



## The Endemicity of Human Fascioliasis in Guilan Province, Northern Iran: the Baseline for Implementation of Control Strategies

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### Abstract

**Background:** The largest global outbreaks of liver fluke disease (Fascioliasis) in humans, caused by species of the genus *Fasciola*, have occurred in Guilan Province of Iran, affecting more than 15000 people. Although, different aspects of fascioliasis have been the subject of various researches during last two decades, nevertheless no community-based study has been performed in endemic regions of Guilan. The aim of present study was to obtain the basic information needed to develop future control strategies.

**Methods:** Fecal and blood samples were collected from 1,984 volunteers in the Bandar-Anzali district, the region where previous epidemics occurred. Fecal samples were examined by Kato-Katz and formalin-ether methods for the presence of *Fasciola* eggs. Sera samples were analyzed by ELISA to detect anti-cathepsin L antibodies.

**Results:** Twenty-seven (1.36%) individuals were seropositive, 9 (0.45%) individuals were egg positive (mean egg count 50.7 ( $\pm$ 30.36) eggs per gram of faeces) and 30 individuals (1.51%) were positive using both methods. No statistical association was observed between infection and age, gender, location, occupation, educational status and dietary habits. The prevalence of intestinal parasites is also included.

**Conclusion:** Human fascioliasis is hypoendemic in this region and recommends a passive case-finding approach, effective primary prevention measures, health education through mass media and effective veterinary public health measures for control of human disease.

**Keywords:** *Fasciola hepatica*, Human fascioliasis, Epidemiology, Endemicity, Control, Iran

### Introduction

Liver fluke disease, or fascioliasis, of animals and humans is caused by endoparasitic trematodes of the genus *Fasciola*. In temperate zones, *Fasciola hepatica* is prevalent while *F. gigantica* is found in tropical zones. Infection in livestock is hyperen-

demic globally costing the agriculture community US\$3.2 billion annually (1). Human fascioliasis is recognized by WHO as one of the "neglected tropical diseases" with an estimated 2.4-17 million people infected and 180 million at risk to infection (2-6).

A significant number of cases were reported in Andean South America, Egypt and Iran, in some communities in the Bolivian Altiplano a prevalence of up to 72% was reported (7-14). In most countries, incidence rates of human fascioliasis are calculated from sporadic reports of the disease and as a result, the disease is under reported worldwide. The incidence of disease also needs further investigation in many African and Asian countries (15).

*Fasciola* infection in the primary host begins following ingestion of encysted infectious larvae or metacercariae deposited on aquatic plants or floating on surface water. The parasites excyst in the gut and the juvenile worms penetrate the intestinal wall and migrate through the peritoneal cavity towards the liver parenchyma. After several weeks (8-12) the parasites enter the bile duct and become fully mature flukes and acute and chronic disease ensues. In humans, the severity of infection can vary from being asymptomatic to a severe and debilitating disease with extensive tissue damage and bile duct hyperplasia. Eggs produced by the flukes are released through the faeces and hatch to form free-living miracidia that infect the intermediate snail host (16, 17).

Veterinary fascioliasis has been prevalent in Iran for at least fifty years, with high prevalence and intensities especially in southern and northern parts, at the littoral of Persian Gulf and the Caspian Sea (18-23). Despite the higher infection rates of livestock in southern areas, human disease is predominantly reported in northern provinces, especially in Guilan Province because of the environmental conditions (especially high rainfall, temperature and moisture) facilitate snail breeding but also because of the dietary habits in the region (12, 24). Both *F. hepatica* and *F. gigantica*, are involved in animal and human fascioliasis in Iran and the distribution of these two fasciolids overlaps in almost all provinces. This overlap makes it difficult to identify the particular species involved in human infections so that it is often referred to as *Fasciola* sp. Recent studies have also verified the presence of hybrid *Fasciola* forms in endemic regions of Guilan province (25).

The WHO included Iran among six countries that are known to have a serious problem with fascioliasis (26). Prior to 1989, human fascioliasis was reported sporadically in Iran (27-29). In 1989, thousands of individuals in the Guilan Province of northern Iran presented to local health centers with classical symptoms of *F. hepatica* infection, which included epigastric and right upper quadrant pain, fever, chill, sweating, weight loss, urticaria, chest pain and hypereosinophilia. This first Iranian outbreak, which involved approximately 10,000 individuals, was followed by a second outbreak in 1999, which involved 5000 individuals. Bandar-Anzali is the most important endemic zone where most of the human cases occurred during the epidemics and inter-epidemic periods (12-14). Despite the high public health importance of human fascioliasis in Iran, many aspects of the disease still require further clarification.

The aim of the present study was to obtain a clear base-line picture of the disease in Bandar-Anzali in order to propose a scientific and practical strategy for future disease control in endemic areas of Iran.

## Materials and Methods

### *Study population and area*

This study was performed over a three-year period from March 2008 to March 2011, in Bandar-Anzali district of Guilan Province, Northern Iran. The sample size was calculated using systematic random sampling based upon data from the statistical centre of Iran. Overall, 138,004 (68,388 males and 69,616 females) were recorded to live in the study region from which 116,664 individuals live in Anzali city and 21,337 in surrounding villages. We sought families to volunteer their participation in a study using information from the local health center. We randomly selected two individuals from each family and a total of 701 and 1,283 subjects volunteered from the city and surrounding villages, respectively. Individuals under 10 years were excluded because they participated in a coprological study prior to this study (author's unpublished data) and parents had concerns about volunteering their children to provide a blood sample. The volunteers were provided infor-

mation about the impacts of fascioliasis on human health and the benefits of taking part in the study to develop public health measures. An epidemiological questionnaire was distributed by local health officers and completed by each individual in the presence of the family guardian if required. The questionnaire included demographic data, profession, educational status and history of eating fresh water plants.

### **Sample collection**

A wide mouth, screw cap, numbered container was given to each person who were thoroughly briefed on the procedure of stool specimen collection. Individuals were then required to bring their fecal samples to the Bandar-Anzali health center's laboratory where five ml of venous blood was also obtained from each participant. Blood was centrifuged at 3000 X g for 5 min, and the sera were kept in labeled Eppendorf tubes on ice for transportation. Based on the research strategy, only one stool sample per subject was collected from all participants. All egg positive and seropositive individuals were required to provide three additional fecal samples on alternative days under a liver-free diet for verification of infection. All samples were transported to the Guilan University of Medical Sciences (GUMS) for further processing.

### **Ethical approval**

Ethical approval was obtained from the Ethical Committee of the Guilan University of Medical Sciences. Approval was also obtained from the Guilan Province Health Centre. All volunteers were informed of the nature, benefits and risks of the study and an informed consent was obtained from adult participants and in case of subjects under 18 years old, consent from a family guardian was obtained. All infected individuals were offered 10 mg/kg of Egaten donated by WHO.

### **Coprological analysis**

A Kato-Katz slide was prepared from each stool sample using the kits donated by WHO. The slides were examined for *Fasciola* ova 24 hours after preparation and the number of eggs per slide was counted and recorded in positive circum-

stances. The Kato-Katz slides were analyzed for egg counts to determine the intensity of the infection and worm burden in infected subjects. In addition, the fecal samples were also processed by formalin-ether concentration technique for diagnosis of intestinal parasites.

### **Serological Analysis**

The method used for the serological diagnosis was previously validated and published (30, 31). In brief, each plate was coated with 100  $\mu$ l of recombinant *F. hepatica* cathepsin L (FhCL1) antigen (1  $\mu$ g/ml) in bicarbonate/carbonate coating buffer at pH 9.0 overnight at 4 °C. After washing the plate four times with PBS/0.1% Tween 20, excess protein binding sites were blocked at 37 °C for 1 h by adding 100  $\mu$ l of 2% bovine serum albumin diluted in PBS/0.1% Tween 20. After a further washing step, 100  $\mu$ l of sera samples (diluted at 1:100) were added and the plate incubated for 1 h at 37 °C. Following another wash step, 100  $\mu$ l of peroxidase-conjugated anti-human IgG (diluted 1:4000) was added to the wells and the plates were incubated for 30 min at 37 °C. After a final washing step, bound antibodies were detected by the addition of 100  $\mu$ l of TMB. The color was developed for 10 min and the reaction was stopped with 50  $\mu$ l of 0.1 M sulphuric acid. The plates were read on an ELISA plate reader (Stat Fax 2100 Microplate Reader, USA) at 405 nm. Results are reported as the mean values obtained for triplicate samples.

### **Statistical analysis**

The statistical analysis was performed using SPSS 16.0 for windows (Chicago, IL, USA), which contains univariate descriptive analysis. The categorical variables were evaluated by using the chi-square test or Fisher's exact test. A value of  $P < 0.05$  was considered as statistically significant.

## **Results**

### **Population characteristics**

A total of 1,984 individuals, aged 10-80 years (mean  $43.8 \pm 16.8$ ) participated in the study of which 701 subjects (35.3%) were from Anzali City and 1,283

individuals (64.7%) from the surrounding villages. Males represented 36.7% of the samples (n= 728) and females 63.3% (n= 1255) (Table 1).

### Prevalence of infection

A total of 30 individuals were positive for fascioliasis, 6 persons were egg positive and seropositive, 3 persons were egg positive and seronegative while 21 persons were seropositive and egg negative. The prevalence of infection by serology in urban and

rural regions was 1.71% and 1.17 % respectively, while it was 0.57% for urban area and 0.39% for rural regions when using coprological analysis. The overall prevalence of fascioliasis according to gender was 0.55% for males and 0.96% for females in urban and rural areas (Table 1). No statistically significant differences between gender within rural and urban populations were observed ( $\chi^2=0.51, P=0.48$ ).

**Table 1:** Percentage of positive cases according to gender

Sex	Number (%) of studied and positive cases by different laboratory tests									
	Anzali city n (%)	Anzali Villages n (%)		Anzali district (total) n (%)		n (%) in total population				
	Serology	Stool exam.	Serology	Stool exam.	Serology		Stool exam.			
Female	457 (36.4)	9 (1.97)	3 (0.66)	798 (63.6)	8 (1) (0.38)	3 (0.38)	1255 (100)	17 (1.36)	6 (0.48)	19 (0.96)
Male	244 (33.5)	3 (1.23)	1 (0.41)	485 (66.5)	7 (1.44)	2 (0.41)	729 (100)	10 (1.37)	3 (0.41)	11 (0.55)
Total	701 (35.3)	12 (1.71)	4 (0.57)	1283 (64.7)	15 (1.17)	5 (0.39)	1984 (100)	27 (1.36)	9 (0.45)	30 (1.51)

The intensity of infection was calculated using Kato-Katz technique for egg count per gram of faeces (epg) with a mean intensity of infection of 50.7epg ( $\pm 30.36$ ). We also examined the samples for the presence of other parasites and found the following prevalence's: *Giardia lamblia* (2.14%), *Strongyloides stercoralis* (0.9%), *Hymenolepis nana* (0.3%) *Trichostrongylus* spp. (0.1%), *Trichuris trichiura* (0.1%) and *Entamoeba histolytica/E. dispar* (0.1%). The most prevalent non-pathogenic parasitic protozoa observed were *Blastocystis hominis* (6.4%), *Endolimax nana* (3.9%), *Entamoeba coli* (2.6%), *Iodamoeba buetschlii* (0.3%). No association between fascioliasis and intestinal pathogenic parasites could be evaluated as only one individual was co-infected with *Fasciola* and *Entamoeba coli*.

### Prevalence according to age

The prevalence of infection was analyzed according to age (Table 2). No egg positive and/or sero-

positive cases were detected in participants from 10-19 years. The highest prevalence was seen in individuals aged 40-59 years. There was no statistically significant association between age and infection ( $\chi^2 = 4.37 P= 0.63$ ).

### Dietary habits of study population

The majority of studied subjects (93.7%) had the history of consumption of one of these types of plants at least once a month while 67.5% had consumed these plants at least once a week. The highest percentage of infected individuals (2.1%) was seen in those subjects who consumed vegetable everyday followed by those who consumed it at least once a week (1.7%) (Table 3). Despite the higher percentage of infected people in these groups the relationship between prevalence and vegetable consumption was not statistically significant.

**Table 2:** Percentage of positive cases according to age

Age groups	Number (%) of studied and positive cases by different laboratory tests								
	n (%)	Anzali city		n (%)	Anzali Villages		Anzali district (total)		
		Serology	stool exam.		Serology	stool exam	n (%)	Serology	stool exam
10-19	120 (57.4)	0 (0)	0 (0)	89 (42.6)	0 (0)	0 (0)	209 (100)	0 (0)	0 (0)
20-29	119 (37.2)	1 (0.84)	0 (0)	201 (62.8)	2 (1)	0 (0)	320 (100)	3 (0.94)	0 (0)
30-39	118 (24.4)	3 (2.54)	0 (0)	365 (75.6)	2 (0.6)	2 (0.55)	483 (100)	5 (1.04)	2 (0.41)
40-49	132 (30.6)	2 (1.52)	1 (0.76)	300 (69.4)	5 (1.7)	1 (0.33)	432 (100)	7 (1.62)	2 (0.46)
50-59	137 (43)	5 (3.65)	3 (2.19)	182 (57)	3 (1.7)	1 (0.55)	319 (100)	8 (2.51)	4 (1.25)
60-69	55 (34.6)	1 (1.82)	0 (0)	104 (65.4)	2 (1.9)	1 (0.96)	159 (100)	3 (1.90)	1 (0.63)
70-79	20 (32.3)	0(0)	0 (0)	42 (67.7)	1 (2.4)	0 (0)	62 (100)	1 (1.61)	0 (0)
Total	701 (35.3)	12 (1.71)	4 (0.57)	1283 (64.7)	15 (1.2)	5 (0.39)	1984 (100)	27 (1.36)	9 (0.45)

**Table 3:** Percentage of positive cases according to aquatic vegetable and Delar consumption

Vegetable consumption	Number (%) of studied and positive cases according to vegetable and Delar consumption							
	Anzali City		Anzali Villages		Anzali district (total)			
	n (%) studied	n (%) positive	n (%) studied	n (%) positive	n (%) studied	n (%) positive	n (%) positive in whole population	
Everyday	88 (45.4)	2 (2.3)	106 (54.6)	2 (1.9)	194 (100)	4 (2.1)	4 (0.20)	
At least once a Week	357 (31.1)	8 (2.2)	792 (68.9)	12 (1.5)	1149(100)	20 (1.7)	20 (1.00)	
At least once a month	223 (43.2)	3 (1.4)	293 (56.8)	1 (0.3)	516 (100)	4 (0.8)	4 (0.20)	
No consumption	33 (26.4)	0 (0)	92 (73.6)	2 (2.2)	125 (100)	2 (1.6)	2 (0.1)	
Total	701 (35.3)	13 (1.9)	1283 (64.7)	17 (1.3)	1984 (100)	30 (1.5)	30 (1.51)	
Delar consumption								
Yes	557 (33.3)	11 (2)	1118 (66.7)	15 (1.3)	1675 (100)	26 (1.6)	26 (1.3)	
No	144 (46.6)	2 (1.4)	165 (53.4)	2 (1.2)	309 (100)	4 (1.3)	4 (0.2)	
Total	701 (35.3)	13 (1.9)	1283 (64.7)	17 (1.3)	1984 (100)	30 (1.5)	30 (1.51)	

Overall, 1,675 subjects (84.4%) had a history of eating this local appetizer, of which 26 individuals (1.6%) were infected. On the other hand, 1.29% of the people who had no history of Delar consumption were also positive for fascioliasis, which was 0.2% of total population studied (Table 3). Statistical analysis showed no association between prevalence and Delar consumption.

#### **Occupation and education of study subjects**

The prevalence of infection was examined according to occupation (Table 4). Totally, 970 subjects

(48.9%) were housewives, 224 (11.3%) were farmers and 810 cases (39.8%) had other occupations. The highest proportions of infected subjects were housewives (0.86%), followed by farmers (0.3%). There was no association between prevalence and occupation. A high number of infected subjects (80%) were illiterate or had low educational level, the majority of which (77.5%) were living in rural areas (Table 5). Nevertheless, no association with education and infection was observed.

**Table 4:** Percentage of positive cases according to occupation

Occupation	Number (%) of studied and positive cases according to occupation						
	Anzali city		Anzali Villages		Anzali district (total)		
	n (%) studied	n (%) positive	n (%) studied	n (%) positive	n (%) studied	n (%) positive	n (%) positive in whole population
Housekeeper	303 (31.2)	8 (2.6)	667 (68.8)	9 (1.3)	970 (100)	17 (1.8)	17 (0.86)
Farmer	0 (0)	0 (0)	224 (100)	6 (2.7)	224 (100)	6 (2.7)	6 (0.30)
Employee	160 (82.1)	2 (1.3)	35 (17.9)	0 (0)	195 (100)	2 (1)	2 (0.1)
Student	115 (66.9)	0 (0)	57 (33.1)	0 (0)	172 (100)	0 (0)	0 (0)
Others	123 (29.1)	2 (1.6)	300 (70.9)	3 (1)	423 (100)	5 (1.2)	5 (0.25)
Total	701 (35.3)	13 (1.9)	1283 (64.7)	17 (1.3)	1984 (100)	30 (1.5)	30 (1.51)

**Table 5:** Percentage of positive cases according to educational statuses

Education level	Number (%) of studied and positive cases according to education level						
	Anzali City		Anzali Villages		Anzali district (total)		
	n (%) studied	n (%) positive	n (%) studied	n (%) positive	n (%) studied	n (%) positive	n (%) positive in whole population
Illiterate	61 (17.6)	1 (1.6)	285 (82.4)	6 (2.1)	346 (100)	7 (2)	7 (0.35)
Diploma>	261 (25.5)	8 (3.1)	763 (74.5)	10 (1.3)	1024 (100)	18 (1.8)	18 (0.9)
Diploma	234 (52.1)	2 (0.9)	215 (47.9)	1 (0.5)	449 (100)	2 (0.5)	3 (0.15)
Diploma<	145 (87.9)	2 (1.4)	20 (12.1)	0 (0)	165 (100)	2 (1.2)	2 (0.10)
Total	701 (35.3)	13 (1.9)	1283 (64.7)	17 (1.3)	1984 (100)	30 (1.5)	30 (1.51)

## Discussion

This is the first study to examine the prevalence of fascioliasis in a non-epidemic period in the Bandar-Anzali region of Northern Iran. Bandar-Anzali is located at the littoral of the Caspian Sea, 23 meters under sea level and includes Bandar-Anzali City and 32 surrounding villages, covering an area of 275 km<sup>2</sup>. The highest precipitation rates in Iran occur in the Bandar-Anzali region with mean annual rainfall of 1850 mm. It has a subtropical climate with relative humidity from 71 to 97%, the highest in Iran. The mean ambient temperature ranges from 13.2 to 19.2 °C. These environmental conditions are ideal for the successful completion of the *Fasciola* life cycle.

The prevalence of fascioliasis in this region was 1.36% and 0.45%, based upon serological and coprological examination, respectively (Table 2). Since, the prevalence of infection by coprological analysis is less than 1% (0.45%) and the mean egg count was 50.7 epg ( $\pm 30.36$ ), according to the WHO epidemiological classification for human fascioliasis, Iran can be classified as a hypoendemic region (11). We found that some of the older individuals were seropositive but egg negative. It is unlikely that these are resolved infection from previous epidemics as antibody titers decrease slowly and will be detectable for a few years but not for a decade which is the period of the last epidemic. The rate of seropositivity in similar studies performed in various endemic areas

worldwide is significantly higher than coprological rates, which supports the results of present study (32-35).

The disease prevalence observed in this study starkly compares with that obtained during the first outbreak of human fascioliasis in the Guilan Province, which were 50% and 36.5% using serological and coprological examinations, respectively (36). In spite of the low disease prevalence, due to its unique epidemiological characteristics a specific pattern of transmission has been proposed for the Caspian Sea areas, which is named "Caspian Pattern". This is defined as a hypoendemic area with large scale-epidemics sometimes affecting more than 10,000 people (37). Due to the potential for a reoccurrence of large-scale human outbreaks, the WHO considers Iran among six countries, which are known to have a serious problem with fascioliasis, despite the fact that similar to many other countries worldwide, Iran is a hypoendemic region (26).

Several societal factors are associated with human fascioliasis including gender (9, 38), age (9), dietary habits (24, 39), and occupation (34, 39). In hyperendemic areas such as Bolivia and Peru, females shed more eggs than males but prevalence does not differ between genders. While in mesoendemic regions of Egypt the prevalence of liver fluke infection in females is statistically higher than in males (7, 8). This is because females are involved in agricultural tasks mainly in rice fields, meal and salad preparation and washing activities. Studies performed during the first human outbreak in Guilan Province indicate a statistically significant difference between two genders, however, in present study conducted in a non-epidemic situation, no statistically significant differences between genders were observed (14, 36). A similar picture was also indicated in Mazandaran (40), Yasouj (41), and Kermanshah (42), provinces of Iran and in other hypoendemic countries such as Pakistan (43), Turkey (44), Mexico (45), and Bolivia (46, 47).

In this study, a higher number of individuals were infected in the 40-59 age range which is different to the outbreaks in Guilan and Kermanshah provinces where the highest numbers of infected

individuals were observed in the lower age range of 10-19 years in Kermanshah (42), and 10-29 years in Guilan (14, 36). The latter finding correlates with similar observations in other hyperendemic regions (7, 8, 46). The observation in this study may reflect the non-epidemic situation as older adults participate in agricultural or cooking activities that increase their risk of infection. Children on the other hand are at school and have lower contact with contaminated materials and environments. Interestingly, none of 172 (8.7%) students that participated in this study was infected with fascioliasis. On the other hand, during epidemic situations all age groups are at risk of infection through eating large scale contaminated freshwater plants.

Several species of wild aquatic and/or semi-aquatic plants are associated with human fascioliasis in Iran (12, 40, 41, 48). In the Guilan Province, *Mentha pulegium*, *Mentha piperita* and *Eryngium caucasicum* are the main species which have been implicated in transmission of human fascioliasis (24). The villagers collect these plants and sell them in the streets or in urban markets throughout the year. They are very popular and are eaten fresh or in local delicacies such as "Delar" and "Zeitoon-Parvarde". It was demonstrated that these traditional foods could transmit the disease if prepared with fresh aquatic plants presenting attached metacercaria (24, 49). Although, the higher infection rates in the present study were seen among those subjects who had consumed fresh local aquatic plants and Delar, the differences in prevalence by plant and Delar consumption were not statistically significant. This finding is in accordance with studies performed in Mexico and Turkey in which no statistical relationship between seropositivity and consumption of raw vegetables were observed (45, 50).

Fascioliasis is predominantly a rural disease with farmers and ranchers most at risk to infection. This is because they are in close contact with environmental factors such as animal reservoirs, lymnaeid snails, water collections and aquatic plants critical for the completion of the liver fluke life cycle and disease transmission (8, 51). However, in this study the highest numbers of human

cases were reported from urban areas. Although this difference was not statistically significant this is an interesting finding that may be due to the vicinity of rural areas to the cities in Guilan Province, sometimes less than 5 km. The short distance means that farmers will travel to town to sell aquatic plants contaminated with metacercariae in the streets throughout the year. Of the 970 housewives that participated, 17 were infected (prevalence 1.75%) while 6 of the 224 farmers that participated were infected (prevalence 2.68%). Many of Iran's neighboring countries such as Turkey, Pakistan, Iraq and Turkmenistan have also reported cases of human fascioliasis. The majority of published data concerns Turkey where sero-epidemiological studies in different regions indicate a prevalence of 0.79 to 6.1%, which is higher than the levels reported in this study (44, 50, 52, 53). Only a small number of studies are reported from Pakistan, principally a coprological study in the rural area of Lahore where 0.31% of the examined population was infected (41, 54). The situation of human fascioliasis in Iraq is also not clear however recent reports indicate increasing numbers of human cases mainly from Sulaimaniyah governorate of Kurdistan region in Northern Iraq (55- 58). Sulaimaniyah has common borders with the Iranian western provinces of Kermanshah where 34 cases of human disease have been documented from 1998 to 2008, which verifies the presence of similar epidemiological conditions in this adjacent area (42).

## Conclusion

According to the results obtained in present study and using the WHO epidemiological classification, human fascioliasis is "hypoendemic" in the Guilan Province of Iran (11). Taking into consideration the cultural and epidemiological characteristics of the region and WHO guidelines, the following primary health care measures are proposed: (i) a passive case finding approach, which contains treatment of infected people who are tested positive by coprological or serological analysis (ii) establishment of effective veterinary public health

measures, including regular treatment of livestock with Fasinex® to reduce exposure of humans to metacecariae (iii) education programmes run by local health centers to inform individuals about the disease and the necessary steps to inhibit transmission (particularly highlighting the danger of eating raw, uncooked aquatic and semi-aquatic plants). Where possible broadcast by mass media to decrease the risk of human infections, especially in high-risk seasons would reduce the risk of infection as demonstrated during previous outbreaks in the region and finally (iv) encourage families to grow fresh water plants in areas that are fenced off from livestock.

## 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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