



Detection of *Fasciola hepatica* infection in cattle and *Lymnaea truncatula* snails in Dakhla Oasis, Egypt

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Abstract:

Fasciolosis in Dakhla Oasis, Elwadi El-Gadid, Egypt was investigated in its final host, cattle, and the intermediate host, *Lymnaea truncatula* snail. The study was conducted in 7 localities of Dakhla Oasis, Egypt. Fecal samples from 300 cattle were collected and examined, liver of slaughtered animals were inspected for flukes in abattoir. Moreover, Lymnaeidae snails were harvested from water streams and examined also. Flukes mitochondrial DNA-targeting PCR assay was carried out to amplify the extracted genomic DNA from both of infected snails and adult worms. *F. hepatica* eggs were found in 31.33% of examined cattle. Moreover, abattoir liver inspection revealed that 11.56% harbored adult flukes. The collected snails were identified as *Lymnaea truncatula* and *Lymnaea cailliaudi*. Furthermore, larval stages of *F. hepatica* (sporocysts, rediae and cercariae) were reported in 41/731 (5.61%) examined snails. However, *L. cailliaudi* snails were uninfected. Using the conventional PCR, *F. hepatica* specific amplicon size of 1030 bp was detected in isolates obtained from different localities. Detection of *F. hepatica* in the infected snails with mitochondrial DNA-targeting PCR was validated with the specific amplicon size. Based on the presence of snail intermediate host and the conventional PCR findings, *Fasciola* species in Dakhla Oasis were identified as *F. hepatica*. Accordingly, zoonotic importance and hygienic measures must be taken in consideration and highlighted by veterinarians and authorized agencies in both urban and rural areas.

Key words: *Fasciola hepatica*, cattle, infection, *Galba truncatula*, Dakhla Oasis, PCR

INTRODUCTION

Globally, fasciolosis is one of most important liver parasitic affections of herbivores caused by *Fasciola* spp. worms. The disease is a worldwide including a wide host range of grass-grazing animals such as sheep, goats, cattle, buffaloes, horses and rabbits (Soulsby, 1982). The Egyptian Academy of Scientific Research and Technology Report, highlighted on animal losses due to fascioliasis (about 190 million Egyptian pounds annually) (Eshazly et al., 2006). Moreover, Soliman (2008) mentioned that among patients admitted to hospitals suffering from fever of unknown origin, 4% had *F. hepatica* infection. *F. hepatica* has a wider range than its tropical counterpart, *F. gigantica*, but their geographical distribution overlaps in many African and Asian countries and sometimes in the same country, although in such cases the ecological requirement of flukes and their snail host are distinct (1980, Mas-Coma et al., 2005). In Egypt, the predominant species of *Fasciola* are *Fasciola gigantica* and *Fasciola hepatica* (Abdel-Nasser and Reffat, 2010; Dar et al., 2012).

Species of family Lymnaeidae are known for their role as intermediate hosts in the life cycle of *Fasciola* spp.

(Soulsby, 1982). The most predominant and widespread (Europe, Asia, Africa and North America) intermediate host of *F. hepatica* is *L. truncatula* (Soulsby, 1982). Furthermore, Dar et al. (2005) found *Lymnaea truncatula* naturally infected with *F. gigantica* in Egypt and several snail species might contribute to the spread of fasciolosis in Egypt. In Ismailia Governorate, Egypt, *L. truncatula* and *L. cailliaudi* snails were recorded for the first time from East of Suez Canal (Hassan, 1999). An experimental infection of the snail with *F. hepatica* was done (Dar et al., 2014), suggesting that it is an important intermediate host for the induction of fasciolosis in Egypt. Egyptian isolate of *F. hepatica* miracidia could be easily developed in French *L. truncatula* snail showing a very low mortality and producing metacercariae higher in number than those noted in sympatric infections (Dar et al., 2012). Even though, there is paucity in information about reporting of *F. hepatica* in *L. truncatula* snails in Egypt.

The current study aimed to identify *Fasciola* spp. infection in cattle and to investigate its molluscan intermediate host with mitochondrial DNA-targeting PCR assay in Dakhla Oasis, El wadi Elgadid, Egypt.

MATERIALS AND METHODS

2.1. Cattle

2.1.1. Fecal samples

Fecal samples of 300 cattle (107 males and 193 females) were collected from seven districts (El-Rashda, El-Hindo, El-Masra, El-Sheikhwali, Azab-Elqasr, El-Qasr and El-Aweyna) in the Dakhla Oasis, El-Wadi Elgadiid Governorate Egypt. Samples were directly collected from the rectum into plastic bottles with gloved hands, labeled by required data (age, sex, clinical signs, etc.) and brought to Dakhla Animal Health Research Laboratory (AHRL Dakhla). In the later, coproscopic examination was performed to detect the presence of *Fasciola* species eggs by concentration sedimentation technique (Soulsby, 1982).

2.1.2. Liver specimens

In abattoir of Mout, Dakhla province, 285 cattle (135 males and 150 females) were subjected to post mortem inspection to investigate presence of different *Fasciola* species worms during the period from January to December 2014. Flukes were collected in saline and were transferred in an ice tank to the laboratory for further examination. Collected flukes were again washed in saline, fixed and stained according to Drury and Wallington (1980) and were identified morphologically according to Soulsby (1982). For PCR assay, some intact adult flukes were preserved at 70% ethanol and stored at -20 °C till the DNA extraction.

2.2. Lymnaeidae snails

Water source and snails collection

Lymnaeidae snails (Fig. 1) were collected in clean bottles from the same districts in Dakhla province. The bottle cover was perforated to allow aeration for snails, and then

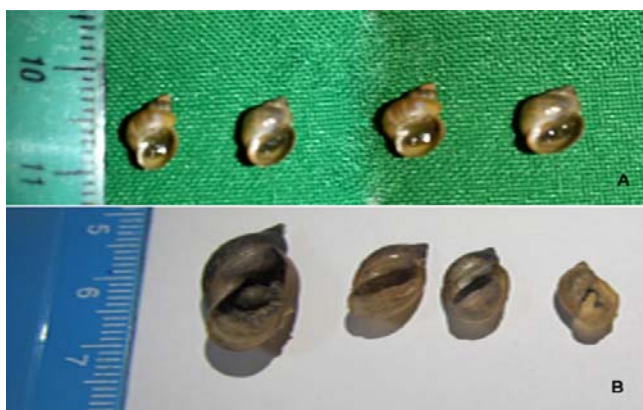


Figure 1: Lymnaeidae snails found in Dakhla Oasis, El-Wadi Elgadiid governorate, Egypt

A. *Lymnaea truncatula*

B. *Lymnaea cailliaudi*

snails were transported to the laboratory for investigation. Collected snails underwent microscopy for the presence of different stages of *Fasciola* spp. infection by exposure technique (three to five snails were placed in a Petri dishes half filled with dechlorinated tap water and daily exposed to a direct light using 100 watt electrical lamp for a period of 2 hours (Abd El-Ghany, 1955). An alternative technique was used by crushing fresh snails directly in suitable Petri dishes with a few amount of water under dissecting microscope, where available parthenatae were recorded (Jackson, 1958). Snails were identified according the key provided by Prof. Santiago Mas-Coma, WHO, Madrid, Spain.

2.3. Molecular identification of *Fasciola* species

2.3.1. Genomic DNA extraction

DNA was extracted from infected snails and adult flukes collected from the different localities in Dakhla province. Microscopically infected snails were properly crushed. Moreover, five adult flukes from each region were thorough washed and their cone shape projections were cut with a sterile scalpel. Prior to DNA extraction, cone shape projections were crushed and homogenized and DNA was extracted by PureLink® Genomic DNA Kits (Invitrogen, USA) according to manufacturer instructions.

2.3.2. DNA amplification

Fasciola spp. mitochondrial DNA-targeting PCR assay was carried out to amplify the extracted genomic DNA from both infected snails and adult worms (Le et al., 2012b). The PCR mixture for *Fasciola hepatica* consisted of 1 µl of the 10 pmol of FHF (GTTTTTTAGTTGTTTGGGGTTTG), and 1 µl FHGR (ATAAGAACCGACCTGGCTCAC), 3 µl of DNA template, 12.5 µl master mix (Biomatic®). The reaction was completed to 25 µl by adding 7.5 µl nuclease free H₂O. The PCR mixture for *Fasciola gigantica* consisted of 1 µl of 10 pmol FGF (TGTTATGATTCATTGTTTGTAG) and 1 µl FHGR (ATAAGAACCGACCTGGCTCAC), 3 µl of DNA template, 12.5 µl master mix (Biomatic®).

The reaction was completed to 25 µl by adding 7.5 µl nuclease free H₂O. Amplification was carried out by initial denaturation at 95°C for 3 min, then 35 cycles, including denaturation at 95°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 2 min, and then a final extension for 7 min at 72°C to complete the amplification. The amplicon yielded by the FHF/FHGR primer pair is 1,031 bp, and that yielded by FGF/FHGR is 615 bp after visualization on 2% agarose.

RESULTS

3.1. Fecal examination

Examination of 300 fecal samples revealed that 94 harbored *Fasciola* spp. eggs. Among those, 39.4% (64/163) of examined females were infected (Table 1). It has been found that El-Rashda district was the highest infected among the 7 examined districts (Table 2).

3.2. Findings of post mortem inspection

It has been found that 33 (11.56%) out of 285 examined animals had adult flukes in their bile ducts. Males less than 5 years had infection rate of 4.17%. Meanwhile, cows exceeded 5 years showed the highest infection rate (18.0%) (Table 1). Adult flukes were morphologically identified as *Fasciola hepatica* in all examined animals.

3.3. Infection in snails

Species of snails collected were identified as *Lymnaea truncatula* and *L. cailliaudi*. Snails were identified by WHO labs in Madrid through Prof. Mas-Coma. *L. cailliaudi* had no infection. *L. truncatula* was found to be infected by *Fasciola* spp. parthenatae. El-Rashda district showed the highest infection in *L. truncatula* snail (Table 2 and Fig. 2).

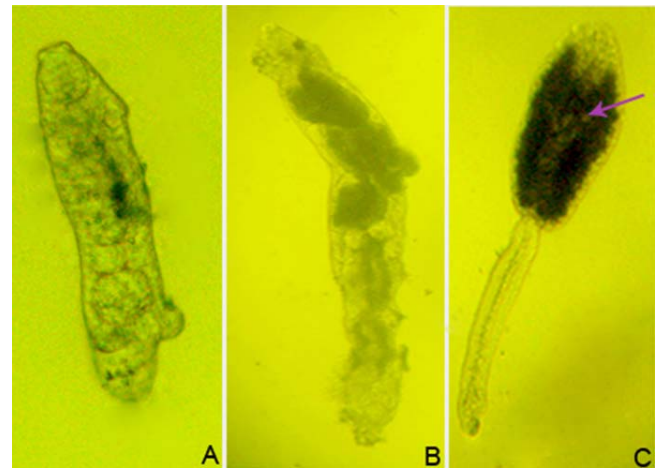


Figure 2: Larval stages of *Fasciola hepatica* obtained from *L. truncatula* snails

A. Immature redia

B. Redia

C. Cercaria

3.4. Findings of conventional PCR

Fasciola spp.-infected *L. truncatula* snails showed specific bands for *F. Hepatica* at 1,031 bp. Pooled samples obtained from the 7 districts revealed *F. Hepatica* showing specific bands of 1,031 bp. Control positive for *F. gigantica* was used to differentiate between *F. hepatica* and *F. gigantica*.

Table 1: Prevalence of *Fasciola* spp. infection in slaughtered animals and fecal samples in Dakhla Oasis relative to age and sex

Age	Sex	Liver inspection		Fecal samples	
		Examined No.	Infected No. (%)	Examined No.	Infected No. (%)
< 5 years	Male	120	5 (4.17)	107	20 (18.70)
	Female	15	1 (6.67)	30	10 (33.33)
> 5 years	Male	0	0.0	0	0.0
	Female	150	27 (18.00)	163	64 (39.40)
Total		285	33 (11.56)	300	94 (31.33)

Table 2: Distribution pattern of *Fasciola* spp. infection in slaughtered animals in seven districts of Dakhla Oasis

Area (Dakhla center)	Liver inspection		Fecal samples		<i>Lymnaea truncatula</i>		<i>L. cailliaudi</i>	
	Exam. No.	Inf. No.(%)	Exam. No.	Inf. No. (%)	Exam. No.	Inf. No. (%)	Exam. No.	Inf. No. (%)
El-Rashda	75	12 (16.00)	85	33 (38.82)	110	7 (6.40)	90	0 (0)
El-Hindo	74	11 (14.90)	75	28 (37.33)	100	6 (6)	70	0 (0)
El-Masra	35	4 (11.43)	40	12 (30.00)	150	10 (6.66)	100	0 (0)
El-Shiekhwali	33	2 (6.10)	32	9 (28.13)	120	8 (6.66)	80	0 (0)
Azab-Elqasr	29	2 (6.90)	28	7 (25.00)	80	5 (6.25)	50	0 (0)
El-Qasr	20	1 (5.00)	20	3 (15.00)	95	3 (3.20)	40	0 (0)
El-Aweyna	19	1 (5.30)	20	2 (10)	76	2 (2.32)	35	0 (0)
Total	285	33(11.60)	300	94 (31.33)	731	41 (5.61)	465	0

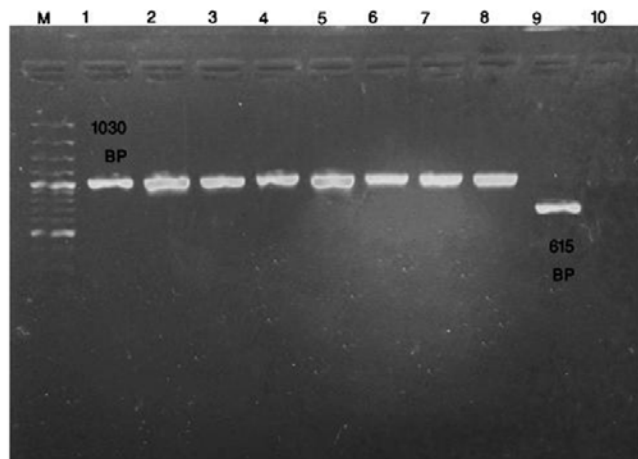


Figure 3: PCR amplification results for recovered flukes and infected *L. truncatula* snails

M is ladder of 500 bp

1: *Fasciola* infected *L. truncatula* snails

2-8: are samples of the different districts

9: *F. gigantica* control positive

10: Control negative

DISCUSSION

Ascending clinically *Fasciola* spp.-infected cases in both domestic animals and humans throw a heavy light on public health and veterinary services, particularly in the under developed countries. Because of their significance referred to substantial economic losses in the livestock industry, effective methods for rapid and accurate detection of each parasitic stage as well as identification of *Fasciola* spp. species are highly needed for epidemiological surveys, clinical management, and infection control (Mas-Coma et al., 2009).

The prevalence of *Fasciola* spp. in livers of infected cattle in slaughterhouses, and in collected snails in Dakhla Oasis, Elwadi El-Gadid, Egypt carried out in the present study. In Mout abattoir, *F. hepatica* infection rate was 11.56%. It was higher in cows than in bulls, and it was higher in senile animals (> 5 years) than younger. Similar results (11.97%) were recorded by Jolanta and Agnieszka (2013) in Poland, Biuet et al. (2013) (14.8%) in Maiduguri, Nigeria. In the current investigation, the infection rate was lower than that obtained by Atallah (2008) in Egypt (18.5%), On the other hand, it was higher than that detected by Ghoneim et al. (2011) who found an infection rate of 6.67% in Kalyobia, Egypt and Kadir et al. (2012) who revealed infection rate of 1.27%.

Examination of 300 fecal samples showed 31.33% infected with *Fasciola* spp. eggs. Females >5 years had higher (39.4%) infection rate than males (18.7%). Similar results

were revealed by Adedokun et al. (2008) in Nigeria, Avcioglu et al. (2014) in Erzurum Province, Turkey. On the other hand, lower infection rates were detected by Fufa et al. (2010) (4.9%). Variations in infection rates could be related to geographical distribution, grazing system and different strategic control of helminthes.

Lymnaea truncatula is the most common intermediate host for *F. hepatica* worldwide (Bargues et al. 2012). Collected snails were identified as *L. truncatula* and *L. cailliaudi*. It has been found that 5.61% of *L. truncatula* snails were infected with *Fasciola* spp. Meanwhile, *L. cailliaudi* snails had no infection. In Egypt, *L. truncatula* was previously recorded uninfected from the Nile Valley and Delta (El-Shazly et al. 2012); Baharia, Dakhla, and Kharga Oases (Frandsen, 1983); the New Valley (Abdel-Ghani 1965, 1976); and Sinai (El-Kady et al., 2000). However, Egyptian populations of *Lymnaea* spp. snails were found naturally infected with *Fasciola* spp. (El-Shazly et al., 2012). Successful experimental infections with *F. hepatica* and *F. gigantica* were obtained under laboratory conditions (Dar et al., 2013).

Based on morphological criteria of the present flukes, they were identified as *F. hepatica*. Morphologic, morphoanatomic, morphometric and chemotaxonomic data were used to identify *Fasciola* spp. in Egypt (Lotfy and Hillyer, 2003; Periago et al., 2008; Abdel-Nasser and Reffat, 2010).

The common species of *Fasciola* in Egypt are *Fasciola gigantica* and *Fasciola hepatica* (Lotfy and Hillyer 2003; Dar et al., 2005; WHO, 2007; Dar et al., 2012). Moreover, Periago et al. (2008) investigated fasciolosis in Nile Delta, Egypt, and revealed the presence of *F. hepatica*, *F. gigantica* and intermediate forms (*Fasciola* sp.). Abdel-Nasser and Reffat (2010) in Qena, Egypt, differentiated morphologically 3 *Fasciola* species; *Fasciola gigantica*, *Fasciola hepatica* and two phenotypically different worms of *F. hepatica*.

To be identified, *Fasciola* spp. obtained from examined snails and from livers in abattoirs underwent mitochondrial DNA-targeting PCR assay. DNA was properly extracted from apical parts of pooled samples obtained from collected flukes and from infected *L. truncatula* snails. PCR amplification of the amplified samples revealed the presence of *F. hepatica* with specific amplicon size of 1031 bp. It is worthy to mention that,

mitochondrial DNA targeting PCR succeeded to detect *F. hepatica* in the infected *L. truncatula* snails. Morphological, immunological, molecular, and combined approaches have been developed, including conventional PCR and multiplex PCR to identify *Fasciola* species. Previously developed PCR/real-time/multiplex PCR methods (Magalhães et al., 2004) used nuclear rather than mitochondrial targets for genetic characterization. Mitochondrial DNA is probably a better choice for a multiplex PCR application, due to its stability and the likely higher copy number even for a single egg (Le et al., 2012a). A mitochondrial duplex PCR have successfully developed and proved for identification of *Fasciola* spp. (Le et al., 2012a,b).

In conclusion, *Fasciola hepatica* and its intermediate host *G. truncatula* snails were recorded in seven districts of Dakhla Oasis, El-Wadi El-Gadid Governorate Egypt. Irrigation system and water sources, wells, were probably to be endemic with *L. truncatula* snails. Mitochondrial DNA-targeting PCR succeeded to detect and identify *F. hepatica* during liver inspection in abattoirs and its parasitic larval stages inside the infected intermediate host, *L. truncatula* snail. Special attention and safety precautions towards water used for both human and animal purposes must be considered to reduce the worm burden, minimize the infection and to overcome the zoonotic risk.

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