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ANTI-*BORRELIA* ANTIBODIES IN RODENTS: IMPORTANT HOSTS IN ECOLOGY OF LYME DISEASE

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Abstract: Data presented in this study focuses on the presence of anti-*Borrelia* antibodies in small mammals from Eastern Slovakia during 2000-2003. The total seropositivity observed was 18.78% in rodents. Amongst all species, the total seroprevalence in *Apodemus flavicolis* was the highest (20.87%), followed by *Apodemus agrarius* (19.58%) and *Clethrionomys glareolus* (11.11%). However, the prevalence in *Apodemus flavicolis* during the year 2000-2001 was higher (26.72%), which reduced to 10.60% in 2002-2003. To compare the year range of seroprevalence in other small mammals was not feasible due to the small sample number. Area-wise distribution of anti-*Borrelia* antibodies was even (18.75% to 20%) in this study, except in the Boťany province (0%). This confirms the equal distribution of *Borrelia* spirochetes in the other 3 localities. Prevalence of anti-*Borrelia* antibodies during summer was significantly higher than during autumn and early spring. The overall study also reviews the importance of small mammals in Lyme disease ecology.

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INTRODUCTION

Lyme borreliosis is a tick transmitted disease of humans as well as of animals caused by the genospecies *Borrelia burgdorferi* [3], characterized by a unique skin lesion, erythema chronicum migrans (ECM), often followed by rheumatic, neurological and cardiac symptoms [24]. The relationship between the tick *Ixodes ricinus*, the principal European vector of *Borrelia burgdorferi sensu lato* [13] and its hosts defines the transmission dynamics of Lyme borreliosis. Research workers in Europe have recently concentrated on rodents for the enzootiological and ecological study of Lyme disease [23, 25]. According to Mather *et al.* [21], mice are quantitatively the most important reservoir host for *B. burgdorferi* nevertheless, the relative importance of different vertebrate species, including other rodents and birds, should not be neglected as potential amplifying host. Rats (*Rattus norvegicus* and *R. rattus*) [22] are similarly competent and locally important hosts of *B. burgdorferi*. Nine small mammal species, besides other animals, appear to be capable of transmitting spirocheates to ticks and thus participating in the natural circulation of *B. burgdorferi s.l.* in Europe. The house mouse, *Mus musculus* is strongly suspected of reservoir competence, and many other small rodent species, particularly in

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eastern Europe and Russia, have been implicated [10]. In short, rodents of the *Apodemus* species are also important in the ecology and dynamics of the circulation of *Borrelia* spirochetes in Europe via *I. ricinus* tick vector. The importance of these animal species as an amplifying host depends on both their abundance and quantitative relationship with *I. ricinus*, which may vary seasonally as change in tick activity and tick/host contact rate [5].

The present study reflects minor serosurvey in small mammals from different parts of Eastern Slovakia, as well as species-wise comparison for seropositivity against *Borrelia burgdorferi*.

MATERIALS AND METHODS

The study was carried out in 4 different parts of Eastern Slovakia, namely, Rozhanovce, Šebastovce, Zemplinske Hradište and Boťany, during 2000 and 2003 (Fig. 1). A total of 330 rodents of different species were trapped and screened for anti *Borrelia* IgG antibodies (Tab. 1). The ecological system in the above-mentioned areas is favourable for tick activity, with an abundance of small mammals and the presence of other reservoir as well as non-reservoir competent hosts. Small rodents were trapped alive using wooden traps at 8-10 m intervals in a wooded area. Rodents were anaesthetized and approximately 1 ml of blood was collected from the inner canthus of eye.

The sera were examined repeatedly by modified ELISA as described previously [29], with some modifications. In brief, whole cell sonificated *Borrelia* antigen (mixture of strains *Borrelia burgdorferi sensu stricto* + *Borrelia afzelii* - V 123, *Borrelia garinii* Ir 112 Eastern Slovakia) was fixed in microplate wells. 100 µl of serum diluted at 1:200 in phosphate buffer with 0.05% tween 20 and 1% BSA were added to each well and incubated at 37°C for 30 min. After a triple washing, 100 µl of anti-mouse IgG antibodies labelled with peroxidase (Sigma), diluted at 1:500 was added per well. After 30 min. of incubation and subsequent washings, 100 µl of substrate solution (Orthophenylene diamine) was added per well (pH 5.0). The reaction was stopped with 5% H_2SO_4 after 15 min. of incubation. Absorbance was measured at a wavelength of 492 nm. Experimentally infected mouse sera and sera from naturally infected small mammals, which were positive in repeated titrations, served as positive controls. Mouse sera that proved negative in repeated titrations, with their absorbance value less than 0.4, were used as negative controls. Cut-off was determined as a value of 3 standard deviations above the mean optical density (OD) for negative serum samples. Sera with the absorbance value higher than 0.5 were evaluated as positive. The reproducibility of ELISA: Panel sera samples were repeatedly examined (10 times) with the absorbance value in ranges: > 1.0; 0.5-0.8; < 0.4. The χ^2 test was used for statistical evaluation of 2 × 2 contingency tables.

Figure 1. Geographical distribution of localities in Eastern Slovakia from which the rodent sera were obtained.

RESULTS

A total prevalence observed was 18.78%, irrespective of area and species of rodents. Amongst different rodents, the highest prevalence was observed in *Apodemus flavicollis* (20.87%) followed by *Apodemus agrarius* (19.58%) and *Clethrionomys glareolus* (11.11%) (Tab. 1).

During the year 2000-2001, seroprevalence observed in *Apodemus flavicollis* was higher than in 2002-2003. We could not compare the differences of the seropositivity in other rodent species due to a very low number of samples during 2002-2003.

Table 1. Presence of anti-*Borrelia* IgG antibodies by species of small mammals and years.

Species	2000–2001			2002–2003			Total		
	No of examined	No of positive	Prevalence (%)	No of examined	No of positive	Prevalence (%)	No of examined	No of positive	Prevalence (%)
A. flavicollis	116	31	26.72	66	7	10.61	182	38	20.88
A. agrarius	94	19	20.21	3	0	0	97	19	19.59
A. microps	15	1	6.67	0	0	0	15	1	6.67
M. arvalis	8	1	12.50	0	0	0	8	1	12.50
Cl. glareolus	26	2	7.69	1	1	100	27	3	11.11
Mus musculus	1	0	0	0	0	0	1	0	0.00
Total	260	54	20.77	70	8	11.43	330	62	18.79

Table 2. Anti-*Borrelia* IgG antibodies in small mammals by seasons of sampling.

Month	No. of examined	No. of positive	Seropositivity (%)
March	32	2	6.25
April	56	12	21.43
May	55	13	23.64
June	43	15	34.88
July	16	6	37.50
August	42	7	16.67
September	70	5	7.14
October	16	2	12.50
Total	330	62	18.79

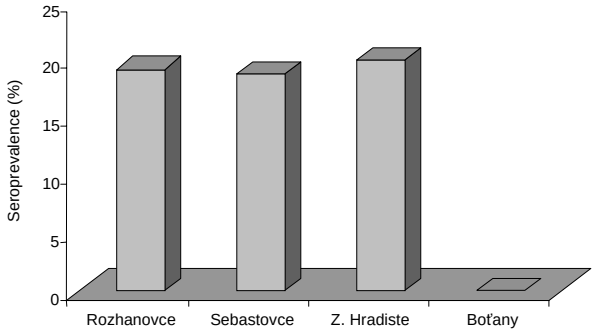

Figure 2. Anti-*Borrelia* IgG antibodies in small mammals, according to the localities studied.

Seasonal difference in the seroprevalence was obviously found in the study. The increasing trend of prevalence was observed from April until July, with the highest prevalence recorded in July (37.50%) (Tab. 2). No statistically significance difference ($p>0.05$) was observed between seroprevalence recorded in different regions (Rozhanovce, Sebastovce and Zemplinske Hradiste), except Boťany province ($p<0.05$) (Fig. 2).

DISCUSSION

Seroprevalence in the small mammals studied confirms their active exposure to the ticks infected with *B. burgdorferi*. In the study, comparatively higher prevalence was found in *Apodemus* in comparison to *Clethrionomys glareolus*, which parallels the findings reported by Aeschlimann *et al.* [2] and Travniček *et al.* [33]. According to Hovmark *et al.* [12], *Clethrionomys glareolus* is also one of the important species in which *B. burgdorferi* was detected. Nevertheless, Humair *et al.* [13] stated that even if the *A. flavicollis* and *A. sylvaticus* proved to have the greatest infectivity potential in Europe, the contribution of voles is not negligible in the maintenance of the Lyme disease spirochete. A previous study in Slovakia [33] reported a higher rate of infestation of *A. flavicollis* by *I. ricinus* than of *C. glareolus*. A similar observation was reported in Danish rodents [8] and also in rodents from Eastern Slovakia [11] wherein, mice were more heavily infested with ticks than bank voles. Moreover, a higher proportion of mice was infected with spirochetes than voles. However, prevalence of antibodies to *Borrelia burgdorferi s.l.* rodents in Poland showed higher seropositivity rates in the species *C. glareolus* in comparison with *A. flavicolis* and *M. arvalis* [25]. *Apodemus* species belong to the group of mice-like rodents with a wide home range, therefore there are more chances of contact with *I. ricinus*. On the other hand, *Clethrionomys* species lives mostly underground, and the possibility of contact with ticks like *I. ricinus* is less likely. This could be one explanation for the antibody prevalence in *Clethrionomys* species in our group of rodents being lower than in *A. flavicolis* and *A. agrarius*. It is also known that *C. glareolus*, but not *A. flavicolis*, progressively acquires resistance to consecutive infestations by *I. ricinus* [6].

Differences in seroprevalence among different species of rodents might be due to variations in immune response. Althought strains of *B. burgdorferi s.l.* seems to have numerous common antigens, mammals may not immunologically recognize a multitude of common epitopes in the same way [18]. Population changes, that is, fluctuations in birth and death rates, can also be an influence [25].

The seroprevalence of anti-*Borrelia* antibodies in mice is connected with the activity of ticks and small mammals. Humair *et al.* [13] found a higher number of infective rodents in late summer, after the peak abundance of nymphs, than in autumn. Rodents became infected with *B. burgdorferi* in mid-summer when *I. ricinus* nymphs fed actively. They further stated that the decline in numbers of infective rodents in autumn was due to the peak appearance of young *Apodemus* in rodent population. Our observations are in accordance with the above explanation. A significant increase in infected animals was observed in summer ($p<0.05$), with a decline in autumn months. A gradual increase in the infected rodent population was noticed in May, June and July (Tab. 2). Histories of tick bites and initial times of exposure of the small mammals are unknown in most instances [18]. The evidence of IgG antibodies can only serve as an indicator of mere contact with the agent of Lyme borreliosis, but it will not determine whether a titre represented current or past infection. It is well known that anti-*Borrelia* antibodies in dogs with subclinical infections or reinfections were found to persist for 1–2 years, or even more [17, 4]. It is also assumed that titres of anti-*Borrelia* antibodies in horses and cattle in endemic areas persist as a result of frequent reinfections [24, 34]. As regards the duration of spirochaetemia and persistance of antibodies in small mammals, it can be assumed that antibodies could be persist for to 2 years, but short life span and other ecological factors - individual susceptibility, size of infective doses, primal infection or reinfection, etc., - limits the antibody persistance. Experimental studies with white-footed mice (*Peromyscus leucopus*) inoculated with *B. burgdorferi* produced IgG antibodies, beginning 5-7 days after inoculation and lasting until 84 days after inoculation, when the experiment was terminated [31]. It is known that some species of rodents can harbour Borreliae during all

seasons, and Anderson *et al.* [1] found *P. leucopus* spirochetes throughout the winter.

The seroprevalence is also extensively influenced by the occurrence of infected ticks in the particular territory. Infected *I. ricinus* ticks are prevalent in almost all territories of Slovakia, with minor differences between individual localities [14, 15, 26, 27]. No significant difference ($p>0.05$) in the seroprevalences among the localities, except Boťany province, was observed in this study (Fig. 2). It seems that our observation reflects an even distribution of *B. burgdorferi* spirochete in these localities.

Borrelia infectivity rate in small mammals depends on the particular genospecies of *B. burgdorferi s.l.*, occurring in a given locality, at a certain time, and the susceptibility of small mammals. According to data in literature, there is a relationship between individual genospecies and animal species, with small mammals acting as a main reservoir of *B. afzelii*, whereas birds serve as a host preferentially to *B. garinii* and *B. valaisiana* [16]. Although the preference is not strict [20], while using antigen from *B. garinii* isolate from Eastern Slovakia in serological diagnostics of small mammals, we were able detect very weak anti-borreliae IgG antibodies (data not published). However, the study of isolation of *Borrelia* genospecies from the tissues of small mammals and their identification using genotype analysis is on-going. Tresová *et al.* [32] detected the first isolates *B. burgdorferi s.l.* from *I. ricinus* in Eastern Slovakia; later, Štěpanová *et al.* [30] identified *B. burgdorferi s.s.* for the first time and other *B. garinii* and *B. afzelii*. Derdáková (personal communication, 2003), using genotyping analysis, confirmed the great representation of *B. burgdorferi s.s.* in Eastern Slovakia, and also detected *B. garinii*, *B. afzelii*, as well as *B. valaisiana*.

In conclusion, rodents - especially the *Apodemus* species - has great importance in the ecology and dynamics of the circulation of *Borrelia* spirochetes in Europe via *I. ricinus* tick vector. The ability of infected rodents to remain infective for ticks for up to 7-40 months [7, 9] proves that they are a reservoir, and that rodents have an amplifying nature.

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REFERENCES

1. Anderson JF, Johnson RC, Magnarelli LA: Seasonal prevalence of *Borrelia burgdorferi* in natural populations of white-footed mice, *Peromyscus leucopus*. *J Clin Microbiol* 1987, **25**, 1564-1566.

2. Aeschlimann A, Chamote E, Gigon F, Jeanneret JP, Kessler D, Walther C: *Borrelia burgdorferi* in Switzerland. *Zbl Bakt Hyg A* 1986, **263**, 450-548.

3. Burgdorferi W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, davis JP: Lyme disease - A tick borne spirochetosis? *Science* 1982, **216**, 1317-1319.

4. Bushmisch SL: Lyme borreliosis in domestic animals. *J Spirochet Tick-Borne Dis* 1994, **1**, 24-28.

5. Craine NG, Randolph SE, Nuttall PA: Seasonal variation in the role of gray squirrels as hosts of *Ixodes ricinus*, the tick vector of the Lyme disease spirochete. *Folia Parasitol* 1995, **42**, 73-80.

6. Dizij A, Kurtenbach K: *Clethrionomys glareolus*, but not *Apodemus flavicolis*, acquires resistance to *Ixodes ricinus* L., the main European vector of *Borrelia burgdorferi*. *Parasite Immunol* 1995, **17**, 177-183.

7. Donahue JG, Piesman J, Spielman A: Reservoir competence of white-footed mice for Lyme disease spirochetes. *Am J Med Hyg* 1987, **36**, 92-96.

8. Frandsen F, Bresciani J, Hansen HG: Prevalence of antibodies to *Borrelia burgdorferi* in Danish rodents. *APMIS* 1995, **103**, 247-253.

9. Gern L: *Contribution a la connaissance de l epidemiologie des babesioses de micromammeferes et de bovines en suisse*, 232. Thesis. Institute de Zoologie, Universite de Neuchatel, Switzerland 1985.

10. Gern L, Estrada-Pena A, Frandsen F, Gray JS, Jaenson TG, Jongejan F, Kahl O, Korenberg E, Mehl R, Nuttall PA: European reservoir hosts of *Borrelia burgdorferi sensu lato*. *Zentralbl Bakteriol* 1998, **287**, 196-204.

11. Hanincova K, Schafer SM, Etti S, Sewell HS, Taragelova V, Ziak D, Labuda M, Kurtenbach K: Association of *Borrelia afzelii* with rodents in Europe. *Parasitology* 2003, **126**, 11-20.

12. Hovmark A, Jeanson TGT, Asbrink E, Forman A, Jansson E: First isolation of *Borrelia burgdorferi* from rodents collected in Northern Europe. *Acta Path* 1988, **96**, 917-920.

13. Humair PF, Turrian N, Aeschlimann A, Gern L: *Borrelia burgdorferi* in a focus of Lyme borreliosis: epizootiologic contribution of small mammals. *Folia Parasitol* 1993, **40**, 65-70.

14. Kmety E, Řeháček J, Vyrosteková V: Investigations of ticks for the presence of *Borrelia* in Czechoslovakia. *Zbl Bact Hyg A* 1986, **263**, 468-470.

15. Kmety E, Řeháček J, Vyrosteková V, Kociánová E, Guryčová D: Studies on infestation of *Borrelia burgdorferi* and *Franciscella tularensis* with ticks in Slovakia. *Bratisl Lek Listy* 1990, **91**, 251-266 (in Slovak).

16. Kurtenbach K, Peacey M, Rijpkema SGT, Hoodless A, Nyttall PA, Randolph SE: Differential transmission of the genospecies of *Borrelia burgdorferi sensu lato* by game birds and small rodents in England. *App Environ Microbiol* 1998, **64**, 1169-1174.

17. Magnarelli LA: Serologic testing for Lyme disease. *Postgr Med* 1990, **87**, 149-156.

18. Magnarelli LA, Anderson JF, Johnson RC: Analyses of mammalian sera in enzyme-linked immunosorbent assay with different strains of *Borrelia burgdorferi sensu lato*. *J Wild Dis* 1995, **31**, 159-165.

19. Mateička F, Kozáková D, Rosa PA, Kmety E: Identification of *Borrelia burgdorferi sensu lato* tick isolates from Slovakia by PCR typing with 16S rRNA primers. *Zentralbl Bakteriol* 1997, **286**, 355-361.

20. Mateička F, Kmety E: Lyme borreliosis: Open questions and problems at the turn of the millenium. *Bratisl Lek Listy* 2000, **101**, 166-169.

21. Mather TN, Wilson ML, Moore SI, Ribeiro JMC, Spielman A: Comparing the relative potential of rodents as reservoir of Lyme disease spirochete (*Borrelia burgdorferi*). *Am J Epidemiol* 1989, **130**, 142-150.

22. Matuschka FR, Endepols S, Richter D, Ohlenbusch A, Eiffert H, Spielman A: Risk of urban Lyme disease enhanced by the presence of rats. *J Infect Dis* 1996, **174**, 1108-1111.

23. Michalik J, Hofman T, Buczek A, Skoracki M, Sikora B: *Borrelia burgdorferi s.l.* in *Ixodes ricinus* (Acari: Ixodidae) ticks collected from vegetation and small rodents in recreational areas of the city of Poznań. *J Med Entomol* 2003, **40(5)**, 690-697.

24. Parker JL, White KK: Lyme borreliosis in cattle and horses: review of the literature. *Cornell Veterinarian* 1992, **82**, 253-274.

25. Pawełczyk A, Siński E: Prevalence of IgG antibodies response to *Borrelia burgdorferi s.l.* in populations of wild rodents from Mazury Lakes District region of Poland. *Ann Agric Environ Med* 2000, **7**, 79-83.

26. Petko B, Cerny V, Jurasek V: Parasite-host relationships of the ticks *Ixodes trianguliceps* Bir. and coincidence of its ecological niches with those of (*Ixodes ricinus* L.). **In:** Dusbabek F, Bukva V (Eds): *Modern Acarology, Vol. 2*, 455-460. Prague, Academia and The Hague, SPB Academic Publishing bv 1991.

27. Peťko B, Štefančíková A, Tresová G, Peterková J, Škardová I, Prokopčáková H, Čisláková L: Common tick (*Ixodes ricinus*) as a source of infection of human and dogs with the agent of Lyme borreliosis in Slovakia. *SlovVet Čas* 1996, **21**, 313-318 (in Slovak).

28. Steer AC, Malawista SE, Snydman DR, Shope RE, Andiman WA, Ross MR, Steele FM: Lyme arthritis: An epidemic of oligoarticular arthritis in children and adults in three connecticute communities. *Arthrit Rheum* 1977, **20**, 7-17.

29. Štefančíkova A, Tresová G, Peťko B, Škardová I, Sesztaková E: ELISA comparison of three whole–cell antigens of *Borrelia burgdorferi sensu lato* in serological study of dogs from area of Kosice, Eastern Slovakia. *Ann Agric Environ Med* 1998, **5**, 25-30.

30. Štepánová-Tresová G, Peťko B, Štefančíková A, Nadzamová D: Occurrence of *Borrelia burgdorferi sensu stricto*, *Borrelia garini* and *Borrelia afzelii* in the *Ixodes ricinus* ticks from Eastern Slovakia. *Eur J Epidemiol* 2000, **16**, 105-109.

31. Schwan TG, Kime KK, Schrumpf ME, Coe JE, Simpson WJ: Antibody response in white-ffoted mice (*Peromyscus leucopus*) experimentally infected with the Lyme disease spirochete (*Borrelia burgdorferi*). *Infect Immun* 1989, **57**, 3445-3451.

32. Tresová G, Mateička T, Peťko B, Kozárová D: Preliminary identification of *Borrelia burgdorferi* isolates from ticks *Ixodes ricinus* in eastern Slovakia. *Epidemiol Mikrobiol Imunol* 1997, **46**, 36-38.

33. Travniček M, Štefančíková A, Nadzamová D, Stanko M, Čislaková L, Peťko B, Mardzinová S, Bhide M: Serological evidence of IgG antibodies to *Borrelia burgdorferi* in game animals and small mammals in eastern Slovakia. *Rev Sci Tech Off Int Epiz* 2003, **22.3**, 1039-1041.

34. Tuomi J, Rentamäki LK, Tanskanen R: Experimental infection of cattle with several *Borrelia burgdorferi sensu lato* strains; immunological heterogeneity of strains as revealed in serological tests. *Vet Microbiol* 1998, **60**, 27-43.