



## NOTE

Parasitology

# High probability of pet dogs encountering the sylvatic cycle of *Echinococcus multilocularis* in a rural area in Hokkaido, Japan

Takao IRIE<sup>1)\*</sup>, Kyoji YAMADA<sup>2)</sup>, Yasuyuki MORISHIMA<sup>3)</sup> and Kinpei YAGI<sup>1)</sup><sup>1)</sup>Medical Zoology Group, Department of Infectious Diseases, Hokkaido Institute of Public Health, North 19, West 12, Kita-ku, Sapporo, Hokkaido 060-0819, Japan<sup>2)</sup>Yamada Veterinary Clinic, Midorimachi-Kita 1-8-3, Nakashibetsu, Hokkaido 086-1165, Japan<sup>3)</sup>Department of Parasitology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan*J. Vet. Med. Sci.*

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**ABSTRACT.** Surveillance of *Echinococcus multilocularis* in 98 pet dogs kept in a rural area of Hokkaido, Japan, from March 2018 to March 2019 suggested infection in seven dogs (7.1%) by *E. multilocularis*-specific copro-DNA examination, and one of them excreted *E. multilocularis* eggs that were identified by sequence analyses. Among the infected dogs, three were not allowed to run free when outdoors. Based on detection of *E. multilocularis* eggs in fox feces collected from roadsides in the same area, dogs kept in rural areas may have a high probability of becoming infected after preying on infected voles along such roadsides, even in domesticated settings. Therefore, examination along with periodic deworming administration is considered necessary to prevent transmission from dogs to owners.

**KEY WORDS:** dog, *Echinococcus multilocularis*, epidemiology, Japan, zoonosis

Every year, several human cases of alveolar echinococcosis caused by *Echinococcus multilocularis* (Cyclophyllidea: Taeniidae) are reported in Hokkaido, Japan [12]. Human infection occurs through accidental ingestion of parasite eggs excreted in feces of carnivorous definitive hosts such as foxes and dogs. In Hokkaido, the tapeworm is maintained through a fox–vole sylvatic cycle with high endemicity (30–40% infection rate in the red fox) [8]. In endemic regions, pet dogs may also be infected with *E. multilocularis*, and are a potential source of infection of humans [1, 3, 9]. Dogs become infected with the tapeworm only when they prey on a metacystode-infected vole. Canine infection is assumed to be mostly dependent on a dog's behavior towards voles (which is mainly based on individual habit and/or discipline by the owner) and habitat, especially the distance from infected wild voles, rather than the dog's biological background (e.g., breed, age, or gender).

Dogs infected with *E. multilocularis* are mostly asymptomatic [2] making it difficult to detect infection through normal health checkups alone. Therefore, the risk of transmission from infected dogs to humans through shedding of parasite eggs remains a significant concern. Necropsy examination of stray/sheltered dogs (conducted in Hokkaido by the government since 1966) demonstrated 1.0% prevalence of *E. multilocularis*, but this may be an underestimate as the number of dogs examined has decreased in recent years [8]. A mass coprological study of pet dogs in Hokkaido in 1997–2007 revealed that *E. multilocularis* eggs were excreted in 0.3% of city dogs and 0.7% of dogs from towns and villages [14]. Study of shelter dogs in Sapporo city, Hokkaido, in 2013–2017 indicated infection in 1.9% of dogs by *E. multilocularis*-specific copro-DNA examination [7]. However, data for rural areas, where *E. multilocularis* is endemic among wild animals, is lacking. We therefore assessed pet dogs living in rural towns of eastern Hokkaido for *E. multilocularis* using copro-DNA and fecal egg examinations, along with interviews of owners regarding the dog's living conditions.

From March 2018 to March 2019, 98 dogs kept by 79 owners who brought their dogs to an animal clinic in eastern Hokkaido for care were included in this study. Stool sample collection and owner interviews were conducted by one of the co-authors (Yamada K., DVM). Dog came from at least six towns near Nakashibetsu, Shibetsu District, eastern Hokkaido, where land use is mainly pasture for dairy farming and the population density is 10.0–34.7 persons/km<sup>2</sup>. To increase the sensitivity of the tests, diagnostic deworming was conducted before fecal collection; dogs were administered the deworming agent praziquantel (5 mg/kg body weight) by the veterinarian and feces deposited the day after administration was collected for examination [4, 6]. Feces were

\*Correspondence to: Irie, T.: tkirie@iph.pref.hokkaido.jp

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heated to 70°C for 12 hr to inactivate *Echinococcus* eggs and then stored at -30°C until examination. Based on the results of a survey conducted at the time of fecal collection, the following information related to pathways of infection was obtained from the dog owners: 1) land use around the home (town, suburb, or farming area); 2) placement of dog's bed (always indoors or always/sometimes outdoors); and 3) history of running outdoors (always leashed when outdoors, accidentally broke free, allowed to run free, or unknown).

Copro-DNA examination specific for *E. multilocularis* was performed on 3 g of feces, as previously described [6], followed by confirmation by sequencing analysis of the cytochrome *c* oxidase subunit 1 (COI) gene [13]. Fecal egg examination was performed using 1 g of feces with a modified sucrose flotation method (specific gravity of 1.27) [5]. For species identification, eggs were collected from the supernatant of the centrifuge tube on a nylon mesh (pore size 20 μm), and the COI and nuclear U1 spliceosomal RNA (U1 snRNA) genes were analyzed as previously described [7].

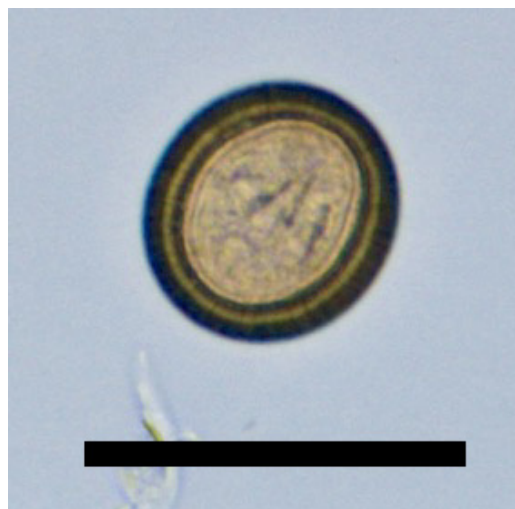
Copro-DNA examination identified 7 of 98 dogs (7.1%) as being positive and *E. multilocularis* was confirmed in all positive samples by sequencing analysis; these dogs were strongly suggested to be infected with *E. multilocularis*. Taeniid eggs (Fig. 1) were detected by the flotation method (6 eggs per gram of feces) in one dog, which was also copro-DNA positive, and the eggs were identified to be from *E. multilocularis* by sequencing of the COI and U1 snRNA genes. Owner survey results concerning exposure of dogs to infection risk are shown in Table 1. Dogs that tested positive for copro-DNA were from homes with each type of land use. The prevalence in surveyed dogs was higher than that reported recently in sheltered dogs in Sapporo city using the same coprological methods [7]. The odds ratio of canine infections in the present study to those in that study was 3.9 (95% confidence interval 0.99–15.5), and the difference in infection risk was suspicious but not significant, consistent with a previous study by Nonaka *et al.* [14].

In the same area that was home to the surveyed dogs, fox feces was collected, following the methods of Morishima *et al.* [11], from areas that foxes are likely to use, such as the edges of agricultural fields and along paths or roads. Fecal collection was conducted twice, in July 2018 (n=73) and October 2018 (n=69). Fox feces were heat-treated as described above and subjected to copro-DNA and fecal egg examination. In the fox feces, 45 of 142 (31.7%) samples were positive for copro-DNA and 23 (16.2%) were positive for *E. multilocularis* eggs. The positive rate by copro-DNA examination in fox feces was compatible with the recent report of *E. multilocularis* prevalence in foxes in Hokkaido by necropsy [8]. Moreover, it was clearly shown that *E. multilocularis* eggs are included in fox feces deposited along roadsides in the study area, and that could serve as an infection source for the intermediate host wild voles (mainly *Myodes rufocanus bedfordiae* in Hokkaido), which inhabit pastures.

From questionnaires to owners of the dogs, 42 (42.9%) of the dogs included in our study were suspected of having a greater chance to capture an infected wild vole because they were permitted to run free outdoors. Four dogs with suspected infections had been allowed to run free on walks. When allowed to run free, even for only a few hours, dogs may catch infected voles when out of sight of their owners [10]; therefore, permitting dogs to run free should be avoided in *E. multilocularis*-endemic areas. However, among the other three dogs that were *E. multilocularis*-specific copro-DNA positive, two had a bed that was always placed indoors and were kept on a leash when outdoors. Considering that 16.2% of fox feces collected along the roadside contained detectable tapeworm eggs, infection of voles could occur in such locations. Although the home range of the vole *M. rufocanus bedfordiae* varies by population density, season and vegetation, it has been estimated at approximately 30 m [15]. Therefore, it is possible for dogs to encounter infected voles at the side of roads where owners take their pet dogs for walks, and the owners of dogs that

**Table 1.** Land use, exercise habits of pet dogs, and numbers of dogs with copro-DNA positive findings confirming *Echinococcus multilocularis* infection

Category	Item	Number of dogs	
		Surveyed	Copro-DNA positive (%)
Land use of the home			
	Town	54	2 (3.7)
	Suburb	11	1 (9.1)
	Farming area	33	4 (12.1)
Placement of dog's bed			
	Always indoors	66	2 (3.0)
	Always/sometimes outdoors	32	5 (15.6)
History of running free outdoors			
	Always leashed when outdoors	20	3 (15.0)
	Accidentally broke free	6	0
	Allowed to run free	42	4 (9.5)
	Unknown	30	0



**Fig. 1.** Egg of *Echinococcus multilocularis* detected in feces of a pet dog. The oncosphere was observed in the embryophore. Scale bar=50 μm.

became infected despite being always leashed reported walking their dogs in areas with thick grass, such as along roadsides near their houses.

In fecal egg examination, eggs of *Toxocara canis* were detected in one dog, and those of Capillariidae in two other dogs. The latter eggs might be from voles infected with adult worm of *Calodium hepaticum* (syn. *Capillaria hepatica*) and passed through the intestines of dogs that preyed on vole. Detection of capillariid eggs in dog feces supports the suggestion that dogs can catch wild voles and thus are at risk of infection with *E. multilocularis*. In fact, seven dogs in our study had been seen by their owners to have interest in and/or to catch a wild vole, one of which dogs was copro-DNA positive, although the total number of dogs that had such habits could not be evaluated due to most owners unfortunately not responding to our questioner concerning this point.

In this study using evaluation of *E. multilocularis* infection by copro-DNA and fecal egg examinations, a high prevalence of *E. multilocularis* was found in pet dogs in a rural area in eastern Hokkaido, Japan. In rural areas where *E. multilocularis* is endemic, pet dogs could more easily encounter the sylvatic cycle of *E. multilocularis* than in urban areas; transmission of the tapeworm from fox to vole and from vole to dog may be expected to occur easily. Therefore, owners should avoid allowing their dogs to run free, and should carefully observe their dog's activity on walks, even if the dogs are leashed. Moreover, regular screening of pet dogs for the tapeworms is recommended, and periodic administration of deworming agents is considered an important measure to prevent transmission from dogs to owners.

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