Potential remedy against *Echinococcus multilocularis* in wild red foxes using baits with anthelmintic distributed around fox breeding dens in Hokkaido, Japan

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SUMMARY

The effect of bait-delivered anthelmintic to reduce the prevalence of *Echinococcus multilocularis* in wild red foxes was evaluated in Koshimizu, in the eastern part of Hokkaido, Japan. The study area (200 km²) was divided into baited and non-baited sections. The anthelmintic baits were distributed around fox den sites in the baited section every month for 13 months. After 1 year of the anthelmintic bait distribution, the prevalence of *E. multilocularis* in foxes, evaluated either by the parasite egg examination (from 27·1 to 5·6 %) or coproantigen ELISA (from 59·6 to 29·7 %), decreased in the baited section contrasting to that in the non-baited section (parasite egg: from 18·8 to 24·2 %; ELISA: from 41·9 to 45·8 %). The prevalence of *E. multilocularis* in grey red-backed vole *Clethrionomys rufocanus*, caught around fox dens, born after bait distribution also decreased and was significantly lower than that in non-baited section. However, within the study periods, the coproantigen-positive rate in fox faeces sporadically increased, while egg-positive rate constantly decreased. Since coproantigen ELISA can detect pre-patent infection, this observation indicates that reinfection pressure in the baited section was still high even after the 13 months of anthelmintic bait distribution. Therefore, the bait distribution longer than our study period is required for the efficient control of *E. multilocularis* in wild red fox population.

Key words: *Echinococcus multilocularis*, bait, praziquantel, *Vulpes vulpes*.

INTRODUCTION

Echinococcus multilocularis is one of the most serious zoonotic parasites in the northern hemisphere. In Japan, the endemic area was restricted to Hokkaido, the northern island of this country although some sporadic human cases were reported in other islands (Takahashi *et al*. 1986; Doi *et al*. 2000). A total of 392 infected patients have been confirmed in Hokkaido and 5–19 (mean = 11) new patients have been reported every year since 1982. In 1999, however, finisher pigs infected with *E. multilocularis* were found in Aomori, and this was the first occurrence of echinococcosis in the local resident animals of the main island of Japan (Kamiya & Kanazawa, 1999). Accordingly, the endemic area was believed to be expanding to other islands. Although many counter measures for echinococcosis, such as population control of red foxes, development of public water supply and intensive sanitary education were conducted in Hokkaido, the eradication of human echinococcosis has not been achieved yet (Minagawa, 1997). Recently, oral chemotherapy of definitive hosts, using bait containing praziquantel, was conducted in southern Germany, and resulted in the decrease of prevalence of *E. multilocularis* in wild red foxes (Schelling *et al*. 1997). In this German study, however, the reduction of *Echinococcus* infection in the red foxes at the perimeter of the treatment area was not so conspicuous compared with the central part, possibly because resident foxes which hunted in the treatment area were replaced by infected foxes from the outside. If the anthelmintic-treated foxes were still alive and stayed in the treated area, a more effective reduction of prevalence of *E. multilocularis* in the red foxes would be expected. Coproantigen detection makes it possible to detect *Echinococcus* infection in red foxes without disturbing the resident fox population, the normal social life and the local predator-prey balance maintained between foxes and their prey, and have been successfully applied to several *Echinococcus* surveys at Koshimizu (Morishima *et al*. 1999*a*), Shiretoko (Nonaka *et al*. 1998), and Sapporo (Tsukada *et al*. 2000). In this study, therefore, we conducted continuous anthelmintic treatment of the resident foxes, and evaluated the

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effectiveness of the treatment by coproantigen detection (Nonaka *et al*. 1998; Morishima *et al*. 1999*a*; Tsukada *et al*. 2000).

MATERIALS AND METHODS

Study area

The experiment was carried out in and around Koshimizu Town, in the eastern part of Hokkaido, Japan (43°50′N, 144°28′E; Fig. 1). A previous study showed that 53·4% of collected fox faeces contained coproantigen of *E. multilocularis* in this area (Morishima *et al*. 1999*a*). The dominant landscape of the study area is pasture and crop land where white potato, wheat and sugar beet are cultivated. The 200 km² study area was divided into 2 sections: the Yambetsu River flowing in the centre of the town forms the boundary between the baited (90 km²) and the non-baited sections (110 km²).

Distribution of fox den sites

In the study area, 35 fox den sites were located in spring, 1997 by investigation on foot and by questionnaire to the local people (Morishima *et al*. 1999*a*). After re-examination of these den sites and investigation of new den sites, 38 fox den sites were confirmed in June 1998 (Fig. 1). Although the greater part of fox den sites could be located, insufficient information about fox denning and the mountainous terrain were an obstacle on the way of finding fox den sites, especially in the southern part of the study area. A total fox family density was about 0·19 fox families/km². The study was conducted on all these den sites: 18 and 20 den sites in the baited and non-baited sections, respectively.

Anthelmintic bait distribution

Bait preparation. Some commercial fish sausages (90 g, 2 cm diameter × 12 cm long) containing fish meats, lard, gelatin and some spices were used as bait, because it was easily obtained and it had been observed in other regions that foxes were attracted by these sausages (Tsukada personal observation). Half of a Droncit® tablet (commercially available anthelmintic, 50 mg praziquantel per tablet; Bayer Co.) was embedded in each small piece of fish sausage (cut into 1·5 cm long).

Bait distribution. Anthelmintic baits were distributed to each resident fox family in the baited section. Five shallow holes (15 cm diameter × 30 cm depth, called 'bait hole' later, 1 in front of the fox den and 4 around the den) were prepared within 100 m from each active fox den to increase the chance of more than 1 fox belonging to a family eating the bait at the same time. To each bait hole, 2 pieces of the anthelmintic bait and 2 pieces of lure fish sausages (same size with anthelmintic bait) were put on the bottom and covered with some leaves or grasses to minimize the loss by scavenging birds such as crows. A total of 90 bait holes for 18 resident fox families were prepared in baited section. To check the bait uptake by foxes, a smooth slope about 30 cm long and 20 cm wide was made in front of each bait hole. Disapperance of the bait and fox footmarks left on the slope were considered to indicate bait uptake by the fox. Bait distribution was conducted every month from May 1998 to May 1999. In the first day, all baits were set in each bait hole. Baits and footmarks were checked every day until the fourth day, and new baits were added if the original baits disappeared.

Monitoring bait uptake. Every morning during bait distribution, footmarks left on the slope were checked to identify which animals had contacted the bait. The bait uptake rates were calculated in each family as a function of the bait hole-night, which is the number of bait hole-nights with the bait uptake by the fox divided by the total number of prepared bait hole-nights. If the bait holes were destroyed by some accidental reasons, such as ploughing by a farm tractor or collapse from heavy rain, these bait holes were excluded from the calculation.

Monitoring for prevalence of E. multilocularis *in the definitive host*

The effect of anthelmintic distribution on the prevalence of *E. multilocularis* in the red foxes was evaluated by comparing the results of faecal examination for taeniid eggs and coproantigen detection assay between the baited and non-baited sections.

Faecal sample collection and pre-treatment. Fox faeces were collected every month from April 1998 to May 1999 by searching along agricultural fields, windbreak forests, roads, and along fox tracks, especially in the snowy season, within an area of about 500 m apart from each fox breeding den (18 and 20 dens in the baited and non-baited section, respectively). These faeces were carefully put into 100 ml polypropylene bottles and soaked with 1% formalin solution, after sterilizing by incubation at 70 °C for 12 h. Then, faecal suspensions containing 0·5 g of faeces were aliquoted into 15 ml centrifuge tubes and 1% formalin containing 0·3% Tween 20 was added to final volume of 15 ml. The faecal suspensions were then 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.

Faecal examination for parasite eggs. Parasite egg examination was conducted by the sucrose centrifugal flotation technique (Ito, 1980) using sucrose solution with a specific gravity of 1·27. Because eggs

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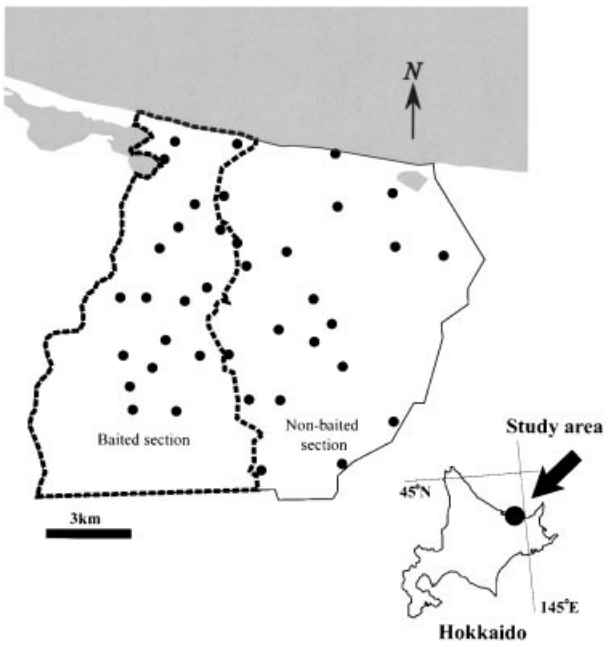

Fig. 1. Distribution of fox den sites in the study area where faecal sampling was conducted. Closed thin and bold dashed lines indicate the boundary of the study area and the baited section, respectively. Shaded areas indicate water surface. Each circle represents the location of fox den site.

of *E. multilocularis* could not be discriminated from those of other *Echinococcus* or *Taenia* species morphologically (Thompson, 1995), species identification was not conducted. The number of taeniid eggs was counted, then the egg intensity was classified into the following 5 grades; grade 4: > 1000 eggs, grade 3: 1000–101 eggs, grade 2: 100–11 eggs, grade 1: 10–1 eggs and grade 0: no eggs. To evaluate the level of parasite egg contamination, the faecal egg intensities were calculated by averaging the score (0–4) of the grade of all faeces collected in each section every month.

Coproantigen detection

Coproantigen detection was performed by a sandwich enzyme-linked immunosorbent assay (ELISA) using a mAb, EmA9, raised against adult *E. multilocularis* somatic antigen (Kohno *et al*. 1995). The basic procedure was previously described by Morishima *et al*. (1999*a*). In this analysis we used 32 fox faeces collected from uninfected wild foxes hunted in the Sapporo area (Morishima *et al*. 1999*b*) as negative controls. A cut-off OD value (mean + 3SD) calculated from the negative samples was 0·173.

Monitoring of prevalence of E. multilocularis *in the intermediate host*

The effect of anthelmintic bait distribution was evaluated by monitoring *E. multilocularis* prevalence in the intermediate hosts. Trapping and necropsy of wild rodents were conducted during May, July, September, and November 1998, and May and June 1999. Because a relatively high prevalence of *E. multilocularis* among rodents was observed around active fox dens (Kamiya *et al*. 1977; Takahashi *et al*. 1989), all traps were set around breeding fox dens where faecal sampling was conducted. Forty sunflower seed-bait Sherman® traps (H. B. Sherman Traps Inc., Tallahassee, Florida, USA) were set around 10–14 den sites each month, arranged in 2 lines with 10 stations each containing 2 traps, > 1 m apart from each other. If we could not find any rodents infected with *E. multilocularis*, nor catch any arvicolid rodents, which are suitable intermediate hosts of *E. multilocularis* (Ohbayashi, 1996), we changed fox breeding dens for the traps on subsequent occasions. All captured rodent species excluding the large Japanese field mouse *Apodemus speciosus* were necropsied and these internal organs (mainly lung and liver) were examined macroscopically for the presence of larvae of *E. multilocularis*. Tissues with suspected lesions were fixed in 10% formalin solution. These tissues were dehydrated in an ethanol series, and paraffin sections were made. Sections were stained with either haematoxylin-eosin (HE) or periodic acid Schiff (PAS) stain for histological examination. The ages of grey red-backed voles, *Clethrionomys rufocanus*, infected with *E. multilocularis* were estimated by the examination of the shape and root ratio of teeth (Abe, 1976). In May and July 1998 and May and June 1999, the age of voles uninfected with *E. multilocularis* were also estimated for comparing age distributions between the infected and uninfected animals.

Statistical analyses

All prevalence and the rate of bait uptake were calculated with 95% confidence intervals using a relationship between the F distribution and the binominal distribution (Zar, 1999). Differences in the prevalence between the baited and the non-baited sections were analysed using Fisher's exact probability test. The difference in the egg intensity between the baited and the non-baited sections was analysed with Mann-Whitney's U test. In tables of statistical tests the sequential Bonferroni technique was applied to avoid rejecting a true H_0 hypothesis (Rice, 1989).

RESULTS

A total of 2656 fox faeces was collected through the whole study period. The mean numbers of faeces collected every month were 86·0 (57–155) and 103·7 (71–151) in the baited and the non-baited sections, respectively. That was relatively stable during the non-snowy season, except from April to June, but decreased almost half of that in the snowy season

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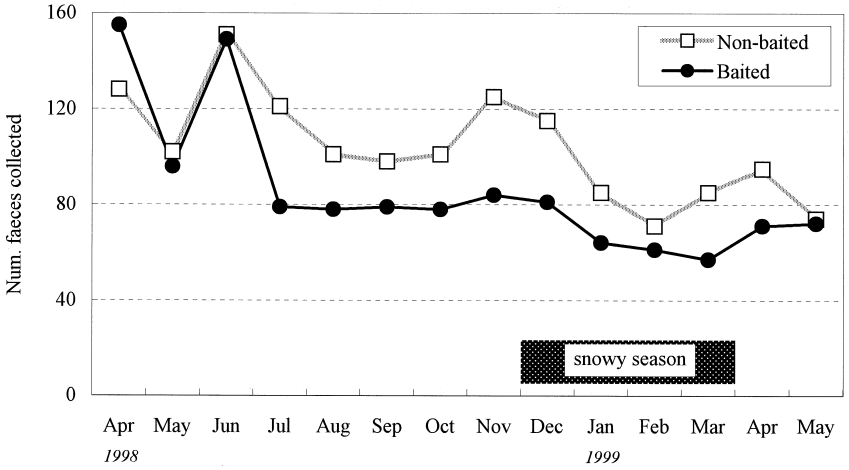

Fig. 2. Number of fox faeces collected in each month at Koshimizu, Hokkaido, Japan.

Table 1. Comparison of prevalence of taeniid egg-positive rate in the fox faeces collected before (April 98) and after (April 99) 1 year of monthly anthelmintic bait distribution between the baited and the non-baited sections at Koshimizu, Hokkaido, Japan

(Statistically significant levels (*: 5 %; **: 1 %) in the sequential Bonferroni test ($k = 2$).)

	Before bait distribution	After bait distribution	
Section	April 98	April 99	Fisher's exact probability test
Non-baited	18·8 %	24·2 %	$P = 0{\cdot}03$*
(95 %CI)	(12·4–26·6)	(16·0–34·1)	
n	128	95	
Baited	27·1 %	5·6 %	$P = 0{\cdot}0003$**
(95 %CI)	(20·3–34·8)	(1·6–13·8)	
n	155	71	
Fisher's exact probability test	$P = 0{\cdot}12$	$P = 0{\cdot}00005$**	

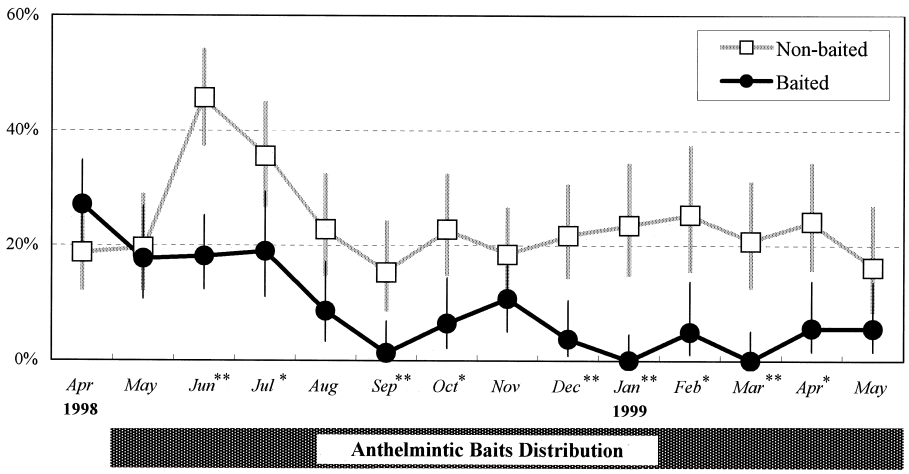

Fig. 3. Change in the taeniid egg-positive rate in the collected fox faeces in the anthelmintic bait baited and the non-baited sections at Koshimizu. Anthelmintic bait distribution was conducted during May 1998 and May 1999. Asterisks show the statistically significant difference in the rate of parasite egg-positive between the baited and the non-baited sections. Error bars are 95 % confidence intervals. Fisher's exact probability test with corrected significance levels in the sequential Bonferroni test ($k = 14$): *$P < 0{\cdot}05$; **$P < 0{\cdot}01$.

(Fig. 2). Fox faeces were usually collected along roads and fields, but 67 faeces (2·5 % of total) were collected in front of bait holes after foxes had consumed the baits.

E. multilocularis *infection among red foxes*

Faecal examination for parasite eggs. The percentage of taeniid egg positive faeces (egg-positive rate)

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Table 2. Comparison of the mean taeniid egg intensity index (±S.E.) in the fox faeces collected before (April 98) and after (April 99) 1 year of monthly anthelmintic bait distribution between the baited and the non-baited sections at Koshimizu, Hokkaido, Japan

(Statistically significant levels (*: 5%; **: 1%) in the sequential Bonferroni test ($k = 2$).)

	Before bait distribution	After bait distribution	
Section	April 98	April 99	Mann-Whitney's U test
Non-baited	0·30±0·06	0·40±0·08	$P = 0·33$
n	128	95	
Baited	0·39±0·05	0·14±0·07	$P = 0·0003$**
n	155	71	
Mann-Whitney's U test	$P = 0·14$	$P = 0·002$**	

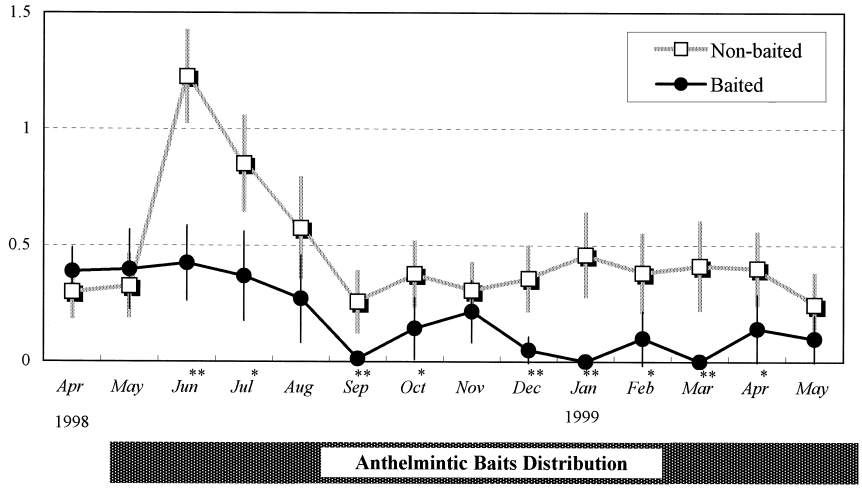

Fig. 4. Change in the mean taeniid egg intensity index in the fox faeces collected in the anthelmintic-baited and non-baited sections at Koshimizu. The number of taeniid eggs in 0·5 g of fox faeces was counted, then the egg intensity was classified into the following 5 grades; grade 4: > 1000 eggs, grade 3: 1000–101 eggs, grade 2: 100–11 eggs, grade 1: 10–1 eggs and grade 0: no eggs. The faecal egg intensities were calculated by averaging the scores (0–4) of the grade of all faeces collected in each section every month. Asterisks show the statistically significant difference in the taeniid egg intensity index between the baited and the non-baited sections. Error bars are 95% confidence intervals. Mann-Whitney U test with corrected significance levels in the sequential Bonferroni test ($k = 14$): *$P < 0·05$; **$P < 0·01$.

in fox faeces collected before anthelmintic bait distribution (April 1998) and after 1 year of the monthly bait distribution (April 1999) was calculated and compared between the baited and the non-baited sections (Table 1). The egg-positive rate in the baited section was comparable to that in the non-baited section before the bait distribution (April 1998; Table 1). After 1 year of bait distribution (April 1999) the egg-positive rate in the baited section decreased significantly although that in the non-baited section increased significantly (Table 1). The egg-positive rate in the baited section was significantly lower than that in the non-baited section (Table 1).

Figure 3 shows the monthly change in the egg-positive rates in the two sections. After 1 month of the bait distribution the effect of anthelmintic became obvious. The egg-positive rate in the baited section did not increase after May 1998 (17·7%) but gradually decreased (0·0–19·0%), contrasting to that in the non-baited section; it increased in June 1998 (45·7%) and decreased gradually until September 1998 (15·3%), but was stable after that. The egg-positive rates differed significantly between the baited and the non-baited sections since June 1998, except for August 1998, November 1998 and May 1999 (Fisher's exact probability test, $P < 0·05$ corrected with the sequential Bonferroni method, $k = 14$).

The faecal egg intensities in fox faeces collected before anthelmintic bait distribution (April 1998) and after 1 year of the monthly bait distribution (April 1999) were calculated and compared between the baited and the non-baited sections for assessing the degree of parasite egg contamination (Table 2). Before bait distribution (April 1998), the faecal egg intensities were almost same between the two sections (Table 2). After 1 year of monthly

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Table 3. Comparison of the coproantigen-positive rates in the fox faeces collected before (April 98) and after (April 99) 1 year of monthly anthelmintic bait distribution between the baited and the non-baited sections at Koshimizu, Hokkaido, Japan

(Statistically significant levels (*: 5 %; **: 1 %) in the sequential Bonferroni test ($k = 2$).)

	Before bait distribution	After bait distribution	
Section	April 98	April 99	Fisher's exact probability test
Non-baited	41·9 %	45·8 %	$P = 0·59$
(95 %CI)	(33·2–50·9)	(35·6–56·3)	
n	129	96	
Baited	59·6 %	29·7 %	$P = 0·00004$**
(95 %CI)	(51·5–67·4)	(19·7–41·5)	
n	156	74	
Fisher's exact probability test	$P = 0·003$**	$P = 0·04$*	

anthelmintic bait distribution (April 1999), however, the faecal egg intensities in the baited section significantly decreased contrasting to the increase of that in the non-baited section (Table 2). The faecal egg intensities between the two sections differed significantly after 1 year of bait distribution (April 1999).

Figure 4 shows the monthly change in the faecal egg intensities between the two sections. In the non-baited section, faecal egg intensities in April and May 1998 were stable, but increased to 1·23 in June 1998, then decreased around 0·24–0·46 during September 1998 and May 1999. On the other hand, faecal egg intensities in the baited section did not increase in June 1998 (0·42), and showed lower values than that of the non-baited section after bait distribution with lowest values of 0·01, 0 and 0 in September 1998 and January and March 1999, respectively. The seasonal change in the faecal egg intensities showed similar patterns to that in the egg-positive rates. The faecal egg intensities in the baited section were significantly lower than that in the non-baited section after 1 month of bait distribution except for August and November 1998, and May 1999 (Mann-Whitney *U* test, $P < 0·05$ corrected with the sequential Bonferroni method, $k = 14$).

Coproantigen detection. The percentage of coproantigen positive faeces (the coproantigen-positive rate) in total faeces collected before anthelmintic bait distribution (April 1998) and after 1 year of the monthly bait distribution (April 1999) were calculated and compared between the baited and the non-baited sections (Table 3). Before bait distribution (April 1998) the coproantigen-positive rate in the baited section was significantly higher than that in the non-baited section. After 1 year of monthly bait distribution (April 1999), however, the coproantigen-positive rate in the baited section decreased significantly and was significantly lower than that in the non-baited section (Table 3).

Figure 5 shows the monthly change in the coproantigen-positive rates between the two sections. Monthly change in the coproantigen-positive rates was more prominent than that in the egg-positive rates in both sections. Two peaks in the coproantigen-positive rates were observed at the non-baited section in June 1998 (74·2 %; 95 %CI: 66·4–80·9) and October 1998 (71·3 %; 95 %CI: 61·4–79·9). Although the coproantigen-positive rate in the baited section was significantly higher than that in the non-baited section before the bait distribution (Fisher's exact probability test, $P < 0·05$ corrected with the sequential Bonferroni method, $k = 14$), the coproantigen-positive rate in the baited section became significantly lower than that in the non-baited section after 1 month of the bait distribution except for July, August and November 1998, and March, April and May 1999 (Fig. 5). Comparing to the result in the egg-positive rates, the coproantigen-positive rates in the baited section conspicuously increased in November 1998 and May 1999.

Prevalence of E. multilocularis *in the intermediate hosts*

Sixty two (4·5 %) out of 1380 small mammals (436 grey red-backed voles, *Clethrionomys rufocanus*; 34 northern red-backed voles, *C. rutilus*; 413 small Japanese field mice, *Apodemus argenteus*; 367 large Japanese field mice, *A. speciosus*; 8 Korean field mice, *A. peninsulae*; 42 Norway rats, *Rattus norvegicus*; 4 house mice, *Mus musculus*; 76 *Sorex* species) that were captured and examined were infected with *E. multilocularis*. Only 3 species, grey red-backed vole, northern red-backed vole and small Japanese field mouse were infected although a total of 7 rodent species and some insectivore species were caught and examined. The prevalence in each species was 13·3 % (95 %CI: 10·3–16·9 %), 2·9 % (95 %CI: 0·1–15·3 %) and 0·7 % (95 %CI: 0·2–2·1 %) in *C. rufocanus*,

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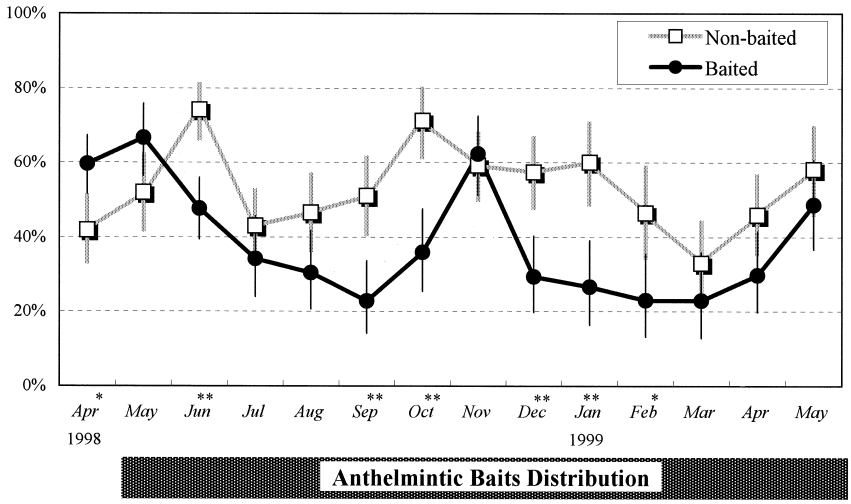

Fig. 5. Change in the coproantigen-positive rates in fox faeces collected in the anthelmintic-baited and the non-baited sections at Koshimizu. Asterisks show the statistically significant difference in the coproantigen-positive rates between the two sections. Error bars are 95 % confidence intervals. Fisher's exact probability test with the corrected significance levels in the sequential Boneferroni test ($k = 14$): $*P < 0{\cdot}05$; $**P < 0{\cdot}01$.

Table 4. Comparison of prevalence of *Echinococcus multilocularis* in grey red-backed voles (*Clethrionomys rufocanus*) between the anthelmintic-baited and the non-baited section in Koshimizu, Hokkaido, Japan

	1998				1999	
Section	May	July	Sept.	Nov.	May	June
Non-baited	33·3 %	22·4 %	25·9 %	10·0 %	7·7 %	0·0 %
(95 %CI)	(4·3–77·7)	(11·8–36·6)	(11·1–46·3)	(3·3–21·8)	(0·2–36·0)	(0·0–5·1)
n	6	49	27	50	13	57
Baited	22·2 %	10·4 %	53·3 %	16·2 %	0·0 %	4·3 %
(95 %CI)	(6·4–47·6)	(3·5–22·7)	(26·6–78·7)	(6·2–32·0)	(0·0–12·7)	(0·5–14·5)
n	18	48	15	37	22	47
Fisher's exact probability test	$P = 1{\cdot}0$	$P = 0{\cdot}17$	$P = 0{\cdot}10$	$P = 0{\cdot}52$	$P = 0{\cdot}37$	$P = 0{\cdot}20$

Table 5. Comparison of prevalence of *Echinococcus multilocularis* in grey red-backed voles with different birth dates (before or after bait distribution) between the baited and the non-baited sections in Koshimizu, Hokkaido, Japan

(Statistically significant levels (*: 5 %; **: 1 %) in the sequential Bonferroni test ($k = 2$).)

	Voles born	
Section	Before bait distribution	After bait distribution
Non-baited	8/18 (44·4 %)	12/89 (13·5 %)
Baited	17/44 (38·6 %)	1/60 (1·7 %)
Fisher's exact probability test	$P = 0{\cdot}78$	$P = 0{\cdot}02*$

C. rutilus and *A. argenteus*, respectively. The grey red-backed vole accounts for 93·5 % of all the infected animals.

The prevalence of *E. multilocularis* in *C. rufocanus* was compared between the baited and the non-baited sections (Table 4). Lower prevalence was observed in both sections during the second half of the study period and the prevalence did not differ significantly between two sections before and after the bait distribution. To exclude the effect of the timing of infection among these voles, the captured voles were divided into two age groups with their estimated birth dates, before and after the bait distribution, and their prevalence of *E. multilocularis* compared (Table 5). Among the voles born after bait distribution, the prevalence in the baited section (1·7 %) was significantly lower than that in the non-baited section (13·5 %) although the voles born

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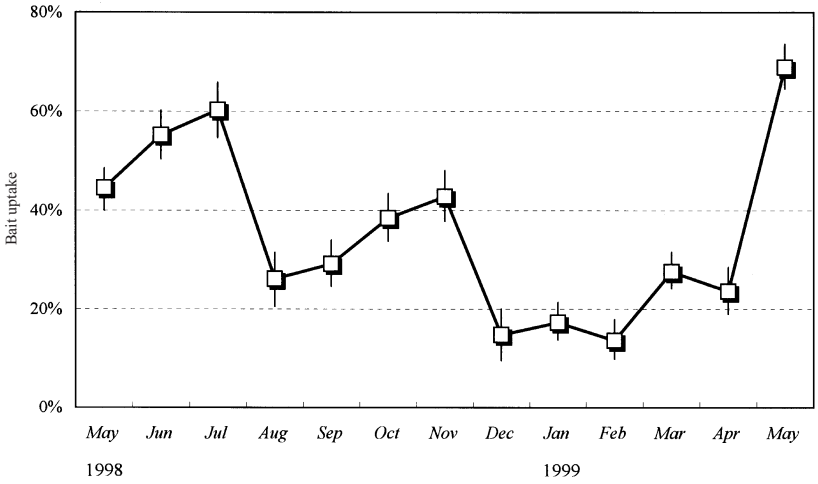

Fig. 6. Change in the rate of bait uptake by red foxes in the anthelmintic-baited section at Koshimizu. The bait uptake rates were calculated as the number of bait hole-nights, which had lost baits divided by the total number of prepared bait hole-nights. Error bars are 95 % confidence intervals.

before the bait distribution showed no difference in their prevalence of *E. multilocularis* between the baited (38·6 %) and non-baited (44·4 %) sections.

Bait uptake by foxes and other animals

Foxes consumed the anthelmintic baits in 1890 bait hole-nights out of 4937 effective bait hole-nights prepared. Although the baits were consumed or disturbed by many animals, such as free-roaming dogs and cats, crows, rodents, martens and insects, most of the baits were consumed by red foxes. Small pieces of anthelmintic tablets chewed and left by foxes were observed around 6·9 % of bait hole-nights consumed by foxes. The monthly rate of bait uptake by foxes showed seasonal fluctuation (Fig. 6). It fluctuated with 3 peaks in July 1998 (60·3 %), November 1998 (42·8 %) and May 1999 (68·9 %). During the snowy season, from December 1998 to March 1999, a relatively low bait uptake by foxes (around 20 %) was observed.

DISCUSSION

Control of *E. multilocularis* is considered extremely difficult because of its sylvatic cycle (Eckert & Deplazes, 1999). Anthelmintic bait (containing praziquantel) distribution against the wild definitive host is one of the most useful counter measures. In Germany, two different field studies (northern and southern Germany) are currently evaluated (Eckert & Deplazes, 1999; Eckert, Conraths & Tackmann, 2000). In the preliminary results of the southern Germany study, the reduction of *Echinococcus* prevalence in the red foxes at the central part of the study area was observed after the distribution of anthelmintic bait (Schelling *et al*. 1997). In our study, the prevalence of *E. multilocularis* in wild red foxes could be reduced by almost 1 year of monthly anthelmintic bait distribution. Especially, excretion of the parasite eggs in fox faeces collected in the baited section gradually decreased after the beginning of the bait distribution and retained a low level in the last half of this study compared with that in the non-baited section. Although egg-positive results mean the possibility of the infection of some *Taenia* species except for *E. multilocularis*, such cases were rare in parasitological and coproantigen surveys in Hokkaido (Kamiya & Ohbayashi, 1975; Morishima *et al*. 1999*a*; Nonaka *et al*. 1998; Sakai *et al*. 1998; Tsukada *et al*. 2000; Yorozuya *et al*. 1968). Therefore, these results mean that the egg contamination by *E. multilocularis* and the potential risk for human echinococcosis could be reduced by the anthelmintic bait distribution.

In contrast to the German study of anthelmintic bait distribution, the reduction of *Echinococcus* infection in the red foxes was more obvious in this study although our study area was too small (about one sixth of the German study) to exclude the perimeter effect of the bait distribution area as in the case of the German study. The river between the baited and non-baited sections cannot be an obstruction to foxes' movement because of $> 1{\cdot}5$ bridges/km on the river and the river freezing in winter. One possibility for the differences between the German study and ours is due to the difference of the examination of *Echinococcus* infection in the red foxes. Since red foxes usually live in exclusive territories (Tsukada, 2000), shooting of the resident foxes as in the case of the German study would cause the intrusion of neighbouring foxes (Tsukada, 1997). On the other hand, anthelmintic-treated resident foxes in this study could be a kind of natural barrier to the intrusion of non-treated foxes into the baited area since the coproantigen detection makes it possible to detect *Echinococcus* infection in the red foxes without killing them. On the other hand,

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another possibility for the difference between two studies is due to the difference in the sampling methods. In the German study, bait distribution as well as collection of foxes for the parasitological follow-up was performed at random. In the present study, however, data were collected in restricted areas around individual dens where baits were distributed. These differences might reflect the different aspects and scales of the observed phenomenon between two studies.

Foxes in the baited section were supposed to be re-infected with *E. multilocularis* after being dewormed by monthly bait distribution because the coproantigen-positive faeces in the baited section were not so dramatically reduced compared with those in the non-baited section, as was observed in the egg-positive faeces. Coproantigen ELISA can detect the early stage of *E. multilocularis* infection even after 4–6 days p.i. while parasite egg examination can not detect these early infections (Nonaka *et al*. 1996). Therefore, coproantigen-positive but egg-negative faeces were supposed to be excreted by the foxes with the pre-patent stage of infection in the baited section. The observed increase in the coproantigen-positive rates in June and October would be explained by high infection pressure in this section because it was reported that high fox predation on voles was facilitated by less grass or snow cover in the study area (Yoneda, 1983).

Especially in June 1998, both the egg-positive rate and mean taeniid egg intensity index were highest in the non-baited section. Morishima *et al*. (1999*a*) reported the same increase in the egg-positive rates and intensities of collected fox faeces in June and considered the reason to be due to the high worm burden in the juvenile foxes compared with the adult. In this study the juvenile fox faeces ($n = 54$) collected in June 1998 showed a significantly higher egg-positive rate (juvenile: 64·8 % *vs* adult: 38·5 %; Fisher's exact probability test, $P = 0{\cdot}02$) and mean taeniid egg intensities excluding grade 0 (juvenile: 2·0 *vs* adult: 1·4; Mann-Whitney's U test, $P = 0{\cdot}049$) than that in the adult faeces ($n = 39$). Most of the juvenile fox faeces were located only around the fox breeding dens because the juvenile fox activities were restricted within this area during this month. This means that the breeding dens of infected foxes are extremely contaminated with *Echinococcus* eggs and are important locations where *Echinococcus* transmissions from the red foxes to the voles occur. Relatively high *Echinococcus* infection foci in voles around fox breeding dens were also reported in other areas of Hokkaido (Kamiya *et al*. 1977; Takahashi *et al*. 1989).

This study showed that bait distribution around fox dens significantly reduced the prevalence in voles born subsequently. However, even after 1 year of anthelmintic treatment, the number of infected voles could not be reduced to the level at which transmission of *E. multilocularis* could not be maintained. Actually, the prevalence of *E. multilocularis* in voles (including voles born before and after the bait distribution) captured in November 1998 and June 1999 did not differ between the baited and non-baited sections. This indicated that we must continue our bait distribution for more than 13 months to decrease the infection pressure of *E. multilocularis* in wild red foxes by the accumulated effects of the treatment. Since most voles cannot survive over 1 year (Yoneda, 1982), reduced infection pressure in the foxes could be observed in the following year. The mathematical model of the effect of chemotherapy treatment for *E. multilocularis* infection among foxes in France showed that the rate of the prevalence of *E. multilocularis* under control, to the prevalence without control decreases linearly in proportion to the ratio of the control effort to the required effort to achieve eradication although some simple assumptions were included (Roberts & Aubert, 1995). To obtain a clear effect of our treatment, including the accumulated effects, more control effort would be required.

Anthelmintic bait acceptance by foxes in this study fluctuated seasonally. The decrease of bait acceptance by foxes reduces the effectiveness of anthelmintic upon the infective foxes and increases the coproantigen-positive rate and the egg-positive rates in fox faecal samples. In this study, the baits were intensively distributed around fox den sites throughout a year, which could effectively reduce the parasite egg contamination at the location where *Echinococcus* transmission from the red foxes to voles possibly happened. The utilization of dens by the red foxes increases during pup-rearing, April–June and dispersal seasons, October–November (Nakazono & Ono, 1987; Uraguchi & Takahashi, 1998) that was comparable to the increase of bait acceptance by foxes. These fox activity patterns in relation to their pup-rearing and den maintenance might cause the observed change in bait acceptance by foxes in the study area. Low bait acceptance observed in winter could be explained by the limited diffusion of smell of fish sausage at low temperature. In the study area, the average temperature is below −3 °C during December and March. Some fox tracks on the snow apparently showed that foxes passed through the bait holes without stopping by.

Some of the anthelmintic tablets were not totally consumed by foxes, but were left behind as small pieces. PZQ tastes very bitter so that foxes might refuse to consume the tablet. In the case of domestic dogs, the feel of the praziquantel tablet on their tongues is believed to be associated with their rejection of the tablet fed with yeast (Gemmell, Johnstone & Oudemans, 1982). For an effective eradication programme, the bait distribution strategy should be improved depending on the seasonal change in the intensity of re-infection, the fox

movement pattern and climate conditions, and by improvement of the taste of baits which are more palatable for foxes (e.g. the industrialized bait used in Germany).

The faecal coproantigen detection method made it possible continuously to monitor the effects of anthelmintic on the infected foxes under field conditions (Nonaka *et al*. 1998; Morishima *et al*. 1999*a*; Sakai *et al*. 1998; Tsukada *et al*. 2000). This method has advantages over the conventional necropsy survey in the following points, such as that the fox societies investigated do not need to be disturbed, and that a large number of faecal samples can be obtained and checked. On the other hand, this method should be carefully conducted under certain conditions, because identification of fox faeces is difficult where small feral or free-roaming dogs also live because their faeces are similar in size and shape to those of foxes, and because collection of fox faeces is difficult due to the difficulty in locating fox den sites where juvenile fox faeces are clumped and because of their rapid decomposition through the activity of dung beetles or heavy rainfall.

This study required intensive and laborious field work, such as locating fox breeding dens, preparing hundreds of manufactured baits, distributing these baits around fox dens monthly, and collecting many fox faeces. From an economic standpoint we must optimize our anthelmintic programme in a more cost-effective manner. Therefore, we are now planning to improve our anthelmintic programme in a much simpler manner, and assess its effectiveness.

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