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Evidence Suggesting that *Fasciola gigantica* Might be the Most Prevalent Causal Agent of Fascioliasis in Northern Iran

K Ashrafi[1], J Massoud[1], K Holakouei[2], M Mahmoodi[2], MA Joafshani[3], MA Valero[4], MV Fuentes[4], M Khoubbane[4], P Artigas[4], MD Bargues[4], S Mas-Coma[4]

[1]Dept. of Medical Parasitology and Mycology, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Iran
[2]Dept. of Epidemiology and Biostatistics, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Iran
[3]Dept. of Social Medicine, School of Medicine, Gilan University of Medical Sciences, Rasht, Iran
[4]Dept. of de Parasitologia, Facultad de Farmacia, Universidad de Valencia, Avenida Vicent Andres Estelles s/n, 46100 Burjassot, Spain

Abstract

Following the occurrence of two large outbreaks in Gilan province, northern Iran, the public health importance of human fascioliasis has increased significantly. The mixed infections of *Fasciola gigantica* and *F. hepatica* within a given domestic animal individual suggest a very complicate picture of possible circulation ways of the parasite and the possibility for humans to be infected with both species. Elucidating these circulation ways is very critical for understanding the epidemiology and transmission of the disease and being ascertain how animals and humans enter the different liver fluke circulations in this endemic zone. The main objective of the present study was to investigate the distribution and natural infections of local lymnaeids, environmental characteristics related to the disease transmission and determining the most prevalent fasciolids and definitive hosts in human endemic areas of Gilan province. Bandar-Anzali and Rasht are the most important endemic areas with most of the cases of human disease during the epidemics and inter-epidemic periods. Sheep raising is not normal in these regions, while cattle is the most common definitive host. According to the data obtained from slaughterhouse observations in Bandar-Anzali and Rasht, the main fasciolid in local cattle is *F. gigantica*. Of 928 adult liver flukes collected from 13 infected livers of cattle, in Rasht and Bandar-Anzali slaughterhouses, 91.1% were diagnosed as *F. gigantica* and 8.9% as *F. hepatica*. *L. gedrosiana* and *L. palustris* were the most prevalent lymnaeid snails in this endemic zone. It appears that *L. truncatula* is not prevalent in Bandar-Anzali and Rasht and surroundings of these endemic cities. Of 4830 different snails studied, only seven *L. gedrosiana* were found to be infected with larval stages (rediae and cercariae) of *Fasciola* sp. Experimental infections of 15 common laboratory mice by metacercariae, obtained from those naturally infected snails, were carried out and all trematodes recovered at necropsy, 8 weeks post-exposure, appeared to be *F. gigantica* based on morphology. The high temperature, moisture and rainfall during the year, especially in Bandar-Anzali, support the establishment and transmission of the disease in the zone. Although the species involved in human infections in endemic areas of Gilan has been usually referred to as *F. hepatica*, the results of this study as well as some interesting epidemiological evidences related to the disease, support that *Fasciola gigantica* might be the main causal agent of fascioliasis in this important endemic zone.

Keywords: Fasciola gigantica, Lymnaea gedrosiana, *Fascioliasis*, *Gilan, Iran*

***Corresponding authors:** E-mail: k_fashi@yahoo.com

Introduction

During the last few decades the public health importance of human fascioliasis has significantly increased, including several areas of true human endemics ranging from low to very high prevalences and intensities (1, 2). Two *Fasciola* species, *F. hepatica* and *F. gigantica*, are involved in both animal and human fascioliasis (3). Fascioliasis due to *F. hepatica* is a great health problem in many countries with temperate climates such as in Europe, the Americas and Australia, whereas the major endemic areas for *F. gigantica* are large tropical regions of Africa, and many areas of Asia including Uzbekistan, Turkmenia, Iran, Iraq, India and Pakistan, etc. (4-6). In Asia and Africa the distribution of *F. gigantica* and *F. hepatica* overlaps and this overlap makes it difficult to identify the particular species involved, so that it is often referred to as *Fasciola* sp. (2). Generally, in tropical countries when both species coexist, *F. gigantica* is usually endemic in lower regions while *F. hepatica* is endemic in the highlands (7).A similar picture occurs in Iran, where the distribution of *F. gigantica* and *F. hepatica* overlaps in most areas, including northern Iran. In endemic areas of human fascioliasis, as Gilan province, both *F. gigantica* and *F. hepatica* occur in the same cattle and buffaloes.

Concerning humans, the fluke species involved in human fascioliasis remains to be identified, having been referred to simply as *F. hepatica.*

Sabbaghian et al. (8) reported high prevalences and intensities of fascioliasis among domestic animals in Khuzestan province, southern Iran, with 57% for buffaloes, 54% for cattle and 35% for sheep. Infection rates of, 91.4%, 49.2%, 29% and 11.2% in buffaloes, cattle, sheep and goats have been reported by Sahba et al (9) in Khouzestan, respectively. In these studies, infection by *F. gigantica* appeared to be more prevalent than that by *F. hepatica*. Sabokbar (10) has shown prevalences of animal fascioliasis as high as 21.5% in Gilan and 12% in Mazandaran provinces in the northern part of Iran. Although the prevalences of fascioliasis among domestic animals in the southern part of Iran are higher than those from the northern part, the number of human disease cases reported is significantly higher in the Northern provinces at the shore of the Caspian Sea, especially in Gilan province (11, 12). The characteristics of Gilan province, including flat lands under sea level, numerous irrigation canals, agricultural tradition (mainly rice), temperatures higher than 20º C, high rainfall (1300-1800 mm annually), high moisture, short dry season, etc., are very appropriate for liver fluke transmission and lymnaeid existence (6).

In Iran, human fascioliasis was sporadic until 1987, when an outbreak occurred in Gilan and affected more than 10000 people (13, 14). The second outbreak occurred some 10 years later in which several thousands of people were again infected (15). Reports of several hundred cases of human disease during inter-epidemic periods and then after, show that Gilan province has become an important endemic area for human fascioliasis. Bandar-Anzali and Rasht appear to be the most important endemic areas including most of the human cases during the epidemics and inter-epidemic periods.

The coexistence of *F. hepatica* and *F. gigantica* in livestock in Gilan suggests a very complicate picture of possible circulation ways of the causal agents. Elucidating the different circulations of the fasciolids in this endemic zone becomes very important for understanding the epidemiology and transmission of the disease. The main objective of the present study was to investigate the distribution and natural infections of local lymnaeids, environmental characteristics related to the disease transmission and determining the most prevalent fasciolids and definitive hosts for elucidating some circulation ways of fasciolids in those two endemic areas of human fascioliasis in the Gilan province.

Materials and Methods

Investigating the natural infections of local lymnaeids Snail collections were carried out monthly in Bandar-Anzali and Rasht water bodies, rice fields and rivers during a one-year period (July 2001 to July 2002). Snails classified as lymnaeids according to shell morphology, were collected by hand or by a 40 × 40 cm net. Those snails with a shell length of less than 5 mm were not examined for larval trematode infections. Efforts were made to obtain as much snails in each sampling as possible. Snails were put in plastic screw capped containers in natural water with etiquettes outside and taken to the laboratory for examination.

In the laboratory all snails were crushed between two glass slides, under a dissection microscope and examined for the presence of rediae and cercariae. The positive specimens were transferred into glass petri-dishes containing natural water for metacercariae formation. The metacercariae were stored at 4°C in water for at least 2 weeks prior to experimental infections of laboratory animals.

Experimental infections of laboratory animals Fifteen common laboratory mice were inoculated orally, each with 20 metacercariae, which were kept at 4º C in water for at least 2 weeks prior to inoculation. The mice were kept in animal house under daily observation, during which all deaths were recorded. All mice were killed 8 weeks post-exposure and their livers, abdominal cavities and peritoneum wall were checked for adult flukes.

Slaughterhouse observations for determining the most prevalent fasciolid For the determination of the parasite species involved in animal fascioliasis, one slaughterhouse in Bandar-Anzali and three in Rasht were visited several times during our study. The infected livers were transferred to the laboratory, and all parasites found collected in normal saline and counted according to the fluke species. We used the morphometric parameters proposed by Sahba et al (9) for discriminating between *F. hepatica* and *F. gigantica* recovered from infected livers. The average length/width ratio is 4.39-5.20 in *F. gigantica*, while it is 1.88-2.32 in *F. hepatica*.

Environmental characteristics of the endemic zone The metereological data including annual temperatures, humidity and rainfall were obtained from the Central Metereological Office of Gilan province. The number and kind of livestock in the endemic zone were obtained from the Gilan Veterinary Office. Observations on livestock in pastures and rice fields in all flatlands of Gilan were also performed for the study of the common domestic animals in the endemic zone. The type of the water bodies inhabiting by the snails, pH and temperatures of the water were studied by visiting different regions in the zone.

Studies on distribution of lymnaeid intermediate hosts For determining the most prevalent lymnaeid intermediate hosts, snail collections were attempted from different habitats in Bandar-Anzali and Rasht when the population of the snails was at the highest level during the year, usually by the end of spring. Snails were classified as lymnaeids according to shell morphology.

Results

Of 4830 snails examined, only 7 *L. gedrosiana* specimens were found to be infected during this one-year period study. The infection rate for *L. gedrosiana* was 0.35%. Three snails from Bandar-Anzali were infected with large numbers of mature cercariae and two mainly with rediae and small numbers of cercariae. Two *L. gedrosiana* from Rasht district, Khoshkebijar and Lashte-Nasha, were found to be infected with immature rediae and cercariae. No cercariae or rediae were detected in *L. palustris* or *L. truncatula*-like snails in this study. The infected sails were found in November 2001(late fall) and January 2002 (early winter).

Of 15 mice used for experimental infections, 2 died on days 12 and 23 post-exposure. No he-

patic lesions were seen at necropsy and no fluke was recovered. From the rest of the mice, three were found to be infected and three immature flukes were recovered, one from each. Two trematodes were recovered from the peritoneal cavity and one from the liver. Three mice showed some pathological evidence but no flukes were found. According to the data from the Gilan Central Veterinary Office and our direct observations, cattle was found to be the most prevalent livestock in Bandar-Anzali and Rasht and certainly has the most important role in the distribution of the disease in these endemic zones, whereas sheep or goat raising is not a normal rule in Rasht and Bandar-Anzali. The slaughterhouse observations indicated that *F. gigantica* is the main fasciolid in local cattle. Of 928 adult parasites collected from 13 infected livers from Gilan flat lands, mainly Rasht and Anzali, 91.1% were diagnosed as *F. gigantica* and only 8.9% as *F. hepatica* based on morphology. The most prevalent lymnaeid snails in these zones were *L. gedrosiana* and *L. palustris*. *L. truncatula*-like snails were only isolately collected from Rasht and Bandar-Anzali. It seems that *L. truncatula* is not prevalent in endemic regions of human fascioliasis in Gilan province. The water bodies in endemic regions are mainly stagnant water collections, rice fields and numerous irrigation canals, rich in vegetations.

The average temperature of these water bodies, which were checked monthly during our study, was about 18° C and the pH ranged between 6-7.5. The water temperature during 8 months of the year was between 15-27° C, which is very appropriate for lymnaeid existence and liver fluke transmission. The average of minimum and maximum temperatures during years 2000-2003 in Bandar-Anzali were 5°C and 29.8° C, respectively. In Rasht, the average of minimum temperature during these three years was 2.3° C and the average of maximum temperature was 31.9°C. The amount of annual rainfall in Bandar-Anzali was between 1415 and 2013 mm (two times higher than the mean rainfall in whole country), whereas in Rasht it was lower and between 1150 and 1427 mm. The averages of minimum and maximum humidity during these years were 66% and 98%, respectively. These climatic conditions are very appropriate for liver fluke transmission and lymnaeids existence in low lands of Gilan.

Table 1: Number and species of lymnaeids examined

No. of snails	Lymnaied snails		
	L. gedrosiana	*L. palustris*	*L. truncatula like*
examined	2028	1998	804
infected	7*	-	-

*Three snails infected with mature cercariae and four with rediae and cercariae

Table 2: Number of infected animals and trematodes recovered post-exposure

No. of				
Animals exposed	Cysts inoculated	Death before necropsy	Animals infected	Trematodes recovered
15	20	2	3	3*

*One trematode recovered from liver and two from peritoneal cavity

Discussion

Whereas in Europe, the Americas and Australia only *F. hepatica* is concerned, the distribution of *F. hepatica* and *F. gigantica* overlaps in many areas of Africa and Asia. This overlap has even become the basis of an already long controversy on the taxonomic identity of the *Fasciola* species in these regions, where the presence of both species as well as intermediate forms has been reported (16). The same picture occurs in the endemic zone of human fascioliasis in Gilan province, where both liver fluke species frequently appear in the same infected livestock individual. The coexistence of *F. hepatica* and *F. gigantica* in local livestock suggests that humans can be infected by both species, as it has been reported from different regions of Asia and Africa (2). Hamond (17) believes that human infections with *F. gigantica* may be more common than is thought, but has not been reported more frequently because it has not been looked for adequately.

The characteristics of epidemiology and transmission of fascioliasis in Gilan appear to be complicated and experimental and cross-infection assays are needed to ascertain how animals and humans enter the different liver fluke circulations. Three lymnaeid snails, including *L. gedrosiana*, *L. palustris* and *L. truncatula* have been reported from Bandar-Anzali (18). The susceptibility of lymnaeid snails to infection with *F. gigantica* and *F. hepatica* has been the subject of several studies (19-21). The results of all experiments verify the role of *L. gedrosiana* as intermediate host for *F. gigantica*, and there is still controversy regarding its role in the transmission of *F. hepatica*.

The finding of 7 naturally infected *L. gedrosiana* specimens from water collections and rice fileds around Bandar-Anzali and Rasht indicates that the life cycle of *F. gigantica* is occurring in these regions and the presence of high incidence rates of fascioliasis by *F. gigantica* in local cattle verifies this fact. Bandar-Anzali and Rasht, with most of the reported human cases, are not sheep raising areas so that cattle is the most common definitive host and certainly has the most important role in the distribution of the disease in these endemic zones. Worth mentioning is the low prevalence and intensity of *F. hepatica* besides high ones by *F. gigantica*. Of 928 adult liver flukes collected from 13 infected livers of cattle, in Rasht and Bandar-Anzali slaughterhouses, 91.1% were diagnosed as *F. gigantica* and 8.9% as *F. hepatica*. All the adult worms recovered from experimentally infected animals in present study, appeared to be *F. gigantica* based on morphology (length /width ratio) and this also is in agreement with slaughterhouse observations, in which *F. gigantica* has been demonstrated as the most prevalent fasciolid in Bandar-Anzali and Rasht.

The absence of fasciolid larval stages in *L. palustris* and *L. truncatula*-like snails in present study does not rule out the possibility of infection in these snails, but anyway it indicates the important role of *L. gedrosiana* in the transmission of fascioliasis at least at animal level in Gilan. The role of *L. palustris* as a second intermediate host for *F. hepatica* has been shown by experimental and natural infections (2, 22, 23). Similar studies are needed in endemic regions of Gilan province for understanding the relationship between this snail species and local fasciolids.

Although recent DNA-based techniques for the detection of larval stages of *F. hepatica* and *F. gigantica* have recently increased the efficiency, sensitivity and specificity of infected snail screening (24, 25), results always show that fasciolid prevalences in lymnaeids are very low, even in high endemic areas. This means the necessity of examining very large number of snails for obtaining only a very few infected ones. Studies in Australia and Louisiana have shown that very low snail infection rates were sufficient to produce major infections in mammalian hosts (26, 27). Only two infected snails were found during a 3-year period study in Morocco (28).

F. gigantica is a parasite which is adapted to tropical areas and to aquatic snails and to an aquatic environment for its transmission (29). The characteristics of the Gilan zone including flat lands mainly under sea level, numerous stagnant water collections and irrigation canals rich in aquatic vegetation, agricultural tradition, mainly rice, temperatures higher than 20° C, high humidity and rainfall, presence of cattle as the main definitive host and *F. gigantica* as the most prevalent fasciolid, all suggest an appropriate condition for *F. gigantica* transmission.

The presence of all necessary conditions for disease transmission, obtaining naturally infected snails in water bodies and rice fields surrounding these cities, frequent growth of sylvatic vegetables in most regions of Gilan including endemic areas and local people habits for eating these wild grown aromatic vegetables, which have been implicated as the main source of human infections in Gilan (13, 14), suggest that *F. gigantica* might be the parasite species most involved in human fascioliasis in Gilan.

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