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日本産シュルツェマダニとヤマトマダニにおけるライム病ボレリアの経卵巣感染の検討

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Research Note

Negative finding in detection of transovarial transmission of *Borrelia burgdorferi* in Japanese ixodid ticks, *Ixodes persulcatus* and *Ixodes ovatus**

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Transovarial transmission of Lyme disease spirochetes *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt et Brenner has been observed experimentally in the ixodid ticks, *Ixodes dammini* Spielman, Clifford, Piesman et Corwin (Burgdorfer *et al.*, 1988; Magnarelli *et al.*, 1987), *Ixodes pacificus* Cooley et Kohls (Lane and Burgdorfer, 1987), *Ixodes ricinus* (L.) (Burgdorfer *et al.*, 1983), and *Ixodes persulcatus* Schulze (Du *et al.*, 1990). However, the transovarial passage to larval *I. dammini* is of limited importance in maintaining *B. burgdorferi* in nature, because of the rarity of transovarial infection in the field-collected larvae (Piesman *et al.*, 1986). In Japan, the authors demonstrated that the prevalence of spirochetal infection in host-seeking adults of *I. persulcatus* and *Ixodes ovatus* Neumann ranged from 10 to 50% (Miyamoto *et al.*, 1992; Uchikawa *et al.*, 1991). The transmission dynamics of *B. burgdorferi* between ticks and reservoir hosts in Japan still remain to be interpreted. In this study, we examined whether larvae of *I. persulcatus* and *I. ovatus* are infected transovarially with *B. burgdorferi*.

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Unfed adult ticks were collected by flagging or dragging vegetations from the following locations in Hokkaido. *I. persulcatus*: Furano (43°14′N, 142°24′E), Sibecha (43° 21′N, 144°37′E) and Nemuro (43°15′N, 145°24′E). *I. ovatus*: Furano, Samani (42°08′N, 142°59′E) and Asahi (44°08′N, 142°44′E). These adult ticks were allowed to feed on ears of noninfected rabbits (Japanese White) purchased from Ichikawa Laboratory Co., Ltd (Tokyo, Japan). The same species of ticks from each location was fed on a rabbit, and the number of ticks per rabbit was 20–50 females and 20–50 males. Engorged females with intact hypostomes, palps and legs were placed individually in a 60×30×15 mm plastic box, and held at 25°C in a saturated humidity with a photoperiod of 12:12 (L:D). After eggs were deposited, the triturated internal tissues (mainly midgut and ovary) of each female were cultured in BSK medium by the methods of Miyamoto *et al.* (1992) to demonstrate spirochetal infection. After larval emergence, the larvae were examined for spirochetal infection by the following two methods.

Culture

The larvae were washed in 3% hydrogen peroxide and then dipped in 70% ethanol for 1 min. The larvae were dissected individually in BSK medium by using a sharp needle, and the whole internal tissues were triturated and inoculated in 2 ml BSK medium within a mini-vial. After 4 weeks, the cultures were examined for spirochetes with a dark-field microscope.

Indirect immunofluorescence assay (IFA)

The whole internal tissues of larvae were triturated and smeared individually onto one of 12 wells on a glass microscope slide (Matsunami, Japan). For positive control, the cultured spirochetes of *B. burgdorferi* B31 strain (ATCC 35210) were smeared onto the slide. The smeared tissues were air-dried and fixed in 100% methanol for 5 min. These preparations were then treated with monoclonal antibody (MAb) H5332 against the outer surface protein A of *B. burgdorferi* (Barbour *et al.*, 1983), and the bound antibody was detected with fluorescein isothiocyanate-labeled anti-mouse IgG

Table 1 Detection of spirochetes in female *I. persulcatus* and the first filial (F_1) generation larvae by culturing and IFA staining of their internal tissues.

Collection sites	Female		Larvae (F_1 progeny)	
	No.	Culture	Culture	IFA
Furano	1	+*	0/10**	NT***
	2	+	0/48	0/48
	3	−	0/11	NT
	4	+	0/50	0/48
	5	−	0/10	NT
	6	−	0/10	NT
	7	+	0/10	NT
	8	+	0/10	NT
	9	−	0/10	NT
	10	+	0/10	0/12
	11	+	0/10	NT
	12	+	0/10	NT
	13	+	0/10	0/12
	14	+	0/10	0/12
	15	+	0/10	0/12
Sibecha	1	−	0/10	NT
	2	+	0/10	NT
	3	+	0/10	0/12
	4	+	0/10	NT
	5	+	0/10	NT
	6	+	0/10	NT
	7	+	0/10	NT
	8	−	0/10	NT
	9	+	0/10	0/12
	10	−	0/10	0/12
	11	−	0/10	NT
	12	+	0/10	0/12
Nemuro	1	+	0/10	NT
	2	+	0/10	0/12
	3	+	0/10	NT
	4	+	0/10	0/12
	5	+	0/10	0/12
	6	+	0/10	NT
	7	+	0/10	NT
	8	+	0/10	NT
	9	+	0/10	0/12
Total (36 females, 28 infected)			0/439	0/240

* Positive culture. ** Number of positives/number of larvae tested. *** Not tested.

Table 2 Detection of spirochetes in female *I. ovatus* and the first filial (F_1) generation larvae by culturing and IFA staining of their internal tissues.

Collection sites	Female		Larvae (F_1 progeny)	
	No.	Culture	Culture	IFA
Furano	1	C*	0/10**	0/12
	2	C	0/10	0/12
	3	C	0/10	0/12
	4	C	0/10	0/12
	5	C	0/10	NT***
	6	C	0/10	NT
Samani	1	C	0/10	0/12
	2	C	0/10	0/12
	3	C	0/10	0/12
	4	C	0/10	0/12
	5	C	0/10	NT
	6	C	0/10	NT
Asahi	1	C	0/10	0/12
	2	C	0/10	0/12
	3	C	0/10	0/12
	4	C	0/10	0/12
	5	C	0/10	NT
	6	C	0/10	NT
	7	C	0/10	NT
	8	C	0/10	NT
Total (20 females)			0/200	0/144

* Positive and negative could not be determined by severe contamination. ** Number of positives/number of larvae tested. *** Not tested.

(Zymed, U.S.A.). The stained spirochetes were observed with a fluorescence microscope (Olympus BH2-RLF, Japan). MAb H5332 was supplied as hybridoma supernatant from Dr. Alan G. Barbour (University of Texas Health Science Center, San Antonio, Texas, U.S.A.).

No spirochetes could be detected from the first filial (F_1) generation larvae of *I. persulcatus* by the culture and IFA methods, although spirochetes were prevalent in the maternal ticks (Table 1). The spirochetes isolated from maternal ticks were identified as *B. burgdorferi* due to the positive reactivity with MAb H5332 by immunoblotting (Data

not shown). In *I. ovatus*, the F_1 larvae also harbored no spirochetes (Table 2). The spirochetal infection in *I. ovatus* females that laid eggs could not be confirmed, because all the cultures were severely contaminated with unknown bacteria. However, several maternal ticks of the *I. ovatus* examined were probably infected, since the same lots of unfed females used in this study were highly infected (42.9% of 14 females in Furano and 16.7% of 18 females in Samani) with spirochetes. The same lot of unfed females from Asahi was not tested for spirochetal infection.

These results strongly suggest that the transovarial transmission of *B. burgdorferi* does not occur in Japanese ixodid ticks, *I. persulcatus* and *I. ovatus*. Therefore, we conclude that the transovarial transmission in these ticks is of minor epidemiologic importance in maintaining *B. burgdorferi* in nature. It seems that the larval or nymphal ticks of these species acquire *B. burgdorferi* more efficiently by feeding on reservoir hosts.

Du *et al.* (1990) observed that the transovarial transmission of *B. burgdorferi* occurred experimentally in Chinese *I. persulcatus*, as contrasted with our negative result in Japanese *I. persulcatus*. One possible explanation of this discrepant finding may be the geographic difference in biological properties of ticks and/or spirochetes between Japan and China.

References

Barbour, A. G., S. L. Tessier and W. J. Todd (1983): Lyme disease spirochetes and ixodid tick spirochetes share a common antigenic determinant defined by a monoclonal antibody. *Infect. Immun.*, **41**: 795–804.

Burgdorfer, W., A. G. Barbour, S. F. Hayes, O. Peter and A. Aeschlimann (1983): Erythema chronicum migrans—A tick borne spirochetosis. *Acta Trop.*, **40**: 79–83.

Burgdorfer, W., S. F. Hayes and J. L. Benach (1988): Development of *Borrelia burgdorferi* in ixodid tick vectors. *Ann. N. Y. Acad. Sci.*, **539**: 172–179.

Du, Y., S. Y. Tang, X. M. Wu and Q. E. Zhang (1990): Dissemination and transovarial transmission of *Borrelia burgdorferi* in *Ixodes persulcatus* (Acari: Ixodidae). *Chin. J. Vector Biol. Cont.*, **1**: 367–369 (in Chinese).

Lane, R. S. and W. Burgdorfer (1987): Transovarial and transstadial passage of *Borrelia burgdorferi* in the Western black-legged tick, *Ixodes pacificus* (Acari: Ixodidae). *Am. J. Trop. Med. Hyg.*, **37**: 188–192.

Magnarelli, L. A., J. F. Anderson and D. Fish (1987): Transovarial transmission of *Borrelia burgdorferi* in *Ixodes dammini* (Acari: Ixodidae). *J. Infect. Dis.*, **156**: 234–236.

Miyamoto, K., M. Nakao, K. Uchikawa and H. Fujita (1992): Prevalence of Lyme borreliosis spirochetes in ixodid ticks of Japan, with special reference to a new potential vector, *Ixodes ovatus* (Acari: Ixodidae). *J. Med. Entomol.*, **29**: 216–220.

Piesman, J., J. G. Donahue, T. N. Mather and A. Spielman (1986): Transovarially acquired Lyme disease spirochetes (*Borrelia burgdorferi*) in field-collected larval *Ixodes dammini* (Acari: Ixodidae). *J. Med. Entomol.*, **23**: 219.

Uchikawa, K., K. Muramatsu, K. Miyamoto and M. Nakao (1991): An extensive prevalence of *Borrelia burgdorferi*, the etiologic agent of Lyme borreliosis, in Nagano Prefecture, Japan. *Jpn. J. Sanit. Zool.*, **42**: 293–299.

摘　要

日本産シュルツェマダニとヤマトマダニにおけるライム病ボレリアの経卵巣感染の検討

日本産シュルツェマダニとヤマトマダニの未吸血成虫は高率（10～50%）にライム病ボレリアを保有する．この要因を明らかにするため，マダニ類における病原体の垂直伝播（経卵巣感染）を検討した．北海道各地で採集したシュルツェマダニとヤマトマダニの雌成虫をウサギで飽血させた後に個別に産卵させた．ふ化後の幼虫から，培養法と間接螢光抗体法を併用してボレリアの検出を試みたが，経卵巣感染を証明することはできなかった．したがって，自然界では保菌動物を介してマダニ幼若虫がボレリアを受け取り，成虫期までボレリアを伝達していると考えられた．