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Sero-Diagnostic Potency of Hydatid Fuid and Protoscoleces Partially Purified Fractions of Both Camel and Equine Origin

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Abstract: The specificity and sensitivity of Enzyme-linked Immunosorbent Assays (ELISA) and Western immunoblot (IB) analysis were performed. Two purified peaks were obtained from each crude extract (HFCp1, HFCp2, HFDp1, HFDp2 PCp1, PCp2, PDp1 and PDp2). Fractionation of crude antigens through SDS-PAGE showed common shared protein bands at the level of 66, 55, 45 and 29 KDa. While partially purified antigens demonstrated that the shared bands between peaks 1 of HFC, HFD, PC and PD were 117 and 73 KDa, while 8 and 16 KDa were shared common epitopes present in peak 2 of HFC, HFD, PC and PD. 39 KDa is a common shared antigen between peaks 2 of PC, PD and HFD and peak 1 of HF C. Finally, 20 KDa is a shared common antigen between peaks 2 of HF C, HFD and P C. The results demonstrated that the sensitivity of ELISA was 100% using PCp1 and HFDp2 in serodiagnosis of hydatidosis in camel and donkey. The specificity of ELISA was 97.6 and 95.9% in camels and donkeys, respectively. Western blot analysis recognized two polypeptides at 80 and 150 KDa which might be diagnostic bands in the two intermediate hosts. While the epitopes at 21 and 59 KDa might be specific for camel hydatidosis. These variations may reflect the antigenicity of these proteins and a suitable guide for comparison or differentiation of the different strains. The common shared reactive band at the level of 138 KDa might be specific for hydatidosis in donkeys.

Key words: Hydatidosis · camel · donkey · SDS · ELISA · Western immunoblot

INTRODUCTION

Echinococcosis/hydatidosis is an economical and zoonotic important disease. True hydatid prevalence in human could not be based on visible surgical cases as hydatid cyst frequently causes no symptoms during early stage especially if localized in the liver [1]. [2] studied the comparative immunodiagnosis of canine echinococcosis by coproantigen ELISA and IgG serum antibody test. [3] used DEADE-cellulose and Sephadex G-200 chromatography for purification of hydatid fluid antigen for serodiagnosis of echinococcosis. The electrophoretically pure antigen was found to be sensitive and specific for *E. granulosus*. [4] evaluated ELISAs for the detection of serum antibodies in sheep infected with *E. granulosus*. ELISAs were developed and validated, using crude protoscoleces and purified 8 KDa hydatid fluid protein which was purified by SDS-gel filtration chromatography. The protoscoleces-ELISA was the most effective immunological method of those assessed for detection of infection with *E. granulosus* in sheep. [5] evaluated the Enzyme-linked Immunoelectrotransfer Blot (EITB) for serodiagnosis of ovine hydatidosis relative to age and cyst characteristics in naturally infected sheep.

The present study aimed at purification and immunochemical characterization of *E. granulosus* antigens obtained form camel and equine origins. Evaluation of crude and partially purified antigens in the diagnosis of hydatidosis in camels and equines by (ELISA) and (IB) to determine the specific antigens of *E. granulosus* which might have a great importance in diagnosis and vaccine candidate production

MATERIALS AND METHODS

Protoscoleces antigens: The protoscoleces antigens of both camel (PC) and donkey (PD) origins were prepared as procedures described by [6].

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Hydatid fluid antigen: Hydatid fluid from both camel and donkey hydatid cysts were centrifuged separately at 12000 rpm for 15 min at 4°C. The supernatant fluids were dialyzed and concentrated according to [7]. The fluids are designated as (HFC) and (HFD) for hydatid fluid antigen originated from camel and donkey origin respectively.

Gel filtration chromatography: The partially purified protoscoleces and hydatid fluid antigens were obtained by gel filtration on Sephadex G100 according to [8]. The protein concentration of all antigens was determined using the modified Lowry's method [9].

Hyperimmune antisera: Sera against both crude hydatid fluid and protoscoleces antigens were raised in rabbits according to [10].

Serum samples: They were collected from camels, 65 naturally infected with hydatidosis, 27 naturally infected with trypanosomiasis, *Homonchus longstipes* and 14 apparently normal non-infected camels. Serum samples were collected from donkeys, 40 naturally infected with hydatidosis, 60 naturally infected with fascioliasis and/or *Gastodiscus aegyptiacus* and/or *Parascaris equorum...etc* and 10 apparently normal non-infected.

Enzyme linked immunosorbent assay: ELISA was preformed in two steps; the first step was carried out as a screening test to select the antigens of choice which were used in the second step of ELISA test and (IB) technique. The test was carried out as described by [11].

Electrophoresis and immunoblot techniques: The four types of crude antigens as well as the partially purified peaks were fractionated using SDS-PAGE according to [12]. Hydatid fluid peak1 of camel (HF C p1) and donkey origin (HF D p1) antigens were characterized by SDS-PAGE technique. The protein bands were electrophoretically transferred for SDS-PAGE to a nitrocellulose sheet using the modified [13].

RESULTS

Electrophoretic pattern of antigens derived from crude hydatid fluid and protoscoleces of both camel and donkey are displayed in the Figs. 1 and 2. On the other hand, Figure 3 revealed the immunoblot profile of the partially purified antigens.

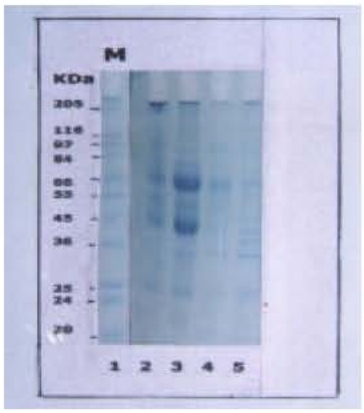

Fig. 1: Electrophoretic profile of crude hydatid fluid and protoscoleces derived proteins (KDa) from camel and donkey origin.Track 1,molecular weight marker, track 2, PC; track 3, HCF; track 4,PD; track 5,HFD

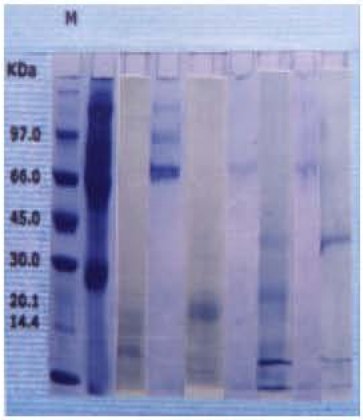

Fig. 2: Electrophoretic profile of crude hydatid fluid and protoscoleces derived proteins concentration (%) from camel and donkey origin. Track 1, low molecular weight; track 2, HFC p1; track 3, HFC p2; track 4, PC p1; track 5, PC p2; track 6, HF Dp1; track 7, HF Dp2; track 8, PD p1; track 9, PD p2

Figure 4 demonstrated the results of sensitivity and specificity of ELISA.

DISCUSSIN

The use of partially purified antigens in immunodiagnosis using ELISA resulted in identification of specific peaks for serodiagnosis of hydatidosis. Protoscoleces peak 1 of camel origin (PCp1) gave 100% sensitivity and 97.6% specificity when used with ELISA test. Also hydatid fluid peak 2 of donkey origin

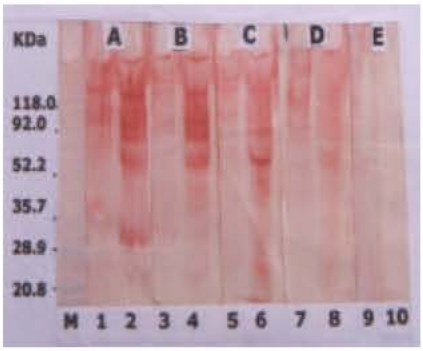

Fig. 3: Western blot analysis:1,HFD p1;2 HFC p1;3,HFD p1;4 HFC p1;5,HFD p1;7,HFD p1;8,HFC p1;9, HFD p1;10,HFC p1 and M,low molecular marker. A-Rabbit hyperimmune anti-sera against hydatid fluid of camel origin ;B, Rabbit hyperimmune anti-sera against protoscoleces of camel origin; C-Rabbit hyperimmune anti-sera against hydatid fluid of donkey origin; D-Rabbit hyperimmune anti-sera against protoscoleces of donkey origin; E-Negative control sera

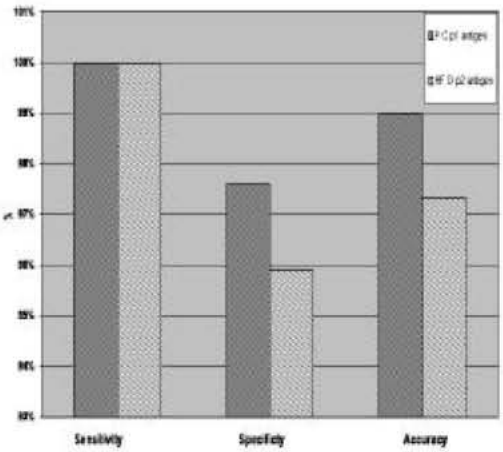

Fig. 4: The senstivity, specificity and accuracy of P C p1 and HF D p2 antigens in serodiagnosis of camel and equine hydatidosis using ELISA

(HFD p2) gave 100% sensitivity and 95.9% specificity for diagnosis of hydatidosis in donkeys. Moreove, (HFCp1) and (HFDp1) gave positive results with sera of both camel and donkey infected with hydatidosis. Both antigens might contain diagnostic antigens for hydatidosis in both of camel and donkey origins, so they were used in (IB) technique for further characterization.This was in agreement with [14] who studied the specific immunodiagnosis of larval cestode infections (*T. saginata, T. hydatigena* and *E. granulosus*) of cattle and sheep using antigens purified by affinity chromatography. These antigens may be useful in the production of species specific immunodiagnostic antigens. Moreover, [15]) found that the fractionated first peak antigen (F1) from hydatid fluid gave more sensitive and specific results for hydatidosis in buffaloes. Also [16, 17] concluded that the use of purified antigens from sheep hydatid fluid in Western blot proved to be highly useful in the diagnosis and post-surgical monitoring of human hydatidosis. The results disagreed with [18] who concluded that the crude antigen was more sensitive than the purified antigen in all tests applied for immunodiagnosis of hydatid disease in human. In this study, ELISA technique indicated that HFCp1 and HFDp1 might be containing diagnostic antigens that could help in serodiagnosis of hydatidosis in both camels and donkeys. The test was based on partially purified hydatid fluid. ELISA technique indicated that PCp1 might contain diagnostic antigens for camel hydatidosis and HFDp2 might be the antigen of choice for serodiagnosis for donkey hydatidosis. Both antigens gave a sensitivity of 100% but the specificity was 97.6% and 95.9% respectively. These results agree with [19,20] who demonstrated that the sensitivity of ELISA was (91.2%) significantly higher than IHA and the ELISA technique detected all IHA negative CE cases, so they decided that the ELISA was the test of choice for diagnosis of CE in human using sheep HF. Moreover, [21] evaluated the ELISA-IgG test using a purified antigen in the diagnosis of human hydatidosis. They found that the sensitivity of ELISA-IgG was 94.3% for hepatic cysts and 92.9% for pulmonary cysts.

Five protein bands were found relatively similar among patterns of crude protoscoleces protein in both species but only PD had protein bands at 35.284 and 96.268. analysis of protoscoleces protein had been used previously as a criterion to evaluate intraspecific variation in *E. granulosus* for ovine and equine protoscoleces [22]. Such results agreed with those obtained by [23] when they studied the electrophoretic variations between ovine and swine populations. However, [24] found that there was a close similarity between *E. granulosus* of horse, goat and pig origins whereas *E. granulosus* derived from cattle showed less homology with any of the former by SDS-PAGE and western blotting. SDS-PAGE technique revealed two common protein bands at 73 and 117 KDa between HF C p1, HF D p1, P C p1 and P D p1, while HF C p2, HF D p2, P C p2, P D p2 had common shared protein bands at 8 and 16 KDa. HF C p2, HF D p2 and P C p2 had common shared protein bands at 20 KDa. These results

agree with those of [25] who recognized bands of 38 and 116 Kda in SDS-PAGE profiles to be present in bovine hydatid cyst fluid (BHCF) from fertile cyst (FC) only. In contrast to [26] who observed active antigenic fractions had higher molecular weight than those obtained in the present study (of 100 to 300 KDa MWs and higher). It could be concluded that SDS-PAGE electrophoretic analysis might be used as a criterion to evaluate intraspecific variation in *E. granulosus*.

(IB) technique revealed that the antigenic bands at 80 and 150 KDa in HFCp1 and HFDp1 might be sensitive antigenic bands for serodiagnosis of hydatidosis in both camels and donkeys. However, the antigenic bands at 21 and 59 KDa in HFCp1 might be specific for camel hydatidosis. While the antigenic bands at 138 KDa in HFDp1 might be specific for donkey hydatidosis. Moreover, [27] used immuno-electrophoresis in the diagnosis of hydatidosis in Sudanese camels. They found that immuno-electrophoresis had sensitivity of 36% and specificity of 84%. However, [28] used (IB) technique for characterization of immunogenic protein from *E. granulosus*. They found that the highest reactivity corresponded to 80, 66 and 56 KD proteins, while in reducing conditions only one 36 KD protein was detected. Also, [29] used Western blot for detection of specific diagnostic antigens of *E. granulosus*. They found that 8 KDa antigen corresponding to the specific *E. granulosus*. Bands of 21, 30 and 92 KDa appeared also specific. So the present results suggest that Western blot could be useful for the diagnosis of hydatidosis Also, [30] used (IB) for evaluation of 100 and 130 KDa antigens in camel hydatid cyst fluid for serodiagnosis of human cystic echinococcosis in Libya. They found that the 100 and 130 KDa antigens were strongly recognized by sera from cystic echinococcosis patients. [31] studied the immunodiagnostic potential of protoscolex antigens in human cystic echinococcosis and the possible influence of parasite strain. They found that the results of SDS-PAGE and western blotting revealed 10-125 KDa antigens were recognized by sera from human cases of cystic echinococcosis. Some of the latter antigens (79, 59, 45, 38, 31 and 29 KDa) exhibited cross-reactivity with sera from humans with other parasitic infections. Finally, IB is a technique more specific than ELISA but much knowledge concerning the identification of the immunodominant peptides is expected. Studies on the purification of specific peptides may obviate the problem of cross-eactivity and their use at appropriate concentrations can improve the ELISA efficiency in the diagnosis and a positive result could be confirmed by the IB procedure.

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