



Evaluation of crude Antigens for Serological Diagnosis of Hydatidosis in Man and Camel in Sudan.

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Abstract

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Echinococcosis/hydatidosis is a zoonotic disease. is caused by adult worms and larval (metacestode) stages of the taeniid cestode *Echinococcus granulosus*. The life cycle is completed into two hosts. The final host usually carnivore e.g. dog, and the intermediate host usually herbivorous and man. Objective: evaluation and comparison of the diagnostic value LA, IHA, ELISA, CCIEP and AGID using prepared antigen in the laboratory, in immunodiagnostic of cystic hydatid disease, (CHD). Prepared Crude and purified antigens from camel hydatid cyst fluid. Were collected 14 patients serum attended hospitals of Khartoum state, 50 sera from healthy subjects, and 84 sera from individuals infected with other than hydatid disease, and also serum collected sera from infected camel at Tambool slaughter house. Sera were analyzed by the five serological tests using crude antigen. Latex agglutination LA, IHA, counter current immunoelectrophoresis (CCIEP), Agar gel immunodiffusion (AGID) and specific IgG enzyme-linked immunosorbent assay (ELISA) tests was the most sensitive test 85.7% and the least sensitive of camel sera 57%. LA, IHA, ELISA, CCIEP and AGID was 80.0%, 80.0%, 85.7%, 66.7% and 57.1% respectively, and specificity 77.8%, 88.9%, 71.4%, 87.5% and 71.4%. The sensitivity in camel sera was 93.0%, 88.3%, 57.0% and 89.8% respectively and specificity was 90.5%, 86.9%, 44.4%, 78.0% and 82.0%. It Conclude the immunodiagnostic is an important tool in diagnosis of CHD. It may also be an important element to control, surveillance and early diagnosis of infection. Conventional serology of CHD is based primarily on sensitive test Echinococcosis such as ELISA, employing hydatid fluid antigen and a subsequent confirmation test such as IHA, CCIEP and immunodiffusion.

Keywords: Hydatidosis, Immunodiagnosis, Human and Camel antigen.

INTRODUCTION

Cystic (CE), which is caused by larval stage of *Echinococcus granulosus*, is one of the most important parasitic diseases in the world and eastern Mediterranean [1]. Human infections occur during the natural transmission of the parasites between the canid as definitive host, and domestic livestock, as the intermediate host. Diagnosis of CE is mainly a positive serological test. Along with an imaging by CT scan and ultrasonography WHO [2]. Among different serological techniques ELISA has been reported to be a relatively reliable test for diagnosis of human hydatidosis. However using crude hydatid cyst fluid (CHF) as an antigen in ELISA reduces the specificity of this test since CHF contains various metabolites of the host and the parasites [3], However counter current immunoelectrophoresis (CCIEP) using crude hydatid cyst fluid has been used for many years in different centers for serodiagnosis of hydatid cyst [4] – [5]. IHA was performed with sheep RBC that were sensitized by various concentrations of crude antigens and antigen B. The best result was obtained by IHA with applying antigen B (10µg/ml) for 40 min. at 37°C or 60 min. at room temperature. It is suggested that the IHA as serological assay, is a valuable method with high diagnostic efficiency for diagnosis of hydatid disease, when performed by purified antigen B. It is a rapid diagnostic assay with any needs neither expensive instruments nor expert personnel so it is useful for seroepidemiological studies and field trial in endemic areas [6]. Agar Gel Immunodiffusion (AGID) test was used for diagnosis of hydatid disease. Sera were obtained from suspected patients before surgery and the antigens of human and camel hydatid fluid were used as crude or purified antigens, ELISA was performed essentially as described by [7]. Microtitration plates were coated by incubation with 100µl of both antigens (crude antigen and AgB) solution (10µg/ml protein concentration) per well and serum samples were diluted 1:256 in phosphate buffer saline. Our study was designed to assess ELISA method using purified antigen from camel hydatid cyst for immunodiagnosis of human hydatidosis. Furthermore the study aimed to compare the validity of the ELISA, IHA, and CCIEP AND AGID for the diagnosis of hydatidosis.

The other work of this study includes the collection of samples from the camels at slaughter house at Tambool area for serological tests. The sensitivity of LA, IHA, ELISA, CIE, and AGID were 93%, 88.3%, 57%, 86.9% and 89.8%, and respectively, and the specificity were 90.5%, 86.9% 44.4%, 78% and 82.0% respectively. To an understanding the problem of hydatidosis/*Echinococcus* in the Sudan, describing and evaluating data from different parts of the Sudan might be of help in an effective control programme. The results of our slaughterhouse survey are in agreement with previous survey in the same region. [8], reported infection rates of 4%, 8%, 3% and 35% in cattle, sheep, goats and camels, respectively, [9]. reported prevalence rate of 49% in camels. Given the fact that in our study, only 68% (68 out of 99) of cysts from camels were found to be fertile (compared to 22% and 24% of cysts from cattle and camels respectively, the principle transmission in this region seems to be based on camels and secondarily on cattle, with sheep only playing a marginal role in the life cycles. This finding was previously documented by [10]. who encountered only calcified or under calcified cysts in sheep. They reported a fertility rate of 24.4% and 29% in camel and cattle cysts; respectively this is in contrast with other regions of Africa including parts of southern Sudan, Kenya and countries of Maghreb, where sheep are heavily involved in the transmission of *E. granulosus* [11].

MATERIALS AND METHODS

Study area

The study was conducted in Tambool town market (Central Eastern of Sudan) which is located 150Km South East of Khartoum. And Khartoum state carried out at hospitals in and specimens were taken from patients who have been already operated or suspected to have hydatidosis. (Figure1).

Figure 1: Map of the Sudan showing Gezira state and Tambool area (red colour) where liver and lung samples were collected.



Study design

The present study was observation- cross-sectional survey carried out in Tambool area (central eastern Sudan and Khartoum State) during December 2011 up to July 2013.

Sampling

The current was observation and cross- sectional research was periodically conducted from December 2011-July 2013. On infected organs (liver and lung and others organs) of camels with cystic hydatidosis. The samples 200 slaughtered camels, and fourteen aspirated samples from patients. Furthermore infertile cyst was further calcified, sterile or calcified, sterile hydatid cysts characterized by their smooth inner lining with slightly turbid fluid in its contents. Typical calcified cysts produce a gritty sound feeling up on incision [12], [13].

Samples collection

A total of 200 carcasses were examined in abattoirs, during routine meat inspection, for presence of cystic echinococcosis. Each carcass was carefully examined. Cysts when found were taken to a laboratory, where their diameters and fertility were recorded. And 14 aspirate samples collected from 4 hospitals in Khartoum state. Collections of protoscoleces were checked for its viability by Eosin exclusion test [3] and observing flame cell activity [4]. Crude and purified antigen was obtained from camel hydatid cyst fluid. Serum samples were collected human and camels, Confirmed CHD patients, others parasitic disease, patients with malignancies and normal individuals respectively, and Sera were analyzed by the five serological tests using crude antigen. Protein content of the sample was determined by protein method assay, [14].

Ethical consideration

Ethical approval for the study was obtained from the Ethical Committee of the federal ministry of health, and permission was provided from all hospitals where investigation was conducted.

Statistical analysis

Epidemiological data were analyzed by using the Statistical Package for Social Science (SPSS).

RESULTS

The use of the serological tests in diagnosis of hydatidosis in human

The results showed that the sensitivity and specificity rates of the latex agglutination (LA) test in detecting hydatid cyst antibodies were 80.0% and 77.0% respectively, (Table 1). The indirect haemagglutination test: The results showed that the sensitivity and specificity rates of the indirect haemagglutination test (IHA) in detecting hydatid cyst antibodies were 80.0% and 88.9% respectively, (Table 1), Results of IHA, ELISA and CIE tests in patients with hydatidosis, healthy individuals and others parasitic infections in(table 2). Agar gel Immunodiffusion (AGID) test: The results showed that the sensitivity and specificity rates of agar gel Immunodiffusion (AGID) test in detecting hydatid cyst antibodies were 57.1% and 71.4% respectively, (Table 1, figure 2)

Table 1: comparison of sensitivity and specificity of serological tests in detection of human hydatidosis.

Types of test	LA	IHA	ELISA	CIEP	AGID
Positive	4	4	4	4	4
Negative	7	8	8	7	5
Sensitivity	0.0%	80.0%	85.7	66.7%	57.1%
Specificity	77.8%	88.9%	71.4%	87.5%	71.4%

Table 2: Results of IHA, ELISA and CIE tests in patients with hydatidosis, healthy individuals and others parasitic infections

Factor	Positive reaction	Negative reaction			Sensitivity			Specificity				
		No.se	IHA	ELISA	CE	IHA	ELISA	CIE	IHA	ELISA	CIE	
Hydatidosis	14	4	4	4	8	8	7	80	85.7	66.	88.9	87.
								%	%	7		5
Healthy	50				50	50	50					
Other diseases	84				84	84	84					
Total	148	4	4	4	58	58	57					

Figure 1: show the precipitated line when positive by CIE

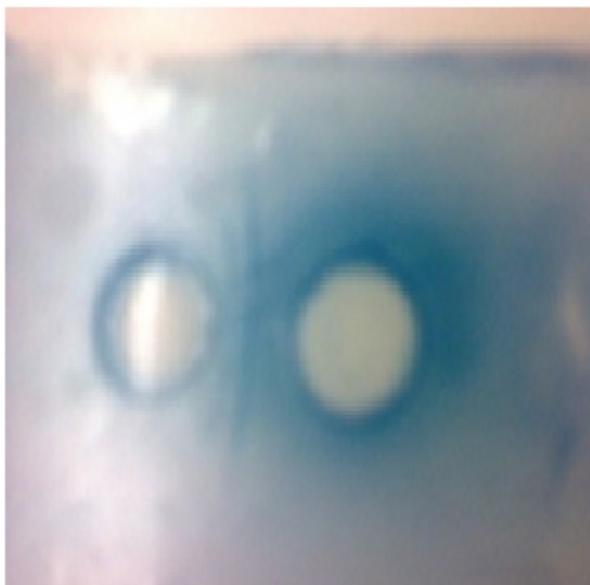


Figure 2: shows the precipitated one line or more when positive by AGID.

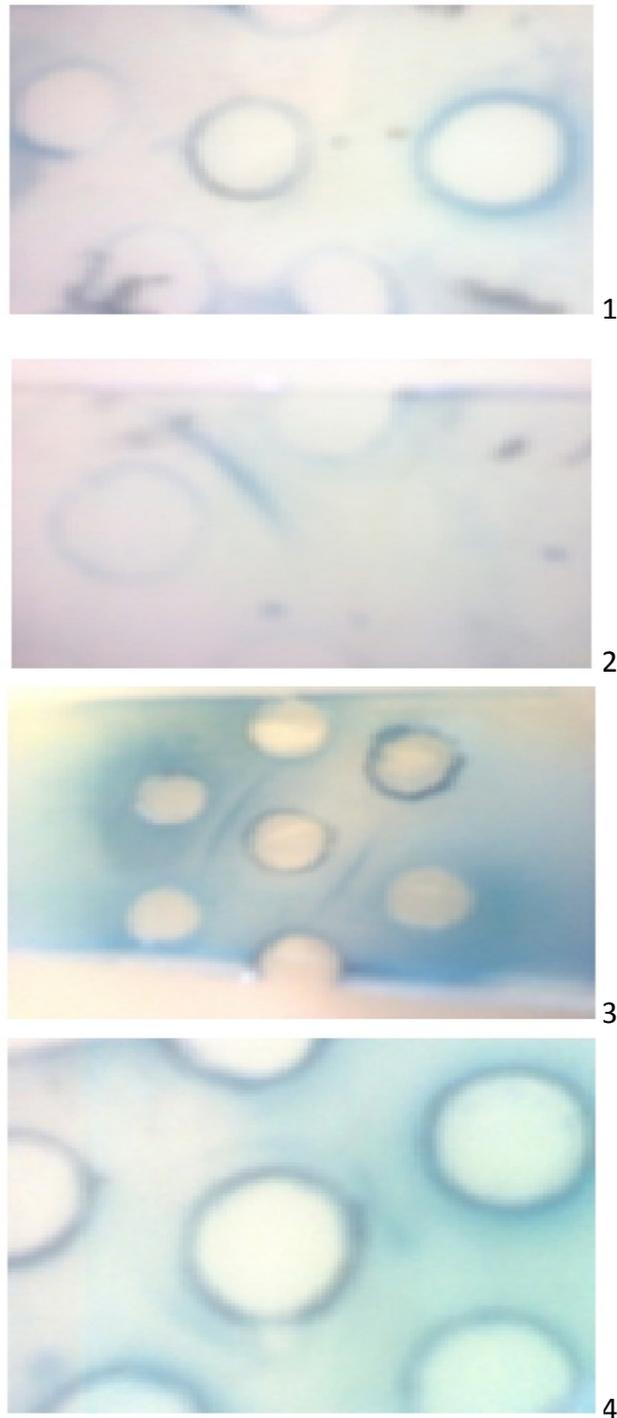


Figure2: 1: Negative **fig.2-2:** one line precipitated **fig.2-3:** two lines precipitated **fig.2-4:** three lines precipitated by AGID.

The use of the serological tests in diagnosis of hydatidosis in camels

The results showed that the sensitivity and specificity rates of the latex agglutination (LA) test, the indirect haemagglutination test(IHA), enzyme linked immunosorbent assay (ELISA), Countercurrent immunoelectrophoresis (CCIEP) test, Agar gel Immunodiffusion (AGID) test

in detecting hydatid cyst antibodies were 93.0% , 90.5%, 88.3% , 86.9%,57.0% , 44.4%, 86.9% ,78.0%,and 89.8% ,82.0% were respectively, (Table 3).

Table 3: comparison of sensitivity and specificity of serological tests in detection of camel hydatidosis.

Types of test	LA	IHA	ELISA	CIEP	AGID
Positive	53	53	53	53	53
Negative	57	52	12	46	5
Sensitivity	93.0%	88.3%	57%	86.9%	89.8%
Specificity	90.5%	86.9%	44.4%	78.0%	82.0%

DISCUSSION

The diagnosis of CE mainly depends on radiological and immunological procedures. Imaging methods are sometimes limited by small size of the lesion and atypical images which are not easy to be distinguished from abscesses or neoplasm. Routine laboratory diagnosis of CE is dependent on detection of specific antibody response. Serum is generally used for detection of specific antibody although some studies show the detection of antibody in urine might also be a good alternative [15]. Hydatid cyst fluid (HCF) is considered the main antigenic source for immunodiagnosis of human CE. For clinical practice, examination of crude HCF has a high sensitivity, ranging typically from 75% to 95% [2]. However its specificity is often unsatisfactory and cross-reactivity with sera from patients infected with other cestode, nematode and trematode species is commonly reported [16]. The results of the serological tests including LA, IHA, ELISA CCIP and AGID indicated that the crude and purified hydatid fluid can be usefully applied in serological diagnosis, as an antigen. They have determined that the antigen gave a sensitivity of 80.5%, 85.7% and 71.4% respectively, which is in agreement with other reports (85.0% - 92.0%[17], [18],[19].The specificity 88.9%, 71.4% and 87.5 respectively is the same as previous studies [20], [21].The serum of all normal persons gave negative result in LA, IHA, ELISA, CCIP and AGID and cross reaction were the same in both [22] and[23].

Malignant tumor shows a more serious clinical picture and the computed tomography, ultrasonography and imaging through magnetic resonance makes the difference. Polycystic kidney disease is always bilateral and the renal function and appearance of arterial hypertension are almost present. Renal abscess comes from skin infection and it ultrasonographically reveals a hipoechoic content of this lesion, [24]. Demonstrated that the ELISA was able to detect hydatid antibodies either by purified or crude hydatid fluid antigens. For screening and epidemiological study of hydatid disease the sensitivity and specificity of ELISA could be comparable to the AGID test [20], [19] ELISA has not been routinely established as a diagnostic method in all laboratories, because of its requirements, and a reliable test has not yet been established for definitive Echinococcal antibodies in hydatidosis. In conclusion, according to this and other comparative studies, the IHA and AGID are suggested for diagnosis of hydatid disease and screening of serum samples in epidemiological studies in high risk population. The lethality of CE is considerably high up to 60% in patients without surgical intervention [25].

In this study show that the serological tests still need more perfection of CE for the diagnosis. This subject needs further investigations. In this study the histopathology of hydatid cysts in different organs of human and camels were also attempted. However, it did not add new to the literature.

CONCLUSION

We suggest that the CHFAg –ELISA which exhibit a relatively high diagnostic sensitivity is only convenient for primary screening test for human and is poorly sensitivity in camel. And LA, IHA, CIE and AGID having high specificity for subsequent confirmatory test.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS` CONTRIBUTION

AAMA collected hydatid cyst samples, prepared crude and purified antigens, and carried out the serological tests. M.B.S. designed the experiment and prepared the final manuscript, M.E.T. edited and helped with experimental designed, N.T.O. analyzed the sequences and designed the study, M.E. and. E.A.D. edited the sequences and helped with experimental design, Teaser designed the experiment. All authors read and approved the final version of the manuscript.

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