Serodiagnosis of *Oesophagostomum Columbianum* infection in Goats using indirect ELISA

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Abstract

To determine the seroprevalence of *Oesophagostomum columbianum* antibodies in goats in Kashmnir, a study was conducted in Srinagar and Ganderbal Districts, located in central parts of Kashmir valley. ELISA was standardized and evaluated to detect goat Oesophagostomosis in experimental and clinical cases using somatic whole adult antigen of *Oesophagostomum columbianum*. Plate ELISA was standardized using 5µg/well antigen concentration with 1:100 and 1:1000 of sera and conjugate dilution. Indirect plate ELISA was able to demonstrate the antibody titer at different weeks post infection in experimental goats. A comparison of plate ELISA on suspected field sera and faecal sample examination by floatation method revealed that 68 (34.34%) samples were found to be positive using ELISA and twelve by faecal examination. The highest prevalence was found in lower age groups in both the seasons, with the increase in age, the infection level decreased. The prevalence was higher in wet season as compared to dry season. The results were stastically significant ($P < 0.05$). No sex predisposition was seen. Sensitivity and specificity of ELISA was 80.0%, and 21.42%, respectively. This test is therefore quite sensitive for clinical cases; an early diagnosis, however lacks specificity of faecal examination were 10.81% and 90.0%, respectively.
Key Words: ELISA, *Oesophagostomum columbianum*, Goat, Somatic antigen

Introduction

Gastrointestinal nematode infection is arguably the most serious constraint affecting small ruminant production worldwide. Economic losses are caused by decreased production, the costs of prophylaxis and treatment, and the death of the infected animals (Barger, 1982; Donald and Waller, 1982).

Oesophagostomum columbianum is a common and widely prevalent parasitosis with significant economic importance in small ruminant livestock (Olivares *et al*., 2001) throughout the Asian sub-continent (Khan, 1989; Mohanta *et al*., 2007).

Caprine oesophagostomosis, like most of the helminth infections, is insidious and chronic in nature and the migratory prepatent stages of the parasite are involved in its major pathogenic effect. Reliable detection of the active infection of *O. columbianum* is usually based upon evaluation of clinical signs and faecal examination, which have their inherent limitations. Clinical signs usually become apparent only when the infection is heavy and the eggs are passed in the faeces after the prepatent period of approximately 41 days (Soulsby, 1982) when the infection is much advanced and the major damage is already done. In order to circumvent these limitations there is an acute need for developing a reliable serological assay like Enzyme Linked Immunosorbent Assay (ELISA) for early detection of the infection. Detection of serum antibodies against parasite by ELISA is a rapid and simple test with which a considerable number of samples could be processed at the same time. Furthermore, seroepidemiological studies involving examination of large group of animals might also benefit from a reliable ELISA. Usually such a test, in contrast to faecal examination, is less time consuming.

Therefore an indirect ELISA based on crude somatic antigen of *O. columbianum* was standardized and evaluated under field conditions as a diagnostic tool for detection of anti-*O. columbianum* antibodies in sera of infected goats.

Materials and Methods

Experimental animals: Four health local goats, 30–45days old, were maintained under intensive rearing conditions precluding accidental parasitic

infections. At the age of five months two goats were used for experimental infection with L3 of *O. columbianum*.

Collection of *O. columbianum* L3 and experimental infection : The infective L3 were obtained by culturing (Soulsby, 1982) the eggs separated from the adult female worms recovered from the caecum and colon of slaughtered goats. The L3 at the dose rate of 600 per kg body wt. were orally administered in one of the 8 helminthes free goats, after overnight withdrawal of feed, to serve as donors for sufficient number of monospecific *O. columbianum* eggs for artificial infection. After the patency of the infection the faeces of the donor goats was cultured (Soulsby, 1982) and the L3 were harvested (Anon, 1971). These L3 were used for artificial infection of two goats as stated earlier keeping the remaining two goats as uninfected control. Serum samples of the infected as well as the control goats were collected on every third day post-infection (DPI) till 33 DPI, following the standard methods and they were preserved at -20 °C for use in the assay.

Antigen Preparation: For preparation of crude somatic antigen (CSAg) *O. columbianum* from the caecum and colon of freshly slaughtered goats was collected (Johnson *et al*., 2004) in a Petri dish containing 0.15 M phosphate buffer saline (PBS), pH-7.2. The worms were washed 3 times in the same buffer and finally 200 worms were homogenized in 10 ml of cooled 0.15 M PBS (pH-7.2) containing 25 mM phenylmethyl sulfonyl fluoride (PMSF) and 24 mM ethylene- diamine tetraaceticacid (EDTA) in a glass tissue homogenizer followed by sonication (Soniprep-150). The disintegrated parasite extract was centrifuged at 4°C at 10000 *g* for 15 minutes and the supernatant was collected as the CSAg with a protein concentration of 3.48 mg/ml specified according to Lowry *et al.* (1951). The crude somatic antigens were stored at - 20 °C for use in the assay.

Standardization of the assay: Plate ELISA was performed in 96 wells polystyrene microtitre plates with all incubation times previously determined by checkerboard titration following Hudson and Hay (1989). The antibody detection was performed according to the method of Voller *et al*. (1976) with some modifications. The optimal concentration of ELISA reagents including the concentration of the coating antigen (5µg/well), dilution of the positive and negative reference sera (1:100) as well as rabbit anti-goat IgG-horseradish peroxidase (HRP) conjugate (1:1000) and the optimal test conditions, respectively were determined by checkerboard dilution assay using flat-bottom 96-well micro-ELISA plate. The absorbance (optical density; OD) of the wells was measured at 492 nm by an ELISA reader (Biorad II). The mean OD plus three times the standard deviation of the negative control sera was taken as the cut-off value for considering a sample as test positive (Lejon *et al*., 2005).

Performance of the assay: The sensitivity, specificity and accuracy of the ELISA were determined (Thrusfield, 2003) using 198 serum samples of field goats. The parasitological status of animals with regard to nematodes trematode and cestodes was carefully examined by floatation technique and recorded. The sensitivity, specificity, positive predictive value and negative predictive value were calculated from the two way analysis of ELISA test and faecal examination using following formulas:

Sensitivity = Sample positive by both tests / Samples positive by reference test (ELISA) X 100

Specificity = Sample negative by both tests / Samples negative by reference test (ELISA) X 100

Positive Predictive Value = Sample Positive by both tests / Samples Positive by Target test (ELISA) X 100

Predictive value = Sample negative by both tests / Samples negative by target (ELISA) X 100

Results

From a total of 198 goats, 68 (34.34%) were positive for *Oesophagostomum columbianum.* After pooling the data, age wise epidemiological observations were made which revealed highest prevalence rate in lower age groups in both the seasons, with the increase in age, the infection level decreased (Table 1). The prevalence was higher in wet season as compared to dry season. The results were statistically significant ($P < 0.05$). No sex predisposition was seen.

The highest OD value (1.23) in the sera of experimentally infected goats was observed after 33 DPI and the cut-off OD value for the standardized ELISA as determined was 0.168. *O. columbianum* antibodies in all experimentally infected goats were detected as early as 18 to 27 DPI. The seroconversion was relatively long before the patency of the infection, which in this study was on 42 DPI. Specific antibodies were consistently present during the subsequent observation period i.e. till 33 DPI.

A total of 198 goat samples suspected for Oesophagostomosis were examined by indirect plate ELISA. Sera of control group (negative goats) and experimentally infected (positive goats) were raised and were taken as reference sera for ELISA. A cut off value of means of negative controls ±3 SD was taken into account for the detection of each positive case.

Table-1. Seroprevalence of *Oesophagostomum columbianum* in different seasons and age groups (N = 198)

Age	Dry Season		Wet Season	
	Examined	Positive (%)	Examined	Positive (%)
<1 year	40	21 (52.50)	31	20 (64.51)
1-4 years	34	8 (23.52)	24	7 (29.16)
>4 Years	36	6 (16.66)	33	6 (21.21)
	P = 0.04		P = 0.03	
Total	110	35 (31.81)	88	33 (37.50)

Sensitivity of plate ELISA was found to be 80.0% whereas specificity was 21.42%, indicating that this test is quite sensitive for clinical cases: an early diagnosis however lacks specificity. The plate ELISA positive and negative predictive values were found to be 10.81% and 90.0%, respectively.

A marked difference was observed between the proportion of ELISA positive and faecal examination (floatation) negative samples. Sixty eight samples were found to be positive by ELISA but only twelve samples were found positive by faecal examination indicating that ELISA is significantly more sensitive method.

Discussion

Diagnosis of gastrointestinal nematode infections has conventionally relied upon detection of the clinical signs, aided by qualitative detection of the eggs in the faeces of suspected animals. Effectiveness of these methods is however since clinical signs are apparent only in heavy infection and the faecal eggs are detected in Oesophagostomosis infection only after its patency on approximately 41 days (Soulsby, 1982) when the major damage is already done by the parasites. It is thus imperative to detect the infection at this stage for minimizing the associated economic losses, especially in weaned kids and lambs.

The standardized ELISA using easily available crude somatic antigen yielded promising results for detection of prepatent and patent Oesophagostomosis in goats. In experimentally infected goats the infection was detected between 18 – 27 days after the infection and it was obviously much earlier than the time required for the infection to reach the patent period (42 days in the present study). ELISA is known for its potential to detect the antibodies at a quite early stage of the infection. Nevertheless, it could detect the artificially induced Oesophagostomosis only after 17 days of the infection. The indirect ELISA was evaluated on field sera and the results were compared with the post-mortem findings with regard to the actual parasitological status. The assay proved high sensitivity (80.20%), specificity (21.42 %) and holds considerable promise for its exploitation in seroepidemiological studies of this economically significant helminthosis. Sequestration of antibodies and the formation of circulating immune complexes (Gasser *et al*., 1993) and immune evasion mechanisms of the parasite (Spinelli *et al*., 1996) might be responsible for wide variations in the antibody level in the necropsy positive samples as shown by indirect ELISA. Two false negative results in the present assay might be due to low worm burden or poor immune response of the host (Gasser *et al*., 1994). Besides, host nutritional status (Jenkins *et al*., 1991; Gasser *et al*., 1992) and physiological and environmental factors like re-infection or co-infection with other parasites (Carmena *et al*., 2005) might also have an impact on the antibody levels. False positive result of the assay with postmortem negative samples might be due to the persistence of antibodies to the past infection, which might have been eliminated by anthelmintic medication. False positive result is probably due to cross reactivity of *O. columbianum* with other helminths. Cross reactivity amongst different helminths is a common and limiting factor in the development of serological tests against helminth infection (Philipp & Rumjaneck 1984; Cuquerella *et al*., 1994; Molina *et al*., 1999). The short comings of antibody ELISA in the diagnosis of helminth infections would best be overcome using antigen detection ELISA. Copro-ELISA assay could be the best possible tool for detection of current infection of gastrointestinal parasites and attempts in this regard have already been made (Johnson *et al*., 2004; Jas *et al*., 2010). The standardized assay offers the potential for its development as one of good diagnostic tools for Oesophagostomosis. The use of purified and recombinant antigens may minimize the cross reactivity with other helminthes, thus these antigens might be a good candidate for immunization and diagnosis of Oesophagostomosis in ruminants.

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