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Research Paper

Contributions on human fascioliasis and its snail intermediate host in Nile Delta, Egypt

Mohamed M. El-Bahy [1], Abeer M. A. Mahgoub [2]*, Eman E. Taher [3]

[1] *Department of Parasitology, Faculty of Veterinary Medicine, Cairo University*
[2] *Department of Parasitology, Faculty of Medicine, Cairo University*
[3]*Research Institute of Ophthalmology, El-Giza, Egypt*
**Corresponding author E-mail: goubya@hotmail.com*

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Abstract

The present study spotted some light on human fascioliasis in Kafr El-Sheikh Governorate in the west of the Nile Delta in Egypt, its species, its intermediate host (IMH) snail and tried to answer previous questions about development of *Fasciola* (*F*.) species in new snail hosts other than that known for animal fascioliasis in Egypt.
The study recorded a percentage of infection by *Fasciola* eggs reached up to 6.02% in 1810 randomly collected human stool samples from 6 climatically selected sites in this governorate using fluke-finder technique. The incidence was high in Sedi Salem and Motobus than in the other study sites. Micrometry measuring of 100 eggs from each locality showed that egg size cannot be used as a main criterion in differentiation between *F. hepatica* and *gigantica*. Wide range of egg size varied between 130-162.5 µ X 75-87.5 µ with a mean length and width of 144.24 ± 11.33 µ X 80± 6.55µ, was recorded. Upon dissection of 1972 *Lymnaea* (*L.*) *cailliaudi*, 268 *L. alexandrina*, 502 *Bulinus* species, 11316 *Biomphalaria alexandrina*, 1398 *Cleopatra* species, 8520 *Physa acuta*, 420 *Melania tuberculata*, 2132 *Vivipara* (*Bellamya*) *unicolar*, 144 *Neritina nilotica* and 1570 *Planorbis philippi*, *Fasciola* parthenitae were not detected in snails other than *L. cailliaudi* (the known IMH of *Fasciola* in Egypt). The results proved that there is no accommodation has occurred in any of the surrounding snails to transmit *Fasciola* to man. The present study proved that human fascioliasis in the study sites was due to *Fasciola gigantica* not *F. hepatica*. This appeared in its tendency to develop in *L. cailliaudi* not in other tested snails with successive radial generations as that described previously for *F. gigantica*. Moreover, early mature flukes extracted from laboratory infected rabbits by the produced encysted metacercariae had the characteristic features described previously for *F. gigantica*.

Keywords: *Cercariae- Egypt- Fasciola gigantica- Human- L. Cailliaudi.*

1. Introduction

Fascioliasis, an infection due to the food and water-borne trematodes *Fasciola hepatica* and *F. gigantica*, is of the most neglected of the neglected tropical diseases [1]. Worldwide, more than 90 million people are at risk of fascioliasis and between 2.4 and 17 million individuals are infected with *Fasciola* [2]. Fascioliasis in human took special interest during the last several years in the Nile Delta region in Egypt. This is mainly due to increase the percentage of infestation from 8 cases only in Dakahlyia in 1972 [3], to 2.38% in Cairo and Giza Governorate, Egypt [4] after stool examination. The percentage increased to 15.03% and 18.12% in the above two governorates respectively, after using ELISA [4], [5].
There has been speculations about the origin of the species that occurred in human, is it *Fasciola hepatica* or *Fasciola gigantica*? With no records for the presence of *Lymnaea truncatula* in the Nile Valley in previous study sites, and is there another type of snail accommodates to help in completing the cycle of the parasite to infect human as mentioned by Farag and El-Sayed [6].
In the present study field and laboratory work aimed at better defining of *Fasciola* as a zoonotic problem with special interest to determine IMH snail responsible for spreading of infection to human. Kafr El-Sheikh governorate was selected as a study area, as it is considered to be an ideal habitat for different snails in the Nile Delta [7].

These field efforts aimed at determining the status of *Fasciola* infection in human in 6 selected regions in Kafr El-Sheik governorate. All of the water ways present in the study sites was examined; samples representing from all types of snails present were collected and examined to clarify their role in transmission of *Fasciola*. In the laboratory snail IMH of human fascioliasis was experimentally determined. Miracidia produced from *Fasciola* eggs isolated from infected cases were tested to develop in *L. cailliaudi, L. truncatula, Bulinus truncatus* and *Biomphalaria alexandrina* snails followed by further development of the produced EMC experimentally in rabbit.

2. Material and methods

This study was assessed and approved by Faculty of Medicine Cairo University Ethics Committee, after taking consents from local health authorities and all participants and have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. This study was conducted between March 2012 and February 2013.

2.1 . Field works

2.1.1. Selection of the study sites

Available national satellite data cleared an inverse relation between diurnal temperature difference (dT) in the lower Nile River Valley and the prevalence of *Schistosoma* infection. This thermal difference values reflect regional hydrologic conditions that can be used as a predictor of environmental risk assessment which depends for some extent on the persistence of the trematoda snail's intermediate hosts [7]. According to this satellite temperature and hydrological data, Kafr El-Sheik governorate has 3 main zones as wet (dT from 12-14 ºC), moist (dT from 14-16°C) and dry (dT from 16-20°C).The study sites were selected to represent these 3 different zones, as Motobus and Sedi Salem represent the wet zone, Shenno and Abioka represent the moist zone while El-Halafy and El-Kafr El-Sharky represent the dry zone.

2.1.2. Collection and examination of samples

Through 1-2 visits per month for the local health units and some schools in each of the study sites, a total of identified 1810 human stool samples were collected during this study. Samples were examined in the Department of Parasitology Faculty of Veterinary Medicine, Cairo University for the presence of *Fasciola* eggs using double sieve system "fluke finder" technique [8] with the aid of methylene blue stain for detection of the eggs under the microscope. The percentage of infection in the examined samples was recorded.
One hundred *Fasciola* eggs isolated from the infected stool samples were identified [9]. Length and width of each egg were measured using the graduated micrometer slide and eye piece [10].

2.1.3. Examination of the different water ways

In cooperation with the Ministry of Health (M.O.H.) Snail Eradication Office in Kafr El-Sheik governorate, samples from all types of snails present in the different water ways within a circle of 2 km diameter around housed area in each of the study sites were collected and brought to the laboratory. Snails were collected [11] by taking of 2 net samples (at 10 and 30 cm depth from the water surface) each 50 regular step for each water way. In the laboratory snails were identified [12], [13] and maintained in the laboratory [14] for 7-15 days.
All collected snails were examined for trematoda infection by light exposure and crushing techniques [15]. Different trematodes parthenitae detected were identified [11], [16]; then recorded for each snail species separately

2.2 . Laboratory experiments

This part of the study was done to determine the snail IMH of *Fasciola* that infect human through testing the ability of their miracidia to develop in *Lymnaea cailliaudi* snail (the known intermediate host for *F. gigantica* in Egypt), *L. truncatula* (the known intermediate host of *F. Hepatica*) as well as in two of *Schistosoma* snail IMH , *Bulinus* spp and *B. alexanderina*. This was followed by infection of rabbits by the produced encysted metacercariae (EMC) for obtaining on mature fluke.

2.2.1. Laboratory breeding of snails for experimental infection

Lymnaea cailliaudi, Bulinus truncatus and *Biomphalaria alexandrina* egg masses were collected from the aquarium of the field collected snails and transferred to another separate one where they were reared [17]. Similarly; large numbers

of *L. truncatula* were collected from a site at the end of El-Mansoryea drain near Kerdasa, Giza and established in the laboratory as above. The aquaria were supplied by fine sand brought from their original habitat.

2.2.2. Separation of *Fasciola* eggs and production of miracidia

Fasciola eggs were separated from stool of the infected human patients by successive sieving using the fluke finder after washing by compressed tape water then collected into a separate cylinder. The washed eggs were kept in the refrigerator in distilled water and embryonated in water in the laboratory for production of miracidia.
Embryonation of eggs was done in an incubator at 28ºC [9]. Eggs were checked daily under the microscope from the 9th day of embryonation after exposure for ½ an hour to direct light. The day of hatching was defined as that in which 50 % or more of the eggs hatched at the same time.

2.2.3. Artificial infection of snails

Laboratory prepared snails of 4-5 weeks old were tested as IMH. Each snail was exposed overnight to 2-5 miracida in a separate well of tissue culture plate (12 mm diameter) [14]. Snails were reared in the laboratory and fed green lettuce. Two infected snails from each group were crushed from the 6th day post- infection (d.p.i.) and every two to three days after this depending on the suspected state of parthenita development inside the snail until the time of first cercarial shedding. Encystment of shed cercariae occurred on cellophane covered containers. Collected encysted metacercariae (E.M.C.) were stored under refrigeration (4° C) for 5-8 days in water. The species of the EMC was determined by experimental infection of rabbits.

2.2.4. Infection of rabbits

To determine the species of *Fasciola* eggs extracted from human, the EMC produced after laboratory infection of the snails were used for production of fluke in rabbits (two rabbits for each host origin) as follows:,
Four Boscat rabbits (1.5-2 kg each) each was infected by 15-20 EMC. The EMC dose was wrapped in a piece of lettuce and deposited over the base the tongue, keeping the animal's mouth closed by hand till it was swallowed. Animals were maintained on dry ration and water free choice until the end of the experiment.
Two rabbits died at 45 days post infection. The remaining two were slaughtered on this day also. The liver surface was examined for the presence of necrotic migratory furrows. Necrotic areas were examined for fluke recovery using 2 surgical needles for extraction of the juvenile migrating flukes. The liver was sliced into small pieces where all flukes present in the liver and the peritoneal cavity were collected [14]. The recovered flukes were fixed and mounted on slides [10]. Fluke species was identified [18].

3. Results

3.1. Incidence of *Fasciola* infections and egg size

Results of examined human stool samples collected from 6 selected places in Kafr El-Sheikh governorate for the presence of *Fasciola* eggs were listed in table (1). The mean percentage reached up to 6.02% of human who shed *Fasciola* eggs in their feaces. Infection rates were higher in the summer season, reaching 7.69%. The highest number of patients shed *Fasciola* eggs in their feaces was in Sedi Salem (8.46%) and Motobus (7.08%). Results suggested that infection in human is not sex or age dependent.
Measuring the length and width of 100 eggs randomly isolated from infected stool samples revealed a wide size ranged from 130-162.5 X 75-87.5 µ with a mean length and width of 144.24 ± 11.33 X 80± 6.55µ (Figure 1-A). These eggs were in six sizes range with different percentages including; 30% of these eggs with size of (130-135 X 75-80 µ), 20% of size (137.5 X 80 µ), 20% of size (150 X 80 µ), 10% of size (152.5 X 87.7 µ), 10% of size (157.5 X 80 µ) and 10% of large size (162.5 X 82.5 µ).

3.2. Trematoda infection in snails collected from study sites

This part of the study focused on examination of different snail species present in each of the study sites to determine if any of them can act as probable IMH for *Fasciola* species in these localities.
Collection and examination of samples from the available snails species found during the year in the different study sites in Kafr El-Sheikh governorate included 6152 *L. cailliaudi* snails, 268 *L. alexandrina* snails, 502 *Bulinus* species , 11316 *Biomphalaria alexandrina* , 1398 *Cleopatra* species, 8520 *Physa acuta* , 420 *Melania tuberculata*, 925 *Vivipara* (*Bellamya*) *unicolar*, 144 *Neritina nilotica* and 1570 *Planorbis pheleppi*. Table (2) revealed a mean infection rate reached 32.05 %, 0.0%, 11.75%, 64.74%, 3.36%, 14.82%, 6.66%, 23.03%, 0.0% and 3.05% by different trematoda species respectively.

Results showed that *L. cailliaudi* snails were distributed in all of the study sites and harbored trematoda larvae in rate of 36.18%, 12.5%, 24%, 38%, 41.8%, and 22.5% in Shenno, El-Halafy, El-Kafr El-Sharki, Sedi Salem, Motobus and Abioka, respectively.

Results displayed in table (3) revealed that there was marked trematoda-snail specificity, especially for major parasites such as *Fasciola*, *Paramphistomum* and *Schistosoma*. *Fasciola* species cercariae (Fig. 1-G) were not detected in snails other than the examined *Lymnaea cailliaudi*. The rate of infection in this snail species reached 45.17%. *Schistosoma* species cercariae were shed only from *Bulinus* and *Biomphalaria* species. The percentage of infection by this parasite in the examined snails reached to 39.8% and 65.06%. Three forms of *Xiphidio* cercariae (Fig. 1-E) were found as common parasite present in most of the examined snail species including *L. cailliaudi*, *Bulinus* spp. and *B. alexandrina*. It worthies to be mentioned that each infected snail harbored only one type of infection in its hepatopancreas. Moreover EMC of *Xiphidio* type were detected in the tissue of the snail foot (Fig. 1-I) in case of *L. cailliaudi*, *Bulunus* spp. and *Physa acuta* inspected snails (Table 3).

These data showed that cercariae of *Fasciola* or *Shistosoma* species were not detected from snails other than their known intermediate hosts (*L. cailliaudi*, *Bulinus* and *B. alexandrina*).

Concerning the other cercariae species found, Paramphistomatidae species cercariae and redia (fig. 1-D) were detected in *Bulinus* species while *Echinostoma* species cercariae were shed from *Physa acuta* snails. During the study, 3 types of *Xiphidio* cercariae (Fig.1-E) were detected that showed low snail specificity; one type was present in most of the examined snails and was previously described as *Xiphidio*- cercariae type I , the second type shed from *L. cailliaudi*, *Cleopatra* species and *Bellamya unicolar* snails has been identified previously as *Xiphidio* cercariae type II; the third type was shed from *L. cailliaudi*, *B. alexandrina* and *Bellamya unicolar* and identified previously as *Xiphidio* cercariae type C. Moreover type of encysted cercariae (Fig 1-I) were diagnosed in tissue of dissected snails (Table 3).

3.3. Identification of *Fasciola* species present in human

To determine the *Fasciola* species developed from the eggs isolated from human, enough amounts of the collected eggs were embryonated separately under 26ºC. Most eggs hatched after 16 days. The eggs showed a moderate degree of hatchability that reached to 63%. Miracidia produced were used to induce experimental infection in four separate groups (30 snails each) of 4-5 weeks old laboratory breed *L. cailliaudi*, *L. truncatula*, *Bulinus truncatus* and *B. alexandrina* snails during June 2012, using an exposure number of 3-5 miracidia / snail.

Dissection of two infected snails from each group at day 6 post infection (PI) revealed the presence of low numbers of senile sporocysts as well as low numbers of juvenile first generation radiae (1st G.R.) between tissue fragments of the dissected *L. cailliaudi* snails only. At day 9 PI, the 1st G.R. was extracted from the hepatopancreas of desiccated snails. The 1st G.R. appeared larger in size and the germ cells began to collect together, forming germ balls. Further growth for these germ balls was recorded in snails dissected at day 12 PI. Germ balls inside the 1st G.R increased in size and became more elongated, without constriction at their posterior third, to form daughter rediae (D.R) in snails dissected on 15th day PI.

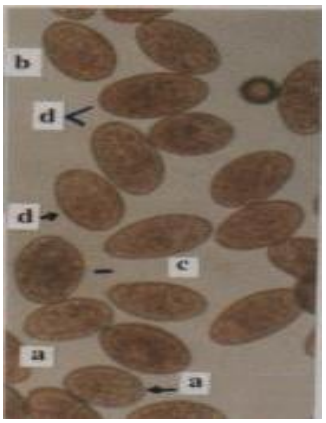
The second G.R. left first generation rediae and migrated to the snail hepatopancreas at 18th days PI. It appeared small in size (Fig 1-B). At the 22th day PI, the germ balls present inside the first G.R. may be differentiated into elongated form without constriction at their ends, these may be considered as the remnant of germ cells that will produce rediae, while other spherical balls appeared as cercarial embryo with presence of constriction (beginning of tail differentiation) at the end of some of the balls which developed earlier than the small spherical form.

Cercariae in different stages of growth were detected in the large size rediae (mainly of 1st generation group) in infected snails dissected on the 28th day PI (Fig 1-C). On day 28, 2nd G.R. was observed that had grown in size and contained early developed cercariae also.

Natural cercarial shedding began on day 41 post infection. Snails dissected at this time contained 3 different sizes and structures of rediae; the first contained developed cercariae shed in the dish. The second one containing daughter radiae shed also in the dish while the 3rd one appears as growing 3rd G.R. redia. It is worthily to be mentioned that the cercariae shed after dissection of snails failed to encyst, while in the naturally shed cercariae the cyst wall developed more rapidly and it is usually completed in a period of 4-8 minutes producing transparent EMC (Fig. 1-H). Mortality in the infected snails was 12% from the period of parthenitae development until shedding of cercariae.

It was worthily to be mentioned that dissection of *L. truncatula*, *Bulinus truncatus* and *B. alexandrina* snails that were exposed to the same type of *Fasciola* miracidia simultaneously with *L. cailliaudi* snails did not show any trematoda parthenita inside it. Also, no mortality was recorded in these snails during the same period.

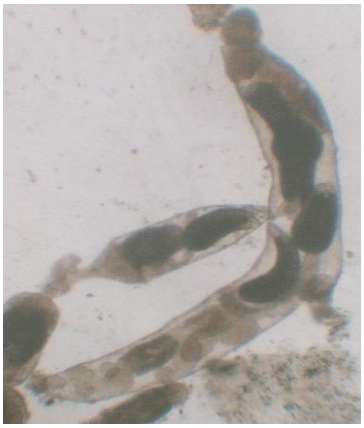
Concerning *Fasciola* worms recovered from infected rabbits died or slaughtered at 45th day PI. Seven worms were extracted. All of them had the characteristic features of *Fasciola gigantica*: an elongated body with parallel body margins and clear difference between the size of the oral and ventral suckers (Fig.1-F).



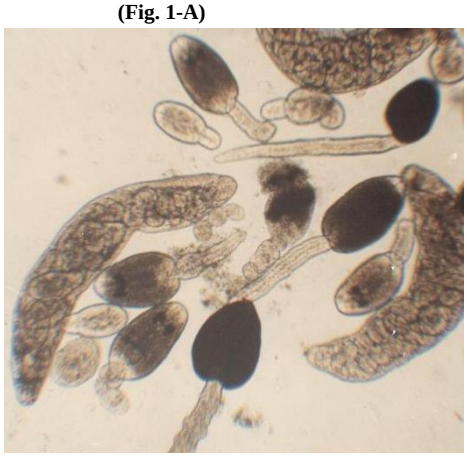
(Fig. 1-A)



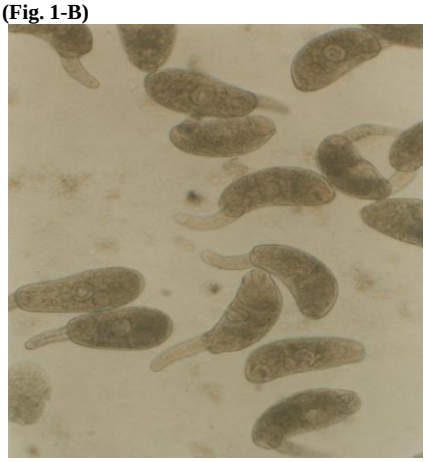
(Fig. 1-B)



(Fig. 1-C)



(Fig. 1-D)



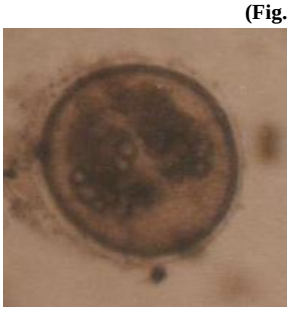
(Fig. 1-E)



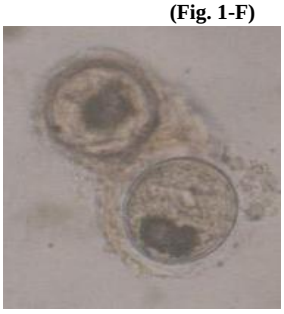
(Fig. 1-F)



(Fig. 1-G)



(Fig. 1-H)



(Fig. 1-I)

Fig. 1: Eggs and Parthenitae Extracted From Examined Laboratory and Field Collected Snails (Fresh Non Mounted Specimens)
(1-A)- *Fasciola* eggs of different sizes, very small (a), medium spherical (b), elongated (c) and large one (d) present in one field collected and purified from stool (X 125).
(1-B)– Three radial generations from experimentally infected *L. cailliaudi* before formation of cercaria at 18th day post infection (X 40).
(1-C) – Formation of cercariae in the 1st generation radia at 28th day P.I. (X40).
(1-D)- *Paramphistomum* Spp. Radia and Cercaria pigmentata (X 125)
(1-E)- Different views of *Xiphidio* cercariae type from field collected snails (X 125).
(1-F)- Immature *F. gigantica* worm 45 days old extracted from rabbit, mounted specimen (X20).
(1-G)- *Fasciola* cercaria shed from laboratory infected *L. cailliaudi* (X 125).
(1-H) -Rapid encysment of shedded cercariae showing transparent cyst wall (X125).
(1-I) - Type of encysted metacercariae extracted from field collected snails (X100).

Table 1: Incidence of *Fasciola* Infection among the Examined Stool Samples during the Year.

District	Kafr El-Sheik				El-Hamool		Sedi Salem		Motobus		Desouk		Total		
	Sheno		El-Halafy		El-Kafr El-Shrki		Sedi Salem		Motobus		Abioka				
Season	No. Exam.	% of Fasci o Inf	No. Exam.	% of Fasci o Inf	No. Exam.	% of Fasci o Inf	No. Exam.	% of Fasci o Inf	No. Exam.	% of Fasci o Inf	No. Exam.	% of Fasci o Inf	No. Exam.	No. +Ve	% of Fascio. Inf
Winter	60	0.0	80	3.75	80	2.5	60	10	60	8.33	40	7.5	380	19	5.0
Spring	120	2.5	100	6.0	100	5.0	60	6.66	60	10	60	6.66	500	28	5.66
Summer	100	7.0	60	3.33	120	5.0	80	8.75	80	7.5	80	6.25	520	40	7.69
Autumn	100	6.0	80	5.0	70	5.7	60	8.33	40	0.0	60	5.0	410	22	5.36
Total	380	6.0	320	4.68	370	4.59	260	8.46	240	7.08	240	6.25	1810	109	6.02

*No.Exam. = number of examined fecal and stool samples.
* % Fas. Inf. = % of samples showing Fasciola Species eggs.

Table 2: Percentage of Trematoda Infection in the Different Snails Collected From the Study Sites

Name of District	Kafr El-Sheikh				El-Hamool		Sedi Salem		Motobus		Desouk				
	Sheno		El-Halafy		El-Kafr El-Shrki		Sedi Salem		Motobus		Abioka		Total		
Type of snail	No. coll-ect	% of Inf. snail	No. col-lect	% of Inf. snail	No. collect	% of Inf. snail	No. coll-ect	% of Inf. snail	No. coll-ect	% of Inf. snail	No. coll-ect	% of Inf. snail	No. collect	No. infec-ted	% of Infected snail
Lymnaea cailliaudi	1600	36.18	520	12.5	636	24	1192	38.0	1164	41.8	1040	22.5	6152	1972	32.05
Lymnaea alexandrina	76	0.0	10	0.0	0.0	0.0	70	0.0	52	0.0	60	0.0	268	00	0.0
Bulinus species	148	11.4	20	10.0	52	9.6	105	12.3	101	4.8	76	9.2	502	59	11.75
Biomphalaria alexandrina	2800	54.0	596	38.08	556	36.15	2534	74.04	2480	79.03	2350	66.0	11316	7327	64.74
Cleopatra species	290	0.0	98	0.0	200	0.0	300	9.0	250	8.0	260	0.0	1398	47	3.36
Physa acuta	2200	15.7	920	12.06	1300	9.0	1700	21.0	1400	23.0	1000	18.0	8520	1263	14.82
Melania tuberculata	55	5.45	28	7.14	48	12.5	115	6.95	115	0.0	59	0.0	420	28	6.66
Vivipara (Bellamya unicolar)	200	22.0	105	20.0	110	20.0	190	24.2	200	26.0	120	19.16	925	2132	23.03
Neritina nilotica	42	0.0	0.0		0.0	0.0	36	0.0	42	0.0	12	0.0	144	0.0	0.0
Planorbis philippi	250	0.0	230	0.0	260	0.0	300	0.0	300	0.0	230	0.0	1570	48.0	3.05

*No.collect. = number of snails collected.
**No. infec = number of snails showing trematoda infection + % of Inf.snail = % of infected snails.

Table 3: Percentage of Different Types of Trematoda Larvae Detected in All of the Infected Snails Collected During the Year.

Types of the examined snails	Types and % of infection by specific cercarial species				Types and % of infection by nonspecific cercarial species			
	Fasciola cercariae	*Schistosoma* cercariae	*Paramphistomatidae* cercariae	*Echinostomatidae* cercariae	*Xiphidio* cercariae (type I)	*Xiphidio* cercariae (type II)	*Xiphidio* cercariae (type C)	Tissue EMC
Lymnaea cailliaudi	45.17%				9.79	44.4	14.91	14.12
Bulinus species		39.8%	8.33%		26.75			26.75
Biomphalaria alexandrina		65.06%			21.18		11.25	
Cleopatra species					47.4	2.58		
Physa acuta				29.76%	70.22			29.56
Melania tuberculata					50			
Vivipara (Bellamya unicolar)						14.10	85.8	
Planorbis philippi					75%			

4. Discussion

There are several important morpho-biological and epidemiological differences between *F. hepatica* and *F. gigantica*. The most important aspect is that *F. gigantica* has a prolonged migration period in the liver tissue (90-100 days) before reaching the bile duct and laying eggs [19] as compared to *F. hepatica* (56-61 days) [20]. Pathological changes that occurred during the liver migrating phase of the fluke were more economically and immunologically dangerous in case of *F. gigantica* in comparison with that of *F. hepatica*, from the aspects of decreases body resistance, and increases their susceptibility to infection by other diseases as well as marked decreasing in general productivity.

The common imported drugs usually are tested against early mature (6-7 weeks old) *F. hepatica*, while maturation period for *F. gigantica* occurs after 10-12 weeks which may lead to failure in efficacy of the used drug. Some drugs such as Clorsulon, affect flukes depending on enhancing the production of Gamma Amino Butyric acid (GABA) at the nerve ganglia. This neuro-transmitting material is present in *F. hepatica* from the 6-7th weeks of life and in *F. gigantica* at 8-9 weeks old flukes [21]. *F. gigantica* in Egypt develops in *L. cailliaudi* and the recorded IMH for *F. hepatica* is *L. truncatula*. The two snails differ in their morphology and their habitat characteristics [22].

All these facts cleared that it is important to know if infection in human is due to *F. gigantica* or *F. hepatica*; to allow accurate early diagnosis and selection of suitable time for treatment especially if this drug was not used against *F. gigantica* before. Moreover, several authors such as Farag and El-Sayed [6] demonstrated that IMH snails for

Schistosoma were accommodating to transmit human *Fasciola*. Also Dar et al. [23] mentioned that *Radix natalensis* is considered to be an essential IMH for *F. gigantica* in Egypt.

The present study was done to spot some light on human fascioliasis in Nile Delta; its species, its IMH snails and tried to answer previous questions about development of *Fasciola* species in new snail hosts other than that known for animal fascioliasis.

The present study followed diagnosis by direct detection of eggs in stool, this aimed to isolate pure eggs of human origin to complete the other objectives of the work. Recorded *Fasciola* infection by this way reached up to 6.02% in the randomly collected samples. This came in agreement with the previously recorded levels in other surrounding districts as 5.6 % in Abis, Alexandria [24] and 10.9% in El-Sharkia Governorate [25]. Infection rate has no sex or age limits but it depends mainly on the feeding habits of the people and their general hygienic measures.

The high percentage of human infection in Motobus and Sedi Salem agreed with the fact published by Malone [7] where these two sites had low diurnal temperature difference (difference in temperature between day and night) which means that there is more moisture in the area; the condition which helped the continuous persistence of the IMH snail. This rate of infection will be more investigated via another running work that will be published by the same authors in the near future depending on serological and immunological techniques.

Concerning *Fasciola* egg size, the present study cleared that *Fasciola* egg size could not be considered as a main point for differentiation between *F. hepatica* and *F. gigantica*, where different egg sizes could be detected in each host. Moreover, some abnormalities could be recorded also. On the other hand, smaller eggs than that previously described for *F. hepatica* as well as larger eggs than that described for *F. gigantica* were extracted. This was supported by the data of previous authors [26], where the egg size for *F. hepatica* (130-150 X 63-90 µ) can be included with *F. gigantica* egg size determined by Watannabe [27] (150-173 X 75-88 µ) and both of them was lower than the size mentioned by Soulsby [19] as *F. hepatica* egg size was 130-145 X 70-90µ, and that of *F. gigantica* was 156-197 X 90-104 µ.

On the other hand Malek [20] described strain of *F. hepatica* in Netherlands which can be distinguished by its markedly large eggs that reached to 146-179 x 80-100 µ in sheep and 143-180 x 88-102 µ in cattle. El-Zayyat [28] in Egypt measured *Fasciola* eggs from 28 patients in the same district and determined *Fasciola* egg size from 130-173X 75-90 µ.

Concerning the wide range of egg measurements recorded in the study cases with absence of specific morphological criteria, this revealed that egg size could not be considered as a basic criteria for identification of human *Fasciola* eggs from that of animal origin. Small size eggs (125-135 X 78-80 µ) as well as large size ones were present in other hosts as large sized eggs of human or sheep corresponded to similar sized eggs in buffaloes and donkeys. In the author's opinion the host size and capacity can be considered as a main factors that affect size of fluke and their eggs where a close size relation was clear between eggs of human and that of some animals like sheep also that from large hosts as buffaloes and donkeys, with presence of chance for abnormal egg formation in each case.

Concerning types of trematoda infection recorded in all types of snails collected from the same study sites, the study cleared that there was marked trematoda-snail specificity especially towards the main parasites as *Fasciola*, *Paramphistomum* and *Schistosoma*, where their cercariae were not detected in snails other than their previously known IMH. This came in agreement with two previous studies in the Nile Delta by [11], [16], and in the contrary with the data published by Farag and El-Sayed [6] and Dar et al. [23] about the detection of *Fasciola* radiae and cercariae in *B. alexandrina* snails naturally infected in Abis, Alexandria.

In the present study *Paramphistomum* species redia and its cercaria pigmentata were diagnosed in the examined *Bulinus* species. The parthenitea of this common ruminant parasite differs from that of *Fasciola* as its redia has no caudal processes and its cercaria has eye spots, posterior suckers and pigments in its body [19]. This can misdiagnosed with that of *Fasciola* species if did not exposed to specific diagnosis.

Really development of trematoda in snails isn't considered as an easy way where Bayne et al. [29] mentioned that trematoda immunity in mollusks is cell mediated depending on presence of plasma factor (s) in the resistant snails able to inactivate and lyses the toxic factors released by sporocysts. This factor rendered the sporocysts unable to recognize the nature of the cells encountered and consequently it fails to mount their attack. This factor also facilitates sporocyst damage. In the same respect El-Bahy [30] concluded that the snail overcomes any foreign trematoda infection through stimulation of different types of its haemolymph cells, mainly granulocytes and hyalinocytes-like cells.

These cells form amebofibrous capsules which led to destruction of the foreign invaders at the end with possible involvement of humoral factors also. These previous facts as well as the present study excluded any chance for adaptation or accommodation between snails and new parasites especially with the fact that both of them were present in the same conditions since long time before, and each trematoda species still has its own IMH. On the other hand; the study proved presence of *L. cailliaudi* and *Fasciola* infection all over the year with marked increase during summer and autumn. Also it was cleared that with the increase of percentage of infection in *L. cailliaudi* by *Fasciola* larvae in some of the study sites the level of fascioliasis in the surrounding animals and man increased. Moreover, the fact cleared that there is no need for sharing new snail as IMH in the cycle of the disease.

The detected *Xiphidio* cercariae and their ability to unspecific encystement in the foot of most fresh water snails in the same aquarium were previously described [11], [16].

Concerning species of *Fasciola* infect human in this localities, the present study cleared that *Fasciola* species detected in human in the study sites was *Fasciola gigantica*. This conclusion depends on several facts including that, the hatched miracidia from the separated *Fasciola* eggs after incubation period were belonging to *F. gigantica* species and this comes in agreement with that previously recorded by Soulsby [19] for *F. gigantica*.

These miracidia grew successfully in laboratory breed *L. cailliaudi* (the known *F. gigantica* IMH in Egypt) and in the same time didn't grow in the other exposed snails (*L. truncatula, B. truncatus* and *B. alexandrina*) under the same laboratory conditions. This came in agreement with that mentioned by Mas-Coma et al. [13] as lymnaeid intermediate hosts of *F. gigantica* are distinguishable from those of *F. hepatica*, morphologically and as for the habitat requirements. *Fasciola gigantica* snail IMH considered as true aquatic type need permanent water bodies of moderate water current and rich in aquatic vegetations. They added that in central, south and West Africa, *L. natalensis* is the transmitter, while the synonymous *L. cailliaudi* is the IMH in East Africa and both of them were closely related to *L. auricularia rufescens*. The rate of parthenitea development inside the snail and the sequence of radial and cercarial generations came in agreement with that previously described [14] for *F. gigantica*. Also the early mature fluke extracted from rabbits infected by the produced EMC had the known characters of *F. gigantica* previously described by Soulsby [19].
In the author's opinion; the recorded cases of human fascioliasis in the non-sandy soil areas of Egypt as El-Mansora and Kafer El-sheik cities with no record for the presence of *L. truncatula* snail, must be due to an infection by *F. gigantica*, this came in agreement with Malek [20] where he mentioned that both species of *Fasciola* have been found in human.

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