An enzyme-linked immunosorbent assay for diagnosis of *Fasciola gigantica* infection in cattle and buffaloes

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Abstract The enzyme-linked immunosorbent assay (ELISA) was evaluated for the diagnosis of *Fasciola gigantica* infection in cattle and buffaloes. The excretory–secretory (E–S Ag) antigen of *F. gigantica* adult flukes obtained after invitro incubation was used as an antigen. The test was conducted with 276 sera collected from cattle and buffaloes which included 22 sera each from naturally infected cattle and buffaloes (known positive serum) and with similar number of samples with healthy cattle and buffaloes (known negative serum). The positive results were observed in 18 and 19 of the sera from naturally infected cattle and buffaloes with sensitivity of 81.8 and 86.3 % respectively. Out of 188 serum samples which were found negative on faecal examination 32 (34 %) sera of cattle and 40 (42.5 %) sera of buffaloes were found positive by ELISA respectively. The sensitivity of the test was found to be 91.6 and 95.6 % in cattle and buffaloes respectively.

Keywords *Fasciola gigantica* · Excretory–secretory antigen · Sensitivity

Introduction

Fasciolosis in ruminants caused by *Fasciola gigantica* is known to be widely prevalent in the Indian subcontinent resulting in immense economic loss due to high morbidity and mortality. Diagnosis of fasciolosis is usually done by routine faecal examination, which is not a very sensitive method as it fails to detect immature fasciolosis and sometimes even clinical and sub-clinical infection.

Diagnosis is not possible by parasitological tests because eggs are not found in the faeces until flukes reach maturity. Therefore it would be necessary to diagnose the disease by other means such as imunodiagnosis at an early stage. Some researchers have evaluated serological tests such as AGPT, CIEP, IHAT and ELISA to detect fasciolosis in animals (Kadhim and Al-attar 1974; Swarup et al. 1987a; Hillyer and Sanchez 1985).

Among various serodiagnostic tests, Enzyme linked immunosorbent assay has been shown to be very sensitive in detection of infection as early as 2 weeks post infection (Farrell et al. 1981; Zimmerman et al. 1982). In the present study enzyme linked immunosorbent assay was standardized as a serodiagnostic test for the detection of fasciolosis in naturally infected cattle and buffaloes.

Materials and methods

Test sera

A total of 276 (138 cattle and 138 buffalo) sera samples were collected which included 22 samples (each from cattle and buffalo) from naturally infected with liver flukes in their liver. The sera from 22 animals (each from cattle and buffalo) which had no liver flukes and other parasitic

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infections during liver examination of slaughtered animals at KMPMCL slaughterhouse Bangalore. Similarly A total of 188 (94 cattle and 94 buffalo) test sera samples were collected from animals which were found negative on faecal examination.

The sera collected from infected animals were considered and mentioned as positive control sera and the sera collected from healthy animals were considered as negative control sera and they were stored at -20°C till use.

Excretory–secretory antigen (E–SAg)

The E–SAg was prepared as per the methodology described by Yadav and Gupta (1996) with slight modifications. Briefly, live *F. gigantica* flukes were washed thoroughly with PBS (pH 7.2) and incubated (20 flukes/10 ml PBS) at 37°C for 6 h. After incubation, the medium was collected and centrifuged at 10,000 rpm in refrigerated centrifuge for 45 min. The supernatant was designated as excretory–secretory antigen. It was aliquotted at -20°C for further use. The protein concentration of the antigen was estimated as per the method described by Lowry et al. (1951).

Enzyme linked immunosorbent assay

Indirect ELISA was performed as per the method described by Sarkar et al. (2003) with some modifications. ELISA plates were coated with antigen $5\text{ }\mu\text{g/well}$ diluted in coating buffer (0.05 M carbonate-bicarbonate buffer pH 9.6). The plates were incubated at 4°C overnight. Then the antigens were discarded and the wells were blocked with $50\text{ }\mu\text{l}$ of blocking buffer (1 % skimmed milk in PBS, pH 7.2) and incubated at 37°C for 2 h. The wells were washed four times using washing buffer, PBS-T (PBS pH 7.2, containing 0.05 % Tween 20). Test sera were diluted in blocking buffer at the ratio 1:100, added in the wells and allowed for incubation at 37°C for 2 h. Again the plates were washed four times and $50\text{ }\mu\text{l}$ of anti-bovine Ig G

immunoglobulin conjugate (Horse-radish peroxidase, Bangalore Genei[®]) was added at the dilution of 1:5,000 and the plates were incubated at 37°C . After 2 h, the plates were washed with PBS-T and $50\text{ }\mu\text{l}$ substrate solution (*O*-Phenylene diamine 10 mg, (Sigma[®]), hydrogen peroxide 30 % v/v in 25 ml of citric acid phosphate buffer) was added into each well. The plates were incubated in dark for 10 min. The reaction was arrested with 1 N H_2SO_4 . The OD Values were recorded by ELISA reader (Labsystems multiskan) at 492 nm wavelength.

Results

The antigen was standardized by checkerboard titration with serum samples of naturally infected animals and it was found to be $5\text{ }\mu\text{g/well}$ of ELISA plate and 1:100 dilutions of serum was found to be optimal. The cut of value was calculated as mean OD values $\pm$ three standard deviation of negative control sera and it was found to be 0.30. Considering this mean optical density as standard, the test sera showing above were read as positive reactants and those below it, as negative reactants for *F. gigantica* infection.

Out of 22 (each from cattle and buffalo) naturally infected positive serum samples examined 18 (81.8 %) and 19 (86.3 %) sera of cattle and buffaloes showed positive reaction respectively. Out of 22 (each from cattle and buffalo) serum samples of animals with no flukes in the liver (known negative serum) showed 02 sera of cattle (9.1 %) and 01 sera of buffaloes (4.5 %) showed positive reaction respectively. The remaining 188 (94 cattle and 94 buffalo) bovines serum samples which were found negative on faecal examination 32 (34.0 %) sera of cattle and 40 (42.5 %) sera of buffaloes were found positive by ELISA (Table 1). The results were found to be statistically significant by Chi square test. The sensitivity of ELISA was found to be 91.6 and 95.6 % in cattle and buffaloes respectively which were positive on liver examination.

Table 1 Enzyme linked immunosorbent assay for the detection of fasciolosis in naturally infected cattle and buffaloes

Particulars of serum	Species	No. of serum samples tested	Positive	% positive
Animals with adult liver flukes in the liver (known positive serum)	Cattle	22	18	81.8 [®]
	Buffalo	22	19	86.3
Animals with no liver flukes in the liver (known negative serum)	Cattle	22	02	9.1
	Buffalo	22	01	4.5
Animals negative on faecal examination (test serum)	Cattle	94	32	34.0
	Buffalo	94	40	42.5

Discussion

Sensitivity of the ELISA standardized in the present study for detection of fasciolosis was 91.6 and 95.6 % in cattle and buffaloes, respectively. Swarup et al. (1987b) reported almost similar results indicating 92.2 and 94.2 % with adult hydro soluble homogenate and partially purified antigen respectively in buffaloes, during the comparative study of ELISA, AGPT and CIEP test in naturally infected bubaline fasciolosis. In the present study a slightly higher sensitivity was observed with excretory–secretory antigen.

Compared to the present study Ibarra et al. (1998) reported almost similar sensitivity of 96.5 % in indirect ELISA. Similarly Mandal et al. (1999) also reported 96.0 % sensitivity in goats with somatic antigen while Garg et al. (2003) reported 93.6 % sensitivity in buffaloes when bile examination was taken as a standard and 95.0 % when faecal examination was taken as a standard.

From the present study it was concluded that the Enzyme linked immunosorbent assay with excretory–secretory antigen of *F. gigantica* was sensitive, reliable and useful in detection of immature and sub-clinical fasciolosis in naturally infected conditions.

References

- Farrell CJ, Shen DT, Wescott RB, Lang BZ (1981) An ELISA for diagnosis of *Fasciola hepatica* infection in cattle. *Am J Vet Res* 42:237–240
- Garg R, Ram H, Banerjee PS, Yadav CL (2003) Comparative evaluation of ELISA in respect to some Parasitological tests for detection of bubaline fasciolosis. *Indian Vet J* 80:851–853
- Hillyer GV, Sanchez ZD (1985) Immunodiagnosis of bovine fasciolosis by ELISA and immunoprecipitation methods. *J Parasitol* 71:449–454
- Ibarra F, Montenegro N, Vera Y, Baulard C, Quiroz H, Flores J, Ochoa P (1998) Comparison of three ELISA tests for seroepidemiology of bovine fasciolosis. *Vet Parasitol* 77:229–236
- Kadhim JK, Al-attar (130) Preliminary observations on antigen activity of *Fasciola gigantica* by immunodiffusion test in sheep. *Br Vet J* i:iv–vi
- Lowry OH, Roserbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265–275
- Mandal S, Yadav SC, Sharma RL (1999) An Evaluation of seroreactivity of antigenic preparations of *Fasciola gigantica* in goats. *Indian J Anim Sci* 69(11):875–879
- Sarkar S, Mandal A, Manna AK, Roy S, Joardar SN, Dasgupta CK (2003) Detection of anti-fasciola antibodies of buffalo sera using DID and ELISA. *Indian J Anim Hlth* 42(1):26–30
- Swarup D, Pachouri SP, Sharma B (1987a) Serodiagnosis of *Fasciola gigantica* infection in buffaloes. *Vet Parasitol* 24:67–74
- Swarup D, Pachouri SP, Sharma B (1987b) Comparative efficacy of sero-diagnostic tests in buffaloes naturally infected with *Fasciola gigantica*. *Indian J Anim Sci* 57(5):373–376
- Yadav SC, Gupta SC (1996) Identification of immunodiagnostic antigens of *Fasciola gigantica*. *J Parasitc Dis* 20:201–202
- Zimmerman GL, Hjen LW, Cerro JW, Farasworth, Wescott RB (1982) Diagnosis of *Fasciola hepatica* infection in sheep by an ELISA. *Am J Vet Res* 43:2097–2100