

Activities specifically related to the mandate of OIE Reference Laboratories

4. International harmonisation and standardisation of methods for diagnostic testing or the production and testing of vaccines

None.

5. Preparation and supply of international reference standards for diagnostic tests or vaccines

None.

6. Collection, analysis and dissemination of epizootiological data relevant to international disease control

- 6.1 Deworming program in wild red foxes in surrounds of Otaru city (Hokkaido, Japan) – the originally produced baits containing 50 mg praziquantel were distributed from May to November 2004. The prevalences of *E. multilocularis* in definitive and intermediate hosts were monitored by the coproantigen ELISA detection method, taeniid egg counting and post-mortem examination of red foxes.
- 6.2 Diagnostic service for the domestic dogs and cats has been provided with the cooperation of the Forum on Environmental Animals (Center for Advanced Science and Technology, Hokkaido University), the Hokkaido Small Animal Veterinary Association.
- 6.3 Research on the environmental contamination by *E. multilocularis* eggs – fox home range, movement of individual foxes was monitored.
- 6.4 Experimental infection of domestic cats with protoscolices of *E. multilocularis*.
- 6.5 Survey on the prevalence of *E. multilocularis* in foxes in Koshimizu town, by faecal egg examination and coproantigen detection. Epidemiologic survey was done after period of control measures against alveolar echinococcosis in that area to check the rate of infection in red fox population.

7. Provision of consultant expertise to OIE or to OIE Member Countries

Professor M. Kamiya and Dr S. Ganzorig visited Kazakhstan and Kyrgystan to attend at the International Symposium on echinococcosis in Central Asia and to provide consultant expertise.

8. Provision of scientific and technical training to personnel from other OIE Member Countries

In 2004, training on coproantigen and copro-DNA diagnostic methods was provided for veterinary personnel from China.

9. Organisation of international scientific meetings on behalf of OIE or other international bodies

In October 2004, the joint Japan-Taiwan symposium entitled “Infectious diseases of Animals and Quarantine” was organised in Sapporo (Japan) by Prof. M. Kamiya. In the symposium, the problems on eradication, diagnosis and risks factors for alveolar echinococcosis in Japan have been discussed.

10. Participation in international scientific collaborative studies

The laboratory participate in international collaborative projects on the control of echinococcosis/hydatidosis with Dr. J. J. Chai and Dr. J. Wei, National Hydatid Research Center in Urumqi (China), Prof. H. K. Ooi, National Chung Hsing University in Taichung (ROC), Dr. C. Carmona, the Parasite Biology Unit, Institute of Hygiene (Uruguay), Prof. B. Shaikenov, Institute of Zoology, Academy of Sciences (Kazakhstan), Prof. P. Giraudoux and

Prof. D. A. Vuitton, Institute of Environmental Science and Technology, WHO Collaborating Center for Prevention and Treatment of Human Echinococcosis, University of Franche-Comte (France) and Prof. B. Gottstein, Institute of Parasitology, University of Bern (Switzerland). The projects have been supported by the Japanese Ministry of Education, Science and Culture, and by the Ministry of Health, Labour and Welfare associated with HSF.

11. Publication and dissemination of information relevant to the work of OIE (including list of scientific publications, internet publishing activities, presentations at international conferences)

■ *Presentations at international conferences and meetings*

Kamiya M., Nonaka N., Ganzorig S. & Oku Y. (2004). - Effective countermeasures against alveolar echinococcosis in red fox population of Hokkaido, Japan. International Symposium "Echinococcosis in Central Asia: Problems and solutions", Cholpan-Ata, Kyrgystan.

Oku Y. (2004). - Echinococcosis: An attack on the source of transmission. Japan-Taiwan Symposium "Infectious diseases of Animals and Quarantine", Sapporo, Japan.

■ *Scientific publications*

Kamiya M., Nonaka N., Ganzorig S. & Oku Y. (2004). - Effective countermeasures against alveolar echinococcosis in red fox population of Hokkaido, Japan. In: "Echinococcosis in Central Asia: Problems and solutions", Almaty-Zurich, Daur, 273-288.

Kamiya M. (2004). - Echinococcus - invasion from nature. *Pharma Medica*, **22**: 17-20. (In Japanese)

Kamiya M. (2004). - Prevalence of alveolar echinococcosis in Japan - Expectation for urgent remedy against its source of infection. *Koushusei (Public Health)*, **68** (11): 874-877. (In Japanese)

Kamiya M. (2004). - Hydatid and alveolar echinococcosis. In: *Parasitic zoonosis*, VII: 401-404. (In Japanese)

Kamiya M. (2004). - Zoonosis with numerous host animals: Echinococcosis. *Kusuri-no-chishiki*, **55** (3): 13-16. (In Japanese)

Kamiya M. (2004). - Travelling parasite: Present situation and tendency of echinococcosis. *Labio*, **21**, 15: 23-27. (In Japanese)

Kamiya M. (2004). - Echinococcosis. *Chikusan-no-kenkyu*, **58** (1): 161-166. (In Japanese)

Kamiya M., Ooi H.K. & Y. Oku (2004). - A new era after the Japan-Taiwan Symposium on "Infectious Diseases of Animals and Quarantine". Emerging Infectious Diseases, accepted.

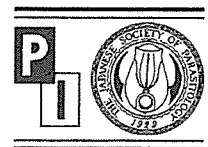
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Oku Y., Malgor R., Benavidez U., Carmona C. & Kamiya H. (2004). - Control program against hydatidosis and the decreased prevalence in Uruguay. *International Congress Series*, 1267: 98-104.

Oku Y., Nonaka N., Yagi K., & Kamiya M. (2004). - Canine echinococcosis. *Japanese Journal of Veterinary Parasitology*, **3** (1): 17-19. (In Japanese)

Shimada M., Akao N., Ishiwata K., Oku Y., Okuzawa E., Takeuchi Ts., Nawa Y., Nishiyama T., Hara M., Hamada A. & Horio, M. (2004). - Parasite information systems that useful for clinicians. *Japanese Journal of Therapy*, **86** (10): 09-034. (In Japanese)

Yokohata Y. & Kamiya M. (2004). - Analyses of regional environmental factors on the prevalence of *Echinococcus multilocularis* in foxes in Hokkaido, Japan. *Japanese Journal of Zoo Wildlife Medicine*, **9** (2): 91-96.



Towards the control of *Echinococcus multilocularis* in the definitive host in Japan

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Abstract

Echinococcus multilocularis is distributed all over Hokkaido, the northern island of Japan. The prevalence in foxes has been around 40% in the last decade. Three trials of anthelmintic bait distribution have been conducted in Hokkaido to reduce the prevalence in foxes. In those trials, bait distribution was done along roads in the study area using cars and/or around fox breeding dens by hand. Changes in the prevalence in foxes were evaluated either by necropsy of captured foxes or by coproantigen and egg detection of faeces collected in field. All of the trials showed bait distribution was effective for the reduction of the prevalence in foxes; however, it was also suggested that a frequent and continuous baiting program is necessary for effective and stable control of the prevalence in foxes. As observed in some cities in Europe, urban foxes infected with the parasite were also recognized in Sapporo. A survey of pet dogs showed that 0.4% of surveyed dogs were determined infected. In addition, a dog which was transported from Hokkaido to the main island of Japan was found excreting *E. multilocularis* eggs. The results raised the public recognition of canine infections, which in turn lead to the modification of a Japanese law for infectious diseases and to the enforcement of a national reporting system of dogs infected with *E. multilocularis* by veterinarians.

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Keywords: *Echinococcus multilocularis*; Foxes; Dogs; Control; Bait distribution; Japan

1. Introduction

High prevalence in foxes with *Echinococcus multilocularis* infection is now recognized in Hokkaido (78,500 km², 5,660,000 inhabitants), Japan. To monitor the change of prevalence, the Hokkaido government has performed necropsy surveys of foxes captured in winter at various sites, showing an overall prevalence of 19.1% in 23,852 foxes surveyed during 1966–2003. However, since the mid-1980s, the prevalence in foxes has tended to increase and has been around 40% in the last decade. Our recent necropsy surveys conducted at the suburbs of the city of Sapporo showed similar high prevalences in foxes [1,2]. In those surveys, 6 raccoon dogs (*Procyonoides necturetes*) 1 live raccoon dog was found excreting taeniid eggs should be intact taeniid eggs were found in the rectal faeces in one of the raccoon dogs.

Infections in domestic dogs were also found in Hokkaido. According to the necropsy survey conducted by Hokkaido

government, 99 dogs (1.0%) were found infected out of 9,907 dogs during the period 1966–2003. However, most of the dogs were examined before 1990 and only <15 dogs on average per year were examined over the last decade. Therefore, the data did not provide up-to-date infection status for dogs in Hokkaido.

Accordingly, effective counter-measures against high prevalence in foxes and precise evaluation of infection status of pet dogs are necessary in Hokkaido. In this paper, recent trials and their achievements toward the control of the infection in foxes and pet dogs in Hokkaido are reviewed.

2. Baiting against *E. multilocularis* in foxes

The first deworming trial against *E. multilocularis* infection in foxes was conducted in Germany in a study area of 566 km² [3]. Baits containing 50 mg of praziquantel were repeatedly distributed in the study area. The deworming effect was most pronounced in the central part of the baiting area; however, the reduction of the prevalence was moderate in marginal areas. In that campaign, high hunting pressure for foxes (2.2 foxes/km²)

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for the prevalence evaluation created free niches in the baiting area to which foxes residing outside the baiting area migrated. The observed higher prevalence in the marginal part was, therefore, due to the effect of those migrating foxes. This deworming trial showed that a bait distribution is effective for reducing the prevalence in foxes; however, it also suggested that the scale of operation must be large enough to have the core area covered, in order to evaluate a true effect of bait distribution if the prevalence in foxes is evaluated by necropsy.

Use of coproantigen detection techniques in field studies enables surveys with minimal disturbance in the local ecology because the prevalence in foxes could be estimated from faeces [4–6]. In our laboratory, a monoclonal antibody (EmA9) based sandwich ELISA was developed for coproantigen detection [7]. Using this method, a deworming trial was conducted in Koshimizu [8]. After a preliminary survey of the prevalence in foxes from 1997 to 1998 [9], the study area (200 km²) was divided into two parts (Fig. 1), one (occupied by 18 fox families) with bait distribution and the other (20 fox families) without bait distribution. Baits containing 25 to 50 mg of praziquantel were repeatedly distributed around each fox breeding den. The changes of the prevalence in foxes were evaluated by the coproantigen and fecal egg examination of field collected fox faeces. It was observed that egg containing faeces was rapidly reduced in the bait distributed area. In contrast, coproantigen positive faeces was not dramatically reduced in the first year, indicating that foxes were readily re-infected by ingesting the intermediate hosts, which had been infected before the bait distribution. However, obvious reduction in the number of coproantigen positive faeces was recognized from the second year probably due to the decrease of infected rodents. The results suggested that longer-term

strategic bait distribution would be required for the efficient control of *Echinococcus* infection in foxes.

Based on these results, a new deworming program was conducted in 2001 to 2002 for covering the whole study area in Koshimizu. In the program, baits were distributed along roads in the town (20 baits/km) or at the cross sections of roads and wind-shielding forests (20 baits/km²). This 2-year bait distribution resulted in the successful reduction of environmental egg contamination. However, the proportion of egg containing faeces has been gradually increasing after completion of the campaign.

In the evaluation of prevalence in foxes using faeces collected in field, identification of fox faeces were critically important. Most of the studies have been conducted with ambiguous identification of the origins of faeces, which was based on the size, shape, color and odor of the faeces. Accordingly, none of the criteria alone or combinations could perfectly distinguish fox faeces from other carnivore faeces. Therefore, the survey results were always accompanied with a certain level of bias. Recent advancement of molecular techniques have enabled the identification of the origin of faeces from fecal DNA, which is derived from sloughed intestinal mucosal cells excreted with faeces [10]. In our laboratory, a multiplex PCR system for the identification of carnivore animals should be for the identification of foxes and other carnivore animals in Hokkaido (raccoons, raccoon dogs, weasels, dogs and cats), which excrete faeces resembling fox faeces has now been developed and applied to field study. With this kind of technique, it will be feasible to evaluate more precisely the prevalence in individual carnivore species.

Another approach for evaluation of baiting has been conducted since 2001 in Otaru. In this trial, tetracycline (TC)

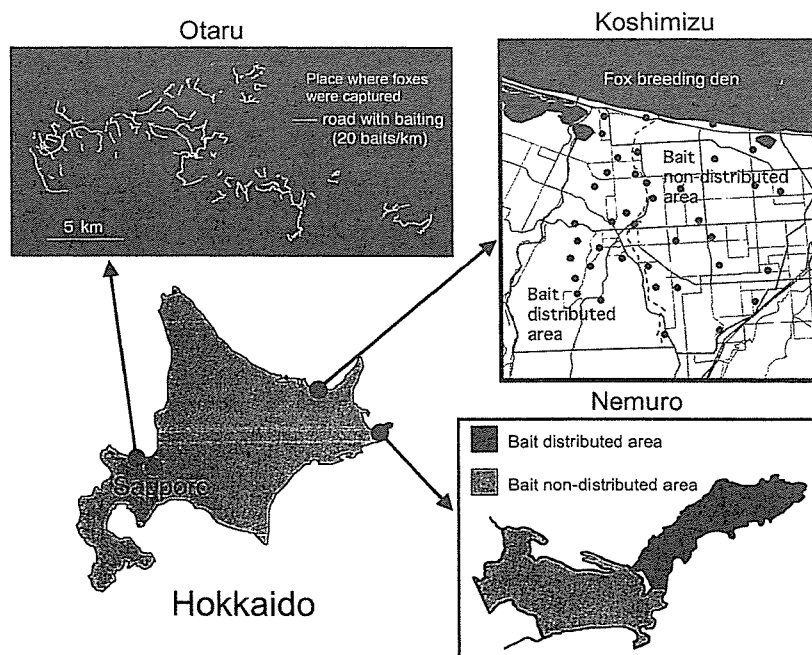


Fig. 1. Places where anthelmintic bait distribution trials were conducted in Hokkaido.

was put into the anthelmintic baits as a biomarker so that consumption of the baits by foxes could be detected by the examination of the TC line in the canine teeth. The baits were distributed along roads in the study area (120 km², 20 baits/km) (Fig. 1). The prevalence in foxes was evaluated by the necropsy of the animals captured by local hunters. Majority of the foxes having a TC line on teeth did not harbor the parasites, indicating foxes consuming the baits were effectively dewormed. Detailed report of this study is now being prepared.

In Nemuro, another baiting trial has also been conducted by Hokkaido Institute of Public Health since 1999 to 2004 [11]. The study area has a geographical advantage for the trial because the bait distributed area (135 km²) is a protruding peninsula with a lake almost crossing its base part so that animal movement to and from the bait distributed area is restricted to minimum (Fig. 1). In this trial, single to multiple bait distributions along roads (15 baits/km²) and around fox breeding dens were conducted annually and the prevalence of foxes were evaluated by necropsy. According to the preliminary data up to March 2002, a tendency of the reduction in the prevalence in foxes was observed in the bait distributed area.

The bait distribution trials in Hokkaido seemed to be effective for reduction of *E. multilocularis* prevalence in foxes. In Koshimizu, bait distribution has started again in 2004 and a new campaign is also planned in Kucchan, both of which are conducted by local residents with support by our laboratory and Forum of Environmental Animals. Although the scales of the campaigns are small (local town base), participation of local residents in the control measure against *E. multilocularis* infection in foxes is a new movement in the control strategy. There are many hurdles to clear, however, we hope this movement will be a driving force to a large scale control program in near future.

3. A possible urban cycle in Sapporo

There are increasing observations that foxes inhabit urban areas [12]. Infected foxes with *E. multilocularis* have been reported in several European cities and a risk of urban residents to get the infection has been increasing. An active urban cycle of the parasite was recognized in Zurich where counter-measures against the infected foxes were conducted [6].

In Sapporo, coproantigen positive fox faeces were found in the parks or woodlands of the urban area where foxes had their dens [13]. Infected foxes with *E. multilocularis* were found by necropsy in four out of six fox families inhabiting in northeastern region of Sapporo (unpublished data). In those studies, arvicolid rodents were captured at the urban fringe, although none were infected with *E. multilocularis*, arvicolid rodents were captured at the urban fringe. Those studies suggested that the urban fringe offers a potential condition for the maintenance of *E. multilocularis* life cycle.

For preparing a future bait distribution in Sapporo, bait uptake by foxes in Sapporo were evaluated by a photo-trap system [14]. Foxes were photo-trapped at seven out of eight baiting sites where foxes were previously observed by local residents or where the inhabitation of foxes could be suspected

Table 1

Criteria for diagnosis in the national reporting system for dogs infected with *E. multilocularis*

- | |
|--|
| (1) Finding the parasite body, which can be morphologically identified |
| (2) Detecting the parasite DNA from eggs or a part of the parasite body |
| (3) Detecting the parasite coproantigen, which should turn to be negative due to deworming treatment |

from the local environment. The result suggested that, by collecting local information (witness) of foxes, bait distribution to foxes could be efficiently conducted in the urban area of Sapporo.

4. Infections in pet animals and enforcement of national reporting system of infected dogs

In central Europe, domestic dogs were infected with *E. multilocularis* with prevalence between 0.3% and 7% in endemic regions [12]. It was estimated that more than 10% of dogs would be infected at least once in their life even in the low prevalent regions [15]. In some endemic areas such as in Gansu, China and in St. Lawrence Island, USA, dogs play important roles both in the maintenance and in the transmission of echinococcosis to humans [12].

In Hokkaido, little studies have been done on the recent prevalence of pet dogs (number of registered dogs in 2003 was 248,149). In our laboratory, a survey of pet dogs has been conducted since 1997 using a diagnostic system composed of coproantigen, faecal egg and faecal egg DNA examinations. The detailed survey report is now prepared for publication, but briefly, up to June in 2004, 15 (0.4%) dogs were determined infected, among which 8 dogs were confirmed excreting *E. multilocularis* eggs by PCR. In addition, a dog which was transported from Hokkaido to the main island of Japan (Honshu) was found excreting *E. multilocularis* eggs.

The survey result raised the public recognition of canine infections, which in turn lead to the modification of a Japanese law for infectious diseases and a national reporting system of dogs infected with *E. multilocularis* by veterinarians has been enforced from October in 2004 to monitor the occurrence of the canine infections by the country base (Table 1).

Finding of an infected dog which moved from Hokkaido to the main island of Japan raised attention for the risk of animal movement in disease introduction to non-endemic areas. The issue was emphasized in Europe [12] and some countries such as UK and Norway have actually enforced some regulations for transporting animals to prevent introduction or spreading of the disease. In near future, if the prevalence in foxes in Hokkaido will continue to be high, establishment of similar regulation may be necessary in Japan.

5. Conclusion

As represented by the appearance of urban foxes, human–fox contacts have been frequently observed in the human living environment. Accordingly, infection pressures to pet animals are increasing. Considering the high prevalence in foxes and

potential risk for pet animal infections with *E. multilocularis*, management of the disease in wildlife and pet animals is now ultimately required. At present, a large-scale efficient control measure for wildlife has not been completely established. Nevertheless, risk control by individuals with baiting foxes coming in contact to each individual or with proper management of their pet animals could be immediately started. Such an effort for the disease control with individual or small scale applications would reduce the local risk of echinococcosis and moreover lead to a regional and national program for the risk control of alveolar echinococcosis.

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Parasitic helminths from feral raccoons (*Procyon lotor*) in Japan

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Summary

An epidemiological survey of 1688 free-ranging raccoons (*Procyon lotor*) captured on the Japanese main islands of Hokkaido, Honshu and Kyushu was undertaken to determine whether *Baylisascaris procyonis*, which provokes fatal neurological larva migrans was present; however, the worm was not detected in any of these individuals. A helminthological investigation was carried out on 229 of the captured raccoons and the following worms obtained: *Toxocara tanuki*, *Porrocaecum* sp., *Molineus legerae*, *Ancylostoma kushimaense*, *Aonchotheca putorii*, *Centrorhynchus* sp., *Centrorhynchus bazaleticus*, *C. elongatum*, *Plagiorhynchidae* gen sp., *Hemiechinostoma* sp., *Metagonimus takahashii*, *M. miyatai*, *Euparyphium* sp., *Plagiorchis muris*, *Brachylaima* sp., and *Taenia hydatigena*. These were the first records of *Porrocaecum* sp., *M. miyatai*, *Brachylaima* sp. and *T. hydatigena* obtained from Japanese feral raccoons. Scanning electron microscopic and/or molecular analyses were performed for both *T. tanuki* and *T. hydatigena* as these helminths both have a zoonotic counterpart amongst their families.

Key words: parasitic helminths; *Procyon lotor*; Japan

Introduction

Feral raccoons (*Procyon lotor*) are an invasive alien species in Japan and present a risk for parasitic helminth disease outbreaks. One of the most severe pathogenic agents provoked by the raccoons is the fatal neurological larva migrans caused by *Baylisascaris procyonis* (Nematoda: Ascarididae) (Kazacos, 2001). As raccoons have spread throughout Japan, a large scaled epidemiological investigation of natural helminth infection is extremely important. Initially, a screening survey that involved a naked-eye examination to determine the presence/or absence of this worm was undertaken in 1688 captured individuals. Second, 229 of these raccoons underwent a detailed helminth

examination to detect minute zoonotic helminths such as genera *Strongyloides* (Nematoda: Strongyloidae) and *Echinococcus* (Cestoda: Taeniidae).

Materials and Methods

Naked-eye examination for detection of Baylisascaris procyonis

The Hokkaido Government authorized the capture of 1688 raccoons for research purposes in the Ishikari, Sorachi and Hidaka Districts (from 43°20' N to 42°30' N and from 141°15' E to 142°10' E) between April 1999 and September 2005 (Fig.1). The raccoons were transported to the Wild Animal Medical Center (WAMC) at Rakuno Gakuen University (RGU) where they were euthanized. Their bodies were measured and maturity determined by the morphological characteristics of their skulls and canines (Grau *et al.*, 1970). Both specimens from the raccoons collected in Hokkaido were registered and preserved as voucher specimens in the WAMC (RGU), Japan (Matoba *et al.*, 2006).

Entire intestinal tracts were removed from all 1688 raccoons and either stored at -20°C or fixed in 70 % ethanol prior to helminthological examination. Among the tracts, small intestines were examined by naked-eye for the presence/or absence of *B. procyonis*.

Precise helminth examination of gastro-intestines

The intestinal contents of 171 raccoons captured in Noppo Forest Park (NFP), Ishikari District, and immediately adjacent areas of the park (43°03' N, 141°21' E), Hokkaido Island, 27 captured in Karuizawa (36°25' N, 138°38' E), Nagano Pref., Honshu Island, 13 captured in Kobe (34°35' N, 135°13' E), Hyogo Pref., Honshu Island, and 18 captured in Sasebo (33°01' N, 129°44' E), Nagasaki Pref., Kyushu Island (Fig. 1) were examined with a dissecting microscope to detect minute helminths such as genera *Strongy-*

loides and *Echinococcus* that are less than ca. 3 mm in size. The raccoons collected from NFP were chosen as representing those from the north of Japan for this analysis as the park is located in Sapporo -the population of Sapporo is about 1,850,000 and is the capital city of Hokkaido- and is an area of natural woods that is a favored ecotourism area on Hokkaido.

Collection of intestinal samples was performed at the WAMC. Once collected, the helminths were placed in lacto-phenol solution and examined morphologically. A camera lucida (OLYMPUS Model BH2-DA) was used to measure the helminths and then voucher specimens were stored at the WAMC (RGU), Japan (Matoba *et al.*, 2006). To achieve positive identification of male ascarid specimens, especially *Toxocara* or *Baylisascaris*, the key morphological characteristics of the cloacal region were observed. A scanning electron microscope (SEM) was also used for positive identification of this genera. Some ascarids were prepared using standard procedures (see Wiger *et al.*, 1978) and observations were made using a JEOL JSM-SI electron microscope. Important morphological characteristics of the perianal region of these ascarids were documented photographically.

Molecular examination

Positively identified toxocarids and taeniids were used for molecular biological examination. Total DNA was isolated using an Easy-DNATM isolation kit (Invitrogen) with some modification of the manufacturer's instructions. The partial mitochondrial 12S rRNA gene was amplified by PCR from the genomic DNA using the oligonucleotide primers P60F: 5'-TTAAGATATATGTGGTACAGGATTAGATACCC-3' and P375R: 5'-AACCGAGGGTGACGGGCGGTGTGTACC-3' as reported by Dinkel *et al.* (1998). This primer set was designed for the detection of DNA of *Echinococcus multilocularis* and related tapeworms.

For the detection of DNA from genera *Toxocara*, *Toxoscaris* and *Baylisascaris*, the primers LC1F:5'-CGAGTATCGATGAAGAACGCAGC-3' and HC2R: 5'-ATATGCTTAAGTTCAGCGGG-3' were used (Yagi *et al.*, 1999). The PCR reaction (50 µl) was performed for 45 cycles, each cycle consisted of denaturation for 1 min at 92°C, annealing for 1 min at 52°C and elongation for 1 min at 72°C. 1.25 units of AmpliTaq GoldTM (Perkin Elmer Co) were used for Taq Polymerase in each reaction. The PCR products were electrophoresed in 1.5 % agarose gel. The DNA fragment was then extracted from the agarose gel, purified using glass beads and sequenced for both DNA strands using a dye-termination kit and model 377, DNA sequencer (Perkin Elmer Co). The nucleotide sequence obtained was aligned with the sequences of the five or six known species of ascaroid nematode reported by Jacobs *et al.* (1997), Zhu *et al.* (1998) and Yagi *et al.* (1999).

Results and Discussion

1. Naked-eye detection of *Baylisascaris procyonis*

The raccoon roundworm (*B. procyonis*) was not isolated

from the gastrointestinal contents of the 1688 individuals collected in the present survey. This worm was also not found in studies done by Miyashita (1992) and Sato and Suzuki (2006) who investigated 25 individuals captured in Osaka Pref. and 531 free-ranging raccoons captured in Wakayama Pref., Honshu Island, respectively. Thus, no raccoon roundworms have been found in the 2244 free-ranging raccoons examined to date. However, the prevalence of roundworms in captive raccoons in Japanese zoos is very high, and outbreak of the fatal neurological larva migrans caused by roundworms in captive mammals has occurred in zoos (Miyashita, 1993; Furuoka *et al.*, 2003; Sato *et al.*, 2002, 2003, 2005b). It is not uncommon for taxa parasites to occur in free ranging raccoons in Japan: *T. tanuki* and *Porrocaecum* sp. were obtained in the present survey and *Contraecum rudolphii* (syn. *C. spiculigerum*) was obtained from 5 individuals in a study performed by Sato and Suzuki (2006).

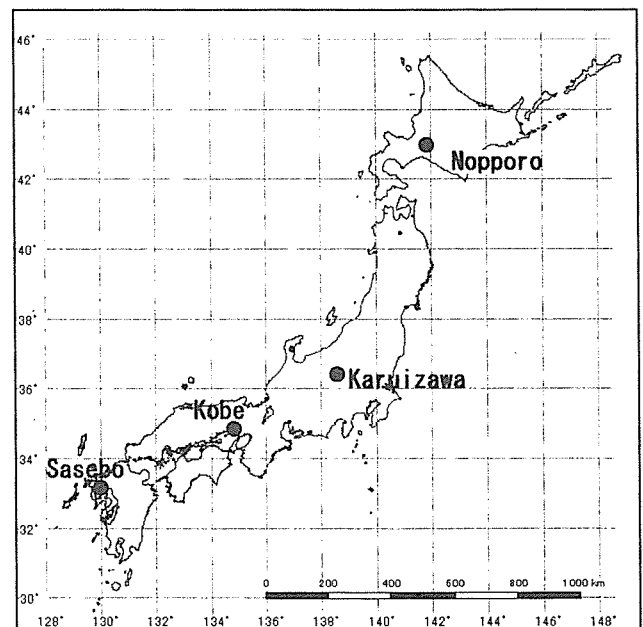


Fig.1. Geographic distribution of feral raccoons examined in the present study

2. Positive identification of toxocarid and taeniid specimens

Although *B. procyonis* was not found in the present survey, two ascarid nematodes and an immature taeniid were detected in the small intestine of free-ranging raccoons captured in NFP. As the Ascariidae and Taeniidae families are included in the classification of severe zoonotic helminths, their presence was considered as a positive identification.

Ascarid specimens

One of the ascarids obtained was a mature male: body 33.7 mm in length, 0.8 mm in width; anterior extremity of the body lacked interlabia; oesophagus 3.2 mm in length with



Fig.2. SEM micrograph of *Toxocara tanuki* from a feral raccoon collected in Hokkaido, Japan

cimen with five or six species of ascarid (Figs 3 – 4), this ascarid was completely identical in sequence to *T. tanuki* obtained from a raccoon dog (*Nyctereutes procyonoides*) captured in the Miyazaki Pref. and also a raccoon captured in Sapporo (Yagi *et al.*, 1999).

The other ascarid specimens were in larval form, the presence of intestinal cecum meant that they were identical to the genus *Porrocaecum*. However, DNA extraction from these larvae was unsuccessful.

Ascarid nematodes from free-ranging raccoons in Japan have been reported; for example, Sato and Suzuki (2006) reported *Contracaecum rudolphi* (syn. *C. spiculigerum*) from 5 individuals. However, a method for the positive identification of ascarids should be established to support future epidemiological surveys.

Taeniid specimen

This specimen consisted of a scolex and several immature segments but was without genital organs: strobila approximately 4.2 mm in length and 1.2 mm in width; scolex with four suckers, 0.26 mm in diameter and rostellum 0.038 mm in diameter (Fig. 5), two rows of large and small hooks on the rostellum (Fig. 5), the large hooks 15 in number, 0.2 – 0.22 mm in length, and the small ones 15 in number, 0.14

ascaroid nematode from raccoon	GCAGACACAT	TGAGCACTAA	AATTTCGAAC	GCACATTGCG	CCATCGGGTT	50
<i>Toxocara canis</i>	*****	*****	*****	*****	*****C***	50
<i>T. cati</i>	*****	*****	C*****	*****	*****C***	50
<i>Toxascaris leonine</i>	*****	*****	*****	*****	*****C***	50
<i>Baylisascaris procyonis</i>	*****	*****	**A*****	*****	*****C***	50
<i>B. transfuga</i>	*****	*****	**A*****	*****	T*****	50
<i>Toxocara tanuki</i>	*****	*****	*****	*****	*****	50
ascaroid nematode from raccoon	CATTCCCGTT	GGCACGTCTG	GCTGAGGGTC	AGA		83
<i>Toxocara canis</i>	*****	*****	*****	**T		83
<i>T. cati</i>	*****	*****	*****	**		83
<i>Toxascaris leonine</i>	*****	*****	*****	T GA*		83
<i>Baylisascaris procyonis</i>	*****	*****	*****	T GA*		83
<i>B. transfuga</i>	*****	*****	*****	T GA*		83
<i>Toxocara tanuki</i>	*****	*****	*****	**		83

Fig. 3. Alignment of the partial 5.8 rDNA sequences of an ascaroid nematode collected from a raccoon and 6 known species of ascaroid nematodes

The nucleotide sequences of *Toxocara canis*, *T. cati*, *Toxascaris leonina* and *Baylisascaris procyonis* are quoted from Zhu *et al.* (1998) and *B. transfuga* and *Toxocara tanuki* are quoted from Yagi *et al.* (1999). The ascaroid nematodes from the raccoon, *Toxocara tanuki* are determined in this study. Asterisks (*) indicate sequence similarity with the raccoon ascaroid nematode. The nucleotide sequence of the ascaroid nematode has been deposited in the DDBJ/EMBL/Genbank nucleotide sequence databases with the accession number AB245964.

ventriculus, but lacked appendices or intestinal caecum in its base; spicules over 2.3 mm in length and lacked tips; cloacal region without a rough area (Fig. 2). Although this species appeared to be identical to *Toxocara tanuki*, we performed molecular analysis on this specimen. According to an alignment of the nucleotide sequences of the partial 5.8SrDNA gene and ITS-2 rDNA from the present spe-

– 0.16 mm in length, respectively. Measurements and morphological characteristics of the scolex of the present specimen were almost the same as those recorded for *Taenia hydatigena* (Abuladze, 1964). Although DNA extraction from this sample was unsuccessful and therefore, this sample could not be molecular biologically identified, the present species was identified as *T. hydatigena*.

ascaroid nematode from racoon	ATAT-G-GAA	GTACGATC-T	G-C---T-FA	TGTATTACGG	AATG---C--	37
<i>Toxocara canis</i>	ATATTAGGGA	GTATGAT-G	GCACGC-CA	-AT-FTATG	AATG---TGA	42
<i>T. cati</i>	ATATGGAGAA	GTACGATCGT	GCACGCCTA	CCT---ATCG	AATG---TGA	44
<i>Toxascaris lenonina</i>	ATATCG-GAA	--A--A----	GC-AC--GCA	CCT-FTATGA	ATGACTC-A	36
<i>Baylisascaris transfuga</i>	ATATTC---A	--A--A----	GA-AT-TGC-	CAT-FTATGA	A-----	26
<i>Toxocara tanuki</i>	ATAT-G-GAA	GTACGATC-T	G-C---T-FA	TGTATTACGG	AATG---C--	37
.....
ascaroid nematode from racoon	TTAATA-C--	AAGCTTCCAG	TGGTGCATC	GCTCACA-G	ATGCATTCCG	83
<i>Toxocara canis</i>	TTAACO-CGC	AAGGTT----	--GTG-----	-----G	-TGCATTCCG	70
<i>T. cati</i>	TTAACO-CGT	AAS-FTC---	TGGTGCATC	TTTCGCAACG	-TGCATTCCG	89
<i>Toxascaris lenonina</i>	TT--TGTG-	AA-----CGC	T---CA-T---	ATAACG	-GCAT-----	62
<i>Baylisascaris transfuga</i>	TT--T-TC--	AA-----CA-	FGG--CA-T-	---ATG-CG	---AT-----	48
<i>Toxocara tanuki</i>	TTAATA-C--	AAGCTTCCAG	TGGTGCATC	GCTCACA-G	ATGCATTCCG	83
.....
ascaroid nematode from racoon	CAGGCTATGT	TGGTAGTTGG	CTA-T--ATA	--ATG-----	ATT-----	116
<i>Toxocara canis</i>	TGAGCTATGC	TGOT-GT-GG	-TAATGATA	TTGTGC--A-	ATT---G-TA	110
<i>T. cati</i>	TGAGCTACGC	CGGT-----	--AATCGATG	TTGTGTGAA-	ATT---A-TA	125
<i>Toxascaris lenonina</i>	-A--CT----	CGGT-G-AG	C-----TA	---TG-GAA-	ATTCTTATG	90
<i>Baylisascaris transfuga</i>	-AAGCTATGA	TGOT-G-AG	C-----GA-A	---TG-AAAG	A-----AGTA	79
<i>Toxocara tanuki</i>	CAGGCTATGT	TGGTAGTTGG	CTA-T--ATA	--ATG-----	ATT-----	116
.....
ascaroid nematode from racoon	CCAGCATACC	CTGCC--AAG	---T--C-T	GTAT--CGGA	CAAG-----T	150
<i>Toxocara canis</i>	C-AGGTCACC	TTGCC--AAG	-GA-----A-	ATATTGCG-A	CAAGA-AA-T	147
<i>T. cati</i>	CCA-CGTACC	TTGCC--AAG	--BC---TAT	GTAT--GC-A	CAAGA-AA-T	162
<i>Toxascaris lenonina</i>	CTATTGTACC	TTACC--AAG	CGGTAAATAT	---T---C-A	C---AT--C-	125
<i>Baylisascaris transfuga</i>	CTATCCTACC	TT-CTTA-G	CA-T--AAT	G-AT--GC-A	---ATACT	116
<i>Toxocara tanuki</i>	CCAGCATACC	CTGCC--AAG	---T--C-T	GTAT--CGGA	CAAG-----T	150
.....
ascaroid nematode from racoon	CGCTGTCTTT	TGCTCA--TG	A-A---GAGG	CGAAAT--G	-GCCATTG--	189
<i>Toxocara canis</i>	CGCTGTCTTT	TGCTCG--TA	A-A---GAGG	C-AAAAT-TG	-GCCAT-OBG	187
<i>T. cati</i>	CGCTGTCTTT	TGCTCG--TG	A-A---GAGG	CGAAAT--G	-GCCATTG-G	202
<i>Toxascaris lenonina</i>	CAFTATCATT	TGCTCAATG	AGATGTGAA-	-----ATA-	-GCCAT-ABG	165
<i>Baylisascaris transfuga</i>	CGTGTCTATT	TGCTCAACG	AGATGTGAA-	AGAAATATA	TGT-ATCAAG	165
<i>Toxocara tanuki</i>	CGCTGTCTTT	TGCTCA--TG	A-A---GAGG	CGAAAT--G	-GCCATTG--	189
.....
ascaroid nematode from racoon	C-----TGT-	--GTTCCTTC	ACGA---TAG	GGCCTCCAGC	ATA-CGTTGT	227
<i>Toxocara canis</i>	TG--TATGT	GGGTTCCTTC	ACGA---TAC	GGCCTCCAGC	A-ACGTTGT	231
<i>T. cati</i>	CG--TATGT	--GTTCCTTC	ACGA---TAT	GGCCTCCAGC	A-ACGTTGT	243
<i>Toxascaris lenonina</i>	CGA-T-T----	-----GCTCT	ATAA---TGC	GATTCCAGC	AT-GTATTG-	200
<i>Baylisascaris transfuga</i>	-AACT-TATC	GCG--GCTCT	-TAAAGT-C	GATT-CCAGC	GT-GTATTGT	207
<i>Toxocara tanuki</i>	C-----TGT-	--GTTCCTTC	ACGA---TAG	GGCCTCCAGC	ATA-CGTTGT	227
.....
ascaroid nematode from racoon	T--GT-G-TT	TG-TTGC-TT	GTTGACCAA	---AGGTTGG	AAGG-AACG-	266
<i>Toxocara canis</i>	T-TATG-TT	TGTTG---T	GCAGCA---	TCCAGTTGG	A-GGTGCCGT	272
<i>T. cati</i>	T-TOTT-TT	--GCT---T	GCAGCAAAA	TCTAGTTGG	A-GGTAACTT	284
<i>Toxascaris lenonina</i>	-AC-----TC	TAT-ACATT	A-TGGCTAA	T---GGTTGA	AGA-T--TG-	235
<i>Baylisascaris transfuga</i>	TATG--GATC	TAGC-A-AT-	A-TG--TCA-	T---AGTTGG	AAA-----	238
<i>Toxocara tanuki</i>	T--GT-G-TT	TG-TTGC-TT	GTTGACCAA	---AGGTTGG	AAGG-AACG-	266
.....
ascaroid nematode from racoon	--TCGCGCC	TTGAA-G----	GAG-GAAT--	ACGG---AAT	G-GTTG-ACA	302
<i>Toxocara canis</i>	ATCGGTCGC	TTGAA-G----	A-G-GAATC	ATCGG-GAAT	G-GTTG-AAA	314
<i>T. cati</i>	CATCGGTCGC	TTGAA-G----	AG-GAATC	CGG-CTGAAT	G-GTTG-ACA	326
<i>Toxascaris lenonina</i>	CAT-AGC---	-TAAATATC	GAGG---C	A-A---TAA-T	G-ACATA-A	271
<i>Baylisascaris transfuga</i>	-A-AA-----	-AAGT-TA-	-ACCA-T-	A-A---TGA-T	GTA-TATA-A	267
<i>Toxocara tanuki</i>	--TCGCGCC	TTGAA-G----	GAG-GAAT--	ACGG---AAT	G-GTTG-ACA	302
.....
ascaroid nematode from racoon	T-A-ATT-TT					309
<i>Toxocara canis</i>	TGAGAT-TT					323
<i>T. cati</i>	TCAATT-TT					335
<i>Toxascaris lenonina</i>	T-GAAT-TT					279
<i>Baylisascaris transfuga</i>	--AGTTCTT					273
<i>Toxocara tanuki</i>	T-A-ATT-TT					309
.....

Fig. 4. Alignment of the ITS-2 rDNA sequences of an ascaroid nematode collected from a racoon and 5 known species of ascaroid nematodes. The nucleotide sequences of *Toxocara canis*, *T. cati* and *Toxascaris lenonina* are quoted from Jacobs *et al.* (1997) and *Baylisascaris transfuga* and *Toxocara tanuki* are quoted from Yagi *et al.* (1999). The racoon ascaroid nematodes, *Toxocara tanuki* are determined in this study. Asterisks (*) indicate alignment of bases that are common to the racoon ascaroid nematode and the 5 other ascaroid nematode species. The nucleotide sequence of the ascaroid nematode collected from a racoon has been deposited in the DDBJ/EMBL/Genbank nucleotide sequence databases with the accession number AB245965

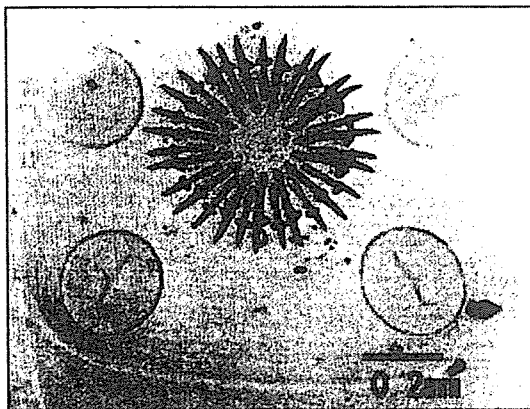


Fig. 5. Scolex of *Taenia hydatigena* obtained from a racoon in Hokkaido

This is first report of this taeniid species collected from a racoon in the world; however, Matoba *et al.* (2003) reported *Taenia taeniaeformis* from a free-ranging racoon in NFP in 2001. The Taeniidae family includes the zoonotic pathogens *Echinococcus* spp. and *Taenia* spp. (Abuladze, 1964); hence, making this report important.

3. Other helminths obtained from free-ranging racoons
Another 9 helminth species were collected from Hokkaido include: namely, 3 nematode species, *Molineus legerae* (Molineidae), *Ancylostoma kushimaense* (Ancylostomidae) and *Aonchotheca putorii* (Capilariidae); 1 acanthocephalan species, *Centrorhynchus* sp. (Centrorhynchiidae); 5 trematode species, *Metagonimus takahashii* (Metagonimidae), *M. miyatai*, *Euparyphium* sp. (Echinostomidae), *Plagiorchis muris* (Plagiorchidae) and *Brachylaima* sp. (Brachy-

Tab.1. Gastro-intestinal helminths of free ranging raccoons in Nopporo, Hokkaido (maturity and sex of host was distinguished)

	Juvenile						Adult					
	Female(N=23)			Male(N=22)			Female(N=80)			Male(N=46)		
	Infection rate (%)	Average of parasites	Infection intensity	Infection rate (%)	Average of parasites	Infection intensity	Infection rate (%)	Average of parasites	Infection intensity	Infection rate (%)	Average of parasites	Infection intensity
NEMATODA												
<i>Toxocara</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
Nematoda larva (<i>Porrocaecum</i> sp.)	4.3	1	1	5	1	1	6.25	6.4	1-27	2.2	1	1
<i>Molineus legerae</i>	0	0	0	0	0	0	12.5	1.6	1-3	19.6	3.3	1-9
<i>Ancylostoma kusimaense</i>	0	0	0	5	1	1	12.5	2	2	0	0	0
<i>Aonchotheca putorii</i>	0	0	0	5	1	1	10	1.5	1-3	10.9	1.8	1-6
ACANTHOCEPHALA												
<i>Centrorhynchus</i> sp.	0	0	0	0	0	0	1.25	1	1	0	0	0
<i>Plagiorhynchus ogatai</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Porrorchis oti</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hemiechinoma</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
TREMATODA												
<i>Metagonimus</i> sp.	0	0	0	15	27.3	2-66	21.3	62.4	1-457	15.2	10.5	1-29
<i>Euparyphium</i> sp.	17.4	2.5	1-4	10	63	6-120	36.3	107.3	1-1500	41.3	23.4	1-183
<i>Plagiorchis muris</i>	0	0	0	0	0	0	2.5	1	1	0	0	0
<i>Brachylaima</i> sp.	3	9	1-25	5	3	3	0	0	0	6.5	39	1-73
CESTODA												
<i>Taenia taeniaeformis</i>	0	0	0	5	1	1	0	0	0	0	0	0
<i>Taenia hydatigena</i>	0	0	0	0	0	0	1.25	1	1	0	0	0

Tab.2. Gastro-intestinal helminths in free-ranging raccoons in Nopporo, Karuizawa, Kobe, and Sasebo, Japan

	Nopporo (N=171)			Karuizawa(N=27)			Kobe(N=13)			Sasebo(N=18)		
	Infection rate (%)	Average of parasites	Infection intensity	Infection rate (%)	Average of parasites	Infection intensity	Infection rate (%)	Average of parasites	Infection intensity	Infection rate (%)	Average of parasites	Infection intensity
NEMATODA												
<i>Toxocara</i> sp.	0.6	0.5	1	0.0	0	0	0	0	0	0	0	0
Nematoda larva (<i>Porrocaecum</i> sp.)	4.1	4.8	1-27	0.0	0	0	0	0	0	0	0	0
<i>Molineus legerae</i>	11.2	1.1	1-9	3.7	2	2	15.4	1	1	6.25	1	1
<i>Ancylostoma kusimaense</i>	1.2	1.5	1-2	0.0	0	0	0	0	0	6.25	1	1
<i>Aonchotheca putorii</i>	8.2	1.6	1-6	0.0	0	0	0	0	0	0	0	0
ACANTHOCEPHALA												
<i>Centrorhynchus</i> sp.	0.6	1	1	3.7	1	1	7.7	1	1	25	4.75	1-13
<i>Plagiorhynchus ogatai</i>	0.0	0	0	0.0	0	0	0	0	0	12.5	1	1
<i>Porrorchis oti</i>	0.0	0	0	0.0	0	0	0	0	0	6.25	1	1
<i>Hemiechinoma</i> sp.	0.0	0	0	0.0	0	0	0	0	0	6.25	1	1
TREMATODA												
<i>Metagonimus</i> sp.	16.5	43.5	1-457	0.0	0	0	0	0	0	6.25	32	32
<i>Euparyphum</i> sp.	31.8	68.4	1-1520	11.1	4.7	2-9	0	0	0	31.3	31.8	1-107
<i>Plagiorchis muris</i>	1.2	1	1	0.0	0	0	0	0	0	0	0	0
<i>Brachylaima</i> sp.	4.1	21	1-73	0.0	0	0	0	0	0	0	0	0
CESTODA												
<i>Taenia taeniaeformis</i>	0.6	1	1	0.0	0	0	0	0	0	0	0	0
<i>T. hydatigena</i>	0.6	1	1	0.0	0	0	0	0	0	0	0	0

laimiidae) (Tab. 1). *T. tanuki*, *Euparyphium* sp. and *M. takahashii* have previously been reported by Asakawa *et al.* (2000), however, our study is the first report of the other helminth species in Hokkaido, and the first report of *Porocaeum* sp., *M. miyatai*, *Brachylaima* sp. and *T. hydatigena* in Japanese feral raccoons.

The following helminths were collected from raccoons from Honshu and Kyushu: *Molineus* sp., *Metagonimus takahashii*, Echinostomidae gen. sp., *Centrorhynchus bazaleticus*, *C. elongatum*, Plagiorhynchidae gen. sp., and *Hemiechinomsoma* sp. (Tab. 2). Although Sato *et al.* (2005a) reported 6 acanthocephalan species that principally infest avian species in Wakayama Pref., Honshu Island, they did not find *Hemiechinomsoma* sp.. The genus *Hemiechinomsoma* that was collected from the present study is also a typical avian acanthocephalan species (Ryzhikov *et al.*, 1985). Previous reports of parasitic helminths collected from feral raccoons captured in Wakayama Pref. have shown that *Strongyloides procyonis* and *Physaloptera* sp. are prominent (Sato & Suzuki, 2006). As *S. procyonis* is a zoonotic nematode (Little, 1965), it was important to examine our specimens for the presence of this nematode; however, our findings were negative.

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SCID マウスとスナネズミにおけるアジア条虫の幼虫の発育と人および代替終宿主に対するその感染能

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Larval development of *Taenia asiatica* in scid mice and gerbils and their infectivity to humans and alternative definitive hosts

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【目的】アジア条虫 *Taenia asiatica* は無鉤条虫に近縁な条虫で主な中間宿主は豚で、その肝臓に囊虫が寄生するがその寿命は短い事が特徴である。アジアで多くの人体寄生例が報告され、有鉤条虫や無鉤条虫とならんで公衆衛生上重要な条虫である。我々は SCID マウスとスナネズミにおける幼虫の発育と、感染後10から48週後に得られた囊虫の感染能力を調べた。

【方法・結果】虫卵の皮下接種後 SCID マウスの皮下から10、24、45週後に囊虫を回収した(表1)。囊虫には少数の遺残的小鉤が顎嚙領域に認められ、10週齢ではこの領域周囲に小型顆粒が多数認められたが、24と45週齢では減少していた。一方、10週齢では石灰小体の数が少

なかつたが、24と45週齢では増加していた。スナネズミにおける発育も同様であった。SCID マウスから回収した10週齢囊虫を経口投与したスナネズミでは全例陰性(0/21例)だったのに対し、20から45週齢の囊虫を経口投与した場合、虫体陽性率(陽性ネズミ数/全投与ネズミ数)は6%から18%で(表2)、ハムスターの虫体陽性率もほぼ同様であった。虫体回収率は0.5-6%であった。また、スナネズミから回収した48週齢の囊虫を経口投与したスナネズミの虫体陽性率は45%(5/11)で、虫体回収率は27%であった。これらのハムスターおよびスナネズミとも部分的な虫体の発育が観察された。一方、SCID マウスとスナネズミから回収されたそれぞれ45と48週齢の囊虫を経口投与したボランティアの人では、それぞれ2/3、2/2に感染し、受胎片節を排泄し、駆虫による虫体回収率は約50%であった(表3)。

表1 アジア条虫の六鉤幼虫を投与したSCIDマウスにおける幼虫の発育

六鉤幼虫*	SCIDマウス		回収幼虫	
	投与経路	投与数	頭数	回収率(範囲)
A 皮下	20,000	5	12,20 週	0.1-1.1%
	腹腔	18,600	5	12,20 週
B 皮下	40,000	3	24,62 週	0.003-1.1%
	皮下	20,000	5	10-45 週

* 幼虫被殻を除去した六鉤幼虫を感染に使用した。

A 2003年12月台湾で受胎片節採取 2004年1月感染実験に使用
B 2004年4月台湾で受胎片節採取 2004年5月感染実験に使用

表2 SCIDマウスから得た各種週齢の囊虫の感染能の検討
-代替終宿主を用いて-

動物	囊虫	陽性動物/全使用動物	虫体回収率*
週齢	投与数	(1-4週に剖検)	
スナネズミ			
A	10 20,40	0/21(0%)	0/640(0%)
B	20 6	2/32(6%)	2/192(1.0%)
C	21 25-40	2/11(18%)	2/403(0.5%)
D	45 2	2/17(11%)	2/34(5.8%)
ハムスター			
A	20 6	3/41(7%)	3/246(1.2%)
B	24 15	3/14(21%)	3/210(1.4%)

* 回収虫体数/全投与虫体数(%)

表3 SCIDマウス(45週齢)およびスナネズミ(48週齢)の囊虫を用いた人への感染結果

人	由来動物	週齢	投与数	駆虫後の回収虫体数
A	SCIDマウス	45	5	0
B	SCIDマウス	45	5	1
C	SCIDマウス	45	5	2
D	スナネズミ	48	5	3
E	スナネズミ	48	5	4

* A-Cの駆虫は感染後7ヶ月、DとEの駆虫は感染後4ヶ月
虫体陽性率は4/5(80%)、虫体回収率は10/25(40%)

【総括】アジア条虫の囊虫の顎嚙領域の小型顆粒と石灰小体の数は成熟の指標として評価できることが示唆され、SCID マウスとスナネズミにおいて感染能を有する囊虫まで発育することを示した。

Keywords, *Taenia asiatica*, alternative host

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四類感染症

エキノкокクス症

Echinococcosis

神谷正男

Key words : エキノкокクス症, 包虫症, echinococcosis, 感染源動物対策, キツネ

はじめに

エキノкокクスは、重い肝臓障害で致死的な病気をもたらす。20世紀初頭まで、我が国には分布していなかった寄生虫で、いわゆる、侵入生物である。人間によって本来の生息地以外の地域に移動した動物(終宿主:キツネなど)に寄生していたが、移動先の新天地に定着し、その土地の動物(中間宿主)に伝播し生活環(life-cycle)が成立、分布を拡大していった(図1)。この寄生虫には、北方圏を中心に分布する多包条虫と世界的に分布する単包条虫が、公衆衛生上、特に重要である。2005年、米国の一般科学誌(SCIENTIFIC AMERICAN)の記事に、飼いイヌならびに野生化したイヌの糞から感染する致死的なエキノкокクス症についての警告がある。‘寄生虫の時限爆弾’という見出しで、東チベット地域を中心に少なくとも60万人の患者と6,000万人の住民が感染の危険に曝されているとの内容である¹⁾。中国ではエキノкокクス症対策を国家プロジェクトとして取り上げている。ヨーロッパでは地域安全保障を担うNATOが、世界のエキノкокクス対策に関する研究集会を開催している。また、世界銀行やWHOが注目しているDALYs(障害調整生存年数)を用いて本症の重要性が論じられるようになっていく²⁾。

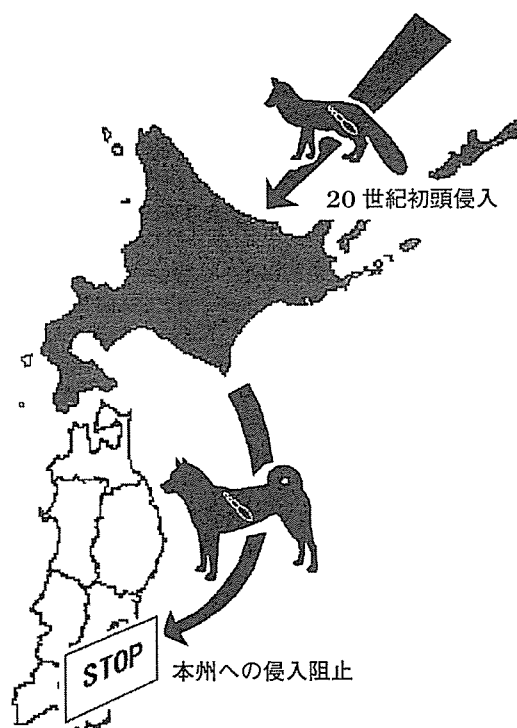


図1 分布拡大の阻止

疾患名 : エキノкокクス症, 包条虫症(幼虫形に包虫症), echinococcosis.

関係法規名 : 4類感染症(直ちに届出: ヒトならびに感染源動物としてのイヌ).

病原体 : エキノкокクス(*Echinococcus*)は,

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5種に分類され、いずれも人獣共通寄生虫である。成虫は体長4mm前後の微小な条虫であるが、幼虫は中間宿主(ネズミ、ヒトなど)の肝臓を中心に無性増殖し、巨大な病巣を形成する。学名は *Echino* (=棘のある) *coccus* (=球状のもの) に由来する。幼虫形(包虫)がそのまま採用されている。

感染経路(生活環): エキノコックスは、終宿主(捕食者:肉食獣)と中間宿主(被食者:草食獣)の間で伝播している。ヒトは中間宿主で、ヒトからヒトへも、ネズミからヒトへも感染しない。ヒトが感染するのは、食物などを介して終宿主動物のキツネやイヌの糞便に排泄されるエキノコックス虫卵を経口摂取する場合にのみ感染する。

疫学: 我が国で人体例(単包条虫による単包虫症)は19世紀末に紹介されている。20世紀中頃から多包条虫による人体例(多包虫症)が、北米アラスカ、ヨーロッパ中央部や北海道など、世界的な流行が問題になってきた。現在、日本、特に北海道で問題となっているのは多包条虫で、主に野ネズミを中間宿主として野生動物間で流行し、北半球に広く分布している。一方、単包条虫の中間宿主は、主に有蹄家畜で、分布は全世界的である。我が国では、輸入牛から、また、人体の輸入症例が散発的に報告されている程度で、多包条虫ほど問題とはなっていない。本稿では、主に多包条虫について述べる。

1. 流行状況

a. 世界における流行状況

多包条虫は北半球に広く分布し、世界中で患者発生率は地域により異なるが、多くは、住民10万人当たり年間罹患率は0.1-10で、ヨーロッパの流行地では1以下が多い。

世界的にアカギツネ(キタキツネはこれに含まれる)が最も終宿主として重要であるが、ホッキョクギツネ(ツンドラ地帯)、コヨーテ(アメリカ合衆国)、オオカミ(旧ソ連、中国)およびイヌも終宿主となる。

キツネの感染率が40%以上の地域でもイヌの感染率は5%以下、多くの場合1%以下であ

る。しかし、アラスカ・セントローレンス島のようにイヌと野ネズミが同居している地域では、イヌでも10%以上の感染率を示す。感染キツネの発見や感染率の推移から、欧米においても本種の分布拡大が問題となっている。

多包条虫の流行域内で中間宿主となる種は限られている。主にげっ歯類で、ヒト、ブタ、イノシシ、ウマ、各種霊長類(動物園)などにも感染する。

b. 日本における流行状況

現在、国内では主に北海道において問題となっているが、日本で初めての単包虫症は熊本で、多包虫症が報告されたのは仙台である。東北地方では流行地に居住したことがない症例も知られている。青森県で1999年に、同じ農家で異なる時期に3頭のブタから多包条虫が検出された。ブタは、中間宿主でありヒトと同じ立場にある。たとえ、豚肉をヒトが生で食べたとしても感染することはない。その後、本州での野生動物の調査では、感染個体は発見されていない。しかし、少数ではあるが感染イヌが北海道から本州に持ち込まれている事実が明らかとなっている。

北方領土から感染したネズミやキツネの移送により北海道に侵入したと考えられる。北海道内において、多包条虫は1935-60年代まで礼文島に、更に1966-80年までは東部に局限しているものと考えられていたが、1980年代に流行地拡大が認識され、1990年代後半には全道に蔓延していることが明らかとなった。

c. ヒトにおける流行状況

海外で感染したと考えられる単包虫症が散発的にみられるが、国内で単包条虫の生活環は確認されていない。多包虫症例は、本州で80人以上の報告がある。ヒトは移動し、かつ、発症するまで10年前後を要し、感染した地域の特定は困難なことが多い。しかし、これまで本州で診断された症例のほとんどは、北海道もしくは海外の流行地に居住した経験がある。

北海道では、かつて、患者の居住地域は、ほぼ北海道東部に限定されていたが、近年ではその他の地域の患者の比率が増加している。更に、

患者は農村部だけでなく都市部から多数、届出られるようになった。北海道で1937年に礼文島出身者から発見されて以来、500例以上の患者が主に病理組織で確認されている。これには血清検査陽性のみの例は含まれない(2003年度受診者数49,976,陽性者数73)。近年、毎年平均20人前後の主に手術で確認される新たな患者が発生している。全道の年間罹患率は10万人当たり0.35と算出される。2003年から3年間の北海道の届出患者数は61人でうち20人(1/3強)は札幌市保健所管内からの届出であった。札幌での年間罹患率は10万人当たり0.45である。また、2006年第46週までの集計では、全道19人中8人は札幌からの届出である。また、北海道南部、特に函館エリアの患者数増加が顕著である。2003年から3年間の函館での届出患者数は9人で、年間罹患率は10万人当たり1以上となる。いずれにしても北海道全域に感染リスクが高まっていると考えられ、今後の患者数の増加が危惧される。

d. 終宿主における流行状況

1) キツネ

多包条虫の伝播において最も重要な終宿主である。流行状況を調べるためにはキツネの調査を行う必要がある。近年、感染率上昇は著しく、1993-97年度では40%近くに、1998年度には57.4%に急増し、その後40%台になっている。2003年度の札幌市内の感染率は64%であった。

養狐業者などのキツネが道外へ移送されることがあるが、道外へ移動する場合は検査・駆虫が必要である。

2) イヌ

北海道で登録されている飼いイヌの数は約23万頭(平成10年度)で、1966-2002年度までの北海道(行政)によるイヌの剖検調査の集計では、平均感染率は1%であった。この調査で陽性例99例(99/9,881)が知られている。この検査対象には飼育状態の不明なイヌが含まれるが、飼いイヌが約4割を占めていた。最近、10年間のイヌの検査頭数は毎年10-20頭と少なく、イヌの感染状況を推察するためのデータは不足している。

近年、終宿主動物の糞便内のエキノコックス成虫に反応する抗原を検出する診断法が開発され、虫卵が排泄される前にヒトへのリスクを図ることが可能になった。この診断法が普及することにより動物病院に来院した飼いイヌ、農家の放し飼いのイヌ、室内犬(散歩には連れ出す)までの様々な飼育状況の感染イヌが見つかる(‘環境動物フォーラム’調べ)。

3) ネコ

北海道において1960-91年の剖検調査で5.5%(5/91)の陽性率であるが、発育は悪く、片節内に成熟虫卵は産生されていなかった。ネコについてはヨーロッパの調査でもイヌより高い感染率が報告されており、ヒトとの接触頻度を考慮すると、重要な多包条虫感染源となり得るので、今後、調査が必要である。

4) タヌキ

終宿主として感受性はキツネより低い、ネコより高い。個体により虫卵を排泄することがある。小樽の2002年の調査で感染率13.3%(6/45)で、虫卵を排出する個体が検出されている。

5) その他

野生化したミンク、アライグマは、中間宿主であるネズミを食べる機会はあると考えられるが、実験的にも野外個体でも感染例は見つっていない。

2. ヒトの多包虫感染

a. 感染経路と予防

ヒトへは、虫卵に汚染された土などを介して、経口的に感染する。キツネの生息しそうな地域における野外活動時に靴や衣服に付着した虫卵が住宅内に持ち込まれることも考えられる。農産物に付着して輸送される可能性もある。畑に排泄されたキツネ糞から虫卵が検出されることがあるので、野菜への虫卵付着・汚染は考えられる。キツネやイヌが排泄した糞便内虫卵による汚染の可能性のある山菜や野菜はよく洗って食べるか、熱を通すことが推奨される。感染キツネの糞便によって作物が汚染されないように努める必要がある。虫卵は加熱に弱い。沢水や設備の悪い井戸水を常用する場合は濾過もしく

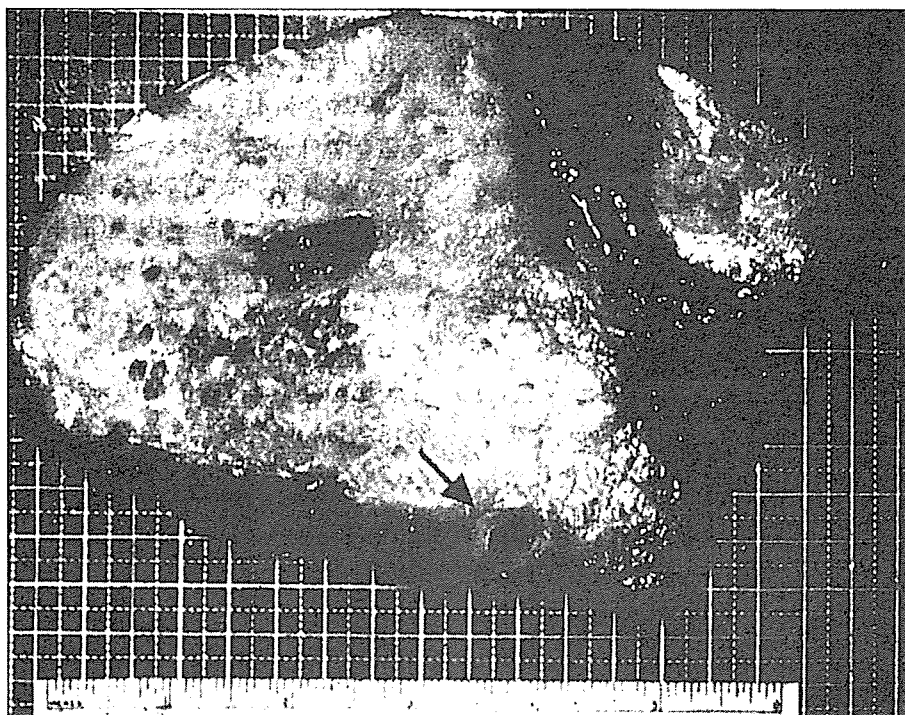


図2 人体エキノコックス症病巣の摘出(肝臓)
→は下大静脈。(北海道大学病院 佐藤直樹博士提供)

は加熱することにより感染を防ぐことができる。しかしながら、様々な虫卵拡散経路が予想され、周囲の環境中に虫卵がある場合、完全に虫卵から隔離して生活することは困難である。したがって、環境全体からリスクを除去する対策が必要である。キツネを駆虫することにより環境中の虫卵を減らす方策が住民により採用され成果を上げている(後述)。

b. 感染後の経過と症状

ヒトが感染すると、多包虫は主に肝臓に寄生し、無性増殖する。病巣の拡大はゆっくりで、症状が現れるまで成人では10年以上を要する。子供ではやや早い。原発巣のほとんどは肝臓であるが、進行すると肺、脾臓、腎臓、脳、腸間膜、骨髄などにも転移する(図2)。

病気の経過は通常以下の3期に分けられる。

(1) 無症状期：成人では感染後10年間ほどで、多包虫の病巣が小さく、感染していても症状の出ない時期。

(2) 進行期：無症状期の後の数年間で、病気の進行につれて、病巣が大きくなり周囲の肝臓

内の胆管および血管を塞ぐために肝臓の機能が悪くなる時期。この時期は更に不定症状期と完成期に分けられる。不定症状期は上腹部の膨満・不快感などの不定症状のみで、肝臓機能障害は検出できない。完成期は肝臓機能不全となり、腹部症状が強く、発熱、黄疸をみる。寄生部位が肝臓以外の場合は、寄生臓器によって症状は異なる。

(3) 末期：通常半年以内で、重度の肝臓機能不全となり、黄疸・腹水・浮腫を合併し、門脈圧亢進を伴う時期。様々な臓器に多包虫が転移し、予後不良である。

c. 診断法

早期診断した場合、病巣は小さく、治癒率(完全な病巣切除率)は高い。一方、自覚症状が現れた後に多包虫症と診断された場合は、多包虫が大きく増殖、転移している例が多く、現在の治療技術でも治癒率は低い。北海道で行っているエキノコックス症の検診は第一次診断としてELISA法の血清診断、第二次診断としてウエスタンブロット法によるELISA法陽性反応の

確認と、問診、腹部の触診、超音波診断、腹部X線撮影などを併用している。

病院からの依頼先は、北海道臨床衛生検査技師会や北海道立衛生研究所疫学部血清科などがある。

d. 治療

最も有効な多包虫症の治療法は、外科手術による多包虫の摘出である。多包虫は小さな嚢胞の集合体で周囲の組織に浸潤しているため、周囲の健康な組織ごと摘出する。完全に摘出しないと、残存した多包虫が増殖し、更に転移する。駆虫薬のアルベンダゾールも治療のために用いられるが、著効を示す例は多くなく、寄生虫の発育を抑える程度の例が多い。治療効果を上げるためには、大量の長期投薬が必要である。化学療法は手術が適応できない場合や手術の補助として用いられている。

3. イヌの多包虫感染

a. 感染経路と予防

イヌの感受性はキツネと同様である。北海道の農村地帯における飼いイヌに関するアンケート調査では約25%のイヌがネズミと接触し、5%が食べたことがあると答えている。イヌの予防には野ネズミを食べさせないことが最も重要である。流行地では放し飼いは禁止し、野ネズミを食べないように注意する必要がある。感染の機会があったと予想された場合は、獣医師に相談し、検査、駆虫を適宜実施する。

b. 感染後の経過と臨床症状

イヌでは小腸粘膜に小型の成虫が吸着するのみで、通常症状は示さないが、まれに下痢・粘血便のみられることがある。

c. 診断法

イヌは、まれに下痢便中に成虫が排泄されていることもあるが、通常、検査しないと感染の有無は判断できない。診断のために、剖検(小腸の成虫検出)が行われてきたが、近年、糞便内抗原検出法やPCR法が利用できるようになった。糞便内抗原検出のためのサンドイッチELISA法および虫卵検査、更に最終確認用のPCRによる虫卵のDNA検出が行われている。

d. 治療

条虫に対する駆虫薬として、アレコリン、ニクロサミド、プラジカンテル(praziquantel (PZQ):商品名ドロンシット)などがある。特にPZQは成虫に対して最も効果的な駆虫薬である。飼いイヌの感染はヒトへの感染が起こる危険性があるため、完全に駆虫する必要がある。通常の投与量(5mg/kg)1回投与でほぼ100%の駆虫効果があるが、PZQは虫卵に対する殺滅効果はないので、虫卵が糞便中に含まれていることを考慮して、3日間は糞便の適正な処置(例えば、熱湯消毒)が必要である。

4. 感染源動物対策の必要性

本症はヒトからヒトへの伝播はないので、ヒト中心の対策のみで危機管理に臨んでも新規患者の増加を止めることはできない。従来、考えられていた以上に、虫卵により感染する可能性のある人口は急増している。今後、増加する患者に対する医療費のみならず、農業などへの社会的コストは大きい。ヒトの‘早期診断・早期病巣切除’のほかに‘感染源対策’が緊急に必要なものである。

2003年11月の法律改正で、虫卵を排出する動物など感染源対策が大幅に強化されることとなった(獣医師によるイヌのエキノコックス症の届出義務、2004年10月施行)。終宿主の糞便に出るエキノコックス成虫抗原を検出して感染を確かめる診断法が確立し、感染源動物が虫卵(=リスク)を排泄する前に把握し、駆虫薬で防除することが可能になった。‘エキノコックス感染源対策を’³⁾と題した論評で提言された‘感染源動物の届出義務を追加し、検疫体制の確立’は、イヌについては実現しつつあるが、自然界での主たる感染源動物であるキツネに緊急に取り組む必要がある。

a. イヌ対策

流行地においてイヌを放し飼いにすると、感染ネズミを食べてエキノコックスに感染し虫卵を排出する。飼いイヌはヒトとの接触が密接で、周囲が虫卵で汚染されるため、飼い主、家族および周辺住民への感染リスクが高くなる。北海