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ADVANCED CONCEPTS IN DIAGNOSIS OF HYDATIDOSIS IN HUMAN AND LIVING ANIMALS

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SUMMARY

Fractionation of fertile hydatid cyst fluid antigens (FHCFA) revealed 12 protein fractions at molecular weights (MW) of 105 kD, 79 kD, 62 kD, 49 kD, 38 kD 28 kD 24 kD 21 kD, 18 kD , 8 kD and 2 bands over 105 kD. The bands at MW of 38 kD, 36 kD, 29 kD 18 kD, 16 kD, 12 kD & 8 kD were reacted specifically with sera of hydatid cysts (HC) experimentally infected rabbits as well as sera of surgically proved HC infected patients using enzyme linked immuno-transfer blot (EITB) technique.

Evaluating the diagnostic efficacy of 3 eluted concentrated sub units of these antigens at MW range of 32-38 kD, 16-18 kD and 8-12 kD, revealed that the fractions in the range of 8-12 kD appear as the most specific (96.66%) fractions. It didn't cross reacted with anti bodies (Ab) in sera of patients infected by *Fasciola*, gastro-intestinal nematodes (G.I.N.) infected camel as well as *Fasciola & Moneizia* infected sheep. It evidenced mild level of cross reaction (10%) with Virus hepatitis "C" & *Schistosoma mansoni* infected patient's sera. These fractions appear more sensitive (100%) for diagnosis of anti HC Ab in sera of surgically proved HC infected patients, HC infected camels and HC experimentally infected rabbits using ELISA technique.

Fertile Hydatid cyst fluid antigen (FHCFA) of 32-38 kD appear less specific (93.33%) than the fraction of (8-12 kD), but this fraction still has absolute sensitivity (100%). The last groups of bands (16-18 kD) appear as the lowest specific (81.66%) and sensitive (92.0%) one.

The protein fractions in the range of 8-12 kD showing marked diagnostic efficacy for hydatidosis infection in random samples of exposed people & pre-slaughtered animals. Diagnosis of infection by ELISA technique was closely related to diagnosis using sonographic examination in human and post mortem examination in animals. So that the former group of antigens identified as a good tool for diagnosis of the disease in human and investigation to infection status in living animals , the matter which is essential to improve the prognosis of the infected patients and breeding valuable animals.

INTRODUCTION

Cystic echinococcosis (CE) or hydatid disease (HD) is recognized as an important worldwide distributed disease from the clinical, economical and zoonotic point of view. The disease in man and other intermediate hosts is caused by ingestion of food or water contaminated with eggs of the dog tapeworm, *Echinococcus granulosus* (Devi and Parija, 2003).

Currently, *E. granulosus* is the only species of the genus echinococcus that is found in the region of the Mediterranean areas, and the domestic dog serves as the only known reservoir for the adult tapeworm, and therefore plays a recognized role as the main source of infection causing both health and veterinary problems (Lamar et al., 2001). Distribution of the disease is normally associated with underdeveloped countries especially in rural communities where man and other domestic animals are maintain in close contact with the dog (Ahmadi, 2004).

In animals, hydatid usually remains as asymptomatic disease producing no clinical symptoms and its course is slow. In domestic animals diagnosis is almost made during postmortem, large unilocular cysts are usually not diagnosed in young animals until middle life or later (Hassan, 1991).

El-Askalany, (1981) recorded infection by Hydatid cysts (H.C.) in slaughtered camels reached to 31.26%, while Derbala and Zayed (1997) found H.C. infection in camels, donkeys, horses, pigs and sheep as 40%, 7.69%, 6.25%, 0.92% and 0.77% respectively.. They failed to detect. infection in slaughtered cows and buffaloes .Serologically El-Baz (1994) mentioned that the infection rates of hydatidosis in camels, donkeys, horses pigs and sheep was 40 %, 7,69%, 6,25%, 0,92% and 0,77% respectively.

At the level of human in Egypt, the impression that hydatid disease was rare existed as accurate epidemiological information was lacking. This was attributed to the concern of medical authorities with the more important disease such as malaria, schistosomiasis and filariasis so that hydatidosis was not a notifiable disease. Also may be due to the slow clinical course of the disease which was not alarming and not contagious (Osman 2006).

Lightowlers and Gottstein (1995) mentioned that, insensitive and non specific tests for diagnosis of hydatidosis include the casoni intradermal test, the complement fixation test, the indirect haemagglutination test and the latex agglutination test been replaced by the enzyme linked immunosorbent assay (ELISA), the indirect immunofluorescence antibody test, immunoelectrophoresis (IEP) & immunoblotting (IB) in routine laboratory application.

There are Varity of antigens can be prepared from Hydatid cysts (HC) include that of internal germinal layer, scoleces, or from cystic fluids either as sterile or fertile Hydatid cyst antigen (FHCA)

(Osman 2006). Hydatid cystic fluid is a complex mixture of parasite-derived and host-derived molecules. It contains several antigens derived from the metabolism of the parasite together with many components from the host (Irabuena et al, (2000).

Fractionation of Hydatid fluid antigen using SDS-PAGE and identification to specific bands by immunoblotting resulted in identification of the arc-5 subunits, including two subunits with relative molecular mass between 37 & 38 KD and 20-22-to 24 KD (Poretti et al (1999). Moreover, Lightowlers & Goltorti (1995) identified another thermostable lipoprotein called antigen B. The major components of this antigen resolved as 3 bands of 8-12 kD, 16 kD and 23-24 kD protein fraction.

Concerning the diagnostic values of these fractions, Poretti et al, (1999) cleared that FHCA of 8 kD and that of 29 kD and 34 kD bands exhibited high specificity than sensitivity, moreover 8-kD showing relatively frequent cross-reactions in tumor patients and in patients with other parasitic diseases but it high specific in differentiation between infection by cystic HC and alveolar one. At the same time, Ito et al, (1999) stated that this fraction (8 kD), revealed no cross-reactivity with any sera from patients with cysticercosis, other parasitic diseases, or with hepatomas, or from healthy controls as genus-specific for echinococcus. According to Dreweck et al, (1997), FHC protein fractions of 26kD, 18kD, 16 kD and 12 kD appear as more specific for detection of IgG antibodies of echinococcosis in general using ELISA technique. They considered as useful approach for post-treatment follow-up of patients at risk of developing recrudescent disease.

As mentioned by Babba et al, (1994), ELISA considered as one of the best sensitive serodiagnostic technique for diagnosis of hydatidosis in man in comparison with latex agglutination, counter immunoelectrophoresis using antigen of 38 kD specially if they associated with Echography and/or chest radiograph, They added that Immunoblotting, succeed in determination of 1- 3 specific protein bands of 65, 12, and 8 kD. The sensitivity of the ELISA did not increase with either the size or type of cyst. Coltorti et al, (1990).

The present study aimed to identify specific and sensitive protein fraction from fertile hydatid cysts of camel lung via SDS-PAGE & EITB technique, and estimate the value of these fractions in diagnosis of hydatidosis infection in suspected human and living animals using ELISA technique.

Materials and methods

Fertil Hydatid cysts fluid antigen (FHCFA) preparation:

-Hydatid cysts (HC) were collected from freshly slaughtered camel lungs (from Cairo slaughter abattoir). They examined from the aspect of viability as well as sample

from their fluid was aspirated and microscopically examined for the presence of protoscolices.

-These cysts were used for collection of fluid antigens and also for induction of experimental infection in young puppies for *Echinococcus granulosus* (Eg) gravid segment (GS) collection which used for induction of infection in rabbit for production of reference sera (versus HC) later on.

FHCFA was prepared according to Ito et al., (1999) where the fluid was collected from fertile cysts and clarified by centrifugation at 5000 rpm for 15 min at 4°C, dialyzed against 5mM Tris-Hcl(pH 7.4) for 48 hr at 4 °C, after determination their protein content by method of Lowry et al. (1951). The antigen was allocated into 1ml vial and stored at –20 °C until use.

Fractionation of FHCFA using SDS-PAGE:-

The prepared antigens were resolved using 1.5 mm thickness, Sodium dodecyl Sulfate-polyacrylamide gel electrophoresis (SDS- PAGE) according to Laemmli (1970) in 12 % polyacrylamide gel slabs in Tris-glycine buffer, pH 8.3 under reducing conditions. The stacking gel consisted of 5 % acrylamide prepared in 12.5 mM Tris-HCL buffer (pH 6.7) (Sigma chemical Co.). Prestained low molecular weigh (MW standard was employed (Sigma SDS-100B). The comb was adjusted as one small well for standard and one large for the sample.

Electrophoretic transfer of protein fractions onto nitrocellulose sheet.

Electrophoresis transfer of fractionated proteins onto nitrocellulose sheet (NC) for electro-immuno transfer blot technique (EITB) was performed according to Towbin *et al.* (1979) using transfer buffer (25 mM tris-base, 192 mM glycine, 20% (v/v) methanol at pH 8.3). Transferring was carried out at 10V, 100 mA overnight at 4°C. Longitudinal strip from the side of the NC membrane containing the MW protein standards and the whole fractionated antigens were cut off, washed by distilled water and stained with ponceau S stain (0.2% ponceau S in 3% trichloroacetic acid, Sigma). The excess dye was washed off by distilled water. The relative M.W. of the visualized bands was estimated from the reference MW standard curve (plate 1).

Determination of FHCFA specific protein fractions using EITB: -

According to Towbin *et al.* (1979), EITB technique was applied in order to determine the specific diagnostic fractions of FHCFA longitudinal NC strips (15 x 0.5 cm) were cut out and allowed to react versus hydatid experimentally infected rabbits (3 samples) and 5 samples of hydatid surgically proved infected human as well as negative control samples at. (1:100 dilution), 0.5ml of sera/ strip. The used conjugate was horseradish peroxidase conjugated goat anti-human and anti-rabbit IgG (Sigma Immunochemicals), at 1:1000 in 3% BSA/PBS. The used substrate is 4-chloro-1-naphthol. Fractions that react versus reference positive sera

and in the same time did not react versus negative control one, considered as specific protein fractions (plate 1).

These strips of NC were returned back to its original position on NC sheet for determination to the sit of the specific reacted kD protein fractions on the whole NC sheet.

Isolation of selected protein fractions from NC by electro elution:

Three transverse pieces of NC membrane corresponding to the protein fractions of 32-38 kD, 16-18 kD and 8-12 kD were cut out horizontally across the whole NC. Each transverse NC strip was transferred separately to elution tube membrane 4-6 MW cut off (Spectrum Medical Inc., Los Angeles, CA 900060).The tube were filled with PBS (pH 7.4) and kept in Bio-Rad elution unit at 10V, 100mA over night at 4 °C. NC materials were removed and the volume was reduced using polyethyleneglycol in molecular porous membrane tubing 4-6 MW cut off according to Katrak et al (1992). The protein contents of the eluted concentrated materials was determined and kept in 1 ml vial at –20°C till use for coating of the ELISA plate.

Indirect ELISA technique:-

ELISA test was done as described by Gottstein *et al.* (1993). Each plate was coated with antigen at its optimal concentration 2 ug/ml coating buffer (adjusted after checkerboard titration). Peroxidase-conjugated protein A (Sigma) was used at 1:2000 dilution. Ortho-phenylenediamidine-OPD was added at a concentration of 340 ug/ml substrate buffer. Absorbance was read at 492 nm using a Titerteck multiskan ELISA reader which calculate the positive and negative value automatically.

The test was applied for determination specificity of 3 fractions (32-38 kD, 16-18 kD & 8-12 kD) versus control & tested sera at 1:100 serum dilution. Specificity of the tested fraction was evaluated according to (Abdel-Rahman *et al.* (1993), as the ability of the tested fraction to detect its target Ab from different antibodies of other parasites using the following equation :

Specificity % = (T – P) / T X100 / 100

Where T = No. of tested sample P = No. of +Ve samples

This at standard serum dilution, while sensitivity is the ability of the tested antigenic fractions to detect its target Ab in samples infected by the parasite at standard serum dilutions (% of positive sera among the total number of the positive samples).

Estimation to the diagnostic efficacy of the selected fractions using ELISA technique:

Collected samples:

In order to estimate the diagnostic efficacy of the selected eluted concentrated FHCFA protein fractions, 3 experiments were designed, one for testing the sensitivity, another for evaluating their specificity and the 3rd one as

application for diagnostic efficacy of these fractions.

To fulfill the requirement of these 3 experiments, special serum samples of known parasitic status were selected from large number of examined samples as the following:

Human samples: A number of 110 stool & blood samples were collected from out-patient clinic in Kasr El Any Medical hospital, Tropical Medicine & Hygiene Dept. (Kasr El-Any street) as well as private clinics from patients complained by digestive disturbances, fever and abdominal pain. Some of these patients were exposed to Sonographic diagnosis of hydatidosis on chest & liver.

Animal samples: A number of 145 sheep & 140 camel fecal and blood samples were collected from these animals at time of slaughtering in Cairo abattoir. Post mortem data of most of these animals could be obtained after evisceration concerning presence of H.C. and different other tissue parasites.

Fecal samples were examined parasitologically in order to determine the parasitic infection in each case, by one or more method include direct smear method according to the technique of WHO (1983), Concentration floatation technique using saturated sodium chloride solution according to Wattal et al. (1986), while the large eggs were diagnosed via two successive sieve system (Fluke finder, Moscow, ID) according to Welch et al., (1987). The collected blood samples were used for separation of the required serum.

From the previous samples and according to parasitic infection in stool or P.M. & Sonographic examination the following serum samples were selected according to the requirements of each experiment:

Selected human sera:

- 10 samples of patients had *Schistosoma mansoni* eggs only in their stool.
-5 serum samples from healthy people (as negative control).

Selected animal sera:

- 10 samples of camels harbor gastro-intestinal parasites (G.I.P.) eggs only in their feces.
- 1 samples of sheep had *Fasciola* eggs only in their faeces.
- 10 samples of sheep feces contain *Moneizia* eggs only.
- 10 sample from hydatid infected Cairo abattoir slaughtered camels (3-15 cysts in lung)
- 5 samples from young camels has parasite free faecal and P.M. (as negative control).
- 5 samples from sheep of parasite free feces and P.M. (as negative control).

Experimental control samples:

1-Sera of hydatid cysts experimentally infected rabbits:

Three rabbits were experimentally infected by *E.granulosus* gravid segment (GS) prepared in other related work (Osman 2006) under supervision of the author. The used GS were collected after scarification of one dog 60 days post experimentally infected by fertile hydatid cysts. The infected rabbits were scarified 60 days post infection where high anti-

hydatid cysts antibodies could be detected in their sera and used as positive control.

2- Three samples of non-infected rabbits.

Samples via personal communications:-

- Five Fasciola and 10 of virus hepatitis (C) infected patients were obtained from Institute of Tropical Medicine & Hygiene, Kasr El-Any street.
- Five surgical proved HC infected human sera (Dept of Parasitology Fac. of Medicine, Cairo University)

For testing the diagnostic efficacy of the selected fractions:-

-Forty serum samples were collected from HC suspected camel at the quarantine pre slaughtering and they were confirming post slaughtering.

- Forty five serum samples were collected from suspected sheep at the quarantine pre slaughtering and they were confirm post slaughtering

-Thirty five serum samples from people exposed to infection (veterinary clinic workers) have available Sonographic data.

These samples were included in sero-diagnosis for anti-H.C. antibodies by ELISA techniques and their data was confirmed by P.M. examination for slaughtered animals or sonographic findings for human to confirm the results.

Results & Discussion

Distribution of hydatidosis is related to underdeveloped countries especially in rural communities where man remains in close contact with the dog (definitive host) and the various domestic animals act as intermediate hosts. Dogs were infected by eating infected carcasses containing the hydatid cyst. The parasite was transmitted to man by ingestion of food or water contaminated with dog faeces containing the infective eggs (Ahmadi, 2004).

In Egypt, El- Askalany, (1981) mentioned that, incidence of human infection call for three existing factors: an existing sheep raising district, the presence of large dog population which has access to the offal of dead or slaughter animals and sanitation that permits a close association between these dogs and human being. All these factors were present in the Egyptian villages where dogs were allowed into houses. Animals shed eggs in stool and children were allowed to play with them.

In human, diagnosis of hydatidosis infection based mainly on imaging studies and immunodiagnostic procedures, Caremani et.al, (1997), while the condition was differ in animals, the infection was diagnosed during P.M. examination of the slaughtered carcasses mainly and numbers from these cysts can reach to dogs by different ways or even pass with the meat for consumers. Presence of an accurate mean for diagnosis of

infection in living animals can prefer a good tool for control the spread of this disease.

As mentioned by De Morilla *et al.*, (1983) & Irabuena et al. (2000), serological diagnosis of hydatidosis, could be considered as beneficial tool in this respect, but cross-reaction between different parasites is still as a questionable point making some difficulties in the accurate evaluation of the infection status of suspect animals, especially under the level of field collected polyclonal sera. They added that, accuracy of these tests was affected markedly by degree of purity and specificity of the used antigens. For this reason Identification of special hydatid fraction could be improve the accuracy of the used diagnostic technique.

The present study was focused on identification of FHCFA specific diagnostic protein fractions using EITB technique. The results in table (1) & plate (1) demonstrate that Ponceau-s stained longitudinal strips, blotted with fractionated *FHCFA* antigen revealed 12 protein fractions were identified using the molecular weight standard curve as 105 kD, 79 kD, 62 kD, 49 kD, 38 kD 28 kD 24 kD 21 kD, 18 kD , 8 kD and 2 bands over 105 kD. Treatment of corresponding NC strips using HC experimentally infected rabbit & surgically proved hydatid infected human sera via EITB test revealed 10 fractions of these bands react versus the above sera, three from these bands were react non-specifically versus negative rabbit and human sera (that over 105 kD, 105 kD & 62 kD), while the other 7 bands could be considered as specific recognized by specific anti-bodies only as in table & plate (1). These specific bands were corresponded to the MW standard of 38 kD, 36 kD, 29 kD, 18 kD,16 kD, 12 kD and 8 kD, with different amount of protein content as in Plate (1) .

The fraction of 29kD could not be detected using human HC infected serum and in the same time, didn't react with the negative control sera. These data was agreed with that mentioned by Ito et al, (1999) & P tti et al, (1999) as FHCF antigens revealed several fractions in the range of 8 kD to over than 105 kD. Failure of 29 kD fraction to react versus human sera may be due to the origin of this antigen as it camel non human origin.

EITB is one of the most specific sero-diagnostic techniques but it considered non-practical for current field application in comparison with ELISA technique (Ibarra *et al.*, 1998). De Morilla *et al.* (1983) mentioned that ELISA technique was a sensitive serological test, able to analyze many samples simultaneously. Moreover, Babba et al, (1994), identify (ELISA) as one of the best sensitive sero-diagnostic technique for diagnosis of hydatidosis in man in comparison with latex agglutination and counter immunoelectrophoresis

Collectively these protein fractions occur among 3 main subunits in the MW zones of 32-38 Kd, 16-18 kD and 8-12 kD. The sites of these 3 groups of bands were cut transversally from the whole NC sheet. The protein

contents of these bands were eluted and concentrated and used for more characterization.

Estimation to the diagnostic efficacy of these 3 sub-unites (32-38 kD, 16-18 kd & 8-12kD) via ELISA technique as in table (2), cleared that the fraction of 8-12 kd appear as the most specific (96.66%) & sensitive (100%) one for diagnosis of hydatid anti-bodies in different serum samples. This group of fractions did not cross react with anti-Fasciola antibodies in infected patients or animals, did not cross react with antibodies produced from G.I.N. infections in camels and *Moneizia* infection in sheep. They cross react with one sample (10%) of *S.mansoni* & Virus hepatitis "C" infected patients. These data came in agreement with Ito et al. (1999) and Babba et al.(1994) as the fraction of 8 kD & that of 12 kD did not cross react with different parasites and liver affections via ELISA & EITB techniques. On the contrary with these authors, these sub-units have low specificity (90%) versus *S.mansoni* infected patients.

The sub unites antigens in the range of 32-38 kD appear of lower specificity (93.33%) and of good sensitivity (100%). The marked diagnostic value of these 2 sub-units fractions was previously mentioned by Babba et al.(1994) and Wen & Craig (1994) using ELISA techniques, as antibodies in sera of HC infected patients were react specifically versus protein fractions of 20, 16, 12 kD, and 38-kD subunit of antigen 5 from FHCA. In the contrary with Dreweck et al. (1997) the band fractions of 16-18 appear lower in specificity (81.66%) and sensitivity (92.0%) than the other tested fractions this may be due to the difference in the strain of the used hydatid fluid antigens. Also with d'Amello et al (1985) where they identify 4 fractions from FHCFA of human origin at MW in the range of 52-13 KD, 67 and 52 KD, as they specifically react versus sera of infected patients.

Hydatidosis is worldwide problem, WHO, 1999 showed that number of yearly infected people with *E.granulosus* was 2.7 millions. Human cystic echinococcosis, is recognized globally as an increasing major zoonotic disease and remains as significant health problem in Arab countries (Dar & Al-Karmi, 1997). In order to this, extraction of special protein fraction which able to induce accurate diagnosis for hydatid infected animals during their life could be facilitate control of the disease as mentioned by El-Askalany, (1981) as the prevalence of the disease in man is conditioned by the degree of infection in sheep, cattle and camel.

The marked specificity & sensitivity of 8-12 kD protein fractions could be demonstrate this antigen as a high valuable purified antigen help in accurate diagnosis of hydatid infected animals in Egypt during breeding and before slaughtering without interest to the other parasitic infections which could be diagnosed in these animals.

Diagnosis of hydatidosis infection in group of exposed people using this fraction as demonstrated in table (3) revealed that the serological incidence of infection using this fraction was (5.71%) which is exactly the same figure by sonographic examination (5.71%). The infection was recorded in suspected older patients (over 35 years old) than in young individuals. Presence of different enteric parasites in both groups of patients (*Giardia, S.mansoni, Entamoeba histolytica & hymenolepis nana*) did not produce cross reactions in diagnosis by these protein fractions. This identifies the value of this fraction for current diagnosis of suspected patients using ELISA technique.

Concerning to pre-slaughtered camels and sheep (table, 4) the incidence of infection was higher by ELISA technique (17.64%) in comparison with the data of P.M. examination of lung and liver (10.58%). This was agreed with El-baz (1994) as the difference between sero-diagnosis and P.M. diagnosis of hydatidosis may be related to presence of hydatid infection in small size which could not be demonstrated by visual examination or the infections in other parts of the body did not accurately investigated. Enteric parasites diagnosed in fecal examination of these animals (*Fasciola, Paramphistomum spp., G.I.N., Moneizia* eggs and coccidian oocysts), previously proved hat they did not cross reacted with these protein fractions.

For conclusion, FHCF fraction of 8-12 kD (camels origin) could be considered as a good tool for early diagnosis of the disease in human and investigation to infection status in living animals. This way facilitated early diagnosis of infection in human and animals the matter which is essential to improve the prognosis of the infected patients as mentioned by Fujimoto et al (2005). Identification of infected animals during their life could be facilitate slaughtering them under special control measures which ensure total condemnations of their infected tissues and eliminate the random arrival of the cysts to dogs the matter which play a role in minimize the infection in dogs and wide spread of the disease. Moreover it is necessary to mention that, control measures of hydatidosis must be carried on paralleled to that of human beings, also against the definitive host and the other intermediate ones.

REFERENCES

Abdel-Rahman, S.M.; O'Reilly, K. L. and Malone, J.B. (1998). Evaluation of a diagnostic monoclonal antibody-bases capture ELISA for detection of a 26 to 28 KDa *F. hepatica* coproantigen in cattle. Am. J. Vet. Res., 59: 533-537.

Ahmadi, N.A. (2004): Using morpheme try of the larval rostellar hooks to distinguish Iranian strains of *E.granulosus*. Ann. Trop. Med. & Parasitol., 98 (3): 211-220.

Babba H, Messedi A, Masmoudi S, Zribi M, Grillot R, Ambriose-Thomas P, Beyrouti I, Sahnoun Y. (1994): Diagnosis of human hydatidosis: comparison between

imagery and six serologic techniques. Am J Trop Med Hyg.;50(1):64-8

Caremani, M.; Benci, A.; Maestrini, R.; Accorsi, A.; Caremani, D.; and Lapini, L. (1997): Ultrasound imaging in cystic echinococcosis. Proposal of a new sonographic classification. Acta trop.,67: 91-105.

Coltorti EA, Fernandez E, Marguet ER, Scozzina JD, Guarnera EA.(1990): Detection of asymptomatic carriers of hydatid cysts: specificity increase of the immunoenzyme assay Rev Inst Med Trop Sao Paulo.;32(4):275-84.

d'Amelio R, Pontesilli O, Dayal R, de Rosa F, Barnet M, Teggi A, Brighouse G, Lambert PH.(1985): Characterization of parasite antigens from human hydatid cyst fluid by SDS-PAGE and IEF. Med Microbiol Immunol (Berl).;174(1):43-50.

Dar, F.K.and Alkarim;T. (1997): Public health aspects of cystic echinococcosis in Arab countries. Acta Tropica, 67 (1-2):125-132.

Devi,C. and Parija; S.C.(2003): Latex agglutination test (LAT) for antigen detection in the cystic fluid for the diagnosis of cystic echinococcosis. Diag.Microbiol.Infect.Dis.45(2):123-6

De Morilla, A.C.; Gomez, A.; Bautista, C.R. and Morilla, A. (1983). Evaluacion de un antigeno somatico y uno metabolico de *Fasciola hepatica* en diferentes pruebas immunologicas par el diagnostico de la fascioliasis en bovinose.Tec. Pecu. Mex.,44:41-45.

Derbala, A.A. and Zayed, A.A. (1997): Prevalence, fertility and viability of cysticercosis and hydatidosis infection in some domestic animals. J. Union Arab Biol., Cairo. Vol. (A), Zoology, 109-123.

Dreweck CM, Lader CG, Soboslay PT, Kern P.(1997): Subclass-specific serological reactivity and IgG4-specific antigen recognition in human echinococcosis. Trop Med Int. Health.; 2 (8):779-87

El-Askalany, M.A.M. (1981): Evaluation of some serological tests on diagnosing hydatid cysts in camels. M.V.Sc., Vet. Parasitology, Fac. Vet. Med. Cairo Univ.

El-Baz, M. A. (1994): Some studies on Diagnosis of hydatidosis in slaughtered animals. M.V.Sc. (Thesis), Fac.Vet.Med. Cairo Univ.

Fujimoto Y, Ito A, Ishikawa Y, Inoue M, Suzuki Y, Ohhira M, Ohtake T, Kohgo Y.(2005) : Usefulness of recombinant Em18-ELISA to evaluate efficacy of treatment in patients with alveolar echinococcosis. J Gastroenterol.;40(4):426-31.

Gottstein B, Jacquier P.,Bresson-Hadni, S. & Eckert J., (1993) : Improved primary immunodiagnosis of alveolar echinoccosis in human by ELISA using the $Em2^{plus}$.-antigen. J.Clin Microbiol 31:373-376.

Hassan, D.A.(1991): Future studies on the possible differences between E.granulosus substrains through biochemical analysis of electrolyte, lipids and other components of the larval and adult stages of the parasite. Ph.D. Thesis, Vet.Parasitology, Fac.Vet.Med., Cairo Univ.

Ibarra, F.; Montenegro, N.; Vera, Y.; Boulard, C.; Flores, J. and Ochoa, P. (1998). Comparison of three ELISA tests for seroepidemiology of bovine fascioliasis. Vet. Parasitol.,77: 229-236.

Irabuena O, Nieto A, Ferreira AM, Battistoni J, Ferragut G, (2000):

Characterization and optimization of bovine Echinococcus granulosus cyst fluid to be used in immunodiagnosis of hydatid disease by ELISA. Rev Inst Med Trop Sao Paulo. , Sep-Oct; 42(5):255-62.

Ito A, Ma L, Schantz PM, Gottstein B, Liu YH, Chai JJ, Abdel-Hafez SK, Altintas N, Joshi DD, Lightowlers MW, Pawlowski ZS.(1999): Differential serodiagnosis for cystic and alveolar echinococcosis using fractions of *Echinococcus granulosus* cyst fluid (antigen B) and *E. multilocularis* protoscolex (EM18). Am J Trop Med Hyg.: 60(2):188-92.

Katrak, K.; Mahon, B.P.; Jones, W.C.; Brautigam, S. and Mills, K.H. (1992). Preparative separation of foreign antigens for highly efficient presentation of T cells in vitro. J. Immunol. Meth., 156:247-254.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of Bacteriophage T 4. Nature, 227:680-685.

Lamar, S.; Kilani,M and Torgerson, P.(2001): Frequency distribution of E.granulosus and other helminthes in stray dogs in Tunisia.Ann.Trop Med.Parasitol., 93:69-76.

Lightowlers, M. & Goltorti, B (1995): Immunodiagnosis of echinococcosis. Thompson RCA, Lymbery AJ,eds. Echinococcus and Hydatid Diseae. Wallingford, United Kingdom: CAB International, 355-410

Lightowlers,M.W.; and Gottstein, B. (1995): Echinococcosis/hydatidosis: antigens, immunological and molecular diagnosis, In: R. C. A., 355-410.

Lowery,O.H.; Rosenbrough, N.J.; A.I. and Randall, R.G.(1951): Protein measurement with Folin-phenol reagents.J.Biol.Chem.193:265-275.

McManus, D.P. & Smyth, J.D. (1986): Hydatid disease (hydatidosis): chang concepts in epidemiology and speciation. Parasit. Today, 2: 163-168.

Osman M.Z. (2006): assessment of role of different hydatid vaccines in protection against animal hydatidosis. PhD Thesis, Dept of Parasitolo. Fac. of Medicine, Cairo univ.

Poretti D, Felleisen E, Grimm F, Pfister M, Teuscher F, Zuercher C, Reichen J, Gottstein B.(1999): Differential immunodiagnosis between cystic hydatid disease and o r cross-reactive pathologies. Am J Trop Med Hyg.;60(2):193-8

Towbin, H.; Stachelin, T. and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedures and some applications. Proc. Nat. Acad. Sci. USA, 76: 4350-4354.

Wattal, C.; N.; Khan, I.A. and Agarwal, S.C. and McManus, D.P. (1986): Diagnostic methods, treatment and control of echinococcosis.Acta Trop.69,36-71.

Welch, S.; Malone, J. and Geaghan, H. (1987). Herd evaluation of *Fasciola hepatica* infection in Louisiana cattle by an ELISA. Am. J. Vet. Res., 48 : 345-347.

Wen, H. and Craig, P.S. (1994): Immunoglobulin G subclasses in human cystic and alveolar echinococcosis . Am. J. Trop. Med. Hyg., 51(6): 741-748

World Health Organization [WHO] (1983): Diagnostic techniques in schistosomiasis control. Report of the schistosomiasis unit of the WHO parasitic diseases program (PDP) . WHO Tech. Rep. Ser., 386.

WHO (1999): How much human helminthiasis is there in the world? Journal of Parasitology 85(3): 397-403.

ADVANCED CONCEPTS IN DIAGNOSIS OF HYDATIDOSIS IN HUMAN AND LIVING ANIMALS

Table (1): Specific and non specific protein fractions of FHCA using SDS-PAGE and Western blot versus reference positive control sera

Band No.	Different protein fractions using Ponseau S stain	EITB Specific protein fractions using reference sera			
		Rabbit +Ve	Rabbit - Ve	Human +Ve	Human - Ve
1	Over 105 kd (two bands)	Over 105 kD	Over 105 kD	Over 105 kD	Over 105 kD
2	105 kD	105 kd	105 kd	105 kd	105 kd
3	79 kD	62 kd	62 kd	62 kd	62 kd
4	62 kD	38 kD		38 kD	
5	49 kD	36 kD		36 kD	
6	38 kD	29 kD		18 kD	
7	28 kD	18 kD		16 kD	
8	24 kD	16 kD		12 kD	
9	21 kD	12 kD		8 kD	
10	18 kD	8 kD			
11	8 kD				

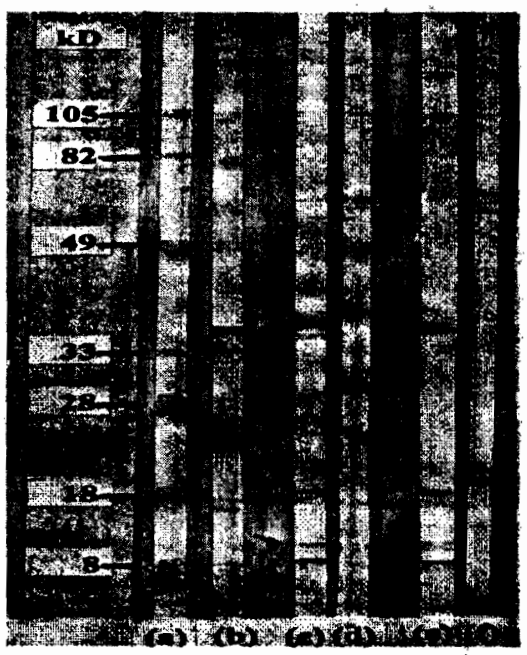

Plate1. Recognition of Fertile Hydatid cysts antigen fractions using Ponceau S stain and EITB technique.

Lane (a) - Low molecular weight marker stained with Ponceau S solution.

Lane (b)-. NC strip demonstrate the different antigenic fractions stained by Ponceau S solution

Lane (c) - NC strip of the fractionated antigen reacted with positive rabbit sera.

Lane (d) - NC strip of the fractionated antigen reacted with negative rabbit sera.

Lane (e)-. NC strip of the fractionated antigen reacted with hydatidodis infected patient sera.

Lane (f)-. NC strip of the fractionated antigen reacted with negative human sera.

Table (2): Specificity & Sensitivity of different eluted FHC protein fractions in diagnosis of infection in selected serum samples using ELISA Technique (1 :100 serum dilution).

Type of tested sera		Tested groups	History of the selected serum samples	No. of samples	Tested eluted concentrated Protein fraction of					
					32 – 38 kD		16 – 18 kD		8 – 12 kD	
					No.+ Ve	%	No.+ Ve	%	No.+ Ve	%
Specificity	Human sera	G - 1	*Fasciola* spp. infected patients	5	0	100	1	80	0	100
		G - 2	Virus hepatitis C infected patients	10	2	80	3	70	1	90
		G - 3	*Schisto.mansoni* infected patients	10	1	90	2	80	1	90
	Camel sera	G - 4	G.I.N. infected camel	10	0	100	1	90	0	100
	Sheep sera	G - 5	*Monaizia infected* sheep	10	1	90	1	80	0	100
		G - 6	*Fasciola* infected sheep	10	0	100	1	90	0	100
	Mean Specificity			55		93.33		81.66		96.66
Sensitivity	Human sera	G - 7	Surgical proved HC infected human sera	5	5	100	4	80	5	100
		G - 8	Sera of healthy persons	5	0	100	0	100	0	100
	Camel sera	G - 9	Hydatid infected at P.M.	10	10	100	8	80	10	100
		G - 10	Parasite free young camel sera	5	0	100	0	100	0	100
	Hydatid experimentally infected rabbit	G - 11	HC Experimental infected rabbit sera	3	3	100	3	100	3	100
	Mean Sensitivity			28		100		92.0		100
Control serum samples		G - 12	Sera of healthy persons	5	0	100	0	100	0	100
		G - 13	Parasite free young camel sera	5	0	100	0	100	0	100
		G - 14	Parasite free young sheep sera	5	0	100	0	100	0	100
		G - 15	Negative rabbit sera	3	0	100	0	100	0	100

Table (3): Value of 8-12 kD FHC protein fraction in Diagnosis of hydatidosis in human and confirmation of the result after sonography on the chest & liver

	Age groups	No.	Number of patients harboring other parasites in their stool samples	Incidence of + Ve cases using ELISA technique		Incidence of + Ve cases after sonography of Lung & liver	
				No.+Ve	%	No.+Ve	%
1	Exposed people less than 25 years old	20	*Giardia* (6), *Schistosoma* (8); Entamoeba. (5) & H.nana (4)	0.0	0.0	0.0	0.0
2	Exposed people over 35 years old	15	*Giardia* (5), *Schistosoma* (7) & Entamoeba. (6)	2	13.33	2	13.33
	Total	35		2	5.71	2	5.71
	Control, healthy persons	5		0.0		0.0	

Table (4): Value of 8-12 kd FHC protein fraction in Diagnosis of hydatidosis in living animals and confirmation of the data post slaughtering

Examined animal	Age groups	No.	Number of animals harboring other parasites in their fecal examination	Incidence of + Ve animals using serological technique At 1: 100 serum dilution		Incidence of + Ve animals after examination of Lung & liver	
				No.+Ve	%	No.+Ve	%
Camel	Imported camels less than 4 years.	25	*Fasciola* (4), *Paramphistomum spp* (6); G.I.N. (11)& *Moneizia* (6)	8	32.0	6	24.0
	Local camels over 5 year	15	*Paramphistomum spp* (5); G.I.N. (10)& *Moneizia* (6)	4	26.6	3	20.0
Sheep	Grazing sheep less than 2 years	25	*Fasciola* (4), *Paramphistomum spp* (4) ; G.I.N. (8) & coccidia (4)	0.0	0.0	0.0	0.0
	Grazing sheep over 5 years	20	*Fasciola* (12), *Paramphistomum spp* (5); G.I.N. (10)& *Moneizia* (4)	3	15.0	0.0	0.0
	Total	85		15	17.64	9	10.58
Control, parasite free camel		5		0.0		0.0	
Control, Parasite free sheep		5		0.0		0.0	