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Comparison of Electrophoretic Patterns of Larval Stages of Taeniidae and Determination of Specific Antigens of Hydatid Cyst by Western Blotting Technique

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Abstract: A large number of mammals are hosts of taeniidae cestodes. Laboratory diagnosis of infections is not common in animals, but is of special importance in humans. The determination of antigenic fractions in larval stages of taeniidae is of a high importance for diagnosis and vaccination. In the present study the antigens from different parts of hydatid cyst, *coenurus cerebralis*, *cysticercus bovis* and *cysticercus taenuicollis* have been characterized using SDS-PAGE technique. In order to determine the specific antigens, sera samples of animals infected with hydatid cyst, coenurus cerebralis and cysticercus taenuicollis were analyzed by western blotting technique. Results showed that in antigenic pattern of the *c. taenuicollis*: scolex had 14 protein bands ranging from 13 to 120 kDa, fluid had 8 protein bands about 12 to 57 kDa and cyst wall had 11 bands about 12 to 100 kDa. A band about 50 kDa was common among the three antigens. For *C.bovis*, only antigens of scolex were analyzed. Six protein bands were determined in the range of 20 to 50 kDa. For hydatid cyst four bands were detected in the protoscolex. The molecular weights of three of them were 12-14, 32-40 and 50-51 kDa. Nine bands ranged between 12 to 60 kDa were observed in the fluid and 5 bands were detected in the laminated layer. For *Coenuruses cerebralis* 6 bands of 13, 20, 23, 30, 35 and, 65 kDa were observed in the scolex, three unclear bands were shown in the fluid of cyst, whereas 6 bands ranged between 13 to 62 were seen in the cyst wall. The results of western blotting analysis showed that in the fluid, protoscolex and laminated layer of the hydatic cyst three bands of 13, 50 and 70 kDa as immunogenic were common. Also two 90 and 110 kDa bands of fluid and cyst wall of C. teanuicollis were detected as diagnostic antigens of hydatid cyst. In the laminated layer and fluid of hydatid cyst two 55 and 40 kDa bands were determined. In conclusion although western blotting is a time consuming and costly method, this is one of the best ways for diagnosis of infections, especially parasitic ones.

Keywords: Larval stages of Taeniidae · SDS-PAGE · Western blotting

INTRODUCTION

A large number of mammals are hosts of Taeniidae cestodes. The larval stages (metacestode) cysticercus,coenurus and hydatid cyst have been found in ruminants and human. Laboratorial testing for diagnosis of larval stages of taeniidae cestodes is not common for domestic animals, but is of great importance for human beings. Immunization against the larval stages of cestodes plays an important role in the epidemic regions [1]. Vast researches have been conducted in order to discover the immune responses against cysticercus and hydatid cyst in human, pig and cattle, namely several procedures for diagnosing immune responses, with the main purpose of developing vaccination methods in order to control the infection with mature and larval stage of cestodes [2].

Today the determination of antigenic fractions in larval stages is an important tool for diagnosis and vaccination. With regard to this fact the following study defined the electrophoretic pattern of antigens in larval stages of the taeneiidae using SDS-PAGE technique and consequently, using Western blotting technique to determine the specific antigens of hydatid cyst [3].

MATERIALS AND METHODS

Organs Infected with hydatid cyst, *Cysticercus taenuicollis*, *Cysticercus bovis* were collected during slaughterhouse investigations. The samples of

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Coenurus cerebralis, were obtained from an infected sheep by Coenurosis, showing clinical symptoms and was transported to our laboratory.

After removing the cyst fluid, it was centrifuged in 5000 (g) for 30 minutes. Then it was dialyzed at 4°C using dialyze bag. The supernatant was stored at -20°C for later use. Scolex and protoscolex of cysts were homogenized with sonicated method at 110 V, 170 W ultrasonic disintegrator (Hielscher, Germany). The samples were centrifuged in 15000 (g) for 20 minutes at 4°C and the supernatant was stored as antigen.

The cyst walls from four metacestodes were removed. The separated tissues were washed several times in PBS (pH=7.4) and then homogenized in a glass homogenizer and sonicated. All antigens were stored at -20°C for later uses after defining concentration using Bradford method [4].

Sera from infected animals with hydatid cyst and *C. taenuicollis* were obtained during slaughtering. The sample of coenurus was obtained from an infected sheep by coenorosis, showing clinical symptoms. Totally, 5 positive sera of hydatid, 5 positive sera of *C. taenuicollis*, one positive serum sample of *Coenurus cerebralis* and five negative sera from uninfected animals were collected and stored at -20°C for later uses.

SDS-PAGE and Western Blotting: The prepared antigens were run on SDS-polyacrylamide gels, composed of 5% resolving gel and 10% stacking gel, under reducing conditions using the discontinuous buffer system [5]. For size estimation, a pre-stained protein marker at range of 15-160 kDa Molecular weight (SM-0671) was used from Fermentase- Chemical. Proteins were transferred in a Bio-Rad Trans-Blot Cell for 12 h in a constant current of 30 V. Blocking in 3% dry skimmed milk in PBS containing 0.1% Tween 20 was carried out 1 h at room temperature. Strips were washed three times with PBS containing 1% Tween 20 for 10 min and incubated with the first antibody diluted in PBS 0.1% Tween 20 for 1 h at room temperature. The strips were washed again for 3×10 min and incubated with the secondary antibody conjugated to horseradish peroxidase (1:1000, Koma Biotech Company) for 1 h at room temperature. After incubation the strips were washed as before in PBS 0.1% Tween 20. 3, 3′-diaminobenzidine tetra hydrochloride (Sigma) substrate was added to 25 ml of PBS and then 10 µl of H2O2 30% was added for exactly 2-5 min at room temperature. Finally the reaction was stopped by the addition of distilled water to strips [6].

RESULTS

The results of this study are presented in two parts including electrophoresis and immunobloting patterns.

In this study electrophoretic pattern of scolex, fluid and cyst wall of *Cysticercus taenuicollis* (in sheep), scolex of *Cysticercus bovis* (in cattle), protoscolex,fluid and cyst wall of hydatid cyst (in sheep) and protoscolex, fluid and cyst wall of *Coenurus cerebralis* (in sheep) were identified, which are described here:

As shown in Figure 1 (columns 1, 2, 3), there were 14 protein bands with the molecular weights ranging from 13 to more than 120 kDa in *Cysticercus taenuicollis*. Usually bands weighing less than 85 kDa were sharp. 8 bands weighing 12 to 57 kDa were observed in the cyst fluid. There were 11 bands from 12 to 100 kDa in the antigen from the cyst wall. There was a sharp band about 50 kDa common in the fluid, scolex and cyst wall (columns 1, 2 and 3).

Electrophoretic pattern of scolex of *C.bovis* is shown in the Fig. 1 (column 4).There were 6 bands ranging from 20 to 50 kDa And the 50 kDa band was well noticeable.

Protein patterns of protoscolex, fluid and cyst layer of hydatid cyst are shown in columns 5, 6, 7, respectively. For the protoscolex, 4 protein bands were observed. In hydatid fluid 9 bands of 12 to 60 kDa were observed and 5 protein bands were observed in the cyst wall.

Electrophoretic pattern of wall, scolex and fluid of *Coenurus cerebralis* were shown in the Figure 2. Six bands were observed in homogenized scolex antigen (columns 1 and 2) with weights about 13, 20, 23, 30, 35 and 65 kDa. There were 3 blurred bands in the cyst fluid, ranging from 20 to 63 kDa bands. In the cyst wall 6 protein bands between 13 to 62 kDa were observed.

In order to identifying the specific antigens of hydatid cyst and *C. cerebralis*, extracted antigens were put under immunobloting next to sera containing antibodies against hydatid cyst and Coenurus cerebralis.

Hydatic Specific Antigen: Results from the western blotting of antigens obtained from *C. taenuicollis*, *C.bovis* and hydatid cyst versus positive hydatid serum are shown in Figure 3.

Bands of about 13, 50 and 70 kDa are common for the three antigens of protoscolex, fluid and cyst wall of hydatid cyst, but in the fluid of the hydatid cyst two obvious bands with molecular weights more than 100 kDa (about 110 and 140 kDa) were observed. For the scolex of C. bovis specific bands were partly blured, but similar to

Fig. 1: Ectrophoretic pattern of: (1); scolex (2); fluid (3); layer of *C. taenuicollis*, (4); scolex of *C.bovis*, (5); protoscoleces (6); fluid (7); layer of hydatid cyst,

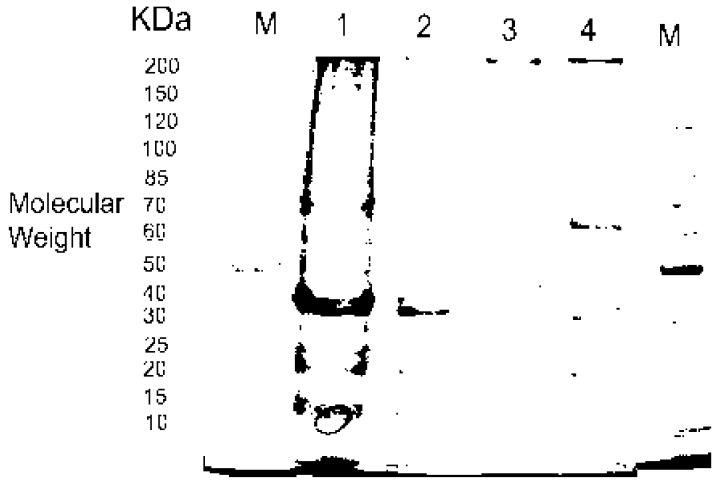

Fig. 2: Electrophoretic pattern of: (1); scolex (2); scolex with concentration=0.1 (3); fluid (4); layer of *Coenuruses cerebralis*

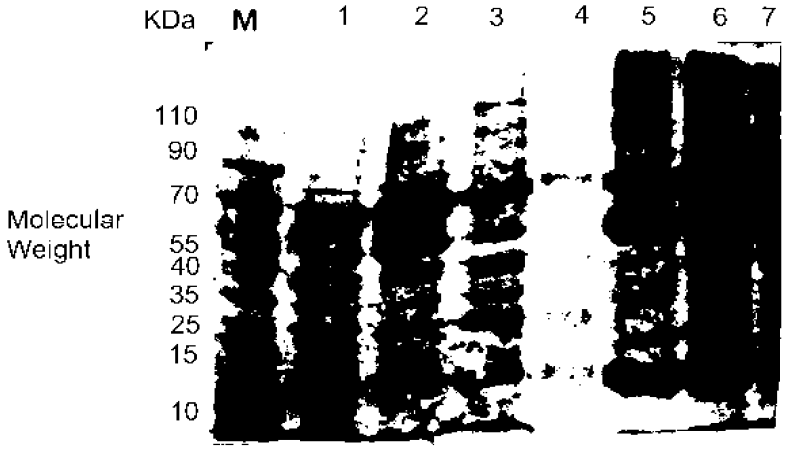

Fig. 3: Western blotting of different antigens versus positive hydatid serum: (1); scolex (2) ;fluid (3); cyst wall of *C.taenuicollis* (4); scolex of c. bovis (5); protoscolex (6); fluid (7);cyst wall of hydatid cyst.

Fig. 4: Immunoblotting of different antigens versus negative serum: (1); fluid (2); cyst wall of *C. cerebralis* (3); scolex of *C. bovis* (4); scolex (5); fluid (6); cyst wall of C. taenuicollis (8); protoscolex (9); fluid (10); layer of hydatid cyst

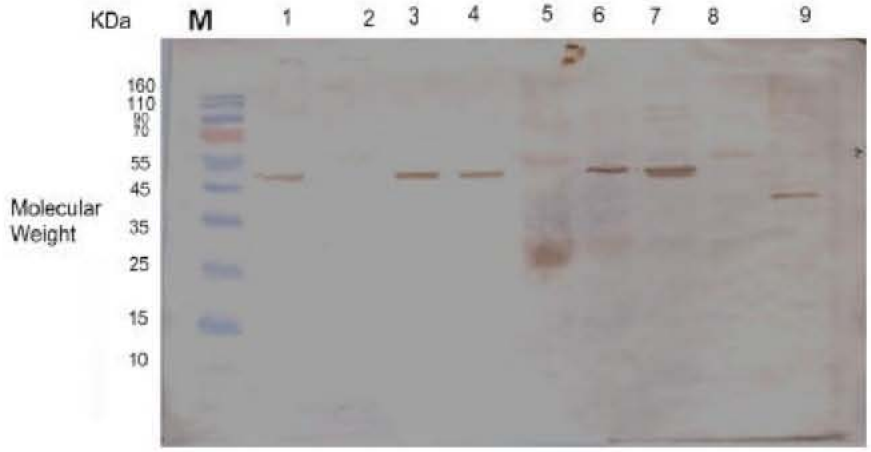

Fig. 5: Immunoblotting of different antigens versus positive serum of C. cerebralis and *C. taenuicollis*: (1); scolex (2) ; fluid (3); cyst wall of c. cerebralis (4); scolex of C. bovis (5); fluid (6); cyst wall (7); and scolex of *C. taenuicollis* (8); cyst wall (9); fluid of hydatid cyst

protoscolex and cyst wall of hydatid cyst. In addition fluid and cyst wall of *C. taenuicollis* were put under immunoblotting next to the anti-hydatid serum.The results are shown in Figure 3 (columns 1, 2 and 3). The existence of two bands about 90 and 110 kDa related to the fluid and, especially cyst wall of *Cysticercus taenuicollis* can be regarded as the diagnostic antigen for hydatid cyst.

In immunoblotting of hydatid antigens with negative serum (without any infection), three bands (37, 110 and 140 kDa) were observed in the antigen, which shows these bands cannot be considered as specific diagnostic bands. It should be mentioned that in other antigens immunoblotting versus negative serum no specific band was observed.

Figure 4 shows different antigens immunoblotting versus negative serum.

The results obtained from western blotting different antigens next to positive sera of C. taenuicollis and C. cerebralis are shown in Figure 5.

As shown in Figure 5, there is one common obvious band for the immunoblotting of protoscolex and cyst wall of *C. cerebralis*, scolex of *C. bovis*, cyst wall and the scolex of *C. taenuicollis* antigens, with the molecular weight of 50 kDa, but the band about 55 kDa in the cyst wall and the band about 40 kDa in the fluid of hydatid cyst (Figure 5, columns 9 and 10) can be regarded as common specific bands for the coenurus and *Cysticercus taenuicollis* cyst.

DISCUSSION

In comparative analysis of electrophoretic pattern of different antigens of larval stages antigens of fluid, scolex and cyst wall for *C. taenuicollis*, *C. cerebralis* and hydatid cyst and antigen of scolex of *C. bovis* were tested and studied for identifying protein profile.

In the present study 14 bands in scolex, 8 bands in fluid and 11 protein bands in cyst wall of *C. taenuicollis* were observed. Vibhav *et al.*[7] in identifying protein profile of different parts of this cyst, using SDS-PAGE method, observed 12 bands in the scolex and three bands in the liquid of this cyst.

Though in identifying protein pattern of cyst wall of *C. taenuicollis* 11 protein bands were observed (Figure 1, column 3) but 8 of them were more obvious. In the study of Also Vibhav *et al.* [7] 22 protein bands were isolated from cyst wall of *C. taenuicollis*.

In this study, three sharp bands were observed in protoscolex of hydatid cyst. Taherkhani and Rogan [8]observed two protein bands (50 and about 40 kDa) in protoscolex of hydatid cyst [8]. Hoseini [9] observed 22 protein bands in this antigenic part using SDS-Page method in defining electrophoretic pattern of hydatid antigens, from which 13 bands in the range of 20-60 kDa and a partly thick band of 60-61 kDa were observed.

Hashemitabar *et al.* [10]observed 12 bands with the molecular weights of 15-16, 20, 24, 30-32, 36, 43-44, 49-50, 84, 88-89 and 120 kDa, which are similar to our findings in this study for the two bands with the molecular weights of 32 and 50 kDa.

In the results obtained from electrophoretic pattern of different parts of hydatid cyst, the hydatid fluid constitutes the highest amount of bands. This part includes 9 protein bands (Fig.1, column 9). Also Burgu *et al.* [3] identified 9 protein bands in hydatid fluid by SDS-PAGE, the molecular weight ranged from 8 to 200 kDa.

For the layer of hydatid cyst 5 bands were observed (Fig.1, column 10) from which four bands about 12-14, 35-38, 50-51 and 60 kDa were of more thickness. In the study of Doiz *et al.* [11] nine protein bands with the molecular weights of 12, 14, 16, 20, 23, 32, 36, 39 and 42 kDa were observed which are in accordance with what was seen in this study. The band of 38 kDa which is representative for antigen 5, was reported in studies by Shepherd and McManus [12], Lightowlers [13], Hoseini [9] and also Taherkhani and Rogan [8], same as what was observed in this study. Hoseini [9] has shown 4 bands in the range of 20 to 30 kDa in germinal layer of hydatic cyst.

In the electrophoresis of antigenic parts of *Coenurus cerebralis*, 6 bands in cyst wall and scolex and 3 bands in the fluid of the cyst were observed which the band of 20 kDa was common in the three parts.

In the scolex of *Cysticercus bovis* 6 bands were observed. Joshua *et al.* [14] identified the c. bovis electrophoretic pattern with regard to the age of the cysts. The bands of 12, 14, 16, 20 and 26 kDa existed in the fluid of all cysts. Additionally, bands of 43 and 185 kDa, respectively existed only in 4 and 8-week old cysts.

Regarding the fact that no comprehensive study has been done on the identification of protein patterns of Coenurus and *Cysticercus bovis*, it should be recommended that this study, introducing protein patterns on mentioned cysts, necessitates supplementary studies.

In antigens immublotting of hydatic cyst wall with negative serum, three bands were observed. Therefore, these bands are considered as unspecific. In immunoblotting of *C. cerebralis*, *C. taenuicollis*, *C. bovis* and H.cyst with sera containing antibodies against coenurus and *C. taenuicollis*, a band of 55kDa in the cyst wall and a band about 40 kDa in the fluid of hydatid cyst can be regarded as common specific bands for the Coenurus and *Cysticercus taenuicollis*.

Altogether, SDS-PAGE and Western blotting have been used as tests studied for diagnosing different infections and their application in the diagnosis of parasitic infections has always been considered important. But the most important challenging point of these methods in the diagnosis of larval stage of the taeneiidae family is standardizing the method of obtaining antigen.

Presently immunoblotting is known as a suitable and assured test for confirming the diagnosis of hydatidose. But supplementary studies are needed for reaching the bands with more specificity and sensitivity. In addition, more attention must be paid to identifying fractions of the antigens from other larval stages of taeneidae cestodes for identifying cross reactions and their differential diagnosis. Also, identifying specific antigens in order to choose immunogenic antigens is of great importance.

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