An extensive prevalence of *Borrelia burgdorferi*, the etiologic agent of Lyme borreliosis, in Nagano Prefecture, Japan

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Abstract: To seize the prevalence of *B. burgdorferi* in Nagano Prefecture, we examined *I. persulcatus* and *I. ovatus* ticks for spirochetal infection in 1990. The ticks swept at 20 sites were tested by culturing their midgut tissues or by an indirect immunofluorescence assay using the monoclonal antibody, H5332. The spirochete was detected in the ticks from 18 sites. The infection rates for the adult ticks of *I. persulcatus* and *I. ovatus* were 21.3% (68/319) and 25.1% (76/303), respectively. Such the high prevalence of the spirochete in the tick vectors was comparable to that in the Western Europe.

INTRODUCTION

It was reported that the first case of Lyme borreliosis from Japan had been contracted in Nagano Prefecture probably by *Ixodes persulcatus* (Kawabata *et al.*, 1987). Horiuchi and Ando (1987) compiled 7 cases of erythema chronicum migrans (ECM), an early clinical marker of Lyme borreliosis, which were preceded by the tick bite in the southeastern region of Nagano Prefecture, and presented later 4 cases serologically diagnosed as Lyme disease in the same region (Horiuchi *et al.*, 1988). In addition to these

reports, ixodid ticks are known to be abundant and human tick bites have been frequently encountered in this mountainous prefecture (Uchikawa and Sato, 1983; Yamaguti, 1980).

Referring to these facts, we recognized it necessary to initiate multifarious studies of Lyme borreliosis in Nagano Prefecture from the view point of community medicine. We set up a study group composed of staff members from Shinshu University and began our study with the propagation of information on Lyme disease to local physicians through distribution of guidelines and a questionnaire regarding this disease (Uchikawa *et al.*, 1990; Nakama *et al.*, 1991). Besides making efforts to find out cases of Lyme borreliosis, we have proceeded to ascertain the extent of the disease in the prefecture. Since this borreliosis is a typical zoonosis, we have diverse means of detecting the etiologic agent itself and

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specific antibodies to it. It seemed, however, to be most efficient to demonstrate spirochetes in unfed ticks from various parts of Nagano Prefecture.

Specimens of *Ixodes persulcatus* Schulze, *Ixodes ovatus* Neumann, *Haemaphysalis flava* Neumann, *Haemaphysalis japonica* Warburton and *Haemaphysalis kitaokai* Hoogstraal were taken and examined for spirochetes in the present survey. Results for the two *Ixodes* ticks are given below, whereas those for the *Haemaphysalis* species should be supplemented with much more data to elucidate the role of these ticks in the natural cycle of *B. burgdorferi*. The present paper includes some of the data already used in Miyamoto *et al.* (1991, and in preparation).

MATERIALS AND METHODS

Tick survey areas were marked beforehand on the map of Nagano Prefecture (Fig. 1). While driving through the 20 appointed survey areas mainly in June, 1990, lanes and tracks in forests at the elevations of 800–2,000 m which appeared to have locally abundant populations of ticks were located. Questing ticks were collected by flagging low vegetations on and along those lanes and tracks. Acquired ticks were kept at 4°C and 100% RH until examination.

For culturing spirochetes, the ticks washed briefly in 70% ethanol were dissected individually under the dissecting microscope with sterile precautions. Midgut tissues triturated by forceps were inoculated into 6 ml of Barbour-Stoenner-Kelly (BSK) medium (Barbour *et al.*, 1983a) containing 50 µg/ml of rifampin, or 150 µg/ml of 5-fluorouracil and 150 µg/ml of phosphomycin, in a culture tube. The cultures were maintained at 32°C and checked weekly for the presence of spirochetes under the darkfield microscope for 4–6 weeks.

The midgut tissues of live ticks not processed to the culture were smeared individually in one of 12 wells on an affluorescent slide (Matsunami, Japan) and allowed to air dry. After acetone-fixation for 10 min, the slides were kept at 4°C until the following indirect immunofluorescence assay (IFA). The smears were treated with the monoclonal antibody, H5332, reactive with the outer

Fig. 1 Location of survey sites in Nagano Prefecture, with indication of sites positive (solid circles) and negative (open circles) for *Borrelia burgdorferi* in *Ixodes* ticks. Dotted lines indicate borders of the national and prefectural parks.

surface protein A (OspA) of *B. burgdorferi* serotype B31 (Barbour *et al.*, 1983b), and then the bound antibody was detected with fluorescein isothiocyanate-labeled anti-mouse IgG (Zymed, USA). The stained spirochetes were observed with the aid of the fluorescence microscope (Olympus BH2-RLF, Japan).

Collection of ticks and preparation for IFA were accomplished by KU and KMR, cultures for spirochetal isolation were done by KMR, KMY and MN, and IFA was completed by MN.

RESULTS

Either or both *I. persulcatus* and *I. ovatus* were taken at all of the collecting sites throughout Nagano Prefecture (Table 1). Acquired ticks consisted of adults, nymphs and larvae in *I. persulcatus*, while all *I.*

Table 1 Prevalence of *Borrelia burgdorferi* in *Ixodes persulcatus* and *Ixodes ovatus* in Nagano Prefecture, Japan.

Site (No.)	Location	Elevation (m)	Date (1990)	<i>Ixodes persulcatus</i>				<i>Ixodes ovatus</i>			
				Male	Female	Total	Nymph	Larva	Male	Female	Total
1	Kayanodaira	1,450	6/19	1/7* (14.3)	1/2 (50.0)	2/9 (22.2)	1/13 (7.7)	—	—	—	—
2	Yamanouchi	840	6/19	0/4 (—)	—	0/4 (—)	—	—	—	4/14 (28.6)	4/14 (28.6)
3	Madarao	900	6/19	0/2 (—)	1/3 (33.3)	1/5 (20.0)	1/6 (16.7)	—	1/2 (50.0)	1/5 (20.0)	2/7 (28.6)
4	Togakushi	1,150	6/19	0/9 (—)	5/15 (33.3)	5/24 (20.8)	0/2 (—)	—	0/4 (—)	0/8 (—)	0/12 (—)
5	Hakuba	680	5/29	—	—	—	—	—	2/15 (13.3)	4/10 (40.0)	6/25 (24.0)
6	Hijiri	900-1,000	6/20	0/1 (—)	1/1 (100.0)	1/2 (50.0)	0/6 (—)	—	3/5 (60.0)	0/1 (—)	3/6 (50.0)
7	Yunomaru	1,300-1,720	6/18	1/12 (8.3)	1/16 (6.3)	2/28 (7.1)	0/3 (—)	—	0/3 (—)	—	0/3 (—)
8	Kamikochi	1,500	6/29, 10/24	0/12 (—)	1/18 (5.6)	1/30 (3.3)	0/50 (—)	0/105 (—)	—	—	—
9	Ohkuchizawa	640	5/9	—	—	—	—	—	2/3 (66.7)	0/3 (—)	2/6 (33.3)
10	Sanjiro	1,400-1,600	5/9, 16	14/40 (35.0)	17/45 (37.8)	31/85 (36.5)	4/27 (14.8)	—	—	—	—
			9/28, 10/25	—	—	—	1/3 (33.3)	—	5/12 (41.7)	5/12 (41.7)	10/24 (41.7)
	Ishikiriba	1,200	5/16	1/2 (50.0)	0/3 (—)	1/5 (20.0)	—	—	—	—	—
	Yamabe	800	5/16, 6/1	0/4 (—)	0/2 (—)	0/6 (—)	—	—	0/9 (—)	2/10 (20.0)	2/19 (10.5)

Table 1 (continued)

Site (No.)	Location	Elevation (m)	Date (1990)	<i>Ixodes persulcatus</i>				<i>Ixodes ovatus</i>			
				Male	Female	Total	Nymph	Larva	Male	Female	Total
11	Mugikusa	1,700-2,100	6/18	0/2 (—)	0/3 (—)	0/5 (—)	0/3 (—)	—	0/1 (—)	0/1 (—)	0/2 (—)
12	Yachiho	900-1,100	6/18	2/12 (16.7)	7/19 (36.8)	9/31 (29.0)	0/2 (—)	—	4/10 (40.0)	3/13 (23.1)	7/23 (30.4)
13	Ontake	1,600-1,800	6/8	2/7 (28.6)	1/9 (11.1)	3/16 (18.8)	—	—	2/9 (22.2)	4/16 (25.0)	6/25 (24.0)
14	Akazawa	1,100	6/8	0/5 (—)	0/1 (—)	0/6 (—)	—	—	0/6 (—)	2/6 (33.3)	2/12 (16.7)
15	Tsumago	500-600	6/6	—	0/1 (—)	0/1 (—)	—	—	0/1 (—)	0/1 (—)	0/2 (—)
16	Ohdaira	1,300	6/6	0/3 (—)	0/5 (—)	0/8 (—)	0/1 (—)	—	1/8 (12.5)	3/9 (33.3)	4/17 (23.5)
17	Iida	1,200-1,300	6/6	1/3 (33.3)	0/9 (—)	1/12 (8.3)	1/3 (33.3)	—	3/5 (60.0)	7/14 (50.0)	10/19 (52.6)
18	Ina	1,000-1,200	6/4	1/3 (33.3)	—	1/3 (33.3)	—	—	1/2 (50.0)	4/14 (28.6)	5/16 (31.3)
19	Hasemura	1,000-1,800	6/4	2/11 (18.2)	4/14 (28.6)	6/25 (24.0)	0/2 (—)	—	4/22 (18.2)	3/17 (17.6)	7/39 (17.9)
20	Kamimura	1,000	6/6	3/10 (33.3)	1/4 (25.0)	4/14 (28.6)	—	—	2/15 (13.3)	4/17 (23.5)	6/32 (18.8)
Total				28/149 (18.8)	40/170 (23.5)	68/319 (21.3)	8/121 (6.6)	0/105 (—)	30/132 (22.7)	46/171 (26.9)	76/303 (25.1)

The site numbers are the same as in Fig. 1. * No. positive/No. examined (percent positive rate).

ovatus were adults. A total of 149 males, 170 females, 121 nymphs and 105 larvae of *I. persulcatus* and 132 males and 171 females of *I. ovatus* was examined for spirochetal infection.

Of those ticks, 78 males and 138 females of *I. persulcatus* and 36 males and 131 females of *I. ovatus* were submitted for culturing of spirochetes. Twelve males (15.4%) and 35 females (25.4%) of *I. persulcatus* and 13 males (36.1%) and 36 females (27.5%) of *I. ovatus* yielded spirochetes. All the isolates were regarded as being identical with *B. burgdorferi* based on the results of IFA.

The remaining 71 males, 32 females, 121 nymphs and 105 larvae of *I. persulcatus*, and 96 males and 40 females of *I. ovatus* were examined for *B. burgdorferi* spirochete by IFA. Sixteen males (22.5%), 5 females (15.6%), 8 nymphs (6.6%) and no larva (0%) of *I. persulcatus*, and 17 males (17.7%) and 10 females (25.0%) of *I. ovatus* were positive for the spirochete.

Table 1 lists all the ticks tested and the identification of *B. burgdorferi* within those ticks by both culturing and IFA. The overall spirochete-infection rates in the adult ticks of *I. persulcatus* and *I. ovatus* in Nagano Prefecture were 21.3% (68/319) and 25.1% (76/303), respectively.

Geographically, *B. burgdorferi* was discovered in almost the entire Nagano Prefecture (Fig. 1). Of 20 collecting sites, only Sites 11 and 15, where limited numbers of ticks, 10 and 3, respectively, were taken and examined, yielded no positive data. Yet the spirochete was cultured or detected frequently from the ticks taken at the sites close to these two (Fig. 1). The infection rates in adult *I. persulcatus* and *I. ovatus* ranged from 20 to 30% in many sites. However, the rates were extremely high at the higher part of Site 10 (Sanjiro) (36.5% for adult *I. persulcatus* and 41.7% for adult *I. ovatus*) and Site 17 (52.6% for adult *I. ovatus*). Half of the adult ticks were infected with the spirochete at Site 6, although the number of ticks examined was small. Compared with infection rates for adult ticks at other sites, those at Sites 7 and 8 were low, recorded as 6.5% (2/31) for ticks of the two species and 3.3% (1/30) for *I. persulcatus* ticks, respec-

tively. Moreover, no spirochete was detected in 50 nymphs and 105 larvae of *I. persulcatus* from Site 8.

DISCUSSION

An extensive prevalence of *B. burgdorferi* in Nagano Prefecture was demonstrated in the present study. Thus, the risk of contracting Lyme borreliosis can be predicted for any part of the prefecture. There was, however, a remarkable difference in the spirochete-infection rates in ticks from different survey sites. Although more collecting and tests are still necessary, it is reasonable to presume at present that there are higher risks of coming in contact with infected ticks in the mountains in the central part of the prefecture including Sites 6, 9 and 12 that yielded the spirochete so frequently (Table 1, Fig. 1).

The next important finding was that *I. persulcatus* and *I. ovatus* were infected with *B. burgdorferi* with the high rates almost equivalent to each other. However, the rate for *I. persulcatus* might be slightly underestimated because of non-reactivity of a part of spirochetes in this tick with H5332 as shown by Nakao *et al.* (in preparation). Miyamoto *et al.* (in preparation) already noted this on the basis of recovery of the spirochete by culturing midgut tissues from 145 adult *I. persulcatus* ticks and 85 adult *I. ovatus* ticks acquired in Nagano Prefecture. We reconfirmed their result in the present study by increasing tick samples. This significant phenomenon has so far been observed only in Nagano Prefecture, since it was reported that *I. persulcatus* ticks yielded many more positive cultures than *I. ovatus* in Hokkaido (Miyamoto *et al.*, in preparation). However, we presume that both *I. ovatus* and *I. persulcatus* play at least equivalent roles in the maintenance of the spirochete in nature in some districts of Japan. Only a single major tick vector has been incriminated in each endemic area of Lyme borreliosis in the northern hemisphere so that the finding of the two significant tick vectors within Nagano Prefecture is noteworthy. Consequently, the ticks responsible for Lyme borreliosis as well as human tick bites should be identified clearly in each case

especially in Nagano Prefecture. Ecologically, *I. persulcatus* occurs above the elevation of 800 m and is more prevalent in the forests around 1,500 m. On the other hand, *I. ovatus* shares the higher elevations with *I. persulcatus* but is not as locally abundant at higher elevations as compared to *I. persulcatus* in Nagano Prefecture. *I. ovatus* extends lower to foothill habitats where activity areas of many local residents are included (Uchikawa and Sato, 1983). Reflecting these distribution patterns of the two ticks, human tick bites in May and June were reported as being caused mostly by *I. ovatus*, and those in July exclusively by *I. persulcatus* in Nagano Prefecture (Nakama *et al.*, 1991). Lyme borreliosis that is preceded by tick bite is likely to be caused by the two ticks with the same seasonality as above.

The spirochete-infection rates ranging from 20 to 25%, on average, in the adult ticks of both *I. persulcatus* and *I. ovatus* in Nagano Prefecture are distinctly higher than those in *Ixodes scapularis* and *Ixodes pacificus* in the Southeast and the Pacific Coast of the United States, respectively. The rates are slightly lower than those in the notorious *Ixodes dammini* in the Northeast of the United States, given in many reports such as Tsai *et al.* (1989). Among the data available so far, the infection rates in *Ixodes ricinus* in the Western Europe are almost equivalent to those found in Nagano Prefecture. In South Germany, 2,400 individuals of *I. ricinus* were examined for *B. burgdorferi* infection, and the infection rates for the adults, nymphs and larvae were recorded as 20.9, 10.6 and 1.1%, respectively (Stanek *et al.*, 1988). In Nagano Prefecture, a total of 121 nymphs of *I. persulcatus* was examined for the spirochete, and only 8 (6.6%) were positive. However, 50 negative nymphs were those from Site 8 where a very low prevalence was recorded in the adult ticks (Table 1). So the infection rate for remaining 71 nymphs from the other sites was 11.3% (8/71) subequal to that for nymphal *I. ricinus* from South Germany. Although 105 unfed larvae from Site 8 yielded no positive data unlike those from South Germany, it is still necessary to examine more individuals of this stage from highly infected populations for the spirochete. Uchikawa *et al.* (1990)

suggested that the epidemiological picture for Lyme borreliosis in Nagano Prefecture might be the same as in Europe based on similarity of ecological properties of tick vectors. It is not contradictory to the above assumption that the same level of spirochetal prevalence in the tick vectors has been confirmed in both Nagano Prefecture and Europe. Moreover, Nakano *et al.* (in preparation) demonstrated the heterogeneity of OspA as well as the non-reactivity with H5332 among some isolates from *I. persulcatus* from Japan as observed so far merely in European strains of *B. burgdorferi*.

Finally, we should pay attention to the locations of our survey sites in Nagano Prefecture and of the prefecture itself in the mainland Honshu. Many sites that yielded the spirochete were located in parts of national and prefectural parks where many people from various parts of Japan get very easily by car in the active season of *I. persulcatus* and *I. ovatus*. Japanese people make close observation of plants and flowers on roadsides and pick up seasonal wild vegetables in forests. So, we are easily brought into close contact with *I. persulcatus* or *I. ovatus* infected with *B. burgdorferi* under these circumstances. On the other hand, Nagano Prefecture is bordered with the 8 other prefectures at the central part of the mainland Honshu. It is highly probable that the prevalence of *B. burgdorferi* in tick vectors will be recorded at the same level as in Nagano Prefecture in some parts of those 8 prefectures.

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摘 要

長野県におけるライム病菌の浸淫状況

長野県におけるライム病菌 *Borrelia burgdorferi* の浸淫状況を把握するために、未吸着マダニ類の感染率を調べた。主として1990年6月に、県下20地点で、標高800~2,000mの範囲の林内でマダニ類の多そうな小道を探し、路上または道沿いの草木上に待機する個体をハタズリ法で採集した。得られたマダニの中腸を用い、分離培養法または単クローン抗体H5332を用いる間接蛍光抗体法によってライム病菌を検出した。

20地点のうち、18地点のシュルツェマダニ、ヤマトマダニの一方または双方からライム病菌が検出され、長野県のほぼ全域に病原菌による汚染のあることが判明した。マダニ属成虫が20~25%の感染率を示し、2種がほぼ同率で汚染されていることが地域的な特色であるとみられた。このように高いマダニ属の感染率は、西ヨーロッパの *Ixodes ricinus* のそれに匹敵するものであった。