Protective Immune Response of Kashmiri Native Sheep to *Haemonchus contortus* Induced by Parasite Membrane Associated Antigens

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ABSTRACT

The membrane associated antigens of *Haemonchus contortus* were analysed for protective immunity in sheep. Sheep were challenged with infective 3rd stage larvae of *Haemonchus contortus* (500/kg bodyweight). Sheep vaccinated with membrane associated antigens showed significant reduction (64.085%) in mean faecal egg counts corresponding to infected control animals. There was general reduction in packed cell volume (PCV) and total serum proteins which was much faster in infected control group compared to vaccinated groups. On the basis of decreased faecal egg counts and haematobiochemical parameters, membrane associated proteins are considered to be the best protective antigens.

Key words: Egg count, immune response, packed cell volume

INTRODUCTION

Sheep are very susceptible to worms due to their close grazing behavior. Almost all sheep are parasitized by helminth parasites. The parasites that cause damage to sheep and goats are stomach worms like *Haemonchus contortus* and *Ostertagia circumcincta. Ostertagia circumcincta* feeds on abomasal contents while as *Haemonchus contortus* feeds on blood. Thus *Haemonchus contortus* (barber pole worm) is of primary concern. It is small about one cm long, blood sucking parasite that bores the lining of abomasum causing blood and protein loss. The barber pole worm is difficult to control because it has short direct life cycle, prolific egg producer and can go to hypobiontic (hibernating) state until environmental conditions are more favorable for its life cycle. Since primary mode of transmission for stomach worms is grazing therefore pasture management and use of anthelmintics are important aspects of controlling the internal parasites. Pasture control

strategies including the use of clean and safe pastures are not readily available. By using anthelmintics the parasite showed resistances to these drugs. Thus there arises a need to develop alternative control strategies like vaccines. Purified gut antigens of *Haemonchus contortus* used as immunogenic afforded a degree of protection against this parasite (Jasmer and Mecuire, 1991; Tavernor *et al*., 1992; Jasmer *et al.,* 1993. Schallig *et al.* (1997) demonstrated that protective immunity in sheep is associated with low molecular weight antigens. The aim the present study was to analyze the protective immune response in Kashmir Native sheep induced by parasite membrane antigens.

MATERIAL AND METHODS

Preparation of Triton X-100 Extracts of *Haemonchus contortus*: Parasites collected from infected abomasum of sheep were washed with 0.01 M phosphate buffer pH 7.2. Frozen parasites were thawed, ground up as a 10% w/v suspension in ice-cold homogenizing buffer (PBS containing 1nM EDTA and 1n M phenylmethyl sulphonyl fluoride), centrifuged (10000g for 20 minutes) and the pellets were homogenized again with buffer containing 0-1 % Teween 20. The last step was repeated and the pellet was resuspended and extracted in ice cold homogenizing buffer containing 2% (w/ver saline for 5 h at) reduced Triton X-100. (Aldrich chemical Co., Inc, St Louis, Mo, USA) without EDTA. The extract was centrifuged (1 h at 10000g) and the supernatant was clarified by passing through a 0-22µm filter and then stored at -20^{o}C. The concentration of crude somatic antigens was determined by Lowry *et al*. (1951)

Experimental design: Eight lambs aged between 5-6 months collected from local breeders of District Anantnag Kashmir , India, were used for the experiment. Lambs were rared and housed under controlled conditions. They were fed on the solid diet of maize and wheat husk. Before inoculation, animals were treated with broad spectrum anthelmintics, Albendazole (Valbazene, 10mg/kg) and Acaricide. At approximately 3 months of age, the animals were randomly allocated to two groups. Five lambs were allocated to the immunized groups and three lambs were treated as unimmunized control group. All the vaccinated animals received 50µg of antigenic solution in PBS in 1 ml Freund's complete adjuvant (Sigma). Each immunization included doses of inoculation (three intramuscularly, three subcutaneously). Both the groups were subsequently challenged with infective 3rd stage larvae (500/kg body weight) of *Haemonchus contortus* obtained from donor animal faeces cultured in an incubator at 27^{O}C for seven days according to the method of Roberts and Oscillvan (1950). Faecal samples were collected from the weak 3rd up to the end of experiment. Faecal egg counts were calculated by employing McMaster's egg counting technique. Throughout the experiment, blood samples were collected after every week from all the animals. Packed cell volume (PCV) was determined by microhaematocrit method. Total serum proteins concentration was estimated by Buiret method (Clinical diagnostic Kit method). The animals were weighed at the time of infection to determine the larval dose, and then every 14 days until the end of infection for the determination of weight gain.

RESULTS AND DISCUSSION

All the animals developed infection which was confirmed by faecal examination. *Haemonchus contortus* eggs were detected in faeces on 28th and 24th day post infection in immunized and unimmunized groups respectively. The peaks of egg counts were found in faeces on day 32nd and 29th day of infection in group immunized and unimmunized groups respectively. The faecal egg counts observed in the present study are presented in Table1. Immunized animals showed 64.08% reduction in faecal egg counts corresponding to unimmunized control. Mean PCV values of both the groups of animals decreased from 4th week of infection (Fig. 1). However, this decrease was more evident in the unvaccinated group corresponding to control group. Mean total serum protein concentration was significantly higher (5.4± 0.3) in immunized group compared to 4.8 ± 0.2 of unimmunized group

Table 1. Weekly faecal egg counts of immunized and unimmunized animal groups

S. No	Week	Immunized	Unimmunized
1	1	0	0
2	2	0	0
3	3	0	0
4	4	200	2550
5	5	850	3800
6	6	2000	3600
7	7	1600	2700
8	8	1200	2400
9	9	800	1900
10	10	400	1700
11	11	200	1400
12	12	100	400
Mean		612.5	1704.16
±		281.85	675.43
Reduction		64.08%	

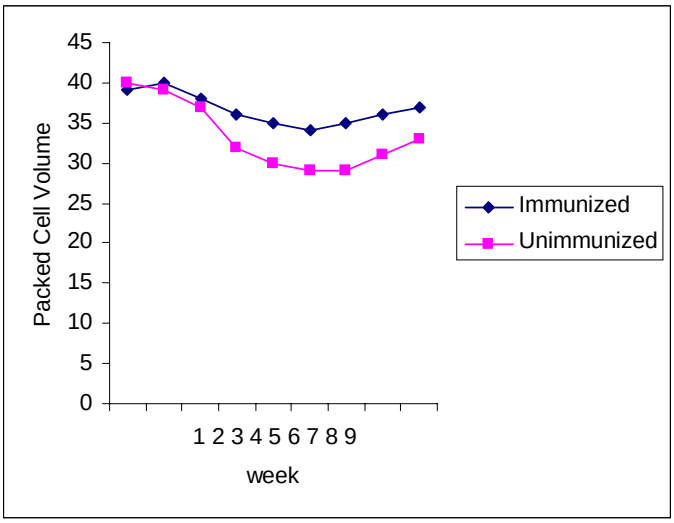

Fig. 1. Variation in packed cell volume (PCV) in immunized and unimmunized groups

The present study demonstrated that *Haemonchus contortus* membrane associated proteins can induce better protective response in sheep. In the present study the degree of protection achieved by vaccination as estimated by the reduction in faecal egg counts was similar to that observed by Munn *et al.* (1997). Values of the PCV observed in the present study were similar to Smith and Smith (1993) and Andrews *et al.* (1995) who observed decreased PCV values in infected animals. Various attempts have been made to induce a protective immune response to blood feeding parasite *Haemonchus contortus* with irradiated larvae, somatic extracts and excretory secretory products of its different stages of life cycle. Our results are in accordance with the findings of Jacobs *et al.* (1999) observed promising protection in lambs against *Haemonchus contotus* when vaccinated with surface antigen of L3 larvae. Considerable efforts have been applied to develop vaccines based on hidden antigens especially proteins molecules associated with the membrane of the gut. The immune response is not induced against these molecules during an infection as they are hidden. Vaccination with hidden antigen preparations has resulted in significant protection against *Haemonchus contortus* in young lambs, sheep and pregnant ewes (reviewed by Newton and Munn, 1999). The protection induced by this type of vaccination is based on the induction of antibodies directed against these hidden antigens (Smith, 1993).

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REFERENCES

Andrews S. J., Hole N. J. K., Munn E. A. and Rolph J. P. 1995. Vaccination of sheep against Haemonchus *contortus* membrane derived protective antigen from the adult parasite; Prevention of the preiparturient rise protective immunity. *International Journal of Parasitology,* **25**: 839–846.

Brandt, J. R. A., Geerts, S., De Deken, R., Kumar, V., Ceulemans, F., Brijs, L. and Falla, N. 1992. A monoclonal antibody based ELISA for the detection of circulating excretory-secretory antigens in *Taenia saginata cysticercosis. International Journal for Parasitology,* **22**: 471-7.

Jacobs, H. J., Wiltshire, C., Ashman, K. and Meeusen, E. 1999. Vaccination against the gastrointestinal parasite *Haemonchus contortus* using a purified larval surface antigen. *Vaccine.*, **17**: 362-368.

Jasmer, D. P. and Mcguire, T. C. 1991. Protective immunity to a blood feeding nematode (*Haemonchus contortus*) induced by parasite gut antigen. *Infection and Immunity.,* **59**: 4412-4417.

Jasmer, D. P., Perryman, L. E., Conder, G. A., Crow, S., and Mcguire, T. C. 1993. Protective immunity to *Haemonchus contortus* induced by immunoaffinity isolated against that show a phylogenetically conserved carbohydrate gut surface epitope. *Journal of Immunology,* **151**: 5450 - 5460.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. I. 1951. Protein measurements with the folin phenol reagent. *Journal Biological Chemistry,* **193**: 265-275.

Mahannop, P., Chaicumpa, W., Setasuban, P., Morakote, N. and Tapchaisri, P. 1992. Immunodiagnosis of human trichinellosis using excretory-secretory (ES) antigen. *Journal of Helminthology.,* **66**: 297-304.

Munn, E. A., Smith, S. T., Smith, H., Jasmer, F. M. and Smith, F. C. 1997. Vaccination against *Haemonchus contortus* with denatured forms of the protective antigens H11. *Parasite Immunology,* **18**: 243-248.

Newton, S. E. and Munn, E. A. 1999. The development of vaccines against gastrointestinal nematode parasites, particularly *Haemonchus contortus*. *Parasite Immunology*. **21**:165-169

Roberts, F. H. S. and Oscillivan, P. J. 1950. Methods of egg counts and larval culture for strongylides infecting the gastrointestinal tract of cattle. *Australian Journal Agriculture Research,* **1**: 99-102.

Schallig, H. D. Van F. H and Leeuwen, M. A. W. 1997. Protective immunity to the blood feeding nematode *Haemonchus contortus* induced by vaccination with parasite low molecular weight antigens. *Parasitology,* **114**: 293 – 299.

Smith, W. D. 1993. Protection in lamb in immunized with *Haemonchus contortus* gut membrane proteins. *Research in Veterinary Science,* **54**: 94 – 101.

Smith W. D. and Smith S. K. 1993. Evaluation of aspects of the protection afforded to sheep immunized with membrane protein of *Haemonchus contortus*. *Research in Veterinary Science,* **55**: 19 – 23.

Tavernor, A. S., Smith, T. S., Langford, C. F., Grahan, M. and Munn, E. A. 1992. Immune response of Clun Forest Sheep to vaccination with membrane glycoprotein from *Haemonchus contortus*. *Parasite Immunology,* **14** : 671 –675.