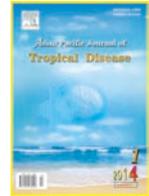


Contents lists available at [ScienceDirect](http://ScienceDirect)

## Asian Pacific Journal of Tropical Disease

journal homepage: [www.elsevier.com/locate/apjtd](http://www.elsevier.com/locate/apjtd)

Document heading

doi: 10.1016/S2222-1808(14)60448-5

© 2014 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

## *Lymnaea stagnalis* (Linnaeus, 1758) snails' infection to trematoda larval stages in Shahrekord city's springs

Shahla Rivaz<sup>1\*</sup>, Vahid Nasiri<sup>1</sup>, Gholamreza Karimi<sup>1</sup>, Mohamad Abdigoudarzi<sup>1</sup>, Habibollah Paykari<sup>1</sup>, Gholamreza Motamedi<sup>1</sup>, Hamidreza Azizi<sup>2</sup>, Khodad Pirali<sup>2</sup>

<sup>1</sup>Parasitology Department, Razi Vaccine and Serum Research Institute, Karaj, Alborz, Iran

<sup>2</sup>Parasitology Department, Shahrekord University, Sharekord, Iran

## PEER REVIEW

**Peer reviewer**

Dr. Mansoor Banani, Department of Biotechnology, Razi Vaccine and Serum Research Institute.  
Tel: 00989381057723  
E-mail: toxocara\_vahidd@yahoo.com

**Comments**

The presence of cercariae in snails in the region and their potential for establishment of zoonotic diseases especially parasitic disease in man and animals may be concerned by health system authorities for surveillance of these fresh water snails.

Details on Page S249

## ABSTRACT

**Objective:** To determine the *Lymnaea stagnalis* snails' infection with trematodes larval stage in one of the springs of the Shahrekord city in Chahar Mahal and Bakhtiari.

**Methods:** To determine the snail infection to trematodes larval stages, the snails were caught from the field, and transferred to the Parasitology Department of Razi Vaccine and Serum Research Institute. Then stimulating of snails by light, tubing and squashing of them were used for detection and identification of the isolated cercariae.

**Results:** Of 400 collected snails from the referred springs, 350 of them identified as *Lymnaea stagnalis*. Observed cercariae were identified and classified as order Plagiorchiida and genus Opisthioglyphe and Plagiorchis.

**Conclusions:** In Chahar Mahal and Bakhtiari Province, due to having more than 10% of water content of country, ecological conditions can play important role to develop sensitive snail especially Lymnaeidae and be considered as a critical and suitable habitat for them.

## KEYWORDS

Fresh water snails, Trematoda, *Lymnaea stagnalis*, Cercariae, Shahrekord

### 1. Introduction

Among parasitic diseases which are transmittable to humans, snail transmitted diseases compose an integral part. Many of freshwater snails are involved in parasitic trematodes cycle and parasitic diseases as intermediate host. In some cases, in addition to medical and health importance for human, they impose considerable damage to livestock and poultry which are one of the important

protein resources<sup>[1]</sup>, and cause decrease in amount of wool, milk, meat and other bestial products that finally lead to an economic detriment.

Knowledge about posture of transmittal of snails population in any zone, and parasitic disease transmitted by them is one of the basic pledges for fighting with snails and increases the health level of the society. Lymnaeidae family snails have place in order of Basomatomorpha and sub-class of Pulmonata from Gastropoda class<sup>[2]</sup>. *L. stagnalis*

\*Corresponding author: Shahla Rivaz, MSC of veterinary Parasitology, Department of Parasitology and Entomology, Razi Vaccine and Serum Research Institute, Karaj, Alborz, Iran.

Tel: +982634570038

E-mail: s.rivaz@rvsri.ac.ir

Foundation Project: Supported by the Razi Vaccine and Serum Research Institute (Grant No.1598).

Article history:

Received 25 Nov 2013

Received in revised form 1 Dec, 2nd revised form 6 Dec, 3rd revised form 11 Dec 2013

Accepted 26 Dec 2013

Available online 28 Jan 2014

(*L. stagnalis*) snail is the biggest intermediate host *Lymnaea* in Iran whose shell has 45 mm length and 25 mm width, contains 7–8 spire and the throat of shell is alike to human's auricle[3]. This snail has been seen in Khoozestan, Khoram abad in Lorestan, Shahrekord and West Azerbaijan zones. This group of snails is involved in establishing of life cycle in at least 71 species of trematodes[4]. Order of Digenea is the most important order of trematodes which in his path of perfection need intermediate host from the snail groups. This group of parasites contains human and animal trematodes which engender variety of pulmonary, digestive, hepatic and blood diseases[5].

Larval phases in trematodes encompass sporocyst, redia and cercaria that create in snail's body and obviously recognition of them is of great importance in line with recognition of parasitic species[6].

In some cases, infection of some species of snails from Lymnaeidae family with miracidium of mammal's and bird's parasitic schistosoma leads to creation of cercaria which is creator of dermatitis in humans[7–9]. Outbreak of pandemic human fasciolosis and the role of snails in economically important trematodes life cycle in veterinarian are the other notable cases[10,11].

Up to now, different cercariae are recognized. Cercariae are created from redia or sporocyst daughter[12]. They often leave their Mollusca (clams) host and have a free life for a while. Then they find another host or place for cystic[13]. Most of the cercariae have a long tail for swimming, but in some cases the tail is short and in other cases there is no tail, so that the creepy movements are recognizable in this group. Anatomy of excretory system is noticeable in classification of cercariae. Excretory sacs can have thick epithelial or thin and non-epithelial walls; this characteristic is also applicable in their classification[14].

## 2. Materials and methods

The snails which were subject to this study were collected from the 'Vaght and Saat' spring in Chaharmahal and Bakhtiyari Province. This spring and its diverted rivers have divided into five parts and during the spring season of 2012, the samples were collected. The snails were put in plastic container and some spring water was added to them and was sent to Parasitology Department of Razi Vaccine and Serum Research Institute. For snail's maintenance, the conditions like the natural habitats were provided. Snails have been placed in preserving aquariums contain color-free water with feeding them by lettuce.

For identifying the snail, the shell height, the spire and the body whorl were measured.

The snails were identified by using the parasitological keys and for confirmation of identification, radula was studied.

The soft parts of snail were placed in 10% KOH and after 4 h were investigated in a Petri dish under a loop. The radula was dissected from the other parts, and after staining with the radula, identifications were be dissected.

For determining the infection to larval stages, snails were investigated by different methods.

At first, the physical stimulation of snails were be done by putting them under the artificial light, by heating them and slight pressure of snails shells.

In the next step, the tubes were used for the detection of cercariae. In each tube, some color-free water has been

purred and a snail has been put in each tube and a screen has been put on their cap for prevention of exiting the snails. These collections were put under the effect of artificial light, and in the next day were studied of the existence of cercariae.

Snails which were studied by the above mentioned methods and without cercariae were crushed between the double glazing surfaces, and were carefully investigated under the dissection loop. Direct wet mount slides were prepared from alive and motile cercariae and their movement, apparent and morphological characters were investigated under light microscopy.

For the fixation, cercariae were heated in the 10% formalin, then (for stabilizing the external shape of the cercariae) were mixed 1 cm<sup>3</sup> of warm formalin with 1 cm<sup>3</sup> of water contain cercariae, and then the cercariae were stained and identified by light microscopes.

## 3. Results

Of 400 collected snails from the referred spring, 350 of them identified as *L. stagnalis*. The average height of the shell was 35 cm, the length of the spire was 15 cm and the body whorl was 20 cm. The length of the spire was shorter than the length of body whorl. Then with the identifying key of the snails and inquiring the *L. stagnalis* were identified which were studied in terms of existence of cercariae and the kind of them.

In collected *L. stagnalis* snails, the infection to trematode larval stages of cercariae in a large number was recognized. During the process of cercarial shedding, the snail started to eject the cercariae; that due to the excessive activity of the cercariae, they were obviously observable under the binocular loop and more than 30% of the under study *L. stagnalis* snails were identified infected by cercariae.



Figure 1. Opisthioglyphe (Plagiorchiidae) of *Lymnaea stagnalis*.

The observed cercariae identified with the mono-branch tail, the stylet in oral sucker, and ventral sucker with the same size or larger than oral sucker, and ventral sucker in the middle of the body. Regarding the mentioned features,

observed cercariae stand in plagiorchoid group of cercariae, Plagiorchidae Family; and with the observed differences in length of cercaria, the shape and the length of the tail and etc. *Opisthioglyphe* (Figure 1) and *Plagiorchis* (Figure 2) were identified.



**Figure 2.** *Plagiorchis* (Plagiorchidae) of *Lymnaea stagnalis*.

#### 4. Discussion

Chaharmahal and Bakhtiyari Province with more than 700 spring, has the most soft and tasty water in the country; in that this province with only 1 percent of Iran's area, it contains more than 10 percent of Iran's water resources. Aquatic habitats in this province in most cases are in the form of springs and in the rare cases in the form of shallow wetlands. Shahrekord city with 1991 meters height from the sea has yearly rain of 248 mm, medium temperature of 12.8 °C and humidity of 33% to 60%. Vaght and Saat spring are located in Shahrekord city with 2 kilometers distance from Shamsabad village, and besides Jahanbin Mountains. Excess of water resources in the area create streams diverted from the spring and stockbreeder come to this streams for grazing their herds and showering their sheep. Moreover, people come to this area for camping and spend some hours of day beside the spring and use its water for washing, eating and cooking.

A survey of sample studies which have done on snails by parasitologists, has been implicated on existence of species of freshwater snails in Iran and their potential ability of creating important diseases like fasciolosis, schistosomiasis and hetrophiyasis has been proven<sup>[15,16]</sup>.

Regarding the study of Shahlapoor (1375) which has done inquiries on Iran's *Lymnaea* regarding spread of fasciolosis disease, it has been identified after the previous theories of researchers. *L. stagnalis* play an important role in transfer of *Fasciola hepatica* and *Fasciola gigantica* to vegetarian animals and humans<sup>[3]</sup>.

The death of birds by infection of this parasite has been

reported. Infection with this parasite causes depression, inhibition of weight increases and eventually the death of the birds<sup>[5]</sup>.

In species of *Plagiorchis*, megalorchis primary intermediate host is *Lymnaea* and the second intermediate host is Nematocera<sup>[1]</sup>.

The infection of birds by *Plagiorchis elegans* and *Plagiorchis maculosus* that be caused by eating dragonfly or insects in larval stage who are the second intermediate host of parasite<sup>[17]</sup>.

In a study by Zbikowska (2005) on fresh water resources which has done in part of Poland also has reported the existence of Plagiorchidae in *Lymnaea* snails<sup>[18]</sup>.

Faltynkova and his colleagues (2007) investigated on the cercariae and metacercariae (Trematoda, Digenea) of the great pond snail *L. stagnalis* in Central Europe. A total of 953 (26.3%) *L. stagnalis* were infected with 24 trematode species comprising 19 species of cercariae and 11 species of metacercariae (six species occurred both as cercariae and metacercariae) of eight families. The dominant cercariae were those of *Opisthioglyphe ranae* (159 hosts infected), *Plagiorchis elegans* (141) (both family Plagiorchidae) and *Echinoparyphium aconiatum* (153) (Echinostomatidae); 14 double infections were found<sup>[19]</sup>.

The Primary intermediate host of *Plagiorchis* is freshwater snails and the secondary intermediate host is fishes, snails and arthropods. Human infections with *Plagiorchis philippiniensis*, *Plagiorchis javensis* and *Plagiorchis harinasutai* have reported in Philippine, Indonesia and Thailand. *L. stagnalis* also harbored sporocysts and shed cercariae of *Schistosomatium douthitti* and a *Plagiorchis*. They also seem to be associated closely with snail life histories in terms of the availability of large snails most often infected by the sporocysts of *Schistosomatium douthitti* and a *Plagiorchis*<sup>[12]</sup>.

Regarding the economic damage emanated from infection of trematodes and that snail have the role of intermediate host in transmission of trematodes, the survey and study of them seem necessary.

The result of this study shows that *L. stagnalis* snails play role in continuance of the lifecycle of some trematodes that regarding the geographic situation of the province, the appropriate whether of the area, existence of proper settlement for intermediate host and the condition was prone for infection definite host. If the definite hosts expose to a large number of cercariae, the possibility of epidemic will engender in the area. Thus, the complimentary studies seem necessary for survey of prevalence of trematodes in this snail all over the province.

#### Conflict of interest statement

We declare that we have no conflict of interest.

#### Acknowledgements

This research was financially supported by the Razi Vaccine and Serum Research Institute (Grant No. 1598).

## Comments

### Background

The snails have fundamental role in maintenance and preparation of complex conditions of parasitic trematoda life cycle and thus developing our knowledge about their behavior and distribution can help us find effective tools to prevent negative side effects of them.

### Research frontiers

Finding new points of view about different aspects of parasitic life cycle by analyzing their snails host is very useful tool to battle with them and this article will open a new insight into basic epidemiological aspect of snail's fauna in this region.

### Related reports

The authors approximately have made good basic background of the main knowledge about the condition of work. It seems that like other similar researches that have been done in other parts of world, this study can change our sight about the ecological condition of snails and their role in maintenance of parasitic disease.

### Innovations & breakthroughs

Although many districts of Shahrekord city have wide water resources, study about the fresh water snails of this region was still obscured. Due to the presence of possible infected snails, water resources could be contaminated by the cercariae and consequently infected the local people. Thus, it shows the importance of study on ecological properties of the snail population of local fresh waters.

### Applications

According to data resulted from this study, we could understand that there is a possible risk of presence of snail transmitted parasitic disease and can lead us to further studies to find complete ecological relationship of possible parasitological life cycle.

### Peer review

The presence of cercariae in snails in the region and their potential for establishment of zoonotic diseases especially parasitic disease in man and animals may be concerned by health system authorities for surveillance of these fresh water snails.

## References

- [1] Żbikowska E. One snail – three Digenea species, different strategies in host–parasite interaction. *Ani Biolo* 2011; **61**: 1–19.
- [2] Kudlai OS. The discovery of the intermediate host for the trematode *Moliniella anceps* (Trematoda, Echinostomatidae) in Ukraine. *Vestnik zoologii* 2009; **43**(4): 11–13.
- [3] Hohagen J, Jackson DJ. An ancient process in a modern mollusc: early development of the shell in *L. stagnalis*. *BMC Dev Biol* 2013; **13**: 27.
- [4] Soldánová M, Selbach C, Sures B, Kostadinova A, Pérez–del–Olmo A. Larval trematode communities in *Radix auricularia* and *L. stagnalis* in a reservoir system of the Ruhr River. *Parasit Vectors* 2010; **3**: 56–68.
- [5] Cichy A, Faltýnková A, Żbikowska E. Cercariae (Trematoda, Digenea) in European freshwater snails – a checklist of records from over one hundred years. *Folia Malacologica* 2011; **19**(3): 165–189.
- [6] Curtis LA. Larval trematode infections and spatial distributions of snails. *Invertebrate Biol* 2007; **126**(3): 235–246.
- [7] Faltýnková A, Nasincová V, Kablásková L. Larval trematodes (Digenea) of planorbid snails (Gastropoda: Pulmonata) in Central Europe: a survey of species and key to their identification. *Syst Parasitol* 2008; **69**: 155–178.
- [8] Żbikowska E, Żbikowski J. Differences in shell shape of naturally infected *L. stagnalis* (L.) individuals as the effect of the activity of digenetic trematode larvae. *J Parasitol* 2005; **91**: 1046–1051.
- [9] Karruki HC, Clennon JA, Brady MS, Kitron U, Sturrock RF, Ouma JH, et al. Distribution patterns and cercaria shedding of *Bulinus nasutus* and other snails in the Msambweni area, Coast Province, Kenya. *Am J Trop Med Hyg* 2004; **70**(4): 449–456.
- [10] Tkach VV. Family Plagiorchiidae Luhe, 1901. In: *Keys to Trematoda*. 2008: 295–325.
- [11] Novobilský A, Ka Ný M, Beran L, Rondelaud D, Höglund J. *Lymnaea palustris* and *Lymnaea fuscus* are potential but uncommon intermediate hosts of *Fasciola hepatica* in Sweden. *Parasit Vectors* 2013; **6**(1): 251.
- [12] Skála V, Bulantová J, Walker AJ, Horák P. Insights into the development of *Notocotylus attenuatus* (Digenea: Notocotylidae) in *L. stagnalis*: from mother sporocyst to cercariae. *Parasitol Int* 2014; **63**(1): 94–99.
- [13] Koprivnikar J, Walker PA. Effects of the herbicide atrazine's metabolites on host snail mortality and production of trematode cercariae. *J Parasitol* 2011; **97**(5): 822–827.
- [14] Yoder HR, Coggins JR. Larval trematode assemblages in the snail *L. stagnalis* from southeastern Wisconsin. *J Parasitol* 1998; **84**(2): 259–268.
- [15] Pokora Z. [Role of gastropods in epidemiology of human parasitic diseases]. [Article in Polish] *Wiad Parazytol* 2001; **47**(1): 3–24.
- [16] Kharoo VK. Studies on the Family Plagiorchiidae Luhe, 1901 with redescription of *Plagiorchis bulbulii mehra*, 1937 a digenetic trematode collected from two different hosts in Kashmir, India, *Indian J Fundam Appl Life Sci* [ISSN: 2231–6345]. 2011 [cited 2014 Jan 20]; **1**(2): 119–126. Available from: t <http://www.cibtech.org/jls.htm>.
- [17] Żbikowski J, Żbikowska E. Invaders of an invader—trematodes in *Potamopyrgus antipodarum* in Poland. *J Invertebr Pathol* 2009; **101**(1): 67–70.
- [18] Żbikowska E. Digenea species in chosen populations of freshwater snails in northern and central part of Poland. *Wiad Parazytol* 2007; **53**(4): 301–308.
- [19] Faltýnková A, Nasincová V, Kablásková L. Larval trematodes (Digenea) of the great pond snail, *L. stagnalis* (L.), (Gastropoda, Pulmonata) in Central Europe: a survey of species and key to their identification. *Parasite* 2007; **14**(1): 39–51.