Seasonal and Geographic Distribution of Cercarial Infection in *Lymnaea gedrosiana* (Pulmonata: Lymnaeidae) In North West Iran

Abbas IMANI-BARAN 1, *Mohammad YAKHCHALI 2, Reza MALEKZADEH-VIAYEH 3, Ali FARAHNAK 4

1. Dept. of Pathobiology, Parasitology Division, Faculty of Veterinary Medicine, Tabriz University, Tabriz, Iran
2. Dept. of Pathobiology, Parasitology Division, Faculty of Veterinary Medicine, Nazlu campus, Urmia University, Urmia, Iran
3. Artemia and Aquatic Animals Research Institute, Urmia University, Urmia, Iran
4. Dept. of Parasitology and Mycology, School of Public Health, Medical Sciences of Tehran University, Tehran, Iran

**Abstract**

**Background:** Trematodes are a diverse group of endoparasites which require molluscan and vertebrate animals as intermediate and definitive hosts in their life cycle. The present study was carried out to determine the diversity and geographic distribution of infection with trematodes’ cercariae in the snail *Lymnaea gedrosiana* from north-west Iran.

**Methods:** A total number of 6759 Lymnaeidae snails were collected from 28 snail habitats; of these *L. gedrosiana* was the prevalent snail (74.37%) which examined for cercarial infection by shedding method.

**Results:** The overall infection rate was 8.03%. The most frequent trematodes cercariae in the snail were xiphidiocercariae (81.98%), furcocercariae (32.26%), echinostome cercariae (5.19%), and monostome cercariae (1.24%). The highest infection rate in *L. gedrosiana* (100%) was with echinostome cercariae from Golestan in autumn.

**Conclusion:** Due to the important role of pond snails in transmission of cercariae to fish as a source of zoonotic diseases, it is essential to estimate the distribution and abundance of the snails and the rate of their infection with different trematodes’ cercariae, and establish control programs in each region.
Introduction

Digenean trematodes have complicated life cycles in which molluscs play the key role as intermediate hosts for part of their developmental stages. In this regard, freshwater snails, in particular those from the order Basommatophora, have substantial contribution to development and transmission of parasitic flukes. For instance, some 20 species of cercariae have been isolated from the lymnaeid snails of Lymnaea peregra (Muller, 1774) (1). Snail-mediated diseases are among the major groups of helminthic diseases caused by trematode parasites. However, the main snail species involving in the transmission of flukes vary in different geographical regions.

Freshwater snails have been studied in different Iranian provinces including Fars, Khoozestan and Mazandaran (2-5). L. gedrosiana (Annandale and Prashad, 1919) has been reported to be a preferred intermediate host for a number of parasitic helminths such as Fasciola gigantica (Cobbold, 1855) (6), Ornitolbilharzia turkestanicum (Skrjabin, 1913) (2, 7), and Trichobilharzia spp. (3). It was also found that L. gedrosiana had a considerable role in the transmission of zoonotic diseases such as cercarial dermatitis (1.1% in South-West and 0.05% in North of Iran), fasciolosis (0.35%), Plagiorchids infections (0.1%), and Clonostomum infections (0.2%) in Iran (3, 6, 8-10). Therefore, examination of the snails makes it possible to gain information about the degree to which they are responsible for infection distribution. This is also the keystone for identifying the trematode fauna in the areas of interest.

To date, no large-scale study has been carried out on the distribution and abundance of L. gedrosiana and its contribution to the transmission of cercarial infection in north-western Iran. Thus, the aim of this study was to elucidate the seasonal and regional incidence of L. gedrosiana and its rate of cercarial infections in the region.

Material and Methods

Study area

West Azerbaijan Province is located in north-west of Iran (35°46’ to 39°58’ in latitude and 44°3’ to 47°23’ in longitude) (Fig. 1).

Excluding the Lake Urmia, this semi-humid and temperate province has an area of 37,608 km² elevating 1,332m above sea level. The climate of the province is largely influenced by the rainy winds of the Atlantic Ocean and Mediterranean Sea; the maximum temperature reaches 34°C in July, while minimum temperature may be –16°C in January. Annual precipitation varies between 300 and 800 mm with large yearly and monthly fluctuations. Generally, the province witnesses two rainy seasons, the first from March to May and the second in October-November (5). Three are numerous water bodies and reservoirs with relatively appropriate environmental conditions in West Azerbaijan province.
where suitable habitats are provided for pond snails (5).

Snail collection
A total of 28 perennial and seasonal freshwater snails habitats were monitored from May to December 2010 (Fig.1). The habitats included various water-body types, i.e. wetlands, ponds, rivers, canals, springs, swamps, pools, streams and ditches, located in both mountainous and low-land areas of north, central and south parts of the province. Snail sampling was undertaken by searching each site for 15 minutes using a standard flat wire mesh scoop with a mesh size of 2mm (11, 12). The collected snails were placed in plastic screw cap containers containing the water of snail habitat and transferred alive to the laboratory for species identification using the morphological keys provided by Mansoorian (13) and Pfleger (1). The identified snails were kept alive in an aquarium at optimal conditions to be investigated for their cercarial infection.

Collection of cercariae from the infected snails
The identified snails as L. gedrosiana were transferred to the Parasitology Museum of the Tehran Faculty of Veterinary Medicine for detailed characterization and verification. The snails were then placed individually in flat-bottomed glass vials (height 7.5 cm, diameter 2.5 cm) containing filtered pond water and exposed to a 100-W light bulb at a distance of 15cm for 4-6 hours for cercarial shedding (14). The snails which did not shed cercariae on the first exposure were re-exposed on the second day. Cercariae were characterized by morphological and biometrical examinations as described by Frandsen and Christensen (15).

Statistical evaluation
Data were analyzed by SPSS statistical program (version 14, SPSS Inc., Chicago, IL, USA) using the non-parametric Chi-square test with confidence interval of 95%. Probability of < 0.05 was regarded as significant.

Results
Snails
Of the total of 6759 collected Lymnaeidae snails, L. gedrosiana was the predominant species (74.37%) observed in 18 out of the 28 investigated water bodies (Fig. 1). The snail was found mainly in the stagnant or slow-moving, clear to slightly turbid waters with aquatic plants cover. During the course of this study, the seasonal and geographical distributions of L. gedrosiana were significantly different ($P = 0.0001$). However, there was no significant differences in the distributions for the snail in Ziveh ($P = 0.816$) and Gogarchinghaleh ($P = 0.677$). The snails counts were significantly higher in summer than in autumn ($P = 0.0001$) (Table 1).

Table 1: Association between regional and seasonal distribution of Lymnaea gedrosiana population in northwestern Iran (n=5026)

| Place          | Season | $P$   |
|----------------|--------|-------|
|                | Summer | Autumn|
| Ziveh          | 38     | 36    | 0.816|
| Najafabad      | 181    | 0     | 0.0001|
| Shorgol        | 156    | 121   | 0.035|
| Ghargologh     | 30     | 15    | 0.025|
| Shabanlu       | 222    | 154   | 0.0001|
| Gogarchinghaleh| 188    | 180   | 0.677|
| Gharahaghaj    | 344    | 406   | 0.024|
| Esmailkandy    | 372    | 175   | 0.0001|
| Marganlar      | 294    | 0     | 0.0001|

Diversity and abundance of cercariae
From the 3673 identified L. gedrosiana snails, 8.03% were infected with cercariae of different trematodes (Table 2). The infections were observed throughout the study period, but the largest number of the infected snails was observed between June and August (Table 3). Identified cercariae and their respective contribution to the total snail infection rate were as follows: xiphidiocercariae 81.98%, furcocercariae 32.26%, echinostome cercariae 5.19%, and monostome cercariae 1.24% (Table 2). Xiphidiocercariae and monostome cercariae...
were found only in the snails sampled from north part of West Azarbaijan province, while echinostome cercariae and furcocercariae were absent from the snails sampled in the central part of the province. All examined snails (100%) from Golestaneh in autumn were infected with echinostome cercariae, and the snails sampled from Gharahaghaj in summer had the highest infection rate with xiphidiocercariae (76.81%) (Table 3).

**Table 2**: Geographical distribution of cercariae infection in examined *Lymnaea gedrosiana* snails of northwestern Iran (n=3673)

| Cercariae            | Place              | No. of examined snails | Snail          | Prevalence (%) |
|----------------------|--------------------|------------------------|----------------|----------------|
| Xiphidiocercariae    | Shorgul            | 277                    | 2.17           |                |
|                      | Gharahaghaj        | 750                    | 81.98          |                |
|                      | Marganlar          | 294                    | 10.2           |                |
|                      | Gharahbaagh (Zanoil)| 278                   | 2.88           |                |
|                      | Gharahbaagh (Jamgoli)| 242                  | 0.83           |                |
| Furcocercariae       | Marganlar          | 294                    | 5.44           |                |
|                      | Gargulug           | 30                     | 10.02          |                |
|                      | Shorgul            | 277                    | 3.61           |                |
| Echinostome cercariae| Zarineh-roud       | 31                     | 32.26          |                |
|                      | Golestaneh         | 318                    | 5.19           |                |
|                      | Shorgul            | 277                    | 3.61           |                |
|                      | Marganlar          | 294                    | 3.43           |                |
|                      | Darlak             | 60                     | 1.67           |                |
| Monostome cercariae  | Gargulug           | 30                     | 1.24           |                |
|                      | Gharahbaagh (Kefi) | 261                    | 1.15           |                |
| Total                | -                  | 3673                   | 8.03           |                |

**Table 3**: Seasonal distribution of cercarial infection in examined *Lymnaea gedrosiana* snails of northwestern Iran (n=403)

| Cercariae            | Place              | Season | No. of examined snails | Snail          | Prevalence (%) |
|----------------------|--------------------|--------|------------------------|----------------|----------------|
| Xiphidiocercariae    | Shorgul            | Summer | 156                    | 3.85           |                |
|                      | Gharahaghaj        | Summer | 344                    | 76.81          |                |
|                      | Gharahaghaj        | Autumn | 406                    | 5.17           |                |
|                      | Marganlar          | Summer | 294                    | 10.24          |                |
|                      | Gharahbaagh (Zanoil)| Autumn| 278                   | 2.88           |                |
|                      | Gharahbaagh (Jamgoli)| Autumn| 242                  | 0.83           |                |
| Furcocercariae       | Shorgul            | Summer | 156                    | 4.49           |                |
|                      | Shorgul            | Autumn | 121                    | 2.48           |                |
|                      | Marganlar          | Summer | 294                    | 5.44           |                |
|                      | Gargulug           | Summer | 30                     | 10.02          |                |
| Echinostome cercariae| Zarineh-roud       | Summer | 31                     | 32.26          |                |
|                      | Golestaneh         | Summer | 75                     | 30.6           |                |
|                      | Golestaneh         | Autumn | 243                    | 100            |                |
|                      | Shorgul            | Summer | 156                    | 3.85           |                |
|                      | Shorgul            | Autumn | 121                    | 3.31           |                |
|                      | Marganlar          | Summer | 294                    | 3.44           |                |
|                      | Darlak             | Summer | 60                     | 1.67           |                |
| Monostome cercariae  | Gargulug           | Summer | 30                     | 6.67           |                |
|                      | Gharahbaagh (Kefi) | Autumn | 261                    | 1.15           |                |

Available at: [http://ijpa.tums.ac.ir](http://ijpa.tums.ac.ir)
Discussion

Lymnaeidae snails are of medical and veterinary importance since they are required, as intermediate hosts, to complete the life cycle of trematode species. They are distributed throughout the world and are known as the vectors of more than 71 species belonging to 13 trematode families (16). A considerable body of research has explored the potential role of lymnaeid snails in transmitting the infectious parasitic trematodes worldwide (17-19). However, intra-molluscan trematode parasitism is frequently associated with the alteration of a host’s growth, fecundity or survival (20), and its susceptibility to trematodes (21). In the present study, L. gedrosiana was found to be a predominant pond snail in the region. This finding was in accordance with previous reports from Iran (4,5,13,22,23). Furthermore, in consistence with several earlier studies (2, 4, 13, 24), in this study the highest population density of L. gedrosiana was recorded in summer.

Until present, only a few studies have been carried out on the diversity and abundance of cercarial infection in the pond snails of Iran. For instance, cercarial infection in L. gedrosiana was reported from Khoozestan province (3, 25), in Galba truncatula (Müller, 1774) from Khoozestan and Kurdestan provinces (26), and in L. gedrosiana and L. palustris (Müller, 1774) from northern Iran (6, 9, 10). The snail L. gedrosiana is found to be a general intermediate host for four groups of cercariae in the studied region. Several studies have confirmed the simultaneous infection of L. gedrosiana with echinostome cercariae (Echinostomatidae), furcocercariae (O. turkestanicum and Trichobilharzia spp.), monostome cercariae (Notocotylidae), and xip-hidocercariae (Plagiorchiidae) (2, 3, 24) in Iran. Sharif et al. (10) found that L. gedrosiana in northern Iran were also infected with the same cercariae types. Loy and Haas (27) isolated the larvae of 18 trematode species from L. stagnalis in Germany. Faltyckova et al. (18) identified 24 trematode species comprising 19 cercariae in L. stagnalis, of which the dominant cercariae were those belonging to three species of Echinoparyphium avoniatum, Opisthogybe ranae, and Plagiorchis elegans. Immani-Baran et al. (28) found the infection of L. auricularia snails in North West Iran with two groups of fluke’s cercariae, i.e. furcocercariae and echinostomecercariae.

Seasonality that is mirrored by changes in environmental variables can intervene in snail’s ecology and influence the larval development of a trematode inside its host snail. It may also affect cercarial shedding (the release of cercariae from the host snail in nature). However, the influence of environmental elements on cercarial shedding is trematode-specific (29). The optimal reproduction of L. gedrosiana in northwestern Iran occurs in early summer (June-July) (2, 4). Similarly, the highest cercarial infection rates in lymnaeid snails of the region were observed between June and September, while Sharif et al. (10) recorded the maximum infection rates in late summer (August-September). Thus, it can be anticipated that both snail’s propagation and their infection with trematodes are correlated with seasonal variations. Farahnak et al. (30) noted that various ecological factors such as season and water temperature, pH and dissolved oxygen influence the emergence of cercariae from the snails and their release inside the water resources.

Conclusion

With regard to the importance of farm animal health in national economy, it is essential to study the diversity, distribution and abundance of the intermediate hosts of infectious trematodes, mainly freshwater snails. L. gedrosiana is a common pond snail in West Azarbaijan province which has shown the capacity for vectoring diverse cercarial species. Results of this study and those of the related investigations can assist in collecting data on the ecological relevance of the snails distribution and the pattern of transmission of digenian trematodes by the snails and finally, in preven-
tion and control of the following disease outbreaks.

Acknowledgments

This study was supported financially by the Urmia University, Iran. The authors acknowledge the support and interest of the technical members of the Artemia and Aquatic Animals Research Institute, Central Lab of Faculty of Urmia Veterinary Medicine and Malacology Laboratory at Urmia University, Iran. The authors declare that there is no conflict of interest.

References

1. Pfleger V. A field guide in colour to mollusks. Aventinum Nakladatelstvi, S.T.O., Polagrafiya, Czech Republic, Prague; 1999, pp. 28-9.
2. Massoud J. Observation on Lymnaea gedroisana, the intermediate host of Ornithobilharzia turkestanicum in Khuzestan. J Helminthol. 1974; 48:133-8.
3. Farahnak A, Essalat MA. A study on cercarial dermatitis in Khoozestan Province, Southwestern Iran. BMC Public Health. 2003; 3: 35-8.
4. Karimi GR, Derakhshanfar M, Paykari H. Population density, trematode infection and ecology of Lymnaea snails in Shadegan, Iran. Arch Razi Ins. 2004; 58: 125-9.
5. Imani-Baran A, Yakhchali M, Malekzadeh-Viayeh R. A study on geographical distribution and diversity of Lymnaeidae snails in West Azerbaijan Province, Iran. Vet J (Pajouhesh and Sazandegi). 2011; 82(4):53-63. (In Persian)
6. Ashrafi A, Massoud J, Holakuei K, et al. Evidence suggesting that Fasciola gigantica may be the most prevalent causal agent of fasciiosis in northern Iran. Iranian J Publ Health. 2004; 33: 31-7.
7. Motamedi GhR, Ghorashi SA, Paykari H, Dalimi AH, Salehi Tabar R, Motamedi N, Karimi GhR. Detection of Ornithobilharzia turkestanicum cercaria (trematoda) by nested-PCR in intermediate host snail, Lymnaea gedroisiana. Arch Razi Ins. 2008; 63(2): 35-40.
8. Ghabadi H, Farahnak A. A faunistic survey on the cercariae of Bellamya bengalensis snail and their zoonotic importance. Iranian J Publ Health. 2004; 33:38-42.
9. Athari A, Gohar–Dehi SH, Rostami-Jalilian M. Determination of definitive and intermediate hosts of cercarial dermatitis-producing agents in Northern Iran. Archives of Iranian Medicine. 2006; 9(1):11-5.
10. Sharif M, Daryani A, Karimi SA. A faunistic survey of cercariae isolated from lymnaeid snails in central areas Mazandaran, Iran. Pak J Bio Sci. 2010; 13(4): 158-63.
11. Canete R, Yong M, Sanchez J, Wong L, Gutierrez A. Population dynamics of intermediate snail hosts of Fasciola hepatica and some environmental factors in San Juany Martinez Municipality, Cuba. Mem Inst Oswaldo Cruz, Rio de Janeiro. 2004; 99(3): 257-62.
12. Gutierrez A, Hernandez DF, Sanchez J. Variations of snail's abundance in two water bodies harboring strains of Pseudosuccinea columella resistant and susceptible to Fasciola hepatica miracidial infection, in Pinar del Rio Province, Cuba Mem Inst Oswaldo Cruz, Rio de Janeiro. 2005; 100(7): 725-7.
13. Mansoorian AB. A practical guidline for identification of Iranian freshwater snails. Iranian J Publ Health. 1986; 15(1-2):41-53.
14. Faltyankoova A, Nasincová V, Kablásková L. Larval trematodes (Digenea) of planorbid snails (Gastropoda: Pulmonata) of planorbid snails in San Juany Martinez Municipality, Cuba. Mem Inst Oswaldo Cruz, Rio de Janeiro. 2004; 99(3): 257-62.
15. Frandsen F, Christensen NO. An introductory guide to identification of cercariae from African fresh water snails with special reference to cercariae of trematode species of medical and veterinary importance. Acte Trop. 1984; 41: 181-202.
16. Bargues MD, Vigo M, Horak P, Dvorak J, Patzner RA, Pointier JP, Jackiewicz M, Meier-Brook C, Mas-Coma S. European Lymnaeidae (Mollusca: Gastropoda), intermediate hosts of trematodiasis, based on nuclear ribosomal DNA ITS-2 sequences. Infect Genet Evol. 2001; 1:85-107.
17. Zbikowska E. Infection of snails with birds shistosomes and the threat of swimmers itch in selected Polish lakes. Parasitol Res. 2004; 92: 30–5.
18. Faltyankoova A, Haas W. Larval trematodes in freshwater molluscs from the Elbe to Danube.

Available at: http://ijpa.tums.ac.ir
rivers (Southeast Germany): Before and today. Parasitol Res. 2006; 99(5):572-82.

19. Faltynkova A, Nasincová V, Kablásková L. Larval trematodes (Digenea) of the great pond snail, *Lymnaea stagnalis* (L.), (Gastropoda, Pulmonata) in central Europe: A survey of species and key to their identification. Parasite. 2007;14:39-51.

20. Sorensen, RE, Minchella DJ. Parasite influences on host life history: *Echinostoma revolutum* parasitism of *Lymnaea edes* snails. Oecologia. 1998; 115:188-95.

21. Kalbe, M., Haberl, B., Hass, W., 1997. Miracidial host-finding in *Fasciola hepatica* and *Trichobilharzia ocellata* is stimulated by species-specific glycoconjugates released from the host snails. Parasitol Res. 83:806-12.

22. Mansoorian AB. Some freshwater Gastropods from Kermanshah province, western Iran. J Fac Vet Med, Uni Tehran. 2000a; 55(2):85-7. (In Persian)

23. Mansoorian AB. Some freshwater Gastropods from Baluchestan and Sistan province, southeast Iran. J Fac Vet Med, Uni Tehran. 2000b; 55(3):49-51. (In Persian)

24. Salahi-Moghaddam A, Mahvi AH, Molavi GH, Hosseini-Chegini A, Masoud J. Survey on *Lymnaea palustris* snail Parasitology and its ecological identification in Mazandaran Province using of Geographical Information System (GIS). J Modarres Medicine Science. 2009; 11(3-4): 65-71. (In Persian)

25. Nourpisheh SH. The biology of *Lymnaea* snail and its role in transmitting of infection to human and animal in Khoozestan province. MS thesis in Medical Parasitology, Health Faculty of Tehran Medical Sciences University, Iran; 1998. (In Persian)

26. Zamini GH. Survey of freshwater snails and identification of intermediate hosts of human and animal trematodes in Khoozestan and Kordestan Provinces, Iran. PhD dissertation in medical parasitology; Health Faculty of Tehran Medical Sciences University, Iran; 1999. (In Persian)

27. Loy C, Haas W. Prevalence of cercariae from *Lymnaea stagnalis* snails in a pond system in South Germany. Parasitol Res. 2001; 87: 878-82.

28. Imani-Baran A, Yakhchali M, Malekzadeh-Viayeh R, Farhangpajuh F. Prevalence of cercariae infection in *Lymnaea auricularia* (Linnaeus, 1758) in North West of Iran. Vet Res Forum. 2011b; 2(2): 121-7.

29. Abrous M, Rondeaud D, Dreyfuss G. *Paramphistomum daubneyi* and *Fasciola hepatica*: influence of temperature changes on the shedding of cercariae from dually infected *Lymnaea truncatula*. Parasitol Res. 1999; 85:765-9.

30. Farahnak A, Vafaie-Darian R, Mobedi I. A Faunistic Survey of Cercariae from Fresh Water Snails: *Melanopsis* spp. and their Role in Disease Transmission. Iranian J Publ Health. 2006; 35(4): 70-4.