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Original Research Article

Sensitivity and specificity of Indirect Enzyme Linked Immuno sorbent Assay (ELISA) for diagnosis of hydatidosis in dromedary camels using hydatid cyst fluid antigens.

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ABSTRACT

Examination of 528 (450 males and 78 females) dromedary camels slaughtered at Cairo abattoir revealed that a total of (93) 17.6 % were infected with hydatidosis. Post mortem examination revealed that infection was restricted only in the lungs and the liver of infected camels. Among the 93 hydatidosis infected camels, lungs were the most frequently infected 88 (94.623%) compared with liver 5 (5.376%). ELISA test using partially crude antigen and purified antigen is important for the early diagnosis of cystic echinococcosis as most cases in the early stages of infection are asymptomatic. Sensitivity of ELISA using the crude antigen was 82.758% while sensitivity of the partially purified antigen was 79.310 %. On the other hand specificity of the crude antigen was estimated as 62.5 % and specificity of partially purified antigen as 75.0 %.

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1. Introduction

Echinococcosis is one of the most important helminthes zoonoses and remains a significant problem worldwide (Shiferaw 2018). It is a neglected public health problem in developing countries, Siracusano et al., (2012). The

definitive host of the parasite, *Echinococcosis granulosus* is dogs which harbor the adult parasite and excrete the parasite eggs along with their feces, while livestock and human are the intermediate hosts (Oku et al., (2004).

The metacestodes usually form fluid-filled cysts (hydatids) located in the liver, lungs and other organs. The disease is distributed worldwide that constitutes a serious health problem for humans as well as significant economic significance in areas where extensive livestock production provides suitable conditions for the cyclic transmission between dogs and livestock animals (Eckert et al., 2000 and Jenkins et al., 2005) and where pastoralism is common and there is a high dog population (Sotiraki et al., 2003). In Africa, the disease is a serious health problem of the nomadic pastoralist tribes of East Africa including Kenya, Tanzania, Uganda, Southern Sudan and Somalia (Macpherson et al., 1986). Other endemic areas include Asia, Europe, Australia and the Mediterranean (Tergut, 2001).

In most cases, the early stages of infection are asymptomatic, and currently, the diagnosis of cystic echinococcosis in the intermediate host is based mainly on necropsy procedures (Zhang et al., 2003). Immunological tests would be desirable for animal import monitoring and also in countries where control programs for the disease are operating (Luka et al., 2009). In comparison with investigations in humans, relatively little research has been directed toward the development of immunodiagnostic techniques for *Echinococcus granulosus* infection in domesticated animals. The identification of new antigen sources and molecules with high diagnostic features always remains an important task that have to be undertaken in order to maintain the progress in immunodiagnosis of echinococcosis.

The present study aimed to investigate hydatidosis in camels clarifying some important knowledge about the nature of such infection in this important animal as well as to prepare different antigens from hydatid cysts of camel origin and evaluate their sensitivity and specificity in the serodiagnosis of camel hydatidosis using ELISA.

2. Material and Methods

2.1. Animals

A total of 528 (450 males and 78 females) of 4–8 years old dromedary camels slaughtered at Cairo abattoir (El-Basatin), were randomly employed in this study. Most of these camels were imported from Sudan and Somalia. During ante mortem inspection, sex and general health condition of each animal was recorded. During post mortem examination, a thorough visual inspection, palpation, and systematic incisions of different visceral organs particularly the lungs, liver, kidneys, heart and spleen were carried out according to the procedures recommended by the FAO/WHO (1994). Hydatid cysts when present were collected individually in plastic bags, labeled and carried to the laboratory for further investigations.

2.2. Blood sera

Collection of camel's blood samples was done at the time of slaughtering in labeled, 10 ml screw-capped tubes. The blood samples were left to clot at room temperature then centrifuged at 1500 rpm / 15 minutes. The sera were then collected, separated in clean dry eppendorff tubes, labeled and kept at -20°C until used. Blood samples of 29 naturally infected and 16 free animals were collected, marked and send to the laboratory.

2.3. Antigens

a. Partially purified hydatid cyst fluid antigen

It was prepared according to Oriol et. al., (1971), Aseptic aspiration of the fluid of fresh fertile camel hydatid cysts was carried out using a sterile plastic syringe, then the collected hydatid fluid was centrifuged at 1500 rpm for 30 minutes. The supernatant was then concentrated using μm 0.5 membrane filter, the concentrated fluid was dialyzed against 0.005 M acetate buffer, PH 5.0, centrifuged at 10.000 rpm for 60 minutes yielding a supernatant containing albumin and pseudoglobulines while the

precipitate contained euglobulines and parasite antigens. This precipitate was dissolved in 0.2 M phosphate buffer, PH 8.0, and then centrifuged at 10.000 rpm for 60 minutes. The supernatant was saved and salted out with saturated ammonium sulphate 40%, then centrifuged at 10.000 rpm for 60 minutes. The supernatant was dialyzed against 0.2 M phosphate buffer, PH 8.0 for 48 hours then against 0.005 M acetate buffer, PH 0.5 and then centrifuged at 10.000 rpm for 60 minutes. The precipitate was dissolved again in 0.2 M phosphate buffer, PH 8.0. The protein content was determined and the preparation was aliquated and stored at -20 °C until be used.

b. Crude hydatid cyst fluid antigen

It was prepared according to Reda et al., (1999) based on the method described by (Maddison et. al., 1989). Hydatid cyst fluid (HCF) was aseptically aspirated from hydatid cysts obtained from camels then centrifuged at 10000 rpm for 10 min at 4 °C. The supernatant was collected and dialyzed extensively, using cellulose membrane against three changes of deionized water. The dialysate was centrifuged again at 10000 rpm for 30 min at 4 °C and the supernatant was collected. The protein content of the antigen was determined according to Lowery et al., (1951). The antigen was aliquated and stored at -20 °C until used.

2.4. Enzyme Linked Immuno Sorbent Assay (ELISA) for antibody detection in camel sera.

Enzyme Linked Immuno Sorbent Assay (ELISA) for antibody detection in camel sera was applied according to (Icona et al., 1980 and Ibrahim et al., 2002). The antigens were diluted in coating buffer at their optimal concentration. Optimal concentration was determined after the preparation of different antigen concentrations and different dilutions of sera. The optimal concentration was 20µg/1ml coating buffer. Each well of 96 micro titter plates with flat bottom was filled with 50µl of the antigen concentration (20µg / 1ml coating buffer) and

the plates were incubated over night at room temperature. The plates were washed 3 times with PBS – T 0.05% to get rid of excess unbound antigen and the remaining free binding sites were blocked with blocking buffer (200 µl/well for 1 hour). Different sera under examination as well as positive and negative control sera (50 µl / well, 1:100 serum dilution in PBS, pH 7.4) were added to the plates and incubated at 37 °C for 2 hours in a shaking water bath. Alkaline phosphatase conjugate (1:1000 in PBS, pH 7.4) was added to all wells and incubated at 37 °C for 1 hour in a shaking water bath. 50 µl of substrate solution was added to all wells. The plates were incubated at 37 °C for 30 minutes in a dark place. The reaction with yellow coloration was stopped by adding 50 µl/well stopping buffer. Optical density (O.D) or absorbance was read at 490 nm in ELISA reader. Cut off value was calculated according to Gonz´alez, G. et al., (2000). The cutoff for positive scores was calculated from the mean absorbance value obtained for the healthy animals plus 3 standard deviations.

3. Results

1. Prevalence of hydatid cyst infection in dromedary camels.

Table 1. The prevalence of hydatid cyst infection in dromedary camels

No. of examined camels	No. of infected animals	Affected organs			
		Lungs		Liver	
528		No.	%	No.	%
	Male (84)	79	94.047	5	5.952
	Female (9)	9	100	0	0
	Total (93) 17.6%	88	94.623	5	5.376

2. Detection of antibodies in sera of camels using ELISA using hydatid fluid antigens

Table 2. Comparison between the results of post mortem findings and indirect ELISA for diagnosis of hydatidosis in camels

Post mortem findings	ELISA				Total
	Crude antigen		Partially purified Antigen		
	Positive	negative	Positive	Negative	
Positive	24 (82.8 %)	5 (17.24%)	23 (79.31%)	6 (20.7%)	29
Negative	6 (37.5%)	10 (62.5%)	4 (25%)	12 (75%)	16
Total	30	15	27	18	45

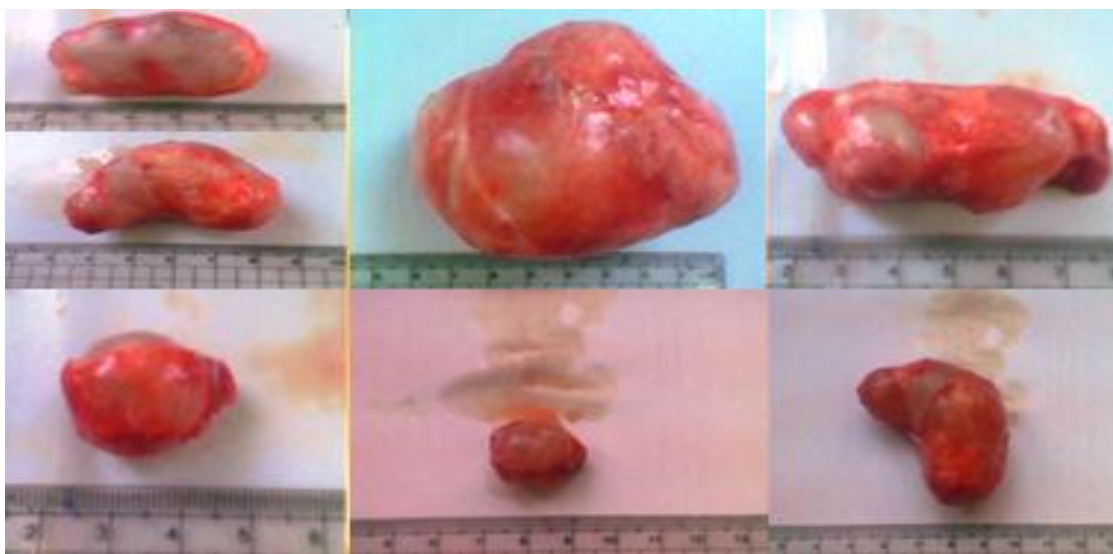


Fig. 1 Hydatid cysts of different sizes detected from liver and lungs of dromedary camels

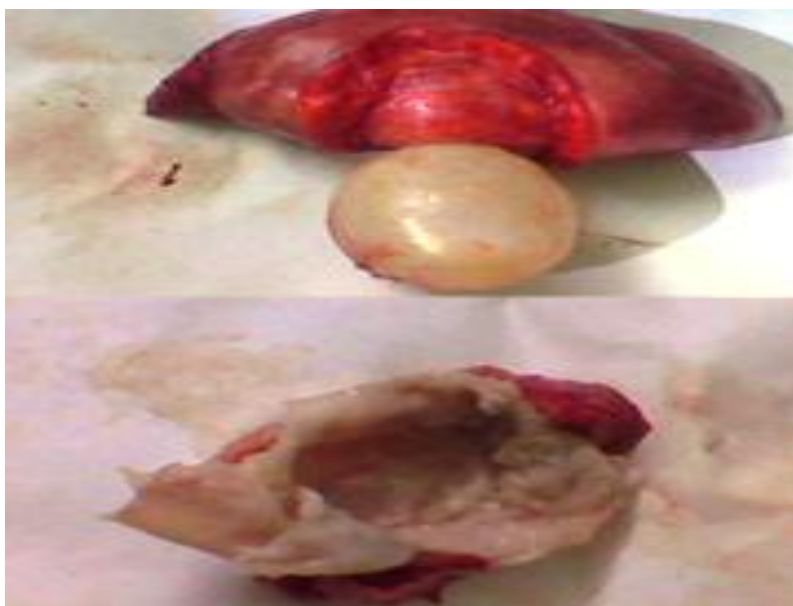


Fig. 2 Hydatid cysts detected from of dromedary camels pre and post their evacuation

3. Calculation of diagnostic sensitivity and specificity

The following definitions were used to calculate the corresponding diagnostic parameters according to Jacobson (1996) and Gonz'alez, et. al., (2000):

True positive (tp): sera from camels with confirmed hydatidosis by postmortem examination showing positive reading.

True negative (tn): sera from healthy camels without hydatidosis by postmortem examination showing negative reading.

False positive (fp): sera from healthy camels without hydatidosis by postmortem examination showing positive reading.

False negative (fn): sera from camels with confirmed hydatidosis by postmortem examination showing negative reading.

Sensitivity = $tp \times 100 / (tp + fn)$.

Sensitivity of crude antigen = $24/29 \times 100 = 82.758\%$.

Sensitivity of partially purified antigen = $23/29 \times 100 = 79.310\%$.

Specificity = $tn \times 100 / (tn + fp)$.

Specificity of crude antigen = $10/16 \times 100 = 62.5\%$.

Specificity of partially purified antigen = $12/16 \times 100 = 75.0\%$.

4. Discussion

Hydatidosis is a global animal and human health problem of increasing economic and public health importance (Lightowlers et al., 2000). It is a helminthic cyclo-zoonotic disease caused by the larval stage (metacestode) of the canid tapeworm *Echinococcus* that requires at least one other vertebrate host for completion of its life cycle. Accidental infection of man occurs during the natural transmission of the parasite between the canid definitive hosts and domestic livestock intermediate hosts (Shiferaw et al., 2018). The disease is endemic in many parts of the world (Willingham, 2003). It is one of the major zoonotic parasitic diseases in the Middle East and Arab North Africa from Morocco to Egypt (Sadjjadi, 2006).

The cestode; *Echinococcus granulosus* is the causative agent of cystic hydatid disease which is recognized as one of the major zoonoses affecting both humans and domestic animals in various parts of the world (Mario et al., 2002) and (Oku et al., 2004). The disease has great public health importance and economic impact in countries where livestock industry is an important segment of the agricultural sector and when livestock production is based mainly on extensive grazing system (Gebretsadik, 2009). In addition, large hydatid cysts formed in the liver and lungs of these domestic animals result in significant economic loss to the meat industry through

condemnation of infected organs (Lightowlers et al., 1984).

As shown in table (1), it was found that the overall prevalence rate of camel hydatidosis was 17.613%, in Egypt other reports on slaughtered camels recorded a higher prevalence rate (Zayed and derbala, 1993) 37.1%, (abdel-Gawad et al., 1981) 31.61% and (Rahman et al., 1992) 31%. Variation in the prevalence of hydatidosis from one area to other may be attributed to the difference in animal husbandry systems like free grazing, incorrect slaughtering of animals, lack of proper removal of infected carcass and the presence of dogs and their relations with animals (Garippa et al., 2004). Regarding the organs affected, it was found that, infection was restricted only in lungs and liver of infected camels. Among 93 hydatidosis infected camels, lungs were the most frequently infected, 88 (94.623%) compared with liver 5 (5.376%). Out of 84 infected males, 79 (94.047%) were detected in the lungs and 5 (5.952%) in the liver. Females infection was only restricted to the liver. The preferential localization of hydatid cysts in the lungs of camels are in accordance with the observations reported by several authors, So our results are in agreement with other studies in camels in Egypt; (Dyab et al., 2005) who found that 100% of infected camels in his study in Assiut Governorate were infected in the lungs, (Zayed and Derbala, 1993) who recorded that the infection was mainly located in the lungs (65.4%), liver (13.5%), lung and liver (17.3%) and heart (3.8%) and with (Rahman et al., 1992, Haridy et al., 2006) who concluded that the predominant site of hydatidosis infection in camels was in lungs.

Necropsy has been considered as the best diagnostic test in the intermediate animal during meat inspection in the abattoir and postmortem examination (Eckert et al., 2001) and (Shiferaw et al., 2018) and is also used as a reference test to estimate the sensitivity and specificity of immunodiagnostic tests.

The development of a sensitive, specific and reproducible serological assay for livestock would provide a useful epidemiological tool for the anti-mortem study and control of hydatid disease (Lightowlers, 1990).

Early diagnosis of cystic echinococcosis can result in significant improvement in the quality of the management and treatment of the disease. In most cases the early stages of infection are asymptomatic but the disease modulates antiparasite immune responses in the hosts with detectable humoral and cellular responses against the parasite (Siracusano et al., 2012), so that methods that are cheap and relatively easy to use are required for large-scale screening of populations at high risk (Zhang et al., 2003). The development of a screening test is important for the identification of cystic hydatid carriers when animals are imported from endemic areas to areas free of infection (Eckert et al., 2001).

Therefore, it was important to evaluate the indirect ELISA using hydatid fluid antigens for the serodiagnosis of camel hydatidosis with the use of postmortem examination as a gold stander. Reviewing the data presented in Table (2), it was found that, for crude antigen, out of 45 serum samples 30 gave positive ELISA results from which 24 were (true positive) as they collected from parasitologically positive camels while the other 6 serum samples were (false positive) as they were collected from parasitologically negative camels. On the other hand, 15 serum samples gave negative ELISA results out of them 10 serum samples were (true negative) as they were collected from parasitologically negative camels while the other 5 serum samples were (false negative) as they were collected from parasitologically positive camels. For partially purified antigen, out of 45 serum samples, 27 gave positive ELISA results from which 23 were (true positive) as they were collected from parasitologically positive camels while the other 4 serum samples were (false positive) as

they were collected from parasitologically negative camels. On the other hand, 18 serum samples gave negative ELISA results out of them 12 serum samples were (true negative) as they were collected from parasitologically negative camels while the other 6 serum samples were (false negative) as they were collected from parasitologically positive camels.

Relatively similar results and comparable higher or lower findings were previously recorded by many investigators during the serodiagnosis of hydatidosis in camels, sheep and humans (Saad and Hassan, 1989) 43% sensitivity and 69% specificity using IHA in Sudanese camels, (Liu et al., 1991) 90.4% sensitivity in sheep, (Mousa, 1992) 86.8% sensitivity and 68% specificity using ELISA in humans. (Ibrahim et al, 2002) in Libya recorded 97% sensitivity by ELISA in camels using native antigen while (Kittelberger et al., 2002) recorded very low sensitivity 11.25% using native antigen in sheep. Zariffard and Khajeh, (2000) recorded 83% sensitivity and 87% specificity in Iranian camels using IHA. (Simsek et al., 2006) used crude and partially purified hydatid fluid antigens for serodiagnosis of hydatidosis in sheep by ELISA and found a sensitivity of 97.1% and 91.6% and specificity of 60.4% and 77.1% for the crude and partially purified antigens respectively.

Our findings are in agreement with (Gatti et al., 2007) who concluded that the total hydatid liquid was the antigen preparation of greatest value and would be the antigen of choice due to its ease of production and availability. On the other hand, others advocated the use of purified antigens (Rogan et al., 1991; Wen and Craig 1994; Mona et al., 2008) and sadjjadi et al., (2007) who concluded that it has become more common to purify components of hydatid fluid such as the lipoprotein antigen B and 5 which are the most appropriate components for immunodiagnosis of cystic echinococcosis as using of the crude hydatid fluid reduces the specificity of the test since it contains various

metabolites of the host and the parasite (Zhang et al., 2003). Such differences in results may be due to the status of the infected cases used in the different studies (Reda et al., 1999)

False positive results may be attributed to recent infection or recently developed cyst (Gatti et al., 2007) and cross-reactivity with antibodies from other infections, but it may also be due to the fact that cystic echinococcosis antibodies can remain in serum for long periods following surgical removal or effective drug treatment of cysts. On the contrary, false negative results may be attributed to low levels of specific IgG, variant Ig antibody expression and/or formation of circulating immune complexes (Zhang and McManus, 2006), in addition, nonspecific conjugate can also play a role in case of camels.

5. Conclusion

Hydatid cysts develop more commonly in the liver and lungs of dromedary camels. ELISA test using crude and partially purified hydatid fluid antigens is an effective and rapid serological test for the early diagnosis of cystic echinococcosis as most cases in the early stages of infection are asymptomatic.

Competing Interests: The authors declare that they have no competing interests.

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