Partially purified fraction (PPF) antigen from adult *Fasciola gigantica* for the serodiagnosis of human fascioliasis using Dot-ELISA technique

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Background: Human fascioliasis has been reported in many countries, including Iran. Various techniques have been evaluated for diagnosis of human fascioliasis using different antigens. We evaluated *Fasciola gigantica* partially purified fraction antigen (PPF) isolated from sheep's liver fluke for the diagnosis of human fascioliasis.

Materials and Methods: Two hundred sixty-one sera were collected from 104 patients living in an area endemic for human fascioliasis, from 89 non-fascioliasis patients living in a non-endemic area, and from 68 healthy individuals. Micro-ELISA was used in the evaluation of the sensitivity and specificity of Dot-ELISA.

Results: With a 1:800 sera dilution as the cut-off titer, the sensitivity of the Dot-ELISA test in diagnosis of human fascioliasis was 94.23% and the specificity was 99.36%.

Conclusion: Dot-ELISA using PPF antigen is a sensitive and specific method for diagnosis of human fascioliasis that is also rapid and inexpensive.

Key words: *Fasciola gigantica*, Dot-ELISA, partially purified fraction antigen, human fascioliasis.

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Fascioliasis is one of the most important zoonotic diseases of sheep and cattle. The disease has a cosmopolitan distribution, being prevalent in most sheep-raising countries. Human fascioliasis has been reported in many countries, including Iran. In 1989, an outbreak of human fascioliasis was reported from Guilan province in the north of Iran (Caspian Sea littoral). More than 10,000 individuals were reported infected in the area, which is still considered endemic for fascioliasis.[1] Human cases are reported frequently.

Both stool examination and serological methods are applied in the diagnosis of fascioliasis. The diagnosis of human fascioliasis based on the detection of the parasite ova in stool is often unreliable because a) egg deposition of the parasite is not regular, b) the incubation phase of the infection is shorter than that of the prepatent period, and c) clinical findings of the disease may appear long before egg can be found in stools.[2] Thus, various serodiagnostic techniques such as immunofluorescent assay (IFA), indirect hemagglutination (IHA), enzyme-linked immunosorbent assay (ELISA) and Dot-ELISA have been developed for diagnosis of human fascioliasis in different laboratories. Unlike IFA and IHA,[2] ELISA is highly sensitive and specific.[3-5] The disadvantage of ELISA technique for field studies is the requirement for a spectrophotometer, whereas most serological tests can be performed easily in the field. A spectrophotometer is not required with Dot-ELISA, a modification of ELISA technique. The Dot-ELISA test has been evaluated for the immune diagnosis of animal or human fascioliasis using different antigens.[6,7] We evaluated the partially purified fraction (PPF) antigen extracted from adult *Fasciola gigantica* for the serodiagnosis of human fascioliasis using Dot-ELISA technique.

Materials and Methods

The methods of Hillyer and de Weil [8] and Mansour et al. [9] were used for preparation of partially purified *Fasciola gigantica* antigen. Briefly, adult *Fasciola gigantica* were collected from the infected livers of sheep slaughtered at an abattoir. The parasites were washed three times with phosphate-buffered saline (PBS), homogenized and centrifuged at $5X10^4$ g for 1 hour at 4°C. The protein content of the supernatant was measured by the Lowry et al.[10] method. Thirty milligrams of the soluble antigen were loaded on a G-200 Sephadex column, then eluted with 0.1 M PBS, pH 7.4. Four milliliters of fractions were collected and their absorbances were measured at 280 nm. Fractions from peak II and III were pooled and dialyzed against TBS (Tris-Buffered Saline) at 4°C and their protein contents were measured and stored at –20°C until used. The method of Zimmerman et al [11] was used for preparation of E/S (excretory/secretory) antigen from *Fasciola gigantica.*

We collected 261 sera from three groups. The first group consisted of 104 patients from Anzali city, Guilan province, North Iran, an area endemic for human fascioliasis. The patients had clinical symptoms of fascioliasis, passed

Table 1. Number and percentage of sera positive for PPF antigen in Dot-ELISA.

Groups	Serum dilution						
	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800
Patients with Fasciola infection (n=104)	104 (100)	102 (98.07)	98 (94.23)	97 (93.27)	82 (78.85)	62 (59.61)	13 (12.50)
Patients without Fasciola infection (n=89)	9 (10.11)	1 (1.12)	1 (1.12)	0	0	0	0
Healthy control subjects (n= 68)	4 (5.88)	1 (1.47)	0	0	0	0	0

Table 2. Sensitivity, specificity and other validity indices of Dot-ELISA for serodiagnosis of human fascioliasis.

Indices	Serum dilution						
	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800
Sensitivity (%)	100	98.07	94.23	93.27	78.85	59.61	12.5
Specificity (%)	91.72	98.72	99.36	100	100	100	100
Positive predictive value (%)	88.89	98.07	98.99	100	100	100	100
Negative predictive value (%)	100	98.72	96.30	95.73	87.71	78.89	63.31

Fasciola eggs in their faeces and had a positive ELISA test. The second group consisted of 89 non-fascioliasis patients from a non-endemic area (Tehran) who were negative in a 3-stool examination and ELISA test. The second group included 27 patients with cystic hydatidosis diagnosed by ELISA and confirmed surgically, 13 with diarrhea caused by either *E. histolytica* or *G. lamblia*, 17 infected with intestinal helminths, 15 with viral hepatitis and 17 with salmonellosis. The third group consisted of 68 healthy individuals from Tehran, who had no symptoms of fascioliasis and who had a negative stool examination and ELISA test for hydatidosis and fascioliasis.

Micro-ELISA was performed on polystyrene plates coated with the E/S antigen of *Fasciola gigantica*.[11] For the Dot-ELISA test, 1 µL of the PPF was dotted on nitrocellulose membrane discs and allowed to air dry thoroughly.[12] The discs were placed into the well of a flat-bottom micrometer plate. Non-specific binding sites were blocked by the addition 100 µL of PBS containing 0.5% Tween 20 in each well. One hundred µL of the serum samples were diluted with PBS-Tween in a double dilution starting at 1:100, added to the discs, and the plate was incubated at room temperature for 1 hour or overnight at 4°C. The discs were washed again with PBS-Tween 20, and 100 µL of the rabbit anti-human IgG peroxidase conjugate was added to each well and the plate incubated for 2 hours. After washing, 100 µL of 0.5mg/mL DAB (diamino benzidine tetrahydrocholoride) and 0.03% H_2O_2 in PBS were added and incubated for 30 minutes. The serum dilution that gave visible brown spots on discs at titers 400 was considered positive. Standard formulas were used for calculation of sensitivity, specificity, percentage of positive predictive value, negative predictive value, false positive rate and false negative rate.

Results

Dot-ELISA detected all positive cases at 1:200 serum dilutions, indicating 100% sensitivity and 91.72% specificity (Table 1). At a serum dilution of 1:800 only one patient with hydatidosis showed a positive reaction. In non-fascioliasis patients and in healthy controls the minimum rate of positive reaction was observed at 1:800 and 1:400 titers, respectively. The sensitivity, specificity and other validity indices of the Dot-ELISA technique using PPF antigen for serodiagnosis of human *Fasciola* infection are shown in Table 2. The best sensitivity and specificity (94.23% and 99.36%) was obtained at the 1:800 serum titer. Thus, for diagnosis of human fascioliasis by Dot-ELISA, the serum dilution of 1:800 can be recommended as the cut-off.

Discussion

Outbreaks of acute human fascioliasis occur throughout the world.[13-19] Various techniques have been evaluated for diagnosis since clinical signs are variable and not efficient.

In the early period of infection, the parasites are not sexually mature and cannot release eggs, so observation of fluke eggs in fecal examination is impossible. Ectopic location of the parasite in extra-hepatic sites may prevent a correct clinical and fecal diagnosis of fascioliasis.

Immunodiagnosis is an important adjunct to clinical findings.[2] The need for immunodiagnostic testing for fascioliasis is much greater for humans than for their livestock.[2] Various immunological techniques have been used in diagnosis of human and animal fascioliasis.[20] Most workers today use ELISA and/or Western immunoblots.[3-5] The value of immunodiagnostic methods depends on the applied antigens. The antigens used for different techniques have been primarily derived from adult worm extracts or excretory/secretory products. Although the preferred antigen is an E/S product, some investigators have used partially purified fraction (PPF) for both ELISA and Dot-ELISA technique.[6,7]

Using ELISA with excretory/secretory Fasciola (FhES) antigen, Espino et al.[21] had positive results in 20 patients with confirmed fascioliasis. No cross reactivity was observed using sera from patients with other parasitic infections, including schistosomiasis. Hillyer and Soler De Galanes [4] used a FAST-ELISA with FhES antigens and found that serum from humans with fascioliasis had an elevated antibody level for the entire 3 years that infection was monitored. Zimmerman et al.[22] used E/S antigens of *Fasciola hepatica* for diagnosis of ovine fascioliasis by Dot-ELISA. In that study, antibodies against the antigens were consistently detected by 4 weeks after the sheep were inoculated. Morilla et al.[23] evaluated Dot-ELISA in naturally and experimentally infected sheep using E/S antigen. The infected sheep gave very high titers, from 1:25600 to 1:204800, which was 1000 to 2000 times higher than with PHT (passive haemagglutination test) or with TIA (thin layer immunoassay). Shaheen et al.[6] applied partially purified antigens from a species of Fasciola at 180 ng protein/dot (2 µL) and serum samples at 1:20 dilution (1 µL) for diagnosis of human fascioliasis. The sensitivity of the assay was 100% and its specificity was 97.8%. The major cause of low specificity was human schistosomiasis, which showed cross reactivity with Fasciola infection.

In a region like the north of Iran, where human schistosomiasis is not prevalent, high specificity with Dot-ELISA can be expected using PPF antigens of Fasciola. However, cross reactivity may interfere with precise interpretation of serologic results. In our study, most cross reactivity was associated with hydatidosis. In both titres of 1:400 and 1:800, only one case of hydatidosis showed a positive reaction, while in 1:1600 the cross reactivity was null. Since serum dilution at 1:800 shows high sensitivity (94.23%) and specificity (99.36%) as well as minimum cross reactivity, we suggest it as the cut-off for this assay. The disadvantage of using a 1:400 dilution is that it has relatively more cross reactivity with other infectious agents even though it had high sensitivity and specificity. In conclusion, by applying PPF antigen in the Dot-ELISA technique, we developed a sensitive and specific method for diagnosis of human fascioliasis that is also rapid and inexpensive.

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References

1. Massoud J. Fascioliasis outbreak of man and drug test (Triclabendazole) in Caspian littoral, northern part of Iran, 1989. **Bul Soc Fr Parasitol.** 1990;8:438.

2. Hillyer G.V. Immunodiagnosis of human and animal. In: Dalton JP. **Fascioliosis.** Oxon, UK: CABI publishing; 1999: 435-449.

3. Hillyer G.V Fasciolosis and fasciolopsiasis In: Balows A, Hausler, WJ, Ohashi, M, Turano A. Eds. **Laboratory Diagnosis of Infectious Disease.** Vol. 90. Springer-Verlag, Berlin; 1986: 856-862.

4. Hillyer GV, Soler de Galanes M. Identification of a 117-kilodalton Fasciola hepatica immunodiagnostic antigen by enzyme-linked immunoelectrotransfer blot technique. **J Clin Microbiol.** 1988; 26: 2048-2053.

5. Hillyer, GV. Serological diagnosis of Fasciola hepatica. **Parasitol Dia.** 1993;17:130-136.

6. Shaheen HI, Kamal KA, Farid Z, Mansour N, Boctor FN, Woody JN. Dot-enzyme linked immunosorbent assay (Dot-ELISA) for the rapid diagnosis of human fascioliasis. **J Parasitol.** 1989; 75(4): 549-552.

7. Youssef F.G. and Mansour N.S. A purified Fasciola gigantica worm antigen for serodiagnosis of human fascioliasis. **Trans Roy Soc Trop Med Hyg.** 1991; 85:535-537.

8. Hillyer GV, de Weil NS. Partial purification of Fasciola hepatica antigen for the immunodiagnosis of fascioliasis in rat. **J Parasitol.** 1977; 63:430-433.

9. Mansour NS, Youssef FG, Mikhail EM, Boctor FN. Use of partially purified Fasciola gigantica worm antigen in the serological diagnosis of human fascioliasis in Egypt. **Am J Trop Med Hyg.** 1983;32:550-554.

10. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. **J Biol Chem.** 1951; 193:265-275.

11. Zimmerman GL, Jen LW, Cerro JE, Farnsworth LL, Wescott RB. Diagnosis of Fasciola infection in sheep by an enzyme-linked immunosorbent assay. **Am J Vet Res.** 1982; 43: 2097-2100.

12. Pappas MG, Schantz PM, Cannon LT, Wahlquist SP. Dot-ELISA for the rapid serodiagnosis of human hydatid disease. **Diagn Immmunol.** 1986;4:271-276.

13. Hillyer GV, Soler de Galanes M, Rodriguez-Perez J, Silva de Lagrava M, Ramirez Guzman M, Bryan RT. Use of Falcon TM assay screening test-enzyme-linked immunosorbent assay (FAST-ELISA) and the enzyme-linked immunoelectotransfer blot (EITB) to determine the prevalence of human fascioliasis in the Bolivian Antiplano. **Am J Trop Med Hyg.** 1992; 46:603-609.

14. O'Neill SM, Parkinson M, Strauss W, Angles R, Dalton JP. Immuodiagnosis of Fasciola hepatica infection (fasciolosis) in a human population in the Bolivian Antiplano using purified cathespin L cystiene proteinase. **Am J Trop Med Hyg.** 1998; 58: 417-423.

15. Esteban JG, Flores A, Aguirre C, Strauss W, Angles R, Mas–Coma S. Presence of very high prevalence and intensity of infections with Fasciola hepatica among Aymara children from the northern Bolivian Altiplano. **Acta Trop.** 1997;66: 1-14.

16. Estebsan JC, Flores A, Angles R, Strauss W, Aguirre C, Mas-Coma S. A population-based coprological study of human fascioliasis in a hyperendemic area of the Bolivian Altiplano. **Trop Med Int Health.** 1997;2:695-699.

17. Bjorland J, Bryan RT, Strauss W, Hillyer GV, McAuley JB. An outbreak of acute fascioliasis among Aymara Indians in the Bolivian Altiplano. **Clin Infect Dis.** 1995;21: 1228-1233.

18. Apt W, Aguilera X, Vega F, Alcaino H, Zulantay I, Apt P, Gonzalez V, Retamal C, Rodriguez J, Sandoval J. Prevalencia de fascioliasis en humanos, caballos, cerdos y conejos silvestres, en tres provincias de chile. **Bol Oficina Sanit Panam.** 1993;115: 405-414.

19. Hassan MM, Moustafa NE, Mahmoud LA, Abbaza BE, Hegab MH. Prevalence of Fasciola infection among school children in Sharkia Governorate, Egypt. **J Egypt Soc Parasitol.** 1995;25: 543-549.

20. Boray JC. Immunology, pathobiology and control of fasciolosis. MSD AGVET. USA: Rheay, New Jersey; 1997:130.

21. Espino AM, Dumenigo BE, Fernandez R, Finlay CM. Immunodiagnosis of human fascioliasis by enzyme-linked immunosorbent assay using excretory-secretory products. **Am J Trop Med Hyg.** 1987; 37: 605-608.

22. Zimmerman GL., Nelson, MJ, Clark CRB. Diagnosis of ovine fascioliasis by a dot enzyme-linked immunosorbent assay. A rapid microdiagnostic technique. **Am J Vet Res.** 1985; 46 (7): 1513-1515.

23. Morilla CA, Paniagua R, Ruiz-Navarrete A, Bautista CR, Morilla A. Comparison of dot enzyme linked immunosorbent assay (Dot-ELISA), passive haemagglutination test (PHT) and thin layer immunoassay (TIA) in the diagnosis of natural or experimental Fasciola hepatica infection in sheep. **Vet Parasitol.** 1989;30:197-203.