COMPARING ELISA USING SERUM ANTIBODIES AND COPROANTIGENS FOR DETECTING EARLY AMPHISTOMOSIS*

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Abstract

Hundred cattle known to be infected with Gastrothylax crumenifer, the most prevalent amphistome species were selected at random for the present study. An indirect ELISA was performed to detect serum antibodies using somatic antigens of the fluke. Fifty one samples were found to be seropositive. Similarly, ELISA was employed to detect coproantigens in faecal supernatants of the same cattle using rabbit hyper immune serum against the somatic fluke antigens. Seventy four samples were found to contain detectable amounts of coproantigens indicating a sensitivity of 74 per cent for the test. The results indicate that coproantigen detection which revealed a higher sensitivity than that of serum antibodies by ELISA is reasonable and practical for the detection of amphistome infections in bovines.

Key words: *Coproantigen, Indirect ELISA, Amphistomes*

Conventional methods of diagnosis of amphistomosis caused by *Gastrothylax crumenifer* in bovines like observation of clinical signs, aided by coprological examination and serological methods have many limitations. (Hafeez, 1981; Johnson *et al.,* 1996; Dumenigo *et al.,* 1996). As a result emphasis has been given on the detection of parasitic antigens in biological samples especially if it does not involve collection of blood samples (Johnson *et al.,*1996). Kandasamy and Devada (2011) ensured that diagnosis of amphistome infection in cattle was feasible using coproantigens in an ELISA. The present study was undertaken to compare the sensitivity of ELISA using serum antibodies and coproantigens in the diagnosis of amphistomosis in cattle.

Materials and Methods

Live flukes from the rumen of infected cattle slaughtered at the Municipal slaughter house, Thrissur, were collected and brought to the laboratory in chilled saline. The most prevalent amphistome species, *Gastrothylax crumenifer* were separated from the rest in the laboratory based on morphology. Somatic antigens were prepared from the above flukes as per Jithendran *et al*. (1996) and its protein content was determined by Biuret method using photometer 5010. Hyper immune sera (HIS) against somatic antigens were raised in rabbits (Johnson *et al.,* 1996) and the sera were tested for antibodies by AGPT (Kagan and Norman, 1976).

Serum samples from 100 cattle with known *Gastrothylax* infection were collected and stored in sterile vials at -20 º C for antibody detection in an ELISA. Faecal samples of the same 100 cattle infected with *Gastrothylax* species, from which the sera were obtained were collected just before slaughter in small plastic vials for coproantigen detection.

An indirect ELISA was performed as per the method describes by Craig *et al*. (1995) and Anderson *et al*.(1999) to detect serum antibodies. The optimum concentration of somatic fluke antigen, test serum samples and rabiit anti-bovine IgG-HRP conjugate (Genei, Pvt, Ltd.) were

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Table: Comparison of results of Serum antibody ELISA and Coproantigen ELISA

		Coproantigen ELISA		Total
		Positive	Negative	
Serum antibody ELISA	Positive	43	8	51
	Negative	31	18	49
Total		74	26	100

standardized by checker board titration.

An indirect ELISA using coproanti gens was performed as described by Ahmad and Nizami (1998) in 96 well flat bottomed microtitre plates. The optimum concentration of coproantigens, anti somatic HIS raised in rabbits and goat anti-rabbit IgG-HRP conjugate (Genei, Pvt, Ltd.) were standardized by checker board titration.

In both the tests, sera from cattle with known *Gastrothylax* infection formed the positive control and those from cattle absolutely free from any amphistome infection formed the negative control. Optimum dilutions of the reagents which gave the highest titre with the positive sample and the lowest titre with the negative sample were selected as the working dilution for the present study. The plates were read at 450 nm in a Multiscan MS ELISA reader. Samples that had an OD value above the sum of the mean of negative controls and three times the standard deviation (cut-off point) were taken as positive.

Results and Discussion

The protein content of somatic antigens prepared for raising HIS in rabbits was found to be 4 mg/ml and that of faecal supernatant containing coproantigens was found to be 1 mg/ml. The optimum concentrations of somatic fluke antigen, test serum and rabbit anti bovine IgG-HRP conjugate for serum ELISA were found to be 1:200, 1:100 and 1: 1000 respectively and those of coproantigens, anti somatic HIS and goat anti rabbit IgG –HRP used for coproantigen ELISA were 1:64, 1:100 and 1:100 respectively.

The cut off point for the samples in the serum antibody ELISA was 0.300 and 51 serum samples were found to have an OD value above this point and marked as sero positive. The sensitivity of the test was 51 per cent. The low sensitivity obtained in the present work, might be attributed to crude somatic antigen used in the present study. According to Craig *et al.* (1995) and Anderson *et al.* (1999) formation of circulating immune complexes, parasite induced immune suppression, nutritional status and antigenic variation could contribute to the lowering of antibody levels.

In the indirect ELISA for detection of coproantigens the cut-off point was 0.225. Using this criterion, 74 faecal samples were found to be positive for coproantigens and the sensitivity of copro antigen ELISA was 74 per cent. Here too, the sensitivity was lower, due to the utilisation of somatic antigens while Ahmad and Nizami (1998) and Rahman *et al.* (1999) were able to obtain a higher sensitivity of 100 percent utilizing excretory /secretory antigens of the parasites.

The results of ELISA using serum antibodies and coproantigens are compared and presented in the table.

Fifty one animals were positive for serum antibodies and 74 for coproantigens out of 100, with known *G. crumenifer* infection. Forty three animals revealed both serum antibodies and coproantigens while 18 animals did not show either of them. Thirty one animals that were negative for serum antibodies were found positive for coproantigens. But eight animals that were negative for coproantigens were found seropositive. The above results indicate the possibility of patent infections since 43 cattle had both the antigens and antibodies. It also signifies the advantages of coproantigens that they are devoid of immune complex formation and immune suppression which naturally interfere with the detection of antibodies. The observation that 8 animals found negative for coproantigens but seropositive suggests the possibility of previous exposure as remarked by Craig *et al*. (1995). The reason for 18 cattle found negative in both the tests reflects the limitations of the assays as discussed above.

References

Ahmad, G. and Nizami,W. A. 1998. Coproantigens: Early detection and suitability of an immunodiagnostic method for echinococcosis in dogs. *Vet.Parasitol*., **77:** 237-244

Anderson, N., Luong, T.T., Vo, N.G., Bui, K.L., Smooker, P.M. and Spithill, T.W 1999. The sensitivity and specificity of two methods for detecting *Fasciola* infections in cattle. *Vet. Parasitol.,* **83**:15-24.

Craig. P. S., Gasser, B., Parada, I., Cabrera, P., Parietti, S., Borgues, C., Acuttis, A., Snowden, K. and Paolillo, E. 1999. Diagnosis of canine echinococcosis: comparison of coproantigen and serum antibody tests with arecoline purgation in Uruguay. *Vet. Parasitol.,* **56**: 293-301

Dumenigo, B.E., Espino, A.M. and Finlay,C.M. 1996. Detection of *Fasciola hepatica* antigen in cattle faeces by a mono clonal antibody based sandwich immunoassay. *Res. Vet. Sci.* **60**: 278-279

Hafeez, M.D. 1981. Studies on certain immunological aspects of amphisto miasis of sheep and goats with special reference to the effect of ionizing radiation. *Ph.D thesis*, Andhra Pradesh Agricultural University, Hyderabad. 300 p.

Jithendran, K. P., Vaid, J. and Krishna, L. 1996. Comparative evaluation of AGPT, CIEP and PHA tests for the diagnosis of *Dicrocoelium dentriticum* infection in sheep and goats. *Vet. Parasitol.,* **61**: 151-156

Johnson, M. J., Behnke, J. M. and Coles, G. C. 1996. Detection of gastrointestinal nematodes by a coproantigen capture ELISA. *Res.Vet. Sci.*, **60**: 7-12

Kagan, I.G. and Norman, L. 1976. Serodiagnosis of parasitic diseases. In: *Manual of Clinical Immunology*. American Society for Microbiology. 1976. pp. 382-409

Kandasamy, A. and Devada, K. 2011. Feasibility of coproantigen detection in amphistomosis. *J.Vet Anim.Sci.,* **42**: In press

Rahman, S. M. A., Reilly, K. L. O. and Malone, J. E. 1999. Biochemical characteri zation and localisation of *Fasciola hepatica* 26-28 kDA diagnostic coproantigens. *Parasite Immunol*., **21**: 279-286.

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