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Short communication

Molecular characterization of *Echinococcus granulosus* in Egyptian donkeys

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ABSTRACT

The present study was carried out during the period from August 2009 to July 2010. The purpose of this study was to identify the genotype of donkeys' echinococcosis in one of the Egyptian governorates. On post-mortem inspection taking place at the zoo of Beni-Suef, Egypt, a total number of 145 donkeys were examined for the presence of hydatid cysts. Ten of these donkeys were found to be infected by hydatid cysts; and location, number and fertility of cysts found were determined. The liver was the predominant site of infection. Molecular identification of these cystic echinococcosis isolates, based on PCR amplification and the sequence of both mitochondrial ND1 and CO1, revealed that they belonged to *Echinococcus equinus* (G4 genotype). An alignment of ND1 and CO1 partial nucleotide sequences with G4 partial nucleotide sequences revealed replacement of G at position 105 with A and replacement of A at position 276 with G respectively. It can be concluded that the donkeys involved in this study were harboring *E. equinus.* For the first time in Egypt, the present work allowed us to record the presence of the *E. equinus* with the molecular tools, and to report new information on the epidemiological status of this parasite in Egypt.
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1. Introduction

Hydatid disease is a zoonotic disease known to spread worldwide. It is caused by larval stages (metacestodes) of tape worm parasite of genus *Echinococcus* that infect different animal species (Rausch, 1995). These domestic animals include sheep, goats, cattle, swines, buffalos, horses, and camels (Bryan and Schantz, 1989). Human beings may also serve as dead-end hosts (Binhazim et al., 1992). *E. granulosus sensu lato* shows intraspecific variation in relation to host, specificity, epidemiology, morphology, developmental biology, biochemistry and genetics (Thompson and McManus, 2002). Based on the genetic characterization which is mostly dependent on the homology of the sequence of the two mitochondrial genes; cytochrome C oxidase subunit 1 (CO1) and reduced nicotinamide adenine dinucleotide subunit 1 (ND1), ten different genotypes, among which G4 (horse strain) have been formerly characterized (McManus, 2002; Lavikainen et al., 2003; Snábel et al., 2009). Recent taxonomic classification suggested the presence of five valid species within the *E. granulosus* species complex. These are named *E. granulosus* s.s. (G1–G3 genotypes), *E. equinus* (G4 genotype), *E. ortleppi* (G5 genotype) and *E. canadensis* (G6–G10 genotypes) and *E. felidis* (Thompson and McManus, 2002; Nakao et al., 2010; Hüttner et al., 2008).

Equine hydatidosis, especially that of donkeys, has been studied in some countries of The Middle East; Lebanon and Jordan (Thompson and McManus, 2002). *E. equinus*

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has been reported from the United Kingdom, Switzerland, Belgium, Italy, Syria and South Africa (Thompson and Lymbery, 1990; Varcasia et al., 2008).

E. equinus (formerly *E. granulosus* G4 or horse strain) appears to be a specific parasite of equids (Jenkins et al., 2005; Nakao et al., 2007; Romig et al., 2006; Thompson, 2008; Saarma et al., 2009; Blutke et al., 2010). Varcasia et al. (2008) identified two species of genus *Echinococcus* infecting horses in Italy; *E. granulosus* s.s. (old G1, sheep strain) and *E. equinus* (old G4 or horse strain). Scant information about this parasite in donkeys is available (Thompson and McManus, 2002). A study in Giza, Egypt, showed the presence of unspecified *Echinococcus* sp. in donkeys (Haridy et al., 2008). However, one of the most recent studies on RFLP patterns of CE isolates from donkeys in Egypt failed to allocate the isolates to any described strain or species (Taha, 2012). Our present work aimed to identify the genotypes of hydatid cysts isolated from Egyptian donkeys, and this was planned to be done by sequencing their PCR-amplified two mitochondrial genes; cytochrome C oxidase subunit 1 (CO1) and NADH dehydrogenase subunit 1 (ND1).

2. Materials and methods

2.1. Parasite samples

This study included 145 donkeys (over five years of age) of local origin. They were raised in different cities of Beni-Suef governorate. Beni-Suef, an Egyptian governorate, located 120 km south of Cairo. The donkeys were brought to Beni-Suef zoo for feeding lions. They were examined for the presence of hydatid cysts on post-mortem inspection at the zoo during the period from August 2009 to July 2010. Examination of all internal organs was also done by using palpation and incision for the detection of hydatid cysts. Intact hydatid cysts, isolated from the infected animals, were put separately in the polythene bags containing ice and brought to Beni-Suef Veterinary College for further processing.

Hydatid fluid was aspirated after washing the cyst with distilled water twice. It was further subjected to centrifugation at 5000 rpm for 5 min, and the sediment was examined under the low power objective of a compound microscope to observe the protoscoleces. Germinal layer (sterile cysts) and protoscoleces (fertile cysts) were randomly collected from the infected animals, and only one cyst from each infected animal was subjected to molecular characterization to assign the status of a single isolate. The material was frozen at −20 °C until further use.

2.2. DNA extraction

DNA was extracted from the germinal layers and/or protoscoleces using the GeneiUltrapure™ Mammalian Genomic DNA Purification Tissue Kit (Bangalore Genei); and according to manufacturer's instructions, the eluted DNA samples were subjected to dryness by putting them in oven at 55 °C for 3 h. The eluted DNA samples were transferred to Japan by air to perform the PCR amplification and DNA sequencing.

2.3. PCR amplification

Two target sequences of the mitochondrial DNA coding for CO1 and ND1 were PCR-amplified by using Takara Ex Taq kit (Takara Biomedicals, Otsu, Japan).

Two conserved primers, JB3 (forward): 5′-TTT TTT GGG CAT CCT GAG GTT TAT-3′ and JB4.5 (reverse): 5′-TAA AGA AAG AAC ATA ATG AAA ATG-3′ (Busi et al., 2007), were used to amplify the mtDNA region corresponding to the part of the CO1 gene.

Two conserved primers, JB11 (forward): 5′-AGA TTC GTA AGG GGC CTA ATA-3′ and JB12 (reverse) 5′-ACC ACT AAC TAA TTC ACT TTC-3′ (Bowles and McManus, 1993; Bowles et al., 1994), were used to amplify the mtDNA region corresponding to the ND1 gene.

The amplification reactions were carried out in a PCR thermal cycler Dice (Takara, Japan); and it was stained with ethidium bromide and photographed. The corresponding amplicons were checked on 1% agarose gel in a Mupid-2× (Advance, Japan).

2.4. DNA sequences analysis

The amplified PCR-products were cut from agarose gels and purified by using GeneiPure™ Quick PCR Purification Kit (Bangalore Genei) according to manufacturer's instructions. The purified PCR products were subjected to automatic DNA sequencing using Rikakan DNA sequencer (Rikakan, Japan). The DNA sequencing was done in both directions by using the forward and the reverse primers for both CO1 and ND1 mitochondrial genes. Nucleotide sequences were first analyzed and edited to check the electropherograms quality by using the software program Finch TV v 1.4.0 (Geospira Inc.©). Using the Basic Local Alignment *Search* Tool "BLAST" search blast.ncbi.nlm.nih.gov, the obtained DNA sequences were subsequently aligned and compared with verified sequences of *E. granulosus* strains available in the Genbank (Table 1). The phylogenetic tree was constructed by using software program "Mega 4". *Taenia saginata* (NC 009938) was used as an outgroup. Bootstrap analyses were conducted using 1000 replicates. Sequences analyzed in the present study were finally deposited in the Genbank and their accession numbers are listed in Table 1.

3. Results

Out of one hundred and forty-five donkeys slaughtered at Beni-Suef zoo for feeding lions, ten animals were found to be infected with hydatid cysts. The infection rate was 6.89%. The hydatid cysts were present only in the livers of the infected animals or in both livers and lungs. Livers showed cysts in all infected cases, while only three of them harbored cysts in their lungs. In the livers, the number of cysts was ten or more in six cases, 8 in two cases, 5 in one case and 4 cysts in the last one. In the lungs, the number of cysts was sharply lower than in livers; as they showed 1 or 2 cysts in each of the infected lungs. The cysts found in the livers and lungs were divided into three types; fertile, sterile and caseous. The fertility of the cysts was determined by the presence of protoscoleces. In the livers, eight cases

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