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Molecular Identification of Both Adult Worm and Larval Stage of *Taenia hydatigena*

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ABSTRACT

The adult worm *Taenia hydatigena* is a tapeworm with definitive hosts, such as canines. In contrast, the larval stage of *T. hydatigena*, known as the *Cysticercus tenuicollis* cyst, is found mainly in small and large ruminant animals and acts as an intermediate host. However, misdiagnosis and cross-reaction with other taeniasis species have prompted the use of molecular identification tactics to accurately diagnose both the metacestode (*C. tenuicollis* cysts) stage and the adult worm. This study addresses these concerns, which have not been previously studied at the molecular level in Egypt. *C. tenuicollis* cysts were collected from the general Matrouh governorate abattoir in Egypt, and adult worms were obtained from an experiment on native Egyptian dogs. The samples were molecularly identified using 12S and 18S rDNA sequences, and a phylogenetic tree was built to identify their relatives in GenBank. The study's results showed that the 18S rDNA gene sequence of the adult worm was approximately 480 bp, similar to *T. hydatigena* with the GenBank accession number OL470118. The 12S rDNA sequencing of the cyst yielded an amplicon of approximately 450 bp that belongs to the *T. hydatigena* larval stage with the GenBank accession number OL470131. It is concluded that carnivorous animals, such as dogs, can pose a risk to small ruminant animals and have a negative impact on them. Therefore, it is recommended that periodic deworming should be performed for stray or farm dogs using different anthelmintic drugs.

Key words: *C. Tenuicollis*, GenBank, *T. hydatigena*, 12S rDNA, 18S rDNA

INTRODUCTION

Sheep and goats are valuable small ruminant animals in Egyptian agriculture, particularly in the Matrouh Governorate, where a total of 288,000 sheep and goat heads are considered to represent approximately 30% of the total agricultural income as the primary source of human protein intake and wool production. Consequently, infection with *T. hydatigena* is a more severe hazard, affecting animals and humans (Abdelsalam et al. 2021). The *T. hydatigena* larval stage significantly impacts intermediate host sheep and goat carcasses, causing damage to infected carcass organs. In addition, *T. hydatigena* larval stage (*C. tenuicollis* cyst) is responsible for production losses and mortality in sheep and goat livestock due to the migration of cysticerci to multiple organs, leading to hemorrhagic and fibrotic tracts, peritonitis, and traumatic hepatitis, resulting in death (Sgroi et al. 2020). In contrast, definitive carnivorous animal hosts, such as dogs, are usually asymptomatic. Nevertheless, significant infections can lead to gastrointestinal disturbances, including diarrhea, retarded growth, a lowered immune response to infectious diseases, and abdominal pain. Additionally, the migration of proglottid segments from the perianal area can lead to anal pruritis (Raza et al. 2018).

T. hydatigena infection carries a high risk of zoonotic disease (McFadden et al. 2016). The close relationship between humans and carnivorous animals, such as dogs, is a significant public health risk for the transmission of zoonotic diseases, including cysticercosis (Emamapour et al. 2015).

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The infection occurs through the contaminated human hand with mature eggs after contact with fecal matter from the infected dog. The human swallows the mature eggs, and the hexacanth embryo penetrates the small intestine, travels, and persists in the liver for 18–30 days, after which the *C. tenuicollis* cyst form is produced. The cyst tunnels into the peritoneal cavity and reaches the viscera (Muku et al. 2020).

The *T. hydatigena* infection requires two hosts in its life cycle: a vertebrate intermediate host, where the infective *C. tenuicollis* cyst develops, and a definitive vertebrate host, which ingests the infective organs containing *C. tenuicollis* cysts. The adult worm is yielded after a 57-day incubation period and has a length of up to 5 meters, consisting of multiple proglottid segments with an armed scolex and 26–44 hooks on the rostellum, arranged in two rows (Gonzalez and Thomas 2018). *C. tenuicollis* cyst is a metacestode or the larval stage of the adult worm *T. hydatigena*. It is found among numerous vertebrate intermediate hosts, including small and large ruminants, horses, and pigs. The metacestode stage is mainly in the omentum, mesentery, and liver (Morais et al. 2017). These intermediate hosts become infected by a mature egg in the feces of infected carnivorous animals, which contains a hexacanth embryo with six hooks (Mokhtaria et al. 2018). The *T. hydatigena* adult worm is a tapeworm that inhabits the small intestine of carnivorous animals, such as dogs and foxes; therefore, they are definitive hosts (Corda et al. 2020).

Traditional techniques for diagnosing Taeniasis species, such as microscopic examination and enzyme-linked immunosorbent assays, lack sensitivity and specificity due to cross-reactions and similar morphological characteristics among Taeniidae species. Thus, molecular identification approaches can overcome these misdiagnoses (Gomez et al. 2019; Ohiolei et al. 2019; Bilal and Musa 2021). Therefore, to prevent hosts' infectivity and apply effective control strategies, it is essential to note that the infection rate of an intermediate host with a *C. tenuicollis* cyst in the Matrouh Governorate, Egypt, was 17.7% (Felefel and Laban 2020). Furthermore, there are many stray dogs and cases of poor hygiene, including unsanitary conditions for condemned organs or illegal slaughter outside abattoirs. These factors lead to the main objective of the current research, which is the significance of molecular identification of the T. hydatigena adult worm and metacestode.

MATERIALS AND METHODS

Ethical Approval

All respective animal protocols were reviewed and approved by the state ethics commission and the ethics committee of Alexandria University, Egypt (serial number 0305892 on 12/15/2022; FWA no. 00018699; and IRB no. 00012098).

Study Settings and Sample Collection

One hundred six cysts of *C. tenuicollis* were collected from the Allam Elrom abattoir in the Matrouh Governorate, Egypt (31.352778°N, 27.236111°E), and two *T. hydatigena* adult worms were yielded experimentally according to (El-Beskawy et al. 2021). The yielded adult worms were preserved in a glass bottle containing 70% ethanol and submitted for identification using a ribosomal DNA approach by the Genetic Engineering and Biotechnology Research Institute (GEBRI) in Alexandria, Egypt (30°50′56″N, 29°36′42″E). The collected *C. tenuicollis* cyst was preserved in a glass bottle containing 70% ethanol and submitted for identification using a ribosomal DNA technique by South Valley University in Qena Governorate, Egypt. Finally, the adult worm and cyst gene sequences were analyzed through nucleotide BLAST at Zagazig University in Zagazig, Egypt.

Study Design

A Cross-Sectional Study

Molecular identification of both adult worms and cysts.

Adult Worms

To extract the genomic DNA from the stretched *T. hydatigena* adult worm and ensure complete disruption of the tissue pellets, the worm was suspended in Amshage lysis buffer and then sonicated for 5min (60–80% amplitude, 20kHz frequency, 0.6s pulse rate). The lysed suspension was then subjected to vigorous vortexing for 5min to obtain a homogeneous mixture. After centrifugation, washing, and drying, the genomic DNA was extracted using the AMSHAG DNA Extraction Kit (Lu et al. 2020). Then, the 18S rDNA universal primers were used to identify the *T. hydatigena* adult worm (Kounosu et al. 2019).

Cysts

The extraction of the mitochondrial DNA from the cyst scolex was performed using the Qiagen DNA Easy Commercial kit per the manufacturer's instructions. The universal mitochondrial PCR primers for the 12S rDNA gene (a homolog of 12S ribosomal RNA in mitochondria) were employed as a molecular marker to identify *C. tenuicollis* cysts from the scolex (Ulziijargal et al. 2019). The thermal cycler conditions were similar, and the following cycle program was used: 95°C for 5min as the initial denaturation step, followed by 30 cycles of denaturation at 95°C for 1min, primer annealing at 54°C for 1min, and elongation at 72°C for 90s; a final extension step of 72°C for 10min (Lorenz 2012).

The PCR was conducted on a Thermo-PCR machine (Thermo Fisher, USA). Subsequently, a 1% agarose gel containing ethidium bromide was used to separate the amplified PCR fragments by gel electrophoresis. The electrophoresis was performed at 100V in a 1xTBE buffer and then visualized using a multi-image gel documentation system. After that, the resultant PCR products were purified with microspin filters, and DNA sequencing was performed using the ABI PRISM® 3100 Genetic Analyzer (Micron-Corp. Korea).

Phylogenetic Analysis

The BLAST online tool of the National Center for Biotechnology Information was used to determine the percentage homology between the obtained sequences and

the sequences of existing species in the database. The sequences were then aligned using the Clustal W program, and published sequences were obtained from GenBank. A phylogenetic tree was created using Clustal W by distance matrix analysis and the neighbor-joining method. The phylogenetic trees were displayed using MEGA X: Molecular Evolutionary Genetics Analysis. Additionally, sequences were submitted to GenBank, and accession numbers were obtained**.** (Wang et al. 2021) The accession number (KJ818276.1) of Fasciola hepatica was considered an out-of-group strain.

RESULTS

Subsequently, DNA templates were used to perform PCR amplification of the 18S rDNA gene for the adult worm (480bp), as shown in (Fig 1). The mitochondrial 12S rDNA for the cyst scolex (450bp) using the primers referred to above (Fig. 2). Both genes produced the same gene lengths and reasonable quantities needed for DNA sequencing reactions after purification with microspin filters. The 18S and 12S rDNA sequences of adult worms and the scolex cyst were closely related to the genus *T.*

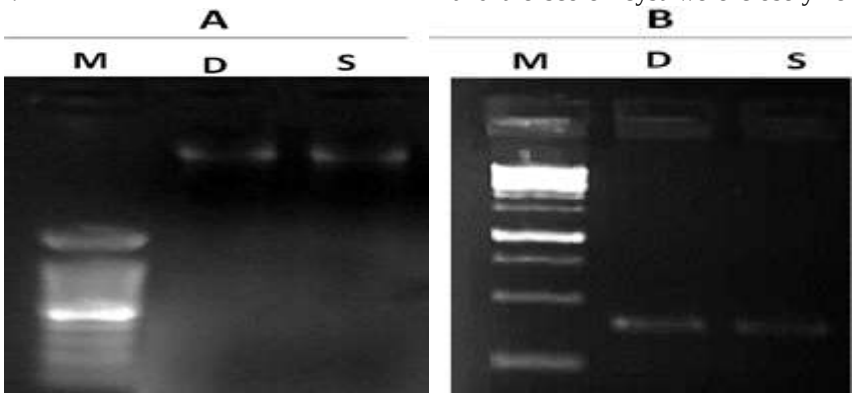

Fig. 1: These are DNA extraction picture (A left) of the genomic DNA of adult worm (D) Using AMSAGE DNA extraction kit. While picture (B right) is the PCR amplification of 18S rDNA of adult worm (D), M in both picture are DNA marker used to measure the length.

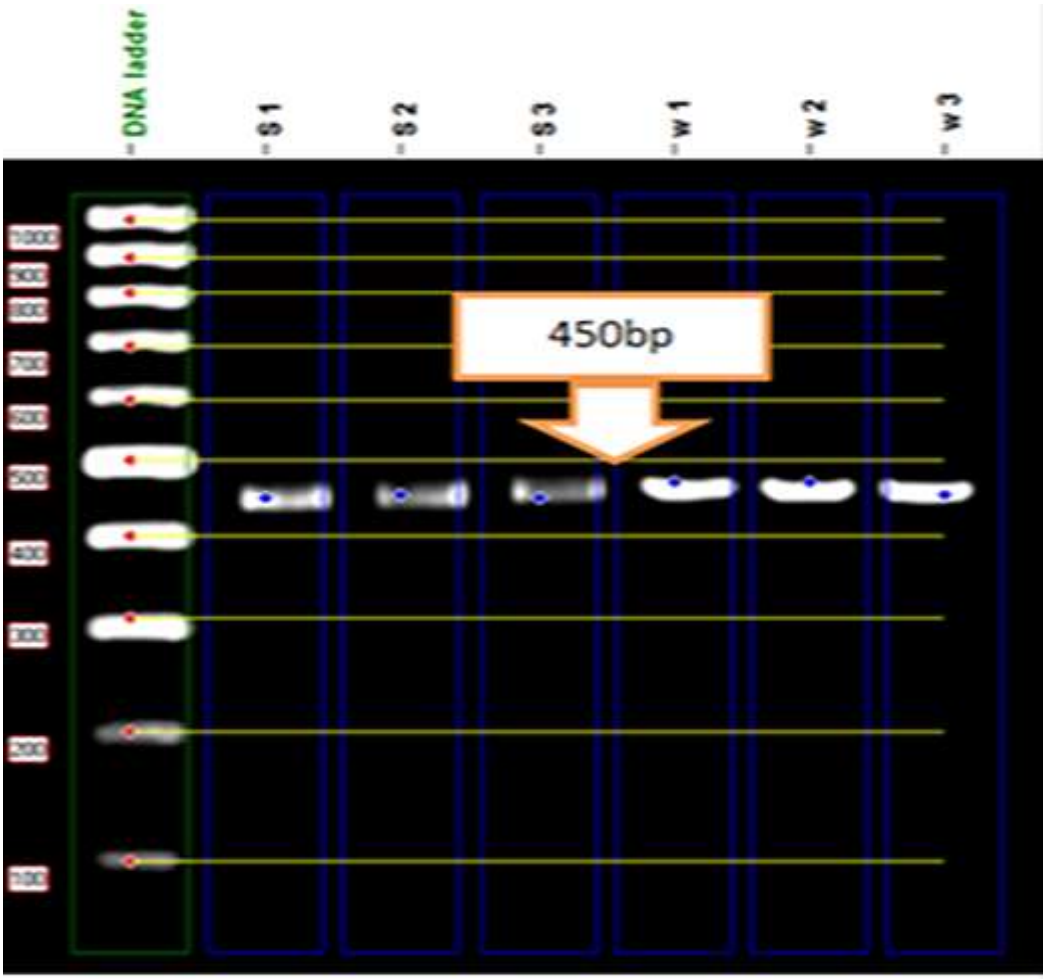

Fig. 2: Agarose gel electrophoresis profile showing the PCR amplification of 12S rDNA of the scolex cyst.

hydatigena, with similarities of greater than 93% and 99%, respectively (Fig. 3). The 18S rDNA sequence of the adult worm was designated *T. hydatigena* TH1 and was submitted to GenBank with the accession number OL470118. The phylogenetic tree clearly shows that *T. hydatigena* TH1 forms a sub-cluster that includes two strains of *T. hydatigena*, one from a goat (Capra hircus) and the other from a sheep, with sequence identities ranging from >98% to >99%.

Regarding the 12S rDNA sequence of the scolex cyst, there was high sequence similarity (>99%) to *T. hydatigena*. It was designated as *T. hydatigena* TH5 and assigned a GenBank accession number of OL470131. According to similarity, identity, and genetic distance, it was closely related to *T. hydatigena* strains JQ71723, KU750812, KX094336, KX681070, and KX094336.

DISCUSSION

There are several advantageous circumstances in the Matrouh Governorate; Egypt to complete the life cycle of *Taenia hydatigena* including the survival of uncontrollable stray dog breeding and the behavior of native personnel in their area, which includes the illegal slaughter of animals out of abattoirs. Hence, dogs acquire the infection by eating infected organs with the mesocestoide of *T. hydatigena* (Oguz et al. 2018).

The 18S gene is a part of the ribosomal functional core and is exposed to similar selective forces in all living organisms. Sequence data from these genes are widely used in molecular analyses to reconstruct the evolutionary history of organisms, as its slow evolutionary rate makes it suitable for reconstructing ancient divergences (Meyer et al. 2010). The 12S ribosomal RNA is the mitochondrial homolog of the prokaryotic 16S and eukaryotic nuclear 18S ribosomal RNAs (Chan et al. 2022).

The mitochondrial genome encodes the ribosomal RNAs 12S rRNA and 16S rRNA, which are required to translate messenger RNAs into mitochondrial proteins. Abigail et al. (2020) emphasized that 12S rDNA mitochondrial genes have numerous benefits as a genetic indicator, including multiple copies, a lack of recombination, and higher mutation rates than nuclear genes. Moreover, they concluded that these molecular attributes could potentially make mitochondrial 12S ribosomal RNA genes effective genetic markers for analyzing phylogenetic relationships in systematic studies. This gene has previously been positively used in phylogenetic relationships among different hosts (Trevisan et al. 2021). The phylogenetic tree (Fig. 3) proved that both genes, 18S and 12S rDNA, as molecular biomarkers to identify the parasite under study, could achieve the study's goal, and they identified the parasite as *T. hydatigena.*

However, they differed in their genetic distance; as a result, strain TH5 (12S rDNA sequence) was a more ancient strain than strain TH1 (18S rDNA sequence). This indicated that the 12S and 18S rDNA genes are valuable for the accurate molecular identification of the same organism (Celik et al. 2021). From the current molecular identification of the adult worm and cyst, it was observed that the identified strains (TH1 for the *T. hydatigena* adult worm and TH5 for the *T. hydatigena* larval stage) were isolated from dogs and sheep, respectively. In a similar study, Omar et al. (2016) successfully amplified approximately 410 bp from the CO1 genes of *T. hydatigena* isolated from goats and sheep from Upper Egypt, with GenBank accession numbers KP641176 and KP641177, respectively. They stated that the phylograms established based on the CO1 gene sequences fit the *T. hydatigena* haplotype of goats (THG = KP641176) and are close to the taxa gi116270720 from India and gi532690879 from Mongolia. The T. hydatigena isolated

Fig. 3: Phylogenetic relationship between TH1 (Taenia hydatigena adult worm) and TH5 (Cysticercus tenuicollis cyst) sequences of 18S and 12S rDNA sequences, respectively and the red square revealed current accession number. The dendrogram was generated by the neighbor-joining method as indicated in the text.

from sheep was close to the taxon gi390195495 from Iran (Omar et al. 2016). Similarly, in line with the present study, Faraj reported that using the 18S rRNA gene, proximity, and genetic dimension among T. hydatigena adult worms isolated from dogs had 99% compatibility values (Faraj and Al-Amery 2018).

Abbas et al. (2021) reported that in small ruminates in the Northern Delta, Egypt, the molecular identification of T. hydatigena larval stage revealed six dissimilar sets of DNA variants subtypes by using 12S rRNA sequences and also discovered 55 subtypes out of 69 isolates, which displayed high haplotype (0.797) and low nucleotide (0.00739) diversities, indicating the residents extension of the parasite. Sarvi et al. (2020) clarified that phylogenetic tree analysis of Cysticercus tenuicollis gives moderate genetic diversity with pairwise sequence distance of larval stage, which is less than 0.5% and higher similarity up to 100%.

Concerning the *T. hydatigena* adult worm, the molecular identification by spending the 18S rRNA gene is considered as a diagnostic tool used to differentiate dog taeniids due to the genetic links between different taeniid species infections (Rostami et al. 2015). In covenant with the current research by Zhu et al. (2019), the adult worm identification at 592bp had the lowest DNA concentration and the highest query cover (93%).

In a study by Jarošová et al. (2022), they established that the multiple set of DNA variants subtypes of *T. hydatigena* are verified in Slovakia, and phylogenetic tree analysis proved the actuality of genetic variation within *T. hydatigena* that contributed to several genotypes, with comparatively low nucleotide pairwise divergence less than 1.3 and 0.2-1.8% for the Hcox and Hnad subtypes, respectively.

Nguyen et al. (2016) stated that a meta-analysis of 26 studies among two different hosts pigs and cattle, which revealed necropsy of *T. hydatigena* larval stage to prove the infection of *C. tenuicollis* larval stage, is a worldwide occurrence. When studying the zoonotic view, the Taenia species is established through significant meta-analysis and accounted for the prevalence of *T. hydatigena* infections in numerous settings.

Boufana et al. (2015) reported that by using two mitochondrial genes, (cox1) and (ND1), they scrutinized the genetic dissimilarity and linked it to several geographical areas, revealing a mutual lineage of *T. hydatigena* adult worms and demographic expansion with variation in both biochemical and morphological traits in Sardegna Island, Italy. On the other hand, for the *C. tenuicollis* cysts collected from small ruminates, the genetic differentiation was absent.

Adwan et al. (2018) detected nine haplotypes with low genetic diversity from 20 isolates of *T. hydatigena* larval stage collected from the northern Palestine. This outcome could help to implement effective control and preventive measures for *C. tenuicollis* cyst infection in Palestine**.**

Conclusion

In order to prevent infection by *T. hydatigena*, the abattoir must implement the HACCP system to perform hygienic measures on the infected organs of slaughtered sheep and periodically deworm dogs with several anthelmintic drugs, thereby breaking the cycle of infection.

Author's Contribution

Desouky Abd-El-Haleem registered the accession number in GenBank and wrote the main manuscript. Marwa Eltarahony conducted practical experiments on the molecular identification of adult worms, including DNA extraction, DNA purification, 18S-rDNA amplification, and purification of the 18S-rDNA amplicon before sequencing and participated in manuscript reviewing. Wael Felefel formed and designed the idea for the research, collected *C. tenuicollis* cyst samples from the Allam Elrom abattoir in the Matrouh Governorate, Egypt, and obtained the *T. hydatigena* adult worm from dogs. Asmaa Gaber Mubarak, Asmaa Gahlan Youseef, and Fatma Ahmed Khalifa conducted investigations on the molecular identification of *C. tenuicollis* cyst, including DNA extraction, DNA purification, and 12S-rDNA amplification. Amany M. Abd El-Ghany analyzed the gene sequences using Nucleotide BLAST and helped write the main manuscript.

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Declarations

The authors declare that they have no competing interests.

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