resources, including local residents for fecal collection and bait distribution, locally produced fish-waste products for bait manufacturing, and local fund-raising to support the deworming program was found to be imperative for the success of a sustainable program for the control and prevention of echinoccocosis. This model is a potential revolutionary tool to control Cryptosporidiosis. Dairy cattle farmers and their cooperatives can be harnessed to lead in intervention planning to reduce risks of environment contamination by *Cryptosporidium*. Furthermore, communication between all professionals involved in this cryptosporidiosis surveillance should be established[7].

Conclusions

Environment-borne cryptosporidiosis poses a tremendous public health threat worldwide. Several cryptosporidiosis outbreaks and alveolar echinococcosis infections have been documented in Japan from contamination of drinking and recreational water and food.

To counter devastating outbreaks of cryptosporidiosis we advocate for sustainable control options against environmental contamination and endogenous development as a tool based on experiences in the control of echinococcosis. The initiative of local NPOs, coupled with the aid of FEA was successful at facilitating control of echinococcosis Fig.4. We also push for continuous surveillance studies and strengthening of public health policies regarding reporting system and cooperation between all local residents, farmers, professionals involved to plan for intervention programs. Overall, we promote to counter these emerging environment-borne zoonoses at the source of contamination to create a healthy environment for a healthy people.

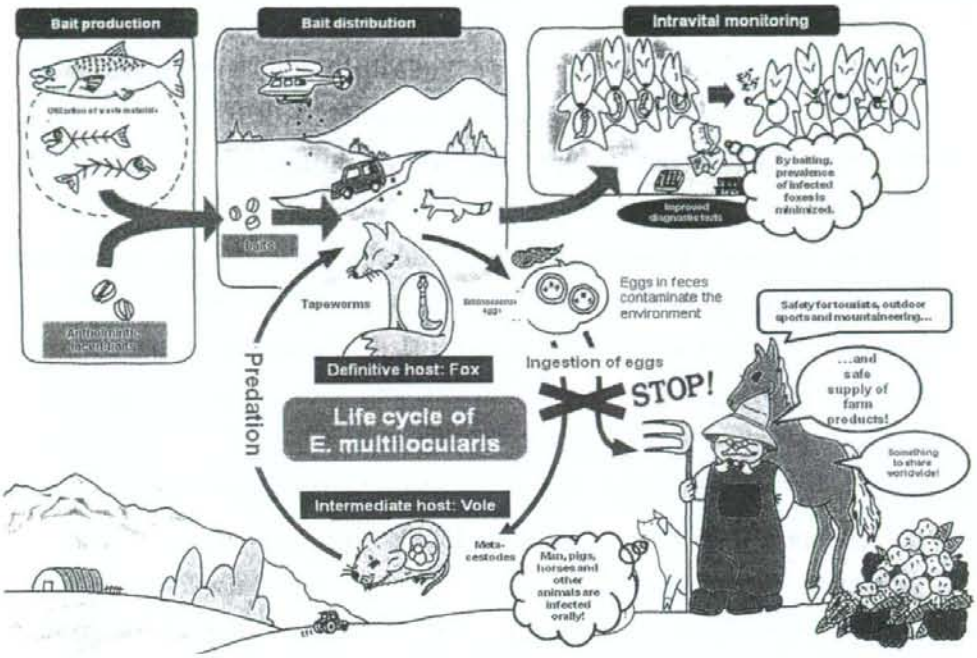

Figure 4. General concept of the countermeasure against the sources of infective agents of echinococcosis.

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水と食物がアブナイ！混入する病原体
クリプトスポリジュムとエキノコックスを例にして

神　谷　正　男
酪農学園大学教授
環境システム学部生命環境学科環境動物学研究室
国際獣疫事務局（OIE）エキノコックス症リファレンス・ラボ

要　旨

飲料水や食物に混入する病原体：クリプトスポリジュムとエキノコックスが問題になっている。これらは、家畜や野生動物の糞便に由来する人獣共通感染症を引き起こし公衆衛生上の脅威となっている。わが国で発生した人体クリプトスポリジュム症（1994～2006年）は、下痢などの比較的軽症な疾病として知られているが、処理の不完全な上水道や水泳プール利用などで集団発生が認められている。感染源や従来の対策について言及した。人体エキノコックス症（多包虫症）は、世界的にも深刻な問題となっているが、北海道で500例以上の手術例が報告されていて、毎年、20例前後の新患者が報告されている。感染源はキツネ、イヌなどの糞に排出される虫卵が食物などに混入する。経口的に摂取されると肝臓などに悪性腫瘍様の病巣を形成し死にいたる病である。健康被害のみならず、地域の農業、観光などへ重大な損害を与えるので、感染源動物であるキツネの駆虫剤（安全性と効果が確認されているプラジカンテル：PZQ入りのカマボコ）を用いて汚染地域の浄化を可能にする技術を、地域の産物（水産廃棄物など）や人材、伝統的な技術を活用する、いわゆる、『内発的発展』例として紹介した。これらの環境由来の人獣共通感染症対策を通して地域住民（健民）のための安全・安心な環境（健土）創りを提唱する。

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Short communication

The first instance of a cat excreting *Echinococcus multilocularis* eggs in Japan

Nariaki Nonaka [a,*], Haruki Hirokawa [b], Takashi Inoue [a,1], Ryo Nakao [a,2], Sumiya Ganzorig [c], Fumio Kobayashi [c], Masakazu Inagaki [a,3], Kentaro Egoshi [a,4], Masao Kamiya [d], Yuzaburo Oku [a]

[a] Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan
[b] Nirenoki Animal Hospital, Obihiro 080-0854, Japan
[c] Forum on Environment and Animals, Sapporo 065-0020, Japan
[d] Faculty of Environment Systems, Rakuno-Gakuen University, Ebetsu 069-8501, Japan

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ABSTRACT

A cat excreting *Echinococcus multilocularis* eggs was recently identified in Hokkaido, representing the first such observation in Japan. The cat was raised free-range and frequently ate rodents. Fecal egg examination revealed eggs of taeniids (EPG: 440) and *Spirometra* spp. (EPG: >1000). PCR targeting part of the mitochondrial cytochrome *c* oxidase subunit I gene of *E. multilocularis* was positive with DNA from 3 single isolated taeniid eggs, and sequence analysis of one amplicon confirmed *E. multilocularis*. The results indicated that the eggs of *E. multilocularis* distributed in Hokkaido can be excreted in cat feces, and suggested the necessity of further studies to clarify whether the eggs excreted in cat feces are infective and thus whether cats can serve as infectious source to humans in Japan.

In Hokkaido, Japan, the prevalence of *Echinococcus multilocularis* in foxes has been approximately 40% over the last two decades, whereas the prevalence in pet dogs has been 0.3% [1]. Since October 2004, a reporting system for canine echinococcosis has been enforced in Japan, and eight cases had been detected as of December 2007.

In contrast to dogs, infection in cats has not been the focus of efforts in Japan, although infections with immature (non-patent) worms were found in five of 108 cats necropsied from 1966 to 2006 (data provided by Hokkaido Government). In our survey (1997 to 2007), nine of 486 cats were positive in a coproantigen test [2], although none of those cats excreted *E. multilocularis* eggs (unpublished data).

In the present work, we describe the first reported instance of fecal *E. multilocularis* egg excretion by a pet cat in Japan. The female, 6-year-old cat was raised free-range in Hokkaido and frequently ate rodents. A fecal egg examination with sucrose (specific gravity: 1.27) flotation technique (2) was performed on 0.5 g of the cat feces, revealing eggs of taeniids (the number of eggs per gram [EPG]: 440) and *Spirometra* spp. (EPG: >1,000). In the preliminary study using dog feces, the method detected 30% of total number of *Taenia taeniaeformis* eggs added in feces. Taeniid eggs found in the cat feces had thick embryophores with oncospheres inside, thus considered as being morphologically mature (Fig. 1). However, a coproantigen test [2] was negative. The reliability of the coproantigen test for cat feces was not fully evaluated: the test detected coproantigen in the feces of cats experimentally infected with *E. multilocularis* [3]; however, in our survey described above, non-specific positive reactions of the test were relatively more frequently observed in cat feces than in dog feces (unpublished data). Therefore, we considered that the test could detect coproantigen in cat feces, but was less reliable for cat feces than dog feces. The reason why the test was negative for the present cat feces is not clear since we do not have sufficient data for evaluating the sensitivity of the test for cat feces. No conditions of the feces which may have affected on the test result were recognized.

Polymerase chain reaction (PCR) was performed on DNA from eggs using the EmSP1-A'/B' primer set (5'-GTCATATTTGTTTAAGTATAAGTGG-3'/5'-CACTCTTATTTACACTAGAATTAAG-3'), designed to amplify a partial fragment (243 base pairs) of the *E. multilocularis* cytochrome *c* oxidase subunit I gene (*cox1*). DNA of 5 isolates of *E. multilocularis* was amplified. Cross-reactivity has not been detected *in silico* with *cox1* sequences registered in GenBank or with DNA extracted from *E. granulosus* (G1 and G6), *E. vogeli*, *Taenia ovis*, *T. pisiformis*, *T. hydatigena*, *T. crassiceps*, or *T. taeniaeformis*.

Abbreviation: *E.*, *Echinococcus*; *T.*, *Taenia*; EPG, eggs per gram; PCR, polymerase chain reaction; DNA, deoxyribonucleic acid; *cox1*, cytochrome *c* oxidase subunit I gene; μl, micro-liter; min, minutes; s, seconds.

* Correspondening author. Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Kita-ku Kita 18 Nishi 9, Sapporo 060-0818, Japan. Tel./fax: +81 11 706 5196.
E-mail address: nnonaka@vetmed.hokudai.ac.jp (N. Nonaka).
[1] Central Institute for Experimental Animals, Kawasaki 216-0001, Japan.
[2] Research Center for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan.
[3] Chubu Shiryo, Chita 478-8502, Japan.
[4] Asahi Kasei, Tokyo 100-0006, Japan.

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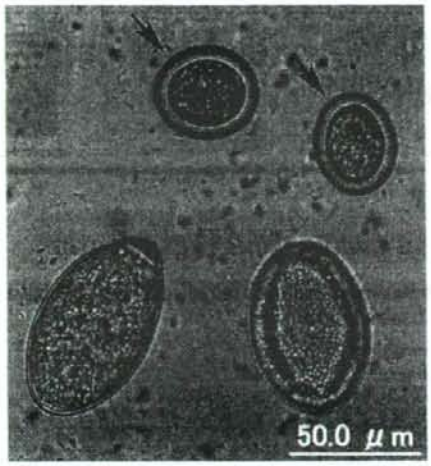

Fig. 1. Taeniid and *Spirometra* eggs found in the feces of a cat. Taeniid eggs (arrows) had thick embryophores and contained oncospheres with six hooks.

Egg DNA was extracted using a QIAamp DNA mini kit (Qiagen). PCR was performed in a GeneAmp® PCR system 9700 (Applied Biosystems) using a HotStarTaq master mix kit (Qiagen). The reaction mixture (20 μl) was composed of the PCR master mix provided in the kit (10 μl), water (6.4 μl), 25 μM of each primer (0.8 μl), and template DNA (2.0 μl). The PCR conditions were pre-incubated at 95 °C for 15 min, followed by 40 cycles of 94 °C for 60 s, 50 °C for 90 s, and 72 °C for 60 s, and a final incubation at 72 °C for 10 min. The amplicon was examined by agarose gel electrophoresis. A negative control without DNA was included in all tests.

To prepare the DNA for the initial PCR, eggs were isolated by a sieving/flotation technique [4]. The DNA extracted from the sieve-trapped eggs (a mixture of taeniid and *Spirometra* eggs) produced a specific product. To confirm that the amplification solely resulted from DNA from taeniid eggs, PCR was performed in addition with DNA extracted from three single isolated taeniid or *Spirometra* eggs. A specific product was observed in the taeniid samples ($n=3$) but not in the *Spirometra* samples ($n=3$).

The sequence of the amplified product from a single isolated taeniid egg was determined with a Beckman CEQ 8000 DNA analyzer using a GenomeLab DTCS Quick Start kit (Beckman Coulter). The sequence obtained was identical to that of *E. multilocularis* registered in GenBank (accession no. M84668) and differed by 11 or more base pairs from the sequences of other canine taeniid cestodes ([5,6] and GenBank accession nos. AB221484, AF216699, AJ239110, DQ309767–DQ309769, M84661–M84667, and M84670–M84671).

The present finding did not definitively indicate infection of the cat. Given that the cat was negative in the coproantigen test, the simple intestinal passage of the eggs after coprophagy (i.e., pseudoparasitism) might be a possibility. However, coprophagy is a very rare phenomenon in cats, thus an *E. multilocularis* infection of our cat nevertheless appears very likely. In Europe, excreted parasite eggs have been identified from naturally infected cats [7,8] and in an experimental infection with a European isolate [9], but the viability of eggs from cat origin could not be confirmed after mouse inoculation [9], and thus the biotic potential of the parasite in this host was considered very low. With Japanese isolates, the parasites could not develop to maturity in experimental infections in cats [3,10,11]. Consequently, it is necessary to investigate the infectivity of eggs excreted from cats.

Unfortunately, the infectivity of the eggs found in this study could not be evaluated. Nevertheless, our finding highlights the potential of cats to excrete *E. multilocularis* eggs in Japan. Although the chance of the parasite to be mature and produce infective eggs in cats is considered to be low, there are no sufficient data to totally deny the possibility. Therefore, further surveys on feline echinococcosis must be conducted to clarify the importance of cats serving as infectious source to humans in Japan. Meanwhile, cat owners and veterinary practitioners should properly manage cats to prevent potential infection.

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A latex agglutination test for the detection of *Echinococcus multilocularis* coproantigen in the definitive hosts

Nariaki Nonaka [a,*], Mari Oka [a], Masao Kamiya [b], Yuzaburo Oku [a]

[a] *Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan*
[b] *Faculty of Environment Systems, Rakuno-Gakuen University, Ebetsu 069-0836, Japan*

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Abstract

A latex agglutination test for detecting *Echinococcus multilocularis* coproantigen in definitive hosts was developed using latex beads sensitized with EmA9 monoclonal antibody raised against somatic antigens of adult *E. multilocularis*. A primary test (LA 1) was performed on 82 fecal samples of necropsied foxes, of which 46 were infected, and resulted in 61% sensitivity and 86% specificity. To increase the sensitivity, 4 ng/mL of excretory/secretory antigens of adult worms was added to the samples in a secondary test (LA 2), resulting in 91% sensitivity and 61% specificity. The positive predictive value of the LA 1 test and the negative predictive value of the LA 2 test were both 85%. The combination of the LA 1 and LA 2 tests is applicable and practical for use in situations that require quick diagnosis or screening based on the following interpretation: the samples that are positive in the LA 1 test are positive; the samples that are negative in the LA 2 test are negative; and the samples that are negative in the LA 1 test and positive in the LA 2 test are classified as suspicious.

Keywords: *Echinococcus multilocularis*; Latex agglutination test; ELISA; Coproantigen; Diagnosis; Definitive host

1. Introduction

Echinococcus multilocularis, one of the most serious zoonotic parasites, is widely distributed in the northern hemisphere, including Hokkaido, Japan (Eckert et al., 2001). Humans are infected by ingesting eggs derived from the feces of definitive hosts. In Hokkaido, the prevalence of *E. multilocularis* in red foxes, the main definitive host, has been approximately 40% over the last two decades. Moreover, infections of domestic dogs, which are another potential infectious source for humans because of their close proximity, have been reported not only on Hokkaido, but also on the main island of Japan (Kamiya et al., 2007; Morishima et al., 2006; Nonaka et al., 2006). In response to the deteriorating situation, a reporting system for canine echinococcosis has been enforced in Japan since October 2004; as of March 2007, seven cases have been detected.

The ability to perform a rapid and on-site diagnosis/screening for infection in definitive hosts would be beneficial for small animal practitioners in their risk management; however, this is difficult to perform with the currently available diagnostic tools. Fecal egg examination is inaccurate for *Echinococcus* species because of the morphological similarity of eggs among taeniid species and the intermittent excretion of eggs even after maturity (Eckert and Deplazes, 2001;

* Corresponding author. Tel.: +81 11 706 5196; fax: +81 11 706 5196.
E-mail address: nnonaka@vetmed.hokudai.ac.jp (N. Nonaka).

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Nonaka et al., 1996; Yamashita et al., 1956). Coproantigen detection methods have been developed for the diagnosis of *Echinococcus* species using either monoclonal or polyclonal antibodies (Deplazes et al., 1999; Sakashita et al., 1995). Although a certain level of cross-reactivity with *Taenia* spp. infections has been recognized (Allan and Craig, 2006; Malgor et al., 1997), the validity of the assays was ascertained in *E. multilocularis* (Deplazes et al., 1999; Morishima et al., 1999), *Echinococcus granulosus* (Malgor et al., 1997), and *Echinococcus vogeli* infections (Matsuo et al., 2000). However, the assays were based on sandwich enzyme-linked immunosorbent assay (ELISA); thus, it takes one to several days to obtain results using a commercial kit or by outsourcing, respectively. Reliable DNA diagnostic methods are available; however, on-site diagnosis is difficult in most cases. We developed a latex agglutination test for detecting *E. multilocularis* coproantigen for on-site diagnosis.

2. Materials and methods

2.1. Parasitological examination and fecal sample collection

The samples used were obtained from 82 foxes shot in and around Sapporo (Ebetsu, Kita Hiroshima, Nanporo and Otaru) during 1997 to 1999 and were frozen at −80 °C for more than 10 days to sterilize infectious eggs of *E. multilocularis*. The intestinal tract of each fox was removed, and parasitological examination was performed on the intestinal contents and the scraping of the mucosa of the whole small intestine and colon under a stereomicroscope. The number of *E. multilocularis* found was counted. Fecal samples from the rectum were mixed with approximately equal volumes of 1% formalin, incubated at 70 °C for 12 h, and kept at room temperature. They were used for latex agglutination tests and sandwich ELISA.

2.2. Sensitization of latex particles

Polybead carboxylate microspheres were coupled with EmA9, a monoclonal antibody raised against adult *E. multilocularis* somatic antigen (Kohno et al., 1995), according to the manufacturer's instructions for the Carbodiimide Kit for Carboxylated Microparticles (Polysciences, Inc.). Briefly, 0.5 mL of a 2.5% carboxylated latex particle (diameter 1.0 μm) solution was washed twice with carbonate buffer by centrifugation at 13,000 × *g* for 6 min, and the supernatant was removed after each wash. The sediment was washed three times with phosphate buffer in the same manner. The sediment was resuspended with 0.6 mL of phosphate buffer and then stirred for 3.5 h on a wave shaker (MINI WAVE, Iuchi) with an equal volume of carbodiimide solution. The mixture was washed three times with borate buffer. After the supernatant was removed, it was resuspended with 1.2 mL of phosphate buffer that contained 60 μg of EmA9 and stirred overnight at room temperature. It was then centrifuged, and the supernatant was removed. One milliliter of 0.1 M ethanolamine was added to the sediment and stirred for 30 min. The mixture was washed, and the sediment was stirred with 1 mL of BSA solution for 30 min in the same manner. After the supernatant was removed, it was resuspended with 1 mL of storage buffer (final concentration: 1.75%) and stored at 4 °C. The latex particles sensitized with EmA9 were used within 2 weeks.

2.3. Latex agglutination tests

Latex agglutination test 1 (LA 1): samples were diluted to 0.13 g/mL with 0.1% Tween 20 in PBS and centrifuged. The supernatant (15 μL) and sensitized latex particles (5 μL) were mixed on a glass slide. Agglutination was checked after 5 min, and the results were classified into three categories by degree of agglutination: −, no agglutination; +, small agglutination masses formed; ++, large agglutination masses formed. Samples that were + or ++ were considered positive for agglutination (Fig. 1).

Latex agglutination test 2 (LA 2): to increase the sensitivity of LA 1, fecal samples were diluted with buffer containing 4 ng/mL of excretory/secretory antigen of adult *E. multilocularis* (EmES antigen) (Sakashita et al., 1995), and a latex agglutination test similar to LA 1 was performed. However, agglutination was checked after 7 min.

2.4. Sandwich ELISA

To compare the results of LA 1 and LA 2 with those of a standard technique, a sandwich ELISA was performed for coproantigen detection using the method of Morishima et al. (1999). A cut-off value to discriminate between positive and negative samples was calculated as 0.111, which was the mean optical density (OD) plus three standard deviations of fecal samples from silver foxes uninfected with *E. multilocularis* (Kaji mink, Fukagawa).

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Fig. 1. Classification of the degree of agglutination. –: no agglutination observed; +: small agglutination masses formed; ++: large agglutination masses formed.

3. Results

3.1. Necropsy

E. multilocularis was found in 46 of 82 foxes necropsied. The intensity of worms ranged from 1 to 408,556, including 6 foxes with <10 worms, 11 foxes with 10–99 worms, and 29 foxes with ≥100 worms.

3.2. Latex agglutination tests

The results of LA 1 and LA 2 were compared with those of necropsy. The sensitivity of LA 1 was 61% (28/46) and the specificity was 86% (31/36), whereas the positive and negative predictive values (PPV and NPV) were 85% (28/33) and 63% (31/49), respectively (Table 1). When the sensitivity of LA 1 was evaluated by the level of worm intensity, the sensitivity became 29% (5/17) for samples with <100 worms found at necropsy and 79% (23/29) for samples with ≥100 worms (Table 2).

All of the 28 positives in LA 1 remained positive in LA 2 (Table 3). Fourteen of 18 samples that were positive in necropsy, but negative in LA 1, were positive in LA 2. However, 9 of 31 samples that were negative both in the necropsy and LA 1 were positive in LA 2 (Table 3). Accordingly, the sensitivity of LA 2 increased to 91% (42/46), but the specificity decreased to 61% (22/36) (Table 1). The PPV and NPV were 75% (42/56) and 85% (22/26), respectively. When the sensitivity of LA 2 was evaluated by the level of worm intensity, it was still low (50%) for samples with <10 worms, but it became higher for samples with ≥10 worms and was 100% for samples with ≥100 worms (Table 2).

3.3. Sandwich ELISA

The results of the sandwich ELISA were compared with those of necropsy. The sensitivity of the sandwich ELISA was 91% (42/46) and the specificity was 94% (34/36), whereas the PPV and NPV were 95% (42/44)

Table 1
Comparison of the results of necropsy with those of latex agglutination tests (LA 1 and LA 2) and sandwich ELISA

	LA 1			LA 2			Sandwich ELISA		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Necropsy									
Positive	28	18	46	42	4	46	42	4	46
Negative	5	31	36	14	22	36	2	34	36
Total	33	49	82	56	26	82	44	38	82
SE/SP[a]	61%/86%			91%/61%			91%/94%		
PPV/NPV[a]	85%/63%			75%/85%			95%/89%		

[a] SE: sensitivity; SP: specificity; PPV: positive predictive value; NPV: negative predictive value.

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Table 2
Sensitivity of latex agglutination tests (LA 1, LA 2) and sandwich ELISA at different levels of *Echinococcus multilocularis* infection intensity

Intensity (number of worms)	Number of tests positive/number of necropsies positive (%)		
	LA 1	LA 2	Sandwich ELISA
<10	2/6 (33)	3/6 (50)	3/6 (50)
10–99	3/11 (27)	10/11 (91)	10/11 (91)
≥100	23/29 (79)	29/29 (100)	29/29 (100)
Total	28/46 (61)	42/46 (91)	42/46 (91)

and 89% (34/38), respectively (Table 1). The sandwich ELISA showed 50% sensitivity for samples with <10 worms, but 91% and 100% sensitivity for samples with 10–99 and ≥100 worms, respectively (Table 2).

4. Discussion

The latex agglutination tests that we developed were simple, and the process could be completed within 10 min using minimal equipment. Effective latex agglutination tests for antigen detection have been developed for *Trichinella* (Choy et al., 1989) and *Trypanosoma* (Nantulya, 1994) infections with sensitivities of 89% and 88%, respectively. The sensitivity of LA 1 was lower than these results. For diseases like echinococcosis, low sensitivity is a serious disadvantage because the definitive hosts of *E. multilocularis* excrete eggs that are infectious to humans. To increase the sensitivity, a small amount of EmES antigen was added to the dilution buffer of the fecal samples (LA 2). As a result, the sensitivity of LA 2 increased to 91%, but the specificity decreased to 61%. A survey has shown that 92% of infected foxes in Hokkaido harbor >10 worms, and the median number of worms detected in infected foxes was 6400 (Yimam et al., 2002). Accordingly, infected foxes harboring <10 worms are of lesser importance in public health, not only because of their lower worm burden, but also because of their smaller proportion in the population. If only samples with ≥10 worms had been considered, the sensitivity of the LA 2 test would have been 97.5% (39/40) (100% with ≥100 worms).

The LA 1, LA 2, and sandwich ELISA produced a range of sensitivities and specificities. LA 1 had low sensitivity, but the specificity was high. Conversely, the sensitivity of LA 2 was high, but the specificity was low. Considering the predictive values, the PPV of LA 1 and NPV of LA 2 were sufficiently high (both were 85%) (Table 1). Therefore, it is effective to combine the two latex agglutination tests for practical diagnosis. In the diagnosis based on the combination of LA 1 and LA 2, samples that are positive in LA 1 are considered positive, samples that are negative in LA 2 are considered negative, and samples that are negative in LA 1 and

Table 3
Consistency in the results of necropsy, latex agglutination tests (LA 1 and LA 2), and sandwich ELISA

Necropsy	Latex agglutination tests			Sandwich ELISA		Number of worms found at necropsy
	LA 1	LA 2	No. of samples	Positive	Negative	
Positive	Positive	Positive	28	28	0	2 – 408,556
	Negative	Positive	14	12	2	4 – 408
		Negative	4	2	2	1 – 32
Negative	Positive	Positive	5	1	4	0
	Negative	Positive	9	0	9	0
		Negative	22	1	21	0

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positive in LA 2 are classified as suspicious. By this way, the chance for detecting infected case as either positive or suspicious in the combination test would become adequately high for preventing an accidental infection, although interpretation of the suspicious results should be carefully informed to dog owners because 39% (9/(14 + 9)) of samples that showed suspicious reaction in the combination test were those of uninfected cases (calculated from Table 3).

The high prevalence of *E. multilocularis* (approximately 40%) in foxes in Hokkaido and the fact that the habitat of foxes has been getting closer to or overlapping that of humans have resulted in the potential risk of infection to humans and companion animals (Eckert et al., 2000; Oku and Kamiya, 2003; Tsukada et al., 2000). Several surveys in Hokkaido showed that canine echinococcosis occurs in 0.3–1% of examined dogs (Kamiya et al., 2007; Nonaka et al., 2006); this, lead to the enforcement of a national reporting system for canine echinococcosis in Japan. Accordingly, a rapid and reliable screening system for canine echinococcosis is required.

Although the proposed assay was evaluated with fox samples, it would provide a rapid screening tool for infection in companion animals with an acceptable reliability. When the assay is performed on companion animals, the animals diagnosed as positive or suspicious must be further examined using more reliable tests such as coproantigen detection ELISA (Morishima et al., 1999) and DNA detection PCR (Dinkel et al., 1998; Trachsel et al., 2007) and should be dewormed to terminate a possible infection with *E. multilocularis*. Therefore, the assay developed here could contribute to the reporting system and to the risk management for echinococcosis. The assay could also be applied for field surveys where easy and quick diagnosis has a great advantage.

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NOTE *Parasitology*

A Vague Understanding of the Biology and Epidemiology of Echinococcosis by Dog Owners in Hokkaido, an Endemic Island for *Echinococcus multilocularis* in Japan

Nariaki NONAKA[1]*, Masao KAMIYA[2] and Yuzaburo OKU[1]

[1]*Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Kita-ku Kita 18 Nishi 9, Sapporo 060–0818 and* [2]*OIE Reference Laboratory for Echinococcosis, Laboratory of Environmental Zoology, Department of Biosphere and Environmental Sciences, Faculty of Environment Systems, Rakuno-Gakuen University, Bunkyo-dai Midori-machi 582, Ebetsu 069–0836, Japan*

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ABSTRACT. A questionnaire survey was conducted by giving 14 statements about echinococcosis to 2,070 dog owners residing in Hokkaido in order to evaluate their understanding about the biology and epidemiology of *Echinococcus multilocularis*. Analysis of the answers revealed that dog owners understood the disease superficially, and there were several points of confusion in their understanding, especially regarding differences in the modes of transmission and disease development in dogs and humans. The results suggest the need for the proper education of dog owners to perform proper prophylactic measures against the disease.

KEY WORDS: canine, *Echinococcus*, epidemiology, parasitic zoonoses, zoonosis.

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Echinococcus multilocularis is distributed in the northern hemisphere, including Hokkaido, Japan. If humans accidentally ingest the parasite eggs and become infected, the parasite metacestodes develop in the liver and occasionally in other organs and cause a lethal disease, alveolar echinococcosis [10]. The parasite is primarily maintained in the sylvatic cycle, with foxes serving as definitive (final) hosts and voles serving as intermediate hosts. In endemic regions, dogs can also become a final host, serving as a potential infectious source to humans [1, 2, 4, 5, 7, 11]. Therefore, prophylaxis for dog infection is of high importance for risk management of the infection to humans, especially for dog owners [6].

In Hokkaido, the prevalence of infection in foxes has been approximately 40% during the last two decades [9]. By 2007, 531 human patients had been identified (data from the Hokkaido government). However, the routes of infection to humans have not been completely clarified [3, 10]. One possible route of infection that should be considered is via infected pet dogs. In our survey conducted from 1997 to 2007 to determine the prevalence of infection in pet dogs, 0.4% of the dogs examined (n=4,768) excreted taeniid eggs that were identified as *E. multilocularis* eggs by PCR examination of egg DNA [8]. To control echinococcosis, the Hokkaido government has been conducting surveys and countermeasures, including annual surveys on the prevalence of infection in foxes and other animals, development of diagnostic and therapeutic measures for human patients, and education of residents through schools and publications.

In this study, we conducted a simple questionnaire survey of dog owners who requested us to test their dogs for *E. multilocularis* infection in order to assess how precisely dog owners who have a potentially high risk of infection understand the biology and epidemiology of echinococcosis.

From 1997 to 2004, the questionnaire was conducted by giving 9 or 14 statements on the biology and epidemiology of echinococcosis to 2,070 dog owners residing in Hokkaido, who were asked to answer whether each statement is Right, Wrong, or Unknown (Table 1). More than 50% of the dog owners answered statements S1 to S8 correctly. Most of those statements contained descriptions of the basic biology and epidemiology of echinococcosis in Hokkaido, and the dog owners seemed to understand well the current situation of the disease in Hokkaido and the general mode of transmission. In contrast, less than 50% of the dog owners answered statements S9 to S14 correctly. In particular, less than 20% answered S13 and S14 correctly and more than 50% answered incorrectly.

Statements S4, S12, and S14 were related to the transmission of the parasite to dogs. Among the dog owners who answered S4 correctly, the percentages of owners that answered S12 correctly and incorrectly were 48.2% and 32.3%, respectively. For statement S14, the percentages were 24.2% and 60.4%, respectively. The results indicate that most dog owners understood that dogs get the infection by ingesting infected rodents; however, their understanding was vague, and many dog owners thought that dogs also get the infection from foxes, presumably by ingesting the parasite eggs excreted from foxes. One possible reason for this misunderstanding could be confusion regarding the two different modes of transmission to dogs and humans. In another words, many dog owners misunderstood an important characteristic of the parasite life cycle: transmission never directly occurs between two final hosts, such as fox and dog.

Regarding their understanding of the transmission to humans, 69.1% of the dog owners understood that humans

* CORRESPONDENCE TO: NONAKA, N. (present address), Laboratory of Veterinary Parasitology, Department of Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, Gakuen-Kihanadai Nishi 1–1, Miyazaki 889–2192, Japan.
e-mail: nnonaka@cc.miyazaki-u.ac.jp

Table 1. Questions about echinococcosis in Hokkaido given to dog owners and the percentage of correct answers

Statement No.	Statement	No. answered	Correct answer	Percentage correct answer	Percentage of incorrect answer
S1[a)]	Infected foxes inhabit Sapporo[b)].	448	Right	87.9	0.4
S2[a)]	The disease is now spread all over Hokkaido.	382	Right	87.2	3.9
S3	The parasite eggs in the feces of infected dogs can be an infectious source to humans.	2013	Right	86.4	3.4
S4	Dogs are infected by ingesting infected rodents.	2008	Right	80.1	10.3
S5[a)]	Rodents playing a role in the transmition of the disease are those found in houses.	445	Wrong	74.6	6.5
S6	Humans get the infection by ingesting the parasite eggs.	1998	Right	69.1	15.9
S7	Infected dogs have to be euthanized in Hokkaido.	1988	Wrong	60.9	3.3
S8[a)]	The parasite eggs can be killed by boiling them.	444	Right	52.7	15.5
S9	Infected dogs can be dewormed completely with an anthelmintic drug.	1985	Right	49.7	17.1
S10	The disease never transmits from human to human.	1995	Right	48.9	26.1
S11	Humans also get infection by ingesting infected pigs.	1986	Wrong	44.6	22.4
S12	Dogs are infected by physical contact with infected foxes.	1997	Wrong	44.5	33.0
S13	Infected dogs have parasitic legions in the liver.	1997	Wrong	19.7	50.5
S14[a)]	Dogs are infected by ingesting the parasite eggs excreted from foxes.	447	Wrong	19.0	62.4

a) Statements S1, S2, S5, S8, and S14 were given to only 451 owners.
b) The capital city of Hokkaido.

get the infection by ingesting the parasite eggs. However, this understanding was also vague, and 26.1% of the dog owners believed that the disease could be transmitted from human to human (see Table 1, S10). Moreover, 22.4% of the dog owners thought that the disease could be transmitted from infected pigs to humans (see Table 1, S11). In Hokkaido, approximately 2,000 infected pigs (prevalence: 0.2%) are detected annually during meat inspections (data from the Hokkaido Government). However, like humans, pigs get the infection by ingesting the parasite eggs and then develop lesions in the liver, thus serve as accidental intermediate hosts. Therefore, pigs never excrete the parasite eggs and transmission from pigs to humans never occurs. The surveyed dog owners misunderstood another important aspect of the parasite life cycle: transmission never directly occurs between intermediate hosts, as from human to human or from pig to human.

Further confusion was elucidated by the answers to statement S13; 50.5% of the dog owners thought that dogs develop parasitic legions in the liver, indicating that the dog owners thought that dogs develop the same legions as humans.

In conclusion, this study revealed that dog owners residing in Hokkaido, an endemic area of the disease, who have a risk of infection by their dogs superficially understood the biology and epidemiology of *E. multilocularis*. Their understanding about the difference in the mode of disease transmission to dogs and humans was not completely clear, leading them to misbelieve that dogs can get the infection in the same way as humans. This vague understanding was presumably due to the complicated nature of the parasite life cycle in that two different hosts play different biological roles, namely, final and intermediate hosts are required for completing the life cycle of the parasite. From the point of view on a risk management, the understanding on it is of primary importance for individual dog owner to perform effective prophylactic measures against parasite infection in his/her dogs. In order to do so, it is paramount that dog owners precisely understand the parasite life cycle and the events related to the risk of infection of dogs and humans. In this context, the role of veterinary practitioners is very important. We hope that the results of this simple questionnaire survey will be used by veterinary practitioners as a reference for explaining and enlightening dog owners of the disease process, then contribute to the performance of proper prophylactic measures against parasite infection by dog owners.

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Echinococcus multilocularis Infection in Pet Dogs in Japan

Nariaki Nonaka,[1] Masao Kamiya,[2,3] Fumio Kobayashi,[3] Sumiya Ganzorig,[1,3] Satoko Ando,[1] Kinpei Yagi,[4] Takashi Iwaki,[1] Takashi Inoue,[1] and Yuzaburo Oku[1]

Abstract

A survey of *Echinococcus multilocularis* infections in pet dogs in Japan from 1997 to 2007 was conducted by testing for coproantigen reactivity, fecal taeniid eggs, and egg DNA. In Hokkaido, the only island where *E. multilocularis* is endemic in Japan, 18 of 4768 dogs (0.4%) excreted taeniid eggs that were positive for *E. multilocularis* DNA by polymerase chain reaction (PCR). Most of the dogs testing positive for egg DNA were kept free-range, but three dogs had been kept inside their owners' houses. In addition, 15 dogs were suspected to be infected based on the results of a coproantigen test. One dog, which was transported from Hokkaido to Honshu, the main island of Japan, was excreting taeniid eggs that were positive for *E. multilocularis* DNA by PCR. These results suggest the importance of proper pet management in disease prevention, even for dogs kept indoors, and they point out a possible means by which the parasite may be introduced into non-endemic areas through transport of infected dogs.

Key Words: Diagnostics; Epidemiology; Parasitology; Zoonosis

Introduction

Echinococcus multilocularis is prevalent in Hokkaido, the northernmost island of Japan, with a prevalence in foxes of approximately 40% in the last two decades. By 2007, 531 human patients infected with *E. multilocularis* were reported in Hokkaido. The parasite is basically maintained in the sylvatic cycle, in which foxes are the definitive host and voles are the intermediate host. However, the habitat of foxes has been getting closer to or overlapping with that of humans, producing a potential risk of infection for humans and companion animals (Eckert et al. 2000, Tsukada et al. 2000, Romig 2002, Oku and Kamiya 2003).

In Central Europe, several studies have revealed that pet dogs were infected with *E. multilocularis* with a prevalence of 0.3%–7% in endemic regions (Deplazes et al. 1999, Gottstein et al. 2001). Deplazes et al. (2004) estimated that more than 10% of dogs would be infected at least once in their life, even in regions of low prevalence. In some endemic areas, such as Gansu and the Tibetan plateau in China and St. Lawrence Island in the United States of America, dogs play important roles both in the maintenance and in the transmission of echinococcosis to humans (Craig et al. 2000, Rausch and Fay 2002, Torgerson and Budke 2003, Budke et al. 2005a and b).

In Japan, despite the recent high prevalence of foxes in Hokkaido and an increasing awareness of the disease as a serious health risk, few data are available for evaluating the current infection rates in dogs in Japan. Infection with *E. multilocularis* in dogs was first recognized on Rebun Island, a small island in Hokkaido Prefecture, in 1954 (Yamashita 1997). In the 1960s, the parasites were also detected in dogs on the island of Hokkaido (Yorozuya et al. 1968). Since then, the local government has conducted necropsy surveys of dogs (including household pets and stray dogs) and has reported that adult *E. multilocularis* were detected in 99 (1.0%) of 9937 dogs necropsied during the period 1966–2006 (data reported by the Hokkaido government). However, most of these animals were examined before 1990 and therefore, the data do not reflect the recent prevalence of infection.

In the present study, to evaluate the current epidemiological status of canine echinococcosis in Japan, a survey of *Echinococcus* infection in pet dogs was conducted by examining coproantigen reactivity and the presence of taeniid eggs and egg DNA in fecal samples.

Materials and Methods

Fecal samples and questionnaire

We obtained dog fecal samples from local veterinarians, who collected them from August 1997 to August 2007. At the same time, dog owners completed a questionnaire to de-

[1]Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan.
[2]Faculty of Environment Systems, Rakuno-Gakuen University, Ebetsu, Japan.
[3]Forum on Environment & Animals, Sapporo, Japan.
[4]Hokkaido Institute of Public Health, Sapporo, Japan.