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Excretory-Secretory Antigens are Better than Crude Antigens for Serodiagnosis of *Haemonchus contortus*

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Abstract: Serodiagnosis of *Haemonchus contortus* by ELISA using adult crude somatic and excretory-secretory antigens of *Haemonchus contortus* was tested in the present study. The diagnostic sensitivity of ELISA using excretory secretory antigens was 87.5% which was significantly higher compared to crude somatic antigen 72.22%. Mean ELISA absorbance value of excretory-secretory antigens was significantly higher corresponding to crude somatic antigens. Excretory secretory antigens showed 92.02% specificity compared to 76.81% of crude somatic antigens ($p<0.05$). The results revealed that excretory secretory antigens are very sensitive and may be useful as a supplementary method for diagnosis of Haemonchosis in ruminants.

Key words: Cross reactivity, ELISA, *Haemonchus*, serodiagnosis, sheep

INTRODUCTION

Haemonchosis is one of the most important diseases of livestock through out the world. The disease has a cosmopolitan distribution but more prevalent in most sheep-raising countries. The parasite inhabits in the abomasa of sheep, bores its walls and feeds on blood. It causes great economic losses in sheep industry including decreased weight gain (Ploeger *et al*., 1990), decreased milk yield Gross *et al*. (1999). Young lambs are very sensitive to *Haemonchus contortus* while as older sheep acquire immunity after continuous or seasonal exposure to the parasite. The mechanism underlying immunity is still not completely understood. Analysis of host immune response to helminth parasites is hampered by two main factors (i) the complexity of antigen profile of parasites (ii) the presence of cross-reactive determinants on antigens (Maizels *et al*., 1987; Gnalzata *et al*., 1988). To identify specific antigens, Excretory-Secretory (ES) products of helminth parasites have received increasing attention. This is due to the fact that excretory-secretory products of helminthes usnally display a relatively simple antigenic composition compared to the somatic worm antigens. Diagnosis of haemonchosis is based on clinical findings as well as laboratory tests. The most reliable method is the finding of egg in the stool of infected animals. This method cannot be used until the parasite attains sexual maturity. Therefore, it is necessary that these parasites should be controlled before they can cause damage to the stomach. Hence serological diagnosis should be preferred because antihaemonchus antibodies can be detected as early as one week post infection and thus can facilitate early chemotherapeutic intervention. Despite of the recent studies involving the diagnosis of haemonchosis using *Haemonchus contortus* antigens in ELISA test, the effectiveness of *Haemonchus contortus* crude somatic antigens and excretory-secretory antigens have not been studied adequately especially in Kashmir as this is the first report in this case.

The present study was carried out from October 2005 to January 2006 and the purpose of this investigation was to compare the specificity of crude somatic and excretory-secretory antigens in ELISA test for serodiagnosis of sheep haemonchosis.

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MATERIALS AND METHODS

Parasites collected from the infected abomasa of sheep were washed with 0.01 M phosphate buffer pH 7.2. Freshly collected worms were incubated in 20 mL phosphate buffer saline for 5 h at 37°C. After incubation, worms were removed and the suspension containing excretory secretory products was centrifuged at 15000 rpm for 15 min at -20°C. The supernatant obtained was concentrated in dialysis bags under moving fan. The concentrated samples were stored at -20°C till further use.

Preparation of Crude-Somatic Antigens

Adult worms were collected from infected animals cleaned in normal saline solution. Later, worms were mixed with Phosphate Buffer Solution (PBS), pH 7.4 homogenized in Teflon tissue homogenizer at 4°C for 1 h. The homogenate was kept undisturbed for 24 h in refrigerator. Supernatant collected form homogenate was centrifuged in cooling centrifuge at 12000 rpm for 15 min. The supernatant was stored as crude solubilized antigen at -20°C till further use. Protein concentration of crude somatic and excretory-secretory antigens was determined by Lowry *et al.* (1951).

Collection of Sera Samples

A total of 432 sera samples collected from sheep confirmed positive for *Haemonchus contortus* by coprological examination were tested for sensitivity in ELISA. To see the specificity of excretory-secretory antigens 138 sera samples were collected from the abattoirs. Out of 138 sera samples, 50 sera samples were collected from the healthy uninfected sheep, treated as control, 88 sera samples were collected from sheep infected with nematode parasites like *Ostertagia circumcincta* (46), *Bunostomum trigonocephalum* (26) and *Trichuris ovis* (18). All the sera samples were stored at -20°C.

ELISA

ELISA was performed in polystyrene EIA micro titration 96-welled flat-bottomed plates. Buffers (carbonate-bicarbonate pH 9.6 and phosphate buffer saline pH 7.2 containing teween 20 were used for coating and washing procedures. Each well of micro titration plate was coated by 1 mL of antigenic solution, protein concentration of which was 2 μg mL^{-1}. The plates were then sealed and incubated at 4°C overnight. After incubation the plates were washed with phosphate buffer saline containing teween 20 (PBST) and then blocked by PBS solution containing 1% bovine serum albumin and 0.5% NaN_3. After over night incubation diluted test samples (1:25) were added to the wells and the plates were again incubated at 37°C for 3 h and then washed with PBST. Thereafter 50 μL of diluted antirabbit IgG conjugated with horseradish peroxide was added to each well and then again incubated for 3 h at room temperature. After incubation the plates were washed three times with PBST. The reaction was developed by adding 100 μL of freshly prepared solution (tetramethlbenzidine and 1%. H_2O_2) to each well. Finally reaction was stopped by the addition of 50 μL of 3 N Na OH solutions. The optical density was recorded at 450 nm on ELISA reader. Samples with an absorbance of <0.25 were deemed negative and those ≥ 0.25 as positive.

Statistical Analysis

The differences observed in the data were statistically analyzed by student t-test and Pearson's chi-square test using SPSS 10.0 for windows.

RESULTS AND DISCUSSION

In order to test the potential diagnostic value of excretory secretory and crude somatic antigens, 432 sera samples of sheep naturally infected with *Haemonchus contortus* were collected from abittors. ELISA analysis showed that excretory-secretory antigens were more sensitive compared to

Table 1: Comparison of sensitivity between crude somatic and excretory-secretory antigens of *Haemonchus contortus*

Antigen	No. of samples	No. of positive	Sensitivity (%)
Crude	432	312	72.22
Excretory-secretory	432	378	87.50

Pearson's chi-square test was applied using SPSS 10.0 for windows, *: $p<0.05$

Table 2: Comparison between mean absorbance of crude absorbance and excretory antigens

		Mean±SD	
Sera samples	No. of sera samples	Crude antigen	E/S antigen
Uninfected (control)	50	0.052±0.04	0.091±0.027
H. contortus	432	0.314±0.13	0.414±0.140
B. trigonocephalum	26	0.271±0.12	0.291±0.098
O. circumcincta	46	0.182±0.06	0.197±0.072
Rabbit hyper immune sera	8	0.421±0.13	0.472±0.210

±(Standard deviation), The differences were statistically analyzed by Students t-test using SPSS 10.0

Table 3: Comparison of specificity between crude somatic antigens and excretory-secretory antigens of *Haemonchus contortus*

		Crude antigen		E/S antigen	
Sera	No. of samples	No. of negitive	Specificity (%)	No. of negitive	Specificity (%)
Uninfected control	50	50	100.00	50	100.00
B. trigonocephalum	26	16	66.67	20	83.34
O. circumcincta	46	26	56.52	41	89.13
Trichuris ovis	18	14	77.78	16	88.89
Total	138	106		127	
Specificity			76.81		92.02

Pearson's chi-square test was applied using SPSS 10.0 for windows

crude somatic antigens. Out of 432 samples collected 378 (87.5%) showed positive reaction with excretory-secretory antigens which was significantly higher corresponding to 312 (72.22%) of crude somatic antigens (Table 1). Chi-Square analysis of the data revealed that variation observed in ELISA sensitivity was significant. Mean ELISA absorbance observed in the present study was significantly higher for the excretory secretory antigens than crude somatic antigens (Table 2). Individual absorbance of the sera of sheep infected with other nematodes showed variation being highest for *Ostertagia circumcincta* infected sera. Diagnostic specificity of ELISA with excretory secretory antigens was increased to 92.02 from 76.81% of crude somatic antigens. Increased specificity of excretory-secretory antigens was statistically significant ($p<0.05$). Cross reactivity of excretory-secretory antigens with the sera samples of other nematodiasis was reduced compared to crude somatic antigens (Table 3). However, high cross reactivity was observed between sera samples of Haemonchosis and Ostertagiasis with both the types of antigens.

The results of the present study revealed that excretory-secretory antigens proved to be better antigens than crude somatic antigen for serodiagnosis of haemonchosis. The diagnostic sensitivity of excretory-secretory antigens observed in ELISA was increased to 87.5 from 72.22% of crude somatic antigen, which was statistically significant. Increased sensitivity of ELISA using excretory-secretory antigens compared to crude somatic antigens may be attributed to strong recognition of excretory-secretory antigens by the sera of infected animals. It has been demonstrated that excretory-secretory antigens elicit strong immune response (Ogilvie and De Savigny, 1982) and may be potential source of diagnostics (Lightowlers and RicKard, 1988; Brandt *et al*., 1992; Mahannop *et al*., 1992) or protective antigens (O' Dounell *et al*., 1989; Savin *et al*., 1990). Somatic antigens have been considered poorly immunogenic compared to excretory-secretory antigens (Parkhouse *et al*., 1987). The excretory-secretory product of *Haemonchus contortus* was found to include antigenic polypeptides of 10, 15,

16, 20, 30, 40, 46, 52, 66, 68 and 93 kDa. (Schalling *et al.*, 1994). The 15 and 24 kDa antigenic proteins present in the excretory-secretory products could be specifically recognized by *Haemonchus contortus* primary sera (Schalling and Leeuwen, 1997). Torgerson and Lloyd (1993) demonstrated that *Haemonchus contortus* antigens with molecular weight less than 30 kDa present in excretory-secretory products can induce strong lymphocyte responses. This might indicate that low molecular weight excretory secretory products have protective value. The diagnostic specificity of ELISA using excretory-secretory antigens was increased to 92.02 from 76.81%. The cross reactivity of excretory secretory antigens with the sera samples of Ostertagiasis, Bunostomiasis and Trichuriasis was reduced compared to that of crude somatic antigens. This reduced cross reactivity may be attributed to high levels of 15 and 24 kDa antigenic proteins present in excretory-secretory products which could be specifically recognized by *Haemonchus contortus* primary sera (Schalling and Leeuwen, 1997).

CONCLUSION

In conclusion the present study demonstrated that E/S products of *Haemonchus contortus* are highly antigenic contains antigenic peptides of potential diagnostic and protective value.

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