Research Article

Prevalence, Molecular Characterization and Phylogenetic Analysis of *Fasciola* spp. Based on ITS2 Gene Sequence from Slaughtered Animals in Abattoirs and its public Health in Qualyobia Province, Egypt

Lobna M.A. Salem, Nashwa O. Khalifa, Marwa O. Abd El-Halim*

Department of Zoonoses, Faculty of Veterinary Medicine, Benha University, Moshtohor 13736, Egypt.

Abstract | Fascioliasis is a serious disease of sheep and cattle worldwide. *Fasciola* species parasitize a variety of mammals, caused by *Fasciola hepatica*, and *Fasciola gigantica*. Traditional morphological techniques to differentiate the two species can be inaccurate, especially when hybrid forms are present. The definitive identification of *Fasciola* species, including their hybrids, has been made possible by advanced molecular techniques. Our study aimed to estimate the prevalence of Fascioliasis and identify the phenotypic features of *Fasciola* that infecting sheep, cattle, buffaloes, goats, and camels in Qualyobia, Egypt. The genetic identity of *Fasciola* species was examined by the analysis of forward and reverse sequences of the ITS2 of the rDNA gene that amplified in 300bp. Out of 286 slaughtered animals {88 sheep, 26 goats, 68 cattle, 25 buffaloes and 79 camels} in the regions of Qualyobia, 51 (17.8%) had *Fasciola* spp. morphologically {27/51(52.9%) were identical to *F. hepatica* while 18/51(35.3%) were identical to *F. gigantica*, 3/51(3.9%) were larval stages and 3/51(3.9%) mixed infections}. DNA from 10 flukes extracted, amplified, and analyzed to identify species using the ITS2 locus. Ten flukes were identified as *F. gigantica* and *F. hepatica*. PCR products from 2 flukes were sequenced for phylogenetic analysis. The sequence of ITS2 gene isolates obtained from the present investigation were compared with GenBank reference sequences of *F. hepatica, F. gigantica*. The phylogeny based on ITS2 revealed two distinct clades separating *F. hepatica* from *F. gigantica* with snaps and indel in one isolate. So concluded that safety measures should be done because the disease has zoonotic effect on public health. The prevalence of Fascioliasis was high in Qualyobia, Egypt, a lot of liver was condemned, which resulted in financial losses for affected farmers. In order to control the disease and raise farmer awareness, it is vital to implement the appropriate preventive measures.

Keywords | *Fasciola* species, Prevalence, Phylogenetic analysis, ITS2 gene, Public health.

Received | September 07, 2022; **Accepted** | September 28, 2022; **Published** | October 20, 2022
***Correspondence** | Marwa O Abd El-Halim, Department of Zoonoses, Faculty of Veterinary Medicine, Benha University, Moshtohor 13736, Egypt; **Email:** marwavet91@yahoo.com
Citation | Salem LMA, Khalifa NO, Abd El-Halim MO (2022). Prevalence, molecular characterization and phylogenetic analysis of *fasciola* spp. based on ITS2 gene sequence from slaughtered animals in abattoirs and its public health in qualyobia province, egypt. Adv. Anim. Vet. Sci. 10(11): 2396-2406.
DOI | http://dx.doi.org/10.17582/journal.aavs/2022/10.11.2396.2406
ISSN (Online) | 2307-8316

INTRODUCTION

Fascioliasis is a parasitic infection with a significant negative impact on animal's productivity and causing widespread human zoonotic disease in several countries including Egypt, Algeria, Tunisia, Botswana, Niger, Vietnam, Mauritania, Korea, and Japan (Robertson and Mochankana, 2018).

Egypt is regarded as one of the Fascioliasis endemic countries in the world and of high parasitic burden in infected animals and humans (Arafa et al., 2018). Fascioliasis caused by *F. hepatica* and *F. gigantica* both utilize animals and humans as final hosts (Amer et al., 2016). Identification of these parasites based on the morphological and morphometric criteria; however, these traditional methods are still unreliable in definite differentiation (Liu et al.,

2014). For that, molecular methods must be used to overcome the challenges of identifying species based on their phenotypic features (Shoriki et al., 2016). When *Fasciola hepatica* flukes mature, they take on the appearance of brown, flatworms in the shape of leaves that are typically 20 to 30 mm in length and 13 mm in diameter. They have two suckers anteriorly; the larger, or acetabulum, located on the ventral side and allows the fluke to adhere to the bile duct wall and stay there, while the smaller, anterior sucker feeds on the bile. *Fasciola gigantica* flatworms can reach lengths of up to 5 to75 mm in length and 12 mm in diameter but are look similar to their smaller cousins. (Mas-Coma, et al., 2014).

In Egypt, *F. gigantica* is common among ruminants along the Nile Valley, while *F. hepatica* is reported in imported sheep and cattle whereas; both species co-exist alongside native livestock animals in Egypt (Mas-Coma et al., 2019). Fascioliasis decreases animal productivity, weight gain, and the output of meat and milk. Due to decreased immunity brought on by chronic Fascioliasis and liver condemnation during postmortem examination in slaughterhouses, it also contributes to moderate icterus, metabolic problems, and secondary infections, while acute Fascioliasis may result in fatalities (Eman et al., 2016).

Fasciola has a complicated life cycle that involves intermediate snail hosts like the common snail and final hosts such as mammals like humans. One miracidium is produced when eggs lost in the stool of the last host and embryonate in fresh water. To spread infection, the miracidium pierces the snail's (*Lymnaeidae* family) tegument. The majority of *Fasciola* transmission worldwide is carried out by snails of the species *Galba* and *Radix*, particularly *Galba truncatula.* The distribution of the intermediate host, which is required to maintain the parasite's viability in endemic areas, is reflected in the distribution of *Fasciola* risk and infection (Vázquez et al., 2018).

The grazing habits of animals on grassland are thought to have caused a variation in Fascioliasis. In general, cattle like to graze close to springs and streams where snails are prevalent. In contrast, compared to sheep, goats often have the lowest infection rate. Goats graze differently than sheep because they naturally prefer to eat leaves and health at elevated areas, opposite to sheep which frequently graze on the ground. The risk of infection in goats may be reduced by this type of grazing because there is less exposure to contagious metacercaria (Manoochehr et al., 2017).

Molecular studies have confirmed that both species can be differentiated by mitochondrial DNA sequencing of NDI and COI or nuclear ribosomal of internal transcribed spacers (ITS) 1 and 2 (Amer et al., 2011). Studies in Thailand have also verified that *Fasciola* hybrid form present in the livestock liver that has been identified as hybrids based on the sequences of ITS1 and ITS2 (Siribat et al., 2018). For molecular and genetic characterization of *F. gigantica, F. hepatica*, and *Fasciola* hybrid form, ITS1, and ITS2 have been renowned and more efficient genetic markers.

Moreover, in Egypt, researchers found the hybrid form that has a morphometric character between both species. The modern techniques of PCR and DNA sequencing ease the species identification, strain clarification (Sumruayphol et al., 2020). The selected gene or sequence needs to be common, extraordinarily moderated within, and sufficiently diverse between taxa. In a perfect world, the variable areas ought to have neighboring preserved areas so that „global" oligonucleotide primers may be chosen (Yuan et al., 2016). Egypt is considered as one of the Fascioliasis-endemic areas in the world (Amer et al., 2016) and the disease burden is high in the Nile Delta region. Both species, *Fasciola hepatica* and *Fasciola gigantica*, are commonly seen present in Egyptian sheep, and the occurrence of the hybrid form has been reported (Amer et al., 2011). This disease is severely affecting the sheep farming industry and causing severe economic loss, especially in the reduction of wool production, meat, and total body weight (Amer et al., 2016).

The objectives of this study were to determine the prevalence of Fascioliasis in slaughtered animals, molecular characterization, and phylogenetic analysis of *Fasciola spp*. worm and its public health in Qualyobia Province, Egypt.

MATERIALS AND METHODS

Study area

The current study was performed in different slaughtered houses of the Qualyobia region, located in Lower Egypt. It is located in the Nile Delta region, north of Cairo. This study was approved by the Department of Zoonoses, Faculty of Veterinary Medicine, Benha University, Egypt. The weather is typically dry, with scorching or extremely hot summer days and warm or mild winter days.

Gross examination of liver and data collection

At the abattoir, physical examination (antemortem) was performed shortly prior to slaughter of 286 animals to determine sex, age, body condition, grazing system and region. Following animal slaughter, the liver was extensively checked post-mortem for the presence of liver flukes. Multiple cuts and subcuts about 1 cm thick were done to check for the existence of *Fasciola* parasites, which made scratchy sounds, exerted brownish fluid, and immature *Fasciola*. Identification of the *Fasciola* species based on the morphological characters of the agent and classify into *F. gigantic* and *F. hepatica* (Urquhart et al., 1996).

Sample collection

The liver was collected from (286) slaughtered animals (88 sheep, 26 goats, 68 cattle, 25 buffaloes, and 79 camels) from June 2018 until March 2021 from El-Ramla abattoir, Toukh abattoir, Sendyoon abattoir, mini-abattoirs in El-Shamut, and Shebeen Al-Qanater abattoir at Qualyobia province, Egypt and were examined for *Fasciola* flukes during the regular postmortem inspection. The obtained worms were washed separately using saline water (0.9%) and repeated washing at least thrice to eliminate the debris, all samples were transported in ice box to laboratory of Zoonoses, Faculty of veterinary medicine, Benha University, then were maintained in ethanol (70%), and kept at – 20˚C for the extraction of genomic DNA.

Morphological examination

Liver flukes were taken out of the 70% ethanol and dyed with Carmine staining method according to (Gibbons et al., 1996). The flukes were washed in tap water to eliminate any remaining ethanol from preservation, and then they were rehydrated using a graduated range of alcohol (70%, 50%, 30%, 10%, and distilled water). The next step was to regressively stain the flukes by soaking them in Aceto Alum Carmine stain for an entire night. The excess stain was then rinsed off with distilled water. To remove excess staining without losing the coloration, they were then immersed in alcoholic acid (2 ml of concentrated HCl in 100 ml of 70% ethanol) for 2-4 hours. The samples were subsequently dehydrated in escalating series of graded alcohols (50% - 70% - 80% - 90% - 100%) and then cleaned with clove oil for 24hours. The specimens were then mounted using Canada balsam and internal organs examined by light microscope.

Genomic DNA extractions and amplification

According to the manufacturer's instructions, the genomic DNA was extracted from each adult worm (10 *Fasciola* flukes) using a QIAamp® DNA Mini Kit from Qiagen in Hilden, Germany. Genomic DNA was eluted in 50 µl elution buffer and stored at –20˚C until used for molecular identification. The primer pair DSJF included a forward primer (5' –ATA TTG CGG CCA TGG GTT AG-3') and a reverse primer known as DSJ3 (5'- CCA ATG ACA AAG TGA CAG CG-3') that was unique for *F. hepatica* was employed. Another species-specific reverse primer DSJ4 (5'- CCA ATG ACA AAG TAA CAG CA-3') specific for *F. gigantica* was also used with DSJF forward primer. These *Fasciola* specific primers were used at a concentration of 50 ρmol for the amplification of approximately 300 bp fragment for *Fasciola* species (Ai et al., 2010). All PCR amplification reactions, including control negative samples, were all based on a 25-ml reaction mixture containing 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 2 mM MgCl2, 200 mM of each deoxyribonucleotide triphosphate, 1.25 U Taq polymerase and 1 ml DNA template. The thermocycler that was set to give 5 min at 94˚C, followed by 30 cycles, each of 30 s at 94˚C, 30 s at 60˚C and 30 s at 72˚C, before a final extension for 5 min at 72˚C. The amplicons (4 ml) from each PCR were separated by electrophoresis in 1.0% (w/v) agarose gel, stained with ethidium bromide, and photographed using a gel documentation system (UVI tec, Cambridge, U.K.).

Sequencing and phylogenetic analysis

The sequencing of random samples of the amplicons occurs using DSJf/DSJ3 from *F. hepatica* samples and DSJf/DSJ4 from *F. gigantica* samples and findings matching the appropriate ITS-2 sequences of *F. hepatica* (GenBank accession MZ396929.1) and *F. gigantica* (MN970008.1). The produced sequences were manually adjusted, then they were compared to equivalent sequences of the *F. hepatica* and *F. gigantica* accessible in GenBank database by the Basic Local Alignment Search Tool (BLAST) application (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=Blast Search). The ITS2 rDNA gene sequences of various *Fasciola* isolates from various geographical locations were obtained from GenBank (www.ncbi.nlm.nih.gov/genbank), and the completed protein sequences were aligned using the MEGA6.0 programme (Tamura et al.,, 2013), using default alignment parameters and manual adjustment. The maximum likelihood approach was used to conclude the phylogenetic analysis. For genetic sequence and correlation analysis of the ITS2 gene in diverse *Fasciola* isolates belonging to different locations, MEGA 6.0 was employed. This programme allowed for the creation of multiple alignments of the ITS2 gene for all *Fasciola* isolates (Li, 2003).

Statistical analysis

Chi square (X^2) test was used to determine association between variables (prevalence, locality, season, sex, age, and rearing system) and their degree of significance. A P value <0.05 was considered significant.

RESULTS

Prevalence of Fascioliasis and its risk factors in slaughtered animals

The infection rate of Fascioliasis in examined slaughtered animals in different abattoirs in Qualyobia was 17.8% (51/286). The specific prevalence of Fascioliasis was 7(28%), 23(26.1%), 16(23.5%), 5(19.2%), and 0 (0%) in buffaloes, sheep, cattle, goats, and camels, respectively (Table-1). So, the higher infection rate was found in buffaloes and sheep then cattle and goats, but this difference was not significant with P value >0.05 (Table-1).

Table 1: Shows the prevalence of *Fasciola* species among slaughtered animals

Livestock species	No. of examined animals	Infected animals	Prevalence rate (%)	X^2 P value
Sheep	88	23	26.1	.7
Goats	26	5	19.2	.8
Cattle	68	16	23.5	
Buffaloes	25	7	28	
Camels	79	0	0	
Total	286	51	17.8	

Table 2: Shows the frequence distribution of *Fasciola* species among positive slaughtered animals:

Positive animal species (51)	F. hepatica		F. gigantica		Mixed infection		Larval stage	
	No.	%	No.	%	No.	%	No.	%
Sheep	12	44.4	8	44.4	-	-	3	100
Goats	4	14.8	1	5.6	-	-	-	-
Cattle	9	33.3	4	22.2	3	100	-	-
Buffaloes	2	7.4	5	27.8	-	-	-	-
Total	27	52.9	18	35.3	3	5.9	3	5.9

Table 3: Shows the infection rate of *Fasciola* spp. among sheep and goats in relation to associated risk factors:

Infection with *Fasciola* spp.									
Species		No. of examined animals		No. of infected animals		Infection rate (%)		X^2 P value	
Risk factors		Sheep	Goats	Sheep	Goats	Sheep	Goats	Sheep	Goats
Locality	Benha	43	14	9	5	20.9	35.7	2.1	7.2
	Shebeen	15	3	6	-	40	-	.35	.02*
	Toukh	30	9	8	-	26.7	-		
	Total	88	26	23	5	26.13	19.2		
Age	1-2year	-	-	-	-	-	-	1.36	.5
	2-3year	69	19	16	3	23.18	15.8	.24	.47
	>3year	19	7	7	2	36.8	28.6		
	Total	88	26	23	5	26.13	19.2		
Sex	Male	63	13	18	5	28.57	38.5	.68	8.1
	Female	25	13	5	-	20	-	.4	.004*
	Total	88	26	23	5	26.13	19.2		
Season	Winter	36	5	6	4	16.66	80	8.7	13.75
	Spring	13	11	4	1	30.76	9.1	.03*	.003*
	Summer	16	3	2	-	12.5	-		
	Autumn	23	7	11	-	47.8	-		
	Total	88	26	23	5	26.13	19.2		
Rearing system	Housed(indoor)	57	10	4	-	7.017	-	30.6	5.58
	Free range(outdoor)	31	16	19	5	61.29	31.3	.00*	.01*
	Total	88	26	23	5	26.13	19.2		
Signs	Bottle jaw	-	-	-	-	-	-	16.5	6.1
	Feces (pasty)	85	15 Pasty +11diarrhea	19	3 Pasty +2 diarrhea	22.4	19.2	.001*	.04*
	Pale mm	21	-	12	-	57.1	-		
	Body condition	16	7	10	2	62.5	28.6		
	Rough coat	11	2	6	2	54.5	100		
Place of slaughter	In shops at villages	36	19	17	4	47.2	21.1	14.3	14.03
	abattoir	52	7	6	1	11.5	14.3	.000*	.000*
	Total	88	26	23	5	26.13	19.2		

Table 4: Shows the infection rate of *Fasciola* spp. among cattle, buffaloes, and camels in relation to associated risk factors

Infection with *Fasciola* spp.												
Species		**No. of examined animals**			**No. of infected animals**			**Infection rate (%)**			**X^2 P value**	
Risk factors		Cattle	Buffaloes	Camels	Cattle	buffaloes	camels	Cattle	buffaloes	Camels	Cattle	buffaloes
Locality	Benha	37	13	-	9	5	-	24.3	38.5	-	.09	4.68
	Shebeen	12	6	4	3	-	0	25	-	0	.9	.09
	Toukh	19	6	75	4	2	0	21.05	33.3	0		
	Total	68	25	79	16	7	0	23.5	28	0		
Age	1-2year	15	-	-	2	-	-	13.33	-	-	1.33	.99
	2-4year	36	18	-	9	6	-	25	33.3	-	.5	.31
	>4year	17	7	-	5	1	-	29.4	14.3	-		
	≥ 8 years	-	-	79	-	-	0	-	-	0		
	Total	68	25	79	16	7	0	23.5	28	0		
Sex	Male	50	15	63	11	6	0	22	40	0	.24	2.95
	Female	18	10	16	5	1	0	27.7	10	0	.62	.08
	Total	68	25	79	16	7	0	23.5	28	0		
Season	Winter	25	10	9	7	3	0	28	30	0	.50	1.7
	Spring	16	5	24	3	2	0	18.75	40	0	.91	.63
	Summer	9	2	31	2	-	0	22.2	-	0		
	Autumn	18	8	15	4	2	0	22.2	25	0		
	Total	68	25	79	16	7	0	23.5	28	0		
Rearing system	Housed (indoor)	15	14	79	3	-	0	20	-	0	.13	15.2
	Free range (outdoor)	53	11	-	13	7	-	24.5	63.6	-	.7	.000*
	Total	68	25	0	16	7	0	23.5	28	0		
Signs	Bottle jaw	-	-	-	-	-	-	-	-	-	12.1	4.8
	Feces (pasty)	59	23	-	16	7	-	27.1	30.4	-	.007*	.2
	Pale mm	24	3	-	2	-	-	8.3	-	-		
	Body condition (Weakness)	13	14	-	5	3	-	38.5	21.4	-		
	Rough coat	9	5	-	6	-	-	66.7	-	-		
	Not respond to pregnancy	7	-	-	5	-	-	71.4	-	-		
Place of slaughter	In shops at villages	29	11	-	14	7	-	48.3	63.6	-	17.7	15.2
	abattoir	39	14	79	2	-	0	5.1	-	0	.000*	.000*
	Total	68	25	79	16	7	0	23.5	28	0		

F. hepatica was the predominant species 52.9% (27/51), *F. gigantica* was 35.3% (18/51), mixed infection was 5.9% (3/51) and larval stage was 5.9% (3/51) (Table-2). Based on the animals species; sheep has 12 *F. hepatica*, 8 *F. gigantica* and 3 larval stages, goats have 4 *F. hepatica* and 1 *F. gigantica*, cattle has 9 *F. hepatica*, 4 *F. gigantica* and 3 mixed infection while buffaloes have 2 *F. hepatica* and 5 *F. gigantica* (Table-2). Based on PCR product, 10 *Fasciola* isolates were corresponding to *Fasciola* hybrid form as they given positive result when used with primer of *F. hepatica*, and *F. gigantica* (Figure-2,3).

Our result revealed that the relation between the incidence of Fascioliasis and gender which revealed that males 40/51 (78.4%) more than females 11/51 (21.6%) animal's carcasses. In all animals' species male was infected more than female except in cattle but was not significant except in goat with P value =004 (Table-3). Locality was not affected on *Fasciola* infection in sheep in contrast to in goats with P value = .02. The incidence of Fascioliasis was higher in age more than 3 years in sheep and goats (Table-3). Fascioliasis was higher in sheep during autumn, spring, winter, and summer 11/23 (47.8%), 4/13 (30.8%), 6/36 (16.7%) and 2/16 (12.5%) respectively and was significantly with P value = .03 (Table-3). In goats, the infection was observed more in winter 4/5 (80%) than spring 1/11 (9.1%) with P value= .003 (Table-3). There was a significant difference in sheep and goats in between different seasons because of the rainy season, presence of fresh green grazing pasturing and spreading of the snail's host. High *Fasciola*

infection recorded in animals reared in free rang system (outdoors) than housed ones (indoors), 44/51 (86.3%) and 7/51 (13.7%) in all animals respectively with P value = .00, .000 and .01 in sheep, buffaloes, and goats, respectively and these were significantly (Table-3,4). Most of infected slaughtered animals suffered from weakness, rough coat, pasty feces, and pale mucus membrane. Moreover, the higher infection found in animals that slaughtered in shops at villages than that slaughtered in abattoir due to lake of meat inspection, 17/36 (47.2%), 6/52 (11.5%) in sheep, 4/19 (21.1%), 1/7 (14.3%) in goats. 14/29 (48.3%), 2/39 (5.1%) in cattle, 7/11 (63.6%), 0/14 (0%) in buffaloes that slaughtered in shops in villages and abattoirs respectively (Table-3,4).

The incidence of Fascioliasis was higher in age 2-4 years in buffaloes (Table-4) and in age more than 4years in cattle (Table-4), this was not significantly with P value >0.05. Moreover, in rainy season than dry ones due to presence of fresh green grazing pasturing, high population of snails and encysted metacercaria. According to seasons, the infection in cattle and buffaloes were 7/25(28%), 3/10 (30%) in winter, 3/16 (18.8%), 2/5 (40%) in spring, 2/9 (22.2%), 0/2 (0%) in summer and 4/18 (22.2%), 2/8 (25%) in autumn respectively, but this was not significantly (Table-4). The differences among the geographical locations could be attributed mainly due to increase in irrigated land masses in the research area and farmers' propensity to feed their livestock in these marshy and wet areas due to a lack of feed. The ecological condition is favorable for the survival and development of the snail intermediate host for species of *Fasciola*.

Macroscopic examination of liver of infected animals with *Fasciola*

Grossly regarding Fascioliasis infection during slaughterhouse postmortem inspection revealing the external smooth liver surface declared multiple white or creamy tunnels ranging from few millimeters to nearly 3 cm (Figure-1, A) represented the postmortem liver fibrosis appear from external liver surface. *Fasciola* tunnels that were observed from undamaged liver surfaces oozing grassy blackish hemorrhagic exudate and declared different took photos of creamy leaf-like *Fasciola* spp. about 1.5-2.0 cm in length and about 1.0 cm in width (Figure-1, B). These reduced the value of the infected carcass and the liver and led to consumer rejection of the liver.

Figure 1A: *Fasciola* tunnels and fibriosis of liver.

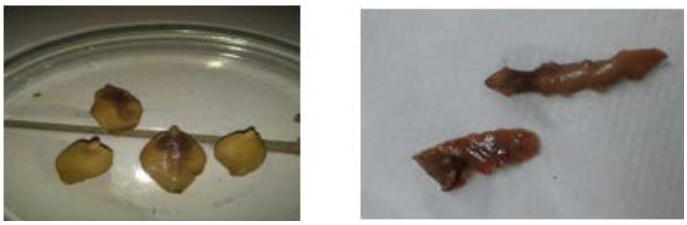

Figure 1B: Macroscopic leaf-like *Fasciola spp*.

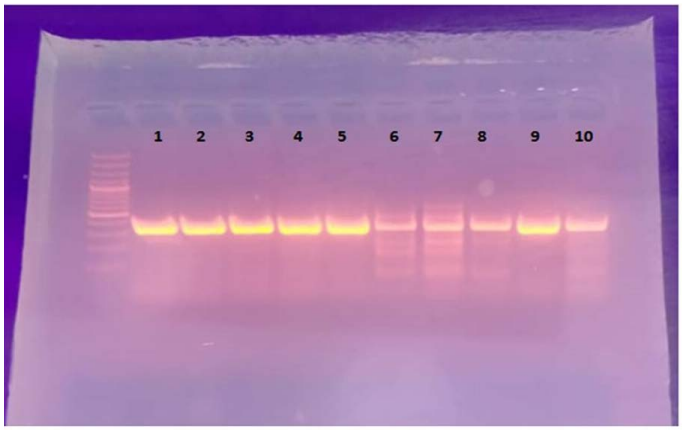

Figure 2: Polymerase chain reaction product 300 bp of partial nucleotide sequences of the ribosomal DNA of ITS2 gen. Lane M: DNA ladder (1000 bp). Lane: 1-10 represent *Fasciola* isolates with DSJf/DSJ3 primer of *Fasciola hepatica*.

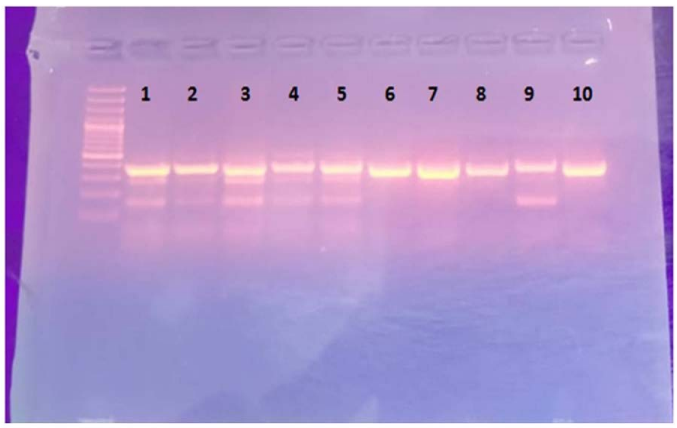

Figure 3: Polymerase chain reaction product 300 bp of partial nucleotide sequences of the ribosomal DNA of ITS2 gen. Lane M: DNA ladder (1000 bp). Lane: 1-10 represent *Fasciola* isolates with DSJf/DSJ4 primer of *Fasciola gigantica*.

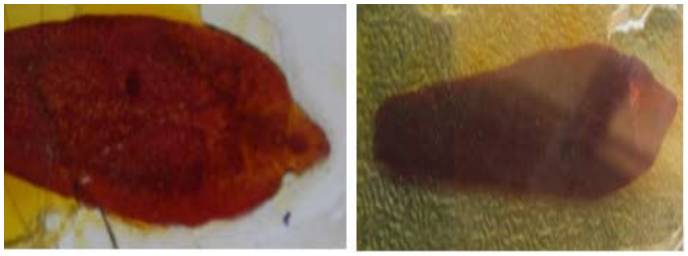

Figure 4A: *Fasciola hepatica* after stained with Aceto Alum Carmine stain.

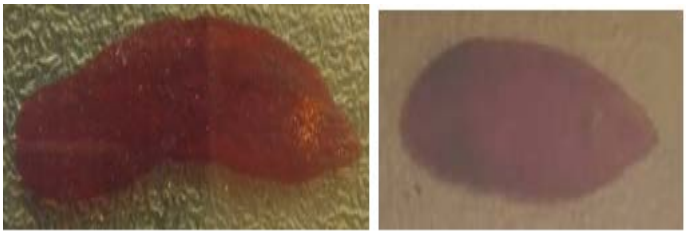

Figure 4B: *Fasciola gigantica* after stained with Aceto Alum Carmine stain.

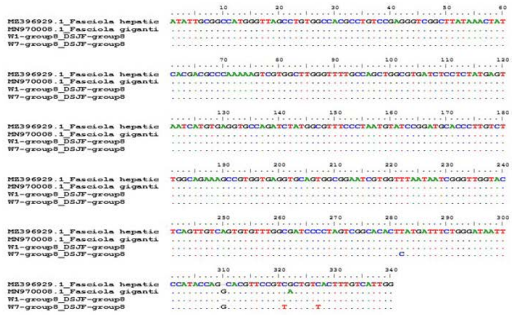

Figure 5: Alignment of ITS2 sequence of *F. hepatica* (GenBank accession MZ396929.1) and *F. gigantica* (MN970008.1) deposited in GenBank with *F. hepatica* and *F. gigantica* that found in livers of infected animals in Qualyobia Province, Egypt, snaps at 282, 321pb and indel at 310 pb in W7 isolate.

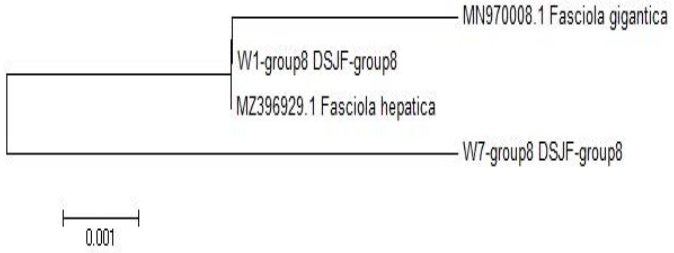

Figure 6: Phylogenetic relationship based on nucleotide sequence of ITS2 gene by phylogeny bootstrap test using neighbor-joining method in MEGA 6.0 software. two isolates cluster in two clades.

PCR AND PHYLOGENETIC ANALYSIS

In this study, 10 isolates of *Fasciola* were investigated from 51 naturally infected sheep, goats, cattle, and buffaloes. Genomic DNA was productively extracted, and the specific primers (DSJf/DSJ3 for *F. hepatica* and DSJf/DSJ4 for *F. gigantica*) were used in this work successfully amplified a fragment of 300 bp of a part of ITS2 gene of *Fasciola* samples (Figure 2,3). 10 isolates give bands at 300 pb when used primers of *Fasciola hepatica* and primers of *Fasciola gigantica* this mean that the ten isolates are intermediate form of *Fasciola*. Alignment analysis through online blast reveals similarity of 100% (isolate W1 GenBank accession OP245092)) and some variability (SNP, INDEL) in (isolate W7 GenBank accession OP245094) of inspected sequence fragment when compared with GenBank database of *F. hepatica and F. gigantica* available reference data (GenBank accession MZ396929.1) and (MN970008.1) respectively. Furthermore, it's clear and interesting that 1 of 2(50 %) of isolates shows SNP (T to C) of the 282nd, (C to T) of the 321st nucleotide of an investigated fragment (Figure 5). Indel found in W7 isolate at 310 pb by deletion or insertion of G nucleotide. SNP may be due to the presence of hybrids in our isolates. The phylogenetic tree revealed the two isolates cluster in two clades (Figure 6).

DISCUSSION

Fascioliasis caused by the *Fasciola hepatica* and *Fasciola gigantica* which is a plant borne trematodes that are hazardous to health and reduce animal productivity and damage economic activities. Consumer rejection of the liver results in a loss for the economy. In addition, an acute infection with *Fasciola* results in weight loss, stunted growth, and infertility. There was a decrease in productivity while the disease was in its chronic phase Mehmood et al. (2017).

The result of this study indicates that the infection rate of Fascioliasis was (17.8%) among the examined slaughtered animals. The reason for this infection could be due to increase in irrigated land masses in the research area and the propensity of farmers and animal rearers to feed their livestock in marshy and damp areas behind the grain because of a lack of feed. Since the intermediate host favours marshy areas with slow-moving water and small streams, which also provide enough moisture for the infective metacercaria to survive Magaji et al. (2014).

The higher infection was found in buffaloes 28% followed by sheep 26.1%, cattle 23.5%, goats 19.2% and camels 0%. The difference of the incidence among slaughtered animals might be attributed to the grazing style of animals but this difference was not significant with P value >0.05 (Table-1). *F. hepatica* was the predominant species 27(52.9%) (12 in sheep, 4 in goats, 9 in cattle and 2 in buffaloes), *Fasciola gigantica* was 18(35.3%) (8 in sheep, 1 in goats, 4 in cattle and 5 in buffaloes), mixed infection was 3(5.9%) in cattle and larval stage was 3 (5.9%) in sheep morphologically (Table-2), our result was lower than that obtained by Usman, (2019) in Nigeria (40.5%) in which higher rate was observed in cattle than sheep and goats, and Ieren et al. (2016) in Nigeria (48%) in which cattle more infected than sheep and goats (males more than females and active rain season than early dry season) and Zemene and Atnaf, (2015) in Ethiopia (21.8%) in which cattle more infected than sheep and goats and in adult animals than young ones, higher infection observed in poor body condition followed by medium body condition. The wide variation

in prevalence between various nations could be primarily attributed to variations in climatic conditions, such as rainfall and temperature Fox et al. (2011) as well as other significant factors like grazing in marshy pastures, having access to streams or ponds, having a large herd, and the type of soil Howell et al. (2015). Additionally, human activity (large urbanization, the growth of irrigated regions, and the transformation of natural wetlands into agricultural lands) is linked to the spread of water and food-borne illness Sabourin et al. (2018).

The present study revealed that the occurrence of Fascioliasis in sheep was 23/88 (26.13%), this result was lower than that recorded by Usman, (2019) in Nigeria which was 39.1%, and female sheep recorded higher infection (44.6%) than males (30.7%) in contrast to our result that higher in males (28.57%) than females (20%). Our result was higher than that recorded by Zemene and Atnaf, (2015) in Ethiopia which was 20.14%, and adult sheep more infected than young ones. Also, was higher than that recorded by Jean-Richar et al. (2014) 23% and Ouchene-Khelif et al. (2018) in Algeria 6.5%, the seasonal infection of *Fasciola hepatica* was highest in winter for sheep.

The infection rate of Fascioliasis was 5/26(19.2%) in examined goats and was higher between older (2-3, >3years) than young goats, in winter than other seasons, among males than females and in freely reared goats (outdoor) than housed animals (indoor). Our result was higher than that recorded by Hassan et al. (2019) in Giza Governorate 0.89%, IrfanUllah et al. (2016) in Pakistan 6.6%, Çelik and ÇELİK, (2018) in Turkey 14.14%, Abah et al. (2019) in Nigeria 6.2%, Abdelazeem et al. (2020) in Egypt 3.5%, Our result also was higher than that recorded by Ouchene-Khelif et al. (2018) in Algeria 2.5%, the seasonal prevalence of *Fasciola hepatica* was highest in summer and winter for goat. While our result was lower than that recorded by Usman, (2019) 35% but in those study the infection was higher in females than males. According to age, sex, season, and rearing system, our results agreed with Abdelazeem et al. (2020) in Assiut and Sohag Governorates, Upper Egypt who said that the highest infection found between older than young goats, in winter than other seasons, among males than females and in freely reared goats than housed animals, but disagreed with Abah et al. (2019) who revealed that female goats more infected than male goats.

The infection rate of Fascioliasis in cattle was 16/68 (23.5%) and was higher in female 5/18 (27.7%) than male 11/50 (22%), more at age > 4year 5/17 (29.4%) than in age 2–4year 9/36 (25%) and 1–2year 2/15 (13.33%), in rainy season than dry ones, in winter 7/25 (28%), 4/18 (22.2%) in autumn, 2/9 (22.2%) in summer and 3/16 (18.75%) in spring. In free rang system (outdoor) 13/53 (24.5%) than housed system (indoor) 3/15 (20%), infected animals had pasty feces, pale mucus membrane, rough coat, weakness and 5 infected females not pregnant. Infection more in Shebeen 3/12 (25%) than Benha 9/37 (24.7%) and Toukh 4/19 (21.05%). Infection rate was higher in cattle that slaughtered in shops in village 14/29 (48.3%) than that slaughtered in abattoirs 2/39 (5.1%). Our result was higher than that recorded by Ejeh et al. (2015) in Nigeria 14.6%, Henok and Mekonen, (2011) in Ethiopia 14.6% in which adult recorded higher infection, while was lower than that recorded by Jean-Richar et al. (2014) in the Lake Chad area of Chad 68% in cattle that grazed at lake water which was the source of infection and all infected with *F. gigantica,* Usman, (2019) in Nigeria 45.7% in which males more infected than females and aged cattle above five years recorded higher infection, Zemene and Atnaf, (2015) in Ethiopia 30.6% in which adult cattle recorded higher infection, Yemisrach and Mekonnen, (2012) in Ethiopia 28.6% but young cattle recorded higher infection than adult ones and Ouchene-Khelif et al. (2018) in Algeria 26.7%, the seasonal infection of *Fasciola hepatica* was highest in summer and winter for cattle.

The infection rate of Fascioliasis in buffaloes was 7/25 (28%) and was higher in male 6/15 (40%) than female 1/10 (10%), more at age 2–4-year 6/18 (33.3%) than > 4 year 1/7 (14.3%), more in spring 2/5 (40%) than winter 3/10 (30%) and Autumn 2/8 (25%). All infected buffaloes were in free rang system (outdoor) 7/11 (63.6%), infected buffaloes had pasty feces, and some had weakness. Infection more in Benha 5/13 (38.5%) than Toukh 2/6 (33.3%). All infected buffaloes slaughtered in shops in villages. Our result was higher than that recorded by Adane et al. (2019) 20.24% in which the higher infection was recorded in female than male and in old animals than young, Mohammed et al. (2016) 24.4% in which the highest infection found in young than adult animals. Our result in large ruminant (cattle and buffaloes) was 23/93 (24.7%) these was higher than that recorded by Fazly et al. (2015) 6/80 (7.50%) in cattle and buffalo samples and *F. gigantica* was only identified in the samples.

The current study revealed the *Fasciola* species that infect sheep, goats, cattle, and buffaloes from Qualyobia, Egypt was *Fasciola* hybrid form (Figures 2 and 3). This agreed with Mosaab et al. (2021) who found *Fasciola* hybrid form in sheep and buffaloes in Aswan, Egypt, but disagreed with the results of Amer et al. (2011) who found that *F. hepatica* was predominant in sheep compared to other hosts, also disagreed with Salihu et al. (2022) who siad that the genotype of *Fasciola* infecting cattle and other ruminant species in Nigeria was *F. gigantica*. Moreover, our result disagreed with Abdelazeem et al. (2020) who recorded that the *Fasciola* species that affected cattle and goats was *Fasciola he-*

patica and *gigantica* based on the sequencing of (ITS-2) of nuclear ribosomal DNA (rDNA). While our result in camels was 0% and this agreed with Ouchene-Khelifi et al. (2018) in Algeria who not found Fascioliasis in camels, and this may be attributed to those camels not grazed as in small and large ruminant.

Molecular characterization of the *Fasciola* species were detected based on partial sequences of ITS2 rDNA and the study revealed that the *Fasciola spp*. sequences from diverse hosts are almost matching to those of earlier available sequences. It has been shown that *Fasciola* hybrid form is existing in Qualyobia, Egypt, the hybrid form was recorded for the first time at south valley university, Egypt. These outcomes meet the full agreement with those found by Amer et al. (2011) and Mosaab et al. (2021) who recorded the presence of the three *Fasciola* species in Egypt including the hybrid form, while the present result differed from that recorded by Arafa et al. (2018) Egypt, Cairo who reported pure *F. gigantica* (isolate from cow and buffalo). However, El-Tahawy et al. (2018) from Nile Delta, Egypt recorded that the *Fasciola* in Egypt concerning the application of PCR for differentiation of two species of *Fasciola* by using certain primers of *F. hepatica*, they recorded that eight samples were found to have positive bands associated to *F. hepatica* and 2 negative bands representing *F. gigantica*. Alignment revealed that our result in W7 isolate (GenBank accession OP245094) was typical identical to found in Egypt (MT423006), (MW600275), (AJ853848), Kenya (MN970008), Nigeria (MN608173), Turkey (KY613944), Iran (KF866247), Germany (KF425321), South Africa (MW793533), Belgium (MW046876), Switzerland (MK321643) and Japan (AB010976) while W1 isolate (GenBank accession OP245092) was typical identical to found in Egypt (MT423007), Kenya (MZ396929), Libya (MT025519), Iran (JF432071), India (MZ413927), Viet Nam (MT429176), Saudi Arabia (MN559388), Spain (MG569979) and China (MH385388).

Alignment analysis confirmed our results and revealed similarity between one isolate (W1) with *Fasciola hepatica* isolate on GenBank when used primer of *F. hepatica* and W7 revealed similarity with some nucleotide differences (SNP, INDEL) with *F. gigantica* on GenBank when used primer of *F. gigantica* and may be due to the presence of hybrids in our isolates, and this outcome may be due to that our isolate is relatively recently introduced to Qualyobia Province as this the first time reported the *Fasciola* hybrid.

CONCLUSION

Fascioliasis is a zoonotic disease caused by parasites of the genus *Fasciola* spp. can result in significant losses for the animal's industry and have an impact on public health because there is a chance that an illness will spread to people. In present study the infection rate of Fascioliasis in slaughtered animals was 17.8% and the hybrid form was common in the study area. This calls for a policy strategy for the prevention and control of this neglected tropical illness. The suggestions below could be useful. The population needs to be made acutely aware of the threat posed by the spread of Fascioliasis in ruminant animals. More effort should be put into meat control because the area has become a popular selling and buying location for animals from crisis zones. It is necessary to use environmentally acceptable molluscides to reduce the intermediate hosts of the parasitic snails. Lack of sanitation facilities at the local abattoirs studied calls for an urgent concern.

CONFLICT OF INTERESTS

The authors have declared no conflict of interest.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Faculty of Veterinary Medicine, Benha University for supporting this work.

AUTHORS CONTRIBUTION

Lobna M. A. Salem, Nashwa O. Khalifa, Marwa O. Abd El-Halim: Planned the study design, collected, and examined samples, drafted and revised the manuscript. Both authors read and approved the final manuscript.

REFERENCES

Abah A, Wokem G, Sounyo I (2019). *Fasciola* infection in goats slaughtered from Port Harcourt metropolis Rivers State, Nigeria. Int. J. One Health. 5: 76-80. https://doi.org/10.14202/IJOH.2019.76-80

Abdelazeem AG, Abdelaziz AR, Khalafalla RE, Mostafa FN, Abushahba MFN (2020). Prevalence and Phylogenetic analysis of *Fasciola* species in Upper Egypt Based on Ribosomal ITS-2 gene Sequencing. EVMSPJ; 16:142-158 https://doi.org/10.21608/evmspj.2020.132162.

Adane Z, Yehualashet B, Anteneh W (2019). Prevalence of Bovine Fascioliasis and Its Economic Loss due to Liver Condemnation at Wolaita Sodo Municipal Abattair, Ethiopia. Vet. Med. Int., vol.2019, 7pp. https://doi.org/10.1155/2019/9572373

Ai L, Dong SJ, Zhang WY, Elsheikha HM, Mahmmod YS, Lin RQ, Yuan ZG, Shi YL, Huang WY, Zhu XQ (2010). Specific PCR-based assays for the identification of *Fasciola* species: their development, evaluation, and potential usefulness in prevalence surveys. Ann. Trop. Med. Parasitol., 104: 65-72. https://doi.org/10.1179/136485910X12607012373713

Amer S, Dar Y, Ichikawa M, Fukuda Y, Tada C, Itagaki T, Nakai Y

(2011). Identification of *Fasciola* species isolated from Egypt based on sequence analysis of genomic (ITS1 and ITS2) and mitochondrial (NDI and COI) gene markers. Parasitol. Int. 60: 5-12. https://doi.org/ 10.1016/j.parint.2010.09.003

Amer S, ElKhatam A, Zidan S, Feng Y, Xiao L (2016). Identity of *Fasciola* spp. in sheep in Egypt. Parasit. Vector. 9: 623. https://doi.org/10.1186/s13071-016-1898-2

Arafa WM, Hassan AI, Snousi SAM, El-Dakhly KHM, Holman PJ, Cragi TM, Aboelhadid SM (2018). *Fasciola hepatica* infection in cattle and freshwater snail *Galba truncatula* from Dakhla Oasis, Egypt. J. Helminthol. 92: 1-8. https://doi.org/10.1017/S0022149X17000086

Çelik ÖY, ÇELİK BA (2018). Investigation of the Prevalence of *Fasciola hepatica* in Small Ruminants in the Siirt Region, Turkey. Iran J. Parasitol. 13, 627.

Ejeh EF, Paul BT, Lawan FA, Lawal JR, Ejeh SA, Hambali IU (2015). Seasonal prevalence of bovine Fascioliasis and its direct economic losses due to liver condemnation at Makurdi abattoirs north-central Nigeria. Sokoto J.Vet. Sci. 13 (2): 50–56. https://doi.org/10.4314/sokjvs.v13i2.7

El-Tahawy AS, Kwan N, Sugiura K (2018). *Fasciola hepatica* infection in water buffalo Bubalus bubalis in three provinces of the Nile Delta, Egypt: a cross-sectional study. J. Vet. Med. Sci. 80: 28-35. https://doi.org/10.1292/jvms.17-0282.

Eman KA, Sherif MB, Reda SF (2016). Molecular characterization of *Fasciola hepatica* infecting cattle from Egypt based on mitochondrial and nuclear ribosomal DNA sequences. Res. J. Parasitol., 11: 61-66. https://doi.org/10.3923/jp.2016.61.66

Fazly A.Z, Muhamad SNNA, Wan NWOB, Geethamalar S, Mohd II (2015). Screening for Zoonotic Fascioliasis in Slaughtered Large Ruminants in Abattoirs in Perak, Malaysia. Trop. Life Sci. Res., 26(2): 121–124.

Fox NJ, White PCL, McClean CJ, Marion G, Evans A, Hutchings MR (2011). Predicting impacts of climate change on *Fasciola hepatica* risk. PLoS ONE, 6, 1, e16126. https://doi.org/10.1371/journal.pone.0016126.

Gibbons LM, Jones A, Khalil LF (1996). Manual for the 8th International Training Course on Identification of Helminth Parasites of Economic Importance. CAB International, Wallingford.

Hassan NM, Farag TK, El Ezz NMA, Abou-Zeina HA (2019). Prevalence assessment of gastrointestinal parasitic infections among goats in Giza Governorate, Egypt. Bull Natl. Res. Cent. 43: 1-7. https://doi.org/10.1186/s42269-019-0151-5

Henok M, Mekonen A (2011). Study on the prevalence and risk factors of Fascioliasis in small ruminants in and around Hirna town, Ethiopia. Global Vet., 7: 497-501.

Howell A, Baylis M, Smith R, Pinchbeck G, Williams D (2015). Epidemiology and impact of *Fasciola hepatica* exposure in high yielding dairy herds. Prevent. Vet. Med., 121, 1–2, 41–8. https://doi.org/10.1016/j.prevetmed.2015.05.013.

Ieren II, Ajanusi OJ, Mbaya PY (2016). Prevalence of liver flukes in cattle, and small ruminants at slaughter in Zaria, Nigeria. Res. Zool., 6(3): 33-36. Https://doi.org/ 10.5923/j.zoology.20160603.01.

IrfanUllah MFN, Jadoon AAK, Tabassum S (2016). Prevalence of *Fasciola hepatica* in domesticated cattle of district Karak, Khyber Pakhtunkhwa, Pakistan. Sciences. 10, 85-91. https://doi.org/10.3923/ajas.2016.85.91

Jean-Richard V, Lisa C, Abbani AA, Ngandolo BN, Helena G, Jan H, Esther S, Jakob Z (2014). Prevalence of *Fasciola gigantica* infection in slaughtered animals in south-eastern Lake Chad area in relation to husbandry practices and seasonal water levels. BMC Vet. Res.10:81 http://www.biomedcentral.com/1746-6148/10/81.

Li KB (2003). ClustalW-MPI: ClustalW analysis using distributed and parallel computing. Bioinformatics. 19:1585-6. https://doi.org/10.1093/bioinformatics/btg192

Lui GH, Gasser RB, Young ND, Song HQ, Ai L, Zhu XQ (2014). Complete mitochondrial genomes of the intermediate form of *Fasciola* and *Fasciola gigantica* and their comparison with *Fasciola hepatica*. Parasit. Vectors. 7: 150. https://doi.org/10.1186/1756-3305-7-150

Magaji AA, Kabir I, Salihu MD, Saulawa MA, Muhammed AA, Musawa AI (2014). Prevalence of Fascioliasis in cattle slaughtered in Sokoto Metropolitan Abattoir, Sokoto, Nigeria. Adv. Epidemiol., Vol. 4, 10 – 15. https://doi.org/10.1155/2014/247258

Manoochehr SK, Kavous S, Belal A, Hamidreza D, Masoud EJ (2017). A reducing trend of Fascioliasis in slaughtered animals based on abattoir data in South of Iran. Vet. World. Vol. 10, EISSN: 2231-0916.

Mas-Coma S, Valero MA, Bargues MD (2014). Fascioliasis. Adv. Experimental Med. Biol. 766: 77-114. https://doi.org/10.1007/978-1-4939-0915-5_4.

Mas-Coma S, Villavicencio AF, Bargues MD, Artigas P, Guamán R, Ulloa SM, Romero J, Osca D (2019). Lymnaeid snail vectors of Fascioliasis, including the first finding of *Lymnaea neotropica* in Ecuador, assessed by ribosomal DNA sequencing in the southern zone close to the Peru border. Acta Parasitolog. 64: 839-849. https://doi.org/10.2478/s11686-019.

Mehmood K, Hui Z, Ahmad J, Rao Z, Muhammad I, Aneela Z, Muhammad H, Mujeeb U, Muhammad K, Yajing W, Hafiz I, Tariq A, Riaz H, Muhammad T, Sadaqat A, Aman U, Jiakui L (2017). A review on epidemiology, global prevalence, and economical losses of Fascioliasis in ruminants Microb. Pathog. 109: 253–262.

Mohammed Y, Nuraddis I, Wubit T, Yosef D (2016): Prevalence of bovine Fascioliasis in municipal abattoir of Haramaya, Ethiopia. Food Sci. Qualit. Manag.48: 38-43.

Mosaab AO, Layla O EL-Majdoub, Alsagher OA, Dalia AI, Shimaa SS, Mohammad A AL-Wabel, Ahmed IA, Mickymaray S, Asmaa MM (2021). Genetic characterization and phylogenetic analysis of *Fasciola* species based on ITS2 gene sequence, with first molecular evidence of intermediate *Fasciola* from water buffaloes in Aswan, Egypt. Ann. Parasitol., 67(1): 55–65. https://doi.org/10.17420/ap6701.312.

Ouchene-Khelifi NA, Ouchene N, Dahmani H, Dahmani A, Sadi M, Douifi M (2018). Fascioliasis due to *Fasciola hepatica* in ruminants in abattoirs and its economic impact in two regions in Algeria. Trop. Biomed., 35 (1): 181-187. Article number: 19.

Robertson ID, Mochankana ME (2018). Cross-sectional prevelance of *Fasciola* trematodiases. Philos Trans. R Soc. Lond. B Biol. Sci. 364: 2763-2776.

Sabourin E, Alda P, Vázquez A, Hurtrez-Boussès S, Vittecoq M (2018). Impact of human activities on Fascioliasis transmission. Trend. Parasitol., 34(10): 891–903. https://doi.org/10.1016/j.pt.08.004.

Salihu MD, Musawa AI, Garba B, Yakubu Y, Bello MB, Magaji AA, Junaidu AU, Jibril A H, Ballah FM, Achi CR (2022). Molecular characterization and species differentiation of *Fasciola* parasite isolated from cattle slaughtered at Sokoto

Modern Abattoir, Nigeria. Nigerian J. Parasitol. ISSN 1117 4145 Volume 43[1]. https://doi.org/10.4314/njpar.v43i1.14

Shoriki T, Ichikawa-Seki M, Suganuma K, Naito I, Hayashi K, Nakao M, Aita J, Mohanta UK, Inoue N, Murakami K, (2016). Novel methods for the molecular discrimination of *Fasciola* spp. based on nuclear protein-coding genes. Parasitol. Int. 65: 180-183. https://doi.org/10.1016/j.parint.2015.12.002

Siribat P, Dekumyoy P, Komalamisra C, Sumruayphol S, Thaenkham U (2018). Molecular identification of *Fasciola* spp. representative samples from Thailand based on PCR-RFLP. J. Trop. Med. Parasitol. 41: 1-7.

Sumruayphol S, Siribat P, Dujardin JP, Dujardin S, Komalamisra C, Thaenkham U (2020). *Fasciola gigantica*, *F. hepatica* and *Fasciola* intermediate forms: geometric morphometrics and an artificial neural network to help morphological identification. Peer J. 8: e8597. https://doi.org/10.7717/peerj.8597.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Molecul. Biol. Evol. 2013:mst197.

Urquhart GM, Duncan J, Armour L, Dunn J, Jennings AM (1996). Veterinary Parasitology. 2nd ed. Blackwell Science, UK. p103-113.

Usman MI (2019). Studies on the prevalence of Fascioliasis among ruminant animals in northern Bauchi state, north-eastern Nigeria. Parasit. Epidemiol. Cont., 3-7.

Vázquez PA, Alda P, Lounnas M (2018). Lymnaeid snails hosts of *Fasciola hepatica* and *Fasciola gigantica* (Trematoda: digenea): a worldwide review. CAB Rev. Perspect Agric. Vet. Sci. Nutr. Nat. Resour. 13:1–15. https://doi.org/10.1079/PAVSNNR20181306210.

Yemisrach Abdulhakim, Mekonnen A (2012). An abattoir study on the prevalence of Fascioliasis in cattle, sheep and goats in Debre Zeit Town, Ethiopia. Global Vet. (3): 308-314.

Yuan W, Liu JM, Lu K, Li H, Duan MM, Feng JT, Hong Y, Liu YP, Zhou Y, Tong LB, (2016). Molecular identification and seasonal infections of species of *Fasciola* in ruminants from two provinces in China. J. Helminthol. 90: 359-363. https://doi.org/10.1017/s0022149x15000383.

Zemene L, Atnaf A (2015). A study on the prevalence, risk factors and financial loss of cattle, sheep, and goats Fascioliasis in Debrezeit Town, Ethiopia. American-Eurasian J. Scient. Res. 10 (3): 126-133, ISSN 1818-6785 © IDOSI Publications, 2015 Https://doi.org/10.5829/idosi.aejsr.2015.10.3.9573.