Bulgarian Journal of Veterinary Medicine, 2019, **22**, No 3, 305–313
ISSN 1311-1477; DOI: 10.15547/bjvm.2136

Original article

STUDIES ON THE SPECIFIC IMMUNODIAGNOSIS OF CYSTIC ECHINOCOCCOSIS IN CAMELS USING ENZYME-LINKED IMMUNOSORBENT ASSAY

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Summary

Kandil, O. M., N. M. F. Hassan, D. Sedky & E. Beshir Ata, 2019. Studies on the specific immunodiagnosis of cystic echinococcosis in camels using enzyme-linked immunosorbent assay. *Bulg. J. Vet. Med.,* **22**, No 3, 305–313.

Cystic echinococcosis (CE) is of increasing public health and socio-economic concern because of the large morbidity rates and produced high economic losses in the livestock industry. The objective of the current research was to study the reliability of indirect ELISA in detecting CE, based on two different types of crude antigens of camel origin; protoscolex and germinal layer antigens from hydatid cyst. Blood samples were collected from 284 (125 slaughtered and 159 live camels). Out of 125 slaughtered camels examined visually, 55 (44%) were found to have hydatid cysts. Of them, 52/125 (41.6%) and 3/125 (2.4%) harboured hydatid cysts in lungs and livers respectively. Fertile lung cysts were 32.8%; 26.9% were sterile, while 40.3% of lung and liver cysts were calcified. The sensitivity of ELISA was 83% and 46.5% when protoscolex and germinal layer antigens were used, respectively. The respective specificity of antigens of protoscolex and germinal layer was 70.3% and 41.7%. The protoscolex antigen showed higher accuracy (73.6%) compared to the germinal layer antigen (52.8%). The cross reactivity of these antigens were evaluated with antigens and hyperimmune sera of CE and *Fasciola* spp. and *Haemonchus contortus* using ELISA. The results showed also weak immunogenic potency of each antigen with *Fasciola* spp. hyperimmune sera at dilution 1:50 while hyperimmune sera of *Haemonchus contortus* did not bind any antigen.

Key words: antigen, camel, cystic echinococcosis, ELISA, *Fasciola, Haemonchus*

INTRODUCTION

Cystic echinococcosis (CE; hydatidosis) is one of the widely distributed parasitic diseases with zoonotic importance. It is caused by ingestion of different *Echinococcus* species eggs (Samorek-Pieróg *et al.*, 2016). The adult worm inhabits the small intestine of dogs as permanent hosts, while the larval stages or hydatid cysts occur in herbivorous intermediate hosts and sometimes in humans (Almalki *et al.,* 2017). The disease can infect different animal species including camels with variable rates of infection (Kandil *et al.,* 2016). Loss of body weight, decreased

fertility rate, and reduction of milk and wool production are the major clinical signs of infection (Torgerson, 2003). Abattoir surveys are important, particularly in the surveillance of many parasitic diseases including CE (Borji *et al.*, 2012). Definite diagnosis of *Echinococcus granulosus* infection in animals is the first step for epidemiological studies and surveillance either in endemic, re-emergent or emergent transmission areas (Craig *et al.*, 2015). No direct parasitological evidence was found for the presence of cysts in organs or tissues and in most cases, the early stages of infection are asymptomatic.

Imaging techniques, for example, ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI) are utilised as often as possible for diagnosing CE. Ultrasound has been used widely because it is simple, noninvasive, and cost-effective (Ozkol *et al*., 2005). However, the accuracy of US-based screening relies greatly on the skills of the ultrasonographer (Yu *et al.,* 2008). Han *et al*. (2016) demonstrated that ultrasonography appears to be the detection modality of choice. Serology could be used for detection of infection in the suspected individuals, especially when it is complicated to differentiate between some cyst stages from the common non-parasitic cysts (Brunetti *et al.,* 2011). The accessibility of suitable serodiagnostic tools including enzyme-linked immunosorbent assay (ELISA) could help in diagnosing many infectious diseases in camels (Al-Ruwaili *et al.,* 2012; Mohamed *et al*., 2013). Indeed, there are few serological studies on camel CE. Unfortunately local commercial anti-camel immunoglobulins are not currently available that is considered a main obstacle to diagnosis of camel antibodies using ELISA. However, it has been obtainable for many various parasitic research studies (Azwai et al., 1995), so sero-diagnostic studies should be directed to this significant and functional aspect.

The present study was aimed at evaluating native crude antigens of protoscolex and germinal layer implemented in ELISA for detection of specific IgG antibodies of CE in camels' serum samples collected from Egyptian abattoirs and markets.

MATERIALS AND METHODS

Ethical approval

All animal experimental procedures were performed in accordance with the recommendations and guidelines stated by the ethical Committee of the National Research Centre under certificate number 17133.

Samples collection

Hydatid cysts were collected from the lung and liver of camels slaughtered at Cairo abattoir (EL-Basatin). One hundred and twenty five animals were visually examined after slaughtering. Infected camels were recorded and infected organs (52 lungs and 3 livers) were collected. Sixty seven hydatid cysts were removed carefully from their host tissue without injuring the cystic wall, washed thoroughly with tape water, rewashed in saline and kept in phosphate buffered saline (PBS) until use (Ahmed *et al*., 2006). The viability of protoscolex was determined by using eosin exclusion 10% solution (Macpherson *et al*., 1985). Adult worms of *Haemonchus contortus* and *Fasciola gigantica* were collected from slaughtered sheep at EL-Basatin abattoir in Egypt. Worm recovery was carried out according to standard procedures (MAFF, 1986).

Antigens preparation

The adult *Haemonchus contortus* worms, *Fasciola species*, germinal layer and protoscolex of hydatid cyst were washed with PBS and subjected to grinding using a homogeniser followed by sonication and high-speed cooling centrifugation (14,000 rpm for 30 min). The supernatant was obtained and the process of centrifugation was repeated twice till no sediment was thrown down (Ahmed *et al*., 2006). The protein content of the different prepared antigens was determined according to Lowry *et al*. (1951).

Serum samples

Blood samples were collected from 284 camels included 125 slaughtered camels at El-Basateen abattoir, Cairo, and 159 randomly selected live camels from the market. Serum samples were prepared and kept at –20 ^{o}C until used.

Hyperimmune sera

Fifteen healthy White New Zealand male rabbits around 1.5–2 kg body weight were grouped in 5 groups (n=3) and immunised with the different prepared crude antigens (germinal layer, protoscolex, *Fasciola species* and *Haemonchus contortus*). One group was kept as a control. Hyperimmune sera were prepared according to Fagbemi *et al*. (1995).

Serological analysis

The potency of protoscolex and germinal layer antigens was evaluated by ELISA which was performed according to Sadjjadi *et al*. (2007). The optimal antigen, serum and conjugate concentrations were determined after preliminary checkerboard titration according to Catty & Raykundalia (1989). The antigen concentration was 20 µg/mL and 40 µg/mL for germinal layer and protoscolex antigens respectively. After coating, blocking with 100 µL per well of 0.1% bovine serum albumin in 0.01 M PBS was done. From the natural infected sera of CE, non-infected sera, random sera from live camels and hyperimmune sera of CE, *Haemonchus contortus* and *Fasciola species* (diluted 1:50, 1:100, 1:200 in PBS), 100 µL were added to each well. One hundred µL of 1:1000 peroxidase conjugate anti-bovine IgG were used. Fifty µL of orthophenylenediamine was used as a substrate. The reaction was terminated with 1M H_2SO_4 and the absorbance values were read spectrophotometrically at 490 nm. Positive samples were assigned according to Rodriguez-Perez & Hillyer (1995) as those with absorbance readings greater than the cut-off value, which was calculated as mean OD of negative sera plus three standard deviations. Sensitivity, specificity and accuracy of ELISA were calculated as described by Timmreck (1994).

Statistical analysis

OD data were expressed as arithmetic mean with standard deviation. The apparent prevalence parameter was analysed using the Chi square test by statistical computer package for social science (SPSS) version 15.

RESULTS

Post mortem findings and CE infection percentage

Out of 125 slaughtered camels examined visually, 55 (44%) camels were infected with hydatid cyst and 70 (56%) were naturally non-infected camels. Fifty two lungs and three livers harboured hydatid cyst with infection percentage 41.6% and 2.4% respectively. The lungs were the most

commonly affected organs with hydatid cysts. The examination of lung cysts demonstrated that 22 or 32.8% and 18 or 26.9% appeared fertile and sterile, while 40.3% of lung and liver cysts were calcified.

Immunogenic reactivity of different CE antigens

Two hundred and eighty four camel's sera were tested by ELISA using the protoscolex and germinal layer antigens to detect CE antibodies. The seropositive samples using protoscolex and germinal layer antigens were 89 (31.3%) and 144 (50.7%) respectively (Table 1). Most of the naturally infected camels were true positive – 28 and 27 using the protoscolex and germinal layer antigens respectively, while 27 and 28 false positive results were respectively recorded. Therefore, the results of ELISA showed that the germinal layer antigen detected the higher prevalence (44.3%) and protoscolex antigen noticed a lower prevalence (8.6%) of non-infected sera from slaughtered camels. Moreover, the germinal layer antigen had a higher diagnostic efficacy (54%) than the protoscolex antigen (34.6%) from random live camel sera. Nevertheless, sensitivity of ELISA was 83% and 46.5% when the protoscolex and germinal layer antigens were used, respectively. The specificity of antigens of protoscolex and germinal layer were 70.3% and 41.7%. The protoscolex antigen showed higher accuracy value (73.6%) compared to the germinal layer antigen (52.8%).

The diagnostic performances of the two antigens used in the study were evalu-

Table 1. Detection of anti-CE antibodies in sera from slaughtered camels compared to live camels findings

Parameter	Animal number	Types of antigens			
		Protoscolex		Germinal layer	
		+	%	+	%
Naturally infected sera	55	28	50.9	27	49.0
Naturally non-infected sera	70	6	8.6	31	44.3
Random sera	159	55	34.6	86	54.0
Total	284	89	31.3	144	50.7

Table 2. Comparison between protoscolex and germinal layer antigens for naturally infected sera (IS), non-infected sera (NIS) and random sera from camels (Chi-square test results)

Groups	Protoscolex antigen		Germinal layer antigen	
	χ^2	Significance	χ^2	Significance
IS *vs* NIS	14.235	<0.001	0.276	0.599
IS *vs* random	8.783	0.003	30.805	<0.001
NIS *vs* random	39.361	<0.001	25.855	<0.001

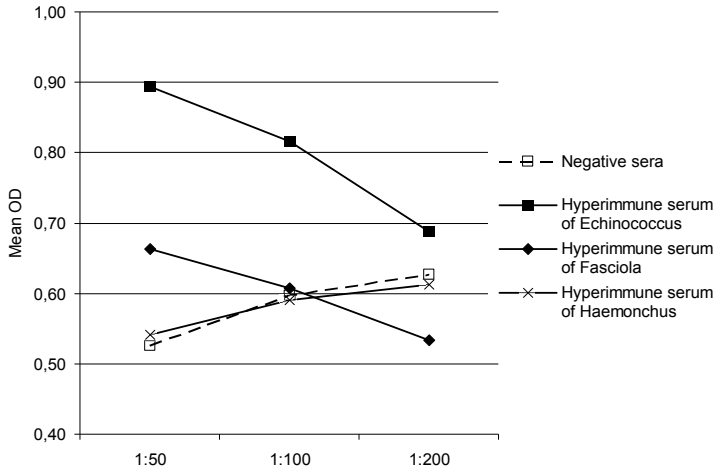

Fig. 1. Cross reactivity of the protoscolex antigen against negative rabbit serum, hyperimmune sera raised against CE as positive control, *Fasciola species* and *Haemonchus contortus.*

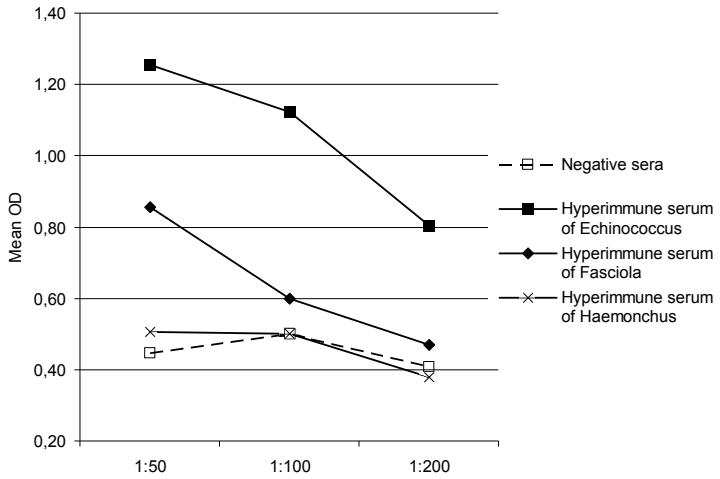

Fig. 2. Cross reactivity of the germinal layer antigen against negative rabbit serum, hyperimmune sera raised against CE as positive control, *Fasciola species* and *Haemonchus contortus.*

ated then statistically compared using a χ^2 test (Table 2). It is shown that the protoscolex antigen was the best antigen used for diagnosis of CE as the statistical analysis using Chi square test found that IS vs NIS, IS vs Random and NIS vs random differed significantly ($P<0.05$), while the germinal layer antigen IS vs NIS showed no significant difference.

The results of cross reactivity of protoscolex and germinal layer antigens with different hyperimmune of CE, *Fasciola spp* and *Haemonchus contortus* showed that the immunogenic potency of each

antigen was weak with *Fasciola* spp. hyperimmune sera (1:50) while hyperimmune sera of *Haemonchus contortus* did not bind any antigen (Fig. 1 and 2).

DISCUSSION

Hydatidosis is considered as an important zoonotic disease, of serious livestock and human health concern in the Mediterranean region and in the world (Adel *et al.,* 2017; Belmamoun *et al*., 2017). Annual losses of animal production due to the hydatid cysts, have been estimated to between 142 and 2190 million US dollars around the world (Moro *et al*., 2011).

The obtained results evidence that out of 125 slaughtered camels examined visually and manually by palpation and incision, 55 (44%) were found to have hydatid cysts (41.6%) in lungs and (2.4%) in liver indicating that lungs are the usual and the most infected organ by hydatid cysts. These results appeared to be in line with that obtained by Rinaldi *et al*. (2008) in Italy and Beyhan *&* Umur (2011) in Turkey, who stated that lungs were the extremely affected organ with CE in buffaloes (80% out of the examined animals). Moreover, the results coincided with that of Debas & Ibrahim (2013) who reported that the cyst could be developed in different parts of animal body but the most predilection sites are the lungs and the liver and at a lesser extent, the spleen and the heart.

Our results disagreed with those obtained by Ahmed *et al.* (2017) who revealed that liver was the most commonly affected organ which might be due to the reflection of the route of parasite entry and seems to support the hypothesis of hepatic portal distribution of oncospheres resulting in liver infection firstly. Out of the total 67 cysts collected, 22 (32.8%), 18 (26.9%) and 27 (40.3%) were fertile, sterile and calcified respectively. Haroun *et al*. (2008) found 6.3% fertile hydatid cyst which is a lower incidence than presented obtained results. Variation in fertility could be attributed to strain differences such as host and organ preference, development rate, infectivity, pathogenesis and antigenicity and drug resistance (Thompson & Lymbery, 1988).

Diagnosis is a basic step in studies of echinococcosis (Barnes *et al.*, 2006). Using specific serological techniques for diagnosis of CE in camels would be helpful for screening the hydatid infection in commercial and domesticated animals and for epidemiological investigations (Han *et al*., 2016). ELISA has been produced for serodiagnosis of animal CE; however, to the best of our knowledge, this is the first study that demonstrates an assay for determination of specific IgG against hydatid antigens in camel sera. Thus, in the present study, the protoscolex and germinal layer antigens of camel origin were successfully used in ELISA to detect specific IgGs in camel sera, determined *post mortem* to be naturally infected by CE. Craig & Rickard (1981) found that hydatid cyst fluid antigen is good for diagnosis of CE because the fluid from fertile cysts has been found to contain high concentrations of diagnostically relevant antigens but also components, mainly immunoglobulins and serum albumin, interfering with diagnostic tests (Sanchez & Sanchez, 1971). The present ELISA results showed 31.3% and 50.7% seropositive samples with CE using protoscolex and germinal layer antigens respectively compared with 44% positivity from the *post mortem* examination. This may be attributed to absence of antibodies production as in case of small cysts, intact cysts, and or calcified cysts existence (Gavidia *et al*.,

2008). However, many serological methods including ELISA, were used for the diagnosis due to advantages in aspect of the collection, storage and transportation (Zhang *et al*., 2012).

The sensitivity for the protoscolex was 83% as compared to 46.5% for germinal layer according to the visual inspection of liver and lungs; on the other hand a negative result was obtained in slaughtered camels judged visually to be not infected with hydatidosis. The specificity of the ELISA was not as high 70.3% and 41.7% for the protoscolex and germinal layer antigens respectively. The obtained false positive results might be secondary to cross reactions with other parasitic infections. Many factors affect IgG production, like cyst number, size, location, and stage (Moro & Schantz, 2009). Moreover, serological techniques' sensitivity is inversely related to the degree of sequestration of the echinococcal antigens inside cysts (Nunnari *et al*., 2012). Moreover, Ibrahem & Criag (1998) showed that antigen B purified from hydatid cyst fluid was highly specific (99%) and sensitive (90%) when used in ELISA to test sera collected from sheep naturally infected with CE. Thus ELISA may be considered a highly sensitive and specific tool for CE diagnosis.

So, the present study concluded that the protoscolex antigen from camel hydatid cyst is a promising antigen for serological diagnosis and screening of CE in camels.

ACKNOWLEDGEMENTS

This work was financially supported by co-project between Egypt and Morocco entitled ''Impact of agricultural wastewaters reuse on human and animals parasites; Diagnosis, cycle and epidemiology of hydatidosis" and funded from Ministry of Scientific Research in Egypt.

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Paper received 02.01.2018; accepted for publication 16.03.2018

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