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Detection of Serum IgG and IgM Antibody in Rats Experimentally Infected with *Echinostoma hortense*

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Abstract: The changes of antibody titer were observed in rats which were experimentally infected with *Echinostoma hortense* metacercaria. Serum levels of IgG and IgM were measured by enzyme-linked immunosorbent assay (ELISA). The mean absorbance values obtained for specific-IgG were from 0.130±0.014 (mean±S.D.) to 0.480±0.073. The peak appeared in the 4th week after infection, then declined slowly during the 5th and 6th week, although mild elevation apperared in the 8th week. The mean absorbance values of specific-IgM were detected from 0.160±0.034 to 0.409±0.084. The peak value (0.409±0.084) was on the 14th day after infection, then declined on the 8th week. Results showed that the assay could be used for detection of *E. hortense* infection in experimentally infected rats or laboratory experiments where evidence of infection is required.

Key Words: *Echinostoma hortense*, Rat, IgG, IgM, ELISA

INTRODUCTION

The numerous species of flukes belonging to the family *Echinostomatidae* are cosmopolitan in distribution and have recorded as part of many kinds of vertebrates, particularly aquatic birds. The most frequent reported species among the group of echinostomes are *Echinostoma trivolis, E. caproni, E. echinatum, E. hortense, E. cinetorchis, E. malaynum, E. revolutum* and *Euparyphium ilocanum*[18,19,20]. Among the 15 echinostomes which have been reported in human infection, *E. revolutum, E. echinatum, E. malaynum, E. hortense,* and *Euparyphium ilocanum* show the highest prevalence rate. These parasites are found in the Far East and South Eastern Asian countries. Infections are acquired by consuming raw snails, tadpoles or freshwater fish containing encysted metacercariae[1,12,16,17].

The infection sources of *E. hortense* are found in various species of amphibia and freshwater fish[2]. The ingestion of raw loach, *Misgurunus angiullicaudatus,* has been confirmed as the main source of *E. hortense* infection[22]. More than 20 cases have been reported in Japan and Korea. An epidemiological study showed high infection rate of *E. hortense* among the residents and intermediate hosts of Chongsong-gun, Kyungsangbuk-do[13]. Echinotomes are ubiquitous intestinal digeneans of aquatic birds and mammals, which are occasionally found in humans who are associated

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with having gastrointestinal distress[10]. Diagnosis of echinostomes from vertebrate hosts is typically done by finding the characteristic operculate eggs through stool examination. A reliable serodiagnostic tool to identify echinostomes in vertebrate hosts would be useful to wildlife biologists and parasitologists concerned with studies on echinostomiasis.

The ELISA method provides early and specific immunodiagnosis of trematode infections in humans and other animals. The main advantage of antibody detection with ELISA is the non invasive detection of host immune responses before the worm expulsion of ova or specific clinical symptoms that appear in the host[9].

To examine the antibody response of the *E. hortense* in rat model, an enzyme linked immunosorbent assay (ELISA) was developed for measuring the level of parasite specific IgG and IgM antibodies in serum.

MATERIALS AND METHODS

1. Isolation of Metacercariae

Metacercariae of *E. hortense* were isolated from the muscle of a raw loach, *Misgurunus angiullicaudatus*, caught from Namhan-gang (River), Munmak, Kangwon-do, Korea. The ground fish was mixed with 10-fold volume's artificial gastric juice (pepsin 0.2 g, HCl 0.7 ml, DW 99.3 ml), and the mixture was incubated at 37℃ for 1 hr. The freed metacercariae were collected under a dissecting microscope, and preserved at 4℃.

2. Experimental Infection

40 Sprague-Dawley male rats weighing about 120 grams were divided into two groups; 20 uninfected control group and 20 metacercariae infected group. Each rat of the infected group was orally infected with 150 metacercariae through a polyethylene capillary tube. Blood was collected from the tails of rats for 8 weeks and allowed to clot at 4℃. The serum was separated by centrifugation and stored at -70℃.

3. ELISA

An enzyme linked immunosorbent assay (ELISA) was developed in order to detect antibodies against *E. hortense* in rats. The ELISA was performed according to the Voller method[24]. All incubations were performed at room temperature in U-bottomed polystyrene mictotiter plates (Nunc, USA). The plates were washed 3 times for 5 min with PBS-Tween (phosphate buffered saline-0.05% Tween 20) during the incubation interval period.

For antigen coating, plate wells were incubated at 37℃ for 1 hour with crude adult worm antigen 100 μl diluted in 0.05 M carbonate-bicarbonate buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6) to a protein concentration of 5 μg/ml. After the following washings, the well were blocked with 200 μl blocking buffer (3% Bovine serum albumin/PBST) for one hour. After washings, the wells were incubated one hour with 100 μl of serum diluted 1:100 with PBS-Tween for measuring IgG and IgM respectively. After washings, the wells were incubated for one hour with 100 μl horseradish-peroxidase conjugated goat anti-rat IgG (Sigma, USA) and horseradish-peroxidase conjugated mouse anti-rat IgM (Serotec, USA) antibodies for measuring IgG and IgM in serum. After final washing, the plates were incubated for one hour with a substrate solution of 100 μl of ortho-phenylenediamine (OPD) 0.5 mg, 30% H_2O_2 10 μl, 0.1 M phosphate citrate buffer (pH 5.0, 50 ml), and then reaction was stopped using 25 μl 2.5 M H_2SO_4.

The absorbance values were read on the ELISA reader (Molecular Devices) at 490 nm. Each sample was examined three times with the results given as the mean absorbance value. The duration of antibody detection from experimental infection rats was from 1th week to 8th week after infection. The serum for control was also measured as a reference on all ELISA plates. Student's t-test was used for sta-

tistical analysis of the ELISA data.

RESULTS

The serum antibody levels measured over 8 weeks are shown in Table 1 and Fig. 1 (IgG and IgM combined). In all types of antibodies studied, significantly higher ($p<0.05$) levels were measured in the infected groups than the control groups. The changes of antibody titer were observed in rats which were experimentally infected with *E. hortense* metacercaria. When serum levels of IgG and IgM antibodies were measured by enzyme-linked immunosorbent assay (ELISA), the mean absorbance values obtained for specific-IgG were increased

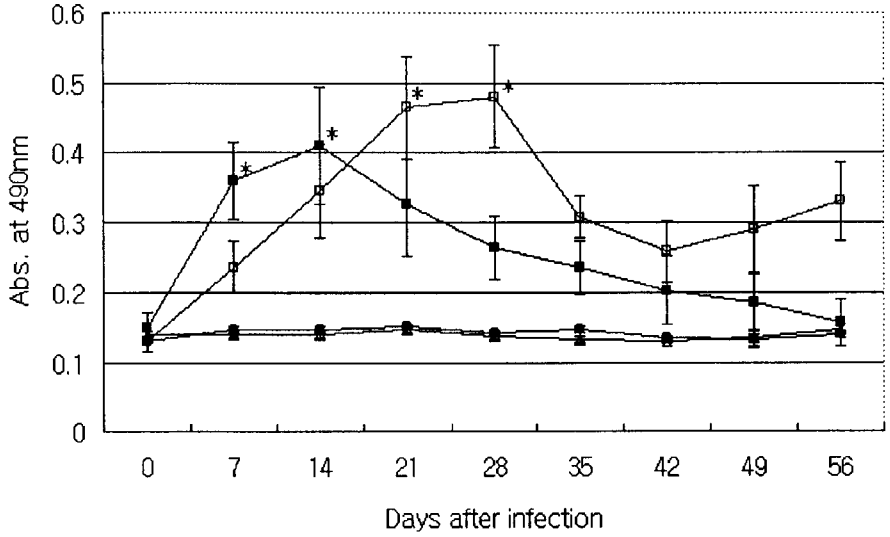

Fig. 1. Changes in the mean absorbance values of ELISA in the rats experimentally infected with *E. hortense*. ●-●: IgG antibody of control group, △-△: IgM antibody of control group, □-□: IgG antibody of experimental group, ■-■: IgM antibody of experimental group (mean±SD, *: $p<0.05$).

Table 1. The mean absorbance values of IgG and IgM to *E. hortense*

Days	Control group		Experimental group	
	IgG	IgM	IgG	IgM
0	0.135±0.004	0.141±0.003	0.130±0.014	0.150±0.022
7	0.147±0.005	0.140±0.002	0.235±0.036	0.359±0.056*
14	0.147±0.005	0.142±0.003	0.344±0.068	0.409±0.084*
21	0.152±0.004	0.148±0.004	0.464±0.07*	0.326±0.075
28	0.132±0.003	0.137±0.005	0.480±0.073*	0.264±0.044
35	0.146±0.005	0.133±0.003	0.307±0.029	0.236±0.038
42	0.135±0.003	0.131±0.002	0.258±0.044	0.203±0.048
49	0.132±0.012	0.135±0.010	0.290±0.063	0.185±0.045
56	0.140±0.006	0.142±0.005	0.330±0.057	0.160±0.034

Mean±SD, *: $p<0.05$

from 0.130±0.014 (mean±S.D.) to 0.480± 0.073, their peak value (0.80±0.073) was detected on the 28th day after infection ($p<0.05$). The mean absorbance values obtained for specific-IgM antibody measured from 0.150±0.022 to 0.409±0.084 and their peak value (0.409± 0.084) was detected on the 14th day after infection and dropped thereafter.

DISCUSSION

The early response showed to be of the IgM class, thereby confirming the nature of this antibody class as being part of the early immunological response in host defence[4,5,8,14,26]. By the following infection with encysted metacercariae of intestinal treamtodes *Echinostoma trivolvis* or *E. caproni* on days 8 and 16 after primary infection with *Nippostrongylus brasiliensis*, ELISA data showed that the IgM titer rose remarkably and plateaued on day 11 after infection[6]. Nyme AK. et al.[15] reported that mice chronically infeced with *Schistosoma mansoni* generated high titers of both IgM and IgG antibodies reacting with Lewis x antigen. The IgM responsed to Le(x) antigen detectable in the 2nd week, whereas the IgG was detectable in the 5th and 6th weeks. Griard et al.[7] (1997) mentioned specific immunoglobulin A, M, and G production in chicken's duodenum and caecum infected by *Emeria acervulina* or *Emeria tenella*. IgG rose in the 1st and 2nd weeks, then continually rose. IgM rose at the 1st week and 2nd week but IgA rose only at the 2nd week. As described by Thaddeus K. Graczyk and Bernard Fried[23], using glycocalyx menbrane crude antigen it was possible to detect anti-*E. caproni* immunoglobulins on day 8 after infection. Markedly different patterns of anti-*E. caproni* antibody titers were observed in experimentally infected golden hamsters and mice[21]. Compared to the infection in mice, golden hamsters showed weak ELISA data dectectable humoral response from the 11th to the 13th week after infection[21]. Chauvin et al.[3] demonstrated the antibody levels in sheep infected by *Fasciola hepatica*. IgM was produced in the 2nd week and the highest value in the 3rd week. IgG elevated from the 2nd week and the levels maintained until the 6th week. Antitibody changes in experimental anisakiasis were also observed in 10 rabbits which were infected with *Anisakis simplex* larvae. Levels of specific-IgM antibody were elevated from the 6th day after infection then reached their peak on the 11th day after infection and serum levels of IgG antibody reached their peak on the 26th day after infection[25]. Specific IgG and IgM antibody levels were observed in experimental paragonimus metacercaria infected dogs by micro-ELISA, using whole worm extract (PwWWE) antigen of 12-week-old *Paragonimus westermani*. Specific IgG antibody to PwWWE began to increase from the 2nd week after infection and continued to increase until the observation period of 13 weeks. Specific IgM antibody to PwWWE increased temporarily for 2~8 weeks after infection[11]. The pattern of anti-*E. hortense* antibody titer was similar to the pattern of anti-*E. caproni*[23], anti-PwWWE[11], anti-*Anisakis*[25] and anti-*Fasicola hepatica* immunoglobulins detected in experimentally infected mice on the 7th day after infection[9].

Our study demonstrated the feasibility of ELISA for the immunodiagnosis of *E. hortense* infection on SD rats. This experiment showed IgM and IgG levels significantly elevated. We think that measurement of antibody level in serum can be used in diagnosis on the infection of *E. hortense*. ELISA is a simple and sensitive method. So it is effective in the measurement of anti-*E. hortense* immunoglobulins. To increase specificity for *E. hortense* infection, antigen which don't show cross reaction to other parasites is in need. Changes of serum antibody level in rats was the same as those of general immune response in hosts, so IgA and IgE levels should be studied for understanding for total humoral immune response in echi-

nostomiasis.

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=국문초록=

호르텐스극구흡충을 실험적으로 감염시킨 흰쥐에서 IgG/IgM 항체가 변화

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본 연구는 흰쥐에 호르텐스극구흡충 피낭유충을 흰쥐에게 실험적으로 감염시켜 감염기간에 따른 항체 생산 유무를 규명하였다. 감염 흰쥐의 혈청을 기간별로 채취하여 IgG 및 IgM 항체가를 ELISA 법으로 측정하였으며, 또한 이의 진단적 이용가치를 평가하고 이들에 대한 상관성을 검토한 결과는 다음과 같다. 즉, 기간별 특이 IgG 항체가는 0.13±0.014 (mean±S.D.)에서 0.4803±0.073까지의 분포를 보였으며 감염 제 28일에 최고치 (0.4803±0.073)를 보였다 ($p<0.05$). 호르텐스극구흡충에 감염된 백서를 통해 매주 얻은 특이 IgM 항체가는 0.16±0.034 (mean±S.D.)에서 0.4093±0.084까지의 분포를 보였으며 감염 제 7일에 증가하여 감염 제 14일에 최고치 (0.4093±0.084)를 보였다 ($p<0.05$). 이상의 결과를 종합할 때 호르텐스극구흡충 감염시 흰쥐 혈청의 IgM은 감염 초기에 급속한 증가를 보였으나 IgG는 감염 이후 계속 증가하는 경향을 보였다.

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