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Surveillance of Helminthes and Molecular Phylogeny of Fasciola Gigantica Infecting Goats in Sadat District, Egypt

ElKhtam A. O.[1], Khalafalla R. E*[2]

[1]Department of Parasitology, Faculty of Veterinary Medicine, University of Sadat City, Egypt

[2]Department of Parasitology, Faculty of Veterinary Medicine, University of Kafrelsheikh, Kafr El-Sheikh, Egypt

ABSTRACT

Helminthes cause health problems in ruminants as anemia, anorexia, weight loss, diarrhea, decreased production and deaths therefore this study was aimed to identify helminthes of goats, their incidence and genotyping of *Fasciola gigantica* recovered from Sadat district (Kafr Dawoud, Wadi Elnatron and ElKhatatba abattoirs) Egypt. The study was applied on 790 animals from March 2014 to March 2015. The overall infection rate with helminthes was 23.54% among slaughtered goats at Sadat district, Egypt. Prevalence in different localities was recorded. The overall prevalence rate of trematode helminth was 9.66% and the recovered trematode species and their associated infection rates were *Paramphistomum cervi* (1.43%), *Fasciola hepatica* (0.41%), *Fasciola gigantica* (5.83%), *Carmyerius gregarious* (1.99%). While the prevalence rate of cestodes was 9% and the recovered species and their correlated infection rates were *Moniezia expansa* (4.28%), *Moniezia benedeni* (0.71%) and *Avitellina centripunctata* (4%). Genotyping of *Fasciola gigantica* of the current study was done by amplifying 18S ribosomal RNA gene (656 bp of nucleic acids), there are no Single Nucleotides Polymorphisms (SNPs) within the sequence of 18S-rRNA gene and when aligned with isolates of the previous studies; the homology was very high (99.9%) and the divergence was very low (0.1).

Keywords: Goat, Helminth, Surveillance, *Fasciola Gigantica*, Molecular, Phylogeny Egypt

I. INTRODUCTION

In Egypt, the incidence of parasitic infection among farm animals varied according to many factors including irrigation, season and frequency of exposure of animal to infection, immune condition of the animal, the geographic location and climatic conditions [1]. Undoubtedly the most pathogenic and economically important helminthes are the liver flukes (*Fasciola gigantica*) where they cause traumatic hepatitis, peritonitis and sudden death in acute fascioliasis.

DNA studies and using of molecular tools aid in the exact identification of helminthes parasites. In ribosomal DNA (rDNA) the Internal Transcribed Spacer 2 (ITS2) gene have proven useful for diagnostic purposes at the level of species [2,3]. ITS2 sequences have also been used to characterize and identify different *Fasciola* spp., [4,5]. While, mitochondrial DNA (mtDNA) genes proves to be excellent markers to differentiate in-between closely-related species and testing the polymorphism of *Fasciola* species [5]. In Egypt, *F. gigantica* and *Paramphistomum* spp are prevalent among livestock in the Nile delta [6], the aim of the present work is to identify helminthes infecting goats, their prevalence, morphology and molecular characterization of *Fasciola gigantica* infecting goats in Sadat district, Egypt.

II. METHODS AND MATERIAL

A. Samples collection and Morphological identification

Samples were collected from 790 goats at Sadat district from local abattoirs in 3 different localities; Kafr Dawoud, Wadi Elnatron and ElKhatatba. Worms were recovered from slaughtered goats from

different organs and during evacuation of gastrointestinal tract. Worms were washed, relaxed, fixed, stained and mounted [7]. Morphological description and identification of the collected worms were done on mounted specimens according to [8].

B. *DNA extraction from Fasciola gigantica and PCR*

Adults of *F. gigantica* were collected from the liver of slaughtered goats and were identified according to morphometric parameters and all other unclear or doubtful samples were rejected [9,10]. These worms were washed several times with PBS and then preserved in tubes that stored at -20°C till DNA extraction [11].

Genomic DNA was extracted using 12 individual worms using the QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. All DNA concentrations were determined using an Epoch spectrophotometer (Biotek, Winooski, VT).

ITS2 region was amplified by polymerase chain reaction (PCR) using the forward primer (3S) 5`-TTTTTTGGGCATCCTGAGGTTTAT-3` and the reverse (A28) 5`-TAAAGAAAG AACATAAT GAAAATAATC-3`, [12,13].

The PCR conditions were as follows: 94°C for 3 min, 50°C for 1 min, and 72°C for 3 min for 30 cycles. Amplification reactions were performed in a final volume of 25 µl containing primers (3.2 pmol), deoxynucleoside triphosphates (dNTPs, 0.2 mM), and Taq polymerase (2.5 U/reaction). PCR products were purified using GFX PCR DNA and Gel Band Purification Kit (GE) according to manufacturer's protocol.

C. *Sequencing and phylogenetic analysis*

PCR products were sequenced using Big Dye Terminator v 3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystems) in a 3100 Automated DNA Sequencer (Applied Biosystems) as recommended by the supplier. Amplicons were sequenced in each direction using the NC5 and NC2 primers, in separate reactions. Bio Edit v7.0.4.1 and DNASTAR Lasergene SeqMan v7.0.0 programs were used for analyzing and editing the DNA sequences.

Sequences of ITS2 regions from different isolates of previous studies were aligned against the isolate of the current study using ClustalW [14] multiple sequences alignment and the Phylogenetic trees were carried out using MEGA5 software [15].

III. RESULTS AND DISCUSSION

A. *Incidence of the recovered helminthes*

The overall incidence of different helminth species among examined slaughtered goats was 23.54% (*186 out of 790*) with variable infection rates in different localities where it was at Wadi El-Natron locality 32% (*96 out of 300*), at Kafr Dawoud was 6.25 % (*70 out of 280*) and at El-Khatatba locality was 9.52% (*20 out of 210*) (see Fig. 1).

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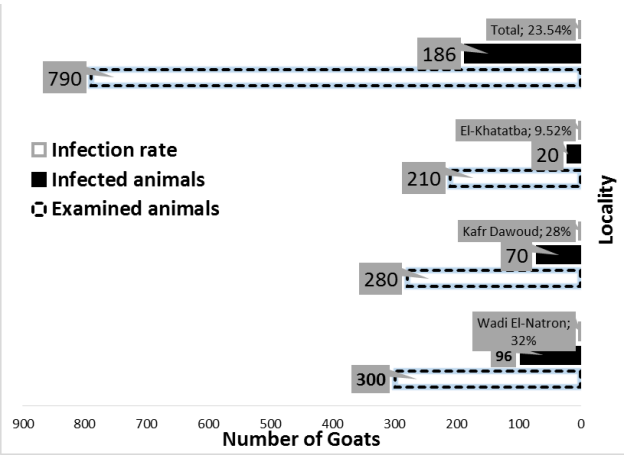

Figure 1: Prevalence of helminth infections of slaughtered goats in different localities (Wadi El-Natron, Kafr Dawood and El-Khatatba) at Sadat District, Egypt

The overall prevalence rate of trematode infection was 9.66% and the recovered trematode species and their associated infection rates were *Paramphistomum cervi* (1.43%), *F. hepatica* (0.41%), *F. gigantica* (5.83%), *Carmyerius gregarious* (1.99%). While the prevalence rate of cestodes was 9% and the recovered species and their correlated infection rates were *Moniezia expansa* (4.28%), *Moniezia benedeni* (0.71%) and *Avitellina centripunctata* (4%) (See Table 1).

Table 1 : Prevalence of different helminth infections in slaughtered goats *(N=790)* from different localities at Sadat District, Egypt.

	Infection rate %
Trematodes	9.66
Fasciola gigantica	5.83
Fasciola hepatica	0.41
Carmyerius gregarious	1.99
Paramphistomum cervi	1.43
Cestodes	9
Moniezia expansa	4.28
Moniezia benedeni	0.71
Avitellina centripunctata	4

The helminthes recovered from slaughtered goats were *F. gigantica, F. hepatica*, *Paramphistomum cervi, Carmyerius gregarius, Moniezia expansa, Moniezia benedeni, Moniezia denticulata* and *Avitellina centripunctata*. The infection rate of *Moniezia expansa*, was agreed with the results recorded in Rudolphi [16] and in Menofia governorate, Egypt [17], but it was disagreed with that recorded by [18] in north Sinai, Egypt.

B. *Genotyping of Fasciola gigantica*

The genotyping of *F. gigantica* which recovered from goat in the current study is based on amplification of 18S ribosomal RNA gene sequence and its characteristics and sequence alignments (Fig. 2).

NNGCNNCNNCCNTCGCTACTACCGATTGATGGTTTAGCAAGGTCCTCGGATTGG
TGCCATTTGCAGTGGCTTCGGCCGCTCGACCGGTGCTGAGAAGACGACCAAACTT
GATCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGG
AAGGATCATTACCTGAAAATCTACTCTTACACAAGCGATACACGTGTGACCGTCAT
GTCATGCGATAAAAATTTGCGGACGGCTATGCCTGGCTCATTGAGGTCACAGCAT
ATCCGATCACTGATGGGGTGCCTACCTGTATGATACTCCGATGGTATGCTTGCGTC
TCTCGGGGCGCTTGTCCAAGCCAGGAGAACGGGTTGTACTGCCATGATTGGTAGT
GCTAGGCTTAAAGAGGAGATTTGGGCTACGGCCCTGCTCCCGCCCTATGAACTGT
TTCATTACTACAATTACACTGTTAAAGTGGTATTGAATGGCTTGCCATTCTTTGTTA
TTGCCCTCAAATGAACCAGGTTGTTGTGGCTGCTCTGCACCGACCACGCCGCCCA
GCGCCTAGTATGGCGGGCAGTCCAGGGACTTTGTTAAACGATCAAACGGACAAC
TCCGACGGGTGGTGTGTACAGAGGGTGGGTCGTATCCAGCCCAAAACCAA

Figure 2. Complete sequences of the 18S-rRNA gene from *Fasciola gigantica* isolate **Y15** of the current study**.**

For comparative purposes, pairwise and multiple alignments of the gene sequences was done with 18S-rRNA gene sequence of Fasciolides from different hosts and geographical regions obtained from Gene Bank to evaluate sequence homology and diversity (Fig.3).

Main variable regions were identified in the 18S-rRNA gene sequences which were characterized by a considerable number of indels (insertion and deletion of nucleotides) as Single Nucleotide Polymorphism (SNPs) with 18S-rRNA gene of *F. gigantica* isolate BDF-Ct.109 (Accession number: KC476170) and *F. gigantica* isolate *ADC53* (Accession number: KJ728737). By using pairwise and multiple alignment of 18S-rRNA gene of *F. gigantica* isolate 124 (FJ756397) with that of our strain, both sequences have revealed a high sequence homology, due to only a few indels and SNPs and shared a high number of identical nucleotides with *F. gigantica* isolate 124 (FJ756397) which was the most similar sequences (Fig.3).

Figure 3 : Sequence characteristics and Sequence comparisons of the 18S-rRNA gene from Fasciola gigantica isolate (Y15 represents the 18S-rRNA gene from Fasciola gigantica isolate of the current study)

Our isolate in the present study was very high homologous (99.9%) with other *F. gigantica* isolates, and the divergence was very low (0.1%), they were the closest inter specific pair, but the homology was much lower among *F. hepatica* and *F. gigantica* isolate ADC53 (KJ728737) which indicates that the ITS2 region allows discrimination between species of Fasciolidae [19].

Inter specific variation (between species) in the regions exceeded that within species. The variation between species ranged between 0.1 and 1.4%, and so, the alignments of the 18S rRNA gene sequence of the different isolates showed that the species differed from each other by single base substitutions and indels, and the first 250 and last 190 nucleotides of the gene were identical in all isolates.

C. *The phylogenetic analysis*

By reading the phylogenetic tree (Fig.4), *F. gigantica* isolate in the present study was clustered with *F. gigantica* isolate 124 (FJ756397) as they was very identical to each other, but somewhat differ from *F. gigantica* isolate BDC-Fsp.Bf.101 (KC424484), isolate BDF-Ct.109 (KC476170) and isolate ADC57 (KJ728738) but it lying faraway from *F. hepatica* isolate Persian 1 (GQ925431).

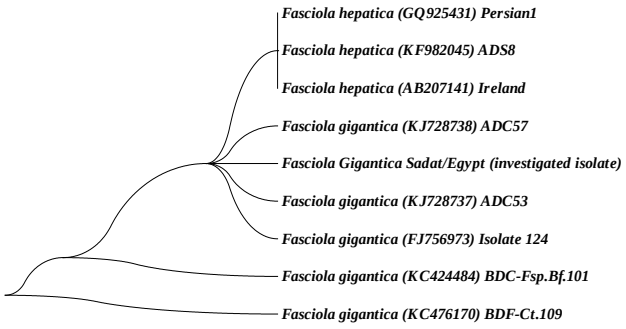

Figure 4. Neighbor-Joining phylogenetic tree [20]. The phylogenetic distances were computed using the Maximum Composite Likelihood method [21] and are in the units of the number of base substitutions per site. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 404 positions in the final dataset. Phylogenetic analyses were conducted in MEGA5 [15].

The phylogenetic tree based on ITS2 region displayed a close relationship between investigated *F. gigantica* isolate from Egypt and other parts of the world (Fig.4).

In Egypt, *F. hepatica* and *F. gigantica* are prevalent among livestock in the Nile delta [7] results showed that identification of Egyptian *Fasciola* based only on morphometric criteria is not a countable as the presence of *F. hepatica*, *F. gigantica* besides the hybrid form in Egypt was confirmed [22]. In a study was done in Egypt [13], nucleotide sequences of the mitochondrial DNA, cytochrome oxidase subunit 1 (CO1) and Internal Transcribed Spacer 2 (ITS2) of the ribosomal RNA gene were used to identify *Fasciola* species that infects cattle in Qena province, Upper Egypt, and they concluded that, to sequencing, amino acids analysis and studying the phylogenetic relationship are pecise tools to identify *Fasciola* species.

IV. CONCLUSION

The results displayed herein indicated that the using of ITS2 region is a powerful tool for identification and discrimination between the Fasciolidae species.

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