



Prevalence and Phylogenetic analysis of *Fasciola* species in Upper Egypt Based on Ribosomal ITS-2 gene Sequencing

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Abstract:

Fascioliasis is an important parasitic zoonosis with an estimated annual economic loss at more than US\$3 billion in animals and infection risk in more than 180 million people worldwide. Given the economic and public health significance of this trematode, a periodical evaluation of its prevalence and the associated risk factors as well as the evolutionary relatedness is needed. Here, 1560 cattle and 1630 goat livers were examined for the presence of adult *Fasciola* among animals slaughtered in slaughterhouses of at Assiut and Sohag Governorates, Upper Egypt. Molecular characterization and phylogenetic relationships were determined based on the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA (rDNA). The overall prevalence of fasciolosis was based on morphological identification of the recovered *Fasciola* adult worms and the prevalence was 5.1% and 3.5% among cattle and goats, respectively. Though the age, gender, and housing system significantly affected the disease in cattle, none of these variables had a significant impact on goat fasciolosis. Nonetheless, the sampled goats were found at increased risk of being infected with *Fasciola hepatica* as the age increases and when the winter season emerges. Phylogenetic analyses showed that both *Fasciola gigantica* and *Fasciola hepatica* sequenced in this study had a common ancestor and they fall in one clade with several previously reported Egyptian and worldwide fasciolae from animals and humans. This study demonstrates that the disease is currently circulating among animals in Assiut and Sohag Governorates that warrants the urgent need for application of the appropriate control measures as well as evaluating the possible ongoing zoonotic burden in people residing such areas.

Keywords: Cattle, goats, *Fasciola*, Prevalence, Zoonosis, ITS2, Molecular characterization, Upper Egypt.

INTRODUCTION

Fasciolosis is a worldwide parasitic (Platyhelminthes: Trematoda: Digenea) zoonosis affecting animals and humans. It was considered as a historical disease for long time; however, the disease has been recently categorized as one of the

reemerging zoonosis with an estimate of up to 2.4 million people are affected by fascioliasis in more than seventy countries across the globe (Mas-Coma et al., 2005).

In mammals, the infection occurs by ingestion of the encysted metacercariae on fresh water plants and water (Mas-Coma et

al., 2005). The immature flukes take several weeks wandering in the liver parenchyma of the affected host, makes the parasite highly tissue destructive, before residing in the bile duct and progressing to the adult stage. To complete the lifecycle, adult flukes produce eggs which are voided with feces and contaminate fresh water where they undergone the necessary developmental stages with the aid of the fresh water snails (Nyindo and Lukumbagire, 2015). Indeed, fascioliasis is a multifactorial disease with limited treatment choices and no vaccine; hence, application of strict measures remains important solution to effectively combat such foodborne zoonotic disease (Knubben-Schweizer and Torgerson, 2015).

The disease is mainly caused by *F. hepatica* Linnaeus, 1758, and *F. gigantica* Cobbold, 1856, both can utilize animals and humans as definitive hosts (Amer et al., 2016). Identification of these parasites is usually based on the Morphological and morphometric criteria, however, these traditional methods remain unreliable in definite differentiation (Liu et al., 2014; Moghaddam et al., 2004). For that, molecular methods are required in resolving the difficulties associated with species identification based on the phenotypic traits (Shoriki et al., 2016). One of these methods widely used to differentiate between *F. gigantica* and *F. hepatica* is the second internal transcribed spacer (ITS-2) of nuclear rDNA which found to have 1.7% sequence difference between the two *Fasciola* species and very low differences within each species (Choe et al., 2011; Hashimoto et al., 1997; Itagaki et al., 2005; Lin et al., 2007; Liu et al., 2014; Marcilla et al., 2002).

Egypt is considered as one of the fasciolosis endemic countries in the world and of high parasitic burden in infected animals and humans (Arafa et al., 2017; Haridy et al., 2006); nevertheless, epidemiological data about the disease in Upper Egypt are scarce and the molecular characterization studies on important hosts like cattle and more particularly goats are severely underappreciated. Therefore, this study was mainly designed to clarify the prevalence *Fasciola* species and associated risk factors of infection among cattle and goats. In addition, the identity, genetic characterization and phylogenetic analysis of nuclear ribosomal internal transcribed spacer gene (ITS-2 region of rDNA) in *Fasciola* species, among slaughtered cattle and goats from Assiut and Sohag abattoirs in Upper Egypt.

2. Materials and Methods

2.1. Research ethics:

Approval of ethical rules was followed after a Research guidance, Publication and Ethics of the Faculty of Veterinary Medicine, Sohag University (2014). All steps of the study were clarified to abattoirs authorities, veterinarians, and owners. The living animals were not included in the study.

2.2. Area of the study

Samples were collected from livers of slaughtered animals at routine postmortem examination in Assiut (27.252°N 31.09°E) and Sohag (26°33'N 31°42'E) abattoirs from January to October 2019.

2.3. Sample size:

Based on a previously published study in El Minia Governorate, one of the most nearest governorates to the survey areas, reported an infection rate of 35% in cattle and utilizing the equation: $n = Z^2 \times P \times q / d^2$ (Charan and Biswas, 2013), a minimum

sample size equal to 350 or 606 animals using 5% or 1% type 1 errors, respectively was sufficient. In goats, with the scarcity of data about the disease in Upper Egypt, we calculated the sample size based on assumption of 50% prevalence and it was found to be 384 or 666 using 5% or 1% type 1 error, respectively. However, to ensure more power for the current study, we considered a greater sample size accounted for 1560 cattle and 1630 livers recruited from slaughtered cattle and goats, respectively.

2.4. The parasite:

During the period from January to October 2019, a total of 3190 livers of slaughtered animals (1560 cattle and 1630 goats) were examined at necropsy and were labeled (animal species, location, age, season, sex, and rearing system). The collected adult *Fasciola* species flukes were transported on ice to the laboratory of the department of parasitology in Assiut university and Sohag University, washed with physiological saline, relaxed, fixed in 10% neutral formalin, stained with alum-carmin and mounted for morphological identification (Soulsby, 1982). Another portion of collected liver flukes was kept in 70% ethanol for extraction of genomic DNA (Huang et al., 2004).

2.5. DNA extraction and PCR conditions:

Ten of morphologically identified positive samples of *F. gigantia* and 10 identified positive samples of *F. hepatica* were selected for DNA extraction afterwards, five adult flukes from each sample were consumed for DNA extraction by using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's guidelines. Specific primers for the amplification of the ITS-2 regions

were used: F: 5'GGTACCGGTGGATCACTCGGCTCGTG3' and R: 5'GGGATCCTGGTTAGTTTCTTTTCTCCG C3' (Luton et al., 1992).

The PCR amplification protocol was performed in a final reaction volume of 25 µl; 12.5µl Master Mix (Promega, Madison, WI, USA), 1 µl forward primer, 1 µl reverse primer, 1 µl extracted genomic DNA (50 ng) and 9.5 µl deionized distilled water (White, 1993). The PCR reaction was performed in Biometra thermocycler (Biometra, Gottingen, Germany) as follows; initial denaturation at 95 °C for 5 min followed by 40 cycles of denaturation at 95 °C for 30 sec, annealing at 60 °C for 30 sec and primer extension at 72 °C for 1 min followed by a final extension at 72 °C for 10 min. Thereafter, using 100 base pair plus ladder (DNA Molecular Weight Marker), the PCR products were electrophoresed in 1.6% (w/v) agarose gels in Tris-acetate-EDTA (TAE) buffer, stained with ethidium bromide and visualized and photographed by UV trans-illuminator.

2.6. ITS2 Sequencing and analysis:

PCR products were purified using Qiagen QIAquick PCR Purification kit following the manufacturer instructions. The purified PCR products of ITS-2 were sequenced by applying the same forward primers in an automated sequencer (ABI PRISM® 310 genetic analyzer, available at Molecular Biology Unit, Assiut University) following manufacturer instructions. The sequences identities were confirmed utilizing BLAST (the online tool provided by NCBI; https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome, Zheng Zhang, et al., 2000). Alignment of the current ITS-2 sequences with corresponding sequences obtained from the NCBI was further

computed using ClustalW (Madeira et al., 2019) and the evolutionary analyses were conducted in MEGA X (Kumar et al., 2018). The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993). Initial trees for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Tamura-Nei model. The analyses involved 15 and 16 nucleotide sequences in cases of *F. gigantica* and *F. hepatica*, respectively. *Fasciola jacksoni* (MN970006.1) and *Fascioloides magna* (KU232369.1) were used as outgroup isolates.

2.7. Statistical analysis

Fisher's Exact test, Odds ratio (OR) with 95% Confidence Intervals (95% CI) in GraphPad Prism software version 6 (GraphPad Software, Inc., La Jolla, CA, USA) were used to compute the categorical variables included in this study. A *P* value <0.05 was considered significant.

3. Results

In the present study, the overall prevalence of *F. gigantica* infection in cattle was 5.06% and *F. hepatica* infection in goats was 3.5%. Cattle's age, gender and rearing system significantly affected the prevalence of fasciolosis with *P* values equal to 0.048, 0.035 and < 0.0001, respectively. OR (Odds ratio) showed remarkable differences between the subcategories of such significant variables. For instance, regarding cattle's age, animals older than 4 years showed the highest OR (OR = 1.93 (95% CI: 1.14-3.28)) followed by 1-2 years old animals (OR = 1.50 (95% CI: 0.80-2.82)). Likewise,

female cattle had a higher odds of exposure (OR = 1.64 (95% CI: 1.04-2.58)) relative to males (OR = 0.61, 95% CI: 0.39-0.96)). Additionally, animals reared under free system were at higher risk of exposure (OR = 2.71 (95% CI: 1.71-4.31)) than those did not (OR = 0.39, 95% CI: 0.23-0.58)). Conversely, in goats, the disease prevalence didn't vary significantly by any of the included variables, however, the obtained OR for each subcategory revealed remarkable variations as noted in the locality, age, season, and rearing system. Regarding locality, animals sampled from Sohag had higher OR (OR = 1.62 (95% CI: 0.92-2.85)) than those from Assiut (OR = 0.62 (95% CI: 0.35-1.09)). Animals with age over 2 years had the highest OR (OR = 1.58 (95% CI: 0.79-3.15)) followed by 1.5-2 years old group (OR = 1.22 (95% CI: 0.58-2.57)) and those sampled at winter season were at highest risk (OR = 2.78 (95% CI: 1.21-6.35)) compared to other sampling seasons (Table 1).

The nucleotide sequences of the ITS-2 of *F. gigantica* and *F. hepatica* isolates reported in this study were deposited in NCBI under accession no MT423006.1 and MT423007.1, respectively. An identity equal to 99.07% was found between the sequences of the two reported isolates.

A 100% match of our *F. gigantica* ITS-2 was found when sequence alignment was conducted along with Egyptian water buffalo (AB553695.1), Burkinian cattle (AJ853848.2) and Kenyan cattle (EF612484.1). However, an identity equal to 99.79% was found with *F. gigantica* ITS-2 reported from cattle in Nigeria (MN608170.1) and Iran (JF432074.1). A range of 99.45%-99.72% identities was found compared to four *F. gigantica* isolates obtained from humans in Vietnam (EU260070.1; EU260059.1; EU260060.1; EU260078.1). On

the other hand, only 92.05% and 93.88% identities were found when our *F. gigantica* ITS2 sequence aligned with the outgroup isolates *Fascioloides magna* and *F. jacksoni* obtained from the American cattle (KU232369.1) and Sri Lankan elephant (MN970006.1), respectively (Fig.1). Comparing *F. hepatica* sequence with another 15 sequences obtained from the NCBI database revealed presence of 100% identity with the Egyptian water buffalo (AB553734.1) and human (KP215282.1) isolates, Algerian cattle (MK212149.1), Australian cattle (MN970007.1), Chinese sheep (MH385386.1), Indian yak (KR911820.1) and Ecuadorian cattle (LC056929.1) ITS-2 sequences. However, lower identities accounted for 96%, 99.63%, 99.34%, 99.76% and 99.80% compared to Egyptian water buffalo (MH385386.1) and sheep (KJ818276.1) and Iranian cattle (KT033697.1), sheep (KT921273.1) and goat (KF866252.1) isolates, respectively. Both Fasciola isolates reported in this study were computed against *Fasciola* hybrid isolates ITS-2 sequences located at the GenBank. Our *F. gigantica* and *F. hepatica* shared identities of 99.23:99.04: 98.62 and 99.04: 99.23: 99.01 with the Egyptian water buffalo (AB553738.1 and AB553737.1) and Japanese cattle (AB207153.1) *Fasciola* sp._ITS-2 sequences, respectively. On the other hand, only 92.03% and 93.49% identities were found when our *F. hepatica* ITS2 sequence aligned with the outgroup isolates *Fascioloides magna* and *F. jacksoni* obtained from the American cattle (KU232369.1) and Sri Lankan elephant (MN970006.1), respectively (Fig.2).

DISCUSSION

Fascioliasis is a parasitic infection with a great adverse influence on animal's productivity and causing a widespread human zoonotic disease in several countries such as Egypt, Tunisia, Algeria, Botswana, Mauritania, Niger, Saudi Arabia, Iraq, Iran, Pakistan, China, Vietnam, Korea and Japan (Agatsuma et al., 2000; Ali et al., 2008; Amer et al., 2011; Amer et al., 2016; Amor et al., 2011a; Ashrafi et al., 2007; Ashrafi et al., 2006; Farjallah et al., 2009; Haridy et al., 2006; Huang et al., 2004; Itagaki et al., 2009; Khalifa et al., 2016; Mochankana and Robertson, 2018; Nguyen et al., 2012; Over et al., 1992; Sharma and Raina, 1989; Wajdi and Nassir, 1983). Hence, the main purpose of the current study was to evaluate the prevalence of *Fasciola* species infection among cattle and goats in Assiut and Sohag Governorates of Upper Egypt. The morphological and morphometrical characterization of *Fasciola* species in Egypt are greatly variable, consequently, the molecular and phylogenetic analysis is greatly needed (Ashour et al., 1999).

The current study revealed an infection rate of 5.1% with *F. gigantica* in cattle and 3.5% with *F. hepatica* in goats. Compared to previous rates reported from different Egyptian governorates, our study came the lowest. In southern Egypt, it was 68.89% and 30.32% for infection with *F. gigantica* and *F. hepatica* in slaughtered animals, respectively in Qena Governorate, (Hussein and Khalifa, 2010) and the prevalence of fascioliasis was 35% in slaughtered cattle in El-Minia Governorate (Dyab et al., 2019). In northern Egypt, a prevalence of 9.77% in cattle were found to be infected with *Fasciola* species in the Delta region (El-Tahawy et al., 2017). Also, the

fascioliasis was recorded as 11.1% in Dakhla Oasis, Egypt (Arafa et al., 2018)

A significant higher infection rates were found in older than younger animals and females than males which disagreed with results of El-Tahawy et al. (2017). This discrepancy could be attributed to the effect caused by regular anthelmintic administration to younger animals that kept for meat production particularly before slaughtering. This strategy does not typically apply for animals of older ages. Likewise, the increased infection among females in this study could be associated with the stress frequently occur for females during different stages of pregnancy and lactations making them more liable to parasitism.

Seasonal variation has been noted for the disease in previous studies (Bloemhoff et

Table 1: Prevalence of *Fasciola gigantica* infection and different associated risk factors in examined slaughtered cattle.

Risk factor	Infection with <i>Fasciola</i> species			Odds Ratio (95% C.I.), P-value	
	No. examined	No. infected	Infection rate (%)		
Localit y	Assiut	720	30	4.2	0.70 (0.44-1.12)
	Sohag	840	49	5.8	1.42 (0.89-2.27)
	Total	1560	79	5.1	P=0.164
Age	1-2 years	343	18	5.4	1.50 (0.80 to 2.82)
	2-4 years	646	23	3.6	Reference
	> 4 years	571	38	6.7	1.93 (1.14 to 3.28)
	Total	1560	79	5.1	P=0.048
Season	Winter	394	29	7.4	2.21 (1.13 to 4.32)
	Spring	401	21	5.2	1.54 (0.76 to 3.12)
	Summer	390	16	4	1.19 (0.56 to 2.51)
	Autumn	375	13	3.5	Reference
	Total	1560	79	5.1	P=0.069
Sex	Male	893	36	4	0.61 (0.39 to 0.96)
	Female	667	43	6.4	1.64 (1.04 to 2.58)
	Total	1560	79	5.1	P=0.0355
Rearin g	Housed system	993	32	3.2	0.39 (0.23 to 0.58)
	Free range system	567	47	6.4	2.71 (1.71 to 4.31)
	Total	1560	79	5.1	P< 0.0001

Table 2: Prevalence of *Fasciola hepatica* infection and different associated risk factors in examined slaughtered goats.

Risk factor	Infection with <i>Fasciola</i> species			Odds Ratio (99% C.I.), P-value	
	No. examined	No. infected	Infection rate (%)		
Locality	Assiut	690	18	2.6	0.62 (0.35 to 1.09)
	Sohag	940	39	6.3	1.62 (0.92 to 2.85)
	Total	1630	57	3.5	P=0.105
Age	1-1.5 years	443	12	2.7	Reference
	1.5-2 years	546	18	3.9	1.22 (0.58 to 2.57)
	> 2 years	641	27	4.2	1.58 (0.79 to 3.15)
	Total	1630	57	3.5	P=0.39
Season	Winter	399	21	5.3	2.78 (1.21 to 6.35)
	Spring	422	17	4.0	2.10 (0.89 to 4.92)
	Summer	398	8	2.0	Reference
	Autumn	411	11	2.7	1.41 (0.56 to 3.54)
	Total	1630	57	3.5	P=0.056
Sex	Male	798	28	3.5	1 (0.59 to 1.71)
	Female	832	29	3.5	0.99 (0.58 to 1.68)
	Total	1630	57	3.5	P=1.00
Rearing	Housed system	893	26	2.6	0.68 (0.40 to 1.16)
	Free range system	737	31	4.0	1.46 (0.86 to 2.49)
	Total	1630	57	3.5	P=0.176

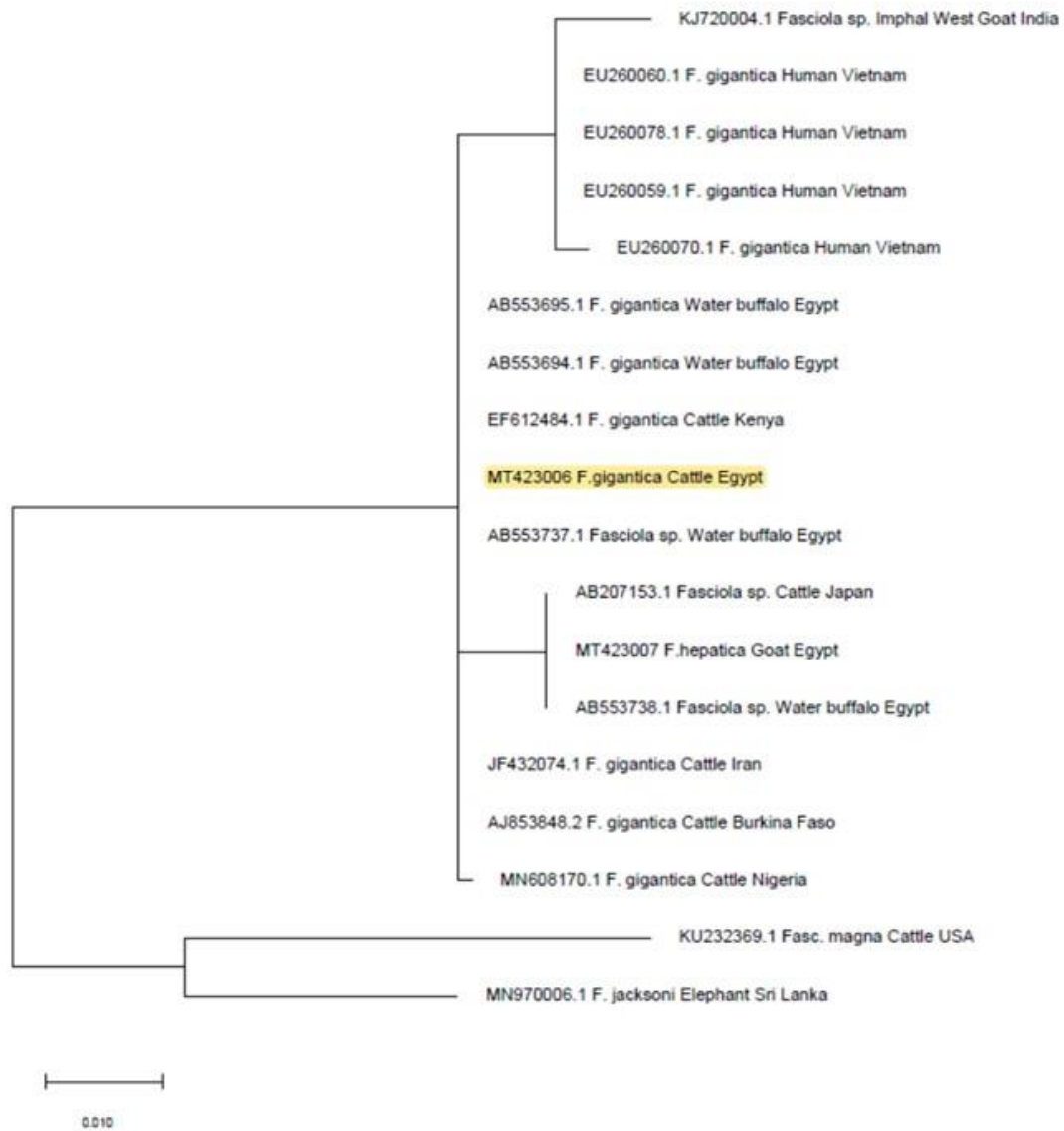


Fig.1. Phylogenetic tree inferred from *F. gigantica* ITS-2 sequences. *F. gigantica* isolate reported in this study is highlighted. A total of 18 different nucleotide sequences were used to construct the tree using MEGAX. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model. Initial trees for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Tamura-Nei model.

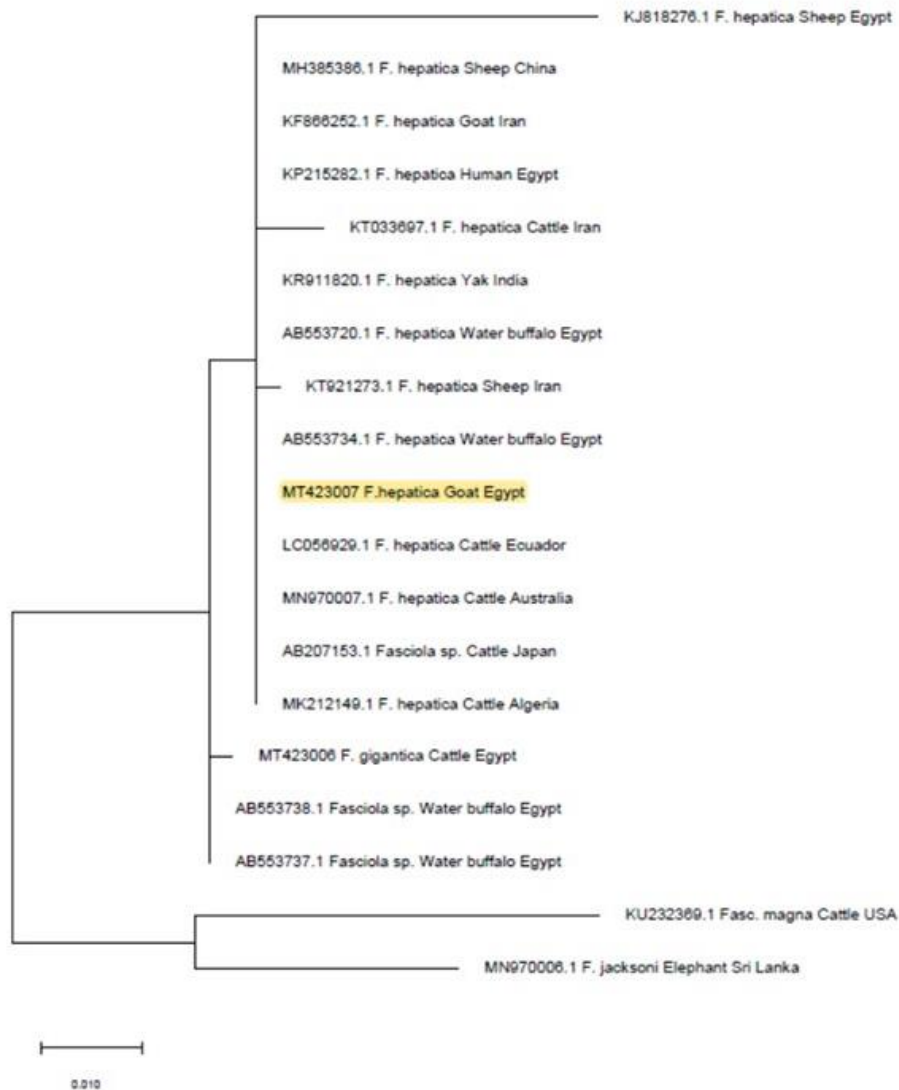


Fig.2. Phylogenetic tree inferred from *F. hepatica* ITS-2 sequences. *F. hepatica* isolate reported in this study is highlighted. A total of 19 different nucleotide sequences were used to construct the tree using MEGAX. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model. Initial trees for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Tamura-Nei model.

al., 2015; Dyab et al., 2019; *cailliaudi*, *Pseudosuccinea columella* and Maqbool et al., 2002; Pfukenyi and *Biomphalaria alexandrina* (Arafa et al., 2018; Mukaratirwa, 2004; Soliman, 2008). Likewise, in our study, a seasonal pattern was observed for the disease in both cattle and goats with increasing infection rate appeared in the winter and spring. Under Egyptian settings, Fasciolae utilize several snails as intermediate hosts such as *Radix natalensis*, *Galba truncatula*, *Lymnaea* (Dar et al., 2005). These snails are widely distributed in Egypt however, their abundance, distribution and role in fasciolosis transmission needs to be re-evaluated in response to the recent climatic changes. A convincing explanation for the documented seasonal pattern of the disease in upper Egypt is required however the fact that

immature stages of the parasites takes long time to reach adult stage since engulfed by the host and also the infected animals keep the adult fasciolae for years in the biliary tract (Robinson and Dalton, 2009), a definite tracking of the timing of the infection likely impossible without a comprehensive multifactorial study.

In the present study, animals reared on free range system were significantly higher than housed cattle because they are highly exposed to the infection on free grazing particularly on sides of canals, banks and rivers and these findings are agreed partially with those obtained by El-Tahawy et al. (2017).

The results of the present studies agreed with those of previous studies in Iranshahr, Iran (Hashemi et al., 2015) where the fascioliasis in Slaughtered cattle was 7.82 % with higher prevalence rates between cattle over 1 year old than younger, males than females, in winter than other seasons and exogenous than indigenous breeds. Also agreed with that conducted in Tonekabon, Iran, where high prevalence rates of fascioliasis in buffaloes (12.76%) and goats (6.97%) (Amor et al., 2011b) were found. Moreover, in eastern Botswana, the prevalence of *F. gigantica* infection in beef cattle was very low (0.74%) in comparison to the current study, but agreed in that, the prevalence of fascioliasis was significantly higher in adult cattle than youngers (Mochankana and Robertson, 2018). Also, in central Vietnam, prevalence of fascioliasis was 45.3 % with a higher prevalence rates observed in adult cattle than calves and in rainy season than in dry season (Nguyen et al., 2012).

The present study revealed that fascioliasis was 3.5% in examined goats and was higher between older than young goats, in winter than other seasons, among males than females and in freely reared goats than housed animals. The prevalence of fascioliasis in goats in Egypt was ranged from low (0.89%) in Giza Governorate (Hassan et al., 2019) to high (14.6%) in Al-Sharqiyah Governorate (El-Azazy and Fayek, 1990). Also, fascioliasis prevalence in goats was 6.6-28.75% in Pakistan (IrfanUllah et al., 2016; Tasawar et al., 2007), 14.14% in Turkey (Çelik and ÇELİK, 2018), 6.2% in Nigeria (Abah et al., 2019) and 6.97% in north region of Iran (Amor et al., 2011b).

In the present work, *Fasciola* isolates of cattle and goats from Assiut and Sohag governorates (Upper Egypt) were genetically identified by sequencing of the ITS2 gene, and the obtained sequences were similar and identical to sequences of *Fasciola* species of previously identified isolates of *F. hepatica*, and *F. gigantica* (Agatsuma et al., 2000; Ali et al., 2008; Farjallah et al., 2009; Itagaki et al., 2005; Periago et al., 2004; Rokni et al., 2010). Interestingly, phylogenetic analysis showed that the isolates reported in this study fall with other animal Fasciolae isolates in the same clade. Similarly, the analysis showed that *F. gigantica* fall in the same clade with four human fasciolae isolates previously obtained from Vietnam. Likewise, *Fasciola hepatica* sequence was found to share one clade with a one isolate recently recovered from human in Assiut Governorate (Khalifa et al., 2016). These similarities demonstrate the close relatedness which indicating that the isolates likely originated from a common ancestor.

In **conclusion**, the present study showed that *Fasciola* species is circulating

among animals in Assiut and Sohag Governorates. Though low prevalence was demonstrated in comparison with other districts in Egypt, appropriate control measures are required to completely eradicate the disease among animal reservoirs in order to avoid any possible public health threatening. The zoonotic burden of the disease among humans residing the survey areas and a further study to determine the contribution of different snails in the disease spread is needed.

5. Acknowledgements

The authors are grateful to the veterinarians of the Assiut and Sohag abattoirs for their help in providing data and sample collection throughout the study.

6. Conflict of interest:

All authors declare that there is no any conflict of interest.

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الملخص العربي

الانتشار والتحليل الوراثي لأنواع المتورقة (الديدان الكبدية) في صعيد مصر بناءً على التسلسل الجيني الريبوسومي ITS-2

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الملخص

داء المتورقة الكبدية (الفاشيولا) هو مرض طفيلي حيواني هام حيث يتسبب في خسائر اقتصادية سنوية تقدر بأكثر من 3 مليارات دولار أمريكي في الحيوانات وكذلك مخاطر العدوى للبشر في أكثر من 180 مليون شخص في جميع أنحاء العالم. ونظرًا للأهمية الاقتصادية والصحية العامة لهذه المتورقة الكبدية ، يلزم إجراء تقييم دوري لانتشارها وعوامل الخطر المرتبطة بها بالإضافة إلى الارتباط التطوري. في البحث المعروض، قمنا بفحص 1560 كبد من الماشية و 1630 كبد ماعز بحثًا عن وجود فشيولا بالغة في محافظتي أسيوط وسوهاج ، صعيد مصر. تم تحديد الخصائص الجزيئية والعلاقات التطورية بناءً على جين (ITS2) للحمض النووي الريبوزومي النووي (rDNA). كان معدل انتشار الفاشيولا 5.1% و 3.5% بين الماشية والماعز على التوالي. على الرغم من أن العمر والجنس ونظام التربية أثرت بشكل كبير على المرض في الماشية ، ولم يكن لأي من هذه المتغيرات تأثير كبير على داء الفاشيولا في الماعز. ومع ذلك ، فقد وجد أن الماعز التي تم أخذ عينات منها معرضة لخطر متزايد للإصابة مرض المتورقة الكبدية مع تقدم العمر وبدء فصل الشتاء. أظهرت تحليلات علم الوراثة المتسلسلة (phylogenetic analysis) أن كلاً من الفاشيولا جايجانتিকা والفاشيولا هيباتিকা في هذه الدراسة لهما سلف مشترك وأنهما يقعان في فرع واحد مع العديد من الفاشيولا المصرية والعالمية التي تم الإبلاغ عنها سابقاً من الحيوانات والبشر. توضح هذه الدراسة أن المرض ينتشر حالياً بين الحيوانات في محافظتي أسيوط وسوهاج مما يستدعي الحاجة الملحة لتطبيق إجراءات مكافحة المناسبة بالإضافة إلى تقييم العبء الحيواني المستمر المحتمل على الأشخاص المقيمين في هذه المناطق.

