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ORIGINAL PAPER

Epidemiology, histpathology, and muscle distribution of Trichinella T9 in feral raccoons (Procyon lotor) and wildlife of Japan

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Abstract The prevalences of *Trichinella* T9 in trapped raccoons (*Procyon lotor*) and several other potential mammalian reservoirs in Hokkaido, Wakayama, and Nagasaki Prefectures were investigated. Muscle samples were collected from 2003 to 2006 from 1,080 raccoons, 113 raccoon dogs including 2 species (*Nyctereutes procyonoides albus* and *N. p. viverrinus*), 41 wild boars (*Sus scrofa leucomystax*), 14 martens (*Martes melampus*), 10 badgers (*Meles meles*), 5 Siberian weasels (*Martes sibirica coreana*), 7 mink (*Mustela vison*), and 1 red fox (*Vulpes vulpes japonica*). The samples were digested, and the prevalence and mean intensity of infection with the *Trichinella* muscle larvae were determined. The prevalence and intensity of the muscle larvae were 0.9% and 93.3 larvae/g (range 0.4–201.8) in raccoons, and 1.6% and 61.6 larvae/g in raccoon dogs, respectively. The infected animals were captured in different areas in Hokkaido Prefecture. These results confirmed that raccoons, which have been introduced from North America since the 1970s, are involved in the sylvatic cycle of *Trichinella* in Japan. In raccoons, the muscle density of *Trichinella* T9 larvae was highest in the tongue, and larvae were not found in the heart muscle or diaphragm. This is the first report of *Trichinella* T9 infection of feral raccoons in Japan.

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Introduction

The raccoon (*Procyon lotor*) is a medium-sized mammal that is widely distributed in North America. Previous studies have indicated that raccoons are one of the reservoirs of the sylvatic *Trichinella* cycle (Smith et al. 1985; Cole and Shoop 1987; Snyder 1987; Richardson et al. 1992). *Trichinella murrelli* have previously been detected in raccoons from Illinois and Indiana (Pozio and La Rosa 2000). Since the 1970s, a large number of raccoons have been imported to Japan as pets. As a result of both intentional release and escape, a well-established feral raccoon population now exists in Japan (Ikeda 2004).

In Japan, although several surveys on the helminth fauna of raccoons have been reported (Miyashita 1993; Asakawa et al. 1999, 2000; Matoba et al. 2002; Sato and Suzuki 2005a,b, 2006), there is no information concerning *Trichinella* infection in raccoons. Infections caused by the consumption of the raw meat of black bear (*Ursus thibetanus*) and brown bear (*U. arctos)* have been reported since 1974 in Japan (Yamaguchi 1991). *Trichinella* infection in the wildlife has also been detected in a raccoon dog (*Nyctereutes procyonoides*) from Yamagata Prefecture

(Saito and Yamaguchi 1985), in red foxes (*Vulpes vulpes schrencki*) from Hokkaido Prefecture (Yimam et al. 2001; Kanai et al. 2006), and in a red fox (*V. v. japonica*) from Aomori Prefecture (Kudo et al. 2001).

Because of the potential public health and epidemiological significance of this parasite, the current study was conducted to assess the prevalence of *Trichinella* infection in feral raccoons and in several other potential mammalian reservoirs in Japan.

Materials and methods

Feral raccoons were trapped from April 2003 to March 2006 in Hokkaido, Wakayama, and Nagasaki Prefectures by each municipality as a part of the local governmental control and eradication programs (Fig. 1). The animals were caught using box traps (Havahart model 1089, Woodstream, Lititz, PA, USA, or handmade by hunters), and were euthanized by humanitarian methods (AVMA Panel on Euthanasia 2001). Tongues or diaphragms were collected from 1,080 raccoons (678 from Hokkaido, 390 from Wakayama, 12 from Nagasaki) and frozen at −20°C at the Wild Animal Medical Center (WAMC) at Rakuno Gakuen University. Individual numbers (AS nos.) were given to animals examined at WAMC. Using 5 g of muscle per sample, the presence of *Trichinella* larvae was determined by an artificial digestion method (Henriksen 1978) with the following changes: Ten individual samples were digested at a time in 0.5 L of 1% pepsin-HCl solution for at least 4 h at 37°C with constant gentle stirring. After the muscle tissues had been digested, the sediment was allowed to settle and was washed several times. The sediment from the last washing was examined for larvae under a dissection microscope. When the sediment contained *Trichinella* larvae, the procedure was repeated using the individual samples.

Portions of the tongue, diaphragm, heart muscle, and superficial pectoral muscle of 469 raccoons from Hokkaido Prefecture were collected and fixed in 10% neutral-buffered formalin for 24 h and processed by routine histological methods for examination by light microscopy. Paraffin-embedded tissues were sectioned at 6 μm, stained with haematoxylin and eosin (H & E) and examined with the aid of a light microscope to assess the host reaction to muscle larvae.

To compare the prevalence of *Trichinella* larvae in raccoons and other animals, tongues were collected from 113 raccoon dogs (*Nyctereutes procyonoides albus* and *N. p. viverrinus*), 41 wild boars (*Sus scrofa leucomystax*), 14 martens (*Martes melampus*), 10 badgers (*Meles meles*), 5 Siberian weasels (*Martes sibirica coreana*), 7 mink (*Mustela vison*), and 1 red fox (*Vulpes vulpes japonica*) in the same study areas, and these were examined using the same methods as for raccoons.

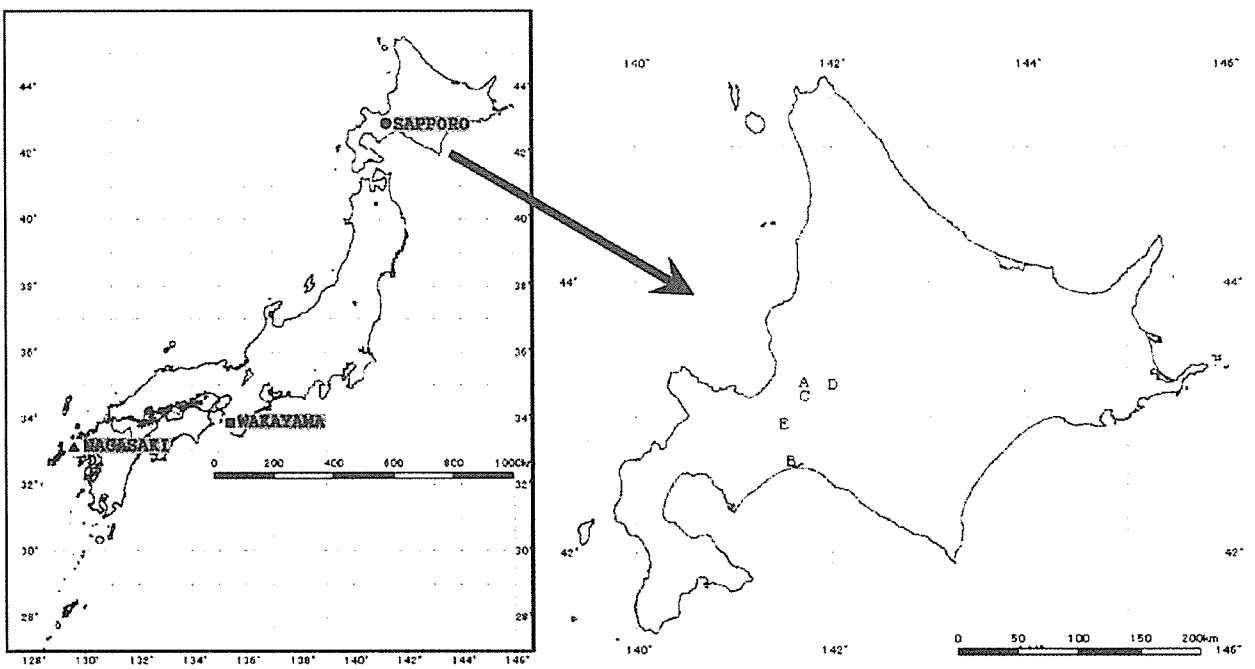

Fig. 1 Locations of the study areas in Japan. The raccoons positive for *Trihinella* were captured in the following areas (no. of the raccoon captured): **a** Kurisawa, 43°7′N, 141°44′E no. 4324; **b** Tomakomai, 42° 37′N, 141°36′E nos. 5342 and 4529; **c** Iwamizawa, 43°11′N, 141°46′E no. 5417; **d** Mikasa, 43°13′N, 141°50′E no.5498; **e** Kitahirosima, 42° 59′N, 141°33′E no.5601

The age of infected raccoons was determined by counting the annual incremental lines that occur in the tooth cementum of canines (Grau et al. 1970).

Results

Trichinella T9 larvae were detected in six (0.8%) raccoons and one (1.6%) raccoon dog in Hokkaido Prefecture (Table 1). The intensity of infection in these raccoons ranged from 0.4 to 201.8 larvae/g of muscle tissue. All the samples from Wakayama and Nagasaki Prefectures were negative for *Trichinella* larvae.

Histopathological examination was carried out on the infected muscle of 469 raccoons. Cysts and brightly staining eosinophilic cyst walls were found in the tongue muscle fibers of four raccoons (AS 4324, 5342, 5417, and 5498; these are same raccoons that were found to be infected using the digestion technique). In the cysts, a single coiled larva and nurse cells were observed (Fig. 2). Inflammatory cells consisted of mononuclear cells, and eosinophils sparsely infiltrated the tongue of the raccoon AS 4324. On the other hand, indistinct capsules and a prominent inflammatory infiltration consisting of eosinophils and lymph cells were observed in raccoon AS 5342. In the other two raccoons (AS 5417 and 5498), the inflammatory infiltrate surrounding the capsules was prominent, and both distinct and indistinct capsules were observed. Muscle larvae were also observed in the superficial pectoral muscle of raccoon AS 4324, but not in the diaphragm and heart muscle of any of the raccoons. No calcification was observed in the investigated sections of any of the raccoons.

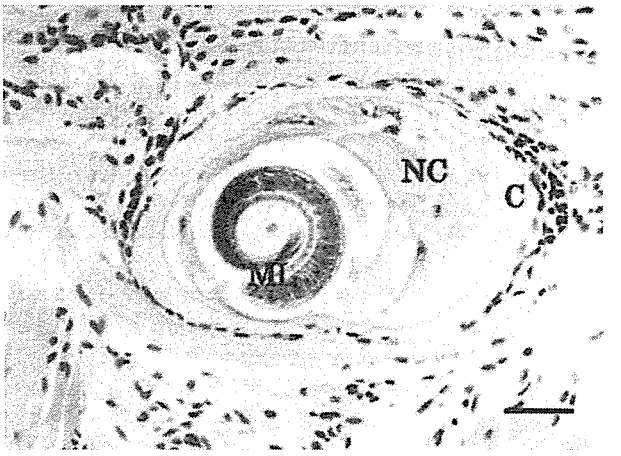

Fig. 2 A *Trichinella* larva encysted in the tongue muscle of a raccoon. Inflammatory cells are observed around the capsule (tongue of the raccoon AS 4324). *C* Capsule, *L* larva, *NC* nurse cell. HE bar = 100 μm

The results of multiplex PCR-test and COI (partial mitochondrial cytochrome oxidase subunit I) sequence analysis (Zarlenga et al. 1999; Kanai et al. 2006) of *Trichinella* isolates in the present study will be reported separately (Kobayashi et al. submitted).

Discussion

Epidemiology and pathology

Although *Trichinella* infection in the wildlife has been reported in Japan (Saito and Yamaguchi 1985; Yimam et al.

Table 1 Prevalence and intensity of *Trichinella* larvae in examined animals from Hokkaido, Wakayama, and Nagasaki from 2003 to 2005

Locality	Host	No. of animals infected/examined	No. of larvae/g (examined muscle)
Hokkaido	Raccoon (*Plocyon lotor*)	6/678	120.0 (masseter), 0.4, 10.0, 184.0, 43.8, and 201.8 (tongue)
	Raccoon dog (*Nyctereutes procyonoides albus*)	1/61	61.6 (tongue)
	Mink (*Mustela vison*)	0/7	–
Wakayama	Raccoon (*Plocyon lotor*)	0/390	–
	Raccoon dog (*Nyctereutes procyonoides viverrinus*)	0/52	–
	Japanese wild boar (*Sus scrofa leucomystax*)	0/41	–
	Japanese marten (*Martes melampus*)	0/14	–
	Eurasian badger (*Meles meles*)	0/10	–
	Siberian weasel (*Martes sibirica coreana*)	0/5	–
	Red fox (*Vulpes vulpes japonica*)	0/1	–
Nagasaki	Raccoon (*Plocyon lotor*)	0/12	–

2001; Kanai et al. 2006; Kudo et al. 2001), this is the first report of *Trichinella* infection in feral raccoons.

Yimam et al. (2001) reported a high prevalence (11.6%) of *Trichinella nativa* in red foxes in Otaru. The distribution of *Trichinella* species in wild animals is strongly influenced by environmental temperature, and therefore, their distribution is bounded by isotherms (Pozio et al. 1992a,b). Geographically, *Trichnella* T9 and *T. nativa* were present in wild animals in Hokkaido.

Histopathologic responses of encapsulated *Trichinella* larvae of raccoons in the present study were different from those of the *T. nativa* in raccoon dogs (Sukura et al. 2002). The raccoon dog is a medium-sized omnivore that is as widely distributed in Hokkaido as the raccoon. In the experimental infection of raccoon dogs with *T. nativa*, a cellular inflammation reaction developed, and cyst walls became thicker over time. In one of the cases of raccoon trichinellosis in the present study, thickened cyst walls along with a minimum host reaction were observed in the same section. Although there have been no reports of experimental infection of *T. nativa* in raccoons, these histopathological features indicate that *Trichinella* larvae from raccoons were distinct from *T. nativa*.

Host ethology and public health

Smith et al. (1985) and Cole and Shoop (1987) reported that *Trichinella* showed a significant prevalence in the male raccoons, and in our study, the infections occurred only in adult males. The reason for this difference between males and females is not apparent, but there are several reports of a relationship between host sex and *Trichinella spiralis* infection (Mankau and Hamilton 1972; Mascaro-Lazcano et al. 1978; Klein et al. 1999). One possible interpretation of the sex differences in *Trichinella* infection is that testosterone may suppress immune function, although empirical evidence is lacking.

The foods consumed by raccoons in the Nopporo Forest Park, Hokkaido have been identified. In winter (November to March), rodents (*Clethrionomys rufocanus bedfordiae*, *Apodemus speciosus*, and *Rattus norvegicus*), which were the most probable paratenic reservoir for *Trichinella*, represented approximately 50% by frequency of occurrence of all food items in the gastrointestinal tract of raccoons (Matoba et al., personal communication). This observation suggests that there is a greater opportunity for infection in winter. In Hokkaido, the mating season for raccoons reaches a peak in February, and offspring are born between March and May (Asano et al. 2003). The explanation for the absence of infected juvenile raccoons is that they were captured and euthanized before their first winter and, therefore, had no chance to acquire paratenic reservoirs. On the other hand, Kanai et al. (2006) examined 344 rodents and 27 insectivores by the artificial digestion method and found no evidence of infection with *Trichinella* larvae. This suggests the existence of another host cycle such as fox–raccoon, raccoon dog–raccoon, or bear–raccoon cycles.

In the present study, all samples from Wakayama and Nagasaki Prefectures were negative for *Trichinella* larvae. This result suggested that there is a focal distribution of this parasite in the Japanese archipelago. In fact, until now, the reports of trichinellosis in wild animals of Japan were limited in Hokkaido Prefecture and Northern Honshu Prefecture (Yamaguchi 1989; Yimam et al. 2001; Kanai et al. 2006; Kudo et al. 2001). However, the expansion of the distribution of feral raccoon populations is remarkable, and well-established feral raccoon population now exists in Japan (Ikeda 2004). In addition, the raccoons utilized the residential areas positively to get food or to keep their covers (Ikeda et al. 2000). Although the prevalence of feral raccoon trichinellosis in Japan is lower than in North America, these habitual characters may increase the area potentially contaminated by this parasite and the risk of human trichinellosis. To better understand the epizootiology of trichinellosis, more information is needed concerning the distribution of this parasite in various regions in Japan.

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Detecting *Clostridium botulinum*

To the Editor: In the October 2005 issue of Emerging Infectious Diseases, Song et al. described a fiber-optic, microsphere-based, high-density array composed of 18 species-specific probe microsensors, used to identify biological warfare agents, including *Clostridium botulinum* (*1*). Although the researchers used multiple probes for *C. botulinum*, we doubt that this approach is suitable for this organism.

C. botulinum comprises a heterogenous group of subspecies that produce botulinum neurotoxin (BoNT); identification and characterization usually rely on animal testing that focuses on antigenetically distinct toxins (*2*). Although strains of *C. botulinum* that do not produce toxins are sometimes isolated from wound infections not related to botulism, some strains of *C. butyricum* and *C. baratii* are also able to produce BoNTs.

The mouse bioassay is currently the accepted method for detecting BoNT. In this assay, mice that receive an intraperitoneal injection containing a sample with more than a minimum lethal dose show symptoms of botulinum intoxication and die. ELISAs, which recognize protein antigenic sites, are still less sensitive than the mouse bioassay (*3*).

Because the mouse bioassay requires euthanizing many animals, and results are not available for several hours, new diagnostic methods are needed. For *C. botulinum*, an organism widely dispersed in the environment, DNA-based methods may not provide the ultimate solution. Rapid methods to detect and differentiate active BoNTs, such as the rapid, mass spectrometry-based, functional method, are promising candidates to substitute for animal testing in the near future (*4*).

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Echinococcus multilocularis in Dogs, Japan

To the Editor: Alveolar echinococcosis in humans is endemic in Japan; however, the causal agent, *Echinococcus multilocularis*, has been restricted to the northernmost insular prefecture of Hokkaido, where the Tsugaru Strait acts as a natural physical barrier against migration to the mainland. Two *E. multilocularis* invasions into Hokkaido have occurred (*1*). The first invasion to the offshore island of Rebun in the mid-1920s was successfully controlled; however, the second invasion, supposedly in the 1940s, led to the current epidemic on the main island of Hokkaido. Both invasions were entirely or partly caused by humans who removed foxes from disease-endemic areas without taking the necessary precautions.

The finding of 19 autochthonously acquired cases of alveolar echinococcosis in prefectures other than Hokkaido (*2*) implies that the parasite exists in other areas, although the source of infection has yet to be identified. In many countries, studies of the increased spread of the parasite have traditionally focused on the contribution of foxes (*3*); however, these cases may also have been spread by domestic dogs from disease-endemic areas. Dogs are susceptible to infection with the parasite from rodents. Although the prevalence of *E. multilocularis* among dogs in Hokkaido is certainly lower than that in foxes (*4–6*), dogs can traverse considerably greater distances by various modes of transport. The number of dogs that travel from Hokkaido to other prefectures has been estimated at >12,000 per year (*7*). Although dogs may carry the parasite to remote areas, surveys of population dynamics have not been undertaken. We therefore studied the extent of *E. multilocularis* infection in dogs being transported by their owners from 4 ferry ports in Hokkaido (Hakodate, Muroran, Otaru, and Tomakomai) from September 2003 through October 2004.

We tested 183 fecal samples from 41 resident (in Hokkaido) and 142 nonresident dogs. We screened for the *Echinococcus*-specific coproantigen by using a commercial enzyme-linked immunosorbent assay kit (CHEKIT-Echinotest, Bommeli Diagnostics, Liebefeld-Bern, Switzerland) and following the manufacturer's recommendations. One dog from each group had the *Echinococcus* coproantigen. To confirm the specificity of the results, these 2 dogs were treated with 1 oral dose of praziquantel, 5 mg/kg.

Subsequent fecal samples were subjected to coproantigen testing and specific PCR amplification according to the method of Dinkel et al. (*8*). The coproantigen test showed a significant reduction in the optical density value for both dogs, which can be interpreted as effective deworming for *Echinococcus*. However, different results were obtained for the PCR test, in which assays of fecal samples from the nonresident dog during the second round of nested PCR produced a single band of the expected size (Figure). Direct sequencing showed that the band was the same as bands obtained for *E. multilocularis* isolates from Hokkaido (GenBank accession no. AB243207). Conversely, fecal samples from the resident dog did not yield any positive PCR results.

The reason for the discrepancy is unclear, but it may be a false reaction in either test. Given that a reduced optical density value was obtained after administration of the taeniacidal drug, the false-positive result of the coproantigen test might have been caused by another taeniid species. Such cross-reaction has been reported previously with this test (*9*). However, no worm debris was found in the fecal samples. Alternatively, sexual maturation or low infection intensity of *E. multilocularis* may produce false-negative results in PCR assays (*8*). Thus, because the owner stated that the dog was allowed to roam freely and frequently preyed on rodents, this coproantigen-positive but coproDNA-negative dog was highly suspected of being infected with *E. multilocularis*.

Infection among wild foxes can spread to domestic dogs by way of highly contaminated rodent hosts (*10*). A nonresident dog became infected with *E. multilocularis* despite staying in Hokkaido for only 5 days and being permitted to roam freely for just a few hours. This finding suggests a high infection pressure of *E. multilocularis* to domestic dogs within the area. In addition, the increased popularity of keeping dogs as companions, greater frequency of dogs' traveling with their owners, and high prevalence in foxes from urban and rural areas in Hokkaido (*5,6*) all contribute to the possibility that *E. multilocularis* could emerge in unsuspected locations. Thus, to prevent this parasite from spreading, measures such as those used by the Pet Travel Scheme of the United Kingdom should be applied to ensure that dogs from disease-endemic areas are pretreated before entry to the main island of Japan.

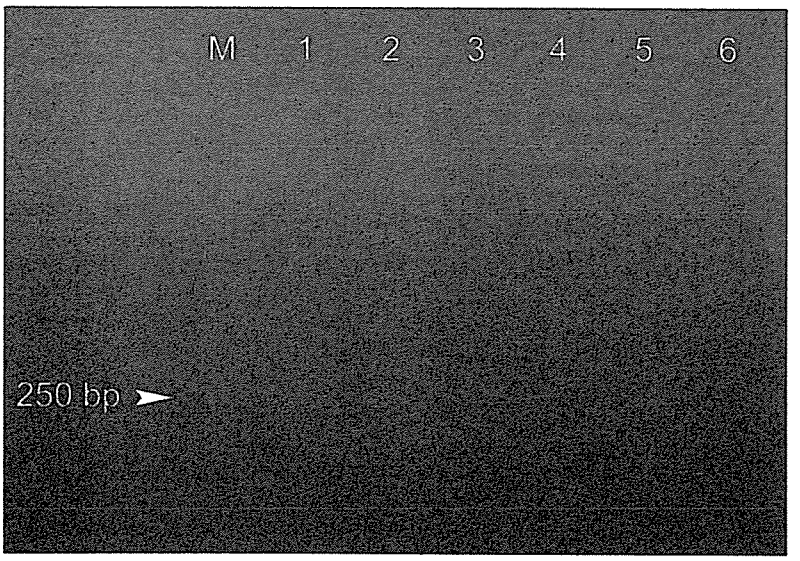

Figure. Nested PCR amplification of coproDNA from 2 coproantigen-positive dogs. Lane M, size marker (100-bp ladder); lane 1, nonresident dog (before treatment); lane 2, nonresident dog (1 day after treatment); lane 3, resident dog (before treatment); lane 4, resident dog (1 day after treatment); lane 5, positive control; lane 6, negative control. Arrowhead shows the expected band in a positive result.

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This investigation would not have been possible without the cooperation of domestic ferry companies. Appreciation is extended to Rikuo Doi for critical review and valuable comments on this manuscript.

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New World Hantavirus in Humans, French Guiana

To the Editor: Hantaviruses are etiologic agents for hemorrhagic fever with renal syndrome in Europe and Asia and for hantavirus pulmonary syndrome (HPS) in the Americas. These viruses belong to the family *Bunyaviridae*, genus *Hantavirus*. The natural reservoir of these viruses is wild or domestic rodents. HPS was first described in 1993 in the Four Corners region of the United States (*1*). It is a respiratory illness associated with the inhalation of aerosolized rodent excreta (urine and feces) contaminated with hantavirus particles. Sin Nombre virus (SNV) was the first etiologic agent of this syndrome. Since 1993, HPS has also been reported and confirmed in 6 countries in South America: Argentina, Bolivia, Brazil, Chile, Paraguay, Uruguay (*2,3*). Several distinct hantaviruses have been associated with HPS, including Juquituba virus in Brazil (*4*), Andes virus in Southern Argentina (*5*), and Laguna Negra virus in Paraguay (*6*).

French Guiana, an overseas French Administrative Unit in the Amazonian forest complex, is located on the northeastern coast of the South America between Brazil and Suriname. Ninety percent of its surface is tropical rain forest; the remaining 10% is a coastal plain, where 90% of the 200,000 inhabitants live. Cayenne and 2 adjacent towns, Remire and Matoury, constitute the main urban centers, with 80,000 inhabitants, ≈40% of the population. People live mainly in individual houses and small buildings. Many houses are built near forests, except those in the center of Cayenne. The outskirts of Remire and Matoury are surrounded by secondary rain forest, and those of Cayenne by wooded hills, where wild mammals such as rodents live in large numbers.

The prevalence of antibodies to New World hantavirus is unknown in French Guiana. Several cases of atypical pneumonia not linked to other etiologic agents (*Coxiella burnetii, Histoplasma boydii*), combined with identification of hantavirus rodent reservoirs in neighboring countries, prompted us to determine the seroprevalence of hantavirus in this area (*7,8*).

To estimate the prevalence of antibodies to New World hantavirus, we conducted a retrospective serologic survey of patients with symptoms compatible with HPS. Patients were from all areas of French Guiana: 64% from the urban centers, 7% from rural regions, and 30% from unspecified regions. From April 2002 through April 2004, a total of 420 serum samples were collected from patients with acute-phase febrile illness, unexplained acute respiratory syndrome, or bilateral interstitial pulmonary infiltrates. Diagnosis of Q fever was excluded by negative serologic results for immunoglobulin M (IgM), IgG, or both to *C. burnetii* (bioMérieux, Marcy-l'Etoile, France).

To detect patients with IgG antibodies to SNV, the ELISA described by Feldmann et al. was used (*9*). Briefly, an SNV-positive serum provided by the Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) was used as a positive control. Negative controls were obtained by random sampling of all previously negative samples. A sample was considered positive if the net absorbance values (after subtraction of absorbance values with and without antigen) were >0.2 for dilutions of 1:100 and 1:400 and the sum of 4 net absorbance values was >0.95. Seropositive samples were confirmed at CDC.

Antibodies reactive with SNV antigen indicate infection with a New World hantaviruses. However, because SNV is broadly cross-reactive with most New World hantavirus, the specific hantavirus cannot be identified.

The seroprevalence of IgG antibody to hantavirus was 1.42% (6/420) in the selected population. Three other samples showed borderline positivity. Antibody prevalence was not significantly different among the 7 age classes used (0–9, 10–19, 20–29, 30–39, 40–49, 50–59, and >60 years of age, $p = 0.36$, degrees of freedom = 6, by χ^2 test) or by sex ($p = 0.22$, by Fisher exact test).

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Laboratory and Epidemiology Communications

The First Reported Case of a Dog Infected with *Echinococcus multilocularis* in Saitama Prefecture, Japan

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Echinococcus multilocularis is a causative agent of human alveolar hydatidosis. The distribution of the parasite in Japan is thought to be limited to Hokkaido, the northernmost insular prefecture. The life cycle of this parasite is predominantly sylvatic. In Hokkaido, the red fox (*Vulpes vulpes*) is a definitive host that excretes *E. multilocularis* eggs into its feces. The gray-sided vole (*Clethrionomys rufocanus*) is a principal intermediate host that becomes infected by ingesting the eggs, which develop into larvae in the host viscera. Domestic dogs also become definitive hosts of the parasite after eating voles harboring the larvae. Since human infections follow the accidental ingestion of *E. multilocularis* eggs, a survey of *E. multilocularis* infection in dogs is a crucial for disease

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prevention. Data obtained from an examination of 9,907 dogs in Hokkaido between 1966 and 2004 by the prefectural government revealed that 99 (1.0%) were infected with the adult parasites. The estimated number of dogs that travel from Hokkaido to other prefectures is over 12,000 per year (1). Some of these dogs might constitute important carriers, transmitting the parasite to remote areas. Morishima et al. (2) recently studied the extent of *E. multilocularis* infection in 183 dogs transported by their owners from Hokkaido to other prefectures and found that two of the dogs were infected. Here we describe the first case of a dog infected with *E. multilocularis* in Saitama Prefecture, which neighbors northern metropolitan Tokyo.

The dogs examined in this survey were abandoned and pound animals. A total of 550 dogs kept in the Saitama Prefectural Pet Owner's Guidance Center were examined between April 1999 and September 2005. Staff members at the center collected fecal samples. These were routinely checked for the presence of helminth eggs and protozoan oocysts by microscopy using direct smear, formalin-ether and sucrose flotation techniques. Taeniid eggs (Fig. 1) were found in a fecal sample from a female mongrel dog that was captured in northern Saitama in June 3, 2005. Because *Echinococcus* eggs are morphologically indistinguishable from those of other tapeworms of the family *Taeniidae* (3), they were examined by PCR according to the method of Dinkel et al. (4). The second round of nested PCR produced a single band of the predicted size at 250 bp. Direct sequencing showed that the band was the same as those found in *E. multilocularis* isolates from Hokkaido (GenBank accession no. AB244598). These results showed that this dog was infected with *E. multilocularis*.

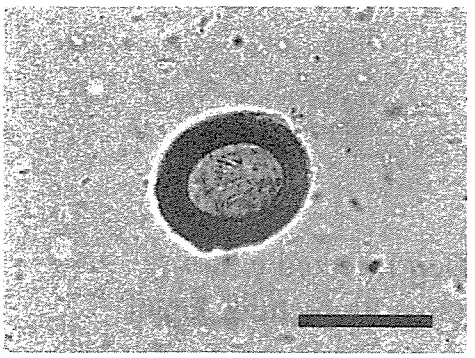

Fig. 1. *Echinococcus multilocularis* egg found in the fecal sample of a dog captured in Saitama Prefecture, Japan. Scale bar equals 30 μm.

The source of infection was not identified. No convincing evidence yet supports the notion that wild animals in Saitama and its neighboring prefectures are infected. Since some dogs that move from Hokkaido to the mainland of Japan are infected (2), the dog described herein seems to have been infected in Hokkaido, then taken to Saitama by her owner, and then either abandoned or allowed to escaped. Regardless, an extensive epizootiological survey should be conducted on wild animals in suspect areas of the prefecture. The Infectious Diseases Control Law of Japan has classified the significance of this disease to public health as Category IV, which has required the mandatory reporting of not only infected humans but also infected dogs since October 1, 2004. In accordance with this law, this is the first reported instance of a dog infected with *E. multilocularis* in a Japanese prefecture other than Hokkaido.

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Intestinal helminths of dogs in northern Japan

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HUMAN beings can become infected by a number of canine intestinal helminths, some of which are considered to be potential public health problems worldwide. However, intestinal worms of dogs currently receive less attention than their protozoan counterparts. This may partly be due to the decline in endemicity reported in developed countries, where dogs are likely to receive more attention and anthelmintic treatment from their owners. To illustrate the importance of veterinary care, a longitudinal study in Japan revealed that progress in prophylactic drug administration against dirofilariosis had reduced the prevalence of several intestinal nematodes in dogs (Asano and others 2004). The low levels of prevalence among canine hosts translate into a reduction in potential zoonotic risk for human beings in a given area. However, endemicity has been found to vary markedly from one region to another, and is also influenced by aspects of the survey protocols, such as subject choice and the diagnostic techniques that are employed (Robertson and others 2000).

This short communication is part of an ongoing study on the zoonotic helminths of Japan. It describes a coprological survey of dogs in Aomori, the northernmost prefecture on the mainland, where no such information is currently available. The analyses focused on hunting dogs, as the way in which they are managed and their activities in fields makes them more susceptible to acquiring a variety of zoonotic helminths, either directly or indirectly from wildlife. Faecal samples were collected from 134 hunting dogs and 86 companion dogs between December 2003 and March 2005. Two different diagnostic techniques were employed: a centrifugal flotation technique (Ito 1980) with a sucrose solution with a specific gravity of 1·27, and a formalin-ethyl acetate sedimentation technique (Young and others 1979).

With the exception of *Trichuris* species and Taeniidae, all eggs were identified to genus or genus and species on the basis of morphological characteristics. The eggs of *T vulpis* are morphologically similar to those of other *Trichuris* species (Bundy and Cooper 1989), and parasitic eggs may be excreted after being acquired from other animals through coprophagy (Traub and others 2002). A total of 100 randomly selected trichurid eggs from positive faecal specimens were therefore re-examined according to the dimensions described by Yoshikawa and others (1989). Given the difficulty of distinguishing between taeniid tapeworm eggs on the basis of shape and size (Thompson 1995), *Taenia* and *Echinococcus* species were differentiated using nested PCR (Dinkel and others 1998). Pearson's χ^2 test was used to assess differences in the egg prevalence of each helminth between both groups. McNemar's χ^2 test was used to compare the diagnostic efficiency of the coprological techniques. All statistical analyses were performed using a statistical package (S-PLUS 6.1J for Windows; Mathematical System).

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The results from both the hunting dogs and companion dogs are summarised in Table 1. *T vulpis* was the most common helminth in both groups, and the prevalence was significantly higher in hunting dogs ($P<0{\cdot}001$). Although comparison of results is difficult because a previous study determined prevalence by postmortem examination (Yagisawa 1978), the occurrence of *T vulpis* in dogs in Aomori appears to have decreased, and the numbers of other intestinal worms have also exhibited an appreciable decline. The reason for the remaining prevalence, particularly in hunting dogs, is uncertain, but these dogs are thought to be reservoir hosts; *T vulpis* is prevalent among wild canids in Aomori (Sato and others 1999a,b). The hunting dogs may have become infected with this parasite in fields that were contaminated by the sylvatic hosts, and have then transmitted the parasite to the local dog population.

In addition, the choice and/or usage of drugs is particularly important. Several anthelmintics are used to prevent canine dirofilariasis. Of these, ivermectin (Cardomec; Mweial) is the most commonly used for this purpose in Japan. However, ivermectin has no effect on intestinal trichuriasis at the usual chemoprophylactic dosage (6 μg/kg bodyweight), and an increase in concentration of more than 16-fold is required (Anderson and Robertson 1982). Ivermectin at this concentration also showed an effect on other intestinal helminths such as *Toxocara canis*.

Regarding the relative sensitivities of each diagnostic method (Table 2), the sedimentation technique had a significantly higher positive rate of trichurid egg detection than did the centrifugal flotation technique ($P=0{\cdot}016$). Notably, the centrifugal flotation technique failed to detect instances of low egg density/abundance (less than 10 eggs per mount in the competitor). Because of the relatively low fecundity of *T vulpis* (Miller 1941), the sedimentation technique appears to be a preferable means of detection. Despite its apparent zoonotic potential (Dunn and others 2002), the prevalence of *T vulpis* has been somewhat overlooked. Consequently, underestimation or underscoring the potential of this parasite is likely to occur if inadequate diagnostic tests are adopted.

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TABLE 1: Number (%) of faecal samples positive for intestinal helminth eggs in 134 hunting dogs and 86 companion dogs from the Aomori prefecture, Japan, by helminth species

Helminth species	Hunting dogs Number (%) positive	95% CI	Companion dogs Number (%) positive	95% CI
Toxocara canis	0 (0)	0-2-2	4 (4·7)	1·3-11·5
Trichuris vulpis	52 (38·8)	31·0-47·3	14 (16·3)	10·0-25·5
Dipylidium caninum	0 (0)	0-2·2	2 (2·3)	0·3-8·2
Taenia species	1 (0·7)	0·02-4·1	0 (0)	0-3·4
Metagonimus species	2 (1·5)	0·2-5·3	0 (0)	0-3·4

TABLE 2: Comparison of the sensitivity of centrifugal flotation and formalin-ethyl acetate sedimentation techniques for the detection of *Trichuris vulpis* eggs in 220 canine faecal samples

		Sedimentation Positive	Negative	Total
Centrifugal flotation	Positive	55	1	56
	Negative	10	154	164
Total		65	155	220

神奈川県におけるアライグマの駆除とアライグマ回虫の調査

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Key Words：アライグマ，アライグマ回虫，神奈川県，外来生物法，監視体制

はじめに

アライグマ回虫（*Baylisascaris procyonis*）は，アライグマの原産地である北米大陸において普通に認められる腸管内寄生虫で，基本的にアライグマ以外の動物では成虫になることはないが，ヒトがその虫卵を経口摂取すると幼虫移行による致死的な中枢神経障害を引き起こすことがある。米国では，1984年と1985年に相次いで幼児死亡例が確認され，最悪の「動物由来寄生虫症」と目されており，現在までに5名の死亡例を含む少なくとも13名の重症例が報告されている[1]。

近年，わが国では展示用動物やペットに由来するアライグマの野生化が，農業被害や在来種の絶滅危惧（生物多様性に及ぼす悪影響）などの問題を引き起こし，特に，北海道，神奈川，岐阜，愛知，和歌山などの各県では深刻な事態となっている。このような状況から，2005年6月に施行された「外来生物法」ではアライグマを「特定外来生物」に指定し，野生個体の積極的な駆除と，輸入，飼育，販売の原則禁止が求められることとなった。それと共に，野生アライグマの駆除作業に伴うアライグマ回虫による健康被害の予防対策も重要となっている。

われわれは1999年からアライグマ回虫に関する全国調査を開始し，動物園等の展示施設のアライグマ，ならびに捕獲された野生アライグマについての調査報告の収集や糞便検査等を実施した[2)3)]。その結果，動物園等での飼育群からはアライグマ回虫の寄生例が少なからず確認されたが，全国の野生アライグマからは，現在のところアライグマ回虫の寄生例は発見されていない。神奈川県は，首都圏にあって最も野生アライグマ問題が先鋭化している地域であることから，この調査を開始して以来7ヵ年間にわたりアライグマの生息状況をフォローすると共に，直接アライグマ回虫の検査を実施してきた。「外来生物法」の施行に伴って野生アライグマの駆除が全国的に実施されつつある現在，神奈川県を国内でのアライグマ回虫問題の一典型として，現在の問題

Surveillance of *Baylisascaris procyonis* Infection among Wild Raccoons in Kanagawa Prefecture, Japan

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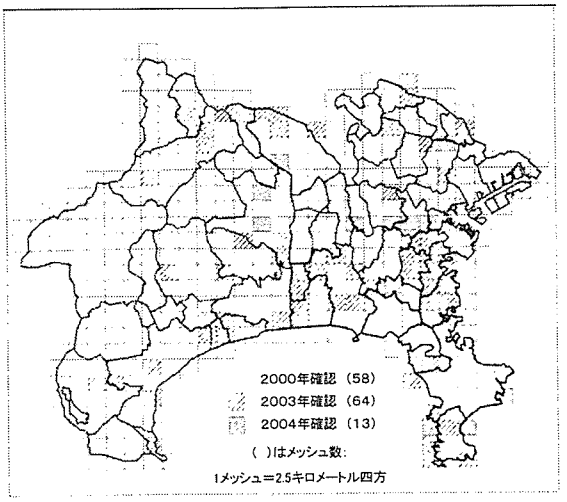

図1　神奈川県における野生化アライグマの生息分布の拡大状況（計画書[5]に依る）

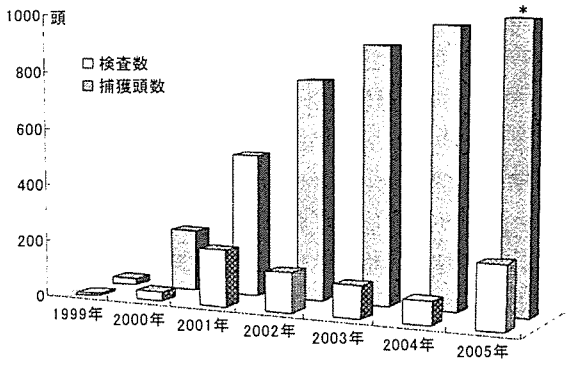

図2　捕獲した野生化アライグマの数と糞便検査実施状況

＊：捕獲頭数は計画書[5]に依り，2005年は推定頭数．

点の整理をする。

県内動物園で展示されているアライグマのアライグマ回虫検査

神奈川県には，社団法人日本動物園水族館協会に所属している動物園が5施設，その他小規模のいわゆる「ふれあい系動物園」が少なくとも14施設存在する。アライグマの展示がなされているのは，このうちの5施設で，個体数は合わせて13頭であった。2001年に，「ふれあい系動物園」の1施設において，飼育されている複数のアライグマからアライグマ回虫卵が検出された。調査の結果，その前年に他県の動物園から導入したアライグマがアライグマ回虫に感染していたものと推定され，その個体によって以前からの飼育アライグマにも感染が広がったものと判明した。この施設では，木造什器の焼却処分，ならびに飼育区域の床，壁などの熱湯処理による虫卵徐染作業を行い，全飼育個体について糞便検査による確認を行いつつ数ヵ月にわたる継続的な駆虫を実施することによって汚染を終息させた。そして，その後も獣医師による定期的な監視が続けられている。

表1　神奈川県における捕獲アライグマ数と糞便検査実施状況

捕獲場所	捕獲年							地区別検査数
	1999	2000	2001	2002	2003	2004	2005	
鎌倉市	8	26	140	80	104	46	73	477
横須賀市				9		4	96	109
藤沢市		3	38	22	5	5	9	82
横浜市			5	4	4	19	20	52
逗子市			5	8		5	23	41
相模原市		1	8					9
三浦市				5		2		7
茅ヶ崎市				5			1	6
城山町			2					2
津久井町		2						2
寒川町		1						1
小田原市			1					1
不明				6				7
年別検査総計	8	33	199	139	113	81	222	796

神奈川県における野生アライグマのアライグマ回虫検査

神奈川県における野生アライグマは，鎌倉市において1988年頃に目撃されたものが最初である[4]。その後分布を拡げてゆき，三浦半島とその周辺域で農業被害が増大し始めたのが1999年であった。さらには，三浦半島から鎌倉，湘南までの地区で高密度に繁殖しつつ，その他の地区でもさまざまな被害情報や捕獲例が増加するなど生息域を急速に拡大していることが確認された（図1）。有害（鳥）獣として捕獲されたアライグマは1998年（平成10年度）では僅かに4頭であったが，以後，急速に増加し，2003年は11市町で903頭，2005年には1,000頭近くになっている。そして2006年3月に発表された「神奈川県アライグマ防除実施計画」では，この時点での県内の野生アライグマの推定生息数を4,030

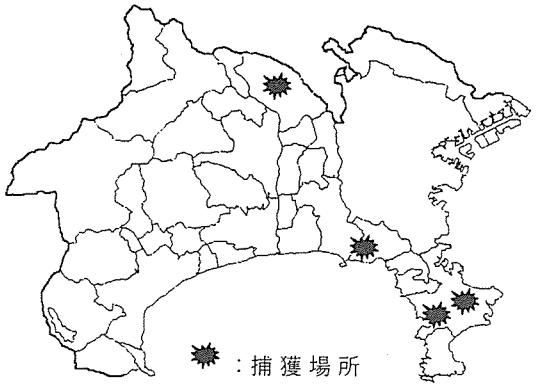

図3　神奈川県内のタヌキ回虫寄生アライグマ捕獲場所

頭としている[5]。

表1は，現在までの捕獲アライグマの糞便検査の実績を地区別にまとめたものである。検査サンプルは，鎌倉市，横須賀市，藤沢市，横浜市，逗子市で捕獲されたものから採取されたものが全体の95%を占める。また，図2はこのようにして実施したアライグマ回虫の検査が，この期間に捕獲されたアライグマに対してどれ程の比率にあるかを示したものである。結論的に言えば，1999年以来，県内で捕獲された野生アライグマは約4,400頭であり，そのうちの約800頭（17%）について糞便検査を実施したが，これらの糞便からはアライグマ回虫卵は検出されなかった。

捕獲アライグマからのタヌキ回虫の検出

これまでの調査において，捕獲アライグマからはアライグマ回虫は検出されなかったが，タヌキ回虫（*Toxocara tanuki*）の寄生例が見つかっている。最初の例は，2001年に相模原市で捕獲された野生アライグマからタヌキ回虫の虫卵と成虫が検出されたものである[6]。アライグマ回虫卵の形態はタヌキ回虫卵と類似しているため，これらの種鑑別には成虫を採取してその形態的特徴を確認することが望ましい。しかし成虫が採集できなかった場合は，虫卵由来のDNAによるPCRを実施し遺伝子を解析することで同定が可能である[7]。タヌキ回虫は，その後，図3に示す様に鎌倉市からの1頭，横須賀市からの2頭を加えて，現在までに捕獲アライグマ4頭からその寄生例が確認されている。

考　察

野生アライグマに関する行政的な対応は，2005年に「外来生物法」が施行されたことにより大きな転換期を迎えた。神奈川県においては，環境省からアライグマ対策モデル事業の指定を受けて2005年の夏に横須賀市内の繁殖地を重点調査域として生態調査を行い，その調査結果を踏まえて2006年3月には10ヵ年で県全域からの完全排除を目標とした「神奈川県アライグマ防除実施計画」が策定された[5]（http : // www.pref.kanagawa.jp/osirase/ryokusei/ysi/keikakuhonbun.）。しかし，この計画は県の環境農政部門が推進し市町村が実施母体となるために衛生行政との連携が不十分であり，アライグマ回虫を始めとする動物由来感染症への対策が十分に実施され難いという問題点がある。

現在まで，神奈川県の野生アライグマからはアライグマ回虫検出例は1例もないが，動物園でアライグマ回虫が検出された事例があり，今後も何らかの機会に感染個体が野生化する可能性を否定できない。アライグマ回虫に関しては，1頭でも感染獣の侵入があれば非感染獣への伝播は容易に起こりうること，しかも虫卵汚染の除去を徹底して行わない限り，容易に再感染を繰返すことが確認されている[3]。また，アライグマ回虫卵は粘着性が強いため除去が容易でなく，また自然環境下での耐熱耐寒性が強く，クレゾールなどの消毒薬にも強い抵抗性を示す上，数年にわたって感染力を保持する。そのため一旦，野生アライグマの中に感染獣が発見された場合，その地域における虫卵汚染対策は困難を極めることが予想され，何よりも迅速な状況把握と対応が求められる。

一方，これまでに捕獲アライグマから4例のタヌキ回虫が検出されたが，これはタヌキの生息域にアライグマが侵入したために起きたことであり，今後はタヌキ回虫の伝播がアライグマ個体間でも容易に起こりうると推定される。タヌキ回虫は，イヌ回虫（*Toxocara canis*）と同じトキソカラ属で，ヒトに対してイヌ回虫程度の幼虫移行症を起こしうると考えられているが，アライグマ回虫ほどの重篤な障害を

起こす可能性は低い。従ってこれからは，捕獲アライグマから回虫卵を検出した際には，行政的な対応の必要性を判断するためにも必ずタヌキ回虫卵との鑑別を行うことが必要になったと云える。これらの事実を踏まえて，現在進行中のアライグマ駆除事業に，自治体レベルでのアライグマ回虫監視と緊急時に対応できる連絡体制を組み込むことが急務であると考える。

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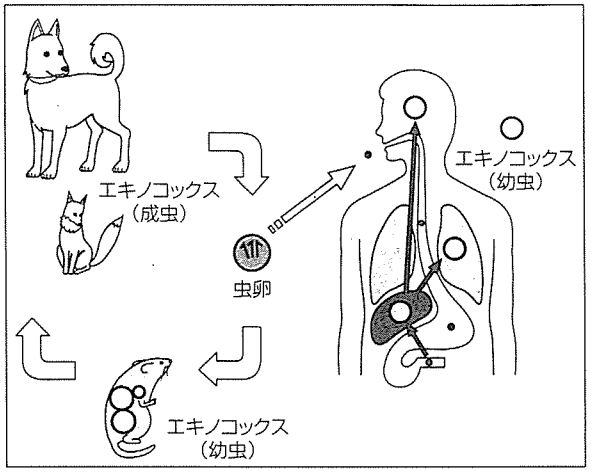

図 1　エキノコックス症の感染経路

埼玉県で捕獲犬一頭からエキノコックス虫卵を検出

2005 年 9 月 9 日，“「エキノコックス」埼玉で確認”，“野犬一頭からエキノコックス，北海道から来る？”といったニュースが，新聞やテレビで報じられたことをご存知だろうか．埼玉県の動物指導センターと衛生研究所が，調査事業のなかで埼玉県北部を放浪していたイヌを捕獲して糞便を検査し，国内では北海道でしかみつからないはずのエキノコックス（多包条虫）の虫卵を検出したというものである．詳しい経過は，国立感染症研究所（感染研）で出している「病原微生物検出情報」で報告されている[1]．

本稿では，イヌのエキノコックスはヒトの病気とどんな関係にあるのか？ということと，近年進歩したイヌのエキノコックス検査法について述べる．1999 年に施行された感染症の予防及び感染症の患者に対する医療に関する法律（感染症法）により，ヒトのエキノコックス症は「4 類感染症」として医師からの届け出を要する疾病となり，2004 年 10 月より，イヌのエキノコックス感染事例も獣医師による届け出を要するものとされているからである[2]．

イヌのエキノコックスはヒトの病気とどんな関係にあるのか？

日本国内では，エキノコックス（多包条虫）は，現在のところ北海道にだけ分布する寄生虫である．成虫はキツネの腸管に寄生し，排泄する糞に混じって外へ出た虫卵が野ネズミに摂食されると，肝臓などで幼虫（包虫）が増殖する．こうした感染野ネズミがキツネに捕食され，腸管内でエキノコックスが幼虫から成虫になることで生活環が維持されている（**図 1**）．

ヒトは野ネズミと同様に虫卵を飲み込むことでエキノコックスに感染するが，中間宿主として好適ではないので幼虫の増殖には長期間を要する．感染から発症に至るのは 5～20 年後であり，肝腫大，腹痛，黄疸などの症状から始まり，有効な治療が施されなければ死亡率は 90%ときわめて高い．

イヌの終宿主としての好適性はキツネと差異はないと考えられるが，生活圏と食性からみて，イヌが野ネズミを摂食することは一般にまれとされていた．それでも最近の調査では，北海道で飼育されているイヌの 1～3%がエキノコックスに感染していることがわかっている．イヌは感染してもとくに症状はなく元気なので，症状からその感染の有無を知ることはできない．感染犬からの虫卵の排出は，感染後 4 週から始まりおおむね半年間に及ぶ．飼育犬はヒトとの関係が密接なので，周囲の人々はこの期間，感染の危険に曝されるの