Immunodiagnosis of *Haemonchus contortus* infection in sheep by counter immuno-electrophoresis using somatic antigen

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Abstract The present study was undertaken for the immuno-diagnosis of *Haemonchus contortus* infection in sheep by counter immuno-electrophoresis (CIEP) using crude somatic antigen. Adult worms of *H. contortus* were found in 57 out of 100 abomasums screened. A total of 250 serum samples which includes, 100 serum from local abattoir in and around Shimoga region from the sheep from which the abomasums were collected for *H. contortus* worms and 150 serum from the migratory sheep were collected to detect the circulating antibody of *H. contortus* by CIEP using crude whole worm somatic antigen. Out of 100 abomasums screened, 18 were heavily infected with adult worms of *H. contortus* and their serum samples showed positive reaction with clear thick precipitin line with crude somatic antigen of *H. contortus* by CIEP and 39 samples showed light precipitin line, which were moderately infected with adult worms of *H. contortus* during necropsy. However, the 12 serum samples showed positive reaction with clear precipitin line, but their abomasums did not show *H. contortus* and the remaining 31 were not harbouring any adult worms of *H. contortus* and no precipitin line observed by CIEP. The sensitivity and specificity of CIEP was found 100 and 72.09 %, respectively. In the present study, 62 % sero-prevalence was observed with CIEP among 150 serum samples from migratory sheep screened for antibodies of *H. contortus* using crude whole worm somatic antigen of *H. contortus*.

Keywords Sheep · *Haemonchus contortus* · Somatic Ag · Immuno-diagnosis · CIEP

Introduction

The ruminants are exposed to many parasitic infections while grazing and exert adverse effects on the health and productivity of animals which are more pronounced in sheep and goats compared to other species of livestock (Iqbal et al. 1993). Various species of parasites affecting sheep and goats includes Fasciola, Moniezia, *Haemonchus*, Oesophagostomum, Ostertagia, Chhabertia, Nematodirus and Trichuris (Husnain and Usmani 2006). Among all, *Haemonchus contortus* is an important blood sucking abomasal parasite of sheep responsible for suppressed production (Ijaz et al. 2009) causing weight losses and even mortality in young animals (Husnain and Usmani 2006).

Haemonchosis is the highly pathogenic strongyle nematode infection of sheep and goats (Soulsby 1982) and is detrimental to their health and productivity. Infection with *H. contortus* constitutes a serious economic constraint to small ruminant production and is known to be the major aetiological agent of parasitic gastroenteritis (PGE) in small ruminants. The adult worms are blood feeders that reside in the abomasum and single female typically producing several thousand eggs per day which pass out of the host in faeces and develop to infective larvae on the pasture. These parasites cost the global livestock industry billions of dollars per annum in lost production and drug costs. Resistance to all the major anthelmintic classes is now common worldwide often leading to failure of treatment and control.

This parasite has its own distinction and comes in a very limited group of important worms since they are voracious

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blood sucker and cause unaccounted mortality and significant loss of productivity and is responsible for anemia, bottle jaw, and death of infected sheep and goats, mainly during summer months in warm and humid climates (Onyenwe et al. 2005). Hence, the present study was undertaken to study the sero-prevalence of *H. contortus* infection in sheep by CIEP using somatic antigen.

Materials and methods

Collection of samples

In the present study, a total of 100 suspected abomasums of sheep were collected from a local abattoir of Shimoga and Bhadravathi region, Karnataka state and brought to the laboratory within 3 h and dissected immediately. The adult worms embedded in the abomasal mucosa and in between the folds of the inner wall of the abomasum was separated using forceps. The worms were collected in Phosphate Buffer Saline (PBS, pH 7.2) and were washed three times thoroughly in PBS along with antibiotics to remove adhered debris on the surface and contamination. The identification of the worms was done based on the morphological characters described (Bowman 2009; Urquhart et al. 1996). Then, the worms were stored in PBS and deep frozen.

Positive sera of sheep naturally infected with *H. contortus*

The blood sample for sera as well as abomasums for *H. contortus* were collected from the same sheep and labeled for identification. In the laboratory, the serum samples were separated by centrifugation, aliquoted and stored in the deep freezer at -20°C . The serum samples of sheep abomasums showed adult worms of *H. contortus* are used as true positive sera.

Field sera

A total of 150 blood samples were collected from migratory sheep without EDTA for serum separation. In the laboratory, the serum samples were separated by centrifugation, aliquoted and stored in the deep freezer at -20°C till further use.

Negative control sera In the study, 0 day lamb serum were used as negative controls.

Preparation of somatic antigen

The somatic antigen was prepared by following the procedure of Prasad et al. (2007) with slight modification.

The adult worms recovered from the abomasum of sheep were washed thoroughly in Hank's balanced salt solution. Then the worms were transferred to a screw capped vial containing 0.15 M phosphate buffered saline (pH 7.2). The segments were triturated using a glass mortar and pestle. The contents were repeatedly frozen and thawed for four times and then disrupted by Sonirep 150 ultrasonication (Sanyo Gallenkamp PLC, UK) three times for 20 s each time at 100 mAmp less than 4°C . The suspension was centrifuged at 12,000 rpm for 30 min in a refrigerated centrifuge (Superspin) at (-4°C) . The supernatant was collected and used as the soluble antigen extract. The protease inhibitor phenyl methyl sulphonyl fluoride (PMSF) (Sigma, USA) was added at the concentration of 2 $\mu\text{l/ml}$ of antigen. As the antigen was prepared in saline solution, the antigen has to be separated from the saline solution and purified before conducting further assays. So, dialysis was conducted to purify the antigen and was aliquoted and stored at -20°C till further use.

Estimation of protein concentration The protein concentration of the somatic antigen was estimated as per the method of Bradford (1976) using protein estimation kit obtained from Bangalore Genei Co., Bangalore.

Counter immunoelectrophoresis (CIEP)

The procedure for the CIEP test was as per the method of D'Souza and Hafeez (1999) with slight modifications. Agarose (M/S HI-Media, Mumbai) was prepared in tris borate buffer with pH 8.2. Half gram of Agarose was added to 25 ml of tris borate buffer and heated. On to glass slides, five ml of molten buffer gel was poured and allowed to solidify. Two wells of four mm diameter were cut with a well distance of six mm and the bases were sealed with molten agar. The well to be located on the cathodic slide was filled with somatic antigen of *H. contortus* and other (anodic well) with serum for evaluation of the test. The electrophoresis was carried out in a suitable trough containing barbitone buffer. The slides were placed on the rack and each end was connected with a filter paper wick dipped in buffer.

A current of 50 mA per slide was applied for 90 min. The observations were recorded before and after staining. The slides were then washed in normal saline and stained with Comassie brilliant blue. The sensitivity and specificity of the serological tests was calculated by using

$$\text{Sensitivity: } \frac{\text{True positive}}{\text{True positive} + \text{False negative}} \times 100$$

$$\text{Specificity: } \frac{\text{True negative}}{\text{True negative} + \text{False positive}} \times 100.$$

Results and discussion

The counter immunoelectrophoresis (CIEP) was evaluated for the diagnosis of *H. contortus* infection in sheep in the present study using somatic antigen for the first time. The protein concentration of the somatic antigen was found to be 0.95 mg per ml antigen. The test was initially standardized with true positive serum collected from the infected sheep with somatic antigen and a clear band could be seen after running the system for 90 min. The test was then evaluated with 100 serum samples of sheep collected from local abattoir of Shimoga region and 150 serum samples from migratory sheep to detect the circulating antibody against *H. contortus* by CIEP with somatic antigen.

In the present study, the overall prevalence of 57 % of *H. contortus* infection was observed in sheep by slaughter house survey of abomasums (Fig. 1, 2). Raza et al. (2009) conducted a study to ascertain the prevalence of *H. contortus* in slaughtered sheep and goats at Multan abattoir and found 37.18 %. Sabbas Attindehou et al. (2012) studied the epidemiology of Haemonchosis in sheep and goats in Benin and found overall prevalence of 55.56 %. The higher prevalence rate of *H. contortus* infection in sheep was reported in other countries includes 82 % in Togo (Bonfoh et al. 1995) and 60 % in Eastern Ethiopia (Sissay et al. 2007). The variation in the prevalence rate of *H. contortus* infection in sheep at different places might be due to number of samples screened, variation in environmental factors and the type management practices followed.

Out of 100 abomasums collected from local sheep abattoir of Shimoga region screened in the present study, eighteen were heavily infected with adult worms of *H. contortus* and their serum samples showed positive reaction with clear thick precipitin line with somatic antigen of *H. contortus* by CIEP (Fig. 3). This was comparable with the results obtained by Ahmad and Nizami (1998) who obtained a maximum of four precipitin arcs using somatic

Fig. 1 *H. contortus* adult worms embedded on the abomasal mucosa

Fig. 2 *H. contortus* adult worms in PBS for somatic Ag preparation

Fig. 3 CIEP showing precipitin arc with somatic Ag of *H. contortus*

Fig. 4 CIEP showing precipitin arc with somatic Ag of *H. contortus*

antigen of *E. granulosus* with both positive sera and hyperimmune serum. Katoch and Singh (1994) used adult worm antigen for detection of echinococcus antibodies in experimentally infected pups by CIEP yielded clear thick precipitin line.

The Thirty-nine serum samples showed light precipitin line by CIEP, which were moderately infected with *H. contortus* adult worms during necropsy (Fig. 4). However, the 12 serum samples showed positive reaction with clear precipitin line by CIEP but their abomasums did not show any adult worms of *H. contortus* and the remaining 31 abomasum were not harbouring any worms and no precipitin line observed with CIEP. The sensitivity and specificity of CIEP was found 100 and 72.09 %, respectively. Mir et al. (2008) studied sero-diagnosis of *H. contortus* by ELISA using adult crude antigen and found 72.22 and 76.81 % sensitivity and specificity respectively. Sultan

et al. (2012) observed 87.5 and 75 % sensitivity and specificity using Indirect ELISA for the diagnosis of natural ovine haemonchosis with crude adult worm antigen. Schallig and Hornok (1995) used ELISA for the detection of *H. contortus* infections in sheep using crude somatic antigens and found sensitivity of 89.2 % with a specificity of 82.7 %. The observed variation in the present study might be due to change in the preparation of the antigen and the type of assay used.

In the present study, the somatic antigen of *H. contortus* was used to detect the circulating antibodies of *H. contortus* in 150 migratory sheep of Shimoga region by CIEP. The test could able to detect the circulating antibodies of *H. contortus* in 93 serum samples with a sero-prevalence of 62 % in migratory sheep of Shimoga region. Whereas, the study conducted by Mir et al. (2008) observed 72.22 % sero-prevalence of *H. contortus* in sheep with crude somatic antigen. The difference could be due to less number of samples included in the present study and also may be change in the preparation of the antigen and the type of antigen used in the assay. It can be concluded that CIEP is a simple, inexpensive and rapid test. Results can be obtained within 90 min and has a potential for field application since it shows high specificity and sensitivity for somatic antigen with positive sera.

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