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Original Article

Contamination of Vector Snails with the Larval Stages of Trematodes in Selected Areas in Northern Iran

Mojgan Aryaeipour [1,2], Allah Bedasht Mansoorian [2], Mohammad Bagher Molai Rad [2], Soheila Rouhani [3], Majid Pirestani [4], Ahmad Ali Hanafi-Bojd [5], Ahmad Daryani [6], Tina Asadi [7], *Mohammad Bagher Rokni [2,8], *Shahabeddin Sarvi [6]

1. *Student of Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*
2. *Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Medical Parasitology and Mycology, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
4. *Department of Parasitology and Entomology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
5. *Department of Medical Entomology and Vector Control, School of Public Health and National Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran*
6. *Toxoplasmosis Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran*
7. *School of Biology, College of Science, University of Tehran, Tehran, Iran*
8. *Research Center for Endemic Parasites of Iran, Tehran University of Medical Sciences, Tehran, Iran*

***Corresponding Author:** Email: roknimoh@tums.ac.ir; shahabesarvi@yahoo.com

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Abstract

Background: Identification of freshwater snails and possible trematodes transmission sites are essential to continue monitoring the potential for disease outbreaks in areas with a history of parasitic infections. We aimed to search some areas in the margin of the Caspian Sea, northern Iran to identify the snail fauna of this area and verify the contamination of vector snails.

Methods: More than 5,308 snails from 51 diverse and permanent habitats were studied from April 2019 to October 2021. Snails were collected randomly and identified using shell morphology. Trematode infection in snails was investigated by the release of cercariae and dissection methods.

Results: Five families of freshwater snails including Lymnaeidae, Physidae, Planorbidae, Bithyniidae, and Viviparidae were investigated in the Caspian Sae Litoral of Iran. Physidae were found as the most prevalent snails (55.1%) followed by Lymnaeidae (29.4%). The parasitize rate was observed as 20% using releasing cercaria technique. Echinostomatoidea (31%), Schistosomatoidea (8%), and Diplostomoidea (21%), and Plagiorchioidea (40%) were seen as detected parasites. Meanwhile, 60% of the studied snails illustrated the other stages of trematodes.

Conclusion: The rate of infection of snails with different cercaria in northern Iran is significant. It needs further deep studies to clarify the situation of zoonoses transmitted by snails in the region. Policy makers should pay attention more to this area in terms of monitoring the snail-transmitted diseases.

Keywords: Vector snails; Trematode; Parasitology; Iran

Introduction

Freshwater snails act as great medical and veterinary important hosts of the first and sometimes second intermediates for parasitic trematodes (1, 2). They have a unique role in facilitating the epidemic of common emerging diseases (3). The significant role of snail studies as a necessity in public health is due to transition diseases such as schistosomiasis, fascioliasis, and echinostomiasis (4-8). Snail-borne diseases form the major parts of parasitic diseases transmitted to humans. The chronic nature of trematode infections in the human and animal hosts makes it difficult to determine when and where transmission occurs (9, 10). Therefore, knowledge about the distribution of snails in the region and parasitic diseases transmitted by them is one of the basic requirements to deal with snails and improve community health levels.

For this reason, in recent years, the attention of indigenous and foreign researchers has turned to the study of various dimensions of Malacology in Iran. The study of intermediate hosts provides critical information about the active transmission sites of trematode infections. For example, the Lymnaeidae family involves in the life cycle of at least 71 species of trematodes (11, 12). In general, the distribution of freshwater snails is based on the biological characteristics of snails, various climatic and ecological conditions of the region (13).

The history of taxonomic research on aquatic snails in Iran shows that it was started in 1920 in Mazandaran Province, northern Iran (14). The freshwater snail fauna seems insignificant in terms of species diversity reported from Iran, and there may still be unknown species in this country. On the other hand, adverse atmospheric events with human intervention have an important effect on changes in the dynamics and population composition of freshwater snails in Iran (3). Anyway, the current reality in the world of snails and their parasitic infestations seems to be different from the past. This necessitates the study of freshwater snails and their parasitic contaminants with more detailed and extensive observations. Guilan and Mazandaran, in terms of agriculture and animal husbandry, are among the most important provinces in the country (14). However, the only comprehensive study in the field of snails in these provinces is related to the year 2000 (14). Since then, there was no updated information on intermediate host distribution and their trematode infections in these areas.

The present study was performed in the margin of the Caspian Sea, as an initial screening province to determine the possible changes on the identification and distribution of freshwater snails with their parasitic contamination using conventional methods. Accurate identification of species with morphological methods is not possible in many cases and requires advanced molecular methods.

Materials and Methods

Study area

Guilan and Mazandaran Provinces are in the northern Iran, lying along the south and east west of the Caspian Sea (Fig. 1). Iran is in the dry zone of the world with an average rainfall of 250 mm. Guilan and Mazandaran climates are humid subtropical and these provinces, by a large margin, have the heaviest rainfall (about 1000 mm/year) in Iran (http://www.irimo.ir/). These areas are a few of the foremost thickly populated locales possessed by 2,530,696 people according to the 2016 census (https://srtc.ac.ir).

The coastal plain along the Caspian Sea in this area is utilized for rice paddies. Hence, climate, environmental, and socioeconomic conditions are favorable for the transmission of trematode disease in these areas. Other features of these areas are tourist attractions, places for fish farming, swimming, fishing, gardening, the habit of drinking spring water, and the consumption of aromatic plants by people. Finally, it is a favorable habitat for the reproduction of intermediate host snails and their trematode parasites. Snails in 50 permanent and temporary stations with different agro-ecological characteristics including low land (LL) and high land (HL) made of L1 (Water fountain), L.2 (grasslands), L3 (wetlands without any grass cover), L4 (river banks), L5 (water canals), L6 (rice field), from a total of 200 stations were studied as preferred habitats for fresh waters nails from 2019 to 2021 (Fig. 2).

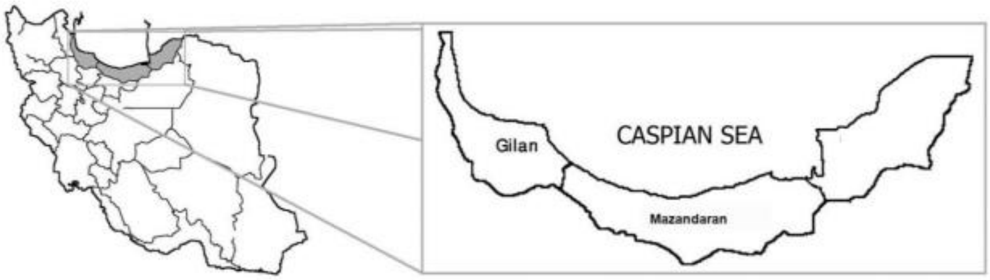

Fig. 1: Two studied provinces along the Caspian Sea during 2019 to 2021 in Guilan and Mazandaran, the northern of Iran

Fig. 2: Area were studied as preferred habitats for freshwater snails during 2019 to 2021 in Guilan and Mazandaran, the northern of Iran a: L1 b: L2 c: L3 d: L4 e: L5 f: L6

Sampling and identification

Depending on the environmental conditions of the study area, we collected the snails by hand net or pliers from some areas in Guilan and Mazandaran Province in the margin of the Caspian Sea using a random sampling method (5). We recorded the geographical coordinates for each collecting sample site using a Global positioning system (GPS) with a UTM unit. The snails were placed individually into plastic bag containers and transferred alive to the Department of Parasitology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran within 1-2 days. We transferred snails with ice cubes in the summer. Based on morphological of shell, the snails were identified to the species level using the identification keys (4). Snails were screened for mature cercarial infections in the laboratory by isolating individually in a 50 ml plate containing filtered water from the site of snails' collection. We held the snails under the light at room temperature for 12 hours to stimulate the production of cercariae (11). Initial identification was performed on live larvae. Then, a more accurate identification was performed using Fall staining. Cercariae were identified according to morphological classification keys (15). Unsuccessful snails in shaded cercariae were dissected to examine other stages of trematodes life.

Ethics approval

This study was reviewed and approved by the Ethical Committee of Research, Mazandaran University of Medical Science (IR.MAZUMS.REC.1399.695) and Tehran University of Medical Sciences (IR.TUMS.SPH.REC.1399.273).

Results

More than 200 sites in the search area were surveyed for snail habitat. A total of 5308 vector snails were collected from 51 locations in these areas (Table 1, 2).

Table 1: location sampling sites (Y: longitude X: Latitude) that were investigated vector snails genera in Guilan, Iran, 2019-2021

Province	Location	
	y	x
Sheikhmahalleh	37.356186	49.557477
Siakal	37.124554	49.687404
Khomam	37.389041	49.669759
Khoshkehbijar	37.371253	49.726179
Kiashahr	37.438109	49.923504
Kelachai	37.07738	50.383023
Lahijan	37.236433	49.97285
Sheikhmahalleh	37.356186	49.557477
Langrood	37.180632	50.133605
Pirbazar	37.356186	49.557477
Roodsar	37.112851	50.230411
Fooman 1	37.205164	49.454789
Fooman2	37.205164	49.454789
Jaddeh saheli	37.386491	50.162059
Bandar Anzali	37.477759	49.359164
Chalandar	36.589237	51.684734
Chamkhaleh	37.190702	50.228517
Dastak	37.386048	50.144262
Dehcola	37.330993	49.420343
Deylaman	36.761551	49.907688
Shaft	37.425693	49.872837

Table 2: Location sampling sites (Y: longitude X: Latitude) investigated for vector snails genera in Mazandaran, Iran, 2019-2021

Location	Y	X
Amol۱	36.33116	52.364594
Amol 2	36.385884	52.334865
Arbekalle1	36.860821	50.680072
Arbekalle2	36.843328	50.707868
Arbekalle3	36.844421	50.70703
Dohezar1	36.842518	50.768956
Dohezar2	36.842518	50.768956
Dohezar3	36.777379	50.814879
Fereidoonkenar1	36.677447	52.533256
Fereidoonkenar2	37.205164	49.454789
Jannatroodbar1	36.793018	50.555152
Jannatroodbar2	36.765931	50.535234
Jannatroodbar3	36.799809	50.738023
Javaherdeh1	36.85652	50.481964
Javaherdeh2	36.866601	50.502146
Javaherdeh3	36.866594	50.50211
Javaherdeh4	36.856419	50.482053
Javaherdeh5	36.854972	50.594959
Jooybar	36.591886	52.840372
Katalom1	36.884377	50.720463
Ramsar	36.884401	50.7203o1
Niasar1	36.857333	50.737101
Niasar2	36.857341	50.737378
Niasar3	36.857327	50.737076
Niasar4	36.857287	50.737516
Noor	36.560172	51.975356
Noushahr	36.595285	51.538376
Rooyan	36.560172	51.975356
Sari	36.575034	52.950431
Sisangan	36.56595	51.956292

The sampling sites were distributed in the different areas of Guilan and Mazandaran provinces. Snails were classified into 5 Family (Genus), including; Physidae (*Physella* sp.) 55.1% (n=2925), Planorbidae (*Planorbis* sp.) 8.2% (n=435), Bithyniidae (*Bithynia* sp.) 2.8% (n= 147), Viviparidae (*Bellamya* sp.) 4.5% (n=241) and Lymnaeidae 29.4% (n=1560) (Table 3) (Fig. 3). Out of the Lymnaeidae, *Stagnicola* sp. accounted for 83.6% (n=1304), *Galba* sp. for 4.8 % (n=68) and *Radix* sp. for 11.6% (n=188) of cases. The highest frequency of vector snails' genera was related to Physidae, followed by lymnaeidae. Snails based on morphological characteristics, such as shell length and width, operculum status, and other characteristics were identified to the species level using the identification keys (Fig. 4).

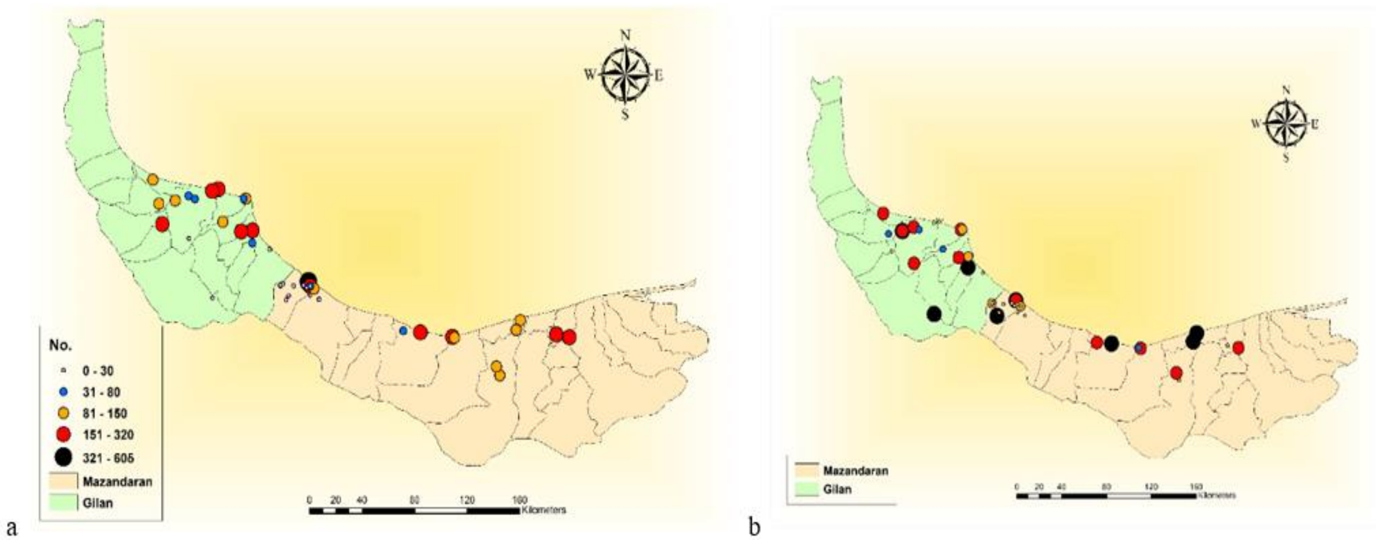

Fig. 3: Map of 50 location-sampling sites investigated for vector snail genera and their distribution in Mazandaran & Guilan, Iran, 2019-2021, a: Total vector snails b: Lymnaeidae snails

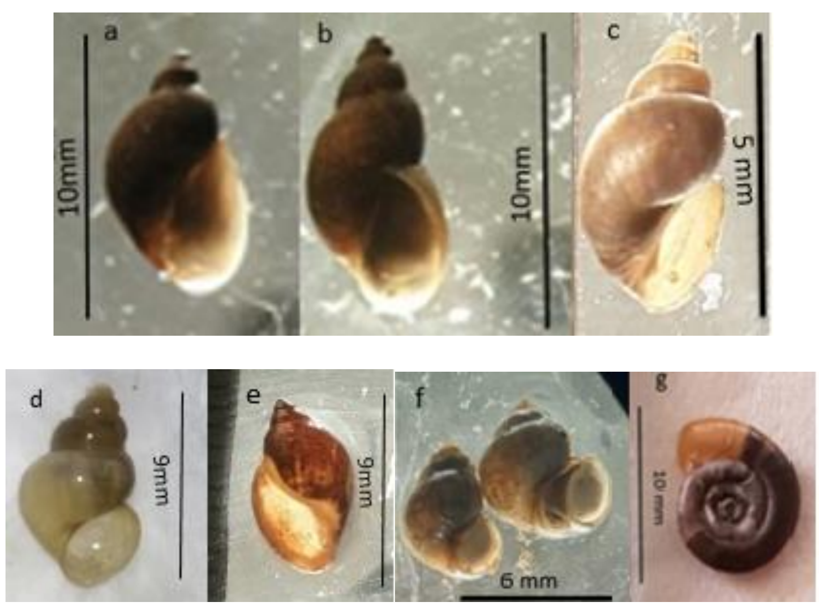

Fig. 4: Vector snail's genera harvested from different agro-ecological zones, Guilan & Mazandaran, Iran, 2019-2021
a: *Radix* sp. b: *Stagnicola* sp. c: *Galba* sp. d: *Bithynia* sp. *e: Phaesa* sp. f:: *Bellamya* sp. *g: Planorbis* sp.

Table 3: Vector snails genera harvested from different agro-ecological zones, Guilan & Mazandaran, Iran, 2019-2021 (N=5308)

Phylum	***Mollusca***						
Class	Gastropod						
Subclasses	Pulmonata					Heterobravchia	
Infraclasses						Caenogastropoda	
Order	Hygrophila					Littorinimorpha	Architaenioglossa
Super family	Lymnaeoidea					Truncatelloidea	Viviparoidea
Family	Lymnaeidae			Physidae	Planorbidae	Bithyniidae	Viviparidae
Genus	*Stagnicola* sp.	*Radix* sp.	*Galba* sp.	*Physella* sp.	*Planorbis* sp.	*Bithynia* sp.	*Bellamya* sp.
Prevalence% (N0.)	24.6(1304)	3.5(188)	1.3(68)	55.1(2925)	8.2(435)	2.8(147)	4.5(241)

The results showed that the distribution of freshwater snails in the different areas was not uniform. In some areas, the presence in some species and the absence of others was observed. To investigate the reason for this discrepancy, sampling was repeated in some habitats as a control in all seasons. In zone (Niasar, Kiashahr) along the river, as a permanent habitat, no differences were observed in different seasons of the year. In two areas including Katalom and Noor, the rice field, we saw an increase in immature snails in rice transplanting time as a temporary habitat. During the use of chemical pesticides, to control plant pests, we saw the destruction of snails.

Plant dependence was not observed in snail habitats. Some snails were found in wetland, completely plant-free soils. According to the results, differences in the distribution of the Lymnaeidae family have been observed even in adjacent locations. The most common distribution in this family was related to *Stagnicaola* sp. In the case of trematodes infection, the results showed that among the snails we were able to survive up to 2 weeks in the laboratory, 20% of snails, excreted cercariae (Table 4) (Fig. 5). Over 60% of the remaining snails studied by dissection method were also identified to other life stages of trematodes (Fig. 6). We also saw simultaneous contamination of Schistosomatoidea with Diplostomoidea and Echinostomatoidea in *Stagnicola* sp.

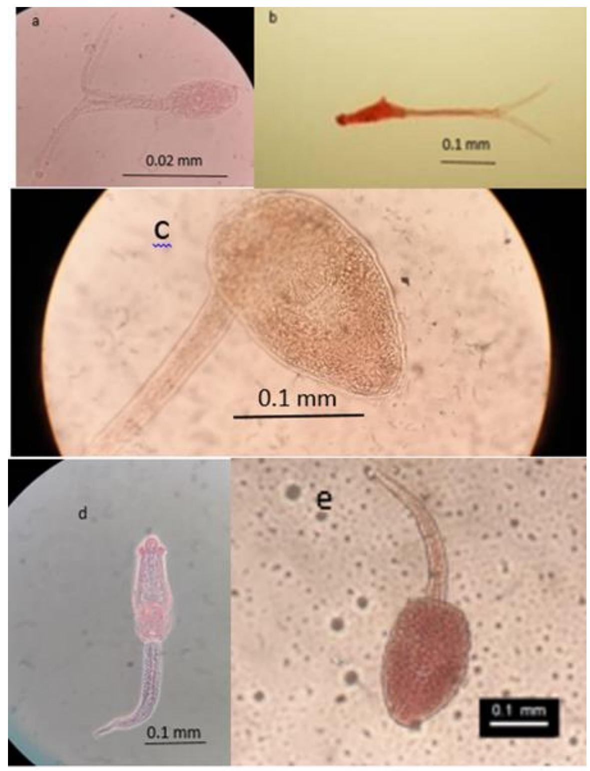

Fig. 5: Trematode cercariae in vector snails, Guilan & Mazandaran, Iran, 2019-2021 a: Diplostomoidea, b: Schistosomatoidea c: Plagiorchioidea, d: Echinostomatoidea, (*Echinostoma* sp), e: Echinostomatoidea (*Fasciola*.sp)

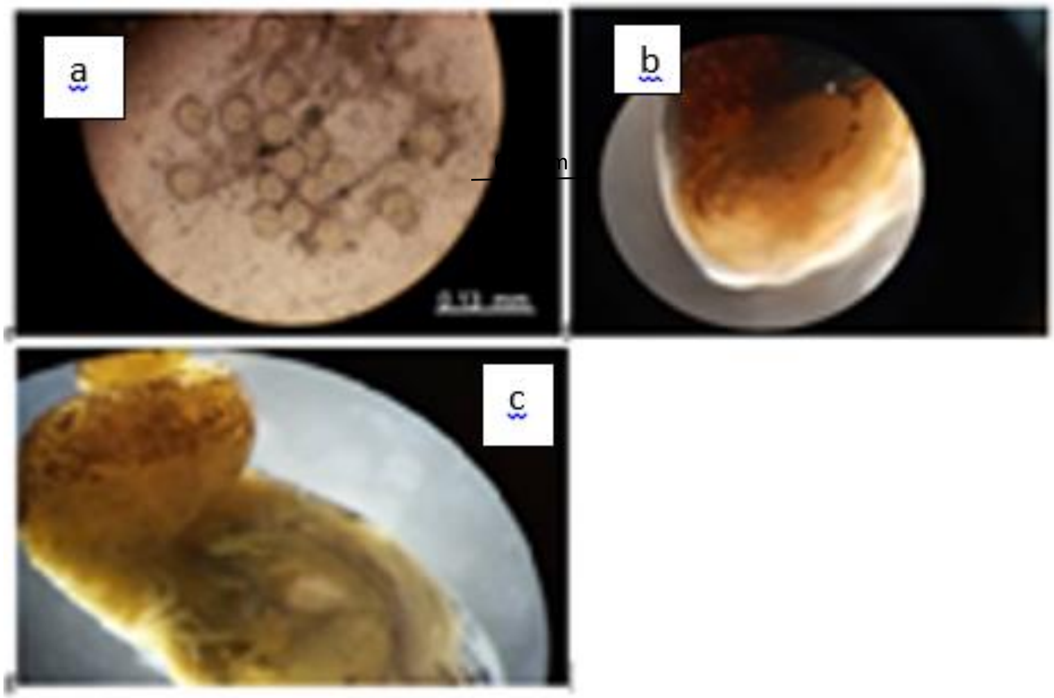

Fig. 6: Trematode metacercaria (a, b) and rediae (c) in tissue of snail, Guilan & Mazandaran, Iran, 2019-2021

Table 4: Trematoda infection in vector snails' genera, Guilan & Mazandaran, Iran, 2019-2021

Class	Subclass	Order	Family	%	Vector snails genera
Trematode	Diene	Echinostomata	Echinostomatoidea	31	*Stagnicola* sp. – *Radix* sp.
		Strigeidida	Schistosomatoidea	8	*Stagnicola* sp. – *Radix* sp.
		Xiphidiata	Plagiorchioidea	40	*Stagnicola* sp. – *Radix* sp. -*Galba* sp. – *Planorbis* sp.
		Diplostomida	Diplostomoidea	21	*Stagnicola* sp. – *Radix* sp. – *Phaesa* sp.

Discussion

Food-born trematodes are infections that have negative socio-economic and adverse health effects on human and animal life (9). Therefore, it is critical to identify the locations and species of trematodes where the disease is endemic (3). Irrigation of domestic animals and entry of farmers without any protective clothing in potentially contaminated waters of springs and swamps, lead to the infectious stages of trematodes (9).

The chronic nature of trematode infection can lead to misdiagnosis and consequently the administration of the wrong drugs and exacerbation of the disease (16). Given the importance of the issue, today, several methods have been designed to increase the sensitivity of diagnostic tests for these diseases (17,18). Human infection with *Fasciola* sp. and other trematodes can occur in native areas of vector snails. In rural and agricultural communities such as the Caspian littoral cities in northern Iran, people regularly use shared water resources with their animals or consume raw plants grown in snail habitats (19, 20). As a result, mapping potential foci of disease transmission is key to the prevention and control of the infection. The ecology of snails is affected by chemical components such as the concentration of few particles and water-soluble gasses, as well as organic variables such as the presence of food, competition, and the relationship between hunting and fishing. Increasing the concentration of heavy metals due to industrial pollutants in water, such as lead, in a certain range, can limit the presence of living organisms (21).

On the other hand, the density of snails in aquatic habitats contaminated with industrial and non-industrial wastewater is high (22). Due to the nitrification process, toxic nitrite from the decomposition of organic matter residues is converted to nitrate, which is one of the main nutrients in the water and is an indicator of nutrition in a water source (16). Another important intervention to disrupt the balance of the environment is the indiscriminate use of pesticides for agricultural and livestock purposes. Organic pesticides are a serious environmental threat due to their widespread use to control insects and field pests (17). Although the consequences on demographic indicators and species composition of organisms such as snails have not been studied, the entry of these toxins into the biological domains of snails is certainly effective. In the study area, a large area of land is under agricultural cultivation. As a result, the type of toxins and the time of their use can cause changes in the snail's fauna (23). Snails can accumulate various environmental poisons in their tissues and distinctive physiological components to neutralize their effects. However, due to their high sensitivity to these pollutants, they can also be used as an indicator of environmental pollution. Environmental pollutants due to heavy rainfall in the region and by surface water can affect a large area of the region (23). Therefore, the snail habitat in these areas is usually not permanent. According to our results, areas such as riverbanks and highlands far from human reach can still be considered permanent habitats. In addition, the population of snails is affected by climate, increasing in wet seasons and decreasing in dry seasons (24). Based on the scientific evidence and arguments, climate change and global warming are now accepted as an emergency in all parts of the world. These changes undoubtedly have adverse effects on all-natural ecosystems and socio-economic organizations and cause changes in the aquatic environment (25). As a result, the composition of snail population species will also change due to this natural phenomenon. Climate change has been suggested as a direct or indirect factor in the decline or destruction of many species (26). This study was conducted to update the identification of species diversity, determine the distribution pattern and frequency of each species of freshwater snails as intermediate hosts of trematodes in northern Iran by random sampling and determination of their contamination. Guilan and Mazandaran provinces have a diverse ecological system consisting of paddy fields, forests, and a variety of water resources. The snails found in this study are different and important intermediate hosts in medicine and veterinary medicine, including; *Stagnicola* sp., *Galba* sp. and *Radix* sp. in Lymnaeidae, *Physella* sp. in Physidae, *Planorbis* sp. in Planorbidae, *Bithynia* sp. in Bithyniidae and *Bellamya* sp. in Viviparidae. In lymnaeidae, the highest frequency was related to *Stagnicola* sp. (83.6%). According to a report, freshwater snails in northern Iran were as follows (14): *A. Jetosto*, *B. Bengalensis, B. Tentaculata*, *B. Truncatus*, *Gy. Charpontler*, *L. Palustris*, *L. Truncatula*, *M. Doriae*, *Ph. Acuta*, *Pl. Planorbis*, *Th. Doriae*, *V. Piscinalis*. Generally, the identification of freshwater snails in Iran compared to most parts of the world has not been completed, and we still need many studies in this area. Snails, such as Lymnaeidae, Planorbidae, Melanoides tuberculata, and Bithyniidae, can infect trematodes (27).

The results showed that 20% of snail species succeeded in discharging cercariae and the others (60%) were contaminated with other stages of life of trematodes such as sporocyst, rediae, or metacercaria. The parasitic infections observed in this study were related to Echinostomatoidea (31%), Shistosomatoidea (8%), Plagiorchioidea (40%), and Diplostomoidea (21.8%) which indicate the presence of infectious stages of trematode in freshwater snails in these areas. Echinostomatoidea cercariae in this study were found in Lymnaeidae snails. *R. auricularia* with infection of Echinostomatidae cercariae, reported in 2010 from Mazandaran (28). *Echinostom* metacercaria occur in the environment or the second intermediate host including snail, amphibian, or fish (19). *Echinostome* cercariae cause echinostomiasis in humans and livestock.

Echinostomatoidea cercariae in dependent families (Psilostomatidae, Philophthalmidae, Cathaemasiidae, Rhopaliasidae, and Fasciolidae) may show changes in emergence, collar, and in the degree of growth of these structures. Gymnocephalus is an important member of Echinostomatoidea, which is very similar to *Echinostom* cercariae lacking collar and collar spines. Metacercaria of *Fasciola* occurs in the environment and stick to plants. Humans can be infected to *Echinostoma* sp, *Fasciola* sp, and *Fasciolopsis buski* because of consumption of undercooked aquatic plants, fish, and snails. Infection of *L. truncatula* to *F. hepatica* has been reported in Guilan (20, 23). In this study, furcocercous cercariae of schistosomatidae were found in Lymnaeidae snails. Schistosomatidae are formed in simple sporocysts. They penetrate directly into the final host (15, 24, 29). In addition, furcocercus cercariae of diplostomoidea were also observed in Lymnaeidae, Physidae, and Planorbidae snails.

Diplostomoidea causes metacercaria in the secondary host. Simultaneous contamination of Schistosomatoidea, Diplostomoidea and, Echinostomatoidea in Lymnaeidae snails were seen in this study. We found Plagiorchioidea cercariae from the order of Xiphidiata similar to another report (24). These cercariae are exposed from Lymnaeidae, Physidae and, Planorbidae, are shown in several unrelated families, including Nanophyetidae, Paragonimidae, and some members of Dicrocoeliidae and Brachylaemidae (15). Lymnaeidae snails have the potential to cause diseases common to humans and animals, such as Schistosomal dermatitis, Echinostomiasis, and Fascioliasis in humans and animals. Snails and cercariae were identified in this study based on morphological features. Based on the morphological features, we could not affirm accurately the species of obtained parasites especially in larval stages. It is necessary to use complementary methods based on the molecular biology to identify the species (20). Our survey shows less diversity of vector snails compared to the previous study (14).

Environmental DNA-based (e-DNA) approach can be used as a promising tool for better quality assessment of the distribution of freshwater snails in water samples collected from farms, and the identification of new environments where parasitic diseases can occur inside and outside in their native area (30).

Conclusion

Industrial agriculture, tourism, and Irregular construction have severely upset the ecological balance of the environment in the margin of the Caspian Sea, northern Iran. As a result, changes in the population of the important intermediate host can be a warning sign for the spread or even recurrence of an epidemic of some parasitic diseases. In general, continuous monitoring of studies, especially in areas where there is a history of occurrence, prevalence, or even epidemic of trematodiases in humans and livestock, is of great importance for updating information. Due to the vastness of the study area and its biodiversity, further studies on other sites are recommended. The use of environmentally friendly methods to control vector snails, water treatment chemicals for domestic use, and regular human and animal disinfection should be encouraged.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interest.

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