Iranian J Parasitol: Vol. 3, No.4, 2008, pp. 19-22

Original Article

Comparison of Excretory-Secretory and Somatic Antigens of *Ornithobilharzia turkestanicum* in Agar Gel Diffusion Test

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(Received 3 Sep 2008; accepted 20 Nov 2008)

Abstract

Background: Ornithobilharziosis as one of the parasitic infections may give rise to serious economic problems in animal husbandry. The Aim of the study was to prepare and compare the somatic and excretory-secretory (ES) antigens of *O. turkestanicum* in gel diffusion test.

Methods: Excretory-secretory (ES) and somatic antigens of *Ornithobilharzia turkestanicum* were prepared from collected worms from mesentric blood vessels of infected sheep. The laboratory bred rabbits were immunized with antigens and then antisera were prepared. The reaction of antigens and antisera was observed in gel diffusion test.

Results: ES antigens of this species showed positive reaction with antisera raised against ES and also somatic antigens. Somatic antigens also showed positive reaction with antisera raised against somatic and also ES antigens.

Conclusion: The antigenicity of *O. turkestanicum* ES and somatic antigens is the same in gel diffusion test.

Keywords*: Ornithobilharzia turkestanicum*, Excretory-secretory, Somatic antigen, Serodiagnosis, Gel diffusion

Introduction

Ornithobilharziosis caused by *Ornithobilharzia turkestanicum* as one of the parasitic infections, may give rise to serious economic problems in animal husbandry. This infection is found throughout Iran affecting all domestic animals (1, 2). According to some reports, this infection is prevalent in north and some central parts of Iran (3, 4). The immunodiagnostic tests could be utilized for the detection of disease even at the prepatent stages before eggs begin to appear in the faeces (5, 6).

Serological techniques such as immunodiffusion test using antigen of the worm for the detection of antibodies against this parasite are sensitive and have been exploited for its serodiagnosis. However the standardization of test under different conditions and with different types of antigens is essential before its practical application and utility under field condition. The present study was therefore aimed to prepare and compare the somatic and excretory-secretory (ES) antigens of *O. turkestanicum* in gel diffusion test.

Materials and Methods

Collection of worms

Adult worms of *O. turkestanicum* were collected in fresh state from the mesenteric vein of naturally infected sheep at Dept. of Parasitology in Razi Vaccine and Serum Research Institute Karaj-Tehran Province, Iran.

The sheep were killed and adult worms were recovered separately from the mesenteric veins

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in saline solution and washed several times in PBS pH 7.2.

Preparation of ES antigen

Freshly collected living worms after proper washing were incubated in 0.01 M PBS, pH 7.2 at 37ºC for 6 h (each worm in 2 ml PBS).The incubation fluid containing ES products of these worms was collected and centrifuged at 20000 g and 4ºC for 30 min. The supernatant was collected and stored at -20 ºC.

Preparation of somatic antigen

Freshly collected worms were washed 3-4 times in PBS and then worm was homogenized in the same medium at the rate of one worm in 3ml PBS. The homogenized worm materials were then sonicated for 15 min under chilled condition and the mixture was centrifuged at 20000g and 4 ºC for 30 min. The supernatant was collected and stored at -20 ºC.

Protein estimation of antigens was done by the procedure described by Lowry (7).

Immunization of rabbits

Laboratory bred, white healthy and rabbits 2-2.2 kg in weight were used for the preparation of antisera. The rabbits were divided into 3 groups comprising 3 animals in each group. Each rabbit received five injections of antigen at interval of 7 days starting from 0.5 ml to a maximum of 2.5 ml. The antigens were used at a protein concentration of 2 mg/ml. The first injections were given with complete Freund´s adjuvant and the second with incomplete adjuvant. All injections were done subcutaneously. Ten days after the last injection, the rabbits were bled intracardially, serum was separated under sterile conditions and was stored at -20 ºC. The serum samples of 3 normal healthy rabbits were also collected simultaneously and stored at-20ºC to be used as control. The ethical approval has been obtained from Ethic Committee in the Institution in which this research has been conducted.

Gel diffusion test

One gram of special noble agar (Difco laboratory),0.1 gram of sodium azid (8%) and 0.09 gram of NaCl were dissolved in 100 ml of distilled water and boiled in water bath for 20 min. pH of agar was adjusted to 7.2. Molten agar was poured into clean small sized Petri dishes (6ml in each plate) and allowed to cool at room temperature.

Central and peripheral wells of 4mm diameter were cut in the gel. The wells were charged with antigens and antisera in different sets. Normal rabbit sera and normal saline was used as control in each set only one type of antigen was applied to the central well and two different types of antisera against each antigen was applied to the peripheral wells. The charged plates were incubated in humid chamber at 37 ºC for 48 h. The plates were then kept at 4 ºC for 24 and 48 h to develop the precipitin lines/ bands.The observations were recorded every 6 h.

Results

Somatic and ES antigens of *O. turkestanicum* were tested with their homologous and heterologous antisera. The results of gel diffusion test are presented in Table1. In all sets, positive reactions were observed in the form of precipitin lines/bands in the agar gel. Normal rabbit sera (control) and normal saline (control/2) did not show any reaction. Somatic antigen of *O. turkestanicum* showed positive reaction with antisera raised against somatic and also ES antigens of this parasite (Table1). ES antigens *O. turkestanicum* showed positive reaction with antisera raised against ES and also somatic antigens of this parasite (Table1). Somatic and ES antigens of the parasite also showed positive reaction with their heterologous antisera.

Table 1: Showing the reaction between homologous and heterologous combination of *Ornithobilharzia turkestanicum* antigens and antisera

Antigen \ Antisera	Somatic antisera	ES antisera	Controls
Somatic antigen	+	+	_
ES antigen	+	+	_

Discussion

Previous studies show that sera of 15 sheep infected with *O. turkestanicum* were tested by immunofluorescent antibody technique (IFA) using the cercaria as antigen, indirect-hemagglutination (IHA) test, using the soluble somatic antigen and ELISA using the ES antigens revealed that IFA may be more reliable, in terms of serological detection of the infection (2).

There is another study that total antibody response to worm extract antigens and to ES were determined in 15 infected sheep also revealed that the immune response reached its maximum level at 4 months post infection. A remarkable level of cross reactivity was observed when *Fasciola gigantica* antisera were used and a low degree of cross reactivity was found with ES antigens of O. *turkestanicum* and *F. gigantica* antisera (8).

Immunization with *O. turkestanicum* from cattle produces a considerable degree of immunity in mice against challenge with *Schistosoma bovis*, *S. haematobium* and *S. mansoni* (9).

In the course of this study, efforts were made to prepare and compare the somatic and ES antigens of *O. turkestanicum* in gel diffusion test. According to the results, somatic antigens showed strong reaction with antisera raised against ES antigens and ES antigens also showed strong reaction with antisera raised against somatic antigens. There was no evidence that a crude ES antigen is preferable to a somatic antigen in the serodiagnosis of ornithobilharziosis in sheep by ELISA (2).

The results of the present study showed that somatic and ES antigens of *Ornithobilharzia* had strong cross reaction with each other. This means that the antigenic materials are common between somatic and ES products of parasite and there is no difference between the antigenicity of crude somatic and ES antigens of *Ornithobilharzia* spp. in gel diffusion test: so both antigens can be used for the diagnosis of the disease in the sera of immunized rabbit (10-13). Finally it has been shown that using the ES antigens of *O. turkestanicum* by ELISA method could be a good diagnostic tool for the illness.

Acknowledgements

The authors wish to thank all colleagues at Department of Parasitology, Razi Vaccine and Serum research institute. This study was financially supported by budget from Ministry of Jehad and Agriculture, devoted to confirmed projects at Razi institute. The authors declare that they have no conflict of interests.

References

1. Abshar N, Paykari H, Karimi GR, Derakhshanfar M. Diagnosis of sheep fasciolosis by use of AGP (Agar Gel Precipitation) test. Iranian Journal of Vete-

rinary Research. University of Shiraz. 2002; 2:150-155.
2. Karimi GhR. Epidemiological studies on *Ornithobilharzia turkestanicum* in sheep and its snail in Shadegan area and evaluation of serological tests for diagnosis of infection. [PhD dissertation]. Ahwaz university; 2003.
3. Eslami A, Sarafrazi M, Hassani TA. Current status of ovine *Ornithobilharzia turkestanicum* infection in Juybar, Mazandaran. Department of parasitology, faculty of veterinary medicine,Tehran university. J Pajouhesh-va-Sazandegi. 1998;35: 106-107.
4. Ghadirian E, Hoghooghi N. The presence of snails of veterinary importance in Isfahan, Iran. British Vet J. 1973;12(2):1-3.
5. Hillyer GV, Galanes MSD, Buchon P, Bjorland J. Herd evaluation by Enzyme Linked immunosorbent assay for the detection of *Fasciola hepatica* infection in sheep and cattle from Altipano of Bolivia. Vet Parasitol. 1996;61:211-220.
6. Hillyer GV, Galanes MSD. Identification of potential serodiagnostic *Fasciola hepatica* antigens by electroelution from polyacrylamide gels. Am J Trop Med. 1988;32: 1210-1217.
7. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurment with the folin phenol reagent. J Biol Chem. 1951; 193:265-275.
8. Karimi GhR, Abshar, N. Derakhshanfar, M. Study on humoral immune-response in sheep experimentally infected with *Ornithobilharzia turkestanicum* by using ELISA test. J Fac Vet Med Univ Tehran. 2005;60 (1):79-86.
9. Massoud J. Nelson GS. Studies on heterologous immunity in Schistosomiasis, 6. Observation on cross-immunity to *Ornithobilharzia turkestanicum*. Bull Wld Hlth Org. 1972;47:591-600.
10. Bautista GR, Lopez-Arellano ME, sanchez A. A new method for serodiagnosis of sheep fasciolosis using helminth excretory-secretory products. Parasitol Res. 1989; 76(2):135-137.
11. Cornelissen JB, Deleew WA, Hejden PJ. Comparison of an indirect haemagglutination assay and an ELISA for diagnosis of *Fasciola hepatica* in experimentally and naturally infected sheep. Vet Quaternary. 1992;14(4):152-156.
12. Malee wong W, Intapan PM, Wongkham C, Tomanakan K, daenseekaew W, Sukeepaisarnjaroen W. Comparison of adult somatic and excretory-secretory antigens in enzyme linked immunosorbent assay for serodiagnosis of human infection with *Fasciola gigantica*. South East Asian. J Trop Med Public Health. 1996;27(3):566-9.
13. Moazeni M, Gaur SNS. Comparison of excretory-secretory (ES) and somatic antigens of *Fasciola* Spp in gel diffusion test.. university of veterinary of Shiraz. Iranian Journal of Veterinary Research. 2003;8:177-180.