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RESEARCH ARTICLE

Genetic Fingerprint of Unilocular Hydatidosis in Egyptian Camels and Humans Using Nested PCR

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The pulmonary and hepatic hydatid cyst fluids were collected from 540 slaughtered camels and 5 human cases in Qalyubia Governorate, Egypt. The prevalence of infection of cystic echinococcosis among camels was 120 (22.2%). The fertility rates of the isolated cysts form camels and humans were 64.5 and 100%, respectively. A nested polymerase chain reaction was used for amplification of mitochondrial NADH 1 gene of *Echinococcus granulosus* complex in fertile cysts obtained from camels and humans, respectively. Two pairs of primers (EGL1 and EGR2) and (EGL3 and EGR4) were used in 2 amplification steps. First, the outer pair of primer originated from a highly conserve region of NADH1 gene generate a primary 435 bp PCR product. Second, a pair of internal (nested) primer (EGL3 and EGR4), designed to the annealing site of primers (EGL1 and EGR2) yield similar diagnostic amplified DNA bands of molecular size marker at 276 bp in all examined cysts obtained from camels and humans indicating a zoonotic relationship. This study confirms similar fingerprinting patterns of *Echinococcus granulosus* complex in camels and humans in Qalyubia Governorate, Egypt. Nested PCR for diagnosis of *E. granulosis* had been used for the first time in Egypt, as far as we know.

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INTRODUCTION

Unilocular hydatidosis or cystic echinococcosis (CE) is an important zoonotic disease caused by the larvae of *Echinococcus granulosus* and represents a major public health problem in many countries around the world including the Mediterranean zone (Pawlowski *et al.*, 2001; Hui *et al*., 2013). CE produces many health problems in livestock and human, with critical economic consequences due to the cost of medical treatment and morbidity for human cases and losses in animal productivities (Torgerson, 2003). Camels could participate with a vital part in the transmission cycle of the parasite and the epidemiology of the disease, especially in rural communities where dogs (definitive host) infected by ingestion of infected camel carcasses containing the hydatid cysts (Moro and Schantz, 2009). Humans are accidental intermediate host infected by ingestion of food or water contaminated with dog feces containing the infective eggs (Naseri-Moghddam *et al.*, 2011). Human hydatidosis is a symptomatic disease because of the slow growth of hydatid cysts, which typically diagnosed in patients using imaging technique as ultrasonography (Sako *et al*., 2002). After primary infection, clinical symptoms become evident after 10 years or more (Sako *et al.*, 2011), but early diagnosis and treatment are very crucial for lessening of morbidity and mortality (Sarkari *et al.*, 2007).

Ten genotypes of *E. granulosus*, EG are recognized worldwide and designated as G1-G10 (McManus *et al.*, 2003). Only 5 strains infect humans, such strains were sheep (G1), Tasmanian sheep (G2), cattle (G5), camel (G6), and pig (G7) strains (Bart *et al.*, 2006). Different molecular strategies and genetic targets have been applied for the identification of *E. granulosus*, examples of these include cox1 (M'Rad *et al.*, 2005), nad1 and cox1 (Abushhewa *et al.*, 2010), and 12sr RNA cox1 and nad1 (Omer *et al.*, 2010; Abdel Aaty *et al.*, 2012; Mogoye *et al.*, 2013). Moreover, NADH dehydrogenase 1 gene (nad1) is the most highly conserved nucleotide sequence among different genotypes of *EG*-complex (Bowles and McManus, 1993). The nested polymerase chain reaction (nPCR) is the highly sensitive and specific assay when compared to the traditional PCR (Osman *et al.*, 2009).

The aims of the present study were to determine the prevalence of hydatidosis, affected organs, and fertility rate of hydatid cysts in camels and humans. After that we used the nested polymerase chain reaction for revealing of mitochondrial NADH dehydrogenase1 (nad1) gene to investigate the DNA fingerprinting of isolates of cystic echinococcosis obtained from camels and humans.

MATERIALS AND METHODS

Survey and cyst collection

Abattoir survey: The study was carried out on 540 camels (5-15 years old) slaughtered at the official slaughterhouses of Toukh and Benha, Qalyubia Governorat, Egypt, during the period from October 2012 to September 2013, through examination of lung and liver of each slaughtered camel was carried out (Salih *et al.,* 2011).

Isolates from humans: Five hydatid cyst fluids from four livers and one lung (Naseri- Moghddam *et al.,* 2011) were recovered from humans (3 males and 2 females, 45-55 years old) admitted to Toukh's hospital and Benha insurance hospital, Qalyubia Governorate, Egypt. The infested samples were sent to the laboratory for further examination.

Parasitological examination: Cyst fluid was aspirated, with a sterile needle, from cysts of infected camels. The contents were examined under a microscope for the presence of protoscolices in the cyst. Cysts with protoscolices were considered fertile. Samples of protoscolices isolated from 25 cysts (20 and 5 camel and human cysts, respectively) were used for genetic characterization (Osman *et al.,* 2009), then stored at -20°C till used.

Genetic characterization

DNA extraction: Genomic DNA was extracted from protoscolices as well as form the camel strain G6 (positive control) via a commercially available QIAamp-tissue kit (Qiagen, Hilden, Germany). DNA extraction and purification were done in harmony with the manufacturer's instructions. Five micro liters of the suspended nucleic acid was run down in the PCR amplification.

DNA of the positive control: Reference *E. graulosus* camel strain (G6), isolated from camels, *Camelus dromedaries,* in Egypt and its GenBank accession number is HG764580 (www.blast.ncbi.nlm.nih.gov/Blast.cgi), was used as a positive control.

Primers selection and PCR amplification: For the first amplification step, a pair of outer primers (EGL 1 and EGR 2) of NADH dehydrogenase 1 gene of *E. granulosus* genotype 6 was used in PCR assay as defined by Bowles and McManus (1993) and Osman *et al.* (2009). Such pair of outer primer was designed for the separation of the primary *E. granulosus* specific PCR product. Primer EGL 1 included bases 32-53 of the positive sense strand (5)-TGA AGT TAG TAA TTA AGT TTAA. EGR2 included bases 447-466 of the complementary strand (5)-AAT CAA ATG GAG TAG GAT TA. Using primers such as EGL 1 and EGR 2 were expected to produce a 435 bp PCR product.

Regarding nPCR amplification, a pair of internal primers (EGL 3 and EGR 4) was designed from the previously mentioned gene sequence. EGL 3 included bases 162-181 of the positive sense strand (5)-TTA TAG TAT GCT TTC TGT GT. EGR 4 included bases 420-437 of the complementary strand (5) - AAC ACA CAC ACC AAG AAT. The specific primers were commercially supplied from (Sigma Scientific Services Co., Cairo, Egypt). A100-bp DNA was used as molecular size marker (Jena Bioscience Co. Jena, Germany).

Polymerase chain reaction: PCR amplification was prepared in 25 µl PCR tube holding 5 µl of target DNA sample, 1 µl of each primer EGL 1 and EGR 2 (100 pmol), and 12.5 µl PCR Master Mix (Jena Bioscience Co. Jena, Germany). Thermal profiles were performed on thermal cycler (T-Biometra) with standard conditions. Every PCR run included positive and negative controls. The positive result is indicative at 435bp.

Nested Polymerase Chain Reaction (nPCR): For the nPCR amplification, 2.0 µl of the primary PCR outcome produced by EGL 1 and EGR 2 were relocated to 25 µl PCR tube having 1 µl of each primer EGL 3 and EGR 4 (100 pmol), and 12.5 µl PCR Master Mix (Jena Bioscience Co. Jena, Germany).The nested pair of primers (EGL 3 and EGR 4) was anticipated to amplify a 276 bp PCR ampilicons, internal to the annealing sites of primers EGL 1 and EGR 2. Thermal profiles were performed on a Thermal cycler (T-Biometra). After amplification, 15 µl from each PCR product were loaded over the 1.5% agarose gel stained with ethidium bromide, and then the nPCR products were seen under UV light. Every PCR run included positive and negative controls.

RESULTS

Among 540 surveyed camels, 22.2% were infected with CE. Post mortem examination revealed that hydatid cysts infect 51.6% lungs and 27.5% livers. Moreover, mixed infections in lungs and livers, 20.83% were also recorded. Out of the 200 isolated cysts, 64.5% were fertile and the fertility rates of the pulmonary, hepatic, and mixed infections were 75.6, 44.2, and 50.0%, respectively. All human cysts were also fertile (Table 1).

The result of nPCR amplification of mitochondrial *nad1* gene of *EG*- complex in fertile unilocular hydatid cysts gained from infected camels and humans indicated that the outer pair of primers EGL 1 and EGR 2 produced a primary 435 bp PCR product (Fig. 1). The nested primers EGL 3 and EGR 4 induced amplification of a 276 bp amplified DNA bands in all examined (fertile) hydatid

Table 1: Prevalence of hydatosis in slaughtered camels

Affected Organ	Infected animals		No. of examined cysts	No. of Fertile cysts	
	No	%		No	%
Lung	62	51.7	123	93	75.6
Liver	33	27.5	43	19	44.2
Mixed*	25	20.83	34	17	50.0
Total	120	22.2	200	129	64.5

The study was carried out on 540 camels (5–15 years old)

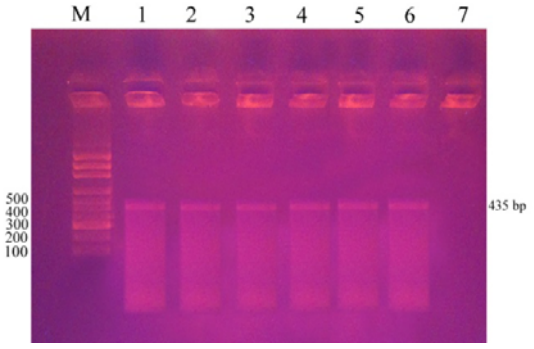

Fig. 1: PCR amplification of the 435bp products of DNA extracted from hydatid cyst isolate for detection of *E. granulosus*- complex using the outer pair primers (EGL 1 and EGR 2). Lane M: a 100bp molecular size marker. Lanes 1-3: hydatid cysts from camels, lanes 4 and 5: hydatid fluids from human patients, lane 6: positive control, and lane 7: negative control.

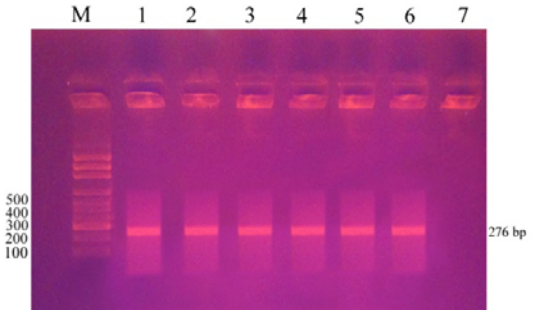

Fig. 2: Nested PCR of the 276 bp products of DNA extracted from hydatid cyst isolate for detection of *E. granulosus*-complex using internal pair of primers (EGL 3 and EGR 4). Lane M: a 100bp molecular size marker. Lanes 1-3: hydatid cysts from camels, lanes 4 and 5: hydatid fluids from human patients, lane 6: positive control, and lane 7: negative control.

cysts obtained from camels and humans, when compared to that of the defined pattern of the positive control DNA of the camel strain G6 *E. canadensis* (Fig. 2).

DISCUSSION

E. granulosus is medically and economically important cyclozoonoses. Our survey indicated that the prevalence of CE was 22.2%. Hydatid cysts have been reported in camels from almost all countries where camels are raised and our result was nearly similar to that detected in slaughtered camels in Algeria, 24.8% (Bardonnet *et al.,* 2003), Ethiopia, 22.6% (Salih *et al.*, 2011), and Uzbekistan, 25.4% (Torgerson *et al.,* 2006). On the other hand, our prevalence was higher than those previously recorded in several governorates in Egypt, 7.7% among camels in Assiut and Aswan (Dyab *et al.*, 2005), 2.5% in Mansora (Haridy *et al.*, 2006), and 16.25% in Qalyubia (Mahmoud, 2012); 3.6% in Libya (Kassem and Gdoura 2006); 6.5% in Tunisia (Lahmar *et al.*, 2004); 12% in Morocco (Azlaf and Dakkak 2006), and 17.5% in Pakistan (Latif *et al.*, 2010). Our results is lower than those reported in in Sudan, 59% and 29.7% (Omer *et al.*, 2010; Ibrahim *et al.*, 2011), Mauritania, 30.1% (Ould Ahmed Salem *et al.*, 2011), Kenya, 61.4% (Njoroge *et al.*, 2002), Saudi Arabia, 32.8% (Ibrahim 2010), Iran, 35.2% (Ahmadi, 2005) and Turkmenistan, 38% (Torgerson *et al.*, 2006).

Considering the slow progression of hydatosis, it would be expected that differences in prevalence rates are more likely owing to changes in the age structure of the livestock populations referred for slaughter, younger animals having typically lower infection rates than older animals (Ibrahim *et al.*, 2011; Salih *et al.*, 2011). The extent of the infection is also influenced by host- related factors, such as sex (Salih *et al.*, 2011), immunity, and presence of concomitant infections (Eckert and Deplazes, 2004). Moreover, other factor influence the prevalence of infection, such as stockbreeding conditions and mode of grassing (Erbeto *et al.*, 2010), environmental determinants (Ibrahim, 2010), or presence of large numbers of nomadic or semi-nomadic camel flocks, and their close contact with dogs, providing the transmission of infection to humans.

Our data indicated that the rate of infection in lungs (51.7%) was higher than that of the liver (27.5%). Our observation is in accordance with those noticed in camels in several African countries, such as Mauritania (Bardonnet *et al.*, 2003), Sudan (Ibrahim *et al.*, 2011), Ethiopia (Salih *et al.*, 2011), and Egypt (Haridy *et al.*, 2006; Mahmoud, 2012). In human cases, hydatid cysts developed mainly in the liver followed by lung. Similar finding was recorded (Pawlowski *et al.*, 2001; Manterola *et al.*, 2008). The fertility of cyst is one of the most important factors in the epidemiology of *E. granulosus*. The fertility rate of hydatid cyst, in the present work, was 64.5% which was higher than that reported in Ethiopia, 50% (Salih *et al.*, 2011) and lower than that recorded in Sudan, 85.4% (Ibrahim *et al.*, 2011). The fertility of cysts varies depending on the hosts and geographical situations (Bardonnet *et al.*, 2003; Ibrahim *et al.*, 2011; Salih *et al.*, 2011). The fertility rates were higher in lungs (75.6%) than those in the liver (44.2%). Our results are in harmony with those reported by other researchers in Egypt (Haridy *et al.*, 2006), Sudan (Ibrahim *et al.*, 2011), and Ethiopia (Salih *et al.*, 2011). Regarding the high fertility rate of cysts in camels, it is indicated that camels in Egypt act as an intermediate host that could maintain transmission of the disease. Camels slaughtered at home without inspection will easily pass on the parasites to dogs and consequently perpetuate the disease in the population. Moreover, all human cysts, used in the present work, were fertile. Similar observation was reported by Mogoye *et al.* (2013).

Molecular genetic study has been carried out to identify nad1 gene for identification of *E. granulosus* (*Eg*) complex for hydatid cyst of infected camels and humans. The outer pair of primers EGL1 and EGR2 generated a primary 435 bp amplified products and the nested primers of EGL3 and EGR4 yield diagnostic amplified DNA bands of molecular size marker at 276 bp. The use of nPCR in the present work revealed absence of variation in amplified DNA in all fertile cysts from camels and humans and having high similarity with DNA fingerprinting of *E. granulosus* G6 positive control indicating a zoonotic association (Azab *et al.*, 2004 and Abdel Aaty *et al.*, 2012) in Egypt. Our results are in harmony with that of Osman *et al.*, (2009) as they indicated that the second amplification step using the nested primer EGL 3 and EGR 4 confirmed the specificity of *EG* complex primary PCR product and increased the

sensitivity of the PCR based assay at least 100 folds. In Egypt, previous molecular representation of human and animal *E. granulosus* was studied through RAPD-PCR (Azab *et al.,* 2004), semi nested PCR, and PCR-RFL (Abdel Aaty *et al.,* 2012; Tawfeek *et al.,* 2009). Consequently, this study is the first work used nested PCR in diagnosis of *E. granulosis* in Egypt, to the best of our knowledge.

Conclusion: All examined fertile cysts, in the present study, from camels and humans yield diagnostic amplified DNA fingerprinting of the mitochondrial marker nad1 gene of the *E. granulosus* species. From a public health and veterinary standpoints, our finding about hydatosis leading to great economic concerns in Egypt and an integrated approach for controlling CE should fulfill actions accommodated to improve public health education and controlling home-slaughter habits, meat inspection services, appropriate disposal of infected viscera and dead animals, dog management, such as treatment and elimination of stray dogs. Consequently, application of standardized recording and reporting systems for livestock CE is highly crucial in Egypt.

Further studies: Further studies are needed to recognize new molecular investigations at the intra-specific level, as well as viability/infectivity studies in infected intermediate hosts. Such studies could help to elucidate the host range and transmission patterns that sustain CE in a given region. More research is required to determine genotypes of *E. granulosus* in humans and animals in Egypt. Further evaluation of the current situation, especially in dogs, humans, and livestock will improve our understanding on the epidemiology of the disease in Egypt and provide the background for the design of cost-efficient control strategies.

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REFERENCES

Abdel Aaty HE, DM Abdel-Hameed, YH Alam-Eldin, SF El-Shenawy, HA Aminou, SS Makled and SK Darweesh, 2012. Molecular genotyping of *Echinococcus granulosus* in animal and human isolates from Egypt. Acta Trop, 121: 125-128.

Abushhewa MH, MH Abushhiwa, MJ Nolan, AR Jex, BE Campbell, A Jabbar and RB Gasser, 2010. Genetic classification of *Echinococcus granulosus* cysts from humans, cattle and camels in Libya using mutation scanning-based analysis of mitochondrial loci. Mol Cell Probes, 24: 346-351.

Ahmadi NA, 2005. Hydatidosis in camels (*Camelus dromedarius*) and their potential role in the epidemiology of *Echinococcus granulosus* in Iran. J Helminthol, 79: 119-125.

Azab ME, SA Bishara, H Helmy, NM Oteifa, LM El-Hoseiny, RM Ramzy and MA Ahmed, 2004. Molecular characterization of Egyptian human and animal *Echinococcus granulosus* isolates by RAPD- PCR technique. J Egypt Soc Parasitol, 34: 83-96.

Azlaf R and A Dakkak, 2006. Epidemiological study of the cystic echinococcosis in Morocco. Vet Parasitol, 137: 83-93.

Bardonnet K, MC Benchikh-Elfegoun, JM Bart, S Harraga, N Hannache, S Haddad, H Dumon, DA Vuitton and R Piarroux, 2003. Cystic echinococcosis in Algeria: cattle act as reservoirs of a sheep strain and may contribute to human contamination. Vet Parasitol, 116: 35-44.

Bart JM, M Abdukader, YL Zhang, RY Lin, YH Wang, M Nakao, A Ito, PS Craig, R Piarroux, DA Vuitton and H Wen, 2006. Genotyping of human cystic echinococcosis in Xinjiang, PR China. Parasitology, 133: 571-579.

Bowles J and DP McManus, 1993. NADH dehydrogenase 1 gene sequences compared for species and strains of the genus *Echinococcus.* Int J Parasitol, 23, 969-972.

Dyab KA, R Hassanein, AA Hussein, SE Metwally and HM Gaad, 2005. Hydatidosis among man and animals in Assiut and Aswan Governorates. J Egypt Soc Parasitol, 35: 157-166.

Eckert J and P Deplazes, 2004. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clinic Microbiol Reviews, 17: 107-135.

Erbeto K, G Zewde and B Kumsa, 2010. Hydatidosis of sheep and goats slaughtered at Addis Ababa Abattoir: prevalence and risk factors. Trop Anim Health Prod, 42: 803-805.

Haridy FM, BB Ibrahim, AM Elshazly, SE Awad, DM Sultan, GT El-Sherbini and TA Morsy, 2006. Hydatidosis granulosus in Egyptian slaughtered animals in the years 2000-2005. J Egypt Soc Parasitol, 36: 1087-1100.

Hui W, M Batur, X Du, S Ma, J Ma and B Jia, 2013. Early antibody, cytokine and chemokine responses during *Echinococcus granulosus* infection in Kazakh sheep with cystic echinococcosis resistance haplotype. Pak Vet J, 33: 205-208.

Ibrahim K, R Thomas, K Peter and RA Omer, 2011. A molecular survey on cystic echinococcosis in Sinnar area, Blue Nile state (Sudan). Chinese Med J (Engl), 124: 2829-2833.

Ibrahim MM, 2010. Study of cystic echinococcosis in slaughtered animals in Al Baha region, Saudi Arabia: interaction between some biotic and abiotic factors. Acta Trop, 113: 26-33.

Kassem HH and NK Gdoura, 2006. Hydatidosis in camel (*Camelus dromedarius*) slaughtered at Sirt abattoir, Libya. J Egypt Soc Parasitol, 36: 1-10.

Lahmar S, H Debbek, LH Zhang, DP McManus, A Souissi, S Chelly and PR Torgerson, 2004. Transmission dynamics of the *Echinococcus granulosus* sheep-dog strain (G1 genotype) in camels in Tunisia. Vet Parasitol, 121: 151-156.

Latif AA, A Tanveer, A Maqbool, N Siddiqi, M Kyaw-Tanner and RJ Traub, 2010. Morphological and molecular characterisation of *Echinococcus granulosus* in livestock and humans in Punjab, Pakistan. Vet Parasitol, 170: 44-49.

M'Rad S, D Filisetti, M Oudni, M Mekki, M Belguith, A Nouri, T Sayadi, S Lahmar, E Candolfi, R Azaiez, H Mezhoud and H Babba, 2005. Molecular evidence of ovine (G1) and camel (G6) strains of *Echinococcus granulosus* in Tunisia and putative role of cattle in human contamination. Vet Parasitol, 129: 267-272.

Mahmoud SM, 2012. Study of some zoonotic parasites in slaughtered animals abattoirs in Kalyobia province. MVSc Fac Vet Med, Benha Univ, Egypt.

Manterola C, F Benavente, A Melo, M Vial and JC Roa, 2008. Description of *Echinococcus granulosus* genotypes in human hydatidosis in a region of southern Chile. Parasitol Int, 57: 342-346.

McManus DP, W Zhang, J Li and PB Bartley, 2003. Echinococcosis. Lancet, 362: 1295-1304.

Mogoye BK, CN Menezes, ML Wong, S Stacey, D von Delft, K Wahlers, M Wassermann, T Romig, P Kern, MP Grobusch and J Frean, 2013. First insights into species and genotypes of Echinococcus in South Africa. Vet Parasitol, 196: 427-432.

Moro P and PM Schantz, 2009. Echinococcosis: a review. Int J Infec Dis, 13: 125-133.

Naseri-Moghddam S, A Abrishami, A Taefi and R Malekzadeh, 2011. Percutaneous needle aspiration injection and re-aspiration with or without benzimidazole coverage for uncomplicated hepatic hydatid cysts. Cochrane Database, Syst Rev, pp: CD003623.

Njoroge EM, PM Mbithi, JM Gathuma, TM Wachira, PB Gathura, JK Magambo and E Zeyhle, 2002. A study of cystic echinococcosis in slaughter animals in three selected areas of northern Turkana, Kenya. Vet Parasitol, 104: 85-91.

Omer RA, A Dinkle, T Romig, U Mackenstedt, AA Elnahas, IE Aradaib, ME Ahmed, KH Elmalik and A Adam, 2010. Molecular survey of cystic echinococcosis in Sudan. Vet Parasitol, 169: 340-346.

Osman AM, IE Aradaib, AK Ashmaig and AA Gameel, 2009. Detection and differentiation of *Echinococcus granulosus*- complex using a simple PCR-based assay. Int J Trop Med, 4: 21-26.

Ould Ahmed Salem CB, F Schneegans, JY Chollet and MH et J, 2011. Epidemiological studies on echinococcosis and characterization of human and livestock hydatid cysts in Mauritania. Iran J Parasitol, 6: 49-57.

Pawlowski ZS, J Eckert, DA Vuitton, RW Ammann, P Kern, PS Craig, KF Dar, F De Rosa, C Filice, B Gottstein, F Grimm, CNL Macpherson, N Sato, T Todorov, J Uchino, W von Sinner and H Wen, 2001. Echinococcosis in humans: clinical aspects, diagnosis and treatment. In: WHO/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern (Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS, eds), Organisation Internationale des Epizooties, Paris, pp: 20-71 (Chapter 2).

Sako Y, D Tappe, K Fukuda, Y Kobayashi and A Ito, 2011. Immunochromatographic test with recombinant Em18 antigen for the follow- up study of alveolar echinococcosis. Clinic Vac Immunol, 18: 1302-1305.

Sako Y, M Nakao, K Nakaya, H Yamasaki, B Gottstein and A Ito, 2002. Alveolar echinococcosis: characterization of diagnostic antigen Em18 and serological evaluation of recombinant Em18. J Clinic Microbiol, 40: 2760-2765.

Salih M, H Degefu and M Yohannes, 2011. Infection rates, cyst fertility and larval viability of hydatid dsease in camels (*Camelus dromedarius*) from Borena, Kereyu and Harar aeas of Ethiopia. Global Vet, 7: 518-522.

Sarkari B, SM Sadjjadi, H Abidi and A Rafati, 2007. Application of western blotting using native antigen B for serodiagnosis of human cystic echinococcosis. Iran J Parasitol, 2: 7-12.

Tawfeek GM, HS Elwakil, NS Awad, M Sarhan, SK Darweesh and WA Anwar, 2009. Genetic variability of antigen B among *Echinococcus granulosus* Egyptian isolates. Korean J Parasitol, 47: 259-264.

Torgerson PR, 2003. Economic effects of echinococcosis. Acta Trop, 85: 113-118.

Torgerson PR, B Oguljahan, AE Muminov, RR Karaeva, OT Kuttubaev, M Aminjanov and B Shaikenov, 2006. Present situation of cystic echinococcosis in Central Asia. Parasitol Int 55 (Suppl): S207-S212.