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Original Article

Investigating the Invasive Contamination of Lymnaeidae Snails with Trematodes According to Species and Sampling Location in Lorestan province, Iran, Middle East

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ABSTRACT

Radicine snails are of considerable medical and veterinary importance as trematode vectors. These snails are responsible for transmission of the zoonotic trematodes including Schistosoma turkestanicum and Fasciola gigantica in Iran. This study investigates Lymnaedae infestation by trematodes, considering the species and sampling locations. 1,700 snails were collected from the suburbs of Borujerd, Khorram Abad, and Dorud in Lorestan, Iran from April to August 2018. Round snails were separated, and Snail species were identified by measuring length, width, spire, and valve, using the shape of the radula as an identification key. To separate the radula, snails soft tissue was removed from the shell using forceps, then incubated in a 7% potassium solution for 24 hours at room temperature. The isolated radula was placed in a 15% acetic acid solution. It was then placed in Mallory's dye solution for 3 minutes and sunsequently washed with an oxalic acid solution. After dehydration with 96% ethanol, the samples were examined under a light microscope. To investigate trematode larvae in snails, 10% of the snail samples (a total of 170 Lymnaeidae snails) were selected and examined using the crushing method on a slide. The morphological results showed that in Dorud and Borujerd, the highest distribution of Lymnaea gedrosiana was 24.09% and 19.72%, while the lowest distribution of Bulinus truncatus was 4.72% and 4.48%, respectively. Lymnaea species were the most abundant in plain villages, whereas Bithynia and Physa were more commonly observed in mountain villages. In Khorram Abad, the highest distribution was related to Lymnaea truncatula (20.15%), while the lowest distribution was related to Lymnaea stagnalis (5.56%). The genera Bithynia and Physa showed a significant increase in mountainous villages of Khorram Abad compared to those in Borujerd and Dorud. The overall infectionrate of Lymneidae snails with trematodes was 32.94%, including 18.23% in Borujerd, 8.23% in Dorud, and 6.47% in Khorramabad. According to the chi-square test ($p<0.05$), a significant difference was observed in the rate of trematode infection in Lymneade snails. In this regard, the Borujerd region exhibited the highest rate of infection, whereas Khorram Abad showed the lowest.

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1. Introduction

The distribution of the snail population in the region and the transmission of parasitic diseases areamong the basic requirements for snail control to improve public health (1). The *Lymnaedeae* snail family, classified under the order *Basomatophora* and suborder *Pulmonata,* is deemed one of the most important groups in this type of study (1).

Freshwater snails play an important role as intermediate host in the life cycles of several parasitic nematodes and trematode species (2). Therefore, these creatures are of significant medical and veterinary importance. Many studies have been performed on freshwater snails and related parasitic infections in Iran, but a reliable and well documented study in this field is still in demand (3). A major part of parasitic diseases that can be transmitted to humans is hosted by snails (3). Therefore, knowing about the distribution of the snail populations in every single region is of paramount importance (3).

Identifying the prevalence of parasitic infections and parasitic worms in various species of freshwater snails, as intermediate hosts, using conventional microscopic methods has been performed in different regions of Iran (4). Current information reveals that many freshwater snail species are widely distributed across the country, while several species are confined to specific regions (5). For example, *Lymnaea truncatula* and *Lymnaea gedrosiana* have been observed in the highlands and plains of considerable parts of the country, respectively (6). At the same time, the geographical distribution of *Bulinus truncatus* seems to be restricted to Khuzestan province (7).

Snails of the *Lymenidae* family belong to the class Gastropoda, suborder Pulmonata, and order Basomatophora. They are hermaphrodite species (8) charachterized by round, triangular prongs. *Lymnaea gedrosiana* and *Lymnaea truncatula* have the widest distribution throughout Iran, while *Lymnaea rufescens* has the lowest distribution (9). These snails are amphibians and capable of living in shallow water for several hours (10). They occasionally move out of the water to rest on the nearby flowers (10). They can survive through the dry months of summer as well as freezing temperatures (10).

Freshwater snails have a wide variety of species across the world, and Iran is no exception (11). However, many ecological and biological aspects of Iran's native species remain unknown (12). Therefore, this study aimed to investigate the contamination of Lymnaeidae snails with trematodes, considering species and sampling locations in Lorestan province (13).

Continuous monitoring and study of snails in areas with a history of occurrence or spread of infections that can be transmitted through snails to humans and livestock are of great health importance

2. Materials and Methods

2.1. Collecting Lymnaeidae Snails

Radisin snails belong to the family Lymnaeidae, a group of freshwater snails with significant medical and veterinary importance, globally. To investigate the level of contamination of *Lymnaea gedrosiana* snails with trematode larvae, as well as their molecular identification and determination of their ancestral origin, 1,700 snails were collected from the suburbs of Borujerd, Khorram Abad, and Dorud in Lorestan, Iran, from April to August 2018. These snails were collected using netted metallic scoops or by hand and identified in the field as *Radix gedrosiana* based on shell morphology, as described in the most recent catalog of freshwater snails from Iran by Gloer and Pe˘si´c (2012). These snails were then preserved in 70-100% ethanol and returned to the laboratory for assessment of trematode larva in snails.

2.2. Study Design

Attention was paid to the potential risks and environmental impact of collectinglarge number of live samples and empty shells. Therefore, the collection of empty shells was carried out on a small and controlled scale (Ethical code: IR.IAU.SRB.REC.1399.051). A total of 1,700 snails were collected. The collection locations are presented in Table 1.

2.3. Fixation and storage of *Lymnaeidae* snails

Snails were identified by measuring the length, width, spire, and valve using a caliper, and by examining the shape of the radula, following the identification key. To separate the radula, the soft tissue of the snail was removed from the shell using forceps and incubated in a 7% potassium chloride solution for 24 hours at room temperature. The isolated radula was then placed in 15% acetic acid. Afterwards, It was placed in Mallory's dye solution for 3 minutes, washed with oxalic acid, dehydrated with 96% ethanol, and then examined under a light microscope.

Table 1. Sampling location and number of samples according to the city and its suburbs in Iran.

City	Village							Number of samples
Borujard	Sarab Zarem	Shirvan	Chegani Kash	Chenaristan	Sheikh Miri	Tudeh Zan	Araban	735
	175	185	60	75	95	82	63	
Khorram Abad	Rig sefid	Ivshan	Taleghan	Zagheh		Goldareh		521
	80	112	115	130		84		
Dorud	Hamyaneh		Zargaran	Zhan	Torshab	Aziz abad		444
	105		95	80	90	74		
Total								1700

To investigate trematode larvae in snails, 10% of the snails samples (a total of 170 *Lymnaeidae* snails) were selected and examined using the crushing method on a slide.

2.4. Radula staining

Radula staining was used to identify *Lymnaeidae* snails. In this method, the buccal mass of the cochlea was separated and placed in a 7.5% potasium chloride solution to dissolve the tissues attached to the radula. Most of the tissue surrounding the radula is dissolved in this way, but small amounts of tissue may remain intact around the radula. Therefore, before staining, w the remaining tissue was removed using a fine and thin brush or via a dissection needle to avoid any problems in preparing microscopic samples.

2.5. Examination of trematode larvae in snails

2.5.1. Petri dish method

In this method, the snails were stimulated individually in a glass petri dish (6 cm in diameter and 2 cm in height) containing chlorine-free water to release the cercaria using light alternation. Then, the water containing released sugars was examined.

2.5.2. Intubation method

In this method, a test tube containing a snail was half-filled with water and exposed to direct light for 5 hours to remove trematode larvae.

2.5.3. Smooth glass surface

The crushed snails were examined under binoculars for the presence of larvae. The number of snails examined by the crushing method to examine trematode larvae is presented in table 2.

3. Results

3.1. Trematode larvae infection in *Lymnaea* snails

Out of 1700 Lymnea snails samples, 10 % evaluated for larvae infection. 73 samples were collected from Borujerd, 45 were from Dorud, and 52 samples reported from Khorram Abad. They were examined by crushing method (Table 3 and Figure 1). According to Table 3, the percentage of contamination infection with trematode larvae in three snail species- Lymnaea gedrosiana, L. auricularia, and L.truncatula- was 32.94%, including 18.23% in Borujerd, 8.23% in Dorud, and 6.47% in Khorramabad samples. Borujerd exhibited the highest infection rate, while Khorramabad showed the lowest (p<0.05). Additionally, the distribution of *L.Gedrosiana* was 44.64%, *L. Auricularia* 14.28%, and *L. Truncatula* 41.07%.

3.2. Distribution of snail species in Dorud villages according to shell charachteristics

In this study, 444 snails were collected from 5 villages in different regions of Dorud city (Hamianeh, Zargran, Zhan, Tarshab, and Azizabad). these samples were subsequently analyzed based on shell characteristics (14) (Table 4). As shown in **Table 4**, the highest frequency distribution was observed in *Lymnaea gedrosiana* (24.09%), while the lowest was recorded in *Bulinus truncatus* (4.72%). *Lymnaeidae* snails were the most abundant species in plain villages; whereas *B. Tinea* and *physa acuta* snails were predominantly found in mountainous villages like Aziz Abad.

3.3. Distribution of Snail Species in Borujerd Villages According to Shell Characteristics

In this study, a total of 735 snails were collected from 7 villages located in different regions of Borujerd city (Sarab Zaram, Shirvan, Chegani Kesh, Chenarstan, Sheikh Miri, Tudeh Zan, and Araban). The specimens were analyzed based on shell characteristics (14) (Table 5). In table 5, the highest frequency was observed in *Lymnaea gedrosiana* (19.72%).

Table 2. The number of snails examined by the crushing method to examine trematode larvae.

City	Village							Number of samples
Borujard	Sarab Zarem	Shirvan	Chegani Kash	Chenaristan	Sheikh Miri	Tudeh Zan	Araban	73
	17	18	6	8	10	8	6	
Khorram Abad	Rig sefid	Ivshan	Taleghan	Zagheh		Goldareh		52
	8	12	10	13		9		
Dorud	Hamianeh		Zargaran	Zhan	Torshab	Aziz abad		45
	11		9	10	8	7		
Total								170

Table 3. The results of investigating the infection of *Lymnaeidae* snails with trematodes based on species and sampling location.

Location	Sample (n)	*Gedrosia* species		*Auricularia* species		*Truncatula* species		Total infected (n)	Percentage of relative abundance
		Tests (n)	Infected (n)	Tests (n)	Infected (n)	Tests (n)	Infected (n)		
Borujard	73	35	16	15	4	23	11	31	18.23
Dorud	45	21	4	11	3	13	7	14	8.23
Khorram Abad	52	28	5	8	1	16	5	11	6.47
Total	170	84	25	34	8	52	23	56	32.94

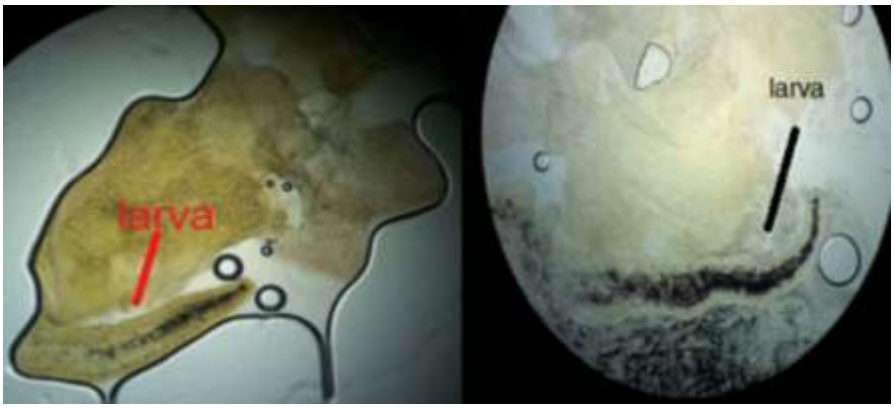

Figure 1. Light microscopy (LM) images of the *Lymnaea gedrosiana*, *Lymnaea truncatula* red color, Optical microscope with 10× magnification (main).

Table 4. Classification of snails based on shells in the study areas of Dorud city.

Snail genus and species	Number	Relative abundance percentage
Lymnaea gedrosiana	107	**24.09**
Lymnaea auricularia	90	**20.27**
Lymnaea truncatula	76	**17.11**
Lymnaea peregra	32	**7.20**
Lymnaea stagnalis	35	**7.88**
physa acuta	38	**8.55**
Bithynia	45	**10.13**
Bulinus truncatus	21	**4.72**

Table 5. Classification of snails based on shells in the study areas of Borujerd city.

Snail genus and species	Number	Relative abundance percentage
Lymnaea gedrosiana	145	**19.72**
Lymnaea auricularia	98	**13.33**
Lymnaea truncatula	115	**15.64**
Lymnaea peregra	70	**9.52**
Lymnaea stagnalis	65	**8.84**
physa acuta	85	**11.56**
Bithynia	74	**10.06**
Bulinus truncatus	33	**4.48**
Gyraulus	50	**6.8**

Lymnaea species were most prevalent in plain villages, while *Bithynia*, *physa acuta*, and *Gyraulus* were predominantly observed in mountainous villages such as Chenaristan and Chegani Kash.

3.4. Distribution of Snail Species in Khorram Abad Villages According to Shell Characteristics

After collecting 521 snails from 5 villages in different regions of Khorram Abad city (Rig Sefid, Ivshan, Taleghan, Zagheh, and Goldera), the shell characteristics were assessed (Table 6). As it is shown in table 6, the highest frequency was observed in *Lymnaea truncatula* (20.15%), while the lowest was recored for *Lymnaea stagnalis* (5.56%). The genera *Bithynia* and *physa acuta* showed a significant increase in mountainous villages of Khorram Abad compared to the plain villages of Borujerd and Dorud.

4. Discussion

Snails of the family *Lymnaeidae* act as intermediate hosts in the biological cycle of Fasciola hepatica, which causes fasciolosis, a parasitic disease of medical importance for humans and animals (11). In many studies, parasitic infestations have been reported mainly at the family and genus levels; therefore, this diagnosis should be advanced to the species level using more accurate methods (11).

Radisin snails belong to the family of large pond snails, *Lymnaeidae*- freshwater snails with exceptional medical and veterinary importance, globally (12). Accordingly, this study assessed the contamination of *Lymnaeidae* snails with trematodes based on species and sampling location in Lorestan province, Iran, as discussed in the following paragraphs.

Lymnaea gedrosiana is highly sensitive to *ornitobilarzia Turkestanicum* and *Fasciola gigantica miracidia* while *L. gedrosiana* is the dominant species in Shadegan region in Khuzestan province, Iran (12).

The diversity and geographical distribution of the *Lymnaea* family in West Azerbaijan Province were also studied. In this research. A total of 3,741 live Lymnaea snails were collected and identified from Northern, Central, and Southern regions of West Azerbaijan Province, Iran. According to the findings of the present study, *Lymnaea* snails inhabits environments with temperatures ranging from 15°C to 34°C across the mountainous and plains of West Azerbaijan Province, Iran. *L. auricularia*, *L. truncatula,* and *L. palustris* found in soils with acidic to slightly alkaline pH, whereas *L. gedrosiana* and *L.stagnalis* were recorded in soils with alkaline pH (8).

Another study investigated the frequency of *Lymnaea* snails in Lorestan Province, Iran. In this study, 1,700 snails were collected from the suburbs of Khorramabad.

The specimens were identified using various morphological identification keys, including shell length, width, and the number of spirals, as well as the direction of shell coiling and the length of the male genital organ. The species diversity of right-rounded snails in the *Lymnaea* family, in the province includes *Lymnaea gedrosiana* 32.08%, *Lymnaea auricularia* 15.25%, *Lymnaea truncatula* 6.25%, and *Lymnaea stagnalis* was 3% (13).

The presence of L. *Gedrosiana*, *L. truncatula*, *L. pregra*, and *L.palustris* in Kermanshah Province has been reported before in another study (15). Another research demonstrated the distribution of *Lymnaea* snails in the Shadgan region of Khuzestan Province. In this research, snails were collected from the aforementioned area and examined for the presence of trematode larvae. The results indicated that 8% of the snails were infected with trematode larvae (12).

The infestation rate of *L. gedrosiana* with trematode larvae in the

Table 6. Classification of snails based on shells in the study areas of Khorram Abad city.

Snail genus and species	Number	Relative abundance percentage
Lymnaea gedrosiana	76	**14.58**
Lymnaea auricularia	83	**15.93**
Lymnaea truncatula	105	**20.15**
Lymnaea peregra	43	**8.25**
Lymnaea stagnalis	29	**5.56**
physa acuta	81	**15.54**
Bithynia	65	**12.47**
Bulinus truncatus	39	**7.48**

waterways and marshlands of Khuzestan Province. In this study, a total of 6,213 snails were examined, and the results showed that 107 snails (5%) were infected/infested with trematode larvae (12).

Another study investigated a broader geographical distribution of various Lymnaea species across different areas of Iran. The authors reported the presence of *L. gedrosiana*, *L.Auricularia*, *L. truncatula*, and *L. stagnalis* in southern of Khozestan Province, while in Isfahan province, *L. gedrosiana*, *L.truncatula*, and *L. palos*tris were found to be the dominant species. They also reported that Chaharmahal and Bakhtiari Province is a natural habitat for *L.gedrosiana*, *L. truncatula*, and *L. stagnalis* snails (13).

The results of the present morphological study conducted in showed that the highest frequency for *Lymnaea gedrosiana* was recorded in Dorud and Borujerd regions (24.09% and 19.72%), while the lowest frequency belonged to *Bulinus truncates* in the same regions (4.72% and 4.48%), respectively. *Lymnaea* species were the most abundant snails in plain villages, while *physa acuta and B. Tinea* were predominantly observed in mountainous villages. In Khorramabad, the highest frequency belonged to *L.truncatula* (20.15%), while the lowest was recorded for *L.stagnalis* (5.56%).

The genera *Bithynia* and *Physa* showed a significant increase in mountainous villages Khorram Abad, compared to those in Borujerd and Dorud. The overall rate of Lymneidae snails infection with termatodes was 32.94%, including 18.23% of samples from Borujerd, 8.23% from Dorud, and 6.47% from Khorramabad. According to chi-square test (p<0.05), a significant difference was observed in the trematode infections rate among Lymneade snails ascross these regions. Accordingly, Borujerd region showed the highest infection rate, while Khorram Abad revealed the lowest. The genera *B. tinea Bithynia* and *physa acuta* demonstrated a significant increase in mountainous villages of Khorram Abad compared to Borujerd and Dorud regions.

The overall percentage of *Lymneidae* snails infection with trematode larvae among the three snail species- *Lymnaea gedrosiana*, *L.auricularia*, and *L.truncatula*- was 32.94%. This includes 18.23% of infected samples from Borujerd, 8.23% from Dorud, and 6.47% from Khorramabad. According to the chi-square test witha 5% significance level (p<0.05), a significant difference was observed in the rate of trematode larvae infection among Lymneade snails Among the regions studied, Borujerd exhibited the highest infection rate, while Khorram Abad recorded the lowest.

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Authors' Contribution

Study concept and design: S, H.
Acquisition of data: R. M.
Analysis and interpretation of data: S. H. R. M, S. H.
Drafting of the manuscript: R. M, S. H, S. S, B. S
Critical revision of the manuscript for important intellectual content: R. M, S. H, S. S, B. S.
Statistical analysis: R. M, S. H, S. S, B. S.
Administrative, technical, and material support: S. H. R. M, S. H.
Study supervision: R. M.

Ethics

There are no human subjects in this study.

Conflict of Interest

The authors declare that they have no competing interests

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Data Availability

All data analyzed during this study are included in this published article.

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