

***Borrelia burgdorferi sensu lato* Infection in Mosquitoes from Szczecin Area**

Danuta Izabela KOSIK-BOGACKA, Wanda KUŻNA-GRYGIEL and Katarzyna GÓRNIK

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The aim of the study was to determine the level of infection in mosquitoes with spirochetes *Borrelia burgdorferi sensu lato* in the woody areas of Szczecin. The mosquitoes were collected from May to September 2003. The spirochetes, *Borrelia burgdorferi* s. l., present in mosquitoes were detected in mosquitoes with indirect immunofluorescence assay (IFA) using rabbit anti-*Borrelia burgdorferi* antibodies and goat anti-rabbit IgG marked with fluorescein isocyanate (FITC). A total of 1557 females and 58 males were collected. They represented the genera *Aedes* (63 %) and *Culex* (37 %). The infection level of the mosquitoes from the area studied amounted to 1.7 %. The results of the present study confirm the potential of these arthropods to spread Lyme borreliosis.

Key words: *Borrelia burgdorferi sensu lato*, IFA, mosquitoes, Poland.

Danuta KOSIK-BOGACKA, Wanda KUŻNA-GRYGIEL and Katarzyna GÓRNIK, Chair and Department of Biology and Medical Parasitology, Pomeranian Medical University, Powstańców Wielkopolskich 72, 70-111 Szczecin, Poland.

E-mail: kodan@sci.pam.szczecin.pl
kuzgryg@sci.pam.szczecin.pl

Since *Borrelia burgdorferi* (BURGDORFER *et al.* 1982) was first isolated and described, numerous centres worldwide have been intensively studying its epidemiology and pathogenicity, and also intra-specific variability of the bacteria. Molecular studies have distinguished at least ten genospecies of *Borrelia burgdorferi* (BARANTON *et al.* 1992; CANICA *et al.* 1993; MARCONI *et al.* 1995; MATHIESEN *et al.* 1997; POSTIC *et al.* 1998; WANG *et al.* 1997; WODECKA & SKOTARCZAK 2000) of which *Borrelia burgdorferi sensu stricto*, *B. afzelii*, and *B. garinii* are etiological agents of Lyme disease (MATHIESEN *et al.* 1997).

A constant threat of contracting Lyme disease persists also due to the wide distribution of *B. burgdorferi* within the animal reservoir in mammals, birds, and reptiles (ANDERSON *et al.* 1986). It has been commonly accepted that the spirochaete spreads within vertebral populations mainly due to ticks. In Europe, the *Ixodes ricinus* tick is the vector of *B. burgdorferi* (BUKOWSKA *et al.* 2003; KARBOWIAK & SIŃSKI 1994; PANCEWICZ *et al.* 1999; SKOTARCZAK 2000; SKOTARCZAK & WODECKA 1998; WEGNER & STAŃCZAK 1995; WODECKA 2003; WODECKA & SKOTARCZAK 2000). Contrary to the immense published material on *B. burgdorferi* prevalence in ticks, reports on the spirochaete infecting other bloodsucking arthropods seem

relatively scarce. The spirochaetes have been detected in deer flies, horse flies, mosquitoes, and fleas (HALOUZKA *et al.* 1999; HALOUZKA 1993; HARD 1966; HUBALEK *et al.* 1998; KOSIK-BOGACKA *et al.* 2002; KUBICA-BIERNAT *et al.* 1998; MAGNARELLI & ANDERSON 1988; MAGNARELLI *et al.* 1987; MAGNARELLI *et al.* 1986; MAGNARELLI 1977; SANOGO *et al.* 2000; TELTOW *et al.* 1991; ZEMAN 1998) as well as in Lycosidae spiders (SURFFRIDGE *et al.* 1999). So far, a few cases of Lyme disease have been reported in association with insect bites (DOBY *et al.* 1987; LUGER 1990), but only one case of *erythema migrans* has been associated with mosquito bites (HARD 1966).

Due to the growing cases of Lyme disease in Poland (NIŚCIGORSKA 1999) it seems reasonable that populations of bloodsucking arthropods should be monitored for *B. burgdorferi* infection prevalence.

The aim of this study was to determine the prevalence of *Borrelia burgdorferi sensu lato* spirochetes in adult mosquitoes (*Culicidae*) in forested areas of northwestern Poland.

Material and Methods

The mosquitoes were attracted to human skin and caught from May to September 2003 in for-

ested areas of the city of Szczecin. Five sampling sites were designated in woody areas located near bodies of water. According to literature data, mosquito activity changes in a diurnal cycle. Since the numbers of mosquitoes increase in the morning and in the evening, the insects were caught by two people between 9 and 10 a.m. as well as between 5 and 6 p.m., each time for 60 minutes.

The collected mosquitoes were kept in a refrigerator at approximately 4°C until the next day. Each specimen was rinsed in 70% ethanol, decapitated, and dried out following the removal of its wings and legs, and finally it was squashed with a glass rod. The material obtained was suspended in 30 µl of PBS buffer. Subsequently, 10 µl of the suspension was transferred to the concavity of a microscopic slide for immunofluorescence (manufactured by bioMérieux). When a preparation dried out it was fixed in acetone for 15 minutes. Spirochetes, *Borrelia burgdorferi* s. l. were detected with the aid of indirect immunofluorescence (IFA) using anti-*B. burgdorferi* rabbit antibodies and goat anti-rabbit IgG. *B. burgdorferi* B31 antigen (bioMérieux) was used as a positive test. The results in the form of glowing complexes of spirochetes-anti *B. burgdorferi* antibodies – marked antibodies, were assessed using a fluorescent microscope (×400) (Axioscop; Opton).

The statistical analysis used the χ^2 test with the Yates correction and a P value less than 0.05.

Results

A total of 1615 adult mosquitoes were caught in the selected sites. The majority of specimens (32%) were caught in September, while only 9.8% in July. The collected mosquitoes belong to the genera *Aedes* (63%) and *Culex* (37%, Fig. 1). Females represented 96.4% of the caught mosquitoes. The most mosquitoes were caught near Kurowo (29.1%), while the fewest 5.1% near Warszewo (Table 1). Only 27 mosquitoes, which represented 1.7% of the population, were infected with *Borrelia burgdorferi* s. l. (Table 1). Most infected mosquitoes were females (26 specimens). *Borrelia burgdorferi* spirochaetes were also detected in an *Aedes* male.

The lowest percentage of infected mosquitoes was found in Dziewoklicz (0.7%), while the highest in Bukowa Forest (2.5%).

Also, differences were found in the infection of mosquitoes by *B. burgdorferi* between months. The lowest number of infected mosquitoes was recorded in May (0.9%), whilst the highest 4% in June (Table 2).

The level of mosquito infection by the spirochetes collected in individual months and sites did not show statistically significant differences ($P > 0.05$).

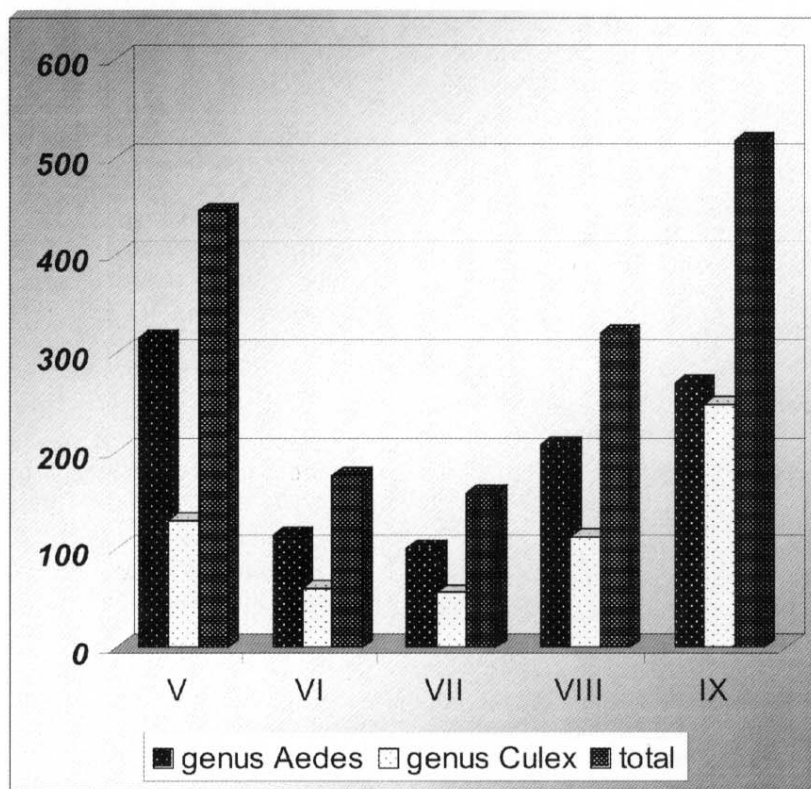


Fig. 1. Number of collected mosquitoes by month.

Table 1

Levels of mosquito infection by spirochetes *B. burgdorferi* in recreational areas of Szczecin

Site	Number of mosquitoes collected	Number and percentage of mosquitoes infected								Total % of infected
		genus <i>Aedes</i>				genus <i>Culex</i>				
		females		males		females		males		
		n	%	n	%	n	%	n	%	
Dziewoklicz	288	2	1.1	–	–	–	–	–	–	0.7
Warszewo	82	1	2.3	–	–	–	–	–	–	1.2
Bukowa Forest	408	5	1.8	1	20	4	3.4	–	–	2.5
Kurów	470	4	1.4	–	–	5	2.7	–	–	1.9
Kurów Forest	367	3	1.6	–	–	2	1.1	–	–	1.4
Total	1615	15	1.5	1	2.3	11	1.9	–	–	1.7

Table 2

Number of collected mosquitoes and infection rates by month

Month	Number of mosquitoes collected and percentage of mosquitoes infected					
	genus <i>Aedes</i>		genus <i>Culex</i>		Total	
	n	%	n	%	n	%
V	316	1.3	129	0	445	0.9
VI	115	2.6	60	6.7	175	4
VII	102	1	56	1.8	158	1.3
VIII	207	1.4	113	3.5	320	2.2
IX	269	1.1	248	1.6	517	1.4
Total	1009	1.4	606	2.1	1615	1.7

Discussion

About 40 mosquito species have been identified and described in Poland. The species belong to the genera *Anopheles*, *Aedes*, *Culex*, *Culiseta*, and *Mansonia* (LACHMAJER *et al.* 1970; LACHMAJER 1954). In Szczecin and its vicinities, 30 species of these insects have been reported (WEGNER 2000). Most of them attack humans, however only six are vectors of human or animal diseases. Literature data show that ecological parameters control the presence of mosquitoes in a given area; these include humidity, temperature, wind, and sun exposure. The sites that were selected were characterised by high ground humidity (proximity of water bodies). The months of the highest mosquito abundance (May, August, and September) were warm but not hot during the collecting period. The population of mosquitoes in June and July declined considerably, which was due to high air temperature and low precipitation (Fig. 1, Table 2). Described cases of Lyme disease observed from July to October coincide with the highest activity of ticks (ANDERSON *et al.* 1986), but also of mosquitoes.

According to LACHMAJER *et al.* (1970) human bait allows catching mainly *Aedes* mosquitoes, while *Culex* species preferring birds are rarely caught this way, which corresponds to our results.

The literature shows that mosquitoes transmit more than 200 viruses that are pathogenic for birds or mammals (LONC & RYDZANICZ 1999), including the virus of yellow fever (JOHNSON *et al.* 2002), West Nile (O'LEARY *et al.* 2004), dengue (ARUNACHALAM *et al.* 2004) and fowlpox. Furthermore, via mosquitoes, a human may contract tularaemia, anthrax, malaria, or filariasis (STOLK *et al.* 2004).

The presence of *Borrelia burgdorferi* s. l. has been confirmed in mosquitoes caught both in Europe and North America (BURGDORFER *et al.* 1982; HALOUZKA *et al.* 1999; HALOUZKA 1993; HUBALEK *et al.* 1998; KOSIK-BOGACKA *et al.* 2002; KUBICA-BIERNAT *et al.*, 1998; MAGNARELLI & ANDERSON 1988; MAGNARELLI *et al.* 1987; MAGNARELLI *et al.* 1986; MAGNARELLI 1977; SANOGO *et al.* 2000; ZAKOVSKA *et al.* 2002). In Connecticut where the incidence of human infections with Lyme disease is particularly high, *Borrelia burgdorferi* was detected in 7-8% of *Aedes* mosquitoes (MAGNARELLI *et al.* 1986). On the other hand, SANOGO *et al.* (2000) have found that 1.9% of *Aedes vexans*, *A. sticticus*, *A. cantans*, and *Culex pipiens pipiens* caught during the summers of 1993-1997 were infected with *Borrelia burgdorferi*, while 5.1% of *Culex pipiens molestus* population was found infected during the winter.

ZAKOVSKA *et al.* (2002) studied the area of Brno during 2000–2001 and, using dark-field microscopy, found that 2.28% of *Culex pipiens pipiens* were infected with *B. burgdorferi* using nested PCR, the authors found 1.14% infected mosquitoes.

KUBICA-BIERNAT *et al.* (1998) carried out studies on the prevalence of the spirochaetes in mosquitoes in north Poland where, using the IFA method, the authors found 0.5% females of the genera *Aedes*, *Culex*, and *Anopheles* infected. Preliminary studies carried out from June until August 2001 have shown that the *Borrelia burgdorferi* infection rate of mosquito females caught at the recreational sites of Szczecin starts from 1.25% (KOSIK-BOGACKA *et al.* 2002). This study has shown that 1.7% of mosquitoes in forested areas of Szczecin are infected. Contrary to previous studies which showed that only *Aedes* females were infected, this study has demonstrated that both *Aedes* and *Culex* mosquitoes are infected (Table 1). The presence of *B. burgdorferi* in an *Aedes* male suggests transovarial transmission of the bacteria in the bloodsucking insects. As in the studies by KUBICA-BIERNAT *et al.* (1998), single *B. burgdorferi* spirochaetes were found in the infected specimens.

The low rate of *B. burgdorferi* infection in mosquitoes, as compared to ticks, is probably due to the short time when the bacteria are able to survive in a mosquito organism. In their experiment, MAGNARELLI *et al.* (1987) have shown that spirochaetes lose their pathogenicity after 2 weeks spent in the alimentary tract of a mosquito.

In the organism of an *Ixodes ricinus* tick, the spirochaetes proliferate intensively and, what is more, are transmitted transstadially and transovarially, which allows them to survive in a tick population for many generations (MROŻEK-BUDZYN 1999).

The confirmed *B. burgdorferi* infection of mosquitoes caught in the forested areas of Szczecin and its vicinities indicate that both *Aedes* and *Culex* mosquitoes may play a role in the epidemiology of Lyme disease. *B. burgdorferi* infection of an *Aedes* male suggests transovarial spreading of the bacteria in mosquito populations.

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