Sero-prevalence of *Haemonchus contortus* infection in sheep by Indirect-ELISA using somatic antigen

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Abstract The present work was carried out to study the sero-prevalence of *Haemonchus contortus* infection in sheep by Indirect-Enzyme Linked Immuno Sorbent Assay (Indirect-ELISA) using somatic antigen. Out of 100 abomasums screened, 57 found positive for *H. contortus* adult worms. A total of 250 serum samples which includes, 100 serum samples from local abattoir in and around shimoga region from the animals from which the abomasums were collected and 150 serum samples from migratory sheep were used to detect the circulating antibody against *H. contortus* by Indirect-ELISA using somatic antigen. Of the 57 sheep harboring adult worms in their abomasums, the serum samples showed positive reaction by Indirect-ELISA with somatic antigen. However, among 43 sheep which are not showing any adult worms of *H. contortus* in their abomasums, but their 21 serum samples showed positive reaction by Indirect-ELISA. The sensitivity and specificity of Indirect-ELISA was found to be 100 and 67.18 %, respectively. Also, the sero-prevalence of *H. contortus* infection was found to be 58.66 % out of 150 migratory sheep serum samples screened for detecting circulating antibodies of *H. contortus* by Indirect-ELISA using somatic antigen in and around shimoga region.

Keywords Sheep · *Haemonchus contortus* · Sero-prevalence · Indirect-ELISA

Introduction

Sheep farming is an attractive feature for farmers in Karnataka state due to low capital input and the ability of the sheep to drive on available pastures. In Karnataka state almost every household in rural areas have sheep and which serve the daily needs of meat, wool, hides and valuable organic manure. But the productivity of sheep is mainly constrained by parasitic infections (Tariq et al. 2008). Among Parasitic infections, *Haemonchus contortus* is the most economically significant parasite of sheep and goats throughout the world, due to the severity of the parasitism and the emerging anthelmintic resistance. Rapid diagnosis, strategic prevention and pasture management, along with a thorough understanding of the pathophysiology of haemonchosis facilitates the development of herd health protocols to control this deadly disease.

Haemonchus contortus favors warm moist climates and most of the animals succumb to haemonchosis in the spring, due to the combined assault of larvae emerging from hypobiosis and the immunosuppression of late pregnancy. This results in the periparturient rise in egg shedding, resulting in numerous infective larvae on pasture at the time when young animals are most susceptible (Soulby 1982). Although all ages of sheep and goats are susceptible to haemonchosis, recently weaned animals are usually the most vulnerable. Besides age, other factors that increase susceptibility include overgrazing, dense stocking rates, and inadequate nutrition, particularly protein intake.

Haemonchus contortus is an economically important, highly pathogenic blood sucking abomasal nematode of small ruminants, especially sheep and goats and causes high mortality and morbidity by affecting health, production and reproduction performance of the animals (Tariq et al. 2008). The development of sensitive and specific

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ante-mortem diagnostic methods for the detection of haemonchosis is important for epidemiological baseline data and for surveillance of control programmes. Therefore, the present study was undertaken for the detection of *H. contortus* specific antibodies in sheep by indirect enzyme linked immunosorbent assay.

Materials and methods

Collection of samples

In the present study, a total of 100 suspected abomasums of sheep were collected in 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 to remove adhered debris on the surface. The identification of the worms was done based on the morphological characters described (Soulsby 1982; Bowmann 2009). Then, the worms were stored in PBS and deep frozen.

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

The blood samples for sera as well as abomasums for *H. contortus* adult worms were collected from the 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, zero day lamb serum and uninfected control sheep sera 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 abomasums 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 worms were triturated using a glass mortar and pestle. The contents were repeatedly frozen and thawed for four times and then disrupted by Sonirep 150 ultra-sonication (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 (4°C) (Superspin). The supernatant was collected and used as the soluble antigen extract. The protease inhibitor phenyl methyl sulphonyl fluoride (PMSF) (Sigma, USA) was added at 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 purify 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.

Enzyme linked immuno sorbent assay (ELISA)

Indirect-ELISA was used to detect antibody of *H. contortus* infection in sheep. The working dilutions of conjugate, antigen and test sera were determined prior to use by checkerboard titrations.

Determination of working strength of anti-ovine IgG conjugate:

To determine the working dilution of anti-ovine conjugate, 100 μl of normal sheep serum (1:1,000) was coated onto 96 well flat bottom polystyrene ELISA plate (Titertrek) by diluting with coating buffer and incubated at 37°C for 1 h. The ELISA plate was washed with washing buffer thrice. The blocking buffer was added to block the non-specific reactive sites and incubated at 37°C for 1 h. The plate was washed with washing buffer thrice. Test conjugate dilutions were prepared in blocking buffer and 100 μl of each dilution was added to the wells in duplicate and incubated at 37°C for 1 h. Further steps were as described in procedure.

Determination of optimal serum dilution:

100 μl of somatic Ag of *H. contortus* in carbonate buffer was added to 24 wells of a 96 well ELISA plate. The plate was incubated overnight at 4°C and washed thrice with

washing buffer. The blocking buffer was added to block the non-specific reactive sites and incubated at 37 °C for 1 h. After washing the plates, 100 µl of positive serum dilutions was added in triplicates and further steps were as described in procedure.

Determination of cut off value:

Ten known negative serum samples were obtained from a zero day lamb serum and uninfected control sheep sera reared in confinement with no access to infection and were regularly dewormed. This was used to determine the cut off value. The cut off value was calculated by taking mean absorbance values of known negative sera plus three standard deviation. Any serum with OD values above the cut off value was regarded as positive.

Procedure:

The flat bottom polystyrene 96 well ELISA plate was coated with 100 µl containing 3 µg of somatic antigen in coating buffer in duplicates. The plate was incubated at 4 °C overnight and washed thrice with washing buffer. The plates were incubated at 37 °C for 1 h after adding 100 µl of blocking buffer (5 % skimmed milk powder with PBS Tween-20) and washed thrice with PBS Tween-20. The positive serum (1:100 dilution) with blocking buffer was added to all wells and incubated for 1 h at 37 °C. The plates were washed four times with washing buffer and 100 µl of 1:1,000 diluted anti-ovine conjugate was added and incubated as above. The plates were washed five times with washing buffer. Then 100 µl of substrate chromogen working solution was added and color reaction was monitored in dark place. The reaction was stopped by adding 50 µl of 2 M H₂SO₄. The absorbance values were read in ELISA reader (Biorad) at 450 nm. Positive control and negative control was included in the assay in duplicate.

Sensitivity and specificity of Indirect-ELISA:

The sensitivity and specificity of ELISA was calculated by the following formula:

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

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

Results

The working dilutions of conjugate, somatic antigen and positive serum were found to be 1:1,000, 3 µg/well and

1:100, respectively by checkerboard assay yielded a mean background absorbance value (x) of 0.298 and a standard deviation of 0.031 for somatic antigen of *H. contortus*. In the present study the cut off OD value was 0.391 (Mean + 3 SD).

In the present study, out of 100 abomasums screened, 57 (57.0 %) found positive for *H. contortus* adult worms. Among positive abomasums, 18 (31.57 %) were heavily infected and 39 (68.42 %) were moderately infected with *H. contortus* adult worms during necropsy and the remaining 43 (43.0 %) were negative.

Out of 100 serum samples screened from local abattoir, 78 % sero-prevalence was observed which includes 57 sheep harboring adult worms in their abomasums, their serum samples showed positive reaction by Indirect-ELISA with somatic antigen. However, among 43 sheep which are negative for *H. contortus*, but their 21 serum samples showed positive reaction by Indirect-ELISA. The sensitivity and specificity of Indirect-ELISA was found to be 100 % and 67.18 %, respectively. The sero-prevalence of *H. contortus* infection in migratory sheep was found to be 58.66 % out of 150 serum samples screened for detecting circulating antibodies of *H. contortus* by Indirect-ELISA using somatic antigen in and around shimoga region.

Discussion

ELISA has emerged as a very useful immunological tool because of which it became one of the most widely used techniques in measuring antibody, antigen and protein (McLaren et al. 1979). In the present study Indirect-ELISA was carried out for the detection of serum antibodies in sheep, specific to *H. contortus* using somatic antigen.

The overall prevalence of 57 (57.0 %) of *H. contortus* infection was observed in sheep by slaughter house survey of abomasums during the study. This is almost in accordance with the Sabbas Attindehou et al. (2012) who studied the epidemiology of haemonchosis in sheep and goats in Benin and found the 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 lower prevalence in comparison of the present study has also been reported at different places (Tariq et al. 2010; Dagnachew et al. 2011 and Qamar et al. 2011). Raza et al. (2009) conducted a study to determine the prevalence of *H. contortus* in slaughtered sheep and goats at Multan abattoir and found 37.18 %. 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.

The assay was standardized with 3 µg/well antigen along with 1:100 and 1:1,000 of sera and conjugate dilution. This is in accordance with the Lone et al. (2012) who used Indirect-ELISA for immunodiagnosis of *H. contortus* infection in both experimental and clinical cases of sheep using somatic whole adult antigen and standardized with 5 µg/well antigen with 1:100 and 1:1,000 of sera and conjugate dilution. The variation in the quantity of antigen used in the assay might be due to type of antigen used in the assay and variation in the methodology followed for preparation of antigen.

Out of 100 serum samples collected from the local abattoir, the assay could able detect *H. contortus* specific antibodies in 78 % with a sensitivity and specificity of 100 and 67.18 %, respectively. The assay detected antibodies in all 57 serum samples which were positive for adult worms of *H. contortus* by necropsy. However, the assay could also detected antibodies in 21 serum samples but their abomasums not showed any worms which indicate the past infection. The false positive reaction of the assay could be due to the persistence of the antibodies of the past infection. Lone et al. (2012) carried out plate ELISA for immune-diagnosis of *H. contortus* infection in sheep and found 80 and 21.42 % sensitivity and specificity respectively. The observed variation in the present study might be due to change in the preparation of the antigen and the type of antigen used in the assay.

However, the higher specificity in the present study might be due to the cross reactivity of the *H. contortus* with other helminth parasites. The cross reactivity among different helminthes is common and one of the limiting factor for the development of serological tests against helminth infection (Molina et al. 1999). The use of more purified antigen may reduce the cross reactivity with other helminth parasites.

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) carried out Indirect-ELISA for the diagnosis of natural ovine haemonchosis using crude adult worm antigen and observed 87.5 and 75 % sensitivity and specificity respectively. (Schallig et al. 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 antigen used in the assay.

In the present study, the sero-prevalence of *H. contortus* infection was found to be 58.66 % out of 150 field serum samples screened using somatic antigen. Whereas, in comparison with the study conducted by Mir et al. 2008, 72.22 % sero-prevalence was observed with crude somatic antigen. The difference could be due to less number of

samples included in the present study and also may be due to change in the preparation of the antigen and the type of antigen used in the assay. It is concluded that, the use of Indirect-ELISA is one of the best serologic technique can be carried out for mass screening of parasitic diseases among population compared to routine copro-logical examination, because ELISA is more sensitive.

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