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

Ramtin Mirfendereski [a], Bahar Shemshadi [a],Saeid Hashemi [b], Salome Shirali [c],

[a]Department of Pathobiology, Science and Research Branch, Islamic Azad University, Tehran, Iran

[b]Department of Parasitology, Borujerd Branch Islamic Azad University, Borujerd, Iran

[c]Department of Biotechnology, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran

***Corresponding authors:** Bahar Shemshadi

Email: bshemshadi@yahoo.com

Abstract

Radicine snails are of considerable medical and veterinary importance as termatodes' vectors such snails are responsible for transmission of the zoonotic trematodes including *Schistosoma turkestanicum* and *Fasciola gigantica* in Iran. This study investigates Lymnaedae infestation with termatodes considering species and sampling location 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 Snails species were identified based on measuring length, width, spire, valve using a shape of the radula as identification keys. To separate the radula, the soft tissue of snails was removed from the shell using forceps and incubated in a 7% potassium solution for 24 hours at room temperature. The isolated radula was placed in 15% acetic acid. Then it was placed in Mallory's dye solution for 3 minutes and washed with the oxalic acid solution. After dehydrating with 96-degree ethanol, it was examined with a light microscope. To investigate trematode larvae in snails, 10% of them (a total number of 170 Lymnaeidae snails) were selected for this investigation by using the crushing method on a slide. The morphological results showed in Dorud and Borujerd, the highest distribution of *Lymnaea gedrosiana* was 24.09% and 19.72%, and

the lowest distribution of *Bulinus truncatus* was 4.72% and 4.48%, respectively. Lymnaea species were the most abundant in plain villages, while *Bithynia* and *Physa* were seen more in mountain villages. In Khorram Abad, the highest distribution is related to *Lymnaea truncatula* (20.15%), and the lowest is related to *Lymnaea stagnalis* (5.56%). The genera *Bithynia* and *Physa* show a significant increase in mountainous Khorram Abad villages compared to the Borujerd and Dorud. The total rate of Lymneidae snails infection with termatodes was 32.94%, which includes 18.23% of samples in Borujerd, 8.23% in Dorud, and 6.47% in Khorramabad. According to chi-square with (p<0.05), a significant difference was seen in the rate of Lymneade snails' trematode infection. In this regard, the Borujerd region showed the highest rate of infection while Khorram Abad revealed the lowest.

Keywords: *Lymnaeidae* snails, trematodes, *radix gedrosiana, Lymnaea auricularial,* Schistosomamiasis

1. Introduction

Distribution of the snail population in the region and transferred parasitic diseases is one of the basic requirements of snail control to improve public health (1). *Lymnaedeae* snails family which are classified in order *Basomatophora* and suborder *Pulmonata* deemed one of the most important species in this type of study (1).

Freshwater snails play an important role in hosting several parasitic nematodes and trematode species' life cycles (2). Therefore, these creatures are of significant medical and veterinary importance. Many studies performed on freshwater snails and related parasitic infections in Iran, but a reliable and 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 snails' population in every single is of paramount importance (3).

Identifying parasitic infections prevalence and parasitic worms in various species of freshwater snails, as intermediate hosts, with conventional microscopic methods has been performed in different regions of Iran (4). Current information reveals that many freshwater snail species have a wide distribution in the whole country while several species are confined to special areas (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* is restricted to the Khuzestan province (7).

Snails of the *Lymenidae* family belong to Gastropoda, suborder Pulmonata, and order Basomatophora. They are hermaphrodite species (8). With 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 can live 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 dry months of summer as well as freezing temperatures (10).

Freshwater snails have a wide variety of species in the world and Iran is no exception (3). But many of the ecological and biological aspects of Iranian native species remained unknown (3). So this study investigates the contamination of Lymnaeidae snails with trematodes considering species and sampling location in Lorestan province (3).

Continuous monitoring and cognitive of snails studies 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 are from the family of the Lymnaeidae, freshwater snails with exceptional medical and veterinary importance globally. To investigate the level of contamination of *Lymnaea gedrosiana* snails with trematode larvae and 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 Peˇsi´c (2012). These snails were then preserved in 70-100% ethanol and returned to the laboratory for assessing the trematode larva in snails.

2.2. Study Design

Attention was paid to the dangers and losses caused by the large collection of live samples and empty shells. Therefore, the collection of empty shells was managed on a small and controlled scale (Ethical code: IR.IAU.SRB.REC.1399.051). The total number of collected snails was 1700. And collection places are reported in **Table 1**.

2.3. Stabilization or fixation ? and storage of *Lymnaeidae* snails

Snails were identified based on measuring the length, width, spire, and valve using a caliper, and the shape of the radula and using the identification key. To separate the radula, the soft tissue of the snail was removed from the oyster with forceps and incubated in a 7% potassium solution for 24 hours at room temperature, and the isolated radula was placed in 15% acetic acid. It was placed in Mallory's dye solution for 3 minutes and washed with oxalic acid and after dehydrating with 96-degree ethanol, it was examined with a light microscope. To investigate trematode larvae in snails, 10% of snails (a total number of 170 *Lymnaeidae* snails) were selected for this investigation using the crushing method on a slide.

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% potash 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 tissues would remain intact around the radula. Therefore, before staining, we removed the remaining tissues with a fine and thin brush or with a dissection needle to avoid any problems in preparing microscopic samples.

2.4. Examination of trematode larvae in snails

2.4.1. 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 remove the circular from them using light alternation. Then, the water containing released sugars was checked.

2.4.2. 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 the trematode larvae.

2.4.3. 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 can be seen in **table 2**.

3. Results

3.1. Trematode larvae infection in *Lymnaea* snails

Out of 1700 snail samples, 10% (170 *Lymnaea* snail samples) 73 samples were from Borujerd, 45 samples were collected from Dorud, and 52 samples reported from Khorram Abad were selected to be

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, including Lymnaea gedrosiana, L. auricularia, and L.truncatula, was 32.94%, which includes 18.23% in Borujerd, 8.23% in Dorud, and 6.47% in Khorramabad samples. Borujerd showed the highest and Khorramabad the lowest rates of infection (p<0.05). Additionally, the percentage of *L.Gedrosiana* was 44.64%, *L. Auricularia* 14.28%, and *L. Truncatula* 41.07%.

3.2. Studying the abundance of snails collected from Dorud villages based on the shape of shells

In this study, 444 snails were collected from 5 villages in different regions of Dorud city (Hamianeh, Zargran, Zhan, Tarshab, and Azizabad). Then these samples were analyzed based on the characteristics of the shells (Gloer, et al., Pesic, 2012) (**Table 4**). As shown in **table 4**, the highest frequency distribution? is related to *Lymnaea gedrosiana* (24.09%), and the lowest is related to *Bulinus truncatus* (4.72%). *Lymnaeidae* snails are the most abundant species in plain villages. While *B. Tinea* and physa acuta snails were mostly seen in mountain villages like Aziz Abad.

3.3. Studying the abundance of snails collected from Borujerd villages based on the shape of shells

In this study, 735 snails were collected from 7 villages in different regions of Borujerd city (Sarab Zaram, Shirvan, Chegani Kesh, Chenarstan, Sheikh Miri, Tudeh Zan, and Araban). They were analyzed based on the characteristics of the shells (Pesic, 2012.) (**Table 5**). As shown in table 5, the highest frequency is related to *Lymnaea gedrosiana* (19.72%), and the lowest is related to *Bulinus truncatus* (4.48%). Lymnaea species are most abundant in plain villages. While *Bithynia*, *physa acuta*, and *Gyraulus* were mostly seen in mountain villages such as Chenaristan and Chegani Kash.

3.4. Studying the abundance of snails collected from Khorram Abad villages based on the shape of shells

After collecting 521 snails from 5 villages in different regions of Khorram Abad city (Rig Sefid, Ivshan, Taleghan, Zagheh, and Goldera) the characteristics of shells were assessed (Gloer, et al., Pesic, 2012.) (**Table 6**). As shown in table 6, the highest frequency is related to *Lymnaea truncatula* (20.15%), and the lowest is related to *Lymnaea stagnalis* (5.56%). The genera *Bithynia* and *physa acuta* showed a significant increase in Khorram Abad mountain villages compared to the plains of Borujerd and Dorud.

4. Discussion and Conclusion

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, and this diagnosis should be advanced to the species level using more accurate methods (11).

Radisin snails are from the family of large pond snails of *Lymnaeidae*, freshwater snails with exceptional medical and veterinary importance globally (12). For this purpose, this study assessed the contamination of *Lymnaeidae* snails with trematodes according to species and sampling location in Lorestan province, Iran, which is discussed in further paragraphs.

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

The diversity and geographical distribution of the *Lymnaea* family in West Azerbaijan province are also studied. Some 3741 live Lymnaea snails were collected and identified In this research, from the three regions of North, Central, and South of West Azerbaijan Province, Iran. According to present study findings *Lymnaea* snails live in habitats with a temperature range of 15°C to 34°C in the mountains and plains of west Azerbaijan, Iran. *L. auricularia*, *L. truncatula*, and *L. palustris* live in soils with acidic to slightly alkaline pH, while *L. gedrosiana* and *L.stagnalis* were recorded in soils with alkaline pH (Imani *et al*., 2019).

Another study investigated the frequency of *Lymnaea* snails in Lorestan province. In this study, 1700 snails were collected in the Khorramabad suburbs. The collected specimens were identified according to some identification keys including snails' shell length, width, and spirals, as well as shells twisting direction and length of the male genital organ. Species diversity of right-rounded snails of the *Lymnaea* family, the idea in the province include *Lymnaea gedrosiana* 32.08%, *Lymnaea auricularia* 15.25%, *Lymnaea truncatula* 6.25% and *Lymnaea stagnalis* was 3% (13).

Mansouriyan (2000) reported the presence of L. *Gedrosiana*, *L. truncatula*, *L. pregra*, and *L.palustris* from Kermanshah province (Mansouriyan, 2000). While another study demonstrated the distribution of *Lymnaea* snails in the Shadgan region in Khuzestan province. In this research, snails were collected from the mentioned area and examined for finding trematode larvae. The obtained results indicate that 8% of snails were infected with trematode larvae (12).

Noorpisheh et al. (2019) investigated *L. gedrosiana* infestation rate with trematode larvae in Khuzestan province waterways/ marshlands. In this study, 6213 snails were examined and the final results showed that 107 snails (5%) were infected/infested with trematode larvae.

Another study determined a wider geographical distribution of various Lymnaea species in some areas of Iran. It indicated the presence of *L. gedrosiana*, *L.Auricularia*, *L. truncatula*, and *L. stagnalis* in the south of Khozestan while in Isfahan province *L. gedrosiana*, *L.truncatula*, and *L. palos*tris were found dominant species. This study also revealed that Chaharmahal and Bakhtiari province is a natural habitat for *L.gedrosiana*, *L. truncatula*, and *L. stagnalis* snails (Rivaz *et al*., 2014).

The results of the morphological study in Iran showed the highest frequency of *Lymnaea gedrosiana* 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 found the most abundant snails in plain villages, while *physa acuta and B. Tinea* were seen more in mountain villages. In Khorramabad, the highest frequency is related to *L.truncatula* (20.15%), and the lowest is related to *L.stagnalis* (5.56%).

In Khorram Abad, the highest distribution is related to *Lymnaea truncatula* (20.15%), and the lowest is related to *Lymnaea stagnalis* (5.56%). The genera *Bithynia* and *Physa* show a significant increase in mountainous Khorram Abad villages compared to the Borujerd and Dorud. The total rate of Lymneidae snails infection with termatodes was 32.94%, which includes 18.23% of samples in Borujerd, 8.23% in Dorud, and 6.47% in Khorramabad. According to chi-square with (p<0.05), a significant difference was seen in the rate of Lymneade snails' trematode infection. In this regard, the Borujerd region showed the highest rate of infection while Khorram Abad revealed the lowest. The genera B. tinea Bithynia and *physa acuta* show a significant increase in mountainous Khorram Abad villages compared to the Borujerd and Dorud because they are mountainous.

The percentage of *Lymneidae* snails infection with termatodes in the three species of snails, *Lymnaea gedrosiana*, *L.auricularia*, and *L.truncatula*, was 32.94% for trematode larvae infection, which includes 18.23% of samples in Borujerd, 8.23% in Dorud and 6.47% in Khorramabad samples. According to the chi-square test with an accuracy of 5% accuracy (p<0.05), a significant difference was seen in the amount rate of Lymneade snails' trematode larvae infection infestation?, so In this regard, the Borujerd region showed the highest rate of infection while and Khorram Abad revealed the lowest infection.

Declarations

Ethics approval and consent to participate

There are no “human subjects” in this study

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

S.H. developed the idea and designed the experiments. R.M., S.H., S.SH., and B.SH. conducted the experiments. R.M. and S.H. analyzed the data. R.M. wrote the manuscript. All authors confirmed the final manuscript before submission.

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٢٤٩ **Figure Legend**

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

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Table Legends

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

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

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

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

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

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

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

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

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

Location	Sample (n)	*Gedrosia* species		*Auricularia* species		*Truncatula* species		Total infected (n)	Percentage of relative abundance
Borujard	73	Tests (n)	Infected (n)	Tests (n)	Infected (n)	Tests (n)	Infected (n)	31	18.23
		35	16	15	4	23	11		
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

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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

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