International Journal of Basic and Applied Sciences, 3 (2) (2014) 93-98
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www.sciencepubco.com/index.php/IJBAS
doi: 10.14419/ijbas.v3i2.2127
Research Paper

Epidemiological and molecular characterization of antigens extracted from Hydatid cysts of camel, cattle and donkeys in Egypt

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Abstract

Cystic echinococcosis or Hydatid disease is recognized as an important worldwide distributed disease from the clinical, economical and zoonotic point of view. In the present work, 180 camels and 90 cattle freshly slaughtered at Cairo abattoir and 120 donkeys scarified at Giza zoo, were inspected for infection by Hydatid cysts (HC) in Egypt. The highest incidence of HC infection was 18.9% in Camel, 14.2% in donkeys and the lowest 3.3% in cattle. Regarding the site of HC infection was 94.3% and 90.2% in Camel lungs and donkeys liver. The fertility of HC was 79.24% and 29.4% from camel and donkeys, while, all inspected hydatid cysts collected from cattle were found calcified. Germinal membranes of fertile HC were used for DNA extraction followed by PCR amplification. It was used for identification of internal transcribed spacer gene1(ITS1)from camel and donkeys by using specific primer. The amplified DNA fragment was further analyzed by PCR mediated restriction fragment length polymorphism (PCR ¬RFLP) using two restriction enzymes (MSP1 and RSA1). The PCR yielded similar amplified DNA band of the same molecular size marker at 1115 bp in different isolates of Hydatid. No band variation of ITS 1 gene could be detected by PCR- RFLP by using two restriction enzymes. Amplification product of ITSI after digestion with MSP1 showed at 661 bp, while those restricted with RSA1 enzyme appeared at 745 bp.

Keywords: *Molecular, Hydatid cyst, Antigens, PCR, Camel and Donkeys, Egypt.*

1. Introduction

Cystic echinococcosis or hydatid disease is one of the most important worldwide distributed disease from the clinical, economical and zoonotic point of view causing sever animal and human health problem [1]. It is a helminthic cyclo-zoonosic disease caused by the larval stage (metacestode) of the canid tapeworm *Echinococcosis* that require at least one other vertebrate host for completion of its life cycle. The disease is endemic in many parts of the world [2]. It is one of the major zoonotic parasitic diseases in the Middle East and Arab North Africa from Morocco to Egypt [3]. 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 [4].
Concerning molecular characterization *of E. granulosus*, the parasite poses a high degree of genetic diversity based on genome pattern, morphology and host specificity have allowed the differentiation of at least Ten different genotypes (G1- G10) among which G4 (horse strain) have been formerly characterized [5] [8] and Camel strain (G6) have been formally in Eastern Africa [9] North Africa [10] and Tunisia [11].
The present study, was conducted on slaughtered camel, cattle and scarified donkeys aiming to determine the incidence rate of Hydatid cysts infection, the site of infection and fertility of cysts. PCR was used for amplification of DNA extracted from fertile HC for identification of ITS1 gene of camel and donkeys followed by further identification by PCR- RFLP using two digestive.

2. Material and methods

This study was assessed and approved by Faculty of Veterinary Medicine, Cairo University Ethics Committee and therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.1. Collected samples

HC were collected from freshly slaughter animals 270 (180 camels, 90 cattle) at Cairo abattoirs and from inspected 120 donkeys on post- at the zoo of Giza Zoo, Egypt. The donkeys were brought to the zoo for feeding lions. They were examined for the presence of HC on post- slaughtered and post- mortem inspection during the period from December 2011 to November 2012. Intact HC, isolated from the infected animals, were put separately in the polythene bags containing ice and brought to Veterinary Medicine of Cairo University for further processing. Examination of all internal organs was also done by using palpation and incision for the detection of HC according to the technique recommended by [12].

2.2. Identification of HC

The suspected infected organs were collected from slaughtered and scarified animals for routine microscopic examination according to [13]. Cyst fluid was obtained from pulmonary and hepatic cysts for demonstration of protoscolices and hooklets. Protoscolices were isolated from the fertile cysts and then washed three times by phosphate buffer saline (PBS), pH 7.2 and preserved in 70% alcohol (v/v) for isolation of DNA [14].

2.3. DNA extraction:

DNA was extracted from germinal layer of fertile HC using Genei Ultrapure TM Mammalian Genomic DNA Purification Tissue Kit (Bangalore Genei). According to manufacturer's instructions, 25µl of the antigen were suspended in 500µl of CTAB buffer and transferred to a microfuge tube, incubated for 15 min. at 55ºC in water bath, then the mixture was centrifuged at 12000 rpm for 5 min. and the supernatant transferred to a clean microfuge tubes. To each tube 250ul of chloroform: IsoAmyl alcohol (24:1) was added and the solution then mixed by inversion. The mixture was spanned at 13000 rpm for 1 min. Here the upper aqueous phase contained the DNA which is transferred to a clean microfuge tube. To each tube 50µl of 7.5 M Ammonium acetate was added followed by 500µl of ice cold absolute ethanol to precipitate the DNA. The precipitate was transferred into a microfuge tube containing 500µl of ice cold 70% ethanol and then centrifuged at 13000 rpm for 1 minute supernatant discarded and the remaining DNA pellet washed by adding 70% ethanol, then centrifuged at 13000 rpm for 1 min. and again the supernatant removed and the DNA re-suspended in DNAse and incubated at 65ºC for 20 min and stored at 4ºC.

2.4. PCR Assay

Amplification of ITS1 gene was done by using of primers described by [15]. The primer was designed as forward 5' GTC GTA ACA AGG TTT CCG TA'3 and reverse 5'TCT AGA TGC GTT CGA A (G/A) TGT CGA TG'3, (Jena, Bioscience, Germany). A100-bp DNA was used as molecular size marker. The amplification reaction was carried in 25µl volume containing 500mM Kcl, 10 mm Tris- Hcl (PH9.0), 1% Triton x-100, 4 mm Mgcl, 100uM DNTPs each, 15-20ng of ITS1 primer, 25ng of DNA and 1.5 units of Tag DNA polymerase. For data analysis PCR assay was performed in thermal cycler (Teche TC – 512UK). The DNA was denaturated for 6 min. at 95ºC. The mixture was then subjected to 30 cycles of denaturation at 94ºC for 45 sec., annealing of primers at 55ºC for 60 sec. and primer extensions at 72ºC for 90 second. The final extension was held at 72ºC for 1 min. PCR products were analyzed after electrophoresis in 1.5% (W/v) Agarose gel and visualized in Ethidium bromide.

2.5. PCR mediated RFLP

PCR product were digested with MSP1 and RSA1 (10u) using buffer recommended by the manufacture (Jena Bioscience, Germany). Restriction fragments were separated by gel electrophoresis through 2%TBE Agarose gel. PCR products were analyzed after electrophoresis in 1.5% (W/v) Agarose gel and visualized in Ethidium bromide.

3. Results

The data demonstrated in table (1) cleared that the total incidence of infection by HC was 18.9%, 3.3% and 14.2% among examined camels, cattle and donkeys respectively. The cyst was diagnosed in 5.7 %, 66.7% and 90.2% of the examined liver respectively, while, it was 94.3%, 33.3% and 9.8% in the examined lung of camels, cattle and donkeys

respectively, table (2). Moreover, the rate of fertile cysts was found to be 42 (79.24%) in camel and 15 (29.4%) in donkeys. while, all cysts collected from the inspected cattle were found calcified. table (3).
Microscopically, the higher incidence of viable motile protoscoleces (60.4%) was found in HC of camel origin, then (23.5%) in HC of donkey origin. The highest incidence of non-motile protoscoleces (18.9%) was found in that of camel origin while it was 5.9% in that of donkey origin, table (4).

Table 1: Incidence of HC Infection in Examined Animals.

Animals	No. Ex.	No. Inf.	%	No. cysts	Mean and average No. Cyst/ animals
Camels	180	34	18.9	53	(1-3)1.55
Cattle	90	3	3.3	6	(1-2)1
Donkeys	120	17	14.2	51	(2-3)3
Total	390	54	13.8	110	

Table 2: Incidence of HC Distributions in Different Site of Infection in the Examined Animals

Infected animals	No. cysts	Site of infection			
		Lung		Liver	
		No.	%	No.	%
Camels	53	50	94.3	3	5. 7
Cattle	6	2	33.3	4	66.7
Donkeys	51	5	9.8	46	90.2
Total	110	57	51.8	53	48.2

Table 3: Incidence of Sterile and Fertile HC in the Examined Animals.

Animals	No. Ex.	No Inf.	%	No. Cyst	Sterile HC		Fertile HC		Calcified HC	
					No	%	No.	%	No.	%
Camels	180	34	18.9	53	3	5.7	42	79.24	8	15.1
Cattle	90	3	3.3	6	0	0	0	0	6	100
Donkey	120	17	14.2	51	17	33.3	15	29.4	19	37.3
Total	390	54	13.8	110	20	18.2	57	51.8	33	30.0

Table 4: Incidence of HC According to Viability of Protoscoleces

Animals	No.Cyst	No. fertile cyst	Fertile cysts with			
			Motile Protoscoleces		Non-motile Protoscoleces	
		No.	No.	%	No.	%
Camels	53	42	32	60.4	10	18.9
Cattle	6	0	0	0	0	0
Donkeys	51	15	12	23.5	3	5.9
Total	110	57	44	40	13	11.9

The genetic characters of HC obtained from camel and donkeys after PCR amplification of ITS1 gene showed similar pattern of PCR product, all amplified DNA products have band of the same molecular size at 1115bp on Agarose gel (Fig.1).
Furthermore, molecular analysis using PCR-RFLP for amplification to the product of ITS1 after digestion with MSP1 showed that all strains sharing in the MW at 661 bp (Fig.2A), while those restricted with RSA1 enzyme appeared at 745 bp ((Fig.2B).

4. Discussion

Hydatid cyst (HC) the larval stage of the dwarfed tape worm of dogs, *Echinococcus granulosus*, develops in the internal organs of human and herbivore intermediate hosts, mainly in the liver and lung [16]. In Egypt the prevalence of hydatidosis still a point under investigation, In the present study, the incidence of HC were 18.9 % and 3.3% in slaughtered camels and cattle from Cairo abattoirs. This result was in agreement with [17-18] who recorded 18.9% & 17.61% in slaughtered camels in Ismailia and Cairo abattoirs, Egypt. In the same time, this result considered to be lower than that recorded by [19] in Assiut governorate, as 7.67% in camels and no infection was recorded in the examined cattle and buffaloes. Presently, incidence of donkey HC infection was (14.2%) this finding considered to be higher than that result by [20]. On the other hands, the low incidence of Cattle HC infection (3.3%) was in agreement with [21] & [19] where, both of them failed to diagnose HC infection in the examined slaughtered cattle.
According to the present study, the rate of infection in camel was higher in Lung (94.3 %) than in liver (5.7 %). An observation in accordance with that noticed in Egypt camel, [18], [22] who found that 100%, 63.7%. And 94.6% in lung camel.

In the present study, the majority of infected donkeys (90.2%) harbored HC in their liver. This result was in agreement with [8], who revealed that the majority of infected donkeys (70%) in their livers at Beni-Suef, Egypt.

From the epidemiological and molecular aspects, fertility of HC is one of the most important factors in the epidemiology of *E. granulosus*. The fertility of cyst varies depending on the hosts and geographical situations [23]. In the current study, fertility rate of HC in camel and donkeys have been found to be 42 (79.24%) in camel lung and 15 (29.4%) in donkey liver. The high rate of fertile cyst may indicate that the cause of infection in investigated animals might be due to camel and donkeys strain (G6 & G4). As such genotype is commonly recognized as a predominating species of *E. granulosus* in Mediterranean countries [24].

In the present work, molecular genetics study has been carried out to identify the genetic characters of HC obtained from the infected camel and donkeys. After PCR amplification of ITS1 gene, similar amplified DNA band of the same molecular size marker at 1115bp were recorded in the different isolates. No band variation of ITS1 gene could be detected by PCR-RFLP after using the two restriction enzymes, MSP1 and RSA1, This meaning absence of ITS1 variant which could not differentiated using these two restriction enzymes. This was in agreement with [25-26]. In the author's opinion and in agreement with [26] absence of variation in amplified ITS1 and indistinguishable genetic character in PCR-RFLP, meaning that the analyzed camel and donkey HC samples are infected with *E. granulosus* of sheep strain. This can be accepted as all examined animals are from the same localities. Moreover more research are continue aiming to further identification of Hydatid infection of camel and donkeys based on PCR amplification and sequence of mitochondrial genes.

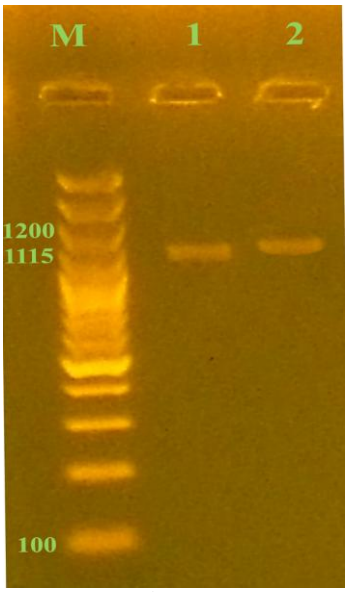

Fig. 1: Agarose Gel Electrophoresis of The PCR-Derived Amplicons of HC Germinal Layer of *E. granulosus* ITS1 Gene, Separated on 2% Agarose Gel and Stained With Ethidium Bromide Lanes: (M) 100 Bp DNA Ladder (Consists of Repeats of 100 Bp Fragment Size, Fermintas), Lane 1Camel DNA & 2 Donkey DNA Containing 1115 Bp.

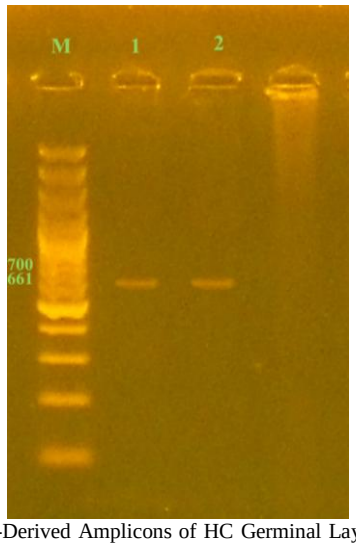

Fig. 2 (A): Agarose Gel Electrophoresis of the PCR-Derived Amplicons of HC Germinal Layer of *E. granulosus* ITS1 Gene After Digestion with MSP1 Separated on 2% Agarose Gel and Stained With Ethidium Bromide Lanes: (M)100 Bp DNA Ladder(Consists of Repeats of 100 Bp Fragment Size, Fermintas), Lane 1Camel DNA & 2 Donkey DNA Containing 661 Bp.

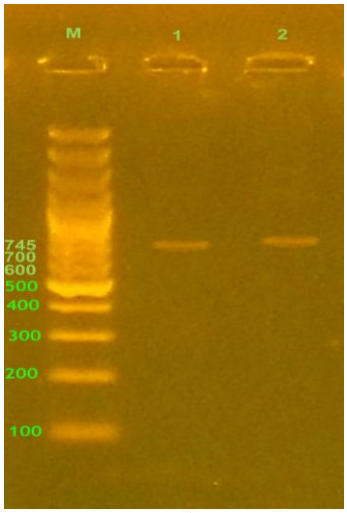

Fig. 2 (B): Agarose Gel Electrophoresis of the PCR-Derived Amplicons of HC Germinal Layer of *E. granulosus* ITS1 Gene After Digestion with RSP1 Separated on 2% Agarose Gel and Stained With Ethidium Bromide Lanes: (M) 100 Bp DNA Ladder (Consists of Repeats of 100 Bp Fragment Size, Fermintas), Lane 1Camel DNA & 2 Donkey DNA Containing 745 Bp.

Acknowledgment

The authors acknowledge Cairo Univ. vice President of postgraduates and research for funding this project. Deep acknowledges for veterinarians and workers of Cairo slaughter houses as well as the staff of Giza zoo for their appreciable help and their permission for the authors to examine the carcass of slaughtered donkeys in the zoo.

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