PERFORMANCE CHARACTERISTICS OF *FASCIOLA GIGANTICA* CATHEPSIN-L CYSTEINE PROTEINASE (FgCL3) BASED DIPSTICK ELISA IN NATURALLY ACQUIRED BUBALIAN FASCIOLOSIS

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**ABSTRACT**

Buffaloes are the important multipurpose farm animals in the Indian sub-continent, contributing significantly to meat and milk production. The humoral immune responses to *Fasciola gigantica* cysteine proteinase in experimentally infected buffaloes have been widely studied. However, scarcely any literature is available on serodiagnosis of bubalian fasciolosis using purified cysteine proteinase under field conditions. In the present study, cysteine proteinase dot–ELISA using dipstick (dipstick - ELISA) was developed for the detection of natural *F. gigantica* infection. Faecal and serum samples were collected randomly from buffaloes (n=100) slaughtered at a local abattoir. Serum samples of buffaloes revealed 54 positive cases in Dipstick–ELISA, out of which only 35 were coprologically positive. The sensitivity, specificity and accuracy of cysteine proteinase dipstick–ELISA under field conditions were 100% whereas corresponding values for coprological examination were 62%, 100% and 80%, respectively. It was concluded that dipstick–ELISA using cysteine proteinase demonstrated a high prevalence of fasciolosis and could be a feasible diagnostic method for detection of natural *F. gigantica* infections in buffaloes.

**Keywords:** buffalo, dipstick – ELISA, *Fasciola gigantica*, FgCL3, Immunodiagnosis

**INTRODUCTION**

Tropical fasciolosis caused by *Fasciola gigantica* in buffaloes is asymptomatic, subclinical and/or chronic form of the disease, adversely affecting their reproductive cycle, weight gain, food conversion efficiency and productivity. The host suffers from unnoticed ill effects of the disease for a prolonged period before the disease is detected at veterinary clinics and/or the abattoir (Edith *et al.*, 2010). The best way of establishing and evaluating control programs for fasciolosis is to have available diagnostic tests that provide information about herds and/or animals in which infection by *Fasciola* causes production losses. This would make it possible to limit treatments to only those animals that really need it, thereby reducing costs.

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avoiding the emergence of resistance, promoting the development of natural immunity to infection and ultimately, optimizing the use of flukicides.

Traditional diagnosis of liver fluke infection is by detection of eggs in faeces or flukes in the liver and bile ducts post-mortem. One disadvantage of the faecal egg counting technique is that it detects the presence of *Fasciola* eggs in the faeces only about 13 weeks post-infection. Serological tests are highly sensitive epidemiological tools for the detection of the disease but their application is limited by cost and expertise in most developing countries. However, over the past 25 years a number of highly sensitive and specific diagnostic tests have been developed that are increasingly replacing the coprological techniques (Dixit et al., 2008). These tests are based on the detection of *Fasciola* antigens in serum and faeces (Mezo et al., 2004, Valero et al., 2009) or on detection of *Fasciola*-specific antibodies in serum (e.g. Salimi-Bejestani et al., 2005, El Ridi et al., 2007). Antibody-detection tests are in widespread use and several commercial tests are available. With the recent development of 28kDa *F. gigantica* Cathepsin-L cysteine proteinase (FgCL3) based dipstick ELISA (Gupta et al., 2011), an important tool has been provided for the diagnosis of fasciolosis in naturally infected animals. The objective of the present study was to compare the diagnostic efficiency of faecal examination with FgCL3 based dipstick-ELISA using the true infection status of the autopsied buffaloes as a gold standard.

**MATERIAL AND METHODS**

**Faecal and serum samples**

Synchronously faecal samples and 5 ml of blood from each buffalo (n=100) were randomly collected from the large ruminant slaughter house, located at Madartekri, Jabalpur. Blood was allowed to clot and serum was separated and brought to laboratory in Eppendorf’s tubes while maintaining cold chain and stored at -20°C. On spot, necropsy findings regarding presence of the fluke (mature as well as immature) were recorded for each autopsied buffalo.

**Coprological examination**

Standard coprological techniques were performed for assessing the infection (Sloss et al., 1994).

**Dipstick-ELISA**

For antigen preparation the standard protocol described previously by Dixit et al. (2002) was followed. Purity of the protein was checked on SDS-PAGE (15%) under non-reducing conditions (Laemmli, 1970). Optimum concentration of the referral antigen, required for dipstick-ELISA has been determined by chequer board titration. Nitro-cellulose membranes attached to plastic sticks (Microdevice, Ambala), was dotted with 2 µg antigen per stick. After drying the sticks in incubator at 37°C for 1 hr, they were stored at 4°C until required.

Dipstick-ELISA was performed according to the method described by Dixit et al. (2002) with a few modifications. The dotted sticks were washed with PBS plus 0.05% (v/v) Tween-20 (PBS/T) and blocked with a solution containing 5% skimmed milk powder in PBS/T (incubation buffer) by incubating at 37°C for 1 h. The sticks were washed three times for 15 minutes with PBS/T (washing buffer) and incubated for 1 hour at 37°C with serum samples diluted 1:200 in incubation buffer. The sticks were given three washings and were again incubated for 1 hour at 37°C with rabbit anti-
bovine peroxidase conjugate at 1:200 in incubation buffer. Finally, three washings were given and the sticks were incubated in the Diaminobenzidine (DAB) substrate buffer (Genei) for the colour development of the dot. After the development of the dot, in known positive samples the reaction was immediately stopped by dipping the strips in the distilled water. The strips are then dried and examined for the development of the dot.

**Sensitivity, specificity and accuracy**

Sensitivity means the ability of a test to correctly identify the percentage of those who have the disease; while specificity means the ability of a test to correctly identify the percentage of those who do not have the disease; whereas accuracy describe the degree to which a measurement reflects the true status of what is being measured and it is used to express the overall performance of a diagnostic test. The gold standard for calculation of the test performance was the true fluke status of the buffaloes. The sensitivity, specificity and accuracy of coprological examination and dipstick-ELISA were calculated using following formulas.

- **Sensitivity** = \( \frac{T_+}{T_+ + F_-} \times 100 \)
- **Specificity** = \( \frac{T_-}{T_- + F_+} \times 100 \)
- **Accuracy** = \( \frac{T_+ + T_-}{T_+ + T_- + TN} \times 100 \)

where; \( T_+ (=\text{true positive}), \) \( T_- (=\text{true negative}) \), \( F_+ (=\text{false positive}), \) \( F_- (=\text{false negative}) \) and \( TN (=\text{total number}) \).

**RESULTS**

**Parasitological results**

Livers of 100 buffaloes were examined for the presence of immature and mature *F. gigantica*. Liver of animals revealing flukes on autopsy constituted Group 1 (\( n = 54 \)) whereas animals failed to demonstrate juvenile and/or adult fluke in hepatic tissue constituted Group 2 (\( n = 46 \)), regardless of infection with other helminth parasites. Coprological examination of buffaloes of both the groups revealed that 89% of the buffaloes harboured helminth infections out of which 87% were mixed infection. Prevalence of amphistome (67%) was highest followed by strongyles (57%), coccidia (43%), *F. gigantica* (34%) and *Strongyloides* spp. (19%).

**Dipstick-ELISA**

It was observed that the immunological findings were positive for Group 10. The results of dipstick–ELISA showed all 54 buffaloes positive for fasciolosis, out of which only 34 were coprologically positive, rest of the 20 buffaloes did not reveal infection in coprological examination. No false positive cases were detected in coprological as well as dipstick–ELISA. Hence the sensitivity, specificity and accuracy of dipstick–ELISA were 100% and of coprological examination were 62%, 100% and 80% respectively.

**DISCUSSION**

Coprological examination revealed a sensitivity of 62% which is in agreement with the previous work of Braun *et al.*, (1995) who reported 68% sensitivity mainly due to discontinuous shedding of eggs. The sensitivity can be improved to 90% when taking three examinations of one sample (Rapsch *et al.*, 2006). However, this rather time consuming approach is unsuitable for diagnosis on the herd level.

Enzymatically active Cathepsin L cysteine proteinase from *F. gigantica* was isolated from adult fluke E/S material and a Dot–ELISA assay
utilizing dipsticks (Dipstick – ELISA) was standardized for its application in field. In our previous studies on 28 kDa cysteine proteinase for the detection of experimental *F. gigantica* infection in sheep, the test evidenced positive reaction at 4 wpi with weekly pooled sera (Dixit *et al*., 2002). These results highlighted for the first time the possibilities and advantages of using pure antigen for the diagnosis of experimental *F. gigantica* infection. Weekly pooled sera of experimentally infected buffaloes when subjected to Dipstick – ELISA revealed antibodies at 2 wpi (Dixit *et al*., 2004). Further, no cross reactivity was observed with weekly pooled sera of buffaloes and goats experimentally infected with *Paramphistomum epiclitum* (Dixit *et al*., 2003).

Varghese *et al.* (2011) reported 90% sensitivity and 100% specificity of cathepsin-L cysteine proteinase based Dot-ELISA in the detection of bubalian fasciolosis. Additionally, sera from 156 *Fasciola*-free buffaloes, yet infected with *Gigantocotyle explanatum, P. epiclitum, Gastrothylax spp.*, *Strongyloides papillosus* and hydatid cyst were all negative, indicating that *F. gigantica* cathepsin-L cysteine proteinase does not cross-react with these helminth parasites in natural infection of the host. Furthermore, no tests currently available can be considered as having both 100% sensitivity and 100% specificity. Recently, the 28 kDa cathepsin-L cysteine proteinase of *F. gigantica* has shown diagnostic potential in goats with 100% sensitivity, specificity and accuracy (Gupta *et al*., 2011). In the present investigation also the sensitivity, specificity and accuracy reached 100% as all the buffaloes which were positive by liver examination, were found positive by dipstick ELISA. These results demonstrate that the prevalence of fasciolosis is higher than previously thought due to low sensitivity of coprological examination.

The serological findings reported herein confirmed earlier work of Varghese *et al.* (2011). Our results indicate that the detection of antibodies in serum samples may provide useful information about the status of *F. gigantica* infection in naturally infected animals regardless of other helminth infection if purified antigen like FgCL3 is used. Thus FgCL3 dipstick ELISA is suitable for routine veterinary diagnostic use as an alternative to plate ELISA. Dipstick ELISA would be more suitable when small numbers of animals are tested. They may be useful for the disease forecasting, assessment of the efficacy of drugs against early stages of the parasite and in time application of control strategies.

**REFERENCES**


