Research Note

Isolation of spotted fever group rickettsiae from *Ixodes persulcatus* ticks in the central part of Hokkaido, Japan

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Abstract: Between 1996 and 1998, a total of 550 unfed ticks were collected from vegetation at 11 sites in the central part of Hokkaido, and were examined for spotted fever group rickettsiae. When 261 ticks consisting of *Ixodes ovatus*, *I. persulcatus*, *I. tanuki*, *Haemaphysalis douglasi* and *H. flava* were examined by inoculating tick emulsions into L-929 cells, two adults of *I. persulcatus* from one site (Hobetsu Town) yielded rickettsial isolates. Serotyping showed that the present isolates (IP-1 and IP-2 strains) were of the unclassified IO-1 serotype belonging to the spotted fever group, which was previously designated by us.

INTRODUCTION

Hokkaido, the northernmost principal island of Japan, had been known as a district which was free from spotted fever group (SFG) rickettsiosis. The only Japanese spotted fever (JSF) caused by *Rickettsia japonica* infection associated with tick bite is an SFG rickettsiosis previously encountered in Japan, and JSF-endemic areas are located along the coast of southwestern and central Japan in a warm climate (Mahara, 1997; Takada, 1995). Yano and Takada (1992), however, detected SFG rickettsiae in *Ixodes persulcatus* Schulze in Hokkaido for the first time by electron microscopy. This finding suggested the presence of SFG rickettsiosis in this northern district. Therefore, the present survey was conducted from 1996 to 1998 to identify SFG rickettsiae in ticks in Hok-

kaido and to estimate SFG prevalence there.

MATERIALS AND METHODS

Unfed ticks were collected by flagging on vegetation at 11 survey sites in the central part of Hokkaido between 1996 and 1998 (Fig. 1). Ticks collected were carried alive to our laboratories for isolation and identification of rickettsiae. To isolate rickettsiae from ticks, mouse fibroblast cells (L-929) cultured in small screw vials were used according to a method described previously (Takada *et al.*, 1994). A cell smear was made to detect rickettsiae by a serologic reaction described below. Two murine monoclonal antibodies, SFG rickettsiae-specific S3 and the *R. japonica*-specific C3 (Oikawa *et al.*, 1993), were used to detect rickettsiae. Serologic reaction was performed by an im-

Fig. 1. A map of survey sites in Hokkaido. 1, Kami-Furano; 2, Nopporo; 3, Kita-Hiroshima; 4, Eniwa; 5, Chitose; 6, Atsuma; 7, Yubari; 8, Hobetsu; 9, Minami-Furano; 10, Shimukappu; 11, Shizunai. Arrowhead indicates the rickettsia-positive site.

munoperoxidase staining method (IP). This procedure was a modification of the method developed by Suto (1983), which has been described in detail elsewhere (Takada, 1990).

RESULTS AND DISCUSSION

A total of 550 ticks comprising two genera and five species were collected (Table 1). Most of them were in the adult stage. *Ixodes ovatus* Neumann was widely distributed and was the most abundant species, followed by *I. persulcatus*. *Ixodes tanuki* Saito, *Haemaphysalis douglasi* Nuttall et Warburton and *H. flava* Neumann were not abundantly collected.

Of the ticks collected, 261 ticks consisting of 204 *I. ovatus*, 49 *I. persulcatus*, 1 *I. tanuki*, 4 *H. douglasi* and 3 *H. flava* were used to isolate rickettsiae, and two adults (♀) of *I. persulcatus* collected in Hobetsu

on June 28, 1996 were positive for rickettsiae (Table 1). These two isolates were designated as IP-1 and IP-2. They propagated well under the repeated subculture of L-929 cells.

This is the first report that SFG rickettsiae has been obtained as a valid strain from ticks in Hokkaido, though Yano and Takada (1992) had previously found SFG rickettsiae in the tissues of *I. persulcatus* in Hokkaido by electron microscopy.

As for the identification of the present two isolates, the SFG-specific monoclonal antibody S3 was reactive with the isolates, whereas the *R. japonica*-specific C3 was not reactive with them (data not shown). According to the standard method (Philip *et al.*, 1978), the antigens and mouse antisera were prepared to assess the cross-reactivity between the present isolates and some known rickettsial strains. These isolates were the same serotype of the IO-

Table 1. Tick collection and isolation of rickettsiae from ticks in the central part of Hokkaido between 1996 and 1998.

Survey site	Total no. collected/no. examined												
	<i>Ixodes ovatus</i>		N	<i>Ixodes persulcatus</i>		<i>Ixodes tanuki</i>		<i>Haemaphysalis douglasi</i>			<i>Haemaphysalis flava</i>		
	♀	♂		♀	♂	♀	♂	N	♀	♂	N	♀	♂
Kami-Furano	12/9	1/1	—	2/1	6/4	—	—	—	—	—	—	—	—
Nopporo	18/10	7/5	4/3	10	8/1	1/1	1	—	—	—	2/1	—	1/1
Kita-Hiroshima	7/2	2	—	1/1	—	—	—	—	—	—	—	1/1	—
Eniwa	2/1	3/1	—	1	—	—	—	—	—	—	—	—	—
Chitose	7/3	4/1	—	—	—	—	—	—	—	—	—	—	—
Atsuma	3/1	2	—	—	1/1	—	—	—	—	—	—	—	—
Yubari	4/2	3/2	—	2/2	1/1	—	—	—	—	—	—	—	—
Hobetsu	98/32	51/40	—	20/12(2)	17/7	—	—	1/1	4/2	1/1	—	—	—
Minami-Furano	4/2	5/3	—	3/1	3	—	—	—	—	—	—	—	—
Shimukappu	8	6/2	1/1	3/1	4/1	—	—	1	1	—	—	—	—
Shizunai	83/35	92/52	4/4	10/7	6/1	—	—	7	—	—	—	—	—
Total	246/97	176/107	9/8	52/25/(2)	46/16	1/1	1	9/1	5/2	1/1	2/1	1/1	1/1

N, nymph. Rickettsia-positive result is outlined and figures in parentheses denote the number of positive ticks.

Table 2. Cross-test for serotyping of the IP-1 and IP-2 strains from *Ixodes persulcatus* in the central part of Hokkaido by indirect immunoperoxidase method.

Mouse antiserum to	Code	Antigen							
		1	2	3	4	5	6	7	8
IP-1	1	<u>1,280</u>	1,280	640	80	80	40	40	80
IP-2	2	1,280	<u>1,280</u>	1,280	80	80	80	80	80
IO-1	3	5,120	5,120	<u>10,240</u>	320	320	160	160	320
<i>Rickettsia japonica</i>	4	80	80	1,280	<u>10,240</u>	640	1,280	1,280	1,280
AT-1	5	160	160	320	<40	<u>10,240</u>	160	160	40
<i>R. sibirica</i>	6	40	40	1,280	640	640	<u>10,240</u>	1,280	320
<i>R. akari</i>	7	160	160	640	640	640	320	<u>5,120</u>	1,280
TT-118	8	40	40	640	640	640	640	640	<u>5,120</u>

Results are shown as a reciprocal of the highest dilution. Homologous titers are underlined and homotypic titers are outlined.

1 strain (Fujita *et al.*, 1994), and were apparently distinguishable from other known Japanese strains and Asian SFG rickettsiae (*R. japonica*, *R. sibirica*, *R. akari*, *R. sp.* TT-118 and *R. sp.* AT-1) (Table 2). The SFG rickettsiae of the IO-1 serotype, including the present IP-1 and IP-2 strains, seemed to be distributed throughout Japan; they have so far been isolated from *I. ovatus* in Honshu (Fujita *et al.*, 1994) and Kyushu (Yamamoto *et al.*, 1995; Fujita *et al.*, 1999b), and from *I. mono-*

spinosus Saito in Kyushu (Fujita *et al.*, 1994). Recent genetic analyses have shown that the IO-1 serotype is identical to that of *R. helvetica* (Fujita *et al.*, 1999a; Ishikura *et al.*, 1999).

In Hokkaido, *I. persulcatus* is the principal carrier of human ixodiasis (Miyamoto and Takahashi, 1990). SFG rickettsia of IO-1 serotype is probably transmitted to humans via tick bites, but it is unclear whether this rickettsia species is pathogenic to humans.

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摘 要

北海道道央部で採集されたシュルツェマダニからの紅斑熱群リケッチア分離例

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1996 年から 1998 年に北海道の道央部 11 地点で得たマダニ類についてリケッチア分離を試みた。培養細胞を用いたリケッチア分離に供したマダニ類はヤマトマダニ、シュルツェマダニ、タヌキマダニ、ダグラスチマダニおよびキチマダニの 2 属 5 種で、このうち穂別町のシュルツェマダニ雌成虫から 2 株の紅斑熱群リケッチアが分離された。これらの分離株 (IP-1 株と IP-2 株) は、従来本州と九州のヤマトマダニならびに九州のヒトツトゲマダニから分離されている不明紅斑熱群リケッチア (IO-1 serotype) と同一の血清型とみなされた。