Preliminary study of the role of red foxes in *Echinococcus multilocularis* transmission in the urban area of Sapporo, Japan

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SUMMARY

In order to assess the infection risk of alveolar echinococcosis among urban residents of Sapporo, the capital of Hokkaido, Japan, a survey was conducted on fox distribution in the urban area and on the prevalence of *Echinococcus multilocularis* among the foxes. The fox distribution, evaluated from fox footprints left on the snow in parks and woodlands, and from locations of fox carcasses recorded by the Sapporo municipality, was concentrated along the border of the urban area and in the southwestern part of the city, facing the mountain. Fox faeces were collected around active fox dens, and analysed by a coproantigen detection assay and parasite egg examination for the *Echinococcus* infection. Thirty-three out of 155 faeces were coproantigen positive. Coproantigen-positive faeces were collected from 11 den sites (57·9% of total den sites), and all except 1 were located in the urban fringe. A high intensity of taeniid eggs (> 100 eggs per 0·5 g) containing faeces were also collected in the 3 sites of them. Although *Echinococcus* infection in rodents was not observed from the necropsy of 23 rodents captured around active fox dens, arvicolid rodents, a suitable intermediate host for *E. multilocularis*, were captured in the urban fringe. Therefore, the urban fringe offers suitable conditions in which the life-cycle of *E. multilocularis* could be maintained. Prompt measures to control echinococcus infection should be taken, even in urban areas.

Key words: *Echinococcus multilocularis*, *Vulpes vulpes*, urban area, coproantigen detection assay, zoonosis, epidemiology.

INTRODUCTION

Echinococcus multilocularis is one of the most serious zoonotic parasites in Hokkaido, Japan. A total of 383 patients with alveolar echinococcosis have been recorded in Hokkaido up to 1999, and 5–19 cases per year are newly detected by the committee for echinococcosis control in Hokkaido. From April 1999, echinococcosis was one of the notifiable infectious diseases by the new Law Concerning Prevention of Infectious Diseases and Medical Care for Patients of Infections in Japan. The main definitive host of *E. multilocularis* is the red fox (*Vulpes vulpes*) and the intermediate hosts are usually wild voles, such as *Clethrionomys rufocanus* and *C. rutilus* (Ohbayashi, 1996). Recently, the prevalence of this parasite among red foxes has drastically increased up to nearly 40% (Kamiya & Sakai, 1998). In addition, red foxes have begun to inhabit the central area of the city. Some foxes have become tolerant to humans and have been fed by urban residents. Under such circumstances, in which urban residents are in close contact with wild red foxes, the occurrence of alveolar echinococcosis among urban residents can be expected. The first infected resident of Sapporo was reported in 1997. Nevertheless, there was little information about the distribution of red foxes in the urban area of Sapporo and the prevalence of *E. multilocularis* among the red foxes. Therefore, we conducted a survey on the distribution of red foxes and the prevalence of *E. multilocularis* among the red foxes in the urban area of Sapporo and discuss the infection risk of this parasite among urban residents.

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MATERIALS AND METHODS

Study area

This survey was conducted in Sapporo, the most populated city in Hokkaido. It has a population of about 1·8 million, and the size of the urban area is about 242 km^2. It is located at the mouth of Ishikari River. The northeastern part of the urban area is relatively flat and, in contrast, the southwestern part of the urban area is mountainous.

Urban area

The definition of urban area used in this study is that the area that was already urbanized or primarily

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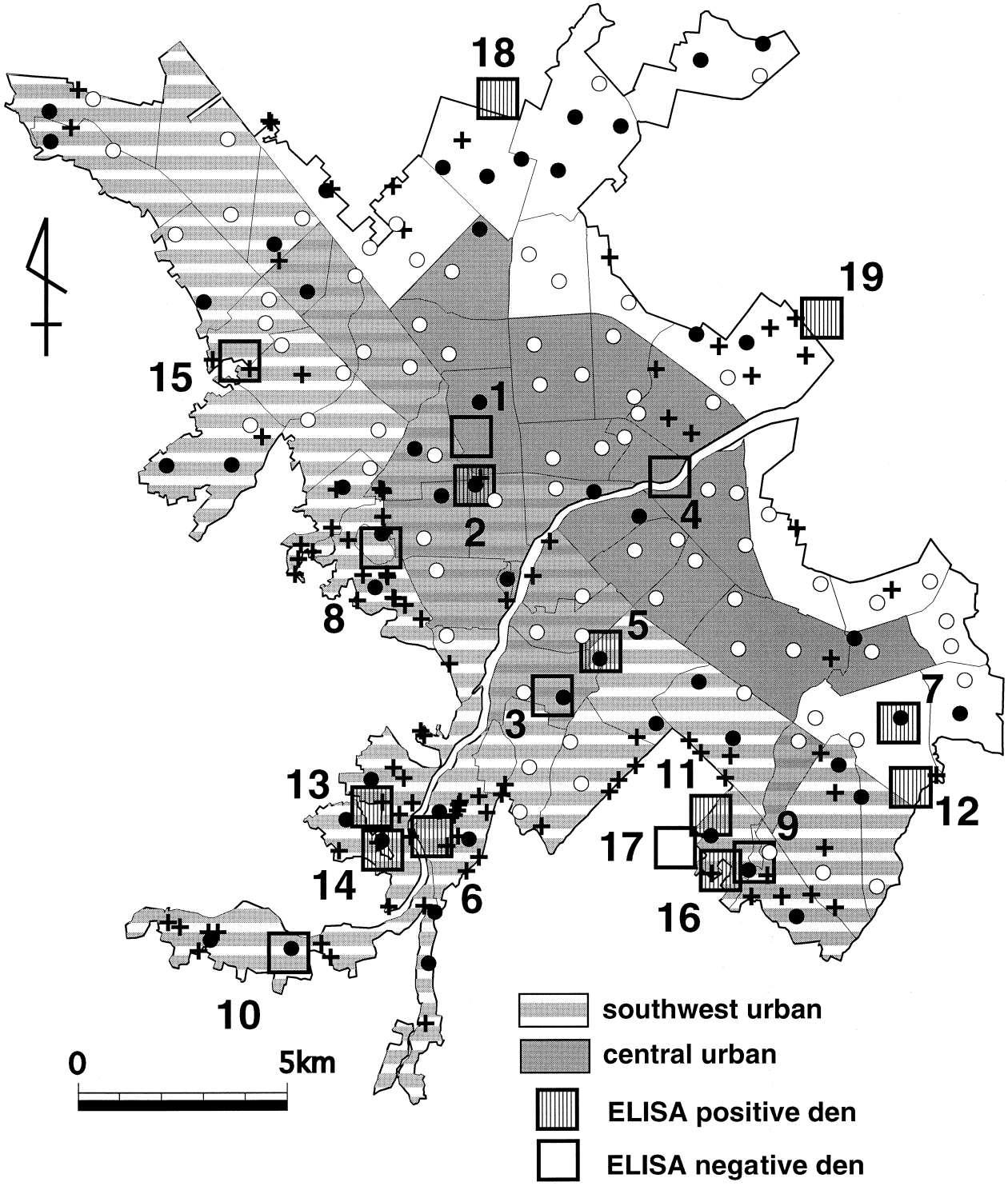

Fig. 1. Distribution of parks and woodlands investigated, fox carcasses recorded and fox den sites where faecal samples were collected in the urban area of Sapporo are shown. Fox footprints could be observed at filled circles, and not at open circle. A cross bar and a large square with number shows the location of each fox carcass and fox den site, respectively. Vertical-striped and open square indicates positive and negative coproantigen-ELISA result, respectively. The detailed explanation of the urban area classification is shown in the text.

planned to be developed within a decade (Sapporo City, 1994). The area surrounded by the urban area, such as large woodlands and parks, military base, and unused areas, is also included into the urban area. The urban area was administratively divided into 61 small districts. These small districts were conveniently allocated into peripheral urban and central urban, and also into southwest urban and northeast urban areas for regional comparison (Fig. 1).

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Fox footprint survey

To clarify the fox distribution in the urban area, fox footprints left on snow were investigated during February to March in 1997. Because the vegetated areas were considered to be preferably used by foxes, we selected 130 parks and woodlands (2·0 km²–0·03 km²), almost evenly located in the urban area, for detection of fox footprints. Fox footprints on fresh snow were investigated in the morning if snowing stopped the night before. Fox footprints were distinguished from dog or cat footprints by their size, the length of the stride, and the pattern of tracks. For regional comparison, the deviations in distribution were tested statistically. Footprint positive parks or woodlands were counted in each regional section, and these positive proportions were compared between peripheral urban and central urban, and also between the southwest urban and the northeast urban areas by χ^2 test.

Fox carcass distribution

The information about fox distribution in the urban area was supplemented by the location of fox carcasses killed by traffic accidents. Fox carcasses were collected by the road-cleaning department of Sapporo municipality and the locations of the carcasses were recorded by the municipal health centre. The information from April 1994 to March 1997 was obtained from the health centre of Sapporo municipality. For regional comparison, the deviations in distribution were tested statistically. Fox carcass density was calculated for each of 61 small districts and mean densities were compared between peripheral urban (48 districts) and central urban (13 districts) and between southwest urban (35 districts) and northeast urban areas (26 districts) by Mann–Whitney *U*-test.

Collection and preparation of fox faecal samples

Nineteen fox den sites were discovered in parks and woodlands in and near the urban area by the field survey and from information from local people. Fox faeces were collected around the active fox dens from April to July in 1997 and from February to August in 1998. In front of the fox dens, many faeces of juvenile foxes were discovered in May and June when the activity of juvenile foxes is restricted around their dens. In other seasons, faeces were collected along the road around the den site. Fox faeces were distinguished from those of other animal species by features, such as size, smell, content and the pattern of deposit. These faeces were carefully put into 100 ml polypropylene tubes, soaked with 1 % formalin solution and sterilized by incubation at 70 °C for 12 h. After sterilization, faecal suspensions containing 0·5 g of faeces were aliquoted into 15 ml centrifuge tubes, and 1 % formalin and 0·3 % Tween 20 in water were added to a final volume of 15 ml. Then the faecal suspensions were centrifuged at 1000 ***g*** for 10 min and the supernatant fraction was used for the coproantigen detection assay. The sediments were used for parasite egg examination.

Coproantigen detection assay

Coproantigen detection was performed by a sandwich enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody, EmA9 raised against *E. multilocularis* somatic antigen (Kohno *et al*. 1995). The basic procedure was previously described by Morishima *et al*. (1999*b*).

Faecal examination for taeniid eggs

Parasite egg examination was conducted by the sucrose centrifugal floatation technique (Ito, 1980) using sucrose solution with 1·27 specific gravity. Because eggs of *E. multilocularis* cannot be discriminated from those of other *Echinococcus* or *Taenia* species morphologically (Thompson, 1995), species identification was not conducted. The number of taeniid eggs per 0·5 g of faeces was counted, then the egg intensity was classified into four grades, such as, > 100 eggs, 100–11 eggs, 1–10 eggs and zero.

Capturing and necropsy of intermediate hosts

Trapping and necropsy of wild rodents were conducted during May and June 1998. Because a relatively high prevalence of *E. multilocularis* among rodents was observed around active fox dens (Takahashi & Uraguchi, 1996; Takahashi *et al*. 1989), all traps were set around 6 fox dens where coproantigen or taeniid egg positive faeces were collected. Forty sunflower seed-bait Sherman traps were set around each den site, arranged in 2 lines with 10 stations containing 2 traps, > 1 m apart from each other. All rodents captured were necropsied and the livers were examined macroscopically for the presence of larvae of the cestode.

RESULTS

Fox footprint distribution

Fox footprints were observed in 38·5 % of total locations surveyed ($n = 130$). Footprint positive parks were not evenly distributed throughout the urban area (Fig. 1). The footprints were also observed in the central urban areas where many high buildings were clustered. Significantly more footprint positive parks and woodlands were located in the peripheral rather than central urban area [peripheral: 47·6 % ($n = 82$); central: 22·9 % ($n = 48$); χ^2

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Table 1. Rodent species and numbers that were caught around the fox dens

Den site	Urban area classification	Number of traps	Cr*	Aa	As	Rn	Total rodents
Loc. 7	P/NE	40	0	0	1	2	3
Loc. 9	P/SW	40	0	0	1	0	1
Loc. 10	P/SW	40	0	0	9	0	9
Loc. 11	P/SW	40	0	1	1	0	2
Loc. 13	P/SW	40	0	0	3	0	3
Loc. 19	P/NE	80	3	1	0	1	5
Total		280	3	2	15	3	23

* Cr, *Clethrionomys rufocanus*; Aa, *Apodemus argenteus*; As, *Apodemus speciosus*; Rn, *Rattus norvegicus*.

test, $P < 0{\cdot}01$], and in southwest rather than northeast urban area [southwest: 28·8 % ($n = 59$); northeast: 46·5 % ($n = 71$); χ^2 test, $P < 0{\cdot}05$].

Fox carcass distribution

One hundred and thirty-eight fox carcasses were recorded in Sapporo for 3 years (1994–1997). These carcasses were not evenly distributed in the urban area (Fig. 1). The density of fox carcasses is significantly higher in the peripheral rather than, the central area (peripheral: 0·0069 foxes/km^2; central: 0·002 foxes/km^2; *U*-test, $P < 0{\cdot}01$) and in the southwest rather than the northeast area (southwest: 0·0083 foxes/km^2; northeast: 0·0026 foxes/km^2; *U*-test, $P < 0{\cdot}01$). A few fox carcasses were collected even in the central area. These spatial features are the same as the results of the footprint survey.

Faecal examination

(*a*) *Coproantigen*. A total of 155 faeces were collected from 19 den sites (1–70 faeces/site), and 21·3 % of the faeces showed sandwich ELISA positive. ELISA positive faeces were collected from 11 den sites, which is 57·9 % of total den sites investigated. Only 1 ELISA-positive den site was located in the central area (Loc. 2, Fig. 1); and the other positive den sites were located in the peripheral area (Fig. 1).

(*b*) *Taeniid eggs*. Taeniid egg-positive faeces were collected from 7 den sites among 11 ELISA-positive den sites. Highest rank of egg intensity (> 100 eggs per 0·5 g of faeces) was recorded only in 3 den sites (Loc. 13, 14 and 19) located in the urban border (Fig. 1).

Capturing and necropsy of intermediate hosts

A total of 23 rodents were captured around 6 fox dens where coproantigen-positive faeces were collected. The species composition of these rodents and the distance of the trapping site from urban border are shown in Table 1. No rodents harbouring *E. multilocularis* were observed. Suitable intermediate hosts, *Clethrionomys rufocanus* were caught at only 1 den site where it was 1 km apart from urban border. Most of the rodents caught in the urban area were *Apodemus* species that are not suitable intermediate hosts.

DISCUSSION

Two methods were used to evaluate fox distribution in this study. The footprint survey was conducted only in winter, so that the distribution of foxes in other seasons was not evaluated. On the other hand, fox carcasses were collected throughout the year, although the accessibility of fox carcasses could vary due to the seasonal conditions. In spite of the difference in methods, both results show that fox distribution in the urban area is dense in the peripheral and near mountainous areas and is scarce and patchy in the central urban area. In the central area, there are many clustering buildings and few woodlands where many prey animals could live, thus, the food availability for the fox should be scarce. In addition, traffic accidents and human disturbance could also obstruct occupancy by foxes in the central area. In some stable urban fox populations in British cities, a lower fox density was observed in the city centre than in peripheral regions (Harris & Rayner, 1986). In Bristol, where fox control was not operated, road accidents accounted for > 50 % of all mortality of foxes (Harris & Smith, 1987). In Sapporo, where fox control was not operated, the number of fox carcasses collected has increased in the last decade. The occupancy by foxes of the urban area seems to be still in progress, so that the habitat preference of fox could not be determined by this study. Continual monitoring of the fox distribution and further investigation may provide more detailed information about this issue.

The coproantigen detection assay, using EmA9, has been effectively applied for many surveys to detect the prevalence of *E. multilocularis* among wild

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fox populations (Morishima *et al*. 1999*b*; Nonaka *et al*. 1997; Sakai *et al*. 1998). EmA9 directed against somatic antigen of adult *E. multilocularis* does not show a cross-reaction with other intestinal parasite antigens (Kohno *et al*. 1995; Sakashita *et al*. 1995; Morishima *et al*. 1999*a*). No taeniid species infection except *E. multilocularis* and *Taenia serialis* among foxes were found in Hokkaido (Kamiya & Ohbayashi, 1975; Yorozuya *et al*. 1968), nor found in and around Sapporo (Morishima *et al*. unpublished observations). Therefore, most or all of ELISA positives can be considered to represent *E. multilocularis* infection.

In the central urban area, ELISA-positive faeces were not found except for Loc. 2 (Fig. 1). In Loc. 2, only 1 ELISA-positive faeces was found among 8 faeces collected in June 1997, and all of 62 faeces collected thereafter were ELISA negative. One adult male fox using Loc. 1 and 2 was attached with a radio collar, and then the ranging behaviour was monitored by the local researcher from March to July in 1998. The fox moved within the range (about 5 km^2) located within the central area, including the botanical garden, university campus and the built-up area, and was frequently observed to feed on offal and garbage (Sawaoka, unpublished data). Within the fox home range, there is little woodland, which is a suitable habitat for *Clethrionomys* spp., the most suitable intermediate host for *E. multilocularis* in Hokkaido. The study on stomach contents of fox carcasses collected in the urban area of Sapporo shows that *Clethrionomys* spp. are less eaten, while *Rattus*, which are not susceptible to *E. multilocularis* infection (Iwaki *et al*. 1995; Webster & Cameron, 1961), are eaten more (Uraguchi *et al*. unpublished data). *Clethrionomys* spp. was not captured in the central zone in this study. Therefore, the life-cycle of *E. multilocularis* is not easily maintained in the central urban area of Sapporo at present.

On the other hand, in the peripheral area, ELISA-positive faeces were collected, and arvicolid rodents were captured at Loc. 19. Although the captured voles were not infected with *E. multilocularis*, egg-positive faeces containing numerous taeniid eggs were collected there. The fox footprint-positive parks and the locations of fox carcasses are concentrated in the peripheral area, suggesting that fox density is relatively high there. These observations suggest that the peripheral urban area is suitable for the life-cycle of *E. multilocularis* to be maintained. The infection risk of this parasite to residents was expected, and was especially high in the peripheral area through sharing the same space with foxes, particularly where the foxes are fed by the residents. At present, countermeasures to reduce the risk of infection to urban residents is not conducted by the local government. However, prompt measures to control the *Echinococcus* infection should be taken in urban Sapporo. In Southern Germany, control measures, such as chemotherapy against wild foxes, were successfully conducted using praziquantel baits spread from the air (Schelling *et al*. 1997). In Sapporo, the distribution of baits by aeroplane is not possible especially in the extremely populated area, while baiting with praziquantel by urban residents should be convenient in the situation that urban fox is tame with the residents and can be fed by some of them. Another possible control programme in the urban endemic areas, would be that praziquantel baits are placed by the residents with great concern about the outbreak of alveolar echinococcosis near locations where foxes appear.

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