Research Note

Detection of *Borrelia burgdorferi* from tabanids and ixodid ticks in Hokkaido, Japan

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Borrelia burgdorferi, the causative agent of Lyme disease, has been detected and isolated from various vertebrate and arthropod hosts in many countries demonstrating widespread infection of this spirochete in nature. Epidemiologic studies on Lyme disease show ixodid ticks are the main vectors of *B. burgdorferi*, e.g., *Ixodes dammini* and *I. pacificus* in the United States, *I. ricinus* in Europe (Schmit, 1985). However, *B. burgdorferi* was identified in some biting flies including tabanids and mosquitoes in Connecticut in the United States where Lyme disease was endemic (Magnarelli *et al.*, 1986; Magnarelli and Anderson, 1988). On the other hand, a recent study for the detection of *B. burgdorferi* from tabanids and muscids (Stomoxyninae) yielded negative results in Brittany where the highest infection rates of *I. ricinus* by *B. burgdorferi* were known in France (Doby *et al.*, 1991).

In Japan, Lyme disease has been considered to be transmitted by the bite of *Ixodes persulcatus* and *I. ovatus* (Uchikawa *et al.*, 1991; Miyamoto *et al.*, 1992) while the role of blood-sucking flies has not yet been studied. This communication aims to give some information on the vector potential of tabanids in transmission of *B. burgdorferi* in Hokkaido where most of the Japanese cases

of Lyme disease have occurred.

Tabanids and ticks were sampled in Furano, Hokkaido (43°14'N, 142°24'E) where *B. burgdorferi* had been isolated from *I. persulcatus* and a rodent, *Apodemus speciosus ainu* (Miyamoto *et al.*, 1991). Host-seeking females of tabanids were collected from May to September, 1991 by using mosquito net traps baited with dry ice. Captured flies were immediately frozen and kept at -20°C until dissection. Within 3 months after capture, all the specimens were dissected and examined for parity. Only parous females of anautogenous species were tested for the presence of *B. burgdorferi*. Foregut and midgut tissues removed from parous tabanids were smeared individually onto one of 12 wells on an affluorescent slide (Matsunami, Japan) and allowed to air dry. These specimens were fixed with methanol for 5 min and kept at 4°C until the following indirect immunofluorescence assay (IFA).

Adult ixodid ticks were collected on May 7, 1992 by flapping vegetation along trails in the forests where tabanid traps had been set up in the previous year. The ticks were transported alive to the laboratory and maintained at 15°C. They were dissected 5 or 6 days after capture. Midgut tissues of male and female ixodid ticks were smeared, dried, fixed and stored in the same manner as the tabanid tissues.

The smears were treated with monoclonal antibody, H5332 supplied by Dr. A. G. Barbour, University of Texas Health Science Center, San Antonio, Texas, U.S.A. (Barbour *et al.*, 1983), and fluorescein isothiocyanate (FITC)-labeled anti-mouse IgG (Zymed, USA), and then examined by fluorescence microscopy.

In total, 1,340 females belonging to 16 species of tabanids were collected. Among them, 193 parous individuals of eight anautogenous species were tested for the presence of *B. burgdorferi*. No spirochetes were found in any of the tabanid tissues (Table 1). These 193 females completed at least one gonotrophic cycle, hence they had taken at least one blood meal from vertebrate hosts. This implies they had had an opportunity to take spirochetes if the hosts from which they had sucked blood were infected with *B. burgdorferi*. Magnarelli and Anderson

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Table 1 Species and number of tabanids tested for the presence of *Borrelia burgdorferi*.

Species	Number of specimens tested
<i>Tabanus chrysurus</i>	67
<i>Chrysops suavis</i>	63
<i>Hybomitra distinguenda</i>	27
<i>T. nipponicus</i>	14
<i>T. trigeninus</i>	13
<i>T. sapporoensis</i>	5
<i>C. japonicus</i>	2
<i>T. pallidiventris</i>	2
Total	193

No spirochetes were found in any of the tabanid tissues. Tabanids were collected in Furano, Hokkaido from May to September, 1991. Specimens tested were parous females of anautogenous species.

Table 2 Prevalence of *Borrelia burgdorferi* in *Ixodes persulcatus* and *I. ovatus* in Furano, Hokkaido collected on May 7, 1992.

Species (sex)	No. infected/No. examined
<i>I. persulcatus</i> (male)	4/27
<i>I. persulcatus</i> (female)	5/28
<i>I. ovatus</i> (male)	8/28
<i>I. ovatus</i> (female)	5/29

Fig. 1 *Borrelia burgdorferi* spirochetes in a female of *Ixodes persulcatus*.

IFA staining by H5332 monoclonal antibody and FITC-labeled anti-mouse IgG, $\times 720$.

(1988) observed nearly all of the tabanids and mosquitoes infected with spirochetes were parous.

Although 9 of 55 (16%) *I. persulcatus* and 13 of 57 (23%) *I. ovatus* were infected with *B. burgdorferi* (Table 2; Fig. 1), 193 parous anautogenous tabanid specimens collected in the same sampling sites did not harbor spirochetes. These findings suggest *B. burgdorferi* in Hokkaido is not infective to tabanids same as that in France (Doby *et al.*, 1991). Consequently, the role of tabanids in transmission of *B. burgdorferi* can be disregarded in Hokkaido.

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摘 要

北海道におけるアブおよびマダニからの ライム病病原体検出の試み

ライム病の伝播におけるアブの役割を明らかにするために、北海道富良野市で採集したアブのうち、真性吸血産卵性種 (anautogenous species) の経産個体

を選び、その消化管内容物の塗抹標本を作製し、単クローン抗体 H5332 を用いた間接蛍光抗体法によって、病原体の *Borrelia burgdorferi* の検出を試みた。同じ場所で採集し、同じ方法で検索したシュルツェマダニとヤマトマダニが、20% 前後の感染率を示したにもかかわらず、検査した 8 種 193 個体のアブからは *B. burgdorferi* は 1 例も検出されなかった。北海道においては、ライム病の媒介者としてのアブの役割は無視してよいと考えられる。