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Studies on antigenic cross-reactivity of *Trichuris ovis* with host mucosal antigens in goat

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ABSTRACT

Objective: To ascertain whether immunodominant antigens of *Trichuris ovis* might share and cross react with host molecule.
Methods: Two crude protein preparations from anterior and posterior parts of *Trichuris ovis* were characterized along with host mucosal antigen by double immunodiffusion, sodium dodecyl sulfate-polyacrylamide gel electrophoresis and western blotting technique. Conventional scanning electron microscopy was performed as per standard procedure.
Results: Sharp and distinct bands of three antigens have been found in double immunodiffusion using hyperimmune serum raised in rabbit indicating the presence of specific antibody against each antigen. All three antigens have shown major and minor bands with molecular weight ranging from 15 to 110 kDa during sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
Conclusions: The antigenic cross-reactivity was thought to result from shared antigens. The existence of paracloacal papillae found in the anterior part of the male was not a unique feature for species differentiation.

1. Introduction

Trichuris ovis (*T. ovis*) infects the caecum and colon of sheep, goats, cattle and other ruminants in all parts of the world but is relatively harmless. However, clinical diseases due to *T. ovis* have been reported in sheep and cattle[1]. Heavy infection may be observed in very young lambs or during drought conditions when sheep offered grain on the ground. In such cases, congestion and edema of the caecal mucosa, accompanied by diarrhoea and unthriftiness are seen and terminally there is weight loss and anaemia. When present in large numbers, the worms cause a haemorrhagic colitis and/or a diphtheritic inflammation of the caecal mucosa. This results from the subepithelial location and continuous movement of the anterior end to the whipworm as it searches blood and fluid.

It appears that this species has merited little attention, probably because of the low numbers normally found in sheep and goat and other ruminants. It also may be that specific identification has not always been made. Immune-mediated expulsion of gastrointestinal nematode from the gut is still a subject of considerable investigation[2]. It is well known that gastrointestinal nematodes release a variety of immunogenic molecules, either excretory or secretory products into the immediate vicinity. But the roles of these immunogenic molecules are not yet established[3].

Recent work has shown that high and low responder strains of rodents show contrasting cytokine profiles, the former being associated with T helper cell response, while the later with Th2 response[4]. Interestingly, low responders appear to switch from Th1 to Th2 during infection, assuming under the influence of worm derived immunomodulatory molecules. If this data can be exploited to the situation in ruminants, it can imply that the outcome of infection is not predetermined but reflects the interaction of parasitological and immunological influence of both host and parasite origin.

The whipworms of ruminants are closely associated with the intestinal mucus barrier. Using established gastrointestinal

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nematode model *T. ovis*, *Trichinella spiralis* and *Nippostrongylus brasiliensis*, several workers demonstrated that mucins are critical in resolving infections[5-7]. Keeping in view that under field conditions, gastrointestinal nematodes can survive for long period of time. It remains to be seen how these parasites interact within the mucosal barrier and suppress the responses against them. In this study, we attempted to investigate the antigenic cross-reactivity of *T. ovis* somatic antigens with mucosal antigens. In addition to this, a scanning electron microscopy (SEM) was also carried out to clearly visualize the adult *T. ovis in situ* as well as to detect unique morphological structure of the posterior part of the male parasite, if any.

2. Materials and methods

2.1. Collection of adult parasites

For collection of *T. ovis*, the caeca of slaughtered goat were collected from the local abattoir. In the laboratory, the individual caecum was incised and the live adult worms, both males and females from the caecal mucosa were collected in 0.15 mol/L phosphate buffer saline (PBS) (pH 7.2) with the help of forceps. The worms were then washed thrice in normal saline followed by five times washings in 0.15 mol/L PBS. Finally, the anterior and posterior parts of the worms were separated and kept in separate vial with 0.15 mol/L PBS for the preparation of somatic antigens.

2.2. Collection of mucosa

To collect the mucus from caecal mucosa, the mucosa was gently flushed with PBS to remove the faecal matter, and then scraped lightly with a scalpel blade and the scraped material was then put into a vial with an equal volume of PBS and was stored at -30 ℃ until required.

2.3. Preparation of crude antigens

The anterior and posterior parts of *T. ovis* as well as collected mucosa were homogenized separately in chilled 0.15 mol/L PBS (pH 7.2) containing phenyl methyl sulfonyl fluoride (25 mmol/L) and ethylenediaminetetraacetic acid (24 mmol/L). Each part was dissolved in 10 mL of PBS solution and homogenized in a glass tissue homogenizer (Remi RQ-127A, India) under strict aseptic conditions.

The homogenizer extracts, each in an ice packed beaker were sonicated for 15 cycles for one minute each in an ultrasonicator (Model-US 50; Nissei Co, Tokyo, Japan). The sonicated materials were spun at 10 000 r/min for 45 min in a refrigerated centrifuge (Remi, C 24, India) at 4 ℃. The supernatant containing the crude antigen was collected in separate vial and labeled accordingly. Protein concentration of each antigen was estimated as per the method[8].

2.4. Raising hyperimmune serum (HIS)

HIS, against each antigen was raised in rabbit as per method described earlier[9] with some modifications that encompassed increased doses. Briefly, three New Zealand white male rabbits weighing 1 200 g were injected intramuscularly with 5 doses of crude antigen, mixed with equal volume of Freund's adjuvant (Sigma, USA) at 10 days interval with increased subsequent doses ranging from 2.5 mg to 500 mg per injection. First dose was given with Freund's complete adjuvant and subsequent 4 doses with Freund's incomplete adjuvant. One rabbit of same breed, sex and weight was also maintained without immunization to collect normal serum.

2.5. Double immunodiffusion test

For monitoring the antibody level in hyperimmunized rabbits against three antigens, the double immunodiffusion test was performed as per the methodology[10] with slight modification. Concentration of the antibody in the HIS was visually assessed by the sharpness and thickness of the bands.

2.6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Crude antigens were analyzed by SDS-PAGE as per method described elsewhere[11] using 12.5% polyacrylamide gel in a vertical mini slab gel electrophoretic apparatus (Atto, Japan). The samples were mixed with sample buffer in a proportion of 1:1 and subsequently the solution was heated at 100 ℃ for 3 min. The amount of protein applied was 50 μg per track. Proteins were run at 18 mA for 150 min. The bands were visualized by staining with coomassie blue R-250. Standard molecular weight marker (PMW-M, Genei, India) was run parallel along with sample proteins to determine the relative molecular weights of the polypeptides.

2.7. Western blotting analysis

Anterior and posterior parts of *T. ovis* and mucus part were separated on a 12.5% SDS-PAGE, and electro transferred onto nitrocellulose membrane on a semi-dry blotting unit (Atto, Japan) as per procedure[12] with some modifications. The blotted proteins were subjected to immunoblot analysis with anti-anterior, anti-posterior and anti-mucous goat polyclonal antibodies and anti-goat horseradish peroxidase antibodies (Genei, India). Standard molecular weight marker (Fermentas) was run parallel along with sample proteins to determine the relative molecular weights of the polypeptides.

2.8. Conventional SEM

Intestinal mucosa embedded with parasites and few adult female parasites were gently removed with the aid of a round brush and fine forceps and immediately transferred to a tube containing a fixative solution that consisted of 2.5% glutaraldehyde and 4% freshly prepared formaldehyde in 0.1 mol/L cacodylate buffer (pH 7.2). Samples were fixed for 1 h at room temperature and 12 h at 4 ℃, then subsequently washed in 0.1 mol/L cacodylate buffer (pH 7.2) and post-fixed in 1% OsO_4 and 0.8% $K_3Fe(CN)_6$. Then

samples were washed in 0.1 mol/L cacodylate buffer (pH 7.2), dehydrated in a graded ethanol series (20°–100° GL) for one hour each step; critical point dried in CO_2, mounted on metallic stubs and coated. The samples were examined under SEM.

3. Results

The subepithelial location of the anterior part of the *T. ovis* was shown (Figure 1). Heavily infected sheep may show reddened and thickened caecal ridges with large numbers of parasites attached to the membrane. In the present study, a large number of *T. ovis* were found with anterior ends deeply embedded in mucosa of caecum and adjacent large intestine but without any prominent lesions on the affected mucosa. The concentration of the crude proteins of anterior part and posterior part of *T. ovis* and mucus were estimated as 1.466 6 mg/mL, 2.408 mg/mL, and 2.396 mg/mL, respectively.

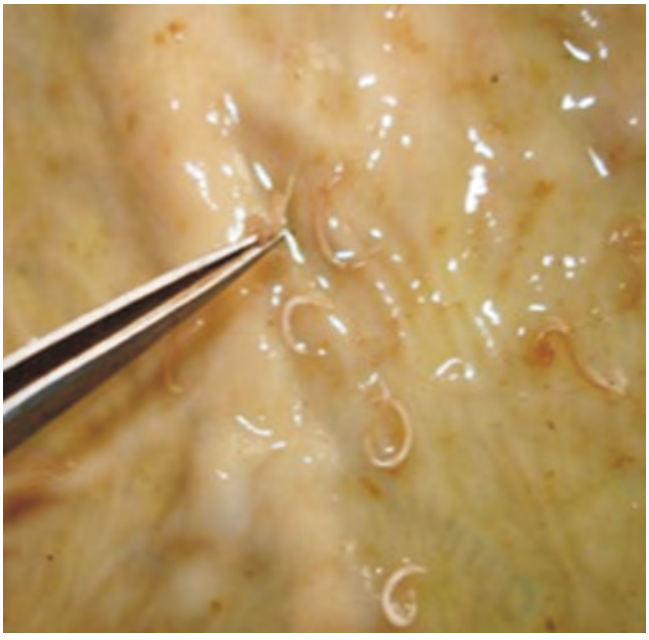

Figure 1. Adult *T. ovis* with its anterior part deeply buried into the mucosa.

3.1. Double immunodiffusion

The central well charged with each antigen along with peripheral wells loaded with the corresponding HIS developed strong precipitation lines against each antigen, indicating that the three antigens under present study have immunogenic properties (Figure 2).

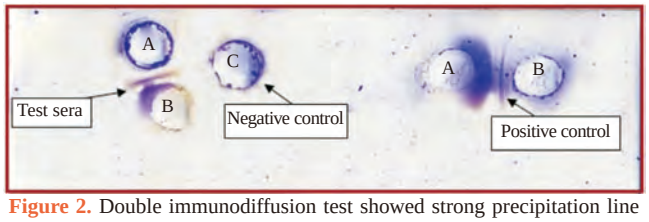

Figure 2. Double immunodiffusion test showed strong precipitation line in test serum sample.

3.2. SDS-PAGE

The SDS-PAGE of the three crude proteins after chemical treatment by urea/thiourea buffer showed several major and minor bands with molecular weight ranging from 15 to 110 kDa. In mucus and posterior part, these bands were less abundant than anterior part (Figure 3). Seven major bands and eight minor bands were observed from the anterior part of *T. ovis*. Five major bands of each from mucosal host antigens and posterior part of *T. ovis*, five minor bands from host's mucosa and four minor bands from posterior part of the parasite were detected in the present study.

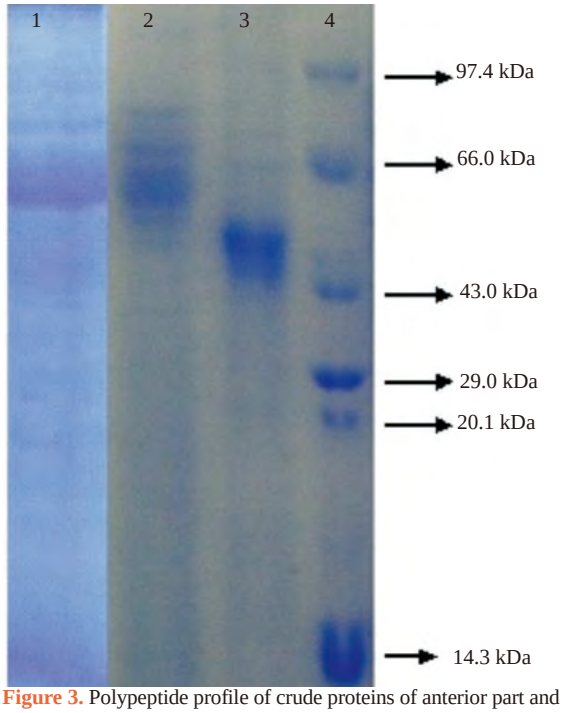

Figure 3. Polypeptide profile of crude proteins of anterior part and posterior part of *T. ovis* and mucosa assessed by SDS-PAGE.
Line 1: Anterior protein; Line 2: Mucous protein; Line 3: Posterior protein; Line 4: Standard marker.

3.3. Western blotting technique

Western blotting analysis was done with the three crude proteins using HIS raised against rabbit. Six immune dominant bands of the anterior parts (Figure 4), ten from mucus (Figure 5), and five from the posterior part (Figure 6) were observed to indict the presence of immune dominant antigens of all proteins.

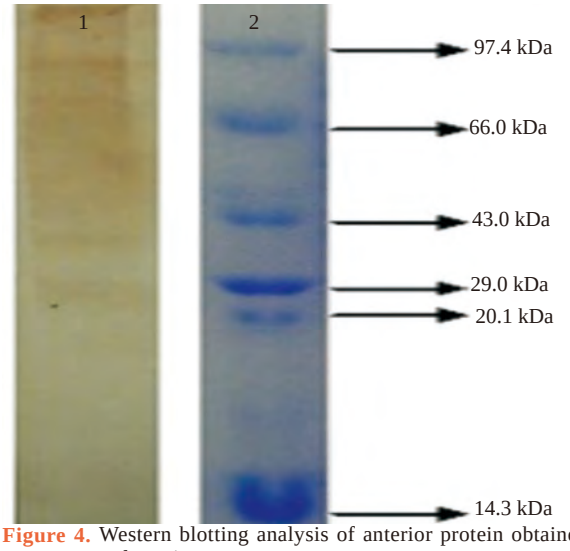

Figure 4. Western blotting analysis of anterior protein obtained from anterior part of *T. ovis*.
Lane 1: Protein fraction; Lane 2: Standard molecular weight marker ranges (14.3–97.4 kDa) (Genie).

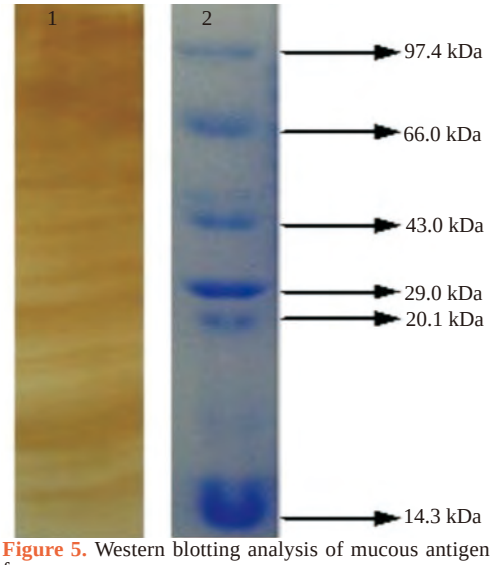

Figure 5. Western blotting analysis of mucous antigen protein obtained from mucosa.
Lane 1: Mucous antigen protein fraction; Lane 2: Standard molecular weight marker ranges (14.3–97.4 kDa) (Genie).

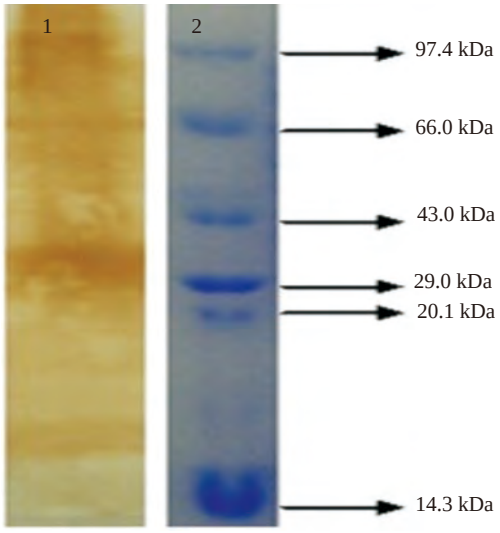

Figure 6. Western blotting analysis of posterior protein obtained from posterior part of *T. ovis*.
Lane 1: Protein fraction; Lane 2: Standard molecular weight marker ranges (14.3–97.4 kDa) (Genie).

3.4. SEM

The caecal mucosa embedded with *T. ovis* and the caudal ends of the male parasite were observed under SEM (Figures 7 and 8). The mucosa contained more mucus than mucosa not infected with parasite. The papillae were easily detected at the hind end of male when the spicule sheath was invaginated.

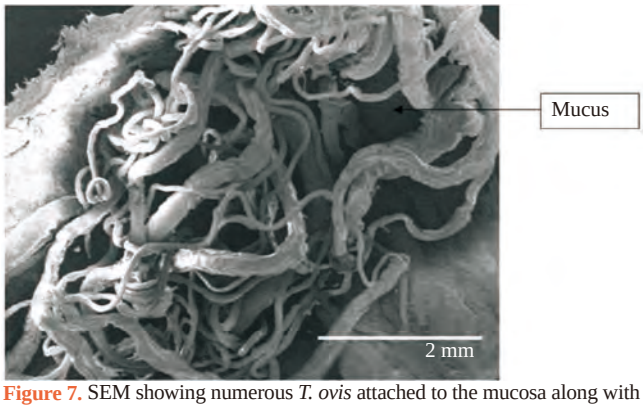

Figure 7. SEM showing numerous *T. ovis* attached to the mucosa along with mucus.

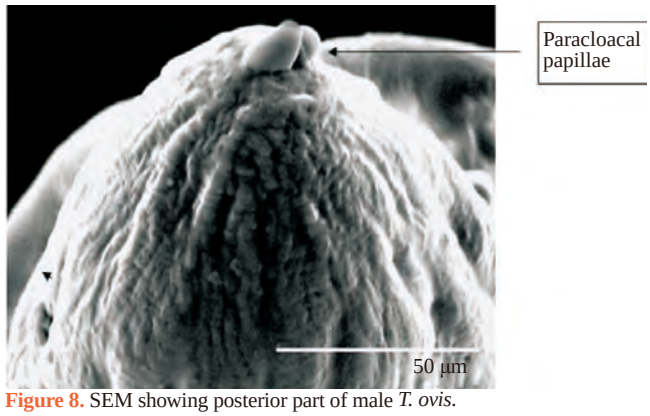

Figure 8. SEM showing posterior part of male *T. ovis*.

4. Discussion

The *Trichuris* nematodes are highly successful parasites within the host because they have the ability to survive in an immune competent environment and can actively suppress the immune responses generated by the host by excreting immune modulatory effects[13]. These nematodes have been found to secrete a complex array of immune modulatory molecules, which are likely to have protective functions particularly in maintaining infection within the host mucosal niche[13]. It has been reported that mucus plays an important role in the Th2 mediated immune responses that result expulsion of the *Trichuris muris* nematode[2,5,6].

In this study, we have tried to demonstrate why *T. ovis* is non-pathogenic despite of its blood feeding capability and deeply penetrating its anterior part into the mucosa. The assumption is that host mucosa might share and cross-react with parasitic antigens. To the authors' knowledge, there is no bibliographic information about antigenic cross-reactivity of sheep and goats infected with *T. ovis*. Difficulty in identifying the potent immunogenic candidate molecules of the metazoan helminthes and their cross-reactivity and stage specific antigenicity are the major limitation for host-parasite immune reaction. In the present study, the antigens corresponding to the homogenates of anterior and posterior parts of *T. ovis* as well as host mucosal antigens were characterized by double immunodiffusion, SDS-PAGE and western blotting technique.

Despite the fact that a complex protein pattern was seen in three different sites when analyzed by SDS-PAGE, few bands were observed in western blotting technique. For the production of antiserum, the rabbits were injected for a long period of time (two and half months) in order to raise antibodies of high affinity that could recognize all the antigenic substances present in the homogenates. However, it is important to remember that the specificity of such antibodies decreases along the immunization period and so cross reaction between the different antigens is more plausible. The six cross reactive antigens of the anterior part and four cross reactive antigens from the posterior part of *T. ovis* having similarity with host mucosa were found in the present study. This finding suggested that keeping in mind the niche in which *Trichuris* inhabits will be a major interaction between

mucosal layer of the host and the parasite particularly the anterior part.

In the present investigation, SEM was undertaken for better visualization of nematodes and their interaction with hosts. The structure of the whipworm spicule sheath was often considered as a useful feature for species determination[14,15]. No paracloacal papillae were found by these authors in any group of *T. ovis* studied. The present finding is in agreement with SEM studies reported by some other authors who pointed out the presence of typical paracloacal papillae[16-18]. Also, collected intestine either fixed or unfixed shows a considerable amount of mucus that both protects the epithelial surface and interacts with the parasite surface.

From the above observation, it can be concluded that all the antigens from different origins have a molecular weight ranging from 15 to 110 kDa but they are distinguishable from each other by their immune electrophoretic mobility. Those antigens from the parasitic part may be considered as functional antigens of the parasite. The existence of paracloacal papillae by SEM is not unique criterion for species differentiation and new criteria are needed for species identification, if any.

Several questions remain to be answered before reaching any conclusion including identification and characterization of the specific host protective antigens, the site of these antigens production and the details of the host-parasite immune responses. Answering all these questions will broaden our knowledge of the host-parasite relationship of widely prevalent and other blood feedings gastrointestinal nematodes of ruminants. Such findings are crucial to not only ascertain the parasitic importance but also confirm what measures are required to counteract these losses.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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