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Ticks and Mosquitoes as Vectors of *Borrelia burgdorferi s. l.* in the Forested Areas of Szczecin*

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The aim of the study was to determine the infection level of adult forms and larvae of ticks and mosquitoes with *Borrelia burgdorferi* in the forested areas of Szczecin. A total of 1699 ticks *Ixodes ricinus,* including 1422 nymphs, 277 adult forms and 2862 mosquito females representing the genera *Aedes* (89.6%) and *Culex* (10.4%) were collected between the years 2004 and 2005. A further 3746 larvae and 1596 pupae of *Culex pipiens pipiens* were colleted from water bodies. *Borrelia burgdorferi s. l.* was detected in the arthropods by the method of indirect immunofluorescence assay (IFA). A positive immunological reaction was detected in 16.6% of the adult forms and in 16.5% of the nymphs of *Ixodes ricinus*. Spirochetes were also detected in 1.7% of mosquito females, 3.2% of larvae and in 1.6% of pupae of *Culex pipiens pipiens*. The results of the present study confirm that contact with ticks constitutes the main risk of contracting Lyme disease, although mosquitoes play a role as vectors as well.

Key words: *Borrelia burgdorferi s. l.*, IFA, *Ixodes ricinus*, mosquitoes, Poland.

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Since the description of *Borrelia burgdorferi* as the etiologic agent of Lyme disease (BURGDORFER *et al.* 1982) it has been well known that ticks play the most important role in transmission of the spirochetes among vertebrates. In Europe, including Poland, the principal vector of *B. burgdorferi* is the common *Ixodes ricinus*. Some studies have shown that other blood-sucking arthropods may also participate in *B. burgdorferi* transmission. Spirochetes were detected in mitgut, malphigian tubules and salivary glands of tabanides flies and blackflies (MAGNARELLI *et al.* 1986, 1988; ZEMAN 1998). Living bacteria were observed also in intestines of *Hybomitra*, *Tabanus* and *Chrysops* (MAGNARELLI *et al.* 1986) and insects of the family Simuliidea (CECHOVA *et al.* 2004) and Culicidae (HALOUZKA 1993; HALOUZKA *et al.* 1998, 1999; HUBALEK & HALOUZKA 1997; HUBALEK *et al.* 1998; KOSIK-BOGACKA *et al.* 2002; KUBICA-BIERNAT *et al.* 1998; MAGNARELLI *et al.* 1986, 1987, 1988). Spirochetes were detected in female and in larval mosquitoes (ZAKOVSKA *et al.* 2002). It is thought that blood-sucking arthropods are a secondary vector and reservoir of *Borrelia burgdorferi*, but their participation in the transmission of these bacteria is little known.

The aim of the present study was to assess the *Borrelia burgdorferi s. l.* infection level. of developmental stages of *Ixodes ricinus* and mosquitoes collected in the forested areas of Szczecin.

Material and Methods

Collection of ticks and mosquitoes

The ticks were collected monthly, from May to September 2004-2005 in forested areas of the city of Szczecin using a flannel cloth of a 1 m^2 surface area. Each sample was collected by two persons dragging a cloth over the underbrush and duff. Each person covered an area of some 100 m^2. This study covered nymphs and adult forms of ticks.

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Tick larvae were not considered due to their short survival period.

The mosquitoes were collected monthly, from May to September 2004-2005 by attraction to a "human lure" between 9 AM and 10 AM. Larvae and pupae of *Culicidae* were collected once a month during the summer season from strongly polluted water bodies.

After collection both ticks and mosquitoes were kept overnight in a refrigerator at approximately 4°C.

Detection of *Borrelia burgdorferi s. l.* in ticks and mosqiutoes

Spirochetes of *Borrelia burgdorferi* were detected in ticks and mosquitoes with the aid of indirect immunofluorescence (IFA). This method had been selected because of results obtained by other authors who demonstrated higher effectiveness of direct methods enabling identification of even single spirochetes. The PCR method is substantially more expensive and it requires at least 130 bacteria for the detection of DNA of *B. burgdorferi* (ZAKOVSKA *et al.* 2002).

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 this way was merged with 30 μl of PBS buffer. Subsequently a 10-μl aliquot of the suspension was transferred to the depression of an immunofluorescence slide. After drying, the preparations were fixed in acetone for 15 minutes and subsequently added to rabbit anti-*Borrelia* antibodies with fluoresceine isothiocyanate (FITC; Sigma)-conjugated goat anti-rabbit IgG. The results of the IFA reaction in the form of growing spirochete-rabbit-antibody-goat-conjugated-antibody complexes were assessed using a fluorescent microscope (400 ×). Infection intensity was taken into consideration while assessing positive reactions in ticks and mosquitoes.

Results

Detection of *Borrelia burgdorferi s.l.* in ticks

In total 1699 ticks *Ixodes ricinus* were collected in the selected sites, including nymphs (83.7%), and adult forms 16.3% (Table 1). *Borrelia burgdorferi* was detected in 16.5% of the collected ticks. The infection level of each developmental stage of *Ixodes ricinus* was from 16.1% in females to 17.2% in males. Single, numerous and very numerous spirochetes were observed in infected ticks.

Detection of *Borrelia burgdorferi s. l.* in mosquitoes

2862 female mosquitoes of the genus *Aedes* (89.6%) and *Culex* (10.4%) were colleted (Table 2). *Borrelia burgdorferi s. l* was detected in 1.7% of females. 3746 of larvae and 1596 of pupae of *Culex pipiens pipiens* were collected from water bodies (Table 3). Spirochetes of *B. burgdorferi* were observed in 3.2% of larvae and 1.6% of pupae. Compared to ticks, mosquitoes showed a lower infestation. Only single bacteria were observed in mosquito specimens.

Table 1

The frequency of occurence of *Borrelia burgdorferi* spirochetes in ticks *Ixodes ricinus* collected between 2004-2005

	Nymphs	Females	Males	Total
No. of ticks examined	1422	155	122	1699
No. (%) infected	235 (16.5%)	25 (16.1%)	21 (17.2%)	281 (16.5%)

Table 2

Appearance of *Borrelia burgdorferi* spirochetes in adult forms of mosquitoes collected between 2004-2005

Species female mosquitoes	No. examined	No. (%) infected
Aedes	2564	44 (1.7%)
Culex	298	5 (1.7%)
Total	2862	49 (1.7%)

Table 3

The frequency of occurence of *Borrelia burgdorferi* in larvae and pupae of mosquitoes collected between 2004-2005

Culex pipiens pipiens	No. examined	No. (%) infected
Larvae	3746	120 (3.2%)
Pupae	1596	26 (1.6%)
Total	5342	146 (2.7%)

Discussion

Studies of the prevalence of *Borrelia burgdorferi* infection in populations of biological vectors have been carried out in many research centers around the world, including Poland. Most of the studies concern the frequency of occurence of *B. burgdorferi* in ticks. The results of studies carried out recently in Poland indicate that the prevalence of *B. burgdorferi* in *Ixodes ricinus* populations depends on the region. STANCZAK *et al.* (1999) revealed tick infection of 37.5% in the Katowice province. A lower infection level (22.6%) was affirmed by NOWOSAD *et al.* (1999) in the forested areas of Poznań. STANCZAK *et al.* (2004) demonstrated 12.4% infection of ticks collected from the region of Gdańsk, Gdynia and Sopot. The lowest level of *I. ricinus* infection (4.1%) in the last years was affirmed by STANCZAK *et al.* (1999) in the vinicity of Białystok.

The infection of *Ixodes ricinus* with *Borrelia burgdorferi* was 4.4% in north-western Poland (WODECKA 2003), and in Szczecin it varied from 9.6% to 11.6% (BUKOWSKA *et al.* 2003). In the present study the level of *I. ricinus* infection with spirochetes was 16.5%. The significant differences in the prevalence of *B. burgdorferii* in ticks in different regions of Poland can be caused by the distinct atmospheric conditions in different years and different numbers and species compositions of animals in the examined sites.

In contrast to many studies of tick infection with *B. burgdorferi*, studies concerning blood-sucking insect infection are relatively few. Spirochetes were detected by SINTON and SHUTE (1939) in intestines of larvae and pupae of *Anopheles maculipennis, Theobaldia spathipualpis* and *Culex pipiens*. *Borrelia burgdorferi* was also detected in the Czech Republic by ZAKOVSKA *et al.* (2002) in 1.6% of *Culex pipiens pipiens* larvae. In this study the percentage of infection of *Culex pipiens pipiens* larvae with spirochetes was twice as high. The percentage of infestation of *Culex pipiens pipiens* pupae was lower than in larvae, at 1.6%.

So far the highest level of infection of adult mosquitoes (7-8%) was revealed by MAGNARELLI *et al.* (1986) in the hiperendemic regions of Lyme disease – in Connecticut, USA. Comparable studies were carried out also in Europe, mainly in the Czech Republic. HALOUZKA *et al.* (1999) identified *B. afzelii* spirochetes in 5% of *Culex pipiens molestus*. HUBALEK *et al.* (1998) revealed the presence of spirochetes in 6 species of mosquitoes: from 1.3% in *Aedes cantans* to 5.9% in *Culex pipienns molectus*. These authors suggest that diverse levels of infection of mosquitoes depend upon the species.

Similar research carried out in Poland shows that the percentage of infected mosquitoes varies from 0.5% in the north-eastern region of Poland (KUBICA-BIERNAT *et al.* 1998) to 1.25% in the region of Szczecin, collected between 2000 and 2001 (KOSIK-BOGACKA *et al.* 2002). In earlier studies between 2000-2001, the prevalence of *Aedes* mosquitoes was lower – 0.8% (KOSIK-BOGACKA *et al.* 2004), while in 2003 it was comparable with the presently reported study (KOSIK-BOGACKA *et al.* 2006).

Some investigations have shown a relationship between the level infection of mosquitoes with *B. burgdorferi* spirochetes and the season of the year. SANOGO *et al.* (2000) revealed that infection with spirochetes is lower (1.9%) in mosquitoes collected in summer than in those collected during winter (5.1%). Similary, HALOUZKA *et al.* (1998) showed a 1.7% and 6% infection level of mosquitoes collected in summer, and winter, respectively. In this study mosquitoes were collected mainly during summer, and the percentage of infection of females corresponds to the results of the aforementioned authors.

The higher percentage of infection of ticks with *B. burgdorferi* spirochetes compared to mosquitoes may be caused by the short period of life of spirochetes in mosquitoes. MAGNARELLI *et al.* (1987) have shown that spirochetes in *Aedes* are infective for 2 weeks post-inoculation. However, these results are inconsistent with results of HALOUZKA *et al.* (1998) and SANOGO *et al.* (2000) since these authors detected the spirochetes in hibernating female mosquitoes collected during the winter, suggesting that spirochetes can survive at least 4 months in the mosquito body. Additionally, the presence of spirochetes in larvae and pupae of mosquitoes may evidence transovarial and transstadial transmission of spirochetes in the population of mosquitoes.

The results of this study confirm the hypothesis that ticks are the principal and competent vector of *B. burgdorferi*, while mosquitoes are secondary vectors.

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