

# Human fascioliasis in nomads: A population-based serosurvey in southwest Iran

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## SUMMARY

Fascioliasis is a human and veterinary concern in Iran. This cross-sectional population-based study was conducted to determine the seroprevalence of human fascioliasis among nomadic people in Kohgiluyeh and Boyer-Ahmad province located in the southwest of Iran. Venous blood samples were collected from 933 nomads in the area. A predesigned questionnaire containing basic epidemiological information was filled out for each subject during the sampling. Sera were evaluated for anti-*Fasciola* antibodies, using excretory-secretory (ES) antigen of *Fasciola hepatica* in an ELISA system. Of 933 recruited subjects, 726 (77.8%) were females and 206 (22.1%) were males. The mean age of the participants was 43.1 ( $\pm 16.7$ ) years old. Most of the

subjects (24.6%) were in the age group of 21-30 years old. Anti-*Fasciola* antibodies were detected in 24 (2.6%) out of 933 cases. Of 24 seropositive cases, 3 (12.5%) were male and 21 (87.5%) were female. The differences between the seropositivity and sex, age, level of education and residence area were not statistically significant ( $p > 0.05$ ). Findings of the current study demonstrated that the seroprevalence of fascioliasis in the studied nomadic population was significant, and that preventive and control measures should be taken to prevent the disease from spreading and causing even greater health and economic problems in this area.

*Keywords:* fascioliasis, seroprevalence, nomads, Iran.

## INTRODUCTION

Fascioliasis is a helminthic disease caused by *Fasciola hepatica* and *Fasciola gigantica*. The disease has traditionally been a concern in veterinary medicine while it was neglected in human health communities until the increase in the number of infected cases in several countries around the world including: Andean countries of South America, northern Africa, the Western Europe countries, and Iran [1-5].

In Iran fascioliasis is a human challenge where

two massive outbreaks of human cases occurred in Gilan province, in the North of the country; the first one in 1989 which infected more than 5000 people and the second one in 1999 which infected more than 10000 people [3]. Although the main focus of human fascioliasis in Iran is the northern part of the country, the disease is still reported from other areas such as Kermanshah and Lorestan in west, Kohgiluyeh and Boyer-Ahmad Province in southwest and Isfahan in central parts of the country [4-8]. Among these areas, Kohgiluyeh and Boyer-Ahmad Province, there is a new focus of the disease where cases of human fascioliasis are continuously reported since 2010 [4, 5]. The disease is common in animals and prevalence of animal fascioliasis in the goats, sheep and cattle were reported to be 7.16%, 11.75% and 12.5% re-

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spectively, in this area. A study by Shafiei et al., in this province revealed that from 58 *Fasciola* samples, isolated from cattle, sheep, and goat, 41 isolates were *Fasciola hepatica*, while 17 isolates (from cattle) were *Fasciola gigantica* [9].

A previous study in rural areas of Kohgiluyeh and Boyer Ahmad province, documented a seroprevalence rate of 1.8% for human fascioliasis [4]. The livelihood of most people in the region is through livestock breeding and farming. Considering the high prevalence of fascioliasis in animals in this region and the fact that most people used to consume the raw aquatic plants, the chance of acquiring the infection is relatively high in the region. In view of the fact that the knowledge about the condition of epidemiological features of fascioliasis are the prerequisite for the control program of this disease, this study was conducted to find out the serostatus and related risk factors of fascioliasis in the nomadic population in Boyer-Ahmad Township in southwest of Iran.

## ■ PATIENTS AND METHODS

### *The study area*

This cross-sectional population-based study was conducted in Boyer-Ahmad Township in Kohgiluyeh and Boyer-Ahmad province, located in the southwest of Iran (Figure 1). Kohgiluyeh and Boyer-Ahmad is one of the 32 provinces of Iran. The province population, based on the census of Iran's Statistical Center in 2016 was 713,052 people, of whom about 50% live in rural areas. The region is

mountainous, and the hills are mainly covered by wild pistachio and oak forest. The nomads of this province make up 70,000 (12%) of the population of the province. Nomads in the region travel with their flocks twice yearly back and forth between the summer highland and the winter pastures on lower (and warmer) lands.

### *Blood sample collection*

This cross-section study was conducted in 2016. Fresh blood samples (about 5 mL) were collected from 933 nomads in the area. Sample size was calculated based on the nomad population in the Boyer-Ahmad district, considering the overall seroprevalence of fascioliasis in the region. All of the participants filled a structured questionnaire during sampling which provided information about their sociodemographic features along with data regarding the fascioliasis risk factors including the source of water supply and eating raw vegetables. The collected samples were transferred, in cold chain, to the School of Medicine at Shiraz University of Medical Sciences (SUMS) and kept at -20° C till tested. The ethical review committee of the SUMS approved the study and informed consent was provided by the participants before sampling.

### *ELISA for detection of anti-Fasciola antibodies*

ELISA was performed in flat bottom 96-well microplates (Nunc, Roskilde, Denmark), using *Fasciola* excretory secretory (ES) antigens. The ES antigen was prepared as previously described [10]. The ELISA microplates were coated in coating



**Figure 1** - Map of Iran and location of Kohgiluyeh and Boyer-Ahmad province and also Boyer-Ahmad Township.

buffer (0.1 M sodium carbonate, 0.1 M sodium bicarbonate, pH 9.6) and the plates were incubated at 4°C overnight. Plates were washed, using phosphate-buffered saline-Tween 20 (PBST, pH 7.4 containing 0.05% Tween 20). Blocking was performed, using 5% skimmed milk solution for 2 hours at room temperature (RT). The sera samples were diluted in PBST (1/100) and, along with positive and negative control sera, were added to the plates and incubated at RT for another one hour. After washing as before, 100 µL of 1/4000 dilution (in PBST) of goat horseradish peroxidase conjugated anti-human antibody (Sigma-Aldrich, St. Louis, Missouri, US) was applied to each well of the plates and then the plates were incubated in

the same way for another one hour. After washing as before, 100 µL of o-Phenylenediamine dihydrochloride (OPD) solution (0.025% H<sub>2</sub>O<sub>2</sub> in 0.1 M citrate buffer, pH 5) was added to each well and the plates were incubated at RT for 30 minutes. The optical density at 490 nm was measured, using an ELISA microplate reader. The cutoff point was set at mean of ODs of negative controls plus two standard deviations.

#### Statistical analysis

Statistical analysis was performed using SPSS (version 18). Chi-squared test was used to find out the association between the seropositivity to fascioliasis and the qualitative variables.

**Table 1** - Demographic characteristics and seroprevalence of fascioliasis among nomadic populations in Boyer-Ahmad district, Southwest of Iran.

Characteristics	Frequency (No.)	Percent (%)	Positive for anti-Fasciola Ab		P value
			No.	%	
<i>Gender</i>					
Male	206	22.1	3	1.5	0.187
Female	726	77.9	31	2.9	
<i>Age group</i>					
1-10	17	1.9	0	0.0	0.474
11-20	51	5.6	1	1.9	
21-30	223	24.6	7	3.1	
31-40	191	21.1	7	3.7	
41-50	146	16.1	1	0.7	
51-60	143	15.8	3	2.1	
>60	135	14.9	3	2.2	
<i>Residence</i>					
Dashtroom	122	13.5	0	0.0	0.06
Zilaei	121	13.4	2	1.7	
Mooshmi	349	38.6	15	4.2	
Margoon	115	12.7	0	0.0	
Bardpahn	80	8.8	2	2.5	
Others	118	13	5	4.2	
<i>Educational level</i>					
Uneducated	419	46.3	8	1.9	0.95
Primary level	212	23.4	6	2.8	
Secondary level	161	17.8	4	2.5	
High school diploma	74	8.2	2	2.7	
University level	39	4.3	1	2.6	

## ■ RESULTS

A total of 933 subjects in nomadic communities were enrolled in this population-based study. Subjects were 726 (77.8%) females and 206 (22.1%) males. Their ages ranged from 1 to 91 years old and the mean age was 43.1 ( $\pm 16.7$ ) year. Most subjects (24.6%) were in the age ~~group~~ of 21-30 years old. Using the ELISA method, anti-*Fasciola* antibodies were detected in sera of 24 out of 933 cases, corresponding to a seroprevalence rate of 2.6%. From 24 seropositive cases, 3 (12.5%) were male and 21 (87.5%) were female. Although the seroprevalence in women was two times higher than the men, still the difference between the seropositivity to fascioliasis and gender was not statistically significant ( $p=0.47$ ). Seropositivity to fascioliasis was more frequent in 21-30 (3.1%) and 31-40 (3.7%) age groups. The differences between seropositivity to fascioliasis and level of education and residence area were not statistically significant. Table 1 shows the demographic features and seroprevalence of fascioliasis among nomadic inhabitants in Southwest of Iran.

## ■ DISCUSSION

Human fascioliasis is a health challenge in several regions of the world including the Andean countries of South America, northern Africa, the Western Europe countries, and Iran in the Middle East [2-4]. As the concern for human fascioliasis in medicine grows, more studies have been carried out and new endemic foci have been discovered. Human fascioliasis is a major concern in northern part of Iran [3]. Moreover, human cases have also been reported from other regions like Kermanshah and Lorestan provinces in the west, and Kohgiluyeh and Boyer-Ahmad Province in the southwest of the country [4, 6, 7]. Our previous study documented a focus of human fascioliasis in Kohgiluyeh and Boyer-Ahmad province in southwest of Iran [4]. Further study revealed that the causative agent of fascioliasis in the area is *F. hepatica*, while both species of *Fasciola* were common in animals in the area [4, 5, 9]. Human infections are usually occurring through ingestion of raw wild freshwater plant such as *Nasturtium microphyllum* and *Mentha logifolia* [11]. Previous studies in Iran indicated that the main

causative agent of human fascioliasis is *Fasciola hepatica* [5, 12, 13]. In keeping with this, Hosseini et al. demonstrated that cases of human fascioliasis in Kohgiluyeh and Boyer-Ahmad Province are caused by *Fasciola hepatica* [5].

The current study was performed more than 5 years after the previous one but in different population group. In the previous study, the population living in five villages around the provincial capital was surveyed and the seroprevalence rate for fascioliasis was found to be 1.8%. In the current study, the nomadic population of the province was studied and 933 of them were surveyed for anti-*Fasciola* antibodies. In the present study, as expected, a higher seroprevalence was observed. This higher prevalence rate of fascioliasis might be linked to the lifestyle of the nomads; their greater contact with nature, the consumption of raw vegetables and in particular the aquatic vegetables, and the lack of access of people to plumbing water. In keeping with this, nomadic communities are considered to be at risk of other parasitic diseases including visceral leishmaniasis and hydatid cyst, due to their particular lifestyle and type of habitual nutrition [14-17].

The prevalence rate reported in the current study is almost the same as that reported in other parts of Iran, including the northern regions of the country [7, 18]. However, the seroprevalence rate is lower than those reported in some of endemic areas of the world, including Bolivia and Egypt [1, 19, 20]. A few studies stated the prevalence of fascioliasis as gender-dependent whereas in the current study no significant correlation was found between sex and seropositivity to fascioliasis [10, 21]. This means that men and women are both equally prone to the infection. It is important to comment on the gender imbalance in sampling in our study, as most of the samples were taken from the female subjects. This is mostly because women were more willing to give the blood sample and they were more reachable than men, as men were usually working in the farming field or were busy with keeping herds during the day and were not simply reachable. This can be considered as a limitation of this study. Since all people in the study positively responded to the question of consumption of raw vegetables, it was difficult to find out a definite association between the consumption of vegetables and seropositivity to fascioliasis.

In conclusion, findings of the current study demonstrated that the seroprevalence of fascioliasis in nomadic population in southwest of Iran is significant and preventive and control measures, including health education, prevention of environmental contamination by infected animals, teaching people about avoiding the use of raw aquatic vegetables, should be taken to prevent this disease from growing and causing even greater health and economic problems in nomadic communities as well as other groups of people in the area. It should also be highlighted that seropositivity does not correspond to true case of active fascioliasis.

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#### Conflict of interest

None

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