



Title	STUDIES ON COPROANTIGEN DETECTION FOR DIAGNOSIS OF ECHINOCOCCUS INFECTION IN DEFINITIVE HOSTS
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Citation	Japanese Journal of Veterinary Research, 44(2), 125-127
Issue Date	1996-08-30
Doc URL	http://hdl.handle.net/2115/2561
Type	bulletin (article)
File Information	KJ00002398251.pdf



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INFORMATION

Hokkaido University granted the degree of Doctor of Veterinary Medical Science (equivalent to Ph. D.) to the following 1 graduate of the Graduate School of Veterinary Medicine on June 28, 1996.

The title of the thesis and other information are as follows:

STUDIES ON COPROANTIGEN DETECTION FOR DIAGNOSIS OF *ECHINOCOCCUS* INFECTION IN DEFINITIVE HOSTS

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Echinococcosis, one of the most important zoonoses distributed throughout the world is principally caused by *Echinococcus multilocularis* and *E. granulosus*. Accurate diagnosis of *Echinococcus* infection in definitive hosts has always been an important component for establishing epidemiological parameters of this disease and preventing human infection. Coproantigen detection, which recognizes excretion/secretion (ES) products of parasites in faeces, corresponds to the presence of worms in the intestine, hence it is a good tool for diagnosis of infected definitive hosts.

To develop a simple and reliable diagnostic method for *Echinococcus* infection in definitive hosts by coproantigen detection, murine monoclonal antibodies (MoAbs) to *E. multilocularis* adult worms were produced and characterized. A selected MoAb was used for coproantigen detection in faecal samples obtained from animals experimentally and naturally infected with *Echinococcus*. The results are as follows:

1. Eleven MoAbs were produced against *E. multilocularis* somatic antigens and one MoAb, designated as EmA9, was selected for the detection of coproantigens in *E. multilocularis* infected definitive hosts because of its specificity to the *E. multilocularis* antigens.

2. Studies on the characteristics of antigen epitope recognised by EmA9 are as follows:

- (1) Antigens recognised by EmA9 are expressed on the tegument and parenchyma of *E. multilocularis* at 2 days postinfection (PI), the reaction becoming very intense at 4 days PI.

- (2) EmA9 recognized epitope may be a carbohydrate moiety of glycoconjugates of the worms because of the heat-stable and proteolysis-resistant characteristics in

addition to its sensitivity to periodate treatment.

(3) This carbohydrate moiety contains mannose, glucose and N-acetylglucosamine residues.

3. A sandwich ELISA using rabbit polyclonal antibodies against ES antigens of *E. multilocularis* and EmA9 was used for detection of coproantigens. Results of studies on coproantigen detection in experimentally infected animals and characterisation of coproantigens recognised by EmA9 are as follows:

(1) In dogs inoculated with 200,000 protoscoleces, coproantigens were detected on as early as 3–5 days PI and increased steadily until autopsy on day 21 PI. Anthelmintic treatment of one dog on day 17 PI by praziquantel resulted in the disappearance of the antigens from faeces. Thus, an accurate diagnosis could be made using this method. The lower limit of antigen detection was 4 ng/g of faeces which was superior to the older methods of coproantigen detection. Detection of circulating antibodies was performed in conjunction with the coproantigen test. Antibody levels of two dogs increased steadily from day 8 PI until day 21 PI, however, antibody levels of one dog which was treated with praziquantel were apparently high throughout experimental infection. From these results, detection of coproantigens using EmA9 provides a good estimate of the actual status of infection in the host.

(2) Studies on the characteristics of the coproantigen showed that heating of faeces did not influence the sensitivity of coproantigen detection by ELISA. This prevents accidental infection, by heating faecal samples before the test is performed. In addition, coproantigen could be extracted easily, and formalin treatment of faeces did not influence the sensitivity of coproantigen detection. Thus long term faecal storage is possible.

(3) When specificity of the test was evaluated using faeces of animals infected with *Taenia taeniaeformis*, *T. crassiceps*, *Trichuris vulpis* and *Toxocara canis*, no cross-reactions were observed. However, there was slight cross-reaction with coproantigen of *T. hydatigena* after excretion of proglottids. This suggests that cross-reactive antigens of *T. hydatigena* may be expressed with the formation of gravid segments.

(4) Experimental infection of *E. multilocularis* in an alternative definitive host, i.e., golden hamsters, was performed to examine whether the amount of coproantigens is dependent on the number of inoculated protoscoleces. The amount of faecal antigens detected by ELISA was dependent on the number of inoculated protoscoleces until day 10 PI and relatively high statistical correlation between ELISA OD values and number of recovered worms was confirmed. These results indicate that coproantigen detection is very useful for monitoring the state of infection in the hosts, although accurate comparison of worm burdens among individual animals is difficult when the number of infected worms fall within the same range.

(5) A sandwich ELISA using polyclonal antibody against ES antigens of adult *Echinococcus granulosus* and EmA9, was used for detection of *E. granulosus* coproan-

tigens in experimentally infected dogs. *E. granulosus* coproantigens could be detected during the initial phase of the infection. Antibody response of dogs against *E. granulosus* antigens was also examined. However, antibody levels of dogs infected with a large number of worms did not rise, whereas one dog infected with few worms showed apparently high antibody levels.

4. Coproantigen detection using sandwich ELISA was applied on faecal samples collected in the field.

(1) Faecal samples were obtained from the rectum of foxes captured at Hokkaido or collected around fox dens and used for the coproantigen detection. The diagnostic probability of a true-positive case in 430 faecal samples from necropsied foxes in 1993 was 87.4% (90/103), and less than 10 worms were detected in 11 coproantigen-negative cases out of 13. In faecal samples collected around fox dens in 1990 and 1992, coproantigen-positive results out of taeniid egg positive cases were 38/40 (95.0%) and 95/97 (97.9%), respectively. In addition, coproantigen was detected in samples stored in 10% formalin over 3 years, suggesting that the antigens detected by this method are quite stable. These results show that detection of coproantigen is very useful not only for diagnosis of infected dogs, but also for field surveys of fox faeces.

(2) Sandwich ELISA was also used for the diagnosis of dogs naturally infected with *E. granulosus* in Uruguay. Faecal samples were obtained from dogs treated with arecoline hydrobromide. ELISA using rabbit polyclonal antibodies against ES antigens of *E. multilocularis* was applied on 82 faecal samples. The predictive value of ELISA in arecoline survey-positive cases was 35.7% (5/14). In addition, ELISA using rabbit polyclonal antibodies against ES antigens of *E. granulosus* was used on 59 samples and the predictive value was 37.5% (3/8). Thus, ELISA sensitivity was unexpectedly low for the diagnosis of dogs naturally infected with *E. granulosus*. In this regard, further improvement on the sensitivity of the test is required in *E. granulosus* infected dogs.

These results indicate that the present sandwich ELISA using monoclonal antibody, EmA9, could detect coproantigen during the prepatent phase of infection in animals experimentally infected with *Echinococcus*. In addition, the sensitivity and specificity of the test are comparatively higher than other diagnostic techniques. Coproantigens recognised by EmA9 are quite stable, so that faecal samples can be heated to render it safe for handling before the test is performed. This test is of great use in gathering prevalence data of animals naturally infected with *Echinococcus* using faeces collected in the field.

Original papers of this thesis appeared in "Japanese Journal of Parasitology", Vol. 44, 404-412, 413-420 & 453-461 (1995), and in "Experimental Animals", Vol. 45, 275-278 (1996).