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THE PREVALENCE OF SPIROCHETE *BORRELIA BURGDORFERI SENSU LATO* IN TICKS *IXODES RICINUS* AND MOSQUITOES *AËDES* SPP. WITHIN A SELECTED RECREATIONAL AREA IN THE CITY OF SZCZECIN

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Abstract: The aim of this study was to determine the prevalence of spirochete *Borrelia burgdorfe*ri *s.l.* in ticks *Ixodes ricinus* and mosquitoes *Aëdes* spp. within the Bukowa Forest, collected between 2000 and 2001. The study covered 215 ticks (193 nymphs and 22 adults) and 947 mosquitoes female of the genus *Aëdes*. Spirochetes of *Borrelia burgdorferi s.l.* were detected in the arthropods studied with the method of indirect immunofluorescence assay (IFA). Positive readings of the immunological reaction were stated in 17.7% of the collected nymphs and adult forms of *Ixodes ricinus*, and in 0.8% of mosquito females of the genus *Aëdes*. The number of *B. burgdorferi* observed in a view field (400×) of microscopic preparations of all infected mosquitoes and about 10% of the infected ticks, ranged from 1–10. This number in 50% of the nymphs was from 11–50 spirochetes. View fields of the preparations of the other 50% of nymphs and adult forms featured more then 50 spirochetes. The observed low values of the prevalence and infection intensity of female mosquitoes *Aëdes* spp. compared to ticks suggest that the former do not pose a serious epidemiological threat in the spreading of Lyme disease.

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INTRODUCTION

Lyme borreliosis is a chronic, cosmopolitan, multisystem, zoonotic disease caused by *Borrelia burgdorferi* sensu lato.

People and animals become infected with those bacteria by ticks of the genus *Ixodes*. In Europe, the principal species transmitting spirochetes of *B. burgdorferi s.l.* is the common tick, *Ixodes ricinus*. According to many authors [2, 9, 22, 26, 27, 30], the persistent, high percentage infection of ticks observed in different regions of Poland poses a serious epidemiological threat of the Lyme disease. These predictions have been confirmed recently by reports of the State Department of Hygiene revealing an increase in the number of cases of borreliosis. Because the spirochetes have also been detected in hematophagous insects, such as horse flies, deer flies, fleas, and mosquitoes [1, 4, 5, 6, 7, 10, 11, 13, 14, 15, 16, 17, 18, 24, 28, 34, 35], the role of those arthropods cannot be ruled out in the epidemiology of Lyme disease.

The aim of the present study was to determine the infection level of *Ixodes ricinus* and *Aëdes* sp.- collected from a selected recreational area - with *Borrelia burgdorferi s.l.*

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MATERIAL AND METHODS

Ticks and mosquitoes were collected monthly, from June to August 2000 and 2001 at one of the most attractive areas of the city of Szczecin - the Bukowa Forest.

The ticks were collected from forest underbrush and duff using a flannel cloth with a surface area 1 m^2. Each sample was collected by 2 persons dragging the cloth over the underbrush and duff. Each person covered an area of some 100 m^2.

The mosquitoes, attracted to a "human lure" between 9:00–10:00 were collected in glass containers.

This study covered nymphs and adult forms of ticks and mosquito females. Tick larvae were not considered because of their short survival period. After the collection, both mosquitoes and ticks were kept until the next day in a refrigerator at approximately 4ºC.

The mosquitoes were identified up to the genus level with the aid of a taxonomic key.

Spirochetes of *Borrelia burgdorferi s.l.* were detected in ticks and mosquitoes using the method of indirect immunofluorescence assay (IFA).

Each mosquito and tick was rinsed in 70% ethyl alcohol, superficially dried, and squashed with a glass rod (the mosquitoes were decapitated and devoid of wings and legs). The material obtained in such a way was merged with 30 µl of PBS buffer. Subsequently, a 10-µl portion of the suspension was transferred to a depression in an immunofluorescence slide. After drying out, the preparations were fixed in acetone for 15 minutes and subsequently added to rabbit anti-*Borrelia* antibodies and with, fluoresceine isothiocyanate (FITC; Sigma)-conjugated goat anti-rabbit IgG. The results of IFA reaction in the form of glowing spirochete-rabbit-antibody-goat-conjugated-antibody complexes were detected under a fluorescent microscope (Axioskop, Opton Austria) (400×). Infection intensity was taken into consideration while assessing positive reactions in ticks and mosquitoes.

RESULTS

A total of 215 ticks, including 193 nymphs (89.8%) and 22 adults (10.2%), were collected between the years 2000 and 2001 (Tab. 1). Indirect immunofluorescence assays revealed infection at the level of 17.7% (jointly for both seasons) (Tab. 1). Spirochetes of *B. burgdorferi* sensu lato were detected in 30 nymphs (15.5%) and in 8 adults (36.4%). Such a high prevalence of *B. burgdorferi* noted in the adult specimens could have been caused by a small number of females and males (a total of 22). Among the collected mosquito females of the genus *Aëdes*, the prevalence of *B. burgdorferi* was 0.8%.

Distinct differences in the numbers of the spirochetes on IFA slides were noted between ticks and mosquitoes. In all the mosquitoes and in about 10% of the infected nymphs only single spirochetes (from 1–10) were visible in microscope view fields. In some 50% of the infected nymphs the spirochetes were more numerous (i.e. 11–50), while in the remaining nymphs and adults there were more than 50 bacteria.

Table 1. Prevalence of infection of ticks *Ixodes ricinus* and *Aëdes* with spirochetes *Borrelia burgdorferi* within the area studied in 2000 and 2001.

	Number of specimens		Prevalence (%)
	collected	infected	
Ticks	215	38	17.7
• Nymphs	193	30	15.5
• Adults	22	8	36.4
Aëdes	947	8	0.8

DISCUSSION

The results of the present study indicate that ticks have the highest potential for infecting humans with Lyme disease. Within the area studied, 17.1% of ticks were infected with *Borrelia burgdorferi* spirochetes. Other authors surveying other regions of Poland revealed lower values of the tick infection. Wegner *et al.*, using also IFA, revealed tick infection of 11.5% in the former Olsztyn province in 1993 [31], and 8.8% at recreational areas of the former Białostockie Province in 1994 [32]. Using the same technique, Stańczak *et al.* [25] demonstrated that 8.1% of ticks collected within the city limits of Białystok, Olsztyn, and Elbląg in 1996, were infected with *B. burgdorferi*. Bukowska *et al.* [2] detected the presence of *B. burgdorferi* in 11.6% of ticks collected in the area of Szczecin in 2000 and 9.6% in 2001, while Michalik *et al.*, collected 16.2% in 1998–1999 in popular recreational areas in Poznań [19].

Other authors, employing PCR method, also obtained lower infection values of ticks. Stańczak *et al.* [25] revealed 13% infection of ticks captured in the proximity of cities: Słupsk, Bydgoszcz, Lublin, and Kraków. The study by Wodecka [33] on the occurrence of *B. burgdorferi* in the population of *I. ricinus* in north-western Poland in 1998-2001 revealed that infected ticks constituted 9.4%. Skotarczak and Wodecka [26], surveying areas of the Zachodniopomorskie Province in 1996, found that 12% of *Ixodes ricinus* ticks were infected. Skotarczak [27] detected the presence of the spirochetes in 8.6% of tick specimens collected from the same areas in 1997. In 2000 and 2001, Pawełczyk and Siński [23] revealed even lower prevalence values among ticks from the Mazury lakes amounting to 6.2 and 2.6%, respectively. On the other hand, values of infection higher than in the present study, were found only in Wielkopolska by Jenek and Głazaczow [8] in 1994–1995 (24.5%), by Nowosad *et al.* [20] in 1997–1998 (22.6%).

Even though the principal vector of Lyme borreliosis are ticks, the role of hematophagous insects in the epidemiology of this disease cannot be underestimated.

Not all borreliosis-affected patients admitted, in their medical interviews, to having contact with ticks [21]. There have been few documented cases of Lyme borreliosis related to insects in Canada [3] and in the USA (Connecticut) [15]. In Sweden, on the other hand, there was a case of erythema migrans observed after a mosquito bite [6].

In Poland there are about 40 species of mosquitoes representing 5 genera: *Anopheles*, *Aëdes*, *Culex*, *Culiseta*, and *Mansonia* [12]. Thirty species of those insects have been reported in Szczecin. Of this number 10 species, including those representing the genus Aëdes, are known to transmit microorganisms pathogenic to humans [29].

In the present study, the collected mosquitoes, lured to a human body, were females representing the genus *Aëdes*, which is consistent with the observations of Lachmajer *et al.* [12], who noticed that those insects attack in shaded areas, among trees and bushes.

Mosquitoes are annoying insects, especially when they occur in mass numbers.

Mosquitoes also transmit various microorganisms pathogenic to humans, among them *Borrelia burgdorferi* was recovered from mosquitoes representing the genera *Aëdes*, *Culex*, and *Anopheles* [1, 4, 5, 7, 10, 11, 15, 16, 17, 18].

Studies on the occurrence of *B. burgdorferi* in mosquitoes have been carried out in a number of research centres in the world. Also in Poland, Kubica-Biernat *et al.* [11] detected *B. burgdorferi* in mosquitoes from Białowieża and the surroundings of Gdańsk - a 0.5% infection rate. In our earlier study [10], carried out at recreational areas of Szczecin, we detected prevalence values between 0.6–3.2% in mosquitoes of the genus *Aëdes*.

It can be concluded from the results of the other authors that the infection frequency of the Lyme borreliosis among humans is related to the percentage of infected ticks or mosquitoes. In the areas of particularly high incidence of human borreliosis (e.g. Connecticut), *Borrelia burgdorferi* was found with the aid of IFA in 36.2% of ticks, *Ixodes scapularis* and in 9.5–11.1% of mosquitoes of the genus Aëdes [16]. On the other hand, in Moravia, Hubalek *et al.* [7] observed 20.4% of *Ixodes ricinus* and 4.1% of mosquitoes infected with *Borrelia burgdorferi*.

The low percentage of infected mosquitoes compared to infected ticks may be related to the 2-week survival period of the spirochetes in the organism of those insects [18]. There has been no report on experimentally proven cases of transovarian or transstadial transmission of the spirochetes, although detection of *B. burgdorferi* in mosquito larvae by Zakovska [34] may indicate their transovarian route.

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