

## Natural Larval Trematode Infections in *Lymnea truncatula* Populations in Elazig Province in Turkey

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**Abstract:** Freshwater snails play a significant role as intermediate host in life cycles of trematodes. In addition to providing these parasites with a site for reproduction, freshwater snails are also carriers to reach them to their next hosts. It is obvious that infected snail carrying cercaria or metacercaria play an important role in the distribution of trematodes to their final hosts including farm animals and human beings. It was aimed to determine the larval trematode infection prevalence in *Lymnea truncatula* populations in Elazig. Total of 191 *L. truncatula* samples were collected from three regions in Icme town and two regions around Hazar lake in Sivrice town making a total of five regions. Shell lengths of *L. truncatula* were 0.6-15 (8.90±4.5) mm and shell widths showed a range of 0.3-0.8 (0.54±0.1) mm. Of the 14 (7.3%) of *L. truncatula* samples studied under stereomicroscope by applying pressure and carrying out dissection, trematode larvae were established. While a significant difference was not observed between shell lengths and the presence of trematode larvae, a significant difference was found between shell width and the presence of trematode larvae.

**Key words:** *Lymnea truncatula*, larval infection, trematode, snail, Elazig, Turkey

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### INTRODUCTION

Freshwater snails play a significant role in life cycles of trematodes. In addition to providing these parasites with a site for reproduction, freshwater snails are also carriers to reach them to their next hosts. Acting as obligatory intermediate hosts for most of the known 18000 trematode species snails have a close relationship with trematodes (Mas-Coma *et al.*, 2005; Saygi, 2009; Zbikowska and Nowak, 2009).

Development of trematodes in a snail begins with swallowing of parasite eggs or with the penetration of miracidium. In both cases, larva stays clear from lashes in the body of a snail and transforms into sporocyst or redia. In the sporocyst period, larvae proliferate and form tens and in some species, hundreds of thousands of cercaria and then leave the snail one after another (Saygi, 2009). Depending on the trematode species, cercaria directly penetrates into the final host or metacercaria is formed, stepping onto the next stage. Metacercaria creates cysts in outer environment or penetrate into the second vertebrate or invertebrate intermediate (including snails) host (Mas-Coma *et al.*, 2005; Zbikowska and Nowak, 2009). It is obvious that infected snail carrying cercaria or metacercaria play an important role in the distribution of trematodes to their final hosts including farm animals and human beings.

In case of high infection prevalence in natural snail populations, larval trematodes may act as snail population regulators (Phiri *et al.*, 2007). It has been reported that some trematodes are responsible for the elimination of snail populations. Studies on larval trematodes may reveal whether there exist trematode species that could be manipulated for biological control of snail-mediated diseases (Phiri *et al.*, 2007). Larval trematode infections are also used as an indicator of environmental quality (Phiri *et al.*, 2007; Mas-Coma *et al.*, 2009).

Trematode infections were reported in humans and animals in studies conducted in Elazig province of Turkey (Kaplan *et al.*, 2002a-c; Kaplan and Baspinar, 2009). As a result of investigations in areas where especially, human cases were recorded, it is believed that the detection of larval trematode infection prevalence in freshwater snail populations in Elazig, predominantly around Hazar lake and Keban Dam reservoir, bears significance for control studies. This study aimed at the detection of larval trematode infection prevalence in *Lymnea truncatula* populations in Elazig.

### MATERIALS AND METHODS

This study was approved by the Firat University Animal Experimental Ethical Committee (09 August, 2010, Approved No.: 77).

**Study region:** Selected as the study region in Elazig were two different water conduits (Icme 1 and 2) in Icme town located on the bank of Keban dam reservoir (38°36'K, 39°33'D, 971 m), swamp areas (Icme 3) with another swamp area (Sivrice 1) in Sivrice town located along the coast of Hazar lake (38°27'K, 39°19'D, 1241 m) and the periphery of a creek flowing into Hazar lake (Sivrice 2) that are among the regions where human fascioliasis cases were established and stockbreeding is common.

**Collection of *L. truncatula*:** One or several regions of 10 m<sup>2</sup> selected in proportion to the sizes of each determined areas were investigated for 15 min and each freshwater snail that were found were put in zip lock bags and labeled. Properties of the area where the specimen were collected were recorded and the samples were then immediately taken to laboratory. The samples were studied morphologically and microscopically in the laboratory.

**Morphological and microscopic examination:** A distinction of species was conducted as defined before (Mandahl-Barth, 1962; Burch, 1982) bearing in mind the morphological characteristics of the shells of collected freshwater snails freshwater snails. Referred to as *L. truncatula* were included in the study and were first investigated in terms of the presence of cercaria release. To that end, the living samples were put in transparent plastic bags each containing 30 mL oxygen water as defined above and maintained there for 15-20 min at 4°C then the samples were placed 1 m away from a 100 W for 24 h (Mendoza *et al.*, 2004).

Plastic bags and water were examined under a stereomicroscope for possible cercaria release. Secondly, etherized snails were extracted from their shells and dissected under stereomicroscope. Pressure was applied with clamps before the dissection procedure and cercaria release was thus checked (Mendoza *et al.*, 2004). Presence of any trematode larvae were investigated during the pressure application and dissection procedures.

## RESULTS AND DISCUSSION

Collected were 191 *L. truncatula* from three regions in Icme town and two regions around Hazar lake in Sivrice town making a total of five regions. Cercaria was not established through a cercaria release study conducted by keeping the samples under artificial light. Of the 14 (7.3%) of *L. truncatula* samples studied under stereomicroscope by applying pressure and carrying out dissection, trematode larvae were established (Fig. 1). While trematode larvae were not found among *L. truncatula* samples collected from 3 regions in Icme

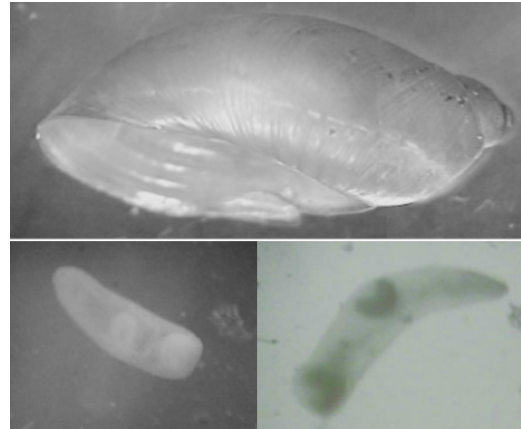


Fig. 1: *Lymnaea truncatula* shell and trematode larvae

town, trematode larvae were established in 9 (17.3%) and 5 (17.9%) *L. truncatula* samples, respectively that were collected from two regions in Sivrice town (Table 1). Shell lengths of *L. truncatula* were 0.6-15 (8.90±4.5) mm and shell widths showed a range of 0.3-0.8 (0.54±0.1) mm. While a significant difference was not observed between shell lengths and the presence of trematode larvae, a significant difference was found between shell width and the presence of trematode larvae ( $p < 0.05$ ) (Table 1).

In addition to trematode infections causing significant economic losses in farm animals, they are also common in human beings. Today, the said infections are prevalent among humans and animals in numerous regions in Latin America, Africa, Europe and Asia (Mas-Coma *et al.*, 2005; Zbikowska and Nowak, 2009). In order to develop appropriate control strategies against trematode infections, it is necessary to establish species and distribution of snails with intermediate hosts, prevalence of larval trematode infection in snail populations to recognize sensitive and resistive species and as a result, to select appropriate methods specific to the region. Conforming to these requirements, World Health Organization implemented individual pilot strategies for controlling fascioliasis specific to different countries and regions where fascioliasis is epidemic (WHO, 2007).

Snails belonging to Lymnaeidae family bear an utmost importance in terms of parasitology. Numerous Helminth species are mostly carried and contaminated by trematodes Trematode infections that are more common in the region with medical and veterinary significance are *Fasciola hepatica* and *Fasciola gigantica* and they can be detected in both humans and farm animals (Kaplan *et al.*, 2002a-c; Kaplan and Baspinar, 2009). In the province, regions where human cases were established, studied and the presence of larval trematode infection was

Table 1: Trematode larvae detection based on sample collection regions and *Lymnaea truncatula* shell sizes

Parameters	Trematode larvae				Total (n)
	Absent		Present		
	n	Percentage	n	Percentage	
<b>Collection region</b>					
İcme 1	7	100.0	-	-	7
İcme 2	6	100.0	-	-	6
İcme 3	98	100.0	-	-	98
Sivrice 1	43	82.7	9	17.3	52
Sivrice 2	23	82.1	5	17.9	28
Statistics $\chi^2$ (p = 0.000)					
<b>Shell lengths (mm)</b>					
0.6	2	100.0	-	-	2
0.7	9	100.0	-	-	9
0.8	6	75.0	2	25.0	8
0.9	24	96.0	1	4.0	25
1.0	48	98.0	1	2.0	49
1.1	41	95.3	2	4.7	43
1.2	28	87.5	4	12.5	32
Statistics $\chi^2$ (p = 0.127)					
13.0	9	81.8	2	18.2	11
14.0	5	100.0	-	-	5
15.0	5	71.4	2	28.6	7
<b>Shell widths (mm)</b>					
0.3	4	100.0	-	-	4
0.4	24	100.0	-	-	24
0.5	69	93.2	5	6.8	74
0.6	56	94.9	3	5.1	59
0.7	19	86.4	3	13.6	22
0.8	5	62.5	3	37.5	8
0.9	1	100.0	-	-	1
Statistics $\chi^2$ (p = 0.039)					

investigated in freshwater snails belonging to Lymnaeidae family, the intermediate hosts of these trematodes. In this study that was conducted for the 1st time in the province, 14 of the entire *L. truncatulas* (7.3%) were established to have trematode larvae.

### CONCLUSION

The results of this study shows that the presence of larval trematode infection in snails is traditionally studied by dissection and microscopic examination. Although, molecular methods are also adopted today, researchers opted for the traditional method based on the current laboratory facilities.

The study is a pre-study and it is the main target to confirm and define freshwater snails established as *L. truncatula* and trematode larvae by molecular methods and also to detect the larval trematode infection presence by molecular methods in addition to traditional methods.

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