Research Note

Diversity of *Borrelia* isolates found in rodent-tick relationship in Fukui Prefecture and some additional areas¹⁾

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Some species of ixodid ticks are recognized as the major vectors of Lyme borreliosis pathogens in North America and the Eurasian continent. *Ixodes persulcatus* Schulze has been regarded as the most likely vector of *Borrelia* to man in Hokkaido, Japan (Nakao *et al.*, 1992) and the mountainous area of central Japan (Kawabata *et al.*, 1987). Recently, numerous strains of the genus *Borrelia* have been isolated mainly from two species of ticks, *I. persulcatus* and *Ixodes ovatus* Neumann, and it is said that

the isolates from *I. persulcatus* are diverse in protein composition, while those from *I. ovatus* show a common protein profile to all isolates in this country (Miyamoto *et al.*, 1992; Nakao and Miyamoto, 1993a; Fukunaga *et al.*, 1993). On the other hand, we recorded *Borrelia*-positive unfed nymph of *Haemaphysalis flava* Neumann (Ishiguro *et al.*, 1992). Also, Nakao and Miyamoto (1993a) have isolated spirochetes from *Ixodes tanuki* Saito, *Ixodes turdus* Nakatsuji and *Ixodes columnae* Takada et Fujita. We report herein the diversity of protein composition of *Borrelia* isolates involved in various relations between ticks and the host rodents in central-western Japan.

In 1991 and 1992, unfed ticks were collected by flagging vegetation in mountainous areas in Fukui Prefecture. Also, fed ticks were recovered from rodents captured by Sherman live trap at a few mountainous sites each in Fukui, Ishikawa, Hyogo and Tokushima Prefectures in 1992 and 1993. To isolate the spirochete from ticks, each block of their internal organs (mainly midgut) was cultured in BSK medium (Barbour, 1984), according to the method (Ishiguro *et al.*, 1992) modified from Miyamoto *et al.* (1992). Earlobe tissues of rodents were cultured to isolate the spirochete, according to the method of Sinsky and Piesman (1989). Antigenicity of each isolate was examined by an indirect immunofluorescence stain (IF; Marjorie and Warren, 1987) for reactivity to either monoclonal antibodies (MAb provided from Dr. A. G. Barbour) H9724 specific to the flagellin protein of genus *Borrelia* and H5332 specific to outer surface protein A (OspA) of B31, the prototype of *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt et Brenner. These protein profiles were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a mini-slab apparatus (Nippon Eido, Japan) by the method of Laemmli (1970). Briefly, the samples containing 1.0 mg protein per ml were placed in an antigen diluent containing 4% SDS and 5% 2-mercaptoethanol, and boiled for 5 min. Each 5 μ l was loaded per 12.0% gel lane and electrophoresed at 12

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Table 1 Typing of *Borrelia* isolates from various origins in central-western Japan based on protein profiles by SDS-PAGE.

No.	Name of isolate	Date	Survey site		Culture source (tick or rodent)	Type of protein profile	H5332	
			Name of mountain	Elevation			IF	WB
1.	Fi01o	Nov. '92	Ho-onji	900	<i>I. ovatus</i> (L) ex Es	Io	32	+
2.	Fi03o	Nov. '92	Johoji	600	<i>I. ovatus</i> (L) ex Es	It	32	+
3.	Fi31p	May '93	Sannomine	900	<i>I. persulcatus</i> (L) ex Es	Io	32	+
4.	Fi31o	Jul. '92	Ho-onji	900	<i>I. ovatus</i> (N) ex As	Io	32	+
5.	Fi81t	Nov. '92	Johoji	600	<i>I. tanuki</i> (N) ex Es	It	32	+
6.	Fi32o	Sep. '92	Johoji	600	<i>I. ovatus</i> (N) ex Cd	Io	32	+
7.	Fi91f	Jun. '91	Sannomine	1,100	<i>H. flava</i> (N) by flg	Io	32	+
8.	Fi62o	Jun. '91	Toritate	1,200	<i>I. ovatus</i> (A) by flg	Io	32	+
9.	Fi05p	Jul. '91	Arashima	1,400	<i>I. persulcatus</i> (A) by flg	Ip	<8	—
10.	Fi01p-04p	May '92	Arashima	1,400	<i>I. persulcatus</i> (A) by flg	Ip ^v	32	+
11.	FiEE1	Aug. '92	Johoji	600	Ear tissue of Es	Io	8	+
12.	HyAE1	May '93	Hyonosen	1,100	Ear tissue of As	Io	32	+
13.	TkAE1	May '93	Gose	200	Ear tissue of As	It	32	+

Isolates Nos. 1–11 obtained in Fukui Prefecture; No. 12 in Hyogo Pref.; No. 13 in Tokushima Pref.; IF, reactivity to MAb H5332 indicated by reciprocal numbers of two-fold serial dilution; Es, *Eothentomys smithii*; As, *Apodemus speciosus*; Cd, *Crocidura dsinezumi*; flg, flagging method; L, larva; N, nymph; A, adult; Ip^v, including four variants of Ip type.

Fig. 1 Comparison of protein profiles by SDS-PAGE among *Borrelia* isolates from various species of ticks and rodents in central-western Japan.

MWS, molecular weight standard; B31, the prototype of *B. burgdorferi*. Headings of lanes are names of isolates as shown in Table 1 (profile of isolate from adult *I. ovatus* is not displayed because of identity with those from immature stages). Closed and open arrowheads indicate *Borrelia* flagellin protein reactive with MAb H9724 and outer surface protein A reactive with H5332 in western blot, respectively.

mA. Separated proteins were stained with Coomassie brilliant blue R-250. Their protein profiles were compared with B31 strain as control. Following SDS-PAGE, proteins were transferred from gels to nitrocellulose membranes for western blots (WB). After incubating with MAb H9724 (1:100 dilution) or H5332 (1:50 dilution), the bound complexes on the membranes were detected by an immunoperoxidase stain; the membrane was incubated with peroxidase-labeled goat anti-mouse IgG (1:100 dilution; Cappel, USA) for 60 min at room temperature, and then treated with a substrate solution (chromogen 4-chloro-1-naphthol and peroxide).

To show the types of *Borrelia* isolates from various sources, the representative cases are listed in Table 1, and those protein profiles are shown in Fig. 1. In addition, the incidences of *Borrelia* types from ticks and rodents in central-western Japan are summarized for the combined survey sites in Table 2. All of the spirochetal isolates were positively identified as genus *Borrelia* based on the reactivity with MAb H9724 in IF and WB. Fi01o was isolated from a larval *I. ovatus* fed on vole *Eothenomys smithii* Thomas. The protein profiles of this isolate were common to every isolate (Fi62o) from unfed adult *I. ovatus*, and therefore designated as Io type. It is said that this type will be described as a new species by Japanese researchers (personal communication from Dr. M. Mori, National Institute of Health). Another one (Fi03o) from an *I. ovatus* larva fed on *E. smithii* was identical with It type mentioned later. Fi31p from a larval *I. persulcatus* fed on *E. smithii* was determined as Io type. Fi31o from a nymphal *I. ovatus* fed on wood mouse *Apodemus speciosus* Temminck was also identified as Io type. Fi81t from a nymphal *Ixodes tanuki* Saito fed on *E. smithii* was designated as It type to discriminate from Io type in protein profile, and it was suggestive that the source tick of this isolate and that of Fi03o (It type) was fed on the same individual of *E. smithii*. Also, Fi32o from a nymphal *I. ovatus* fed on insectivore *Crocidura dsinezumi* Thomas

Table 2 Incidence of *Borrelia* types from immature fed ticks or rodents summarized for combined survey sites in central-western Japan based on protein profiles by SDS-PAGE.

Ticks or rodent ears cultured	<i>Borrelia</i> isolation		
	NP/NE	Io	It
<i>I. ovatus</i> L ex Es	4/12	3	1
<i>I. ovatus</i> L ex As	0/ 4		
<i>I. ovatus</i> L ex Aa	0/14		
<i>I. ovatus</i> L ex Cd	0/12		
<i>I. ovatus</i> N ex Es	3/ 9	3	
<i>I. ovatus</i> N ex As	1/ 7	1	
<i>I. ovatus</i> N ex Cd	1/ 2	1	
<i>I. persulcatus</i> L ex Es	1/ 8	1	
<i>I. persulcatus</i> L ex As	0/ 7		
<i>I. persulcatus</i> N ex As	0/ 3		
<i>I. tanuki</i> L ex As	0/ 4		
<i>I. tanuki</i> N ex Es	1/ 1		1
Ear tissues of Es	2/19	2	
Ear tissues of As	6/22	2	4

NP, numbers of positives; NE, numbers of ticks or rodent ears examined; L, larva; N, nymph; Io, Io type; It, It type; Es, *Eothenomys smithii*; As, *Apodemus speciosus*; Aa, *Apodemus argenteus*; Cd, *Crocidura dsinezumi*. Species (numbers) of rodents examined for fed ticks consisted of Es (38), As (33), Aa (15) and Cd (8).

and Fi91f from an unfed nymph *Haemaphysalis flava* was exactly determined as Io type. Every isolate mentioned above reacted with H5332 in IF, and those OspA (approximately 30 kDa of Io type and 32.5 kDa of It type) reacted with H5332 in WB. Fi05p from an unfed adult *I. persulcatus* was designated as Ip type, because it was isolated from 6 of 10 adults *I. persulcatus* examined. This type isolate, however, did not react with H5332 even in 1:8 dilution in IF; also, those OspA-like bands (approximately 31.5 kDa) did not react with H5332 in WB. Fi01p, Fi02p, Fi03p and Fi04p from each unfed adult *I. persulcatus* were different from one another in protein profiles. To distinguish these types from Ip type (Fi05p), each was designated tentatively as a variant of Ip type. These variants were

reactive with H5332 in IF, and the molecular bands of 30 to 32.5 kDa reacted with H5332 in WB. FiEE1 from earlobe tissue of *E. smithii* captured in Fukui Prefecture was quite similar to Io type. This isolate reacted barely with H5332 (1:8 in IF), while it was reactive obviously with H5332 in WB. HyAE1 from ear of *A. speciosus* captured at 1,100 m elevation in Hyogo Prefecture was identified as Io type. We have found both species of *I. ovatus* and *I. persulcatus* at this site, and Io type isolate from the former but no isolate from the latter (Takada *et al.*, 1994). TkAE1 from ear of *A. speciosus* at a hilly site in Tokushima Prefecture was identified as It type. These isolates from *A. speciosus* were reactive with H5332 in IF, and molecular bands of 30 and 32.5 kDa each reacted with H5332 in WB. The protein profiles of It type obtained in the present survey resemble those of some strains which Nakao and Miyamoto (1993a) have isolated from *I. tanuki* and rodents, *Clethrionomys rufocanus bedfordiae* Thomas and *Apodemus speciosus ainu* Thomas.

In Europe and to the Far East, it is known that *Borrelia* isolates from *I. persulcatus* or *Ixodes ricinus* (L.) possess various types of protein profile. Based on the molecular weights of OspA and OspB, and reactivities with several kinds of monoclonal antibodies, those tick-borne spirochete were classified into three species, *B. burgdorferi*, *B. garinii* and *B. afzelii* (Baranton *et al.*, 1992; Wilske *et al.*, 1993; Canica *et al.*, 1993). According to those reports, Fi05p of our isolates possibly is identical with *B. afzelii*.

On the other hand, *E. smithii* seems a more affinitive reservoir for Io type *Borrelia* involved with immature stages of *I. ovatus* central-western Japan (Table 2). Nakao and Miyamoto (1993b) have reported that *Sorex unguiculatus* is a potential reservoir of Io type *Borrelia* in Hokkaido. Nevertheless, we should take notice of some interesting data; Io type was not only isolated from *I. ovatus*, but also from fed larva of *I. persulcatus* and unfed nymph of *H. flava*, and It type was isolated from both *I. tanuki* nymph and *I. ovatus* larva fed on *E. smithii*, and also from

ears of *A. speciosus*.

Considering the fact that various cases involved in the complicated relationships between ticks and rodents were frequently found in the present survey in spite of the small number of specimens examined, it seems that each type of *Borrelia* is not always maintained through a steady reservoir, namely, we recognize that the actual transmission dynamics of *Borrelia* in natural habitats of Japan may be unexpectedly diverse. However, it seems possible that a certain affinity between *Borrelia* type and tick species plays major role in the transmission dynamics, as Nakao and Miyamoto (1993c) have described an interesting model in a forest area of Hokkaido, the northernmost Japan where *I. persulcatus* and *A. speciosus ainu* predominate. Thus, further comparative studies on vectors and reservoirs of *Borrelia* in various areas is required.

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摘 要

マダニ・野鼠相互関係における *Borrelia* 分離株の多様性, 福井県および 西日本数地区での観察

中・西日本で採集した未寄生および野鼠寄生のシュルツェマダニ, ヤマトマダニ, タヌキマダニおよびキチマダニから, またスミスネズミおよびアカネズミからも *Borrelia* 株を分離した. SDS-PAGE によれば, これらは *B. burgdorferi* B31 株と蛋白組成が多少とも異なり, 便宜的にはシュルツェマダニ由来の Ip type およびその四つの異型 (variant), ヤマトマダニ由来の Io type (全国均一), そしてタヌキマダニ由来の It type の7型に分けられた. このうち, Io type とスミスネズミの親和性が強く示唆されたが, 少なくとも本地域では, *Borrelia* の各 type はマダニと野鼠の寄生関係の中でその伝播経路が多岐にわたる可能性が推測された.