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Evaluation of coproantigen diagnosis for
natural *Echinococcus multilocularis* infection in red foxes

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Abstract

The validity of a coproantigen ELISA for *Echinococcus multilocularis* was evaluated by comparison of three diagnostic methods; autopsy, egg examination and the ELISA. Of 71 foxes, 39 were found to be infected with the cestode at autopsy. The overall mean of worm burdens was 3,451, but the number varied (1-34,522). The ELISA could detect 94.9% (37/39) of the worm positives and there were no false-positives. Two false-negatives were infected with 1 and 4 cestodes, whereas 3 cases with similar worm burdens (2, 4 and 6 worms) were diagnosed as positives. This indicates the detection limit of the assay may be equivalent to less than 10 (in the worm burden). On the other hand, egg examination showed low sensitivity (43.6%, 17/39). These results suggest the ELISA has a potential to replace for the conventional methods.

Key word: coproantigen, diagnosis, *Echinococcus multilocularis*

Alveolar echinococcosis (AE), caused by larval infection of *Echinococcus multilocularis*, is one of the most serious parasitic zoonoses. Humans become infected by ingesting the eggs derived from feces of the definitive host, but the route of transmission has not been clarified. Contaminated food or water supply, or contact with the definitive hosts have been suspected as infectious source of human cases. Identification of infectious source is of primary importance in the prevention of human AE cases and in establishing the control strategies against the parasites. In Hokkaido, northern island of Japan, the red fox *Vulpes vulpes* plays a major role as the definitive host. The postmortem diagnosis of the definitive host is most reliable, but the

method is biohazardous and time-consuming, and requires well skilled for accurate diagnosis. Furthermore it can not be applied to live animals such as domestic dogs and cats serving as potential definitive hosts. For this reason, even previous studies have proposed the importance of domestic dogs and cats as possible infectious source for human AE cases^{6,10,11,14}, there are only few survey for *E. multilocularis* in such domestic animals. It is probably attributable to unavailability of reliable antemortem diagnostic methods. Recently, a number of studies on coproantigen detection assays have been reported for diagnoses of intestinal infection of various cestodes including genera *Taenia* and *Echinococcus*³. In our laboratory, a monoclonal

antibody based sandwich enzyme-linked immunosorbent assay (ELISA) has been developed for the detection of *E. multilocularis* coproantigen in feces of the definitive hosts. The validity of the assay has been proved in experimental infections with *E. multilocularis* to dogs, foxes and laboratory rodents used as alternative definitive hosts^{5,9,13}. This study was conducted to evaluate the validity of the coproantigen diagnosis in naturally infected hosts by comparing the results of three diagnostic methods i.e. autopsy, coproantigen detection assay and fecal egg examination.

A total of 71 red foxes were examined. All of which were shot in Sapporo and its environs (Ebetsu, Kita-Hiroshima, Nanporo) during December 1997 to March 1998. The intestinal tract of each fox was ligated at the pylorus and the end of rectum, cut and carefully placed in plastic bags. The sample was frozen at -80°C for more than 10 days to sterilize the infectious eggs. Thereafter, the material was thawed and the small intestine was divided into six parts. Parasitological examination was performed for scrapings of contents and mucosa of the small intestine and colon placed in tap water under a stereomicroscope. The number of *E. multilocularis* was counted and recorded for each intestinal parts. Feces from the rectums were used for coproparasitological examinations. A monoclonal antibody, EmA9⁵, based sandwich ELISA was employed for the detection of the *E. multilocularis* coproantigen. The basic procedure was previously described by Sakashita *et al.*¹³ except avidin-biotinylated peroxidase complex (ABC) method was applied to the assay for the improvement of the sensitivity. Briefly, changes in the procedure were as follows; the monoclonal antibody was conjugated with biotinylation reagent (ECL, Amersham Life Science, USA) and horseradish peroxidase (HRP) conjugated rabbit anti-mouse IgG was substituted by streptavidin-biotinylated HRP complex (Amer-

sham Life Science). All of the samples were tested in duplicate. The diagnostic cutoff value was calculated by the mean OD value plus three standard deviations of 37 *Echinococcus* uninfected fox samples obtained from a fox farm. A centrifugal flotation technique⁴ with sucrose solution of specific gravity 1.27 was used to fecal egg examination. Because *Echinococcus* eggs cannot be morphologically discriminate from those of *Taenia* species¹⁵, such detected eggs were recorded as taeniid eggs.

Of the 71 foxes, 39 were found to be infected with *E. multilocularis*, resulting in the prevalence of 54.9%. Two immature *Taenia* sp. were recovered from two foxes which also infected with *E. multilocularis*. As shown in Table 1, the tapeworms were mostly found in distal parts of the small intestine, especially in four-and five-sixth parts. The mean of detected worms was 3,451, however, the number varied from 1 to more than 34,000 cestodes; the 39 foxes had a total biomass of 131,083 worms, but 86.8% of them parasitized in only five foxes. Our result is similar to those of European studies^{1,16} in respect to the frequency distribution of the worm intensity and relatively few foxes (<20%) harboring 10 or less worms.

Thirty seven out of the 39 worm positive samples gave coproantigen positive results (Table 2a). The assay sensitivity was thus 94.9% (37 of 39) and the specificity was 100% (37

Table 1. Intestinal distribution of *Echinococcus multilocularis* in red foxes in Sapporo and its environs

Section	Mean	Range	Occurrence % (positives/total)
Small intestine			
I	0.9	0-22	10.3% (4/39)
II	6.3	0-62	33.3% (13/39)
III	180.9	0-3,600	71.8% (28/39)
IV	370.2	0-3,320	89.7% (35/39)
V	1564.6	0-21,500	84.6% (33/39)
VI	1237.7	0-25,137	66.7% (26/39)
Colon	0.5	0- 7	12.8% (5/39)

of 37). No false-positive but two false-negatives were found in very low worm burden cases in which one and four worms recovered. However, coproantigen ELISA showed positive results in similar worm burden cases in which the detected worm numbers were 2, 4 and 6, therefore, the detection limit of the assay in the worm burden would be less than 10. Correlation of ELISA OD values and worm intensities were given in Fig. 1a. There was moderate and significant positive correlation ($r=0.74$, $P<0.0001$).

Taeniid eggs were recovered from 17 of

Table 2. Comparisons of postmortem and antemortem diagnoses for *Echinococcus multilocularis* in 71 red foxes in Sapporo and its environs

	Autopsy		Total
	Positive	Negative	
a) Coproantigen			
Positive	37	0	37
Negative	2	32	34
Total	39	32	71
b) Taeniid egg			
Positive	17	0	17
Negative	22	32	54
Total	39	32	71

the 39 worm positive samples (Table 2b). Although the assay specificity reached 100% (17 of 17), the sensitivity was 43.6% (17 of 39). Among samples with the worm burden of more than 1,000, the sensitivity of the egg examination increased to 90.0% (9 of 10). Among taeniid egg positive samples, weak correlation ($r=0.69$, $P<0.001$) was observed between the number of eggs detected and worm burdens (Fig. 1b). However, there was no significant correlation ($r=0.37$) between ELISA OD values and egg numbers. Because the coproantigen assay detects excretory/secretory products of the worms, the OD values will indicate the number of infected worms. Therefore the number of eggs can theoretically correlate the worm burden, but the OD values cannot necessarily follow the egg numbers. Problematical sensitivity of fecal egg examination has been pointed out in cestode infections^{7,8}). Such low sensitivity of the method is likely to be attributed to periodic absence of eggs in host feces. Not only in prepatent infection but also even in patent period, low or intermittent egg production has been reported^{2,9}).

Annual prevalence survey by Hokkaido gov-

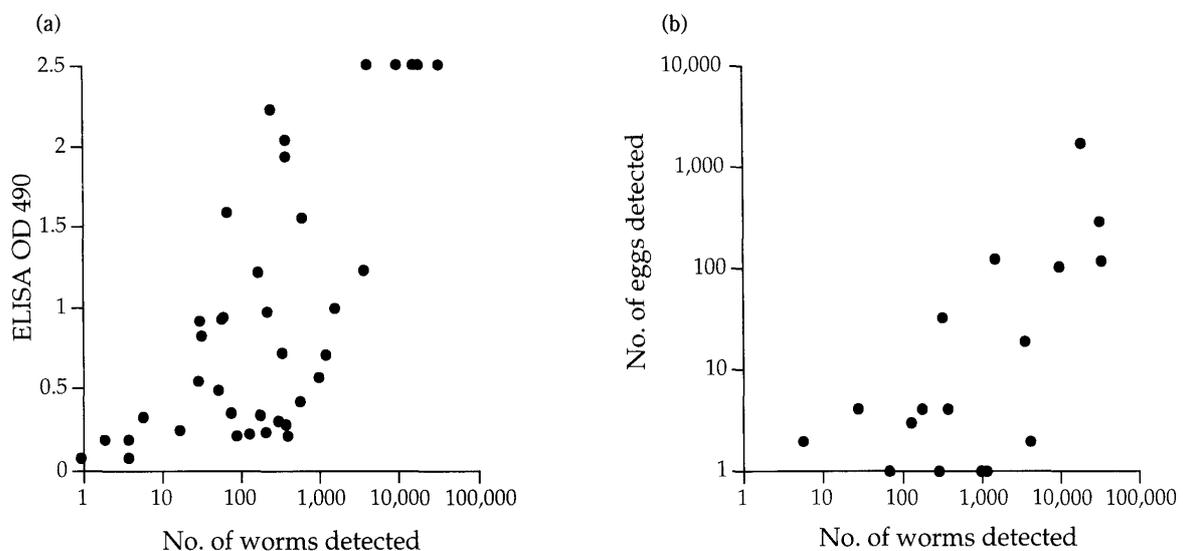


Fig. 1 Correlations between the number of detected worms and the coproantigen ELISA OD values(a)/and the number of detected worms and the number of taeniid eggs(b) observed in red foxes infected with *Echinococcus multilocularis*.

ernment has been performed by examination of the worms only in distal one-sixth part of the small intestines. In our present observation, although the worm distributed intensively at the posterior parts of the small intestine, the worm detection rate was higher in fourth and fifth parts (89.7% and 84.6%, respectively) than distal sixth parts (66.7%) (Table 1). The result suggests the present survey method in Hokkaido may cause misdiagnosis and underestimation of the prevalence. Sakai *et al.*¹²⁾, who used the post-mortem results of Hokkaido government to validate the coproantigen ELISA, noticed the cases giving coproantigen positive but worm negative and argued that these cases could be attributed to the worms parasitized at unexamined parts. In conclusion, whereas methodological simplification such as a dipstick test will be convenient for generalized use, the present coproantigen ELISA has a potential to replace for the conventional postmortem diagnosis.

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