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DETECTION OF *BORRELIA BURGDORFERI* SENSU LATO IN MOSQUITOES (*CULICIDAE*) IN RECREATIONAL AREAS OF THE CITY OF SZCZECIN

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Abstract: The mosquitoes were caught within the period of their highest activity, i.e. June–August 2001, in recreational areas of the city of Szczecin. Spirochetes, *Borrelia burgdorferi* sensu lato were detected in mosquitoes with the aid of the method of indirect immunofluorescence (IFA) using rabbit anti-*Borrelia burgdorferi* antibodies and goat anti-rabbit IgG marked with fluorescein isothiocyanate (FITC). A total of 639 mosquito females representing genera *Aëdes* (99.1%) and *Culex* (0.9%) were collected. The mean value of the infection rate of mosquitoes from the area studied was 1.25%. The highest infection rate was recorded in June (3.2%), while the lowest—in July (0.6%). All mosquitoes infected with *Borrelia burgdorferi* spirochetes belonged to the genus *Aëdes*. The results of the present study confirm a potential role of those arthropods in epidemiology of Lyme borreliosis.

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INTRODUCTION

Domestic and wild animals, as reservoirs of *Borrelia burgdorferi* spirochetes, play a significant role in epidemiology of Lyme borreliosis [9, 8, 22, 24, 28] and by blood-feeding arthropods as their vectors. Birds are principal culprits of spreading the spirochetes to substantial distances. Their blood, in many cases, was tested positively for the presence of *B. burgdorferi* [1, 10, 20, 28].

As demonstrated by hitherto completed studies, along with ticks, the principal vectors of *B. burgdorferi* [2, 3, 9, 10, 21, 22, 26, 28, 29], also blood-feeding insects such as bot-flies, fleas, and mosquitoes can play a certain role in transmitting the spirochetes [2, 4, 5, 6, 7, 11, 14, 15, 16, 17, 18, 19, 23, 27, 29].

The aim of the present study was to determine the frequency of occurrence of spirochetes, *Borrelia burgdorferi* sensu lato in the population of mosquitoes inhabiting recreational areas of the city of Szczecin.

MATERIAL AND METHODS

The mosquitoes were lured to a human skin and caught from June–August 2001 within recreational areas of Szczecin. The procedure was repeated 10 times for 30 min each time by 2 persons, from 12:00–13:00 hs. The mosquitoes collected were kept in a refrigerator at approximately 4°C until the next day. The systematic position of the mosquitoes was determined with the aid of a key [25].

Each mosquito 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 in this way was blended with 30 µl of PBS buffer. Subsequently 10 µl of the suspension was transferred to the concavity of a microscopic slide for immunofluorescence (manufactured by bioMèrieux). When a preparation dried-out it was fixed in acetone for 15 min.

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Spirochetes, *Borrelia burgdorferi* sensu lato were detected with the aid of indirect immunofluorescence (IFA) using anti-*B. burgdorferi* rabbit antibodies and goat anti-rabbit IgG. The results, in the form of glowing complexes of spirochetes–anti *B. burgdorferi* antibodies–marked antibodies, were assessed using a fluorescent microscope (Axioscop; Opton).

RESULTS

The results of the present study are shown in Table 1. All mosquitoes collected belonged to only 2 genera: *Aëdes* and *Culex*. A total of 639 females were collected, of which 633 mosquitoes represented the genus *Aëdes* and only 6 – genus *Culex*. The majority of mosquitoes were collected in July (335) while the fewest – in June (126).

No mosquito of the genus *Culex* was infected with *B. burgdorferi*. Positive immunofluorescence reaction was observed solely in mosquitoes of the genus *Aëdes*. In 2001, the prevalence of females of *Aëdes* spp. was from 0.6% in July to 3.2% in June.

DISCUSSION

In Poland there are some 40 mosquito species representing 5 genera: *Anopheles*, *Aëdes*, *Culex*, *Culiseta*, and *Mansonia* [12, 13].

In the present study, only females of the genera *Aëdes* and *Culex*, with distinct dominance of the former, were lured to human skin. Similar results were reported by Lachmajer *et al.* [13].

Presently demonstrated changes in the abundance of mosquitoes in 2001 are similar to the results related to earlier years in the areas of western Poland, as published by Lachmajer *et al.* [12]. In June, the abundance of mosquitoes was low, whereas it attained its maximum in July, to decrease in August (Tab. 1).

Mosquitoes are annoying insects, particularly when they occur in mass numbers [25]. Bites of females of haematophagous species of mosquitoes may trigger allergies caused by introduction of the salivary gland secretions to the human blood [14]. Mosquito females are also vectors of microorganisms pathogenic to people and animals. It has been known from the relevant literature that over 100 species of known mosquito species transmit over 200 virus species, in most cases, pathogenic to birds and mammals [14]. Mosquitoes are blamed for infecting people with rabbit myxomatosis, tularemia, avian pox, and with anthrax [25]. In Poland, the virus of Tick-Born Encephalitis was detected in females of *Aëdes* spp. and *Anopheles maculipennis* [25]. In Poland, as well as in other countries, the presence of *Borrelia burgdorferi* was stated in various blood-feeding insects, including mosquitoes [2, 4, 5, 7, 11, 16, 17, 18].

The study carried out in the Czech Republic by Halouzka demonstrated 4.1% - infection of *Aëdes vexans* and 3.5–4.3% of *Culex pipiens molestus* with spirochetes *Borrelia burgdorferi* [5]. In the areas of particularly high prevalence of human Lyme borreliosis, i.e. Connecticut, USA, 8% of mosquitoes of the genus *Aëdes* were infected with *Borrelia burgdorferi* [18].

Table 1. Levels of mosquitoes infection with spirochetes *B. burgdorferi* in recreational areas of Szczecin.

Month	Number of mosquitoes collected			Percentage of mosquitoes infected		
	genus *Aëdes*	genus Culex	total	genus *Aëdes*	genus Culex	total
June	126	0	126	3.2%	-	3.2%
July	335	5	340	0.6%	-	0.6%
August	172	1	173	1.2%	-	1.2%
Total	633	6	639	1.3%	-	1.25%

In Poland, studies on the presence of spirochetes in mosquitoes were carried out by Kubica-Biernat *et al.* in the vicinity of Gdańsk. The above authors, using the method of indirect immunofluorescence, revealed 0.5% infection of mosquitoes in the area studied [11].

There has been only one published record of erythema migrans associated with mosquito bite [6].

The presently reported detection of spirochetes in 0.6 to 3.2% of mosquitoes in recreational areas of Szczecin indicates their possible role in the epidemiology of borreliosis. The risk of acquiring borreliosis transmitted by mosquitoes is diminished by the only 2-week survival of spirochetes in the organism of a mosquito [17]. No blood-feeding arthropods, except ticks, were demonstrated to transmit spirochetes transstadially or transovarially.

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