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A Faunistic Survey of Cercariae Isolated from Lymnaeid Snails in Central Areas of Mazandaran, Iran

M. Sharif, A. Daryani and S.A. Karimi

Department of Parasitology and Mycology, Mazandaran University of Medical Sciences, Sari, Iran

Abstract: The aim of this study was to elucidate the species diversity of larva trematodes in the Mazandaran Province, Northern Iran. In this cross-sectional study, a total of 3,266 lymnaeid snails from 3 species were collected from different parts of streams, swamps, rice fields and rivers in the central areas of Mazandaran Province (Sari, Neka, Qaemshahr and Savad-Koh cities), during April to September, 2008. The samples were tested by crushing and emerging methods. From the total of examined snails, 119 (3.6%) were found to be infected with the lymnaeid snails. *Lymnaea gedrosiana* were found to be infected with the Furcocercariae of Diplostomidae, Clinostomidae, Echinostomatidae and also cercariae of the Plagiorchiidae. The latest infection was found to be in *L. palustris*. The Mazandaran Province with its temperate climate is a suitable place for living of snails, particularly lymnaeidae, that could have a significant role as an intermediate host of diseases.

Key words: Faunistic survey, cercaria, lymnaeid snails, Iran

INTRODUCTION

Pond snails (Lymnaeidae) are fresh water gastropoda. Their preferred habitats are stagnant in the slow streaming water with heavy vegetation. These snails are hermaphroditic.

Three species of lymnaeids were reported throughout the Mazandaran Province: *L. (stagnicola) palustris*, a second intermediate host of *F. hepatica*; *Galba (Lymnaea) truncatula*, the main intermediate host of *F. hepatica* and *Radix (Lymnaea) gedrosiana*, a member of the *auricularia* complex transmitting *F. gigantica* (Moghaddam *et al.*, 2004a). Athari *et al.* (2006) reported that infection rate of animal *schistosoma* in *L. gedrosiana* and *L. palustris* in North of Iran was 0.17 and 0.06%. Furthermore, infection rate with other Furcocercariae in these snails was 0.27 and 0.2%, respectively (Athari *et al.*, 2006). In the Khuzestan Province, South Western of Iran, from 2000 *L. gedrosiana* snails collected, 48 (2.1%) were infected with animal *Schistosoma* (Farahnak and Essalat, 2003).

The role of lymnaeid snails as the first intermediate host of animal *schistosoma* has been shown throughout the world (Ferte *et al.*, 2005).

The susceptibility of *L. gedrosiana* to *F. hepatica* under laboratory conditions has been reported in Iran. Lymnaeid snails associated with larvae stages of *Fasciolid* sp. have been reported from Guilan Province (Ashrafi *et al.*, 2004) and Mazandaran Province

(Moghaddam *et al.*, 2004b). In Guilan Province, from the total of 4,830 different snails studied, only seven (0.35%) of *L. gedrosiana* were found to be infected with larvae stages of *Fasciola* sp. (Ashrafi *et al.*, 2004).

The most important and widespread intermediate host of *F. hepatica* in Europe, Asia, Africa and North America was found to be *L. truncatula* (Soliman, 2008) while, in Iran, *L. gedrosiana* was reported to be a proper intermediate host for *F. gigantica* (Ashrafi *et al.*, 2004) and cercarial dermatitis (Athari *et al.*, 2006; Farahnak and Essalat, 2003).

The various studies in many countries indicated that pond snails (Lymnaeidae) were infected with larvae of *Fasciolid* sp. (De kock *et al.*, 2003; Pointier *et al.*, 2006; Bargues *et al.*, 2007; Soliman, 2008; Barragan-Saenz *et al.*, 2009) and trematodes such as *Paramphistomum* (Dryfuss *et al.*, 2004), *Holostom* (Klockars *et al.*, 1928), *Trichobilharzia*, Diplostomidae, Plagiorchiidae, Echinostomatidae, *Monostom*, *Tetracotyle* Metacercariae, Schistosomatidae, Azygiidae, Notocotyidae and Strigeidae (Faltynkova and Haas, 2006). In contrast, Bin Dajem (2009) in his study revealed none trematode immature stages in different snails under study. The lack of comprehensive studies on these snails, especially in relation to rate of infection, in the Mazandaran Province, was the major factor for the decision to conduct the present study. The aim of this study was to evaluate the occurrence of trematode infections in Lymnaeidae and recognize of lymnaeid snails using the crushing and emerging methods.

Corresponding Author: Ahmad Daryani, Department of Parasitology and Mycology, School of Medicine, Mazandaran University of Medical Sciences, PC 48168-95475, Sari, Iran
Tel: +98 151 3241031 Fax: +98 151 3543249

MATERIALS AND METHODS

This study was conducted on 3,266 lymnaeid snails in the central area of Mazandaran Province (Sari, Neka, Qaemshahr, Svad-koh cities) from April to September 2008, by a wooden handle paddle with 1.5 m long and net size of 30×30 cm. Snails were collected from agriculture canals, various streams, swamps, rice fields and rivers. Samples then transferred to Sari Health Research Center. After identification of species, each 1 to 2 snails were placed in a Petri-dish containing dechlorinated water and then, they were placed against light for 3 to 5 h or over night at room temperature. The snails were examined for the presence of cercariae by shedding and crushing methods. If no cercariae shedding were observed, snails were pressed and crushed between two square pieces of 15×15 glasses and studied in order to find cercariae, sporocysts and redia. Collected cercariae were observed carefully and mixed in 90% ethanol and 10% formalin and cleared in lactophenol and/or stained with azocarmine and neutral red. After measuring and drawing different parts of cercariae, the genus of cercariae were identified by a systematic key reference (Frandsen and Christensen, 1984; Christian, 2003).

RESULTS AND DISCUSSION

From a total of 3,266 lymnaeid snails (Fig. 1) examined, 119 (3.6%) were infected with 4 species of trematodes larvae (Fig. 2 a-d, Table 1).

All of furcocercariae and *Echinostom* cercariae were found in *L. gedrosiana* in Sari, center of Mazandaran

Province. *Plagiorchiidae cercariae* were common in all localities (Table 1).

In this study, Sari with 92 (9%) positive cases, showed the most infection, followed by, Savad-koh with 15 (2%) positive cases, Neka with 9 (1.2%) positive cases and Qaemshahr with 3 (0.4%) positive cases (Table 2). Cercariae with 4 families of digenean parasites were recorded from *L. gedrosiana* in which infection rate of this snail was 4.6%. *L. Palustris* was infected only by plagiorchiidae cercariae and its infection rate was 1.7%. Athari *et al.* (2006) in North of Iran showed less prevalence of trematode infection in these snails. This difference may be due to difference in study area. *L. truncatula* showed no infection in the above cities (Table 2). Ashrafi *et al.* (2004) also in a study on *Fasciola* in Guilan Province didn't report any cases of *Fasciola*



Fig. 1: Lymnaeid snails from Mazandaran Province of Northern Iran

Table 1: Snails occurrence from four geographical area in mazandaran province

Cercarial type	Localities								Intermediate host (sec)
	Sari		Neka		Qaemshahr		Savad-koh		
	No. examined (1016)	%	No. examined (750)	%	No. examined (750)	%	No. examined (750)	%	
Diplostomidae ¹	84	8.2	-	-	-	-	-	-	<i>L. gedrosiana</i>
Plagiorchiidae ²	5	0.5	9	1.2	3	0.4	15	2	<i>L. gedrosiana</i> <i>L. palustris</i>
Clinostomidae ³	2	0.2	-	-	-	-	-	-	<i>L. gedrosiana</i>
Echinostomatidae ⁴	1	0.1	-	-	-	-	-	-	<i>L. gedrosiana</i>

¹Cercariae species were as follows: Diplostomidae cercariae (n = 84), ²Plagiorchiidae cercariae (n = 32), Echinostomatidae cercariae (n = 1), ³Clinostomidae (n = 2), ⁴One case of Plagiorchiidae sporocyst was obtained

Table 2: The prevalence of infected snails in central areas of Mazandaran Province

Localities	<i>L. gedrosiana</i>		<i>L. palustris</i>		<i>L. truncatula</i>	
	No. examined	No. infected (%)	No. examined	No. infected (%)	No. examined	No. infected (%)
Sari	839	91 (10.8)	58	1 (1.7)	119	0 (0)
Neka	576	8 (1.4)	24	1 (4.1)	150	0 (0)
Qaemshahr	488	2 (0.4)	89	1 (1.1)	173	0 (0)
Savad-koh	620	15 (2.4)	7	0 (0)	123	0 (0)
Total	2523	116 (4.6)	178	3 (1.7)	565	0 (0)

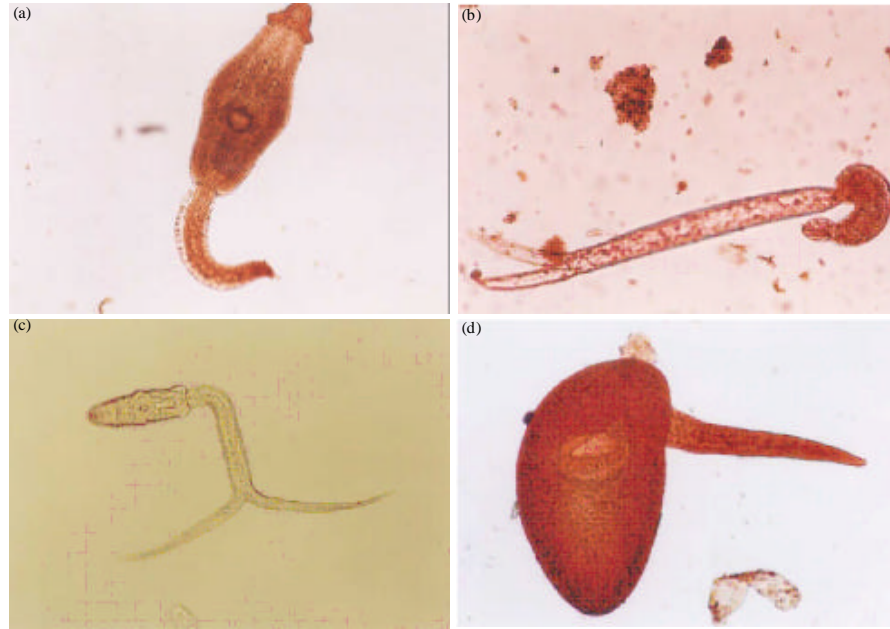


Fig. 2: Kinds of detected cercariae (a) Echinostomatidae, (b) Clinostomidae, (c) Diplostomidae and (d) Plagiorchiidae

cercaria. Prevalence of digenean larva, including Diplostomidae, was extremely high in *L. gedrosiana* from Sari (Table 1).

Trematodes are a diverse group of endoparasite requiring molluscan and vertebrate as intermediate and definitive host in their life cycle. Intramolluscan trematode parasitism is frequently associated with the alteration of a host's growth, fecundity or survival (Sorensen and Minchella, 1998) and snails susceptibility to trematodes is highly specific (Kalbe *et al.*, 1997). The distribution of freshwater mollusks varies with physical, chemical and biological characteristics (Sharpe, 2002). The DNA sequence and isoenzyme studies have been done for the detection of larva stages trematodes and distinction of snails (Ashrafi *et al.*, 2004; Mas-coma *et al.*, 1999).

This study provides to estimate larva trematode parasites among lymnaeid snails and their zoonotic importance in animal or on human health. The characteristics of Mazandaran Province includes high humidity, rain fall, temperature 20-30°C (April to September), agricultural tradition, mainly rice, numerous stagnant water collections and irrigation canals rich in aquatic vegetation. The presence of all necessary conditions above suggest an appropriate condition for snails and all of the examinations performed verify the role of lymnaeid snails as intermediate host for larvae trematodes.

Most prevalence of snails in these regions is May to September. Moreover, in this study, most infection rates

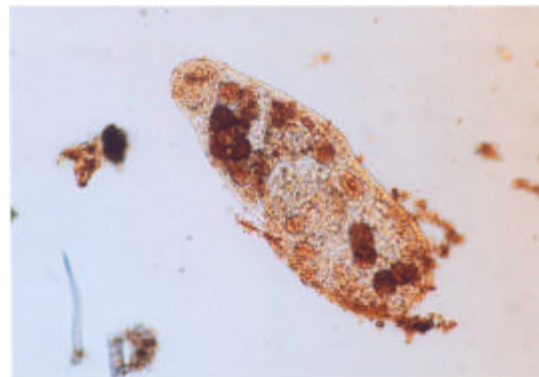


Fig. 3: Sporocyst of Plagiorchiidae

of snails were found in August to September, in Seyedmahale area of Sari.

In the present study, Plagiorchiidae, Diplostomidae, Clinostomidae and Echinostomatidae families cercariae were found by shedding and crushing methods.

In this study, 0.97% of snails *L. gedrosiana* and *L. palustris* were infected with species of Plagiorchiidae (Fig. 3). The first intermediate hosts for Plagiorchiidae are freshwater snails; the second intermediate ones are fish, arthropods, mollusks (snails) and definitive hosts are vertebrates including human (Ghobadi and Farahnak, 2004; Fried *et al.*, 2004; Farahnak *et al.*, 2005). Plagiorchiids are parasite of intestine, gallbladder, bileduct or cloaca of vertebrates (Fried *et al.*, 2004). The

only genus that infects human is *Plagiorchis*. *P. muris* has been reported in natural infections in human (Hong *et al.*, 1996).

Human infections with *P. philippiniensis*, *P. javensis* and *P. harinasutai* have been reported in Philippines, Indonesia and Thailand (Kumar, 1999).

Plagiorchiidae have been reported in snails *Bellamya (Viviparous) bengalensis* (Ghobadi and Farahnak, 2004) and *Mellanooides tuberculata* (Farahnak *et al.*, 2005) from the Khouzestan Province, in South Western Iran. In the study conducted in Germany, this trematode has been reported from *L. palustris* and *Radix auricularia* (Faltynkova and Haas, 2006).

In this study, 0.1% of snails *L. gedrosiana* were infected with Echinostomatidae Cercariae. These Cercariae may infect fish, molluscs (Snails, clams) and amphibians (tadpoles, frogs) (Fried *et al.*, 2004; Farahnak *et al.*, 2005).

Adult Echinostomatidae infect a variety of mammals (including humans) and aquatic birds (Anonymous, 1995). Two separate life cycles were demonstrated in endemic areas, i.e., the human cycle and the Sylvatic cycle (Bundy *et al.*, 1991). In the endemic areas, the disease occurs focally and is associated with common socio-cultural practices (Carney, 1991). In light to moderate infections, anemia, headache, gastric pain and loose stools have been reported. Heavy infections are associated with eosinophilia, abdominal pain, profuse watery diarrhea, anemia, edema and anorexia (Anonymous, 1995; Chattopayday *et al.*, 1990). Farahnak *et al.* (2005) reported Echinostomatidae cercariae in the *Mellanooides tuberculata* from Khouzestan Province, South Western Iran (Farahnak *et al.*, 2005). This trematode has been reported in *L. palustris* and *Radix auricularia* from Germany (Faltynkova and Haas, 2006).

In this study, 8.2% of snails *L. gedrosiana* were infected by Diplostomidae cercariae. In the life cycle of the Diplostomidae, fish, snails, amphibians and reptiles are sources of metacercariae and piscivorous birds and mammals are considered as final hosts (Fried *et al.*, 2004; Cribb *et al.*, 2003).

It should be noted that cercariae of all diplostomatids are fork-tailed (Cribb *et al.*, 2003). Diplostomidae cercariae encyst in a variety of organs, including inside of the eye ball (Machado *et al.*, 2005). Infections with *Neodiplostomum soulensis* have been reported in humans. Examination of stool samples from military soldiers in Korea revealed that the prevalence of infection was 0.4% (Huh *et al.*, 1994). Infections with *F. seolensis* are predominantly reported from Korea (Hong and Shoop, 1994; Chai and Lee, 2002; Chai and Shih, 2002).

In this study, 0.2% of snails *L. gedrosiana* were infected with clinostomidae cercariae. Definitive hosts of

Clinostomidae are piscivorous birds, including, herons, pelicans, cormorants and darters, while the second intermediate hosts are fish. It should be noted that *C. complanatum* develops through species of lymnaea (Cribb *et al.*, 2003). Human infection by *Clinostomum* is considered rare and is known to occur as a result of eating raw fresh-water fish such as carp, cyprinus carpio and crucian carp and carassius ouratus (Chung *et al.*, 1995).

In the literature reviewed, 44 species of *Clinostomum* were recorded; from those, 19 species were recorded in Asia, Africa and America, which were identified as synonyms of *C. complanatum* (Chung *et al.*, 1995).

The first human case of *Clinostomum pharyngitis* was reported in Taegu, Korea (Chung *et al.*, 1995). The first human infection with *C. complanatum* had been reported from Japan, all additional case records have also been reported in Japan (Muller, 2002).

In addition to trematodes mentioned in this study, infection of *L. palustris* to larvae of Azygiidae (Faltynkova and Haas, 2006) and infection of *Radix (Lymnaea) auricularia* to trematodes such as Strigeidae, Schistosomatidae, Sanguinicolidae and Notocotylidae (Faltynkova and Haas, 2006), *Trichobilharzia ocellata* (Zbikowska, 2004) and *T. franki* (Ferte *et al.*, 2005) have been demonstrated in the literature.

CONCLUSION

The suitable humid climate in Mazandaran Province, Northern Iran and presence of naturally infected snails, coupled with other conditions in this geographical area, verify the existence of zoonotic diseases which are transferable by snails.

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