DETECTION OF ETIOLOGICAL AGENT OF LYME BORRELIOsis IN NATIVE MOSQUITOES (DIPTERA: CULICIDAE) POPULATION

*Borrelia burgdorferi* spirochete, the etiologic agent of Lyme borreliosis, is primarily transmitted by hard ticks of genus *Ixodes*. Aside of a few reports, however, that associate the development of erythema chronicum migrans, a skin lesion diagnostic for the disease, with the bite of mosquitoes (HÅRD, 1966), deer flies or horse flies (MAGNARELLI et al. 1986, LUGER 1990, VILJANEN et al. 1992), little is known about the role of hematophagous insects in epidemiology of this illness. Therefore we investigated the presence of *B. burgdorferi* in mosquitoes (Diptera: Culicidae) collected in natural foci of Lyme borreliosis in Poland.

A total of 1103 females of 4 genera: *Aedes* (n 215), *Culex* (n=624), *Culiseta* (n=28), *Anopheles* (n=257) were analysed for borreliae by the two methods:
- indirect immunofluorescence assay (IFA), using anti-*B. burgdorferi*, strain 1B29, polyclonal antibody (PAB 1B29) and FITC-labelled goat anti-rabbit IgG antibody (SIGMA). Positive control preparations, prepared with *B. burgdorferi* B31 (bioMérieux), as well as negative ones, were examined with each set of slides assayed;
- polymerase chain reaction (PCR), using oligonucleotide primers FL6 (5'TTC AGG GTC TCA AGC TTG CAC T3') and FL7 (5'GCA TTT TCA ATT TTA GCA AGT GAT G3') (PICKEN 1992) which flanked a DNA region within the gene encoding *B. burgdorferi* flagellin protein (*fla* gene), resulting in an amplicon of 276 bp. The amplified product was detected by electrophoresis in 1.5% agarose gel containing ethidium-bromide. One negative (DDW) and one positive (*B. burgdorferi*, native strain Ho-44/10) control were included in every PCR run.

Of the 750 mosquitoes analysed individually by IFA, *B. burgdorferi* s.l. was detected in 4 (0.5%). Two of these spirochete-infected females belonged to the genus *Aedes* and were collected on the human bait in the village of Białowieża (Białystok province, north-eastern Poland). Two others, *Culex* sp. and *Anopheles* sp. were caught in a cellar in the village of Brzyno (Gdańsk province, northern Poland). Each of infected specimen contained only a few borreliae.

A total of 227 mosquitoes: 67 individuals and 16 samples pooled by 10 specimens were tested by PCR. DNA of *B. burgdorferi* s.l. was detected in 10 pools. Thereby in each positive pool at least one mosquito was infected. Then the calculated minimal infection rate was 6.25%. All the females tested were hibernating *Culex pipiens* collected in the village of Nadole (Gdańsk province).

The results obtained are in agreement with those reported by HALOUZKA (1993) who detected spirochetes in 4.1% of *Aedes vexans* and in 3.5--4.3% of hibernating *C. pipiens molestus* collected in southern Moravia, Czech Republic. Moreover, HALOUZKA et al. (1997) have made first successful isolation of
Borrelia afzelii from mosquitoes. Thus, these biting insects which often viciously attack men, should be taken into consideration as a secondary vectors of B. burgdorferi in natural foci of Lyme borreliosis.

REFERENCES


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