DOI: 10.7860/JCDR/2017/26066.10086

Review Article

Internal Medicine Section

# Immunodiagnosis of Human Fascioliasis: An Update of Concepts and Performances of the Serological Assays

BAHADOR SARKARI[1], SAMANEH ABDOLAHI KHABISI[2]


## ABSTRACT

Human Fascioliasis (HF) is a foodborne neglected parasitic disease caused by *Fasciola hepatica* and *Fasciola gigantica.* New epidemiological data suggest that the endemic areas of the disease are expanding and HF is being reported from areas where it was previously not observed. Diagnosis of HF is challenging. Performances of parasitological approaches, based on the detection of parasite's egg in the stool, are not satisfactory. Currently serological methods for the diagnosis of HF are mainly based on detection of anti-*Fasciola* antibodies in serum. Although, there have been some improvement in the development of immunological diagnostic tests for the diagnosis of HF, yet these tests suffer from insufficiency in sensitivity or/and specificity.

Detection of antigens, rather than antibodies, seems to be a suitable approach in the diagnosis of HF. Antigen can be detected in sera or stool of the fascioliasis patients. Circulating antigen in serum disappears within a short time and most of the circulating antigens are in immune complex forms which are not freely available to be detected. Therefore, antigenemia might not be an appropriate method for the diagnosis of HF. Detection of antigen in stool (coproantigens) seems to be a suitable alternative method for the diagnosis of HF. Recent data provided convincing evidence that detection of coproantigen improved and simplified the diagnosis of HF.

The present review highlights the new achievements in designing and improvement of diagnostic approaches for the immunodiagnosis of HF. Moreover, current status of the available immunodiagnostic techniques for the diagnosis of HF, their strengths and weaknesses has been discussed.


**Keywords:** *Fasciola hepatica, Fasciola gigantica*, Parasitic disease

## SEARCH CRITERIA AND DATA EXTRACTION

All the related published literature cited within PubMed, ISI web of science, Google Scholar, and Scopus were searched. The search terms were "fasciolosis", "fascioliasis","human fascioliasis", "human fasciolosis", subsequently combined with the search terms "diagnosis", "serodiagnosis", "parasitological diagnosis", "coproantigen", or "antibody detection". A few studies were also found by back tracing of the reference lists of the articles. Data were extracted from those papers which fulfilled our eligibility criteria.

## INTRODUCTION

HF is a food borne neglected parasitic disease caused by *Fasciola hepatica* and *Fasciola gigantica* [1]. Approximately 2.5-17 million people are infected with HF and an increase of HF cases has been reported from many countries [1,2]. The highest prevalence of HF has been reported from highlands of South American (Bolivia, Peru), Nile Delta in Egypt, China, Spain, Vietnam and also Iran [2-5].

Performances of parasitological diagnostic approaches, based on the detection of parasite's egg in the stool sample, are not satisfactory. This is mainly because of the absence of egg in stool sample, which may be due to the inability of *Fasciola* to produce eggs, due to its lack of adaptation to the human host, or encapsulation of eggs in liver granuloma or abscesses and low egg shedding due to low infection burden or old infection [6]. Besides, termination of egg shedding in the advanced chronic phase of fascioliasis is not uncommon. Furthermore, humans are not a suitable host for *Fasciola*; therefore the fluke may not attain maturity in human subjects. This may result in human biliary fascioliasis cases without egg shedding [6]. In fascioliasis cases with scanty eggs shedding; more specific methods such as Kat-Katz is needed to detect the eggs in the stool samples.

Juvenile fluke may sometimes deviate while migrating from the intestine to the liver and enter different other organs such as subcutaneous tissues, peritoneum or infrequently in brain and lung [7]. It is obvious that parasitological diagnosis is not helpful in such ectopic cases.

In France, in a study of 711 fascioliasis cases, eggs were found in only 27.6% of cases [8]. In a study done by De N et al., in Vietnam, only 16.9% of *Fasciola gigantica* infected cases were egg positive [9]. In an outbreak of HF in Kermanshah in the western part of Iran, none of serologically proven fascioliasis cases were egg positive [10]. In a study in Mexico, *Fasciola* eggs were detected in only 14 out of 50 fascioliasis cases [11]. In a series of 23 cases of hepatic fascioliasis in Egypt, confirmed by serological test (HAT) along with imaging and clinical presentation, only two cases were egg positive [12].

Immunodiagnosis, based on antigen or antibody detection, are the appropriate approaches for the diagnosis of HF. This is because in HF, incubation period usually ranges from a few days to 2-3 months, while the prepatent period is 3-4 months or even longer. Therefore, the patient usually present clinical signs or symptoms long before than the egg appears in the stool. However, antibodies to *Fasciola* antigens can be detected in patient's sera two weeks after infection, showing that serological tests are the appropriate techniques for the diagnosis of infection. Furthermore, antigen can be detected in sera or stool of patients about eight weeks after the infection, again long (around two months) before the beginning of egg shedding [6].

Currently available serological methods for the diagnosis of HF are mainly based on the detection of anti-*Fasciola* antibodies in serum. Antibody detection tests are not suitable for post treatment follow up of patients, since antibodies may persist for at least 4-5 months, or may be some years, after successful treatment. In one study, anti-*Fasciola* antibodies became negative after two months of treatment in 40% of successfully treated patients [13]. In another study, IgG

ELISA became negative in more than 80% of cases in first month and in 95% of cases after four months [14].

Detection of antigen, rather than antibodies, seems to be a suitable alternative approach in the diagnosis of a few of parasitic diseases including HF [15-18]. Antigen can be detected in sera, urine or stool of the fascioliasis patients. In fascioliasis, antigenemia develop during the invasive course of infection (as early as two week post infection), and decrease and become undetectable in later phases of infection.

Circulating antigen in serum disappears within a short time and most of the circulating antigens are in immune complex forms which are not freely available to be detected. Above this, there are interfering elements in human sera which reduces the applicability of diagnostic tests which are based on the detection of antigen in human sera. Therefore, antigenemia might not be an ideal method for the diagnosis of HF.On the other hand, detection of antigen in stool (coproantigens) seems to be the most suitable method for the diagnosis of HF.

## Antigen Detection Approaches For the Diagnosis of Human Fascioliasis

Having the following criteria, it appears that coproantigen detection assays are the most appropriate approaches for the diagnosis of HF:

- Satisfactory sensitivity and specificity.
- Ability to evaluate large number of sample for large community survey.
- Ability to detect the antigen in acute, early (about two months before egg shedding) and chronic phases of infection.
- Applicability for post treatment follow up.
- Capability for detection of re-infection.
- Usefulness for surveillance programs.
- Ability to detect the infection in subjects, shedding very low number of eggs.

Several antigen detection assays with diverse sensitivity and specificity have been utilized for the diagnosis of HF, based on detection of *Fasciola* Circulating Antigens (CAs) or coproantigens. The principal approach for the detection of antigen in stool sample is a sandwich ELISA, using polyclonal or monoclonal or both antibodies. Batch to batch variability is one of the main drawbacks of polyclonal antibodies in diagnostic tests [19-21]. This has been improved by development of assays based on monoclonal antibodies, which improved the reproducibility of the assays. Quite a few monoclonal or polyclonal antibodies have been produced against different antigens of *Fasciola hepatica* or *Fasciola gigantica* and have been used in antigen capture ELISA for the diagnosis of HF [19, 20, 22-24].

Utilizing polyclonal antibodies, raised against proteins in sandwich ELISA system for the diagnosis of HF achieved a diagnostic efficacy of 95.52% for coproantigen (sensitivity of 96.4% and specificity of 94.8) and 87.93% (sensitivity of 94.74% and specificity of 84.62%) for detection of CA [25].

Demerdash ZA et al., produced two monoclonal antibodies against Excretory-Secretory (ES) antigens of *Fasciola gigantica* and evaluated their performances in the detection of CA and coproantigens in fascioliasis patients. Capture ELISA, based on these monoclonal antibodies had diagnostic efficacy of 94.3% and 97.1 for detection of antigen in serum and stool, respectively. It was concluded that detection of coproantigens in stool specimens is superior to the serum samples. A positive correlation was found between ova count in stool and the Optical Density (OD) of ELISA [20].

Polyclonal antibody against Fatty Acid Binding Proteins (FABPs) was generated in rabbits and used in sandwich ELISA for detecting of coproantigen in the stool and CA in the sera of *Fasciola* infected patients. The sensitivities of sandwich ELISA test were 96.43% and 94.74%, and the specificities of the test were 94.87% for the detection of coproantigen and 84.62% for the detection of CA [25].

Evaluating the dynamics of antigenemia and coproantigen in HF patients revealed that CA cannot be detected in patients with patent infection while coproantigens were clearly detected in all patients with patent infection [26].

Sabry H and Mohamed S, reported a sandwich ELISA, based on detection of CA, with 92.4% sensitivity and 94.7% specificity [27]. Promising results were obtained in Attallah Am et al., study when they applied a sandwich ELISA for detection of a 27 kDa antigen of *Fasciola gigantica* in human sera. The test provided high degree of sensitivity/specificity and efficiency (>93%). Correlation between Egg Per Gram (EPG) and antigen level was significant in this test [28].

*Fasciola* antigen can also be detected in urine of fascioliasis patients. CA of *Fasciola* in urine and also in serum has been evaluated by a sandwich ELISA, using a monoclonal antibody produced against *Fasciola* antigen. Sensitivity of the assay in detecting CA in sera and urine of fascioliasis patients was 100% while their specificities were 98 and 97%, respectively [29].

ES78 along with MM3 are amongst the most widely used monoclonal antibodies for the diagnosis of fascioliasis. These two monoclonal antibodies have been used in FasiDIG and MM3-COPRO tests, respectively [24,30]. Performance of MM3-COPRO test, for detection of *Fasciola* coproantigen in preserved stool sample (up to 120 days in 37°C) was evaluated, using a CoproGuard preservative diluent. Results showed that using CoproGuard enhances the coproantigen extraction, without affecting the detection limit of the assay [31].

A few commercial kits including Bio-K ELISA and FasciDIG IPK La Havana are now available for detection of *Fasciola* antigens for the diagnosis of animal fascioliasis. The Bio-K ELISA was used in a study by the authors on 99 stool samples from ruminants in Iran and showed a sensitivity of 96.7% and specificity of 100% [22].

MM3-COPRO has been designed for the diagnosis of HF and evaluated for community survey [32]. The assay has been used for diagnosis of HF in children in Bolivia in comparison with Kato Katz, and achieved a sensitivity of 94.68% and specificity of 98.48%. When the assay was used in Peru, a sensitivity of 94.73% and specificity of 93.58% were found for the test, using Kato Katz and rapid sedimentation, together, as standard method. MM3-COPRO was found to be negative in six egg passing cases, one case with EPG as high as 1248 [32].

Controversy exists about the relation of OD in ELISA and intensity of infection. While a few authors reported positive correlation between EPG and ELISA OD [28], few others have not found such correlation [28,31,32]. Detection of circulating immune complexes in serum of fascioliasis patients were found to be correlated with egg shedding in stool [33].

In a study by Valero MA et al., no correlation was found between the OD of coproantigen detection ELISA and EPG, although there was a correlation between these two factors in low burden (<400 EPG) cases [32].

Based on these findings, Mas-Coma S et al., suggested that coproantigen technique appears to be insufficient to evaluate fascioliasis intensity [6].

Although, very valuable, but coproantigen detection based assays have a few drawbacks as follows:

- Subjects shedding eggs but having negative coproantigen test have been reported [32].
- False negative results might be seen in patients with encapsulated worm in liver abscess or ectopic fascioliasis.

- In low burden infection, coproantigen detection test may be negative
- Coproantigen tests, as well as antibody detection assays, are not able to differentiate *Fasciola hepatica* from *Fasciola gigantica*.

Differences in performance of antigen detection assays are contributed to differences in composition of ES antigen, differences in protocol of antigen preparation or variation in ES composition, obtained from different hosts.

## Antibody Detection Approaches for the Diagnosis of Human Fascioliasis

Numerous antibody detection systems have been introduced for the diagnosis of HF and a few of them, including DRG *Fasciola hepatica* IgG ELISA (DRG International, Inc., USA), are commercially available. One of the main advantages of antibody detection assay in fascioliasis is that antibody can be detected nearly two weeks post infection, which is about 2-3 months before the presence of eggs in the faeces. Early diagnosis and appropriate treatment of HF may avoid tissue (liver) damages. Thus, antibody detection is a suitable method for early diagnosis and management of the disease.

Spurious infections may happen by consumption of infected animal livers a short time before sampling. Placing the patient on a liver or even meat free diet and follow up of the patient by repeating the stool examination is the main recommended method for excluding the true fascioliasis. Serological tests can also rule out these spurious infections as these cases have negative serological test.

Performances of the currently available serological antibody detection tests are not satisfactory, since their specificity is far from satisfaction due to cross reaction with other helminthic antigens. This is mainly due to the complexity and huge differences in the immunogenicity of *Fasciola* antigens in humans and also animals.

Serological tests are useful for the diagnosis of acute, when the parasite eggs are still not produced, as well as chronic fascioliasis.

Pitfalls and challenges regarding the antibody based serological assays for the diagnosis of HF include:

- Lack of optimal test system.
- Lack of sensitivity.
- Lack of specificity; due to cross reaction with other helminthes antigen.
- Lack of ability to differentiate between *Fasciola hepatica* and *Fasciola gigantica.*
- Incapability to measure the intensity of infection and parasite burden.
- Unsuitability for follow up of the treatment, as anti *Fasciola* antibodies take time to return to negative and undetectable levels.
- Inability to differentiate between past and present infection.

Cross reactivity with other helminthic infections is a main drawback of antibody detection assays in diagnosis of HF. The use of subunit antigen, purified from somatic or ES antigens, has significantly improved the performances of such immunodiagnostic tests.

Due to its simplicities and appropriate performance, researchers have focused on improving ELISA system for detection of anti *Fasciola* antibodies for the diagnosis of HF. Numerous antigens including crude extract of *Fasciola hepatica* or *Fasciola gigantica*, ES antigens, purified antigen from ES or crude antigen, synthetic and also recombinant antigens have been evaluated in a variety of immunodiagnostic systems for the diagnosis of HF. The most common considered antigens of *Fasciola* which have been utilized for the diagnosis of HF are discussed below:

**ES antigen:** ES antigen is the most common antigenic source which has been used in different diagnostic assays for the diagnosis of HF. It is now more common to purify a component of ES and use it in the diagnostic test. Using ES antigen of *Fasciola gigantica*, anti *Fasciola* antibody was detected in 98% of fascioliasis patient in Mattar MA et al., study [34]. The 27 KDa antigen and cathepsins are the most studied component of ES in both *Fasciola hepatica* and *Fasciola gigantica*.

Antigens of 23 and 17 KDa of ES were found to be reactive with most of HF patient's sera. These two antigens had 95.5% sensitivity and 100% specificity in western blotting, for the diagnosis of HF [35].

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) pattern of ES antigen of *Fasciola hepatica* and *Fasciola gigantica* was reported to be different where 18, 27, 29, 48, and 62 kDa bands were common in both species, bands of 19, 45, 55 and 58 kDa were exclusively detected in *Fasciola hepatica* ES antigen [36]. Rabbit polyclonal antibodies, raised against *Fasciola hepatica* and *Fasciola gigantica* ES antigen, reacted with protein bands of 25, 27, 29, 62 and 67 kDa [36].

**Cathepsin:** Cathepsins, mainly Cathepsin L1 (CL1), are the most important cysteine protease secreted by *Fasciola* spp. and also the most evaluated antigen which has been widely used for the development of serological tests for the diagnosis of HF. Both recombinant and native counterparts of cathepsins provide acceptable performance in diagnostic assays. B cell epitopes of CL1 have also been evaluated for diagnosis of HF with satisfactory results [37,38]. Two synthesized B cell epitopes of *Fasciola gigantica* CL1, have been used in a peptide based ELISA, for the diagnosis of HF. The assay had 100% sensitivity and 97.3% specificity [38].

Gottstein B et al., evaluated two ELISA systems, based on recombinant Saposin-Like Protein 2 (SLP) and CL1 for diagnosis of HF. Superior performance was reported for SLP with sensitivity of 87% and specificity of 99% [39].

Another, ELISA system based on recombinant SLP developed by Gonzales Santala B et al., was found to have a sensitivity and specificity of 99% in diagnosing HF [40].

A lateral flow immunoassay (SeroFluke) for diagnosis of HF was developed based on MM3 monoclonal antibody, as detector antibody, and recombinant CL1 from *Fasciola hepatica* [41]. The test can be performed on whole blood or serum, in comparsion to MM3-SERO, which can be performed only on serum. The SeroFluk test can be done in few minutes, with minimal training, can be done on whole blood, and it allows individual testing of patients.

Utilizing CL1 purified from ES antigen of *Fasciola* in an ELISA system had sensitivity and specificity of 100% for the diagnosis of HF in Guilan province, in Iran [42]. Using CL1 cystein proteinase, O'Neill SM et al., showed that CL1-ELISA that detects IgG4, provided a more conclusive diagnosis [43].

Two cysteine protease of *Fasciola gigantic* (Fas 1 and Fas 2), obtained from regurgitated materials of adult worm were evacuated by Rabee I et al., in an ELISA system. Fas 1 had better sensitivity (91.1%) and specificity (89.1%) for the diagnosis of HF [44].

A Fas 2-ELISA system was developed by Espinoza JR et al., for diagnosis of fascioliasis in children in Peru, with 92.4% sensitivity and 83.6% specificity. No association between ELISA OD and infection intensity was seen [45].

Comparison of cysteine proteinase and adult somatic antigen of *Fasciola gigantica* in an ELISA system revealed that the cysteine proteinase antigen provides higher diagnostic value [46].

Application of recombinant CL1 of *Fasciola gigantica* in an ELISA system for diagnosis of *Fasciola gigantica* infected patients exhibited sensitivity of 100% and specificity of 98.9% [47].

***Fasciola spp*. 27 kDa antigen:** It has been reported that the ES of *Fasciola gigantica* comprises of more than six polypeptides which react with sera of fascioliasis patients. Among these antigens, the 27 kDa antigen was reported to react with all of patients' sera but not with sera of healthy controls [48]. In line with this study, the antigenic components of ES of *Fasciola hepatica* was evaluated by Sampaio-Silva ML et al., and the findings showed that the 27 kDa antigen was recognized by the sera of all of fascioliasis patients [49].

A 27 kDa antigen of *Fasciola gigantica* obtained from SDS-PAGE of adult worm was evaluated for diagnosis of HF in a Dot-Blot assay with 100% sensitivity [50]. In a similar study, a 27 kDa antigen of ES components of *Fasciola gigantica* was purified by HPLC and employed in ELISA for the diagnosis of HF in Vietnam. Results showed a high sensitivity (100%) and specificity (97.6%) [51]. The system then applied for the diagnosis of clinically suspected cases of HF. Antibody was found in 25.9% of cases, of whom only 8% were egg shedding. Proper response of seropositive cases to triclabendazol treatment confirmed the HF in patients [51].

The 27 kDa antigen of both *Fasciola hepatica* and *Fasciola gigantica*, excreted by the parasite, seems to be an immunodominant antigen with a high diagnostic value. The antigen appears to have both N and O-glycan. It has been demonstrated that the major antigenicity of this antigen is due to its protein epitopes since the deglycosylted antigen retain its antigenicity [52].

An ELISA system based on the detection of 27-kDa antigen of *Fasciola gigantica* was developed with high degrees of sensitivity and specificity (>93%). A significant correlation was found between egg count and antigen level in this study [28]. In line with this study, antigen of 27-28 kDa of *Fasciola hepatica* have been purified from the parasite ES antigen, and was reported as a potential candidate for the diagnosis of HF [53].

A dot-ELISA, using the 27 kDa antigen of *Fasciola gigantica*, had 98.2% sensitivity and 100% specificity in the diagnosis of HF [54]. The 27 kDa antigen of *Fasciola hepatica* was evaluated in ELISA and western blotting for diagnosis of fascioliasis patients in Iran with sensitivity and specificity of 91.6% and 100% for western blotting and 100% and 93.6% for the ELISA [55].

**Saposin:** An indirect ELISA, using SLP 2, was developed for detection of IgG antibodies against *Fasciola hepatica* in human sera. Sensitivity of 100% and specificity of 95.6% were reported for this system [56].

Other components of ES or adult worm antigens: Proteomic approach and immunoblotting techniques revealed that the enzyme Leucineaminopeptidase (LAP) and phosphoenolpyruvate carboxykinase are two immunodominant antigens which reacted with sera of HF patients [57]. Using two recombinant antigens of *Fasciola hepatica*, ferritin (FhFtn_1) and a tegument-associated protein (FhTP16-5) in ELISA systems yielded sensitivity of 96.6% and 91.4% and specificity of 95.7% and 92.4%, respectively [58]. A recombinant thioredoxinperoxides of *Fasciola gigantica* has been evaluated for diagnosis of HF with low (66.7%) sensitivity but high (96.8%) specificity [59].

### Detection of Ig Isotypes or IgG Subclasses

Polyspecific responses are a feature of human immunity against fascioliasis that is mainly related to complexity of *Fasciola* antigen. In human, IgG1, and IgG4 are the most abundant antibodies produced against *Fasciola* antigens, including ES, crude, CL1 and tegumental extract. Detection of IgG4 provides higher performance, in comparison to other subclasses of IgG, in diagnosis of HF.

In a study by Morales A and Espino AM, IgG1 and IgG4 were the main isotypes of antibodies detected in HF patients by an ELISA, using *Fasciola hepatica* tegument protein [60]. Two B cell epitopes of *Fasciola gigantica* CL1 have been synthesized as single peptide and used in ELISA for detection of IgG4 antibodies in sera of HF patients. The sensitivity and specificity of 100 and 99.7% have been reported for the assay [61]. In another study, IgG4 provided the highest diagnostic value in an ELISA system, using recombinant *Fasciola gigantica* CL1 antigen [62]. Hassan MM et al., reported that IgG4 has the highest specificity (diagnostic value) in diagnosis of HF. It was reported that the level of antibody was unchanged, one month after the treatment, [63].

## CONCLUSION

Parasitological examinations, although considered as the gold standard and the way for differential diagnosis in some situations, are not useful for the diagnosis of HF during the acute phase of the disease and suffer from the poor sensitivity during the chronic phase. Detection of anti-*Fasciola* antibodies has the main advantages of being positive as early as two weeks post infection, far before presence of eggs in the feces. However, antibody detection assays suffer from the lack of sensitivity and specificity and incapability to differentiate between the past and present infections.

Recent data provided convincing evidence that detection of coproantigen improved and simplified the diagnosis of HF. Antigen can be detected in sera or stool of the fascioliasis patients. It seems that detection of coproantigen fulfill the requirement expected from a non-invasive and reliable technique for the diagnosis of HF. Although a few commercial antigen detection tests are available for the diagnosis of animal fascioliasis, but the need for development and improvement of serological tests for the diagnosis of HF is much greater than for animals. Promising results have been obtained with a few of antigen detection-based assays for the diagnosis of HF. Yet these assays need proper field evaluation.

## ACKNOWLEDGMENTS

Authors want to acknowledge Reza Shahriarirad for technical assistance provided. The study was financially supported by vice chancellor of research of Shiraz University of Medical Sciences (Grant No. 12400).

## REFERENCES

[1] Mas-Coma S, Valero MA, Bargues MD. Fasciola, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Adv Parasitol. 2009;69:41-146.
[2] Mas-Coma S, Bargues MD, Valero M. Fascioliasis and other plant-borne trematode zoonoses. Int J Parasitol. 2005;35(11):1255-78.
[3] Sarkari B, Ghobakhloo N, Moshfea A, Eilami O. Seroprevalence of human fasciolosis in a new-emerging focus of fasciolosis in Yasuj district, southwest of Iran. Iran J Parasitol. 2012;7(2):15-20.
[4] Hosseini G, Sarkari B, Moshfe A, Motazedian MH, Abdolahi Khabisi S. Epidemiology of human fascioliasis and intestinal helminthes in rural areas of Boyer-Ahmad township, Southwest Iran; a population based study. Iran J Public Health. 2015;44(11):1520-25.
[5] Shafiei R, Sarkari B, Sadjjadi SM, Mowlavi GR, Moshfe A. Molecular and morphological characterization of Fasciola spp. isolated from different host species in a newly emerging focus of human fascioliasis in Iran. Vet Med Int. 2014;2014:405740.
[6] Mas-Coma S, Bargues MD, Valero MA. Diagnosis of human fascioliasis by stool and blood techniques: update for the present global scenario. Parasitology. 2014;141(14):1918-46.
[7] Mas-Coma S, Agramunt VH, Valero MA. Neurological and ocular fascioliasis in humans. Adv Parasitol. 2014;84:27-149.
[8] Rondelaud D, Dreyfuss G, Vignoles P. Clinical and biological abnormalities in patients after fasciolosis treatment. Med Mal Infect. 2006;36(9):466-8.
[9] De N, Nguyen V, Dang T, Nguyen D, Le K, Trieu N. Therapeutic efficacy of triclabendazole in treatment of human fascioliasis in Vietnam. J Malaria Parasite Dis Cont. 2005;6:54-62.
[10] Hatami H, Asmar M, Masoud J, Mansouri F, Namdaritabar H, Ramazankhani A. The first epidemic and new-emerging human fascioliasis in Kermanshah (western Iran) and a ten-year follow up, 1998-2008. Int J Prev Med. 2012;(4):266-72.
[11] Zumaquero-Rios JL, Sarracent-Perez J, Rojas-Garcia R, Rojas-Rivero L, Martinez-Tovilla Y, Valero MA, et al. Fascioliasis and intestinal parasitoses affecting schoolchildren in Atlixco, Puebla State, Mexico: epidemiology and treatment with nitazoxanide. PLoS Negl Trop Dis. 2013;7(11):e2553.

[12] Mekky MA, Tolba M, Abdel-Malek MO, Abbas WA, Zidan M. Human fascioliasis: a re-emerging disease in upper Egypt. Am J Trop Med Hyg. 2015;93(1):76-79.
[13] Apt W, Aguilera X, Vega F, Miranda C, Zulantay I, Perez C, et al. Treatment of human chronic fascioliasis with triclabendazole: drug efficacy and serologic response. Am J Trop Med Hyg. 1995;52(6):532-35.
[14] Tawfeek GM, Hussein DA. IgM and IgG cystatin capture enzyme linked immunosorbent assays: a tool for serodiagnosis and assessment of cure of acute fascioliasis after triclabendazole (TCZ; Fasinex) therapy. J Egypt Soc Parasitol. 2000;30(3):679-97.
[15] Ghatei MA, Hatam GR, Hossini MH, Sarkari B. Performance of latex agglutination test (KAtex) in diagnosis of visceral leishmaniasis in Iran. Iran J Immunol. 2009;6(4):202-07.
[16] Sarkari B, Chance M, Hommel M. Antigenuria in visceral leishmaniasis: detection and partial characterisation of a carbohydrate antigen. Acta Trop. 2002;82(3):339-48.
[17] Sarkari B, Hatam GR, Mikaeili F, Sadeghi H, Ebrahimi S. A comparative study of antigen and antibody detection in visceral leishmaniasis using serum and urine-based ELISA. Trop Biomed. 2008;25(2):96-99.
[18] Sarkari B, Rezaei Z. Immunodiagnosis of human hydatid disease: Where do we stand? World J Methodol. 2015;5(4):185-95.
[19] Abdel-Rahman SM, O'Reilly KL, Malone JB. Evaluation of a diagnostic monoclonal antibody-based capture enzyme-linked immunosorbent assay for detection of a 26- to 28-kd Fasciola hepatica coproantigen in cattle. Am J Vet Res. 1998;59(5):533-37.
[20] Demerdash ZA, Diab TM, Aly IR, Mohamed SH, Mahmoud FS, Zoheiry MK, et al. Diagnostic efficacy of monoclonal antibody based sandwich enzyme linked immunosorbent assay (ELISA) for detection of Fasciola gigantica excretory/secretory antigens in both serum and stool. Parasites & Vectors. 2011;4:176.
[21] Sirisriro A, Grams R, Vichasri-Grams S, Ardseungneon P, Pankao V, Meepool A, et al. Production and characterization of a monoclonal antibody against recombinant fatty acid binding protein of Fasciola gigantica. Vet Parasitol. 2002;105(2):119-29.
[22] Abdolahi Khabisi S, Sarkari B, Moshfe A, Jalali S. Production of monoclonal antibody against excretory-secretory antigen of Fasciola hepatica and evaluation of its efficacy in the diagnosis of fascioliasis. Monoclon Antib Immunodiagn Immunother. 2017;36(1):8-14.
[23] Mezo M, González-Warleta M, Carro C, Ubeira FM. An ultrasensitive capture ELISA for detection of Fasciola hepatica coproantigens in sheep and cattle using a new monoclonal antibody (MM3). J Parasitol. 2004;90(4):845-52.
[24] Muino L, Perteguer MJ, Garate T, Martinez-Sernandez V, Beltran A, Romaris F, et al. Molecular and immunological characterization of Fasciola antigens recognized by the MM3 monoclonal antibody. Mol Biochem Parasitol. 2011;179(2):80-90.
[25] Allam G, Bauomy IR, Hemyeda ZM, Sakran TF. Evaluation of a 14.5 kDa-Fasciola gigantica fatty acid binding protein as a diagnostic antigen for human fascioliasis. Parasitol Res. 2012;110(5):1863-71.
[26] Espino AM, Diaz A, Perez A, Finlay CM. Dynamics of antigenemia and coproantigens during a human Fasciola hepatica outbreak. J Clin Microbiol. 1998;36(9):2723-26.
[27] Sabry H, Mohamed S. Diagnosis efficacy of anti-cysteine protease for detection of Fasciola antigen in serum and stool samples. New Egypt J Med. 2007;36:163-9.
[28] Attallah AM, Bughdadi FA, El-Shazly AM, Ismail H. Immunodetection of Fasciola gigantica circulating antigen in sera of infected individuals for laboratory diagnosis of human fascioliasis. Clin Vaccine Immunol. 2013;20(10):1569-77.
[29] Mansour WA, Kaddah MA, Shaker ZA, al Assal FM, Derbala MF. A monoclonal antibody diagnoses active Fasciola infection in humans. J Egypt Soc Parasitol. 1998;28(3):711-27.
[30] Martinez-Sernandez V, Orbegozo-Medina RA, Gonzalez-Warleta M, Mezo M, Ubeira FM. Rapid enhanced MM3-COPRO ELISA for detection of Fasciola coproantigens. PLoS Negl Trop Dis. 2016;10(7):e0004872.
[31] Ubeira FM, Muino L, Valero MA, Periago MV, Perez-Crespo I, Mezo M, et al. MM3-ELISA detection of Fasciola hepatica coproantigens in preserved human stool samples. Am J Trop Med Hyg. 2009;81(1):156-62.
[32] Valero MA, Periago MV, Perez-Crespo I, Angles R, Villegas F, Aguirre C, et al. Field evaluation of a coproantigen detection test for fascioliasis diagnosis and surveillance in human hyperendemic areas of Andean countries. PLoS Negl Trop Dis. 2012;6(9):e1812.
[33] Sampaio-Silva ML, Santoro F, Capron A. Circulating immune complexes in human fascioliasis. relationship with fasciola hepatica egg output. Acta Trop. 1981;38(1):39-44.
[34] Mattar MA, El-Toukhy HM. Evaluation of circulating anti-Fasciola IgA antibodies by ELISA technique using two different Fasciola antigens. J Egypt Soc Parasitol. 2004;34(3):739-49.
[35] Escalante H, Davelois K, Ortiz P, Rodriguez H, Diaz E, Jara C. Western blot technique standardization of the diagnosis of human fasciolosis using Fasciola hepatica excreted-secreted antigens. Rev Peru Med Exp Salud Publica. 2011;28(3):454-61.
[36] Abdolahi Khabisi S, Sarkari B. Detection of Fasciola hepatica and Fasciola gigantica common and uncommon antigens, using rabbit hyper immune serum raised against their excretory–secretory and somatic antigens. J Parasit Dis. 2016; 40(4):1552-57.
[37] Cornelissen JB, Gaasenbeek CP, Boersma W, Borgsteede FH, van Milligen FJ. Use of a pre-selected epitope of cathepsin-L1 in a highly specific peptide-based immunoassay for the diagnosis of Fasciola hepatica infections in cattle. Int J Parasitol. 1999;29(5):685-96.
[38] Intapan PM, Tantrawatpan C, Maleewong W, Wongkham S, Wongkham C, Nakashima K. Potent epitopes derived from Fasciola gigantica cathepsin L1 in peptide-based immunoassay for the serodiagnosis of human fascioliasis. Diagn Microbiol Infect Dis. 2005;53(2):125-29.
[39] Gottstein B, Schneeberger M, Boubaker G, Merkle B, Huber C, Spiliotis M, et al. Comparative assessment of ELISAs using recombinant saposin-like protein 2 and recombinant cathepsin L-1 from Fasciola hepatica for the serodiagnosis of human Fasciolosis. PLoS Negl Trop Dis. 2014;8(6):e2860.
[40] Gonzales Santana B, Dalton JP, Vasquez Camargo F, Parkinson M, Ndao M. The diagnosis of human fascioliasis by enzyme-linked immunosorbent assay (ELISA) using recombinant cathepsin L protease. PLoS Negl Trop Dis. 2013;7(9):e2414.
[41] Martinez-Sernandez V, Muino L, Perteguer MJ, Garate T, Mezo M, Gonzalez-Warleta M, et al. Development and evaluation of a new lateral flow immunoassay for serodiagnosis of human fasciolosis. PLoS Negl Trop Dis. 2011;5(11):e1376.
[42] Rokni MB, Massoud J, O'Neill SM, Parkinson M, Dalton JP. Diagnosis of human fasciolosis in the Gilan province of Northern Iran: application of cathepsin L-ELISA. Diagn Microbiol Infect Dis. 2002;44(2):175-79.
[43] O'Neill SM, Parkinson M, Strauss W, Angles R, Dalton JP. 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. 1998;58(4):417-23.
[44] Rabee I, Mahana NA, Badr AM. Immunodiagnosis of Egyptian human fasciolosis gigantica using Fas 1 and Fas 2 cysteine proteinase antigens. J Egypt Soc Parasitol. 2013;43(3):787-96.
[45] Espinoza JR, Maco V, Marcos L, Saez S, Neyra V, Terashima A, et al. Evaluation of Fas 2-ELISA for the serological detection of Fasciola hepatica infection in humans. Am J Trop Med Hyg. 2007;76(5):977-82.
[46] Rokni M, Massoud J, Hanilo A. Comparison of adult somatic and cysteine proteinase antigens of Fasciola gigantica in enzyme linked immunosorbent assay for serodiagnosis of human fasciolosis. Acta Trop. 2003;88(1):69-75.
[47] Tantrawatpan C, Maleewong W, Wongkham C, Wongkham S, Intapan PM, Nakashima K. Serodiagnosis of human fascioliasis by a cystatin capture enzyme-linked immunosorbent assay with recombinant Fasciola gigantica cathepsin L antigen. Am J Trop Med Hyg. 2005;72(1):82-86.
[48] Intapan PM, Maleewong W, Wongkham C, Tomanakarn K, Ieamviteevanich K, Pipitgool V, et al. Excretory-secretory antigenic components of adult Fasciola gigantica recognized by infected human sera. Southeast Asian J Trop Med Public Health. 1998;29(3):579-83.
[49] Sampaio-Silva ML, Da Costa JM, Da Costa AM, Pires MA, Lopes SA, Castro AM, et al. Antigenic components of excretory-secretory products of adult Fasciola hepatica recognized in human infections. Am J Trop Med Hyg. 1996;54(2):146-48.
[50] Kamel HH, Saad GA, Sarhan RM. Dot-blot immunoassay of Fasciola gigantica infection using 27 kDa and adult worm regurge antigens in Egyptian patients. Korean J Parasitol. 2013;51(2):177-82.
[51] Nguyen TG, Le TH, De NV, Doan TT, Dao TH, Vercruysse J, et al. Assessment of a 27-kDa antigen in enzyme-linked immunosorbent assay for the diagnosis of fasciolosis in Vietnamese patients. Trop Med Int Health. 2010;15(4):462-67.
[52] Tantrawatpan C, Maleewong W, Wongkham C, Wongkham S, Intapan PM, Nakashima K. Characterisation of Fasciola gigantica adult 27-kDa excretory-secretory antigen in human fascioliasis. Parasitol Res. 2003;91(4):325-27.
[53] Antitupa I, Quispe W, Mayo J, Valverde F, Sanchez E. Purification of antigenic fraction 27-28 kDa from the metabolic antigen from metabolic secreted-excreted from Fasciola hepatica. Rev Peru Med Exp Salud Publica. 2014;31(2):288-91.
[54] Intapan PM, Maleewong W, Wongkham C, Tomanakarn K, Ieamviteevanich K, Pipitgool V, et al. Excretory-secretory antigenic components of adult Fasciola gigantica recognized by infected human sera. Southeast Asian J Trop Med Public Health. 1998;29(3):579-83.
[55] Shafiei R, Sarkari B, Sadjjadi SM. Performance of a 27 kDa Fasciola hepatica antigen in the diagnosis of human fascioliasis. J Lab Physicians. 2015;7(1):17-20.
[56] Figueroa-Santiago O, Delgado B, Espino AM. Fasciola hepatica saposin-like protein-2-based ELISA for the serodiagnosis of chronic human fascioliasis. Diagn Microbiol Infect Dis. 2011;70(3):355-61.
[57] Marcilla A, De la Rubia JE, Sotillo J, Bernal D, Carmona C, Villavicencio Z, et al. Leucine aminopeptidase is an immunodominant antigen of Fasciola hepatica excretory and secretory products in human infections. Clin Vaccine Immunol. 2008;15(1):95-100.
[58] Caban-Hernandez K, Gaudier JF, Ruiz-Jimenez C, Espino AM. Development of two antibody detection enzyme-linked immunosorbent assays for serodiagnosis of human chronic fascioliasis. J Clin Microbiol. 2014;52(3):766-72.
[59] Wang Y, Zhou Y, Cheng N, Chen M, Ai L, Liu Y, et al. Cloning, expression and immunodiagnostic evaluation of the Fasciola gigantica thioredoxin peroxidase. Chinese Journal Of Parasitology & Parasitic Diseases. 2015;33(2):81.
[60] Morales A, Espino AM. Evaluation and characterization of Fasciola hepatica tegument protein extract for serodiagnosis of human fascioliasis. Clin Vaccine Immunol. 2012;19(11):1870-78.
[61] Tantrawatpan C, Maleewong W, Wongkham C, Wongkham S, Intapan PM, Nakashima K. Evaluation of immunoglobulin G4 subclass antibody in a peptide-based enzyme-linked immunosorbent assay for the serodiagnosis of human fascioliasis. Parasitology. 2007;134(Pt.14):2021-26.
[62] Wongkham C, Tantrawatpan C, Intapan PM, Maleewong W, Wongkham S, Nakashima K. Evaluation of immunoglobulin G subclass antibodies

against recombinant Fasciola gigantica cathepsin L1 in an enzyme-linked immunosorbent assay for serodiagnosis of human fasciolosis. Clin Diagn Lab Immunol. 2005;12(10):1152-56.

[63] Hassan MM, Mostafa NE, Ramadan M, Nassar A, Hassounah O, Omar O. Anti-fasciola IgG isotypes among patients with fascioliasis before and after treatment. J Egypt Soc Parasitol. 2000;30(2):505-10.

**PARTICULARS OF CONTRIBUTORS:**
1. Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Parasitology and Mycology, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran.

**NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:**
Bahador Sarkari,
Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz-71345-1735, Iran.
E-mail: sarkarib@sums.ac.ir

**FINANCIAL OR OTHER COMPETING INTERESTS:** As declared above.

Date of Submission: **Dec 16, 2016**
Date of Peer Review: **Mar 06, 2017**
Date of Acceptance: **Apr 17, 2017**
Date of Publishing: **Jun 01, 2017**