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Molecular characterization of *Cysticercus tenuicollis* of slaughtered livestock in Upper Egypt governorates

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ABSTRACT

Objective: To present the molecular characterization of *Cysticercus tenuicollis* (*C. tenuicollis*) of *Taenia hydatigena* (*T. hydatigena*) from livestock isolates in Egypt, and to introduce a detailed image of *C. tenuicollis* infection in ruminant animals in Upper Egypt.
Methods: The prevalence rates of *C. tenuicollis* infections among the slaughtered animals from different organs were determined using the amplification of sequencing of the *MT-CO1* gene.
Results: In the present study the infection rates of *C. tenuicollis* were found to be 16% and 19% in sheep and goat samples respectively. Firstly we report one larval stage of *T. hydatigena* detected in the camel liver in Egypt. *C. tenuicollis* infection manifested a higher prevalence in females than in males. Those above two years of age manifested a higher infection rate than younger animals. The preferred site for the infection was the omentum: a 70% preference in sheep and a 68% preference in goats. The molecular characterization using the *MT-CO1* gene of isolates from sheep, goats and camels corresponded to *T. hydatigena*. For this study, molecular characterizations of *T. hydatigena* were done for the first time in Egypt. Molecular tools are of great assistance in characterizing the *C. tenuicollis* parasite especially when the morphological character cannot be detected, because the metacestodes are frequently confused with infection by the hydatid cyst, especially when these occur in the visceral organs. In the present study, *C. tenuicollis* manifested high identity in the goat and sheep samples, while differences were found more frequently in the camel samples (10 base pair).
Conclusions: Clearly molecular diagnosis for *C. tenuicollis* infection significantly helps to differentiate it from such other metacestodes as hydatidosis, which manifests a completely different pathogenicity and requires different control programs.

1. Introduction

Cysticercus tenuicollis (*C. tenuicollis*) is the larval stage of the taeniid cestodes *Taenia hydatigena* (*T. hydatigena*), which has a large footprint both in veterinary science research and in the agricultural and animal husbandry economy. The adult worms are found in the small intestines of canines such as dogs and foxes, while the metacestodes are found in a large number of domestic and wild intermediate ruminant hosts [1,2].

The intermediate host becomes infected through the ingestion of tapeworm eggs in the faeces of dogs in the pasture [3,4]. The *C. tenuicollis* is most often found attached to the omentum, to the mesentery and to the organ surface. Bladder

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worms are often detected in older animals, and in larger numbers [3,5]. Infections may be so severe that they kill the host, but generally infection is mild and localized, with damage confined to the liver, and of little significance to the overall health of the host [6].

Diagnosis of cysticercosis in ruminants is based on morphological and molecular characterizations, the important characteristics for morphological identification being the number and lengths of the large and small hooks, the number of uterine branches and the general structure of the adult [3,7,8]. The molecular tools, including the sequence data of mtDNA genes (*ND1* and *CO1*), are available on GenBank [9].

The aim of this study was to present the molecular characterization of *C. tenuicollis* of *T. hydatigena* from livestock isolates in Egypt, using the amplification of the *MT-CO1* gene sequencing.

2. Materials and methods

2.1. Study area

The animal isolates were collected over the period of January to December 2013 from abattoirs of the Qena, Sohag and Aswan governates of Upper Egypt.

2.2. Prevalence study

Animal isolates consist of 500 sheep, 350 goats and 103 camels, with samples detected from different organs such as the liver, lung, urinary bladder, mesentery and omentum.

2.3. DNA extraction

DNA extraction was performed using a DNA mini kit that was supplied by Qiagen, Germany. The manufacturer protocol for DNA extraction was used for the *C. tenuicollis* samples.

2.4. PCR study

This PCR study was carried out to amplify the 340 bp fragment that corresponds to the mitochondrial *CO1* gene. The primers used are according to Bowles *et al.* [10]. Briefly, 25 μL reaction mixture consisted of 12.88 μL DNase distilled water, 2.5 μL 10× PCR buffer, and 2.5 μL 25 mmol/L $MgCl_2$, 2 μL 1 mmol/L deoxynucleoside triphosphate mixture, 1.25 μL of each primer, 2.5 μL of target DNA and 0.125 μL of TagDNA polymerase. PCR was performed by adding one cycle at 95 °C for 5 min before the 35 cycles of 1 min at 95 °C, 1 min at 50 °C and 1 min at 72 °C, and finally 5 min at 72 °C. Amplification producers were resolved on a 1.5% ethidium bromide stained agarose gel, visualized and photographed on the UV trans illuminator.

The sequences obtained were edited and aligned using MEGA 5.5 software, and were undertaken by BLAST algorithms and databases from the National Center for Biotechnology.

3. Results

3.1. Prevalence and organ distribution of C. tenuicollis

The overall prevalence of *C. tenuicollis* found in slaughtered sheep, goats and camels was 80 (16%), 67 (19%) and 1 (1%) respectively (Table 1). In addition, there were highly significant differences ($P < 0.01$) in infection rates among the infected animal groups. Based on age and sex, there were significant differences in infection rates ($P < 0.01$) among sheep, while among goats the differences were non-significant ($P > 0.05$). Seasonal variation was a non-significant factor ($P > 0.05$) in the infection rates of sheep and goats. Older sheep and goats (> 2 years) had a greater rate of infection than juveniles (< 2 years). In this study, the predominant predilection site for the *C. tenuicollis* cyst was found to be the omentum in sheep (70%) and goats (69%). The study found that the statistical differences were high among various organs (Table 2) in sheep ($P < 0.01$). The detailed data for the infection rate and the organ distribution of the *C. tenuicollis* cyst are shown in Tables 1 and 2.

Table 1
Prevalence rate of *C. tenuicollis* in slaughtered sheep and goats.

Characteristic	Animal type	Infected number [*n* (%)]	*P* value
Overall	Sheep	80 (16%)	0.001**
	Goats	67 (19%)	
Male	Sheep	7 (9%)	
	Goats	13 (18%)	
Female	Sheep	14 (17%)	0.001**
	Goats	14 (21%)	0.654
> 2 years	Sheep	15 (18%)	
	Goats	15 (22%)	
< 2 years	Sheep	9 (11%)	0.001**
	Goats	9 (13%)	0.943

**High significant $P < 0.01$.

3.2. Molecular characterization of C. tenuicollis

The PCR amplification was successful on all isolates for the *CO1* gene. The amplified fragment size was approximately 410 bp. Partial sequences of *MT-CO1* from camels, goats and sheep respectively (GenBank accession numbers: KP641175, KP641176 and KP64117) corresponded to *T. hydatigena*. After the alignment of finding sequences with these sequences (AB792722.1; JQ710601.1) from GeneBank, the 99% identity with the camel sequences and 100% with those from sheep were found.

Table 2
Organ distribution of *C. tenuicollis* in sheep and goats examined *n* (%).

Animal	Liver	Lung	Urinary bladder	Diaphragm	Mesentery	Omentum	*P* value
Sheep	7 (9%)	0	2 (3%)	1 (2%)	9 (11%)	56 (70%)	0.001**
Goats	7 (11%)	0	0	0	8 (12%)	46 (69%)	0.015*

**: High significant $P < 0.01$; *: Significant $P < 0.05$.

In the exact same manner, nucleotide sequence variation of the *CO1* gene was compared with the existing genotypes, on the basis of 396 nucleotides. The overall mean distances of the study sequences with sequences from GenBank are 0.009. It may be noted that the pairwise illustrates the low distances between the sequences from camels and goats, compared with the sequences from GeneBank, as is shown in Table 3.

Table 3
Pairwise distances among the sample and GeneBank sequences.

	THC	THG	THS	AB792722.1	JQ710601.1
THC					
THG	0.050				
THS	0.010	0.010			
AB792722.1	0.050	0.000	0.010		
JQ710601.1	0.016	0.010	0.010	0.010	

THC: From camel; THG: From goat; THS: From sheep.

Accordingly, phylogenetic analysis was conducted on CO1 sequence data to elucidate the similarities and differences of the *T. hydatigena* genotypes. The phylograms based on the CO1, found that *T. hydatigena* haplotype goat (THG = KP641176) was the closest taxon to gi116270720 from India and gi532690879 from Mongolia. The THS = KP64117 from sheep was the closest taxon to the gi390195495 from Iran. *T. hydatigena* haplotype camel (THC = KP641175) forms one distinct clade of its own.

4. Discussion

The present study of the prevalence of *C. tenuicollis* in ruminant groups found the infection rate to be the highest in goats (19%), compared with sheep (16%), and detected one case in camels, which we record here for the first time. Biu and Murtala, reported a mean prevalence of *C. tenuicollis* in sheep (71.6%) and in goats (71.9%) in Nigeria, which is higher than that found in the present study [11]. Moreover, Utuk and Piskin found a 65.6% prevalence rate in sheep and 61.6% in goats in Turkey [12]. In this study, we report the incidence of infection in sheep and in goats as well as one incident of infection in the liver of a camel. Sheep and goats are the most slaughtered animals in abattoirs in Egypt.

Previous studies of *C. tenuicollis* in different regions focus on the prevalence and morphological diagnosis of this disease [11,13,14]. In this study, we used molecular genotyping to refine the understanding of the genealogy of this parasite and what distinctive characteristics distinguish it from others in the field of ruminant pests *etc*. the present study shows *inter alia* that the incidence of infection by this parasite is higher among females than males in the goat and sheep populations. These findings correspond with the study in Nigeria [11]. However our study finds high statistical differences between sheep, contingent on age and sex, but no statistical differences between goats. In contrast, Senlik [4] and Ghaffar [13] found the prevalence rate in males higher than that in females. In this study, the prevalence rate in older animals (sheep and goats) above 2 years was higher than in young animals, a finding that corresponds with previous results [4,5,11]. The infection rate increased significantly ($P < 0.01$) with age in sheep, but non-significantly in goats.

This study found that the dominant preferred site of *C. tenuicollis* infection was the omentum (70%, 69%) in sheep and goats respectively. This finding was similar to the previous findings [6,11,15], in which *C. tenuicollis* in both sheep and goats were prevalently shown on the omentum.

To date, molecular genotyping has successfully distinguished between hydatid cysts and cysticercus features of *C. tenuicollis*. Molecular genotyping does this based on the pairwise distances. The isolates from sheep and goats were compared with sequences from the GenBank. The findings from Iran by Rostami were used to determine the pairwise nucleotide variation between sheep isolates [16]. Significantly, we found *C. tenuicollis* for the first time in camels in Egypt and this was confirmed by molecular levels. In this study, the phylogenetic analysis showed *T. hydatigena* for the first time appearing in the isolates from sheep and goats in the same clade. From previous studies, we know that there is little genetic information now available about the ubiquity of this parasite in ruminant populations [13,16]. Further phylogenetic analysis studies should be carried out in final and intermediate hosts at molecular levels.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Guralp N. [*Helmintology*]. 2nd ed. Ankara: Ankara University Press; 1981. Turkish.
[2] Kaufmann J. *Parasitic infections of domestic animals. A diagnostic manual*. Switzerland: Birkhauser Verlag Basel; 1996.
[3] Kassai T. *Veterinary helminthology*. Oxford: Butterworth-Heineman Publishing; 1999.
[4] Senlik B. Influence of host breed, sex and age on the prevalence and intensity of *Cysticercus tenuicollis* in sheep. *J Anim Vet Adv* 2008; **7**(5): 548-51.
[5] Rao BT, Prasad PVV, Hafeez MD. Prevalence of *Cysticercus tenuicollis* infection in slaughtered sheep and goats at Kakinada, Andhra Pradesh. *J Parasit Dis* 2003; **27**(2): 126-7.
[6] Schineider T. [*Veterinary parasitology*]. 6th ed. Berlin: Parey im MVS; 2006, p. 166-234. German.
[7] McManus DP. Molecular discrimination of taeniid cestodes. *Parasitol Int* 2006; **55**: S31-7.
[8] González LM, Villalobos N, Montero E, Morales J, Sanz RA, Muro A, et al. Differential molecular identification of *Taeniid* spp. and *Sarcocystis* spp. cysts isolated from infected pigs and cattle. *Vet Parasitol* 2006; **142**(1–2): 95-101.
[9] Jia WZ, Yan HB, Guo AJ, Zhu XQ, Wang YC, Shi WG, et al. Complete mitochondrial genomes of *Taenia multiceps, T. hydatigena* and *T. pisiformis* additional molecular markers for a tapeworm genus of human and animal health significance. *BMC Genomics* 2010; **11**(1): 447.
[10] Bowles J, Blair D, McManus DP. Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. *Mol Biochem Parasitol* 1992; **54**(2): 165-73.
[11] Biu AA, Murtala S. Studies on *Cysticercus tenuicollis* infection in slaughtered sheep and goats in Maiduguri, Nigeria. *Cont J Vet S* 2012; **6**(1): 14-8.
[12] Utuk AE, Piskin FC. Molecular detection and characterization of goat isolate of *Taenia hydatigena* in Turkey. *ScientificWorldJournal* 2012; **2012**: 962732.
[13] Ghaffar NM. Tenuicollosis in slaughtered sheep at Duhok abattoir – Kurdistan region of Iraq. *Basrah J Vet Res* 2011; **10**: 1-16.
[14] Yildirm A, Içа A, Beyaz I, Atasaver A. [Acute hepatitis cysticercosa and pneumonitis cysticercosa in a lamb: case report]. *Turk Parazitol Derg* 2006; **30**(2): 108-11. Turkish.
[15] Samuel W, Zewde GG. Prevalence, risk factors and distribution of *Cysticercus tenuicollis* in l organs of slaughtered sheep and goats in central Ethiopia. *Trop Anim Health Prod* 2010; **42**(6): 1049-51.
[16] Rostami S, Salavati R, Beech RN, Babaei Z, Sharbatkhori M, Baneshi MR, et al. Molecular and morphological characterization of the tapeworm *Taenia hydatigena* (Pallas, 1766) in sheep from Iran. *J Helminthol* 2015; **89**(2): 150-7.