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Echinococcus multilocularis Coproantigen Detection in Golden Hamster, an Alternative Definitive Host

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Abstract: Golden hamsters as alternative definitive hosts of *Echinococcus multilocularis* were used for coproantigen detection by means of sandwich ELISA. The test was performed in hamsters infected with approximately 20,000, 4,000, 500, 0 (control) and 100,000 (i.e., group I, II, III, IV and V respectively) protoscoleces. Comparison of mean OD values of each group showed significant differences depending on the number of protoscoleces administered and days postinfection. There was also a relatively high statistical correlation between the number of recovered worms and ELISA OD values (correlation coefficient=0.699, $P<0.05$), although accurate comparison of worm burdens among individual animals was difficult when numbers of infecting worms fell within the same range.

Key words: *Echinococcus multilocularis*, coproantigen, golden hamster

Echinococcus multilocularis is a cestode, which maintains its life cycle by using various carnivores (e.g., fox, wolf and dog) as definitive hosts, and many species of rodents as intermediate hosts [6]. Human infection is acquired by ingesting eggs excreted from infected definitive hosts and larvae (protoscoleces) multiply particularly in the liver, thus making it one of the most important zoonoses. Accurate diagnosis of infected definitive hosts has always been an important component in most hydatid disease control programs. Coproantigen detection is one of the most useful immunodiagnostic tools, because it reflects current intestinal infection of adult worms [1, 2]. Kohno *et al.* (1995) produced monoclonal antibodies (MoAbs) to adult *E. multilocularis* somatic antigens for the diagno-

sis of infected definitive hosts and selected one MoAb, designated as EmA9, for coproantigen detection because of its specificity and high sensitivity to *E. multilocularis* antigens [5]. They further suggested that sandwich ELISA with EmA9 has good potential as an immunodiagnostic tool for detecting *E. multilocularis* infected definitive hosts. Nevertheless, correlation of worm burden with the amount of coproantigens, i.e., ELISA OD values, has not been clarified, so that experimental infection of definitive hosts with different numbers of protoscoleces is required. Recently, immunosuppressed rodents have been established as alternative definitive hosts [3, 4]. This allows experimental infection with *E. multilocularis* adult worms to be easily performed. In addition, experiments employ-

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ing alternative definitive hosts have some advantages, such as the possible use of many animals at the same time, less expense and ease of management, etc. In this study, coproantigen detection in golden hamsters, a suitable alternative definitive host [3], infected with different numbers of protoscoleces and its use for monitoring the state of *E. multilocularis* adult worm infection were carried out.

Sixteen 10-month-old inbred male golden hamsters (ACN strain) kindly provided by Nippon Institute for Biological Science were used. The animals were given commercial pelleted feed (CE-2, Clea Japan, Inc., Tokyo, Japan) and water *ad libitum*. Protoscoleces of *E. multilocularis*, maintained in our laboratory, were removed from gerbils infected 9 months previously. Hamsters in groups I, II, III and IV (4 animals each), lightly anaesthetized with diethyl ether, were administered by stomach lavage with approximately 20,000 (I), 4,000 (II), 500 (III) and 0 (IV; control) protoscoleces respectively. The animals were treated with 5 mg of prednisolone tertiary-butylacetate (Suspension of Codelcortone®-T.B.A., Banyu Pharm. Co., Ltd., Tokyo, Japan) (PTBA) at days -3, 0, 2, 4, 6, 10, 14 and 18 post-infection (p.i.). Hamsters in group I-III were autopsied at day 20 p.i., and worms recovered from the small intestine were counted. Three days after finishing the first experiment, hamsters in group IV were reassigned to group V, given approximately 100,000 protoscoleces and additionally treated with PTBA every 4 days until the autopsy. Two hamsters died during the second experiment. The two remaining hamsters were autopsied at day 20 p.i., and the numbers of worms counted. Feces of all hamsters were collected everyday until autopsy. Fecal suspen-

sion was prepared as described previously [7], briefly, feces were mixed with 1% formalin containing 0.3% Tween 20 to make a ratio of 1:8 [fecal weight (mg): solution (μ l)]. After heating at 70°C for 12 hr, the fecal suspension was centrifuged and the supernatant was used for coproantigen detection. Sandwich ELISA for coproantigen detection was carried out as described previously [5, 8], and summarized briefly as follows: Rabbit polyclonal antibodies to *E. multilocularis* excretory/secretory (ES) antigens were coated onto microtitre plate wells (Greiner, Frickenhausen, Germany) to capture coproantigens in the fecal solution. MoAb EmA9 was added and the reaction was visualized by using peroxidase labelled rabbit anti-mouse IgG+M+A (Zymed Laboratories, San Francisco, USA) and o-phenylenediamine as substrate. The test was read at OD 490 nm.

At autopsy, various numbers of worms were recovered from the small intestine (Table 1). ACN hamster has been considered as relatively resistant to infection with *E. multilocularis* as an alternative definitive host. In this study, the mean percentage of recovery in group I-III was low (1.3%) but a high percentage of recovery (21.02 ± 12.02) was observed in group V which was treated with PTBA as controls in the first experiment. ACN hamster could therefore be used as an alternative definitive host after long-term pre-treatment with PTBA.

Coproantigens in infected hamsters were detected by sandwich ELISA with EmA9. We examined changes in the levels of coproantigens detected postinfection and the correlation of the number of protoscoleces administered with amount of coproantigen detected by ELISA, i.e., ELISA OD values (Fig. 1). Comparison

Table 1. Number of worms recovered from the small intestine of hamsters experimentally infected with *E. multilocularis*

Group*	Number of protoscoleces administered	Number of worms recovered mean $\pm$ S.D. (range)	% recovery mean $\pm$ S.D. (range)
I	20,000	597.50 $\pm$ 744.13 (25-1,600)	2.99 $\pm$ 3.72 (0.13-8.00)
II	4,000	31.25 $\pm$ 31.17 (6-76)	0.78 $\pm$ 0.78 (0.15-1.90)
III	500	0.50 $\pm$ 1.00 (0-2)	0.10 $\pm$ 0.20 (0-0.40)
IV	0	-	-
V	100,000	21,015 $\pm$ 12,020 (12,515-29,515)	21.02 $\pm$ 12.02 (12.52-29.52)

*Number of hamsters are as follows: group I-IV, 4; V, 2. Hamsters in group IV are uninfected controls.

Fig. 1. Coproantigen detection in feces of experimentally infected golden hamsters. Numbers of protoscoleces administered are as follows: Group I ($\square$, 20,000), II ($\blacksquare$, 4,000), III ($\circ$, 500), IV ($\blacktriangle$, 0), V ($\boxtimes$, 100,000). Average OD values for each groups are shown. The error bar is the standard deviation (S.D.) of OD values in each group.

of mean OD values in the groups showed that the OD values differed dependent on the number of protoscoleces administered and the difference became more obvious as days postinfection increased. Variation in OD values among individual samples in the same group was small until day 15 p.i., suggesting that the protoscoleces administered developed normally in hamsters. Larger variation in OD values among individual samples in group I was observed after day 15 p.i. This variation may be due to the elimination of infected worms or the inhibitory action of the host on worm development.

Correlation of the number of worms recovered in hamsters in groups I, II and V with the amount of coproantigen, i.e., ELISA OD values, was analysed by Pearson's correlation coefficient test (Fig. 2). The result showed a relatively high correlation between the number of worms recovered and ELISA OD values (correlation coefficient=0.699, $P<0.05$), although accurate comparison of worm burdens among individual animals was difficult when the number of infecting worms fell within the same range. Worm burdens based on ELISA OD values can be roughly estimated, e.g., worm burden ranges: 10–100, 100–1,000 and 1,000–10,000, etc.

Fig. 2. Correlation analysis of ELISA OD values versus number of worms recovered. OD values show good correlation with the number of worms recovered, as determined by Pearson's correlation coefficient test. Correlation coefficient=0.699 ($P<0.05$).

The prepatent period of cestodes generally varies. *E. multilocularis* matures and egg production commences between 28 and 35 days after infection [10], so that the degree of worm development also varies during the prepatent period. Since the amount of antigens secreted also increases as worms develop [9], the worm burden cannot be estimated on the basis of ELISA OD values. The relationship between the number of infected worms and ELISA OD values should also be investigated during the patent phase when worms are in the same stage of development. Furthermore, fluctuation in coproantigen levels was also previously observed in dogs experimentally infected with *E. multilocularis* [5, 8], suggesting that antigen release from worms during infection is irregular. Further examination is therefore required to estimate the correlation of the worm burden with the amount of coproantigens with due regard to the development of worms and the period of coproantigen release in addition to the number of infected worms.

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