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Original Article

Seroprevalence of Human Fascioliasis in Chaharmahal and Bakhtiyari Province, Southwestern Iran

Kouroush MANOUCHEHRI NAEINI [1], Farnaz MOHAMMAD NASIRI [1], Mohammad Bagher ROKNI [2], Soleiman KHEIRI [3]

1. *Dept. of Parasitology, Mycology and Entomology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran*
2. *Dept. of Parasitology and Mycology, School of Public Health, National Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran*
3. *Dept. of Epidemiology and Biostatistics, School of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran*

***Corresponding Author:** Email: K.manouchehri2014@gmail.com

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Abstract

Background: Fascioliasis caused by *Fasciola hepatica* and *F. gigantica* is a foodborne and waterborne zoonotic disease with a worldwide distribution. The illness occurs in regions with intensive sheep or cattle production. In some parts of Iran the incidence of human infection has increased over the past decades. Since Chaharmahal and Bakhtiyari Province has been known as a main pole of traditional animal husbandry and there was no information about human fascioliasis in this region, the present study was carried out on human population to determine the seroprevalence of this infection.

Methods: In this cross-sectional study 1475 serum samples collected from individuals attended to clinical laboratory of the province were subjected to ELISA to detect anti-*F. hepatica* IgG antibodies. The socio- demographic characteristics of the subjects were also collected through questionnaires.

Results: In spite of expectation, the present study showed that out of the 1475 serum samples examined anti- *F. hepatica* IgG antibodies were found only in two of the subjects (0.135%). Therefore, because of the very few number of positive cases no statistical analysis was applicable.

Conclusion: According to the finding of this investigation, human fascioliasis may not be an important health problem in this region of Iran. However, for a better understanding of situation of this parasitic infection in this area more comprehensive animal and human studies are needed.

Keywords: Human fascioliasis, Seroprevalence, ELISA, *Fasciola hepatica*

Introduction

The cosmopolitan digenetic trematode, *Fasciola hepatica* also known as sheep liver fluke, has long been known as an important parasite of sheep and cattle. Since this pathogenic parasite can cause significant morbidity and mortality in livestock and produce great economic losses, numerous studies have been carried out on it (1). Some of data from coprolites indicate that Europeans have been infected with *F. hepatica* for at least 5000 years (2). *F. hepatica* was the first trematode to be described and the first for which the entire life cycle was defined. Historical evidence show that the first recorded information on infections caused by this parasite was published by Jean de Brie in 1379, and the complete life cycle of *F. hepatica* was established by the investigations of Leuckart and Thomas in 1883, independently of one another (3).

The adult worms of *F. hepatica* reside in large bile ducts of different species of mammalian hosts, in-

cluding human and may live in the host for many years. The produced eggs of the parasite are carried by the bile into the intestinal lumen of the host and passed into the environment with the feces. Within 1 to 2 weeks, the miracidium which develops in water, escapes from the egg and infect the amphibious snail intermediate hosts of the family Lymnaeidae. After 4 to 7 weeks, the progeny cercariae are liberated from the snail. Finally, the liberated cercariae encyst on water vegetation e.g. watercress or water surface to form metacercariae (4). Although the most important definitive hosts of *F. hepatica* are sheep, a wide range of other herbivorous and omnivorous animals, including goats, cattle, horses, camels, vicuna, hogs, rabbits, and deer, are commonly infected with the parasite; even dogs may harbor the fluke (5). Infection of these mammalian hosts and human that occur in 61 countries worldwide, results from ingesting raw watercress, other fresh aquatic vegetation or water contaminated with the metacercariae of the parasite (6-10).

Some estimates indicate that there are about 3 million cases of human fascioliasis, globally. Human fascioliasis has a wide geographical distribution. So that, this infection have been reported from all continents except Antarctica, with the highest rates of infection in Bolivia, Peru, Egypt, Iran, Portugal, and France (8,11,12). During the past three decades two epidemics involving up to 100,000 persons have been reported in northwestern of Iran (8).

Based on the hepatic migratory phase of the parasite larvae and to the presence of adult worms in the bile ducts, human fascioliasis has two distinct clinical phases (5, 6, 13). In areas of the world that fascioliasis is enzootic, this infection is suspected in patients suffering from fever, hepatomegaly, and eosinophilia with a history of consuming freshwater plants. After the establishment of infection in human host, different classes of antibodies, precipitins, and hemagglutinins can be detected in the serum of patients. To confirm the clinical diagnosis of fascioliasis and follow-up of suspected patients, radiographic techniques and magnetic resonance imaging are used. However, stool examinations and a number of serological tests are recommended for presumptive diagnosis of disease (14). During the late stages of fascioliasis, the biliary obstruction and cholangitis caused by the parasite or its eggs can produce a number of symptoms and signs of disease. However, liver biopsy may be very important in diagnosing fascioliasis, particularly for asymptomatic disease (15).

For diagnosis of fascioliasis, different serological tests such as indirect hemagglutinaion, complement fixation, countercurrent electrophoresis, immunosorbent assay, and skin test have been used. While, chronic fascioliasis is diagnosed by finding the characteristic eggs in stools or materials obtained by duodenal or biliary drainage (5, 16).

Due to large numbers of human infections that have been reported from the Northern parts of Iran during recent decades, the country has been considered as an endemic focus of human fascioliasis in the world (8, 17). On the other hand, Chaharmahal and Bakhtiyari Province has known as one of the main poles of traditional animal husbandry in the country, where there is a close association between human and animals. Therefore, the present study was conducted to determine the seroprevalence of human fascioliasis in this region of the country.

Materials and Methods

Study area

Chaharmahal and Bakhtiyari Province with 16532 sq. km. is situated between 31° 09' North latitude and to 32° 48' East longitude in the southwestern part of Iran. About %10 of water sources of the country are in this province. Because of high mountains, the humid Mediterranean winds evacuate all over these places. This region of Iran has various climates. Due to the mountainous region of the province, there will be different regions as matter of temperature. The max absolute temperature has been recorded in Lordegan synoptic station with 47.5 ◦C and the min absolute temperature in Dezzak station with -34.5 ◦C (18).

Study population and sampling method

In this cross-sectional study that carried out during 2013-2015, the population was selected

based on non-randomly simple sampling method among patients who attended to the clinical laboratories of health centers of the province for periodic check-ups or diagnosis of their recent illness. According to statistical calculations and approximate prevalence about 5% that was derived from some of studies in Iran, 1475 samples were determined. The samples were allocated between the seven counties, Shahrekord, Lordegan, Boroujen, Farsan, Kiar, Ardal, and Kohrang based on their population. (Table 1).

Sample collection and Data analysis

After describing the details of the study for the laboratories' staff partners, the collected sera were transported to Resaerch Laboratory of Parasitology, Shahrekord School of Medicine and stored at -20 ◦C until examinations. At the same time, socio-demographic information of the subjects including, age, sex, job, residence, diet, level of education, and clinical signs was recorded through questionnaires. Finally, the data were processed and analyzed by using the SPSS ver. 20 software (Chicago, IL, USA).

Laboratory method

We used an ELISA method for the detection of specific anti- *F. hepatica* IgG antibodies in serum samples. The ELISA was conducted based on the method established in the Laboratory of Serological Diagnostic of Helminthic Diseases, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran (19).

Results

The sera were belonged to 791(53.6%) females and 684(46.4) males aged 5 to 87 yr (mean 31. 26± 17.26). Socio- demographic characteristics of the subjects are shown in Table 1.

Table 1: Frequency distribution of serum samples assigned to the population by socio- demographic characteristics

Variable	Number	Frequency %
Sex		
Male	684	46.4
Female	791	53.6
Age group (yr)		
5-10	151	10.3
11-20	303	20.9
21-30	375	25.2
31-40	257	17.2
41-50	174	11.9
51-60	103	7.1
61-70	56	3.7
≥ 71	56	3.7
Residence location		
City	1033	70
Village	426	28.9
Mobile	16	1.1
Level of education		
Illiterate	307	20.8
Primary school	303	20.6
High school	279	18.9
High school graduate	344	23.3
Collegiate	242	16.4
Vegetable consumption		
Yes	1313	89
No	162	11

Out of the 1475 sera, the specific anti- *F. hepatica* IgG antibodies were found only in two of the samples (0.135%) (CI. 95%, 0.0049, 0.00016). These positive samples belonged to a 63-yr-old illiterate woman who was living in urban areas of Shahrekord, and a 79-yr-old illiterate woman who was living in rural areas of Lordegan County. Despite both the infected subjects had a history of vegetables consumption, none of them had signs or symptoms of fascioliasis.

Discussion

Nearly 40 million people harbor food-borne trematodes, including fascioliasis, globally (20). The pattern of human infections with food-borne trematodes is in a state of change regarding number, geographic range, and economic and clinical significance (21).

Fascioliasis is a cosmopolitan parasitic zoonosis that is found where there is close association of livestock, humans, and snails. Due to huge productivity losses of fascioliasis in livestock, it is very important, economically (22).

According to a number of estimates, about 2.4 million of human cases of fascioliasis are found in 61 countries with 180 million people at risk (23). Since 1980, the increasing prevalence of human infections with *Fasciola* spp. in some parts of the world has changed the epidemiological pattern of infection. So that fascioliasis is now considered as emerging or re-emerging infection (24-26).

In the Middle East, Iran has been considered as one of important foci of human and animal fascioliasis with variable prevalence rates of infection in different regions of the country (27- 31).

Because of significant rainfall and good pastures, Chaharmahal and Bakhtiyari Province is known as one of the main poles of traditional animal husbandry in Iran. However, according to the results of one investigation carried out on slaughtered sheep in Shahrekord abattoirs, the capital of the province, the approximate prevalence of fascioliasis in slaughtered sheep has been reported about 4% (32).

In Iran, infection of cattle, sheep, buffalo and goats with *F. hepatica* and *F. gigantica* were reported from many parts of the country with different prevalence rates. For example, in some parts of Gilan Province, north of Iran, the average rate of infection with *Fasciola* in cows was 32.1% with rate as high as 55.2%. Besides, in some parts of Mazandaran Province, 25.4% of cattle and 7.3% of sheep were infected with the parasites (33).

Despite the prevalence of animal fascioliasis in slaughtered sheep in Shahrekord abattoirs (32), the present study showed that the rate of human infection with *F. hepatica* is much lower compared with the prevalence of animal infection in this region (0.135% vs. 4%). The patterns of fascioliasis in reservoir hosts are not always closely linked with those in humans, since these are determined by distributions of intermediate hosts, whereas human infection is further limited by eating behavior (34).

The present study showed that the rate of human infection in this region was significantly much lower from other parts of the country, particularly the northern parts of Iran (33, 35). Before 1988, about 100 cases of human infection had been diagnosed per year in Iran (30). In the first outbreak of fascioliasis in Gilan Province in 1988, about 10000 individuals from Port of Anzali were infected by *Fasciola*. Besides, in this region, serological examination of 452 inhabitants using ELISA method in 1988 showed infection rate of 50% (35). Between 1984-1996, in different parts of Gilan Province, the number of human fascioliasis cases was 1100, of whom, the highest and lowest rates of infection belonged to Port of Anzali (904 cases) and Rudsar (1 case), respectively (33). In the second outbreak of human fascioliasis, which occurred in 1999 among the population of Anzali Port, the number of infected cases was estimated to be 2465 (30). In Mazandaran Province, 107 human cases were diagnosed between the 1999 and 2002 (30). In addition, 17 cases of human fascioliasis were found in a district of Kermanshah in 2000 (33). In areas with a high endemicity of infection,

different epidemiological situation and transmission patterns have seen (36, 37).

In endemic areas of infection, studies show that local patterns of human fascioliasis are partially determined by social customs and attitudes toward raw foods, including vegetables. The consumption of watercress has been implicated in most human *F. hepatica* infections (38-39). However, in the outbreak of human fascioliasis occurred in Glian Province in 1988, the consumption of product made locally from green wild grown plants of the *Mentha* and *Eryngium* species, called "Khalivash" and "Dallar" were known as the main sources of infection (40).

In this study, in spite of vegetable consumption by majority of the subjects, the rate of human infection was very low and there was no significant correlation between the history of vegetable consumption and rate of infection. The differences present in the rate of human and animal fascioliasis in this region with other parts of Iran, e.g. northern parts of Iran, may reflect the differences in climate and weather, distribution of snail intermediate hosts, social customs, and eating behaviors.

Conclusion

Although the study showed that the prevalence of human fascioliasis in this area is much less than expected, but for a better understanding of the true prevalence of human fascioliasis in this area, it is necessary to design more comprehensive studies using the randomly cluster sampling method with a larger sample size in further works. In addition, due to the favorable geographical and climatic situation of the region for animal husbandry and the economic importance of fascioliasis in livestock, it is suggested, in future, the snail fauna and prevalence of infection in livestock be studied.

Ethical 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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