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Digenetic larvae in Schistosome snails from El Fayoum, Egypt with detection of *Schistosoma mansoni* in the snail by PCR

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Abstract The present study aims to detect the digenetic larvae infections in *Bulinus truncatus* and *Biomphalaria alexandrina* snails and also PCR detection of *Schistosoma mansoni* infection. The snails were collected from different branches of Yousef canal and their derivatives in El Fayoum Governorate. The snails were investigated for infection through induction of cercarial shedding by exposure to light and crushing of the snails. The shed cercariae were *S. mansoni*, *Pharyngeate longifurcate* type I and *Pharyngeate longifurcate* type II from *B. alexandrina*, while that found in *B. truncatus* were *Schitosoma haematobium* and *Xiphidiocercaria* species cercariae. The seasonal prevalence of infection was discussed. Polymerase chain reaction was used for the detection of *S. mansoni* in the DNA from field collected infected and non infected snails. The results of PCR showed that the pool of *B. alexandrina* snails which shed *S. mansoni* cercariae in the laboratory, gave positive reaction in the samples. Pooled samples of field collected *B. alexandrina* that showed negative microscopic shedding of cercariae gave negative and positive PCR in a consecutive manner. Accordingly, a latent infection in the snail (negative microscopic) could be detected by using PCR.

Keywords *Biomphalaria alexandrina* · *Bulinus truncatus* · *Schistosoma mansoni* · Cercariae · PCR

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Introduction

Freshwater snails are obligatory intermediate hosts for the development of parasitic trematodes. The snails belong to the family *Planorbidae* include; genus *Biomphalaria* for *Schistosoma mansoni*, and genus *Bulinus* for *S. haematobium*. The schistosomes also were found in animals like cattle *(S. bovis)*.

Previous studies in Egypt recorded different cercariae from *Biomphalaria alexandrina* and *Bulinus truncatus* from different sites in Egypt (Riffaat et al. 1971; Fathalla 1981; Hassan 1987; Abou Basha et al. 1989; Aboelhadid 2004; Korany 2011).

Schistosomiasis is a disease of different mammalians such as humans and domestic live stock, caused by 'bloodflukes' of the genus *Schistosoma*. Millions of people are reported to be infected and other millions of people at risk (Doumenge et al. 1987; WHO 1993). The WHO recommends that the major of researches on schistosomiasis should focus on the development and evaluation of new strategies and tools for control of the disease (WHO 2004). PCR-based techniques have been reported for the diagnosis of a huge number of infectious pathogens; however its application for the *S. mansoni* detection isn't widespread (Melo et al. 2006).

The diagnosis of infection in *B. alexandrina* depends on; induction of cercariae shedding by exposure to light, crushing of snail then microscopic examined and lastly rearing of snails in the laboratory till shedding occurs. These methods exhaust time and need professional staff to be performed (Hanelt et al. 1997). PCR assay enabled very sensitive and early detection of infection, and the feasibility of large scale examination of snails with minimal efforts (Hamburger et al. 1998; Hanelt et al. 1997). In addition, the PCR assay discriminate *S. mansoni* from other

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parasites that may co-infect snails that hardly discriminated based on morphology (Chingwena et al. 2002; Hertel et a1. 2003; Thiengo et a1. 2004).

The present study aims to detect the digenetic larvae infections in *Bulinus truncatus and Biomphalaria alexandrina,* which inhabit different branches of Youssef canal and their derivatives in El Fayoum Governorate. PCR detection of *Schistosoma mansoni* cercariae infection in *Biomphalaria alexandrina* snails was monitored.

Materials and methods

Collection of snails

Biomphalaria alexandrina snails were found in large number in small ditches and several minor canals at small depth and were scanty in big canals. On the other hand, *B. truncatus* snails were found in main streams and canals. The snails were collected by the method recommended by Mandahl-Barth (1962). 1,245 snails were collected from different localities of the irrigation system of the Nile branch (Youssef canal) and its branches as well as its small ditches in El Fayoum Governorate during the period from October 2009 to February 2011. The collected snails were put in plastic bottles together with pond water and some vegetation and they were brought to the laboratory as soon as possible where they were assorted and identified. Identification of the collected snails was based upon morphological characters as previously described (Mandahl-Barth 1958; Abdella et a1. 1999).

Examination of snails

Exposure technique

Three to five snails from each Species, were placed in a Petri dish half filled with de-chlorinated tap water, were daily exposed to a direct light using 100 watt electrical lamb for a period of 2 h (Abd El-Ghany 1955).

Crushing technique

All freshly snails were crushed directly in a suitable Petri dish with a few amount of water under dissecting microscope, where all available parthenatae were recorded (Jackson 1958).

Characterization of different cercariae shed from the examined snails

Examination of shed cercaria was done directly on the samples after they transferred to a suitable Petri-dish in enough amount of water. Living cercariae were stained by supravitally stain using very dilute solutions of the vital dyes, neutral red and Nile blue sulphate according to Cable (1977). This enabled the cercariae to become relaxed and sufficiently stained for observation within few minutes. All biological characters of the cercariae were recorded.

Identification of cercariae

Identification of cercariea from freshwater snails was performed as previously described by Frandsen and Christensen (1984).

PCR for detection of infection in *Biomophilaria alexanderin*

Snail's samples

Different groups of *B. alexanderina* snails were used for DNA extraction:

(i) Negative and positive (infected by *Schistosoma mansoni* strain) control snails were obtained from that routinely maintained at the Laboratory of Schistosomiasis (Thoedor Bilhars, Egypt) in out bred albino mice and in *B. alexanderina* snails.
(ii) Field collected snails naturally infected, shed cercariae of *Schistosoma mansoni*.
(iii) Field collected snails, its infectious status was screened by crushing and examining snails under microscope and it didn't shed cercariae.

DNA extraction

Genomic DNA extractions form snails were performed using the DNeasy Blood & Tissue Kit (Qiagen), according to the protocol of Vidigal et al. (2000). The purified genomic DNA was stored at −80 °C until use. DNA was amplified by PCR using the primers; forward: (5′-TTACGATCAGGACCAGTGT-3′), and reverse: (5′-CCGGACATCTAAGGGCATCA-3′). It was designed to flank the majority of the region coding for the small subunit (SSU) rRNA gene (Melo et al. 2006). PCR was carried out in 50 µl reactions using 10 mM Tris–HCl, 50 mM KCl, 0.1 mg/ml gelatin, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 50 pmols of each primer and 2.5 U of *Taq* DNA polymerase (Amersham Biosciences, Uppsala, Sweden). Then, 2 µl of the genomic DNA solution was added into the PCR mixture. The amplification program included an initial denaturation of 94 °C for 5 min, followed by 30 cycles of 92 °C for 30 s, annealing at 55 °C for 1 min and extension at 72 °C for 1 min. Several positive (10 pg of *S. mansoni* DNA) and negative (no template) controls were included

PCR products were visualized in 5 µl aliquots after running on a 1 % agarose gel.

Results

Prevalence of snail's infection by digenetic larvae

The investigation of 822 *B. alexandrina* snails revealed that 122 snails were found harbouring cercariae three different types of cercariae were recorded with overall prevalence (14.84 %). The recorded cercariae included: *Schistosoma manasoni*, *Pharyngeate longifurcate* type I and *Pharyngeate longifurcate* type II with incidence (18.03, 40.00 and 42.00 % respectively) (Tables 1, 2). The seasonal variance in infection revealed that the shedding of *Schistosoma mansoni* cercaria was higher in spring (9.50 %) than in winter (0.50 %). *Longifurcate-pharyngeate* cercaria was recorded with no seasonal prevalence with highest incidence (10.30 %) in autumn and. *Longifurcat-pharyngeate* cercaria II was recorded also in all seasons with prevalence (11.00 %) in autumn, followed by spring (7.14 %), summer (5.20 %) and winter (4.50 %) Fig. 1.

Out of 423 examined *B. truncatus* snails, only 13 snails only were found infected; 2 types of cercariae were recorded with overall prevalence (3.07 %) (Tables 1, 2) plate I. These cercariae were; *Schitosoma haematobium* and *Xiphidiocercaria* species with prevalence (69.00 and 30.10 % respectively). Autumn and spring season are the main seasons of cercarial shedding from *B. truncatus* with prevalence (7.00 and 2.17 % respectively)

PCR results

The testing by polymerase chain reaction to detect the infection of *Bimophalaria alexandrina* snails by *Shistosoma mansoni* indicate that one pool of negative snails (microscopically negative when crushed) gave negative PCR (lane no. 2) however; another pool of negative snails microscopically gave positive reading at 710 bp (lane no. 3). On the other hand the field positive snails of lanes (4, 5, 6, 7, 8 and 9) gave positive reading at 710 bp. Also, the positive control in lane 10 gave the same band at 710 bp., and the control negative non infected snail didn't give any bands (lane no. 1) Fig. 2.

Discussion

The prevalence of infection among the examined *B. alexandrina* was 14.84 %.

Table 1 Cercariae types emerged from *Biomphalaria alexandrina and Bulinus truncates*

Snails	*Biomphalaria alexandrina*			*Bulinus truncatus*		
Cercaria species	Ex. No.[a]	Inf. No.[b]	%	Ex. No.	Inf. No.	%
LPD type I	822	49	6.00	423	0	0
LPD type II	822	51	6.20	423	0	0
Schistosoma mansoni cercaria	822	22	2.67	423	0	0
Schistosoma haematobioum cercariea	822	0	0	423	9	2.13
Xiphidiocercria sp.	822	0	0	423	4	0.09

LPD longifurcate-pharyngeate distome cercariae

[a] Means examined number

[b] Means infected number

Table 2 Seasonal variation of cercariae types infection emerged from *Biomphalaria alexandrina* and *Bulinus truncatus*

Season	Snail type						
	Biomphalaria alexandrina				*Bulinus truncatus*		
	Ex. No.	LPD type I	LPD type II	*Schistosoma mansoni cercaria*	Ex. No.	*Schistosoma haematobioum cercariea*	*Xiphidiocercria* sp.
Autumn	155	16 (10.32 %)	16 (10.32 %)	11 (7.09 %)	100	4 (4.00 %)	3 (3.00 %)
Winter	202	11 (5.44 %)	11 (5.44 %)	1 (0.05 %)	55	1 (1.81 %)	0 (0 .00 %)
Spring	42	1 (2.38 %)	3 (7.14 %)	4 (9.52 %)	46	1 (2.17 %)	0 (0.00 %)
Summer	423	21 (4.96 %)	22 (5.20 %)	6 (1.42 %)	222	3 (1.35 %)	1 (0.45 %)
Total	822	49 (0.09 %)	52 (0.06 %)	22 (0.03 %)	423	9 (0.02 %)	4 (0.01 %)

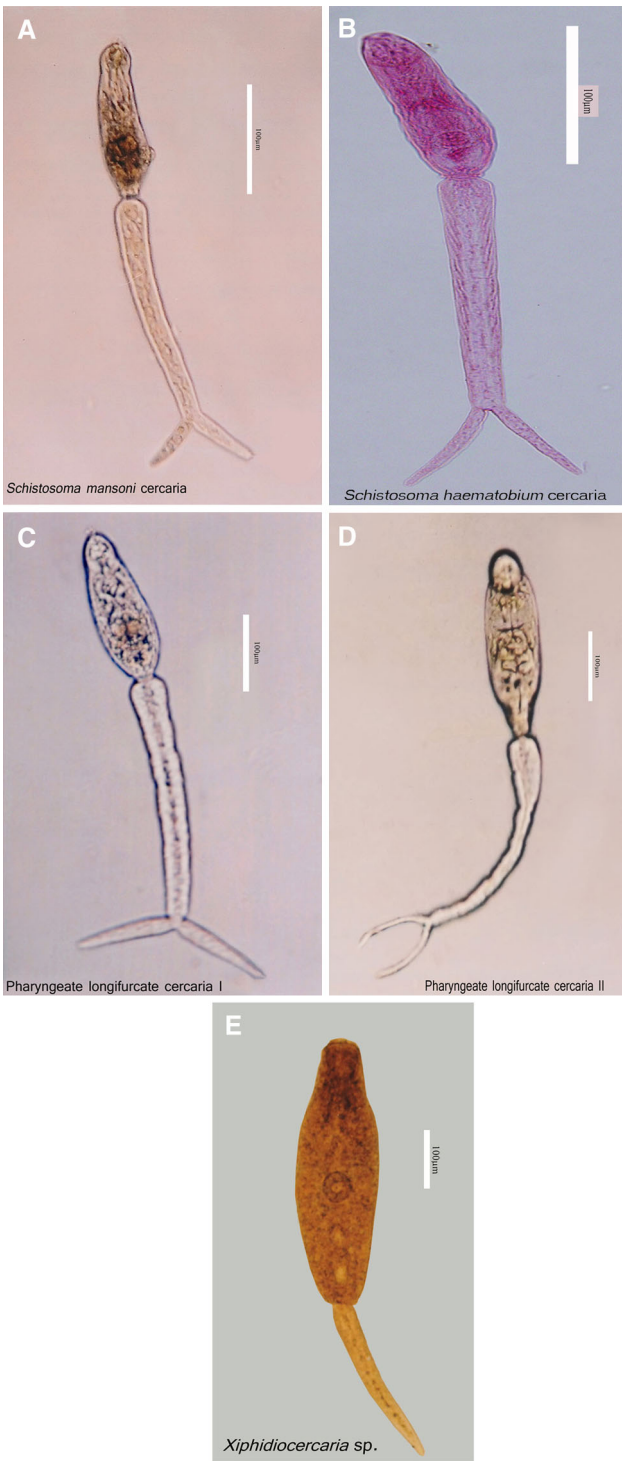

Fig. 1 Cercariae shed from *Biomphalaria alexandrina* and *Bulinus truncatus*. **a** *Schistosoma mansoni cercaria*, **b** *Schistosoma haematobium* cercaria, **c** *Pharyngate longifurcate cercaria type I,* **d** *Pharyngate longifurcate cercaria type II,* **e** *Xiphidiocercaria* species

In addition the obtained cercariae from this snail were; *Schistosoma manasoni*, *Pharyngeate longifurcate* type I and *Pharyngeate longifurcate* type II. This result agrees with that recorded by (Wanas et a1. 1993; Khalifa et a1. 1997;

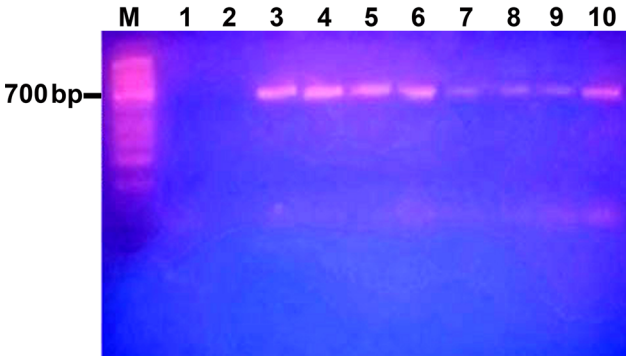

Fig. 2 PCR of *Biomphalaria alexandrina* snails for detecting the *S. mansoni* infection. *Lane M* is the DNA size marker, *lane 1* negative control snail, *lanes 2, 3* field snails negative microscopically, *lanes 4–9* field giving cercaria in lab, *lane 10* control positive

Aboelhadid 2004) by rates of 10.50 % in Giza Province, (0.02 %), 5.50 % in Beni-Suef Province respectively. Completely different prevalence was recorded in Qena Province the author recorded very high prevalence (84.11 %) (Hassan 1987). This variance may be referred to the area of study and recent intensive use of antischistosomal drugs which reflected on the recent surveys of infection.

The seasonal prevalence of infection was nearly similar to the results of Aboelhadid (2004) in which he detected the prevalence of *S. mansoni* cercariae shedding was high in spring (8.3 %). Another result of Korany (2011) failed to detect this cercaria in Beni Suef Province. She also recorded *Pharyngeate longifurcate* type I and *Pharyngeate longifurcate* type II, only in spring, at rat of 4.00 % for each. She recorded (27.66 %) in winter. This variation with our results may be referred to the different locality and may be the methods of examination. The variation may also be referred to the intensive use of molluscicides and anthelminthic drugs.

The obtained cercariae, we categorized it according to Frandsen and Christensen (1984) whom outlined the diagnostic features of this cercaria as the tail longifurcate, pharynx, oral and ventral suckers present, penetration gland cells all of one type and body and furcal finfolds were not present. It is similar to the cercariae that were reported by; Aboelhadid (2004) and Korany (2011) in Beni Suef from *B. alexandrina* . Mean while this cercaria differed with that recorded by Hassan (1987) in Qena, Egypt, who reported that it has 3 pairs of penetration glands; one pair preacetabular and 2 postacetabular while in the present cercaria there are 4 pairs of penetration glands; 2 pair are preacetabular and the other 2 are latero-postacetabular. The present *Xiphidiocercariae* shed from *B. truncatus* is similar to that recorded by Wanas et a1. (1993) and Khalifa et a1. (1997) from Assuit, in morphological and biological characters in which it encysted in the same snail, but there are certain minor differences.

According the result of this investigation, *S. mansoni* remains a problem in which the prevalence in the snail is 2.70 %. This result was from microscopic examination of the snails. So the possibility of prepatent infection is still present and may be the rate of infection increase depending on the results of PCR. Hamburger et al. (1998) adapted a polymerase chain reaction (PCR) assay very sensitive for detecting DNA of *Schistosoma mansoni* cercariae in water and in infected snails of early infection. The PCR assay enabled a clear differentiation between infected and normal snails. Infected snails were detected as early as 1 day after penetration of a single miracidium. The high sensitivity of the test enabled identification of a single infected snail even when its DNA was pooled with material from up to 99 uninfected snails, thus demonstrating the possibility of mass diagnosis in pools of snails.

The PCR used in the current study was proved to possess high level of sensitivity (Melo et a1. 2006) than standard screening of intermediate hosts by cercarial shedding. These PCR protocols have potential to be used as tools for monitoring of schistosome transmission. Depending on the recommendation of the World Health Organization that a major focus of research on schistosomiasis should be on the development and evaluation of new strategies and tools for control of the disease (WHO 2004). So this investigation targeted the use conventional PCR for detecting the infection of snails by *S. mansoni*.

The results concluded that, *S. mansoni* continues to constitute a considerable risk in this area and increase the possibility of distribution as the prepatent infection where the PCR could detect this latent infection.

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