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PREVALENCE OF IGG ANTIBODIES RESPONSE TO *BORRELIA BURGDORFERI* S.L. IN POPULATIONS OF WILD RODENTS FROM MAZURY LAKES DISTRICT REGION OF POLAND

Agnieszka Pawełczyk, Edward Siński

Department of Parasitology, Institute of Zoology, Warsaw University, Warsaw, Poland

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Abstract: Three rodent species: *Clethrionomys glareolus*, *Apodemus flavicollis* and *Microtus arvalis* from Mazury Lakes District of Poland were examined for antibodies to *Borrelia burgdorferi* by enzyme-labelled protein G assay (ELGA). *C. glareolus* had an exceptionally high prevalence of *B. burgdorferi* antibodies - 58%, but *A. flavicollis* and *M. arvalis* also showed significant prevalence of 16.6% and 10.5%, respectively. The ELGA method is highly specific with good reproducibility. Nevertheless, some differences of sensitivity of assessed samples were season dependent. However, high seroprevalence did not coincide with infestation rates of examined rodents by *I. ricinus* ticks. The results indicated that in Mazury Lakes District, naturally infected rodents play an important role as an animal reservoir host for *B. burgdorferi*, and these animals may increase the risk of human infections in some habitats used as recreation areas. Also, this study shows that ELGA method based on the affinity of protein G for IgG of wild animals may be widely used to determine the competent zoonotic reservoir of *B. burgdorferi*.

Address for correspondence: Prof. dr hab. Edward Siński, Department of Parasitology, Institute of Zoology, Warsaw University, Miecznikowa 1, 02-096 Warszawa, Poland.
E-mail: esinski@biol.uw.edu.pl

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INTRODUCTION

The maintenance of *Borrelia burgdorferi* s.l. in nature is dependent on the zoonotic cycle involving tick vectors and vertebrate hosts [7]. In Europe, *Ixodes ricinus* L. is the primary tick vector of *B. burgdorferi* s.l. [5], and within this group of *Borrelia*, three human pathogenic species: *B. burgdorferi* s.s., *B. garinii*, and *B. afzelii* have been recognized in Europe [9]. Very often, both *B. garinii* and *B. afzelii* were also found in rodents, and multiple infections with those two species were observed in some naturally infected rodents as well as in *I. ricinus* ticks. Probably both *B. garinii* and *B. afzelii* are etiological agents responsible for the approximately 10,000 cases per year of Lyme borreliosis in Eurasia [9].

The results of our previous investigations show that in woodland habitats of northern Poland, the rodent species *Apodemus flavicollis* Melchior (yellow-necked mouse) and *Clethrionomys glareolus* Schreber (bank vole) contribute substantially to *B. burgdorferi* infection of vector - *I. ricinus* [12]. Although both species of rodents are important hosts of the immature stages of *I. ricinus*, the relationship between vector and its hosts has not been quantified in many habitats of northern Poland. In Europe however, only a few studies have dealt with the small wild rodents as a reservoir of *Borrelia* spirochetes [4, 6, 8, 13]. No attempt to quantify the relative seroprevalence of these animals to spirochetal infection has yet been reported, especially at sites with high tick abundance, such as wooded sites of northern Poland.

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The aim of the current study was to assess the prevalence of IgG antibody responses to *B. burgdorferi* s.l. in populations of wild rodents from the Mazury Lakes District of Poland and to evaluate the possible local potential of three species of rodents (*C. glareolus*, *A. flavicollis* and *Microtus arvalis*) in maintaining zoonotic reservoir of spirochetes for Lyme disease. This knowledge of naturally infected animals could be very useful for understanding the natural history of *B. burgdorferi* in its reservoir hosts.

MATERIALS AND METHODS

Environmental sampling. The rodents were trapped in the nature reserve surrounding Lake Łuknajno near Mikołajki, Mazury Lakes District of Poland, using wooden life traps. Traps were set out at 15 m intervals from each other in parallel lines, and trappings were carried out on 4 consecutive days, with traps being inspected in the early morning and just before dusk. The animals were collected during 1998-1999, the two main capture periods being April-June and August-September. The rodents were killed humanely using ether, and blood samples of approximately 1 ml were collected from the heart. Sera were stored at -20°C until use. The rodents were examined for the presence of *I. ricinus.*

Enzyme-labelled protein G assay (ELGA). The ELGA assay was performed according to Deruaz *et al.* [2], with some modifications. Sonicated suspension of *Borrelia garinii* (strain isolated from *I. ricinus* ticks which were collected from urban area near Katowice, Poland) in concentration of 10 µg/ml in coating buffer (0.1 M sodium bicarbonate, pH 9.6) was used as antigen. To test rodent sera by ELGA assay polystyrene microplates (Nunc Maxisorp Immuno-plate) were coated with the antigen solution (150 µl per well) by passive adsorption for 18 h at 4°C. Coated plates were washed 3 times with 150 µl per well of PBS/Tween 20 detergent (PBS plus 0.05% Tween 20 detergent). The wells were then filled with 150

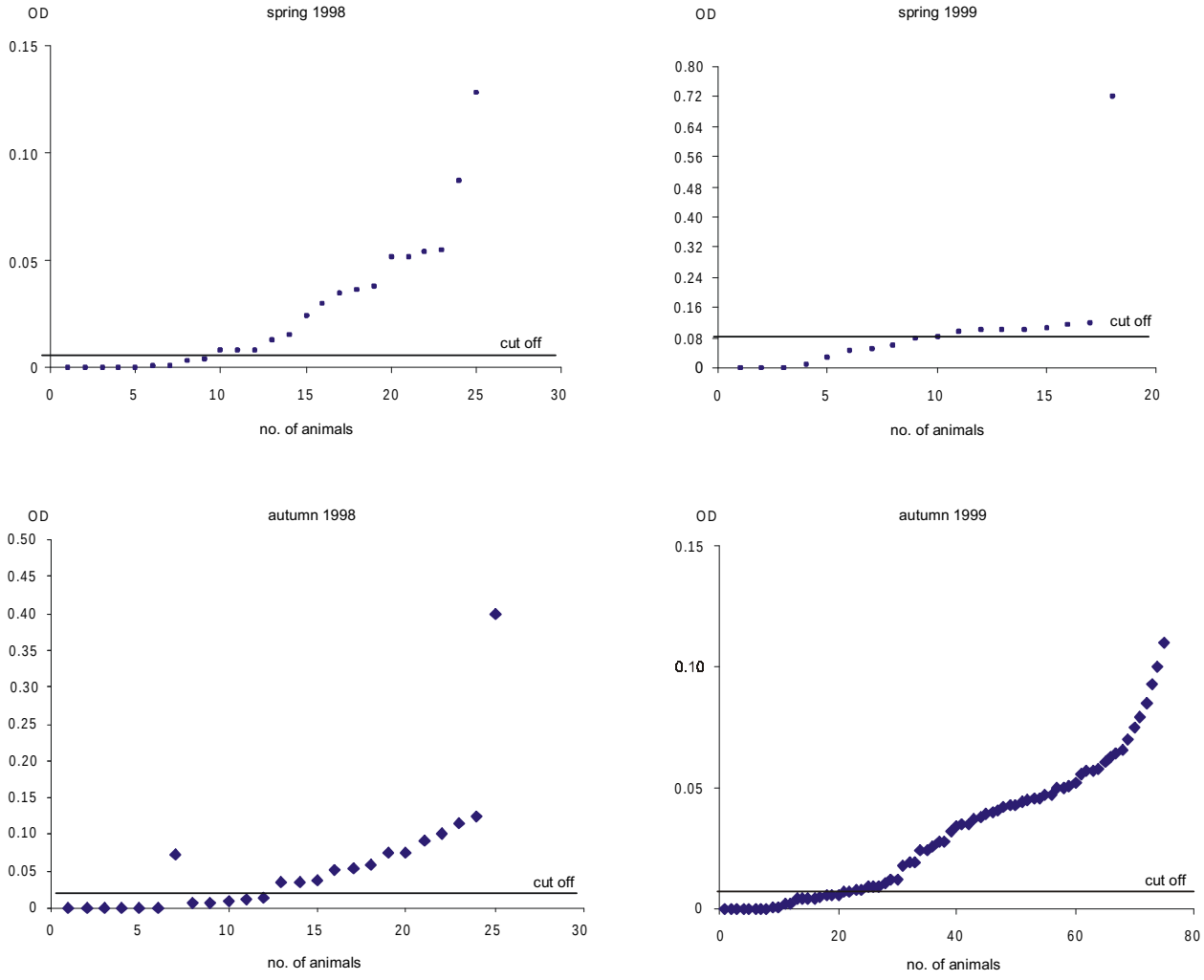

Figure 1. Antibody titers to *Borrelia burgdo*rferi s.l. among *Clethrionomys glareolus* according to the season.

Table 1. Total IgG seroprevalence to *Borrelia burgdorferi* in relation to infestation by *Ixodes ricinus* ticks in population of rodents from Mazury Lakes District during 1998–1999.

Species	Total No. examined	No. (%) seropositive	No. (%) with ticks larvae	Mean No. of larvae/ animals
Clethrionomys glareolus	142	83[(1)] (58.0)	122 [(1a)] (86)	4.7
Apodemus flavicollis	30	5[(2)] (16.6)	24[(2b)] (80)	3.1
Microtus arvalis	38*	4[(3)] (10.5)	12 [(3c)] (31.5)	1.3

*data for 1998; Values [(1)] ve [(2)] not significant; [(1)] ve [(3)] are statistically significant, $p < 0.001$; [(2)] ve [(3)] are not significant. Values [(1a)] ve [(2b)] not significant; [(1a)] ve [(3c)] are statistically significant, $p < 0.001$; [(2b)] ve [(3c)] are statistically significant, $p < 0.0001$ as analyzed by Fisher's Exact Test; ve = versus.

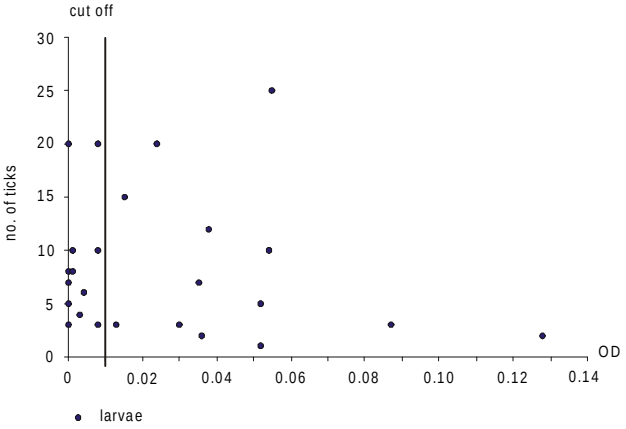

Figure 2. Comparision of IgG antibody titers to *Borrelia burgdorferi* s.l. with the numbers of *Ixodes ricinus* larvae removed from *Clethrionomys glareolus* in spring, 1999.

µl of bovine serum albumin (BSA) blocking buffer (PBS, 0.05% Tween 20, 1% BSA) and left for 1 h at 37°C to block the remaining protein binding sites on the microtiter plate. The remaining fluid was then aspirated. Rodent sera (150 µl per well) were tested at dilution 1/200 in BSA buffer (PBS, 0.05% Tween 20, 1% BSA, pH 7.4) and added to two wells per serum sample. After incubation for 2 h at 37°C the plates were rinsed 3 times in PBS/Tween 20 detergent to remove unbound antibody, and the remaining fluid was then aspirated. After 3 washes, 150 µl of peroxidase-labelled protein G (0.1 µg/ml in wash buffer) was added to each well and the plates were incubated for 2 h at 37°C. After 4 washings, a substrate of 50% 2,2'-azino-di-(3-ethylbenzthiazoline sulfonate) and 50% H_2O_2 was added for 10 min at room temperature and colour development stopped by adding 100 µl of 10% dodecyl sulfate in distilled water. Optical density was analyzed at 405 µm by using automatic microplate reader (µQuant) connected to a computer with Microtek software. The measurements were made against a series of 4 blank wells, which received all the above reagents excepting rodent's serum, which was replaced by PBS.

Statistical evaluation. The average optical density (OD) values for the known negative dilutions were analyzed statistically to determine significant titers for positive reactions. A serum dilution was considered positive if the OD was higher than the value of the average of the OD of all ELGA positive sera, plus 2 standard deviations. The OD below this value was considered as negative (cut off) [2]. To determine significant differences in percentages of positive sera, values were analyzed by an unpaired *t* test as well as by Fisher's Exact Test.

RESULTS

A total of 210 sera from 142 *C. glareolus*, 30 *A. flavicollis* and 38 *M. arvalis*, collected between April

Table 2. Prevalence of *Clethrionomys glareolus* with IgG antibodies to *Borrelia burgdorferi* s.l.

Season	Total No. examined	No. (%) seropositive	Mean IgG antibody titer* (range)	Cut off** (OD)
1998				
Spring	24	13 (52.0)	0.047[(1)] (0.013-0.128)	0.011
Autumn	26	14 (53.8)	0.087[(2)] (0.035-0.040)	0.016
1999				
Spring	18	8 (44.4)	0.180[(3)] (0.082-0.720)	0.080
Autumn	74	48 (64.0)	0.045[(4)] (0.011-0.100)	0.010

* Antibody titers as values of optical density (OD); ** cut off = mean OD + 2 × (±SD); Values [(1)] ve [(2)] are statistically significant, $p < 0.001$; [(1)] ve [(3)] are statistically significant, $p < 0.001$; [(1)] ve [(4)] are statistically significant $p < 0.001$. Values [(2)] ve [(3)] are statistically significant, $p < 0.001$; [(2)] ve [(4)] are statistically significant, $p < 0.001$. Values [(3)] ve [(4)] are statistically significant, $p < 0.001$ as analyzed by unpaired *t*-test; ve = versus.

Table 3. Prevalence of *Apodemus flavicollis* with IgG antibodies to *Borrelia burgdorferi* s.l.

Season	Total No. examined	No. (%) seropositive	Mean IgG antibody titer* (range)	Cut off** (OD)
1998				
Spring	6	2 (33.3)	0.056[(1)] (0.05-0.1)	0.148
Autumn	9	0	0	0
1999				
Spring	6	1 (16.6)	0.07[(2)] (0.03-0.1)	0.127
Autumn	9	2 (22.2)	0.10[(3)] (0.01-0.500)	0.4

* Antibody titers as values of optical density (OD); ** cut off = mean OD + 2 × (±SD). Values [(1)] ve [(2)] not significant; [(2)] ve [(3)] are statistically significant, $p < 0.001$ as analyzed by unpaired *t*-test; ve = versus.

Table 4. Prevalence of *Microtus arvalis* with IgG antibodies to *Borrelia burgdorferi* s.l.

Season	Total No. examined	No. (%) seropositive	Mean IgG antibody titer* (range)	Cut off** (OD)
1998				
Spring	15	2 (13.4)	0.021(1) (0.02-0.079)	0.079
Autumn	20	2 (10.0)	0.023(2) (0.01-0.130)	0.093
1999				
Spring	3	0	0	0
Autumn	nt	nt	nt	nt

* Antibody titers as values of optical density (OD); ** cut off = mean OD + 2 × (±SD). Values (1) ve (2) not significant as analyzed by unpaired *t*-test; nt = not tested; ve = versus.

1998 and October 1999 were assayed for specific IgG antibodies to *Borrelia* antigens. The results for all species are presented in Table 1. In ELGA assay, a positive reaction for IgG against *B. burgdorferi* s.l. was found in all rodents species. Of the *C. glareolus* examined sera, 58% were positive (Tab. 2). *A. flavicollis* and *M. arvalis* showed a prevalence of 16.6% and 10.5%, respectively (Tab. 3 and 4).

In general, ELGA assay is highly specific with a good reproducibility. However, seroprevalence of examined rodents showed a seasonal variation, and according to the season, different sensitivity was observed between positive sera for each species of rodents. For instance, OD for sera of *C. glareolus* ranged from 0.013 in spring 1998 to 1.000 in autumn 1999 (Tab. 2 and Fig. 2).

The mean infestation rate of studied animals was above 66%; however, the seropositivity did not coincide with rate of infestation of examined animals by *I. ricinus* ticks (Tab. 1).

Figure 2 shows that seronegative *C. glareolus* were infested quite intensively by larvae of *I. ricinus* (mean number of larvae per animal was 4.7). Among rodents of *C. glareolus* and *A. flavicollis* that had contact with larvae of *I. ricinus* the prevalence was significantly higher than among *M. arvalis*, those having also such contact (Tab. 1).

DISCUSSION

The European rodent species *C. glareolus*, *A. flavicollis* and *M. arvalis* are natural hosts of the *I. ricinus* ticks, a vector for the spirochetal agent of Lyme borreliosis - *B. burgdorferi* s.l.

The purpose of the present study was to investigate the prevalence of IgG antibodies in selected populations of rodents in order to assess their role as a reservoir for Lyme borreliosis in the region of the Mazury Lakes of northern Poland which are very extensively used for recreational purposes. The results of this study indicate that *B. burgdorferi* is present in the rodent populations in the Mazury Lakes District. Enzyme-labelled protein G assay (ELGA) showed very clearly that the rodents, at least those of *C. glareolus* species, have very high levels of seropositivity. The mean rate of seroprevalence for *C. glareolus* was considerably higher (58%) than those for *A. flavicollis* and *M. arvalis* (16.6% and 10.5% respectively). Disparity in seropositivity rates among these 3 species of rodents might be due to variations in immune responses, population changes (i.e., fluctuations in birth and death rates) and different degrees of exposure to infected ticks. However, the presence of antibody titers in populations of rodents during 2 years shows that the animals in natural conditions have been exposed to *B. burgdorferi* for some period of time. In the experimental studies, there is evidence that white-footed mice (*Peronyscus leucopus*) inoculated with *B. burgdorferi* produced IgG antibodies beginning 5–7 days after inoculation until 84 days after inoculation when the experiment was terminated [11]. It is also well known that some species of rodents can harbur *Borrelia* during all seasons, and Anderson *et al.* [1] reported that in *P. leucopus* spirochetes are present throughout the winter. In the group of *B. burgdorferi* s.l., genomospecies of *B. garinii* and *B. afzelii*, but not *B. burgdorferi* s.s., were found in naturally infected rodents, and also quite often multiple infections with those two species were observed in some rodents [10]. The species of *C. glareolus*, *A. flavicollis* and *M. arvalis* are also the primary hosts for infecting larval and nymphal *I. ricinus* ticks, which during subsequent feeding provide the source of infection to associated human populations. It is also well known that *C. glareolus*, but not *A. flavicollis*, progressively acquires resistance to consecutive infestations [3]. In the present study, all examined species of rodents were infested by larvae of *I. ricinus*., *C. glareolus* and *A. flavicollis* were infested more abundantly with larval *I. ricinus* than *M. arvalis*. Nevertheless, each of investigated species of rodents could contribute as a host species to the horizontal infection of a vector (*I. ricinus*) population. However, for all examined rodent species there was no direct correlation between seropositivity rates of IgG antibodies and prevalence of infestation with *I. ricinus* larvae.

High IgG antibodies prevalence, at least in sera of *C. glareolus*, demonstrate that rodents are an important reservoir for *B. burgdorferi* s.l., and that these animals may increase the risk of human infections in some habitats used as recreational areas. In addition, this study shows that ELGA method based on the affinity of protein G for IgG of wild animals may be widely used to determine the competent zoonotic reservoir of *B. burgdorferi*.

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