Journal of Parasitology. Photon 105 (2015) 212-218
https://sites.google.com/site/photonfoundationorganization/home/journal-of
Original Research Article. ISJN: 3729-2384: Impact Index: 3.56

Journal of Parasitology

Photon

# Epidemiological and serological studies on *Cystic echinococcosis* among camels in Egypt

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**Mousa W.M., Mahdy O. A.*, Abdel-Wahab A.M. and El-Gameel O.A. receive International Parasitological Research Award- 2015**

**Article history:**
Received: 20 April, 2014
Accepted: 25 April, 2014
Available online: 13 February, 2015

**Keywords:**
Epidemiology, serological tests, Hydatid cyst, camels, Egypt

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**Abstract**

Cystic echinococcosis (CE) is an important zoonotic diseases in the Middle East, is acquiring a public health problem in areas where the losses can take the form of a reduction in weight gain; yield of milk, fertility rate and in the value of wool or other products. So we initiated this research to evaluate two type of *Hydatid* antigens (PSCAg and HCFAg) by several techniques (ELISA, SDS- Page and Immuoblotting technique) for its using in diagnosis of CE. The incidence of Cystic echinococcosis (CE) was 7.5% among examined camels in Egypt during the period from January to December 2012. Out of 33 infected camels 87.9% were found to have the infection in their lungs and liver. The collected HC were divided into small (26.60%), medium (44.70%) and large (28.70%). Medium sized cysts were the most prevalent in the infected lungs (45.60%) while hepatic cysts were mostly of large size (50%). Out of 94 examined cysts, 69 (73.4 %) were fertile and 66 (70.21%) were viable. The viability of protoscolices of liver and lung fertile cysts was 50% and 71.11% respectively In ELISA, the sensitivity of Protoscolces antigen (PSCAg) was higher than that of purified *Hydatid* cyst fluid antigen (HCFAg), while specificity and diagnostic efficacy of purified HCFAg was higher than that of PSCAg. The SDS-PAGE analysis of camels purified HCF Ag indicated that 7 specific-protein bands were detected at molecular weight 174.3, 99.0, 70.0, 65.6, 48.0, 26.0 and 18.5 KDa. While the PSCAg showed 4 specific protein bands at molecular weight 181.8, 92.5, 62.3 and 16.6 KDa. The ELTB analysis showed presence of 4 protein bands corresponding to molecular weight standard at 22, 26, 31 and 51 KDa react specifically against sera positive.

**Citation:**
Mousa W.M., Mahdy O. A.*, Abdel-Wahab A.M., El-Gameel O.A. 2015. Epidemiological and serological studies on Cystic echinococcosis among camels in Egypt. Journal of Parasitology. Photon 105, 212-218.


**Photon Ignitor:** ISJN37292384D693913022015

## 1. Introduction

Cystic echinococcosis (CE) is one of the most important zoonotic diseases in the Middle East and Arabic North Africa from Morocco to Egypt (Tappe et al., 2011). Cystic echinococcosis is acquiring a public health or economic significance in areas where livestock animals are still slaughtered traditionally and carcass wastes are easily accessible to scavenging dogs and other wild carnivores. These losses can take the form of a reduction in weight gain; yield of milk, fertility rate and in the value of wool or other products. Diagnosis of Hydatidosis is still difficult, which is based mainly on serodiagnostic techniques. So, in recent years, the validity of serological techniques for diagnosis of Hydatidosis has been improved. A variety of techniques are applied for such diagnosis in different animals and human as enzyme- linked Immunosorbent assay (ELISA) (Rahimi, et al., 2011) and Enzyme-linked immunoelectro transfer blot (EITB) (Simsek and Koroglu, 2004). The serological techniques are still lacking the diagnostic specificity, especially in endemic areas

therefore, evaluation and purification of antigens are needed to increase the sensitivity of these serological techniques for the detection and confirmation of the disease in its early stages (Mousa and El-Massrey, 1999). Immunoblotting has been reported to yield very sensitive and specific results in diagnosis of Hydatidosis (Luka et al., 2009). So, the present study was initiated to determine the incidence of cystic echinococcosis among camels in Cairo governorate. The site, size, cysts fertility and viability of their protoscolices were also determined. Also, immunodiagnosis of Hydatidosis was done by using ELISA and ELTB.

## 2. Objective of Research

The CE is an important medical and veterinary problem in the Middle East. Domestic intermediate hosts such as camels, cattle, sheep and goats are major reservoirs of disease for humans. There is no recent information was recorded about the incidence of CE among camels in Egypt. So we initiated this research to evaluate two type of camels *Hydatid* antigen (PSCAg and HCFAg) by several techniques (ELISA, SDS-Page and Immuoblotting technique) for its using in diagnosis of this disease.

## 3. Materials and Methods

The serological techniques are still lacking the diagnostic specificity, especially in endemic areas therefore, evaluation and purification of antigens are needed to increase the sensitivity of these serological techniques for the detection and confirmation of the disease in its early stages especially in IH such as Camel.
So, the aim of the present study, was initiated to determine the incidence of cystic echinococcosis among camels in Cairo governorate. The site, size, cysts fertility and viability of their protoscolices were determined. Also, immunodiagnosis of Hydatidosis was done by using ELISA and ELTB.

### *3.1 Study animals and sampling methods*

A total of 441 dromedary camels slaughtered at Cairo abattoir (El-Basatin), Egypt during the period from January to December 2012 were examined for Hydatidosis. During the post-mortem examination (PM), a thorough visual inspection, palpation and systematic incision of each visceral organ particularly the liver, lung, kidney, heart and spleen was carried out according to Fathi et al., 2011. Infected organs from each positive animal were collected to detect the total number of *Hydatid* cysts (HC). Collected HC were transfer to the laboratory in ice box to conduct cyst count, size measurement, cyst fertility test and viability of protoscolices.

### *3.2 Examination of HC and viability of protoscolices*

*Hydatid* cyst size and volume were classified into small (1-2 cm, < 6ml HCF volume), medium (3-5cm, 6-20ml) and large (>5 cm, >20 ml) according to Berhe, 2009. The fertility of HC was identified and classified according to Ahmadi, 2005 as fertile and infertile cysts (without any protoscolices). The infertile HC were further classified into sterile, calcified, (degenerated). The viability of HC was determined by taking a drop of sediment and examined microscopically for the amoeboid-like peristaltic movement (flame-cell activity) x40 (Salih et al., 2011).

### *3.3 Antigen preparation*

*Hydatid* cysts were collected from the lung and liver of camels. The cyst fluid was processed according to the method of Maddison et al., 1989. Protoscolices antigen was prepared according to Ahmed et al., 2001.

### *3.4 Serum samples*

Serum samples were obtained from 12 camels naturally infected with HC to determine the sensitivity. While serum was collected from 32 camels with no HC at slaughter to determine the sensitivity, Infection was determined at the point of blood collection by gross identification of HC in the various organs of animals.

### *3.5 Enzyme linked immunosorbent assay (ELISA)*

Procedure of ELISA was carried out as described by De Savgny et al., 1979. The HCAg prepared from camels were optimally diluted in coating buffer (carbonate – bicarbonate buffer, pH 9.6) and used to coat (200 µl/ well). Test sera were added 100 µl / well. Anti- sheep IgG (Sigma) optimally diluted 1:1000 with PBS. The optimal density (OD) values were read in a micro-ELISA reader system at 405 nm.

### *3.6 Electrophoresis and Western blotting*

The HCF Ag from camels was subjected to discontinuous electrophoresis in homogenous polyacrylamide gels using 12.5% mini-gels as described by Laemmli, 1970. The electrophoresed proteins were transferred from unstained gels to 0.45µm nitrocellulose membrane following the standardized procedures of Towbin et al., 1979.

### *3.7 Statistical analysis*

Calculation of diagnostic sensitivity, specificity and efficacy was conducted according to Jacobson, 1996.

**Table 1**: Classification of *Hydatid* cysts according to fertility and viability
*No. = Number of examined HC.*

| *organ* | *Fertile cysts* | | *Viable cysts* | | *Non viable cysts* | | *sterile* | | *degenerated* | | *Total* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *No.* | *%* | *No.* | *%* | *No.* | *%* | *No.* | *%* | *No.* | *%* | |
| *Lung* | *67* | *74.4* | *64* | *71.11* | *3* | *3.33* | *9* | *10.0* | *14* | *15.5* | *90* |
| *Liver* | *2* | *50* | *2* | *50* | *0* | *0* | *0* | *0* | *2* | *50* | *4* |
| *Total* | *69* | *73.4* | *66* | *70.21* | *3* | *3.19* | *9* | *9.57* | *16* | *17.02* | *94* |

**Figure 1**: *Hydatid* cyst of camels a.Closed caseated HC. b:Opened caseated HC.

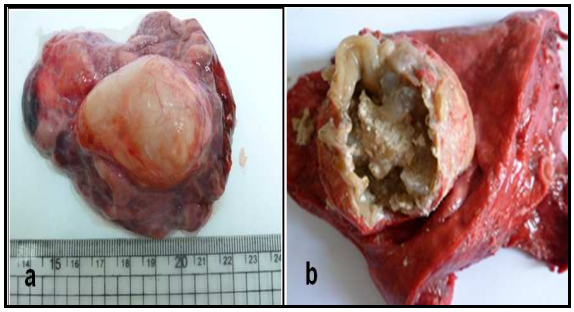
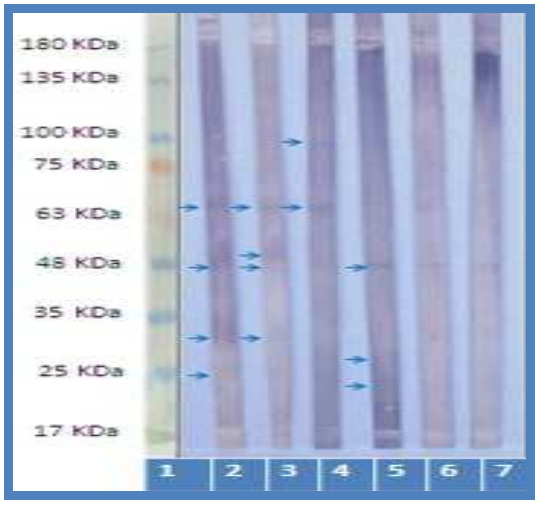



**Figure 2:**
- Specific and non-specific protein fractions of HCF antigen (camels' origin) on NC treated versus positive and negative sera
-Lane no.1-Un stained low molecular weight standard.
-Lane no.2-NC strip treated by serum of experimentally infected rabbit (13W.P.I.) with HC
-Lane no.3- NC strip treated by serum of Rabbit hyper immune serum against HCF antigen.
-Lane no.4- NC strip treated by serum of sheep sera infected with *C. ovis*.
-Lane no.5- NC strip treated by serum of naturally infected sheep with HC.
-Lane no.6- NC strip treated by serum of negative control sera of rabbit.
-Lane no.7- NC strip treated by serum of sheep sera infected with *C. tenuicollis*.




## 4. Results

### *4.1 Incidence HC*

Thirty three out of 441 (7.5%) camels were infected with HC infection. Considering the seasonal incidence of infection was found to be 3.13%, 13.33%, 14.60% and 6.30% at summer, autumn, winter and spring respectively.

### *4.2 Cyst distribution and size*

It was found that 29 (87.9%) and 5 (12.12%) out of 33 infected camels were found to have the infection in their lungs and livers respectively 24 (26.70%), 41 (45.60%) and 25 (27.80%) out of 90 pulmonary cysts, and1 (25.0%), 1 (25.0%) and 2 (50%) out of 4 hepatic cysts were small, medium and large sized cysts respectively.

### *4.3 Cyst fertility and viability*

Out of 94 examined cysts, 69 (73.4 %) were fertile and 66 (70.21%) were viable. The viability of protoscolices of liver and lung fertile cysts was 50% and 71.11% respectively, table 1and figure1.

### *4.4 Enzyme linked immunosorbent assay (ELISA)*

Out of 44 camels serum samples tested against partially purified antigen 19 gave positive results from which 9 were true positive. Twenty five serum samples gave negative ELISA results out of them 22 serum samples were true negative. By using protoscolices antigen out of 44 serum samples 29 gave positive ELISA results from which 12 were true positive while15 gave negative were true negative in camels.
From the previous results it can be concluded that purified HCFAg sensitivity when tested against camels sera was 75% while specificity was 68.7%. While, sensitivity of PSCAg when tested against camels were 100% and specificity was 46.87%.

### *4.5 Characterization of purified HCFAg & PSCAg (camels' origin) using SDS-PAGE*

Electrophoretic analysis of PSCAg gave 4 bands corresponding to molecular weights 181.8, 92.5, 62.3 and 16.6 KDa. While purified HCF antigen separated into 7 bands of molecular weight 174.3, 99.0, 70.0, 65.6, 48.0, 26.0 and 18.5 KDa.

### *4.6 Immunoblotting*

The protein bands of purified HC fluid antigen were electrophoreticaly transferred into nitrocellulose (NC) membrane (figure 2). When purified HCF antigen tested against sera of experimentally infected rabbit with HC infection (13W.P.I.) the reaction cleared that there were 4 bands of molecular weights 65.6, 48.0, 31.8 and 26.0 KDa (lane no 2). While against sera of

naturally infected sheep with HC revealed 3 bands of molecular weights at 48.0, 26.6 and 22.3 KDa (lane no.5). The same antigen was tested against rabbit hyper-immune sera against HCF revealed 4 bands at 65.6, 51.6, 48.0 and 31.8 KDa (lane no.3). On using the same antigen against sheep sera infected with *C. tenuicollis* (lane no. 7) and against negative control rabbit sera (lane no. 6) it gave one band at 48.0 KDa for both sera. By using HCF antigen against sera of sheep infected with *C. ovis* revealed 3 bands ranged from 92.5 to 48.0 KDa. These bands were at 92.5, 65.3 and 48.0 KDa (lane no. 4). This result cleared that there were 4 protein bands corresponding to molecular weight slandered at 22, 26, 31 and 51 KDa react specifically against sera of animals infected with HC as these 4 bands didn't react with any of the negative control sera. While 48 and 65 KDa protein bands found to be non specific bands as they showed positive reaction in both HC positive sera and sheep sera infected with *C. ovis.* Also 48 KDa protein bands gave positive reaction when tested against *C. tenuicollis* infected sheep sera and negative control sera of rabbit.

## 5. Discussion

The CE is an important medical and veterinary problem in the Middle East. Domestic intermediate hosts such as camels, cattle, sheep and goats are major reservoirs of disease for humans (Daryani et al., 2007). It was found that the overall incidence rate of camels hydatidosis was 7.5%. This result is in agreement with Dyab et al. (2005) in Egypt by who recorded 7.67% and in Yemen by Al-Salami et al. (2009). While higher incidence rate was mentioned by Asma, 2009 as 17.29% in Punjab and lower incidence (2.53%) of camels' hydatidosis was recorded in Egypt by Haridy et al., 2006.

This variation in the incidence of hydatidosis from one area to other could be attributed to differences in culture, social activity, attitude to dog in different regions, strain differences, host age factors, sources of slaughtered animals (Ibrahim, 2010), the variation in the environmental conditions, the nature of the pasture and the way of rising and grazing of these animals (Salih et al., 2011).

The seasonal prevalence of infection was found to be the highest at autumn (13.33 %) and winter (14.60%) in camels. Relatively similar results were obtained in Iran by Daryani et al. (2007) who reported that the highest prevalence rate was recorded in winter and autumn. While Ibrahim (2010) recorded that, spring showed the highest prevalence of hydatidosis in camels, cattle and sheep in Saudi Arabia. The seasonal variations may be attributed to age differences among seasons (Ibrahim, 2010).

The infection was restricted on lung (87.9%) and liver (12.1%). These results were in agreement with that recorded in Egypt by Dyab et al. (2005) and El-kattan (2012) who reported that infection was mainly located in the lungs. On the contrary Ibrahim, (2010) found that the most commonly infected organs were liver (48.75%) and lung (32.83%) of the total infected organs. Lungs were the most frequently infected visceral organs in examined camels this might be due to the fact that camels are slaughtered at older age, during which period livers capillaries are dilated and most ionosphere (hexacanth embryo) be able to enter the lymphatic circulation and to be carried via the thoracic duct to the heart and lungs in such way that the lung may be infected before or instead of liver (Fathi et al., 2011).

Concerning the size of the examined cysts, HC collected were divided into small (26.60%), medium (44.70%) and large (28.70%). Medium sized cysts were the most prevalent in the infected lung (45.60%) while hepatic cysts were mostly of large size (50%). These results are in agreement with El-kattan, 2012.

Regarding to fertility and viability of camels HC collected in this study, viability rate was 70.2%, while fertility rate was 73.7%. These results agreed with El-Kattan 2012& Muqbil et al., 2012 who recorded 78% fertility rate in camels in Egypt & Yemen.

According to their fertility in different organs, fertility rate of pulmonary cysts (74.5%) was higher than that of the hepatic cyst (50.0%). This result was in agreement with Salih et al., 2011 who reported that pulmonary cysts fertility (57.24%) was higher than that of hepatic cysts (40%), while viability rate was 66.6% in both.

The difference in fertility and the proportion of viable protoscolices (PSC) from fertile cysts may be related to the difference in immunological response per host, and genotype dependent (Fathi et al., 2011). Also, Salem et al., 2011 reported that the differences between prevalence rates, HC location and fertility depend on the strain(s) of *E. granulosus.*

Sensitivity of purified HCF antigen when tested against camels sera using ELISA was 75% while specificity was 68.7%. While for PSCAg when tested against camels sera sensitivity was 100% while specificity was 46.87%. So these results cleared that, sensitivity of PSCAg was higher than that of purified HCFAg in diagnosis of HC

infection. While, specificity of purified HCF Ag was higher than that of PSCAg for diagnosis of HC infection in camels. This result was in agreement with that of Kittelberger et al., 2002 in New Zealand who recorded high diagnostic sensitivity using crude PSCAg preparation.

The diagnostic efficacy of purified HCF for diagnosis of HC infection in camels was 70.45% while the diagnostic efficacy of PSCAg was 61.36%. From these results it can be concluded that the diagnostic efficacy of purified HCFAg for diagnosis of HC infection in camels was higher than that of PSCAg. These results agreed with Fotoohi et al., 2013 in Iran who recorded that the HCF was the most effective antigen for diagnosis of HC infection in human. Sadjjadi et al., 2007 concluded that, it has become more common to purify components of HCFAg such as the antigen B and antigen 5 which are the most appropriate components for immune-diagnosis of cystic echinococcosis.

The SDS-PAGE analysis of PSCAg gave 4 bands of MW 181.8, 92.5, 62.3 and 16.6 KDa. While, purified HCFAg separated into 7 bands of MW 174.3, 99.0, 70.0, 65.6, 48.0, 26.0 and 18.5 KDa. These results are relatively similar with that obtained by Mousa, 1992 who found 9 protein fractions ranging in MW from 8-150 KDa when analyzed 2 different HCFAg of camels origin using SDS-PAGE and Kittelberger et al., 2002 who recorded that HCF of sheep contained protein bands ranging from 7 to about 150 KDa and the protoscolices preparation at least 10 major protein bands were visible ranging in size from about 15 to more than 100 KDa. On the other hand Sadjjadi et al., 2007 concluded that, it is well known that the nature and quality of antigen B in HC fluid are variable among the host species and this may be one of the reasons why different laboratories obtain different results.

The purified HCFAg was analyzed using SDS-PAGE then transferred to nitrocellulose sheet. The strips of the blotted purified HCFAg were tested against sera from experimentally infected rabbit with HC (13 W.P.I), rabbit hyperimmune sera against HCF, negative control rabbit sera and sheep sera infected with HC, *C. tenuicollis* and *C. ovis.* There were 4 protein bands corresponding to molecular weight slandered at 22, 26, 31 and 51 KDa react specifically against sera infected with HC as these 4 bands didn't react with any of the negative control sera.

These results were in agreement with the result of Al-Yaman and Knobloch, 1989 who mentioned that the 20 KDa band was found to be sensitive for *E. granulosus*. Also Sabry, 2007 recorded that the HCFAg bands corresponding to MW 38, 36, 29, 18, 16, 12 and 8 KDa react specifically against sera of infected rabbits as well as sera of surgically proved HC infected patients. Al-Olayan and Helmy, 2012 mentioned that the clusters of bands at 24-22 KDa were found to give positive in all HC infection cases using camels and sheep HCF extract and didn't react with any of sera from patient with other parasitic diseases and from normal control. But these results were in contrary with Bandyopadhyay and Singh (2000) who found that the western blot of affinity purified buffalo HCF against 12 weeks experimentally infected mice sera showed immunogenic fractions of 66 and 48 KDa. Also in present study 48.0 and 65.0 KDa protein bands found to be non specific bands as they showed non-specific positive reaction in HC positive sera and *C. ovis* infected sheep sera. These results agree with that of (Craig et al., 1986) who mentioned that the cross reactive antigens occurred mainly between 30 and 67 Kda regions.
Such variation in data might be related to the type of antigens, disease which used to determine the specificity, as well as the time of incubation during the experiment (Mousa , 1992).

## Conclusion

We concluded that the diagnostic efficacy of purified HCFAg for diagnosis of HC infection in camels was higher than that of PSCAg. Purified HCFAg has 7 specific-protein (174.3, 99.0, 70.0, 65.6, 48.0, 26.0 and 18.5 KDa). While the PSCAg showed 4 specific protein (181.8, 92.5, 62.3 and 16.6 KDa). ELTB analysis showed presence of 4 protein bands (22, 26, 31 and 51 KDa) reacted specifically against sera infected with HC so these bands were immunogenic can used in vaccine preparation and used in the diagnosis of CH in camels.

## Research Highlights

The purified HCFAg of Camel origin is found more immunogenic for diagnosis of HC infection than that of PSCAg. by using SDS-PAGE, EITB. Moreover, purified HCFAg sensitivity when tested against camels sera was 75% while specificity was 68.7%. While, sensitivity of PSCAg when tested against camels were 100% and specificity was 46.87% by using ELISA.

## Recommendations

Infection by HC originate from dogs and spread to contaminant the nature around other animals and their contact human, the problem tack the chronic natures due to several factors include: No strict hygienic measures applied on the slaughtered

house waste products before transferring from the abattoirs.

Easley arrival of dogs and related carnivorous to pasture, dead animal carcasses and condemned organs.

Lacking of intermediate host specificity for HC development as most animals and human act as suitable intermediate hosts for develop and maintaining HC able to induce infection in dogs.

Ignorance of farmers and people that obliged to still in enemata contact to dogs, slaughter house workers and some veterinarian by the dangerous of dogs as a source for this type of infection.

As infection are considered to be asymptomatic in dogs and in intermediate hosts especially in the early stage of infection, no available drugs able to induce early limitation for infection. This problem can be controlled by the destruction of life cycle of *Cystic echinococcosis.*

## Funding and Policy Aspects

This study is the part of project is funding from Cairo University, Egypt.

## Acknowledgements

The authors acknowledge Cairo University vice President of postgraduates and research for funding this project. Deep acknowledges for veterinarians and workers of Cairo slaughter houses for their appreciable help and their permission for the authors to examine of the slaughtered Camel in Cairo slaughterhouses.

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