. Both dogs and horses in Romania are exposed to infection. The aim of the present study was to assess the seroreactivity against *Borrelia burgdorferi sensu lato* in dogs and horses from different regions of Romania. 276 samples from dogs and 260 samples from horses located in different regions of Romania were analyzed by ELISA and IFA, respectively. The effect of several factors potentially affecting seroreactivity (location, age, gender, occupation, and vector exposition risk) was evaluated using Fisher's exact test (R 2.12.0). The overall prevalence of anti-*Borrelia* antibodies was 6.52% (18/276) in dogs, with a significantly higher positivity (46.15%, 6/13, $p = 0.0005$) recorded in a midcountry region. Seroreactivity was correlated with occupation, with working dogs being more exposed. The results may indicate that Lyme borreliosis foci are restricted to small areas, but further studies on *Borrelia* prevalence in tick populations are needed to confirm this hypothesis. In horses, a global seroprevalence of 11.92% (31/260) was observed. No correlations were found between positive results and age, sex, county, or occupation. This is the first serological survey on antibodies to *B. burgdorferi sensu lato* in Romanian dogs and horses.

Key Words: *Borrelia burgdorferi Sensu Lato*—Dogs—ELISA—IFA—Horses—Seroprevalence.

Introduction

Lyme borreliosis is a vector-borne disease caused by spirochetes of the *B. burgdorferi sensu lato* (s.l.) complex and transmitted by ticks belonging to the *Ixodes* genus. Besides causing a multisystemic disease in humans, it affects a wide range of wild and domestic animals; among these, Lyme disease has been well studied in dogs and horses. Even though only 5%–10% of the exposed individuals show clinical symptoms (Manion and Bushmich 1998, Bhide et al. 2008, Leschnik et al. 2010), data regarding exposure to *B. burgdorferi* s.l. in these species can provide valuable information on the infectious potential for humans in a specific location (Salinas-Meléndez et al. 2001, Duncan et al. 2005). The importance of dogs and horses as sentinel species had been previously underlined (Bhide et al. 2004, Amusategui et al. 2008, Hansen et al. 2010, Maurizi et al. 2010) because of the fact that they are host species for the vector ticks, sharing habitats and being in close physical contact with humans. The presence of *B. burgdorferi* s.l. has been acknowledged in Romania more than 20 years ago (Crăcea et al. 1988). Studies conducted a decade ago have measured *Borrelia* seroprevalence in healthy blood donors and forestry workers (Hristea et al. 2001). Data regarding the pathogen's circulation in enzootic areas is scarce, with limited studies conducted on lizards and their ticks (Majláthová et al. 2008) as well as on unfed ticks collected from the vegetation (Coipan and Vladimirescu 2010). The aim of this study was to assess the presence of antibodies against *B. burgdorferi* s.l. in dogs and horses from various regions in Romania. The effect of several risk factors (age, gender, habitat, exposure to ticks, and preventive acaricide treatments) was also investigated.

Materials and Methods

Animal sera originated from submontane and montane areas of Transylvania and the Dobrogea plateau. A total of 276 dogs from eight counties and 260 horses from four counties were sampled between 2008 and 2010. Animals were selected based on availability and owner's consent, where applicable,

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with samples being obtained from veterinary clinics, shelters, studs, and private homes.

Information regarding age, gender, service, and in dogs, exposure to ticks during the previous year, living conditions (outdoor versus indoor), and application of prophylactic acaricide treatments was obtained for each animal. Subsequently, animals were divided into groups, according to gender, age, and occupation. Dividing of the animals according to their age was done as follows: young: 0–2 years in dogs, 0–5 years in horses; adult: 3–7 years in dogs, 6–15 years in horses; old: 8+ years in dogs, 16+ years in horses. Three levels of occupation were considered: companion/sport, stray/wild, and working animals, including shepherd dogs, hunting dogs, guard dogs, and draft horses. In addition, for dogs, two risk levels (high versus low) were established. High risk was defined by the presence of at least one of the following characteristics: detection of ticks during the past year or at the moment of consultation, living outdoors, and lack of preventive treatment against ticks. No formal randomized selection of the animals was applied, but a good approximation of the general population was aimed at by sampling animals of different sex, age, and use at each site. Animals younger than 2 months were excluded from this study. None of the animals were vaccinated against Lyme disease.

Blood samples were drawn aseptically by cephalic vein puncture in dogs and jugular vein puncture in horses, using tubes without anticoagulant, and in some horses, tubes containing EDTA. Sera and plasma, respectively, were separated and stored at −20°C until processing.

Dog sera was analyzed using a commercial enzyme-linked immunosorbent assay (Lyme Borrelia Canine IgG - ELISA; NovaTec Immunodiagnostica, GmbH) for the detection of specific antibodies of the immunoglobulin G (IgG) class against *B. burgdorferi*, according to the manufacturer's instructions, with sera diluted 1:101. Absorbance values were measured using a PR 3100 TSC™ Microplate Reader (Bio-Rad) at 450 nm wavelength, with 620 nm as a reference wavelength. Interpretation was done by comparing absorbance values of the samples to that of the provided cutoff.

Indirect immunofluorescence assay (MegaScreen® FluoBorrelia horse; MegaCor Diagnosik) was used for the detection of IgG antibodies in horse serum or plasma, according to the manufacturer's specifications, with samples diluted in phosphate-buffered saline 1:64. Slides were read using a fluorescence microscope (Axioskop 40 FL Microscope; Carl Zeiss GmbH) at 400× magnification, comparing each sample to the visual intensity and appearance of the *B. burgdorferi* s.l. fluorescence pattern seen in the positive and negative controls.

Statistical analysis of the results was performed using R.2.12.0 for Windows. Fisher's exact test was used to analyze the effect of each explanatory factor (location, age, gender, occupation, and vector exposure risk) on *Borrelia* seroprevalence. Values of $p < 0.05$ were considered significant.

Results

A total mean prevalence of anti-*B. burgdorferi* IgG antibodies of 6.52% (18/276) ranging between 0% and 46.15% in different counties was observed for dog sera. Detailed results are presented in Table 1. Significant differences in seroprevalence among locations were observed ($p = 0.0005$), dogs from Braşov county showing the highest seroprevalence.

Table 1. Seroprevalence in Dogs and Horses from Romania: Distribution by Counties

County	*Dogs* (n = *276*) n *(%)*	*Horses* (n = *260*) n *(%)*
Alba	—	11/92 (11.95)
Bihor	2/8 (25)	—
Bistriţa-Năsăud	—	9/90 (10)
Braşov	6/13 (46.15)	—
Cluj	5/108 (4.62)	—
Constanţa	0/18 (0)	2/20 (10)
Hunedoara	0/12 (0)	—
Maramureş	2/44 (4.54)	—
Mureş	0/9 (0)	—
Tulcea	3/64 (4.68)	9/58 (15.51)

The presence of antibodies against *B. burgdorferi* s.l. was significantly higher ($p = 0.0441$) in working dogs than in pets (Table 2). Interestingly, no positive serological reactions were detected in stray animals. No statistical associations were found between positive results and age, sex, and risk category, respectively.

The mean seroprevalence of anti- *B. burgdorferi* IgG in horses was 11.92% (31/260). The highest seroprevalence was recorded in Tulcea county (15.51%, 9/58) and the lowest was found in Bistriţa-Năsăud and Constanţa counties (10%, 9/90, and 2/20, respectively). These differences among locations were not statistically significant. No significant correlations were found between age, gender, or service and seroreactivity.

Discussions

Lyme borreliosis has been reported in many countries of Europe. However, data on the epidemiology of Lyme disease spirochetes in Romania are deficient, even if the vector tick, *Ixodes ricinus*, is widespread (Coipan and Vladimirescu 2010). To our knowledge, there has been no survey regarding the presence of anti-*B. burgdorferi* antibodies in dogs and horses in Romania up to this date.

Literature-based data document the exposure of various species of domestic animals to borreliae worldwide. Seroprevalence recorded in dogs in our study was higher than figures reported in Sweden (3.9%) (Egenvall et al. 2000) and close to values obtained in Spain (6.9%) (Amusategui et al.

Table 2. Distribution of Seroreactive Dogs and Horses Regarding Sex, Age, Occupation, and Risk Group (Dogs)

Variable	*Category*	*Dogs* (n = *276*) n *(%)*	*Horses* (n = *260*) n *(%)*
Gender	Male	10/164 (6.09)	23/169 (13.6)
	Female	8/112 (7.14)	8/91 (8.79)
Age	Young	4/121 (3.3)	12/98 (12.24)
	Adult	10/122 (8.19)	16/134 (11.94)
	Old	4/33 (12.12)	3/28 (10.71)
Occupation	Pet/sport	3/103 (2.91)	9/99 (9.09)
	Working	15/148 (10.13)	20/148 (13.51)
	Stray/wild	0/25 (0)	2/13 (15.38)
Risk group	High	10/141 (7.09)	—
	Low	8/135 (5.92)	—

2008) and the Czech Republic (6.5%–10.3%) (Pejchalová et al. 2006, Kybicová et al. 2009), but lower than values from Turkey (27.75%) (Bhide et al. 2008), Bulgaria (22.6%) (Zarkov et al. 2003), Croatia (40%) (Bhide et al. 2004), and Slovakia (33.5%) (Štefančíková et al. 2008b).

In our study, the seroprevalence in horses was higher compared with data from Turkey (6.3%) (Bhide et al. 2008), but below the values observed in Sweden (16.8%) (Egenvall et al. 2000), Slovakia (26.5%) (Štefančíková et al. 2008b), Poland (25.6%) (Štefančíková et al. 2008a), Denmark (29%) (Hansen et al. 2010), or France (33%) (Maurizi et al. 2010).

Low positivity in horses may be due to a lower infestation with ticks of one of the studied groups of racing and leisure animals when compared with the general population (unpublished data). Thus, the obtained results could lead to an underestimation of the real seroprevalence.

In Europe, at least seven species of the *B. burgdorferi* s.l. genospecies complex have been found and the geographical distribution of these species is variable (Poupon et al. 2006). *Borrelia afzelii*, *Borrelia garinii*, and *Borrelia lusitaniae* were identified in ticks from Romania (Majláthová et al. 2008, Coipan and Vladimirescu 2010). The IFA test performed in this study used whole-cell antigen from *B. afzelii*, *B. garinii*, and *B. burgdorferi* Sensu stricto, whereas the ELISA test used purified and recombinant antigens from the same genospecies. All strains are either reference strains or strains isolated from Central Europe. Although no information is available on the extent to which these antigens allow the detection of antibodies against various *Borrelia* species, previous studies indicate that use of local antigens would insure a higher sensitivity of serological tests (Štefančíková et al. 2008a, 2008b).

The evidence of IgG antibodies can only serve as an indicator of a mere contact with the agent of Lyme borreliosis, but do not stand for either acute infection or reinfection (Štefančíková et al. 2008b).

It has been shown that IgG antibodies generally start emerging about 1–2 months past infection or reinfection and can persist up to 1–1.5 years in dogs and between 9 months and 2 years in horses (Bhide et al. 2004, Hansen et al. 2010). In horses, it is thought that detectable titers of antibodies are maintained in endemic areas as a result of frequent reinfestation (Štefančíková et al. 2008b). The short persistence of anti-*B. burgdorferi* antibodies in dogs and horses, as opposed to several years in humans, recommends the use of canine and equine seroprevalence estimates as a sensitive environmental risk indicator of the changes in the epidemiology of Lyme disease, considering that recent reinfection would be needed to attain detectable antibody titers (Goossens et al. 2001).

The presence of IgM-type antibodies would indicate an early infection. In this study, we did not quantify IgM-type antibodies, possibly omitting recently infected animals.

In accordance with the literature, no relationship has been established between seroreactivity to *B. burgdorferi* s.l. and gender (Štefančíková et al. 2008a) or age (Couto et al. 2010), although some authors reported a higher prevalence in older animals (Amusategui et al. 2008). The lack of correlation may be a consequence of the limited persistence of anti-*B. burgdorferi* antibodies, which would explain that older individuals would not show higher seroprevalence as a result of higher opportunities to be infected throughout their lives.

In dogs, occupation was correlated with seropositivity in this study, with working dogs being more likely to have a detectable level of antibodies against *B. burgdorferi* s.l. As working dogs were sampled in all locations and not only in the one with the highest seroprevalence, this correlation is probably the result of a more frequent exposure to infected ticks, considering that this group included shepherd dogs, guard dogs, and hunting dogs. A higher seropositivity in working and hunting dogs was also documented in Slovakia (Štefančíková et al. 2008b).

The significantly higher number of reactive dogs in Braşov county could be explained by the following hypotheses:

(1) On one hand, the presence within the sampling sites of Lyme borreliosis foci, restricted to small areas, where the seroprevalence attains a much higher value than in other regions of the same county, and
(2) On the other hand, the presence of the spirochaete at a higher rate than in 2001, when Hristea recorded in Braşov county a mean seroprevalence of 4.8% in humans, with higher values in other counties.

To confirm one of these two hypotheses, studies regarding tick density and prevalence of infection with *B. burgdorferi* s.l. in questing ticks from multiple areas should be conducted in this county. Similarly, to design balanced samplings with respect to the studied explanatory factors, future serological studies should take into account the notable differences in seroprevalence between various locations.

Dogs act as reservoirs for *B. burgdorferi* s.l., and even if experimental studies on horses are limited, one can assume, based on the lack of borreliacidal efect of horse serum against *B. burgdorferi* Sensu stricto, that this species could also have reservoir potential, at least for some genospecies (Mather et al. 1994, Kurtenbach et al. 1998, Bhide et al. 2008). In humans, no increased risk of seropositivity against Lyme disease was associated with ownership of dogs, but nevertheless, similar seropositivity in hunting dogs and humans frequenting the same areas has been established (Goossens et al. 2001). Such data highlight the practical value of serological surveys on these animals as useful epidemiological tools to establish the emergence of this disease in further regions, to locate endemic foci, and to monitor the changes of the genuine environmental risk of Lyme borreliosis.

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References

Amusategui, I, Tesouro, MA, Kakoma, I, Sainz, Á. Serological reactivity to *Ehrlichia canis, Anaplasma phagocytophilum, Neorickettsia risticii, Borrelia burgdorferi* and *Rickettsia conorii* in dogs from Northwestern Spain. Vector Borne Zoonot Dis 2008; 8:797–803.

Bhide, M, Travnicek, M, Curlik, J, Štefančíková A. The importance of dogs in eco-epidemiology of Lyme borreliosis: a review. Vet Med Czech 2004; 49:135–142.

Bhide, M, Yilmaz, Z, Golcu, E, Torun, S, et al. Seroprevalence of anti-*Borrelia burgdorferi* antibodies in dogs and horses in Turkey. Ann Agric Environ Med 2008; 15:85–90.

Coipan, EC, Vladimirescu, AF. First report of lyme disease spirochetes in ticks from Romania (Sibiu County). Exp Appl Acarol 2010; 52:193–197.

Couto, CG, Leif Lorentzen, L, Beall, MJ, Shields, J, et al. Serological study of selected vector-borne diseases in shelter dogs in Central Spain using point-of-care assays. Vector Borne Zoonot Dis 2010; 10:1–4.

Crăcea, E, Constantinescu, S, Balaci, L, Vizitiu, O, et al. Lyme borreliosis in Romania. Arch Roum Path Exp Microbiol 1988; 47:17–21.

Duncan, AW, Correa, MT, Levine, JF, Breitschwerdt, EB. The dog as a sentinel for human infection: prevalence of *Borrelia burgdorferi* C6 antibodies in dogs from southeastern and mid-Atlantic States. Vector Borne Zoonot Dis 2005; 5:101–109.

Egenvall, A, Bonnett, BN, Gunnarsson, A, Hedhammar, A, et al. Sero-prevalence of granulocytic *Ehrlichia* spp. and *Borrelia burgdorferi* sensu lato in Swedish dogs 1991–94. Scand J Infect Dis 2000; 32:19–25.

Goossens, HAT, van den Bogaard, AE, Nohlmans, MKE. Dogs as sentinels for human Lyme borreliosis in The Netherlands. J Clin Microbiol 2001; 39:844–848.

Hansen, MGB, Christoffersen, M, Thuesen, LR, Petersen, MR. Seroprevalence of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in Danish horses. Acta Vet Scand 2010; 52:3.

Hristea, A, Hristescu, S, Ciufecu, C, Vasile, A. Seroprevalence of *Borrelia burgdorferi* in Romania. Eur J Epidemiol 2001; 17:891–896.

Kybicová, K, Schánilec, P, Hulínská, D, Uherková, L, et al. Detection of Anaplasma phagocytophilum and *Borrelia burgdorferi* sensu lato in dogs in the Czech Republic. Vector Borne Zoonot Dis 2009; 9:655–661.

Kurtenbach, K, Sewell, HS, Ogden, NH, Randolph, SE, et al. Serum complement sensitivity as a key factor in Lyme disease ecology. Infect Immunol 1998; 66:1248–1251.

Leschnik, MV, Kirtz, G, Khanakah, G, Duscher, G, et al. Humoral immune response in dogs naturally infected with *Borrelia burgdorferi* sensu lato and in dogs after immunization with a *Borrelia* vaccine. Clin Vacc Immunol 2010; 17:828–835.

Majláthová, V, Majláth, I, Hromada, M, Tryjanowski, P, et al. The role of the sand lizard (*Lacerta agilis*) in the transmission cycle of *Borrelia burgdorferi* sensu lato. Int J Med Microbiol 2008; 298:161–167.

Manion, TB, Bushmich, SL. Interpretation criteria of Western blots for the serodiagnosis of Lyme disease in horses. AAEP Proc 1998; 44:144–145.

Mather, TN, Fish, D, Coughlin, RT. Competence of dogs as reservoir for Lyme disease spirochetes (*Borrelia burgdorferi*). J Am Vet Med Assoc 1994; 205:186–188.

Maurizi, L, Marié, JL, Aoun, O, Courtin, C, et al. Seroprevalence survey of equine Lyme Borreliosis in France and in Sub-Saharan Africa. Vector Borne Zoonot Dis 2010; 10:535–537.

Pejchalová, K, Zákovská, A, Fucík, K, Schánilec, P. Serological confirmation of *Borrelia burgdorferi* infection in dogs in the Czech Republic. Vet Res Commun 2006; 30:231–238.

Poupon, MA, Lommano, E, Humair, PF, Douet, V, et al. Prevalence of *Borrelia burgdorferi* sensu lato in ticks collected from migratory birds in Swizerland. Appl Environ Microbiol 2006; 72:976–979.

Salinas-Meléndez, JA, de la Garza, SG, Riojas-Valdés, VM, Wong González, A, et al. Antibody detection against *Borrelia burgdorferi* in horses located in the suburban areas of Monterrey, Nuevo León. Rev Lat Am Microbiol 2001; 43:161–164.

Štefančíková, A, Adaszek, Ł, Peťko, B, Winiarczyk, S, et al. Serological evidence of *Borrelia burgdorferi* sensu lato in horses and cattle from Poland and diagnostic problems of Lyme borreliosis. Ann Agric Environ Med 2008a; 15:37–43.

Štefančíková, A, Derdáková, M, Škardová, I, Szestáková, E, et al. Some epidemiological and epizootiological aspects of Lyme borreliosis in Slovakia with the emphasis on the problems of serological diagnostics. Biologia 2008b; 63:1135–1142.

Zarkov, IS, Marinov, MM. The lyme disease: results of a serological study in sheep, cows and dogs in Bulgaria. Rev Med Vet 2003; 154:363–366.

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