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DETECTION OF SPIROCHETES IN, AND ISOLATION FROM, CULICINE MOSQUITOES

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In mosquitoes, spirochetes were first detected in 1907, when Jaffé reported a spirochete in *Culex* sp. and named it *Spirochaeta culicis* (Arch. Protistenkd. 9: 100-107.). Spirochetes were also detected in *Anopheles funestus* and *A. maculipennis* (Sinton J.A., Shute P.G. 1939: J. Trop. Med. Hyg. 42: 125-126) and in the salivary glands of *Anopheles gambiae* (Masseguin A., Palinacci A. 1954: Bull. Soc. Pathol. Exot. 47: 3). The presence of the Lyme disease (LD) spirochete, *Borrelia burgdorferi* has also been reported in mosquitoes and other bloodsucking insects (Magnarelli F.A., Freier J.E., Anderson J.F. 1987: J. Infect. Dis. 156: 694-695). In South Moravia (Czech Republic), spirochetes have been previously observed in mosquitoes (Halouzka J. 1993: Biológia 48: 123-124), and one strain of *Borrelia afzelii* was isolated from *Aedes vexans* (Halouzka J., Postic D., Hubálek Z. 1998: Med. Vet. Entomol. 12: 103-105).

In this study, we examined 2,583 female mosquitoes collected in South Moravia during the years 1993-1997. In the summer season (June to September), 571 mosquitoes were sampled from various localities and habitats: lowland floodplain forest, mixed oak forest with bushes, deciduous mixed forest and shores of a fishpond covered with reedbelts. Three species of the genus *Aedes* – *Aedes vexans* Meigen, 1830, *Aedes sticticus* (Meigen, 1838), *Aedes cantans* (Meigen, 1818) and, moreover, *Culex pipiens* Linnaeus, 1758 biotype *pipiens* were sampled. During the winter period, 2,012 overwintering *Culex pipiens* biotype *molestus* were obtained from cool and humid basement rooms of buildings. The mosquitoes were kept alive at the temperature of about 4°C until dissected. The abdomen content of each mosquito was triturated in a drop of saline and individually examined by darkfield microscopy at a magnification 400×. Specimens with a high number (>100) of motile spirochetes were occasionally inoculated into BSK-H liquid medium (Sigma) supplemented with 5% rabbit serum (Sigma), antibiotics (phosphomycin 100 µg/ml, rifampicin 50 µg/ml), and incubated at 33°C up to six weeks.

The mean prevalence of spirochetes in mosquitoes caught in the summer season was 1.9%. In the winter collection, the mean prevalence of spirochetes in *Culex pipiens molestus* was 5.1% (Table 1). As much as 22 mosquitoes contained more than 100 spirochetes, and 11 of them were inoculated into BSK-H medium. Due to heavy bacterial contamination, only

Table 1. Prevalence of spirochetes in mosquitoes.

Locality	*Aedes vexans*[a]	*Aedes sticticus*[a]	*Aedes cantans*[a]	*Culex pipiens pipiens*[a]	*Culex pipiens molestus*[b]
Lanžhot	1/48*	3/172	0/36	2/83	0/0
Valtice	2/87	0/0	0/27	0/0	31/670
Lednice	0/0	0/0	0/0	2/64	23/566
Mor. Žižkov	0/22	1/32	0/0	0/0	20/354
Šlapanice	0/0	0/0	0/0	0/0	29/422
Total	3/157 1.9%	4/204 2.0%	0/63 0%	4/147 2.7%	103/2012 5.1%

[a] Caught in the summer season
[b] Caught in the winter season
* No. positive/No. examined mosquitoes

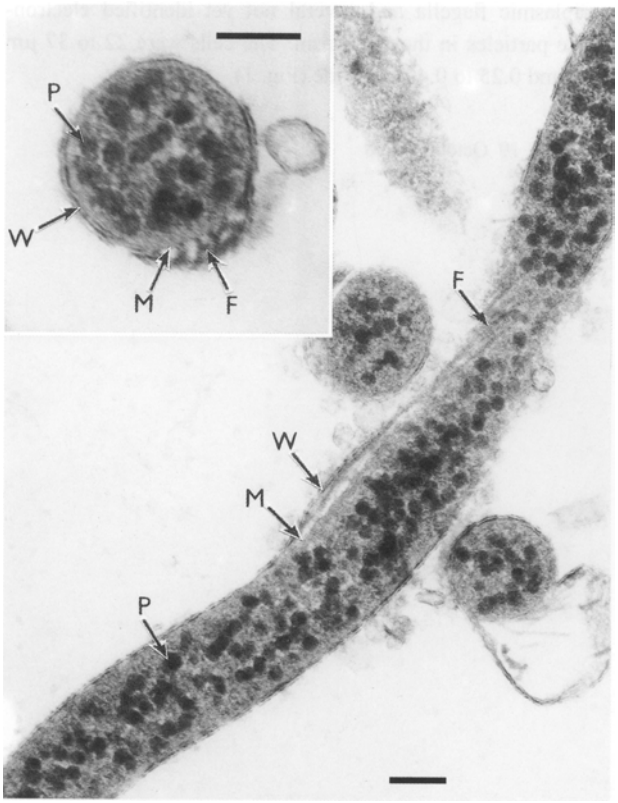

Fig. 1. Transmission electron micrographs of longitudinal and cross sections of spirochetes, strain BR91. F – endoflagella; W – cell wall; M – cytoplasmic membrane; P – electron-dense particles. Scale bars = 200 nm.

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five spirochetal strains were recovered: BR84 (Moravský Žižkov, October 1996), BR85 (Šlapanice, October 1996), BR89 and BR90 (Břeclav, January 1997) and BR91 (Valtice, January 1997). All strains were isolated from *Culex pipiens* biotype *molestus* and have been successfully adapted and cultivated in BSK-H medium. All the strains were found to be susceptible to penicillin: at final concentration of 2000 i.u./ml, their cells lost motility within 24 hours at 33°C.

The strain BR84 isolated from a single female mosquito was identified as *Borrelia afzelii* by OspA serotyping with monoclonal antibodies, genomic fingerprinting by pulsed-field electrophoresis and restriction fragment length polymorphism; the other four strains do not belong to *B. burgdorferi* s.l. (Halouzka J., Wilske B., Stünzner D., Sanogo Y.O., Hubálek Z. 1999: Infection 27: 275-277).

For transmission electron microscopy, the cells were fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.4 at 4°C for 2h, gently mixed with melted agarose, and cut into small blocks. After buffer rinsing, the specimens were postfixed with 2% OsO_4 in 0.2M cacodylate buffer at room temperature for 1h. The material was dehydrated in an ethanol series, saturated by acetone-Durcupan and embedded in Durcupan ACM. Ultrathin sections were cut on an LKB Ultratome III ultramicrotome, contrasted with uranyl acetate and lead citrate and viewed in a Tesla 500 TEM at 70kV. The mosquito spirochetes revealed the presence of about 17 to 30 periplasmic flagella and several not yet identified electron-dense particles in the cytoplasm. The cells were 22 to 37 µm long and 0.25 to 0.40 µm wide (Fig. 1).

Untyped spirochetes were reported (Halouzka J. 1993: Biológia 48: 123-124) and confirmed in the present study in overwintering mosquitoes both at the beginning (October) and the end (February) of the winter season. This means that mosquitoes carry the spirochetes for a relatively long time (more than 4 months). Some of the isolated strains might be commensals of the mosquito midgut.

So far, ixodid ticks are known to be the only biological vectors of LD spirochetes. Nevertheless, according to some reports, a human case of LD was associated with a mosquito bite (Hard S. 1966: Acta Dermatol. Venereol. 46: 473-476) and another case with the bite of a tabanid fly (Stanek G, Flamm H., Groh V., Hirschl A., Kristoferitsch W., Neuman R., Schmutzhard E., Wewalka G. 1986: Zentralbl. Bakteriol. Hyg. A263: 442-449).

The results of this study show that a number of culicine mosquitoes harbour different spirochetal strains including the etiologic agent of LD. However, further biological and ecological characteristics of the isolated strains should be investigated.

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