کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله
The Endemicity of Human Fascioliasis in Guilan Province, Northern Iran: the Baseline for Implementation of Control Strategies

Keyhan ASHRAFI¹, *Farshid SAADAT¹, Sandra O’NEILL ², Behnaz RAHMATI¹, Hadid AMIN TAHMASBI³, John PIUS DALTON⁴, Abolhassan NADIM⁵, Mohsen ASADINEZHAD⁶, Sayed Mahmood REZVANI⁷

¹. Dept. of Microbiology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
². School of Biotechnology, Faculty of Science and Health, Dublin City University, Glasnevin, Dublin, Ireland
³. Dept. of Microbiology, Faculty of Science, Azad University of Lahijan, Iran
⁴. School of Biological Sciences, Queen’s University Belfast, Medical Biology Centre, Belfast, Northern Ireland
⁵. Dept. of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences. Iran.
⁶. Dept. of Biochemistry and Biophysics, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.
⁷. Dept. of Communicable Diseases Control, Guilan province Health Center, Rasht, Iran

*Corresponding Author: Email: fsaadat@razi.tums.ac.ir

(Received 24 Sep 2014; accepted 16 Jan 2015)

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 (±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 hyperendemic 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 baseline 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, 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 baseline 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 circumstances. 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 μl of recombinant *F. hepatica* cathepsin L (FhCL1) antigen (1μg/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 μl of 2% bovine serum albumin diluted in PBS/0.1% Tween 20. After a further washing step, 100 μl of sera samples (diluted at 1:100) were added and the plate incubated for 1 h at 37 °C. Following another wash step, 100 μl 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 μl of TMB. The color was developed for 10 min and the reaction was stopped with 50 μl 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±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 ($\chi^2=0.51, P=0.48$).

| Number (%) of studied and positive cases by different laboratory tests | Anzali city | | | | Anzali Villages | | | | Anzali district (total) | n (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex | n (%) | | | | n (%) | | | | n (%) | | | | | | |
| Female | 457 (36.4) | 9 (1.97) | 3 (0.66) | 798 (63.6) | 8 (1) | 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 (±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. dispers* (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 seropositive 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 | Anzali City stool exam. n (%) | Anzali Villages stool exam. n (%) | Anzali district (total) stool exam. n (%) |
|------------|-----------------------------|----------------------------------|----------------------------------------|
| 10-19      | 120 (57.4)                  | 89 (42.6)                        | 209 (100)                              |
| 20-29      | 119 (37.2)                  | 210 (62.8)                       | 320 (100)                              |
| 30-39      | 118 (24.4)                  | 365 (75.6)                       | 483 (100)                              |
| 40-49      | 132 (30.6)                  | 300 (69.4)                       | 432 (100)                              |
| 50-59      | 137 (43)                    | 182 (57)                         | 319 (100)                              |
| 60-69      | 55 (34.6)                   | 104 (65.4)                       | 159 (100)                              |
| 70-79      | 20 (32.3)                   | 42 (67.7)                        | 62 (100)                               |
| Total      | 701 (35.3)                  | 1283 (64.7)                      | 1984 (100)                             |

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

| Vegetable consumption | Anzali City (n (%) positive) | Anzali Villages (n (%) positive) | Anzali district (total) (n (%) positive) |
|-----------------------|-----------------------------|----------------------------------|----------------------------------------|
| Everyday              | 2 (2.3)                     | 2 (1.9)                          | 4 (2.1)                                |
| At least once a Week  | 8 (2.2)                     | 12 (1.5)                         | 20 (1.7)                               |
| At least once a month | 3 (1.4)                     | 4 (0.8)                          | 4 (0.2)                                |
| No consumption        | 0 (0)                       | 2 (2.2)                          | 4 (0.2)                                |
| Total                 | 13 (1.9)                    | 17 (1.3)                         | 30 (1.5)                               |

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 (%) positive | n (%) studied | n (%) positive | n (%) positive |
| 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 statues

| 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 |
| 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 km². 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 (±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 hyperendemic 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 x pulegium, Mentha piperita and Eryngium canescens 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 metacercariae (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.

Acknowledgement

The research project conducted in rural areas of Bandar-Anzali was supported by funding from Guilan University of Medical Sciences, contract No. P/3/132/4332 and Tehran University of Medical Sciences, contract No. 130/6/4998. The study performed in urban area of Bandar-Anzali was MSc thesis, No. 20230507872025, funded by Azad University of Lahijan in Guilan Province. The Kato-Katz kits used in this study were provided by WHO headquarter in Geneva. Thanks to Dr. Antonio Montresor, public health specialist and Henrietta Allen, technical officer of World Health Organization for providing the kits. The authors would also like to acknowledge the collaboration received from Health Center of Guilan Province and Bandar-Anzali Health Center. Special thanks are given to Dr. Arsalan Dadashi, Mr. Alireza Rasaei and Mr. Mohammad Reza Hadhani for their valuable assistance in performing this study. The authors declare that there is no conflict of interests.
References

1. Spithill TW, Smooker PM, Copeman DB (1999). Fasciola gigantica: epidemiology, control, immunology and molecular biology. In: Fasciolosis. Ed. Dalton JP. CABI Pub. Wallingford, Oxon, UK, pp. 465–525.

2. World Health Organization (2010). Working to overcome the global impact of neglected tropical diseases: first WHO report on neglected tropical diseases, 172 p.

3. Chen MG, Mott KE (1990). Progress in assessment of morbidity due to Fasciola hepatica infection: a review of recent literature. Trp Dis Bull, 87 (4): R1-R38.

4. Rim HJ, Farag HF, Sommarni S, Cross JH (1994). Food-borne trematodes: ignored or emerging? Parasitol Today, 10: 207-209.

5. World Health Organization (1995). Control of food-borne trematode infections. WHO Tech Rep Ser, 849:1-157.

6. Hopkins DR (1992). Homing in on helminthes. Am J Trop Med Hyg, 46: 626-634.

7. Esteban JG, Flores A, Angles R and Mas-Coma S (1999). High endemicity of human fascioliasis between Lake Titicaca and La Paz valley, Bolivia. Trans R Soc Trop Med Hyg, 93 (2): 151–156.

8. Esteban JG, Gonzalez C, Curtale F, Muñoz-Antoli C, Valero MA, Bargues MD, el-Sayed M, el-Wakeel AA, Abdel-Wahab Y, Montresor A, Engels D, Savioli L, Mas-Coma S (2003). Hyperendemic fascioliasis associated with schistosomiasis in villages in the Nile Delta of Egypt. Am J Trop Med Hyg, 69 (4): 429-437.

9. Curtale F, Hassanein YA, El Wakeel A, Mas-Coma S, Montresor A (2003). Distribution of human fascioliasis by age and gender among rural population in the Nile Delta. Egypt J Trop Pediatr, 49 (5): 264-268.

10. Farag HF (1998). Human fascioliasis in some countries of the Eastern Mediterranean Region. Eastern Mediterranean Health J, 4 (1): 156-160.

11. Mas-Coma S, Esteban JG and Bargues MD (1999). Epidemiology of human fascioliasis: a review and proposed new classification. Bull WHO, 77 (4): 340–346.

12. Ashrafi K, Massoud J, Holakuei-Naieni K, Mahmoodi M, Joafshani MA, Valero MA, Fuentes MV, Khoubbane M, Artigas P, Bargues MD, Mas-Coma S (2004). Evidence suggesting that Fasciola gigantica may be the most prevalent causal agent of fascioliasis in the endemic province of Gilan, northern Iran. Iran J Public Health, 33 (4): 31-37.

13. Massoud J (1998). Fascioliasis outbreak in man and drug test (Triclabendazole) in Caspian Sea littoral, Northern part of Iran. Bull Soc Fr Parasitol, 8 (suppl. 1): 438-439.

14. Asmar M, Milaninia A, Amir-Khani A, Yadeegari D, Forghan-Parast K, Nahvarian H, Piazak N, Esmaeili A, Hovanesian A, and Valadkhani Z. (1991). Seroepidemiological investigation of fascioliasis in Northern Iran. Med J Islamic Repub Iran, 5 (1(2): 23-27.

15. Mas-Coma S (2004). Human fascioliasis: Epidemiological patterns in human endemic areas of South America, Africa and Asia. Southeast Asian J Trop Med Publ Health, 35 (S1): 1-11.

16. Rowan SE, Levi ME, Youngworth J, Brauer B, Everson GT, Johnson SC (2012). The variable presentations and broadening geographic distribution of hepatic fascioliasis. Clin Gastroenterol Hepatol, 10 (6): 598-602.

17. Arauco R, Zetola NM, Calderon F, Seas C (2007). Human fascioliasis: a case of hyperinfection and an update for clinicians. Foodborne Pathog Dis, 4 (3): 305-12.

18. Hosseini SH, Jilochani M, Bahonar AR and Elsarni A (2010). Cattle fascioliasis in Guilan province, Iran. Int J Vet Res, 4 (1): 57-60.

19. Elsarni A, Hosseini SH, Meshgi B (2009). Animal fascioliasis in north of Iran. Iran J Public Health, 38 (4): 132-135.

20. Khosravi A and Babaahmady E (2012). Epidemiology of Fasciola hepatica in Iran. Int J Biol, 4 (4): 86-90.

21. Oryan A, Mansourian M, Moazeni M, Nikahval B and Barbard S (2011). Liver distomatosis in cattle, sheep and goats of northeastern Iran. Glob Vet, 6 (3): 241-246.

22. Sahba GH, Arfzaa F, Farahmandian I and Halali H (1972). Animal fascioliasis in Khuzestan, southwestern Iran. J Parasitol, 58 (4): 712-716.

23. Tavakoli HR, Mahmoodzadeh A, Hajia M (2008). A five years study of fascioliasis and dicrocoeliosis in Iran’s slaughterhouses. Asian Pac J Trop Med, 1 (4): 9-13.

24. Ashrafi K, Valero MA, Massoud J, Sobhani A, Solaymani-Mohammadi S, Conde P, Khoubbane M, Bargues MD, Mas-Coma S
(2006). Plant-borne human contamination by fascioliasis. Am J Trop Med Hyg, 75 (2): 295-302.
25. Ashrafi K, Valero MA, Panova M, Periago MV, Massoud J, Mas-Coma S (2006). Phenotypic analysis of adults of Fasciola hepatica, F. gigantica and intermediate forms from the endemic region of Gilan, Iran. Parasitol Int, 55 (4): 249-260.
26. World Health Organization (2007). The "neglected" neglected worms. Action Against Worms, 10: 1-8.
27. Farid-Moayer H (1971). Human infection with Fasciola hepatica and Dicrocoelium dendriticum in Isfahan area, central Iran. J Parasitol, 2: 160.
28. Hanjani AA, Nikakhtar B, Arfaa F, Khakpour M, Rashed MA (1971). A case of infection with Fasciola hepatica with allergic manifestations. Acta Medica Iranica, 14: 149-151.
29. Dowlati Y, Dowlati A and Seyyedi BV (1987). Cutaneous fascioliasis. Med J Islamic Repub Iran, 1: 62-65.
30. O'Neill SM, Parkinson M, Strauss W, Angles R, Dalton JP (1998). Immunodiagnosis of Fasciola hepatica infection (fascioliasis) in a human population in the Bolivian Altiplano using purified cathepsin L cysteine proteinase. Am J Trop Med Hyg, 58 (4): 417-23.
31. Gonzales Santana B, Dalton JP, Vasquez Camargo F, Parkinson M, Ndao M (2013). Diagnosis of human fascioliasis by enzyme-linked immunosorbent assay (ELISA) using recombinant cathepsin L protease. PLoS Negl Trop Dis, 7 (9): e2414.
32. Hassan MM, Moustafa NE, Mahmoud LA, Abbaza BE, Hegab MH (1995). Prevalence of Fasciola infection among school children in Sharkia Governorate, Egypt. J Egypt Soc Parasitol, 25 (2): 543-9.
33. Espinoza JR, Maco V, Marcos I, Saez S, Neyra V, Terashima A, Samalvides F, Gotuzzo E, Chavarry E, Huaman MC, Bargues MD, Valero MA, Mas-Coma S (2007). Evaluation of Fas2-ELISA for the serological detection of Fasciola hepatica infection in humans. Am J Trop Med Hyg, 76 (5): 977-82.
34. Safar E, Mikhaiel F, Bassiouini G, El-Bassiouni S, El-Kholy H (2005). Human fascioliasis in some areas in Cairo and Giza Governorates, Egypt. J Egypt Soc Parasitol, 35 (1): 181-92.
35. Marcos I, Maco V, Terashima A, Samalvides F, Espinoza JR, Gotuzzo E (2005). Fascioliasis in relatives of patients with Fasciola hepatica infection in Peru. Rev Inst Med Trop Sao Paulo, 47 (4): 219-22.
36. Forghani-Parast, Yadegari D, Asmar M (1993). Clinical epidemiology of human fascioliasis in Guilan. J Guilan Univ Med Sci, 2 (6-7): 4-11. [In Persian].
37. Mas-Coma S (2007). Parasitic diseases, global change and the developing world: the example of emerging fascioliasis. Scientia Parasiologica, 1: 10-21.
38. Marcos I, Maco I, Samalvides F, Terashima A, Espinoza JR, Gotuzzo E (2006). Risk factors for Fasciola hepatica infection in children: a case-control study. Trans R Soc Trop Med Hyg, 100 (2): 158-160.
39. Mas-Coma S, Bargues MD, Valero MA (2005). Fascioliasis and other plant-borne trematode zoonoses. Int J Parasitol, 35 (11-12): 1255-1278.
40. Moghaddam A, Massoud J, Mahmoodi M, Mahvi AH, Periago MV, Artigas P, Fuentes MV, Bargues MD and Mas-Coma S (2004). Human and animal fascioliasis in Mazandaran province, northern Iran. Parasitol Res, 94 (1): 61-69.
41. Sarkari B, Ghabakhloo N, Moshfeka AA, Eilami O (2012). Seroprevalence of human fascioliasis in a new-emerging focus of fascioliasis in Yasuj district, southwest of Iran. Iran J Parasitol, 7 (2): 15-20.
42. Hatami H, Asmar M, Massoud J, Mansouri F, Namdaratabar H, Ramazankhani A (2012). The first epidemic and new emerging human fascioliasis in Kermanshah, western Iran, and a ten-year follow up, 1998-2008. Int J Prev Med, 3 (4): 266-72.
43. Qureshi AW, Tanveer A, Qureshi SW, Maqbool A, Gill TJ and Ali SA (2005). Epidemiology of human fascioliasis in rural areas of Lahore, Pakistan. Punjab Univ J Zool, 20 (2): 159-168.
44. Kaplan M, Kuk S, Kalkan A, Demirdag K, Ozdarendeli A (2002). Fasciola hepatica seroprevalence in the Elazig region. Mikrobiyol Bul, 36 (3-4): 337-42. [In Turkish].
45. Martinez-Barbabosa I, Gutierrez-Quiroz M, Romero-Cabello R et al. (2006). Seroenepidemiology of fascioliasis in school children in Mexico City. Rev Biomed, 17: 251-257.
46. Strauss W, Angles R, Esteban JG & Mas-Coma S (1997). Human fascioliasis in Bolivia: serologi-
51. Ozturhan H, Emekdağ G, Sezgın O, Korkmaz M, Altintas E (2009). Seroepidemiology of Fasciola hepatica in Mersin province and surrounding towns and the role of family history of the fascioliasis in the transmission of the parasite. *Turk J Gastroenterol*, 20 (3): 198-203.
52. Kaya S, Demirci M, Demirel R, Arıdogan BC, Ozturk M, Korkmaz M (2006). Seroprevalence of fascioliasis and the difference of fascioliasis between rural area and city center in Isparta, Turkey. *Sandi Med J*, 27 (8): 1152-6.
53. Ali Shah SA, Yasin Khan M, Ahmad J (2010). Fascioliasis- a cause of obstructive Jaundice: a case report. *J Postgrad Med Inst*, 24 (4): 332-335.
54. Hawramy TA, Saeed KA, Qaradaghy SH, Karboli TA, Nore BF, Bayati NH (2012). Sporadic incidence of fascioliasis detected during hepatobiliary procedures: a study of 18 patients from Sulaimaniyah governorate. *BMC Res Notes*, 5: 691.
55. Hassan HA, Majid RA, Rashid NG et al. (2013). Eosinophilic granulomatous gastrointestinal and hepatic abscesses attributable to basidio-bolomycesis and fascioliasis: a simultaneous emergence in Iraqi Kurdistan. *BMC Infect Dis*, 13: 91.
56. Karabuli TA, Shaikhani MA, Karadaghi SH, Kasnazan KH (2009). Education and imaging: Hepatobiliary and pancreatic: fascioliasis. *J Gastroenterol Hepatol*, 24 (7): 1309.
57. Ezzat RF, Karboli TA, Kasnazani KA, Hamawandi AM (2010). Endoscopic management of biliary fascioliasis: a case report. *J Med Case Rep*, 4: 83.
کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله