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Evaluation of dipstick–ELISA using 28 kDa *Fasciola gigantica* cathepsin L cysteine proteinase (FgCL3) for serodiagnosis of fasciolosis in naturally infected goats

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ABSTRACT

In the present investigation 100 goats presented for slaughter were used to evaluate the 28 kDa cathepsin L cysteine proteinase (FgCL3) dipstick–ELISA for the diagnosis of fasciolosis. Presence of *Fasciola gigantica* worms in liver at the time of slaughter was taken as a gold standard for the evaluation of the assay. Faeces, blood and liver were taken from all slaughtered goats. Biochemical parameters and coprological examinations using sedimentation technique were also taken into consideration. Total serum proteins, albumin, mean values of aspartate aminotransferase and alkaline phosphatase of positive group were not significantly different from those of the negative group. The diagnostic sensitivity of dipstick–ELISA was higher than coprological detection of fluke eggs. However, specificity was equal for both the tests. The accuracy of dipstick–ELISA in detecting circulatory antibodies during the course of disease was higher than coproscopic examination. In conclusion, the FgCL3 antigen dependent dipstick–ELISA was more reliable and has better scope for field application in the endemic areas.

1. Introduction

Goats are extremely sensitive and susceptible to natural and experimental *Fasciola* infections (Reddington et al., 1986). In adults, the disease usually appears in a chronic form whereas young stocks (<1 year) suffer from acute course of the disease with high rate of mortalities (Leathers et al., 1982). Conventionally, the diagnosis of this disease in ruminants is relied upon copro demonstration of *Fasciola gigantica* eggs. Coprological examination cannot be used during pre-patent period of the disease (Yadav et al., 1999), which corresponds to the major pathogenic effects. It is therefore desirable to have a simple, sensitive and specific test for the early diagnosis of fasciolosis in goats.

Serum biochemistry of infected animals can be a good indication of the extent of tissue damage and the severity of the infection. Sheep and goats experimentally infected with *F. gigantica* exhibit significantly altered levels of plasma proteins, aspartate aminotransferase (AST) and alkaline phosphatase (ALP) etc. These can be exploited as marker enzymes based upon stages of experimental *F. gigantica* infections (Mbuh and Mbwaye, 2005; Ahmed et al., 2006). There have been limited studies of the serum biochemical changes in goats naturally infected with *F. gigantica* (Swarup et al., 1986).

Another alternative for early detection of fasciolosis during prepatency was demonstration of anti fluke antibodies using cysteine proteinases derived from

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excretory–secretory products (ESP). These molecules elicit strong immune responses during natural infection. 28 kDa *F. gigantica* cathepsin L cysteine proteinase (FgCL3) based ELISA was used in immunodiagnosis of experimental and/or natural *F. gigantica* infections in sheep (Dixit et al., 2002), buffaloes (Dixit et al., 2004; Raina et al., 2006) and cattle (Sriveny et al., 2006). In our earlier attempts to use the protease for pre-patent detection of experimental *F. gigantica* infection in sheep and buffaloes, harbouring different degrees of mature and immature fluke, were successful to detect the *in situ* infection at 2–4 weeks post infection (Dixit et al., 2002, 2004), but the antigen has yet to be assessed in *F. gigantica* infected goats. In experimental *F. gigantica* monoinfection in ovine, bovine and bubalian hosts, the FgCL3 showed 100% sensitivity (Dixit et al., 2002, 2004; Sriveny et al., 2006). However, under field situation of natural *F. gigantica* infection in buffaloes, this sensitivity declined to 97.1% but specificity of the test remained 100%. Furthermore, no tests currently available can be considered as having both 100% sensitivity and 100% specificity.

The aim of this work was to standardize a simple and rapid dipstick–ELISA for diagnosis of natural caprine fasciolosis using 28 kDa FgCL3 of adult flukes. The dipstick–ELISA was evaluated using the true infection status of the autopsied goats as a gold standard and the results were compared to coproscopic and biochemical tests.

2. Materials and methods

2.1. Parasites

Flukes were collected from infected liver of buffaloes slaughtered at Large Animal slaughter House, Jabalpur. Infected liver was brought to the laboratory and viable, intact flukes were collected from the liver and kept in luke warm PBS. Damaged flukes were not processed for preparation of antigen and were discarded.

2.2. Faecal and serum samples

Synchronously faecal samples and 5 ml of blood from each goat ($n = 100$) were randomly collected from the small ruminant slaughter house, located at Madartekri, Jabalpur. Blood was allowed to clot and serum was separated and brought to laboratory in eppendrof 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 goat.

2.3. Biochemical analysis

Biochemical parameters were analyzed using diagnostic kits in auto analyzer (HYCEL diagnostics, Model-Celly 40, SR-C1240).

2.4. Coprological examination

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

2.5. Purification of FgCL3

For antigen preparation the standard protocol described by Dixit et al. (2002) was followed. Extensively washed viable flukes were incubated (one fluke per ml) in RPMI-1640 (pH 7.3) containing glucose, 30 mM HEPES and 40 mg/ml gentamycin. After 3 h of incubation at 37 °C, the culture medium containing *in vitro* released regurgitant was centrifuged at 6300 × *g* at 4 °C for half an hour and then supernatant was frozen at −20 °C until required. The regurgitant was thawed; cold ethanol was added drop by drop until final ethanol concentration of 60% (v/v) was achieved. The mixture was equilibrated at −20 °C for 18 h and pelleted at 6300 × *g* at 4 °C for half an hour. The pellet was discarded and the supernatant was taken up to a final concentration of 75% (v/v) ethanol equilibrated overnight at −20 °C. Again the supernatant was thawed and centrifuged at 6300 × *g* at 4 °C for half an hour. The pellet obtained from 75% ethanol precipitation was washed in absolute ethanol, dried and resuspended in distilled water. The enzyme was aliquoted and stored at −20 °C. Protein estimation of the antigen was done by Nano-Drop Spectrophotometer (Nano-Drop Technology, USA).

2.6. Sodium do-decyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Purity of the protein was checked on SDS-PAGE (15%) under non-reducing conditions (Laemmli, 1970). Protein was electrophoresed at 50 V through the stacking gel and 100 V through the separating gel until tracking dye reached the bottom of the gel. The gel was stained with 0.1% coomassie stain and destained using 7.5% acetic acid.

2.7. Dipstick–ELISA

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 h, they were stored at 4 °C until required.

Dipstick–ELISA was performed according to the method described by Dixit et al. (2002) with 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 min with PBS/T (washing buffer) and incubated for 1 h at 37 °C with serum samples diluted 1:50 in incubation buffer. The sticks were given three washings and were again incubated for 1 h at 37 °C with rabbit anti-goat HRP (Genei) at 1:800 dilutions 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.