



# FEASIBILITY OF COPROANTIGEN DETECTION IN AMPHISTOMOSIS\*

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## Abstract

Detection of antigens in faecal supernatants is a more reliable and suitable method, in cases where coprological examination fails to reveal any parasitic ova. Hundred cattle known to be infected with *Gastrothylax crumenifer*, the most prevalent amphistome species were selected at random for the study. An indirect ELISA was performed to detect coproantigens in faecal supernatants of these infected cattle using rabbit hyper immune serum against somatic antigens of the fluke. Seventy four samples were found to contain detectable amounts of coproantigens indicating a sensitivity of 74 per cent for the test. The results indicate the feasibility of ELISA for the detection of coproantigens of amphistome infections.

**Keywords:** Coproantigen, ELISA, Amphistomes

The Indian livestock industry suffers much due to fatal enteritis caused by flukes of various species (Mukherjee and Deorani, 1962). Nath (1987) reported that *Gastrothylax crumenifer*, a species of amphistome accounted for the highest prevalence (62.64 %) among other 17 species in Kerala. Conventional methods of diagnosis of amphistomes like detection of clinical signs, parasitic ova and necropsy are cumbersome and limited. These hardships are overcome by serological techniques especially detection of parasitic antigens in biological samples (Johnson *et al.*, 1996). As coproantigens are shed by both immature and mature flukes, it paves way for early detection of the disease

condition. This paper ensures the feasibility of coproantigen detection employing an indirect ELISA in the detection of amphistomosis in cattle. This study appears to be the first of its kind from the country in the diagnosis of amphistomosis.

## Materials and Methods

Live flukes from the rumen were collected in chilled saline from infected cattle slaughtered at the Municipal slaughter house, Thrissur. 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 was raised in rabbits (Johnson *et al.*, 1996) and the sera was 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° C. 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.

Coproantigens were prepared by the method described by Ahmad and Nizami (1998). The faecal supernatant was used as the source of antigen. The protein content was determined by Biuret method.

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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. 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.

An indirect ELISA was performed as described by Ahmad and Nizami (1998) in 96 well flat bottomed microtitre plates. They were read at 450nm 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 coproantigens, anti somatic HIS and goat anti rabbit IgG –HRP used in the present work were 1:64, 1:100 and 1: 100 respectively.

In the indirect ELISA for detection of coproantigens, the cut-off point was determined by the sum of the mean optical density (OD) value of the negative controls and three times the standard deviation. A sample was considered positive for coproantigens, if its OD value was higher than the cut-off point. The cut-off point in the present study was 0.225. Using this criterion, 74 faecal samples were found to be positive for coproantigens. The sensitivity of copro antigen ELISA was 74 per cent .

Although the above results were in accordance to Maleewong *et al.* (1997) and Moustafa *et al.* (1998), Ahmad and Nizami (1998) and Rahman *et al.* (1999) were able to obtain a higher sensitivity of 100 percent. The utilisation of somatic antigens of *G. crumenifer* to raise antiparasitic antibodies in rabbits might be a reason for the difference in the sensitivity of the test, when compared to those utilizing excretory /secretory antigens of

parasites. Another factor that could have affected the sensitivity of coproantigen ELISA is the storage of coproantigens for varying periods at varying temperatures.

It is worthy to note that in the present study, coproantigens could be detected in faecal supernatants stored upto eight weeks at -20°C concurring with the results of Deplazes *et al.*(1990) and Craig *et al.* (1999).

The present study indicates that ELISA for coproantigen detection in amphistomosis is relatively easy to handle and appears feasible for field use. The findings encourage research in the detection of coproantigens for the diagnosis of immature amphistomosis when faecal examination does not reveal any ova.

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