Prevalence of Trematode Snails From Different Localities in Fayoum Governorate, Egypt

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Introduction

Snails (Gastropoda) act as intermediate hosts for various species of trematodes (Platyhelminthes: Trematoda) infecting livestock, human populations, birds and fish. Climate effects and utilization of river water for irrigation purposes are the main factors affecting distribution of snails [1]. Fascioliasis is an emerging/re-emerging vector-borne disease with the widest known distribution. Approximately 17 million people are infected around the world. Several snail species may contribute in transmission of Fscioliasis in Egypt. [2]. Schistosomiasis is also caused by digenetic trematodes that belong to genus *Schistosoma*. It cause a major public health problem in rural Egypt. Two species of schistosomes: *S. mansoni* and *S. hematobium* whose intermediate hosts are fresh water snails, *Biomphalaria alexandrina*, and *Bulinus trancatus* respectively are prevalent In Egypt [3]. This study aims to identify the prevalent snail spp. collected from different localities in Fayoum Governorate Egypt., and to identify trematode spp. Infecting them using PCR technique

Methods

- Different snail species were collected by using scoop net from July to September 2012 from 5 cities , 28 different sites in Fayoum governorate, Egypt and preserved in 99% ethanol for further examination. Snails were identified morphologically according to (Brown, 1994).
- DNA was extracted from each snail by Jet Quick® tissue DNA Miniprep Kits according to the protocol of manufacturer.
- PCR tests were performed for each snail (Subsamples of both foot samples and soft tissue samples) using different universal and specific DNA primers to detect possible infections.
- The PCR conditions were as follows for all primer sets : 95°C for 4 min, followed by 40 cycles of 95°C for 45 s, 56°C for 35 s and 72°C for 40 s followed by 72°C for 7 min.
- The PCR product were separated by electrophoresis in a1.5%-agarose gel, and visualized by a UV transilluminator.
- Purified PCR products were directly sent for sequencing (GATC Biotech, Köln, Germany). Identity of sequences was checked by BLAST search using FASTA format.

Figure 1: (a) shows *P. columella* on water hyacinth during collection . (b) shows different *P. columella* size

Results

Pseudosuccinea columella was the most abundant snail type collected from 5 cities, 28 different sites in Fayoum governorate, Egypt as shown in chart (1).
20% of the examined *P. columella* snails were infected with *Fasciola* sp., (figure 3) and the sequencing results revealed infection with *Fasciola gigantica* (figure 4), which can be considered a new finding for the African continent.
Also *Physa* sp. and *Cleopatra* sp. were infected with trematodes with a prevalence of 18% and 21%, respectively.
The overall prevalence was 11.5% considering all collected snails. No snails were found to be infected with *Schistosoma* sp. by PCR tests.

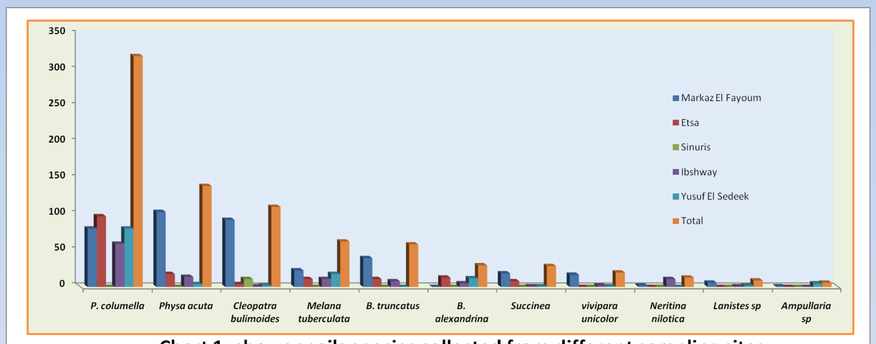

Chart 1: shows snails species collected from different sampling sites

Snail Species	Tested Subsample	Positive Samples	Prevalence of infection	Sequencing Results
P. columella	97	20	20%	*Fasciola gigantica*
Cleopatra bulimoides	14	3	21.42%	*S.sinensium/ Saturnius sp.*
Physa acuta	22	4	18.18%	*Saturnius sp.*
Other snails species	100	-	-	-
Total	233	27	11.50%	-

Table 1: shows prevalence of trematode infection in different snail species.

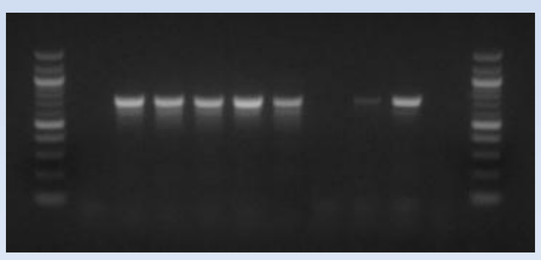

Figure 2: Agarose gel electrophoresis of polymerase chain reaction (PCR) products from snail foot tissues using "Universal" DNA primers to amplify 710-bp fragment of the mitochondrial cytochrome c oxidase subunit I (COX I) gene

Figure 3: Agarose gel electrophoresis of polymerase chain reaction (PCR) products from *P. columella* snail tissue using "Universal" DNA primers (cestrem 8f / cestrem 2r) amplifying part of the ITS2-28S rDNA ((ITS) the internal transcribed spacer regions of the ribosomal RNA gene (rDNA)).

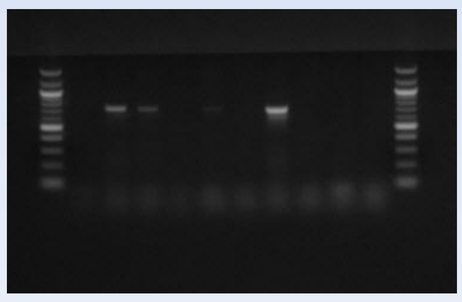

Figure 4: Agarose gel electrophoresis of polymerase chain reaction (PCR) products from *P. columella* snail tissue using specific DNA primers (Fg ITS F/ Fg ITS R)to amplify 716 -bp fragment of the ITS of *Fasciola gigantica*

Conclusions and outlook

Sequencing results of snail foot tissues helps in identification of the snails types.
Microscopic examination of the fixed snail tissues was difficult and time consuming.
Infection with *Fasciola* spp. were found in 21% of examined snails (*Pseudosuccinea columella*). the sequencing results revealed infection with *Fasciola gigantica*
Further sequencing tests is valuable to detect single or/and mixed *Fasciola* infection.
No snail species were found infected with *Schistosoma* spp.
Further examination is required to determine trematode infection in *Cleopatra* and *Physea* spp.
Correlation between snail abundance and water parameters (salinity, pH, Temp.) should be considered.
As a serious zoonotic disease, Fascioliasis is of importance for public health in Egypt and other countries. *F. gigantica* has not been reported before from the invasive snail *P. columella* in Egypt and this may explain the high rate of human infections in the region. Further investigations concerning the potential of *F. gigantica* to produce infective cercariae from this snail host are planned.

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