Seroprevalence of fascioliasis in the eastern region of Turkey: an eight-year investigation

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ABSTRACT

Background/Aims: Fascioliasis is a zoonotic disease and one of the most neglected infectious diseases in humans. Its prevalence has been increasing significantly during the last decades. This study aimed to investigate the prevalence of fascioliasis using direct microscopy and indirect hemagglutination (IHA) technique in a region in Eastern Anatolia of Turkey.

Material and Methods: This study was conducted on the serum samples obtained from 817 patients (372 male and 445 female) between 2011 and 2018, who were suspected to have fascioliasis. IHA was used to investigate anti-*Fasciola hepatica* antibodies in the serum samples. Stool specimens were obtained from the seropositive patients and were examined with the native-Lugol method to identify the parasites.

Results: It was determined that 5.5% (45/817) of all the patients were *F. hepatica* seropositive and 6.4% (52/817) were borderline positive. Positivity was 5.7% (21/372) among males and 5.4% (24/445) among females, and the difference in the infection rates between these groups was not significant ($p=0.913$). The highest number of patients who applied to the clinic was in the "45 and over" age group (317 patients); 270 patients were in the 25-44 age group. A maximum positivity of 10.3% was observed in the 7-14 age group.

Conclusion: Previously, fascioliasis was considered a rare infection in humans; however, it has emerged as an important public health problem in the world. Considering fascioliasis in patients with clinical symptoms, not only with direct observation but also using serological methods, would be effective in early diagnosis and treatment of the disease.

Keywords: *Fasciola hepatica*, seroprevalence, IHA, Turkey

INTRODUCTION

Fascioliasis is a zoonotic parasitic infection caused by the genus *Fasciola* of the liver fluke species. It is a common and serious disease, especially in the domestic ruminants because of the economic losses it causes. Fascioliasis is one of the most neglected infectious diseases in humans. The prevalence of human fascioliasis has been increasing significantly during the last decades; thus, the infection is regarded as a major public health problem today (1).

Fascioliasis is prevalent throughout the world, and cases have been reported in all the 5 continents (2, 3). The disease is endemic in several geographical regions, mainly in Asia and Africa, ranging from low to high prevalence and intensity. Moreover, human cases have emerged in several new regions, and increasing number of cases are being reported in Europe (1). It was stated that up to 2 to 17 million people are infected with *Fasciola hepatica* worldwide (4). In certain regions, the infection prevalence is as high as 90% (5).

Humans are infected through the ingestion of contaminated aquatic plants or drinking water contaminated

with metacercaria, an infective form of the parasite (1). Metacercaria exists in the duodenum and particularly settles in the liver and biliary tract with migrations; however, very rarely, it may also be found in other parts of the body (6).

Symptoms of fascioliasis may appear a few days after the infection, and the clinical course varies according to the number of metacercaria present. Although an infected individual could be asymptomatic, the usual symptoms include fever, abdominal pain, anorexia, weight loss, urticaria, hepatomegaly, anemia, and jaundice (7). Furthermore, metacercaria absorbs vitamin B12, leading to vitamin deficiency.

The definitive diagnosis of human fascioliasis is based on microscopic identification of *F. hepatica* eggs in the stool (1). However, this method is not very effective since the parasite passes the eggs into feces several months after the infection, and egg shedding occurs at irregular intervals. Moreover, the parasite eggs could be observed in rare ectopic fascioliasis cases; hence, carpological techniques with sensitivity limitations might provide false negative

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Table 1. Age distribution of patient groups.

	0-6	7-14	15-24	25-44	45 and above	Total
Positive	2 (3%)	4 (10.3%)	6 (4.8%)	21 (7.8%)	12 (3.8%)	45 (5.5%)
Negative	58	35	108	231	288	720
Borderline	7	0	10	18	17	52
Total	67	39	124	270	317	817

χ^2 , 12.558; p, 0.1279

Figure 1. Antibody test results according to gender.
 χ^2 , 0.0118; p: 0.9133

results, as acute and erratic infections could pass undetected (3). To overcome these diagnostic problems, there is a need for a reliable tool for detection. Serological tests, i.e., detection of antibody responses against the parasite antigens are useful, sensitive, and specific for the diagnosis of fascioliasis (8).

In this study, we investigated the prevalence of fascioliasis with microscopy and indirect hemagglutination (IHA) technique in the Van province of Turkey.

MATERIALS AND METHODS

This study was conducted on the serum samples obtained from 817 patients (372 male and 445 female) who presented with gastrointestinal complaints and were referred to the Faculty of Medicine, Parasitology Laboratory by various outpatient clinics with a suspicion of fascioliasis between January 2011 and September 2018.

Ethics Approval was obtained from the University Non-invasive Ethics Committee prior to conducting the study (2019/04-04).

Approximately 5 mL venous blood was obtained from the patients, and the serum samples were separated by the centrifuge at 3,000 rpm in the laboratory and stored at -20°C until the tests. Furthermore, the stool specimens were obtained from the seropositive patients, and all the stool samples were examined with the native-Lugol method to identify the parasite eggs.

IHA method was used to investigate anti-*F. hepatica* antibodies in the serum samples. The commercial kit for the IHA method (Fumouze Laboratories, France) was used according to the test procedures with a 1/160 titer cut-off value, and 1/320 and higher dilutions were considered positive.

Statistical Analysis

Chi-squared test was conducted for statistical analyses using the Statistical Packages for the Social Sciences (SPSS) for Windows V.11.0 (SPSS Inc., Chicago, IL, USA) software and $p \leq 0.05$ was considered significant. The patients were divided into groups according to their ages (0-6, 7-14, 15-24, 25-44, and 45 years and over) for analyses.

RESULTS

It was determined that 5.5% (45/817) of all the patients were *F. hepatica* seropositive, and 6.4% (52/817) patients were borderline-positive. Positivity was 5.7% (21/372) among males and 5.4% (24/445) among females, and the difference in the infection rates between these groups was not significant ($p=0.913$) (Figure 1). The highest number of patients who presented to the clinics was in the "45 and over" (317 patients) and 25-44 (270 patients) age group, and a maximum positivity of 10.3% was observed in the 7-14 age group (Table 1).

Table 2. Intestinal parasites detected in *Fasciola* seropositive patients.

Parasites	Number
<i>Blastocystis hominis</i>	25
<i>Giardia intestinalis</i>	11
<i>Entamoeba coli</i>	3
<i>Hymenolepis nana</i>	1
<i>Taenia saginata</i>	1
<i>Trichuris trichiura</i>	1
<i>Enterobius vermicularis</i>	1

Figure 2. *Fasciola* seropositivity by age group. **Figure 3.** Number of positive results between 2011 and 2018 χ^2 ; 227.442, p: 0.001, *continue.

The annual number of patient applications varied. The number of applications during the last 2 years was quite high, and in the first 9 months of 2018, this figure reached the highest level with 183 patients. It was thought that

perhaps the lack of awareness about the parasite or the change of physicians in 2012–2013 led to only 10 patients being examined. Whereas the highest infection rate was observed in 2016 (10.4%), the lowest positivity was observed in 2018 with 1.7% in contrast to the number of applications (Figure 2).

Furthermore, *Blastocystis hominis* was found in 25 patients with *F. hepatica* antibodies in their serum samples, while *Giardia intestinalis* was found in 11 and *Entamoeba coli* in 3 patients. *Hymenolepis nana*, *Taenia saginata*, *Trichuris trichiura*, and *Enterobius vermicularis* were found in only 1 patient each. *F. hepatica* eggs were not detected in the stool samples of the examined patients (Table 2).

DISCUSSION

Fascioliasis is a significant disease, especially in the domestic ruminants because of the economic losses it causes. However, it was one of the most neglected infectious diseases in humans until the last decade. It is prevalent throughout the world, especially in Bolivia, Ecuador, Peru, Iran, and Egypt with the status of the infection being unknown in several countries (1). Today, it has become a serious public health threat owing to the increasing number of clinical cases and outbreaks in several parts of the world (2). Furthermore, histopathological lesions in the acute and chronic stages of the disease have led to understanding the pathology of the parasite, thereby determining the appropriate diagnostic methods.

Among the factors that affect the transmission of fascioliasis, ecological factors such as climate, temperature, humidity, chemical structure of soil, aqueous flora, presence of adequate water sources, as well as the presence of living organisms, such as reservoir parasites, distribution of intermediate parasites, nutrition habits, and socio-economic level are significant (5, 6).

The routine diagnosis of *F. hepatica* infection is generally based on the detection of parasite eggs in the stool samples. However, the success of this technique is not satisfactory because of the fact that the parasite lays a small number of eggs with no eggs being found during the acute period of the infection (7). The parasite could be detected with invasive techniques, such as obtaining the duodenal fluid, duodenal aspirates, surgery, histological examinations, biopsies, and noninvasive diagnostic techniques that include radiology, ultrasound, tomography, and magnetic resonance imaging (2, 7).

In recent years, immunological techniques have become more sensitive and specific and are preferred compared to the conventional microscopic examination of the stool specimens (9). A number of immunoassays that identify the antibodies against fluke in serum and antigens in stool are available. These include direct immunofluorescence assays, indirect hemagglutination (IHA), enzyme-linked immunosorbent assays (ELISA), and western blot (3). The clinical symptoms might be apparent for several weeks before the eggs are passed into the stool. Acute infection could lead to fever, hepatomegaly, abdominal pain, weight loss, anemia, eosinophilia, obstructive jaundice, and cholangitis and cirrhosis in chronic infections (10). Thus, serologic tests have advantages, such as early detection and detection of extrabiliary cases; however, they are unable to discriminate between the current and past infections. In this study, IHA method was preferred because it allows the detection of antibody titers that provide information about the severity of the infection. Thus, it is a useful and fast method, which significantly contributes to the follow-up of treatment.

Human fascioliasis is a public health problem in several countries in the America, Europe, Africa, and Asia and is reported in numerous other countries; however, it is infrequent in Oceania. It was estimated that 2.4 to 17 million individuals are infected worldwide (1, 11).

Fascioliasis was reported with different prevalence values in different countries; 0.7% (41 cases per 5861 patients studied) in Chile (12), 3.2% in Portugal (13), 7.3% in Egypt (14), and 8.7% in Peru (15). Human endemic areas could be extended as in Peru with a high prevalence of 34.2% in the Mantaro valley (16). The highest human prevalence was reported in the Bolivian Altiplano, up to 53%, determined using immunological methods (17).

Fascioliasis is a re-emerging disease and common in certain provinces in Turkey. In previous ELISA studies on the seroprevalence of fascioliasis, it was determined that the prevalence was 2.4% in Isparta provincial center, 9.3% in a village in Isparta province (18), 0.55% in patients with no family history of fascioliasis and 1.93% in patients with a family history of fascioliasis in Mersin province, 0.79% among the participants (19), and 2.78% in 540 randomly selected healthy individuals in Elazığ province in eastern Turkey (20). In a recent study conducted in the Van province (Eastern Anatolia Region in Turkey), it was reported that 24 of 92 (26.09%) individuals were positive for fascioliasis with 1 familial outbreak (21).

In the seroprevalence study conducted by Turhan et al. (22) in Antalya province in Turkey, 3% of the participants were fascioliasis seropositive. The number of serological studies on human fascioliasis has increased in the 2000s. Serologically, it was reported that the disease prevalence was 3.01% in Antalya province and between 0.9 and 6.1% in Isparta province, which are located in the Mediterranean region in Turkey (23). ELISA detected fascioliasis in 89 (5.6%) of 1,600 randomly selected individuals (24). It was observed that 30 of the studied 291 (10.3%) individuals were seropositive in 1/160 dilution, and 11 individuals (3.7%) were determined as seropositive in 1/320 dilution (25).

In the study by Bacq et al. (26), it was reported that the authors could not observe the eggs despite repeated stool inspections in a group of patients; however, between 1/160 and 1/20480 dilutions, seropositivity was determined with the IHA method, and they reported that eosinophilia and IHA tests were adequate to determine the efficacy of treatment. Pulpeiro et al. (27) reported that 12 patients without eggs in their stool were determined as positive with the IHA method. In this study, 1/320 and above dilutions were evaluated as positive. On the basis of these criteria, it was determined that seropositivity was 6.5%, and 16.6% of the cases were determined as borderline positive. These results reflect epidemiological data, which demonstrated that human fascioliasis has a major regional significance in Turkey. In contrast, *Fasciola* eggs were not detected in patient stool samples with the native-Lugol examination. This could be because of the fact that the parasite eggs are heavy and large; thus, more accurate results could be obtained with the formol-ether concentration technique.

The correlation between the prevalence rates of fascioliasis and age varies according to the human endemic and non-endemic areas. In endemic areas, children under 15 years usually exhibit the highest prevalence rates (28), in contrast to the current status in the non-endemic areas (1, 11). It was found that the infection was most frequent in the 10-14 age group, and the prevalence was 8% in 15-year-old children and 15% in 5-14 years old (29). Tas Cengiz et al. (24) reported that the differences of fascioliasis incidence between males and females and between the age groups were statistically significant (24). In our study, the number of females with fascioliasis was higher than the males, which was most probably because of the higher watercress consumption rates among females compared to male individuals. It was also determined in this study that all the patients with fascioliasis were chil-

dren. It was considered that adults were immune to *F. hepatica* since most could have been infected with this parasite earlier.

In conclusion, fascioliasis is a parasitic disease, which significantly impairs the welfare of patients with serious pathological effects; thus, it is a significant health hazard in Turkey. Considering the patients with fascioliasis with clinical symptoms, the diagnosis should be conducted not only with direct observation but also using serological methods, which would be effective in early diagnosis and follow-up treatment. Fascioliasis was previously considered a rare infection among humans; however, it has emerged as an important public health problem with the development of new diagnostic methods, increased knowledge on pathogenesis, and high incidence of the disease globally and in Turkey. Therefore, effective epidemiological prevention methods and necessary education programs should be conducted, particularly in the endemic areas.

Ethics Committee Approval: Ethics Committee Approval has received for this study from the Ethics Committee of Van Yuzuncu Yil University, School of Medicine Non-invasive Ethics Committee.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

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