

Immunization of Arabian sheep with whole gut homogenate of *Haemonchus contortus*

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Summary

Ten female lambs of 7-month-old were divided into two equal groups and raised under a helminth-free conditions. Animals in group 1 were immunized two times by whole gut homogenate (WGH) of *Haemonchus contortus* emulsified in Freund's adjuvant. In group 2 (control), animals were injected by phosphate buffered saline emulsified in the same adjuvant. Animals were challenged by 10000 third-stage larvae (L3) of *Haemonchus contortus* on day 33 after the first immunization and then humanely killed on day 42. Animals were tested for serum antibody and eggs per gram of faeces (EPG) throughout the study and nematodes in their abomasom were counted after necropsy. The results indicated that animals immunized with WGH showed a higher level of serum antibodies. A significant difference was observed in mean optical density of sera in ELISA between the two groups ($P<0.05$) and a 77 and 78% reduction in EPG and nematode counts at necropsy, respectively ($P<0.05$).

Key words: *Haemonchus contortus*, Vaccination, Whole gut homogenate, Arabian sheep

Introduction

Haemonchosis, a disease of sheep, cattle and goats, is of considerable economic importance throughout the world. Severe infections in sheep lead to morbidity and death of weak animals. Even relatively light infections in adults cause various degrees of anaemia, loss of production and agalactia (Munn *et al.*, 1987). Control of haemonchosis at present depends largely on repeated application of anthelmintics (Newton and Munn, 1999). Chemical residues and the selection of parasite populations resistant to many anthelmintics are problems associated with this strategy. Therefore, development of an effective anthelmintic vaccine would be of great value (Munn *et al.*, 1993).

In blood-feeding nematodes like *Haemonchus contortus*, the parasite gut provides a potential source of protective

antigens (Jasmer and McGuire, 1991). In fact, substantial protection can be induced against *Haemonchus contortus* by immunizing lambs or goat kids with protein fractions isolated from the gut of this parasite. Such proteins are often known as hidden antigens because they are not recognized serologically by sheep which have acquired immunity following infection.

Vaccination with the hidden antigen H11, a membrane glycoprotein with microsomal aminopeptidase-like activity isolated from the intestinal brush border of adult *H. contortus*, is known to protect adult sheep and young lambs against haemonchosis (Andrews *et al.*, 1995). Substantial protection has also been achieved by immunizing sheep with a glycoprotein fraction isolated from the intestinal membranes of the parasite. This fraction has been termed *Haemonchus* galactose-containing glycoprotein (H-gal-

GP) (Smith and Smith, 1996).

These studies have been limited to the western part of the world with host strains and parasites in climate conditions related to that part. The present investigation was undertaken to evaluate the protective potential of whole gut homogenate (WGH) of local strains of *Haemonchus contortus* in the Arabian sheep against infection challenge.

Materials and Methods

Animals

Ten female lambs of 7-month-old, weighing 24-30 Kg, were housed in a concrete floored indoor and raised in helminth-free conditions during the study. Before the outset of experiment, lambs were treated with a combination of Albendazole (10 mg/Kg), Levamisole (8 mg/Kg) and Ivermectin (0.2 mg/Kg) for two weeks as suggested by Kabagambe *et al.* (2000).

Preparation of WGH of *H. contortus*

Haemonchus contortus WGH was prepared from adult nematodes recovered at necropsy in slaughterhouse. Nematodes were immediately placed in a protease inhibitor buffer (PIB; 50 mM Tris base, 50 µg/ml Aprotinin, 5 mM EDTA, pH = 7.4) and chilled on ice. Nematode intestinal tracts, with esophageal and rectal tissues removed, were dissected from individual worms, placed in chilled PIB plus 10% glycerol and stored at -70°C. To prepare WGH, 200 intestinal tracts were removed from the freezing medium, manually homogenized in 1 ml ice-cold PIB (Siefker and Rickard, 2000) and subsequently sonicated at 5 × 10 sec. WGH was analysed by a 7.5% polyacrylamide gel and Coomassie blue staining and its protein concentration was determined using Bradford method (Harlow and Lane, 1988), before storing at -70°C.

Experimental design

Lambs were divided into two equal groups. On day zero, animals in the first group (vaccinated group) were immunized with 100 µg WGH, diluted in 1 ml of phosphate buffered saline (PBS) and emulsified in 1 ml Freund's adjuvant.

Animals in the second group (control group), received 1 ml PBS emulsified in 1 ml of the same adjuvant. The dose was divided equally and administered intramuscularly in the semimembranous muscles of each hind leg. Booster immunization was performed subcutaneously in the shoulder region on day 21. Freund's complete adjuvant was used for the first injections and Freund's incomplete adjuvant was used for booster immunizations. On day 33, each lamb was challenged with approximately 10,000 *Haemonchus contortus* third-stage larvae (L3) obtained from a donor sheep, maintained at a separate place. Until challenge, the animals were bled with 10-day intervals and then weekly until the end of study. EPG count was made weekly prior to challenge and biweekly after challenge by sugar floatation method (Clayton-lane method). Lambs were necropsied 6 weeks post challenge, nematodes were recovered from abomasa and identified characteristically. Having determined the EPG and the number of female worms at necropsy, fecundity ratio (FCR) of female worms was calculated by the following formula (Boisvenue *et al.*, 1991):

$$\text{FCR} = \frac{\text{EPG at necropsy}}{\text{number of female worms at necropsy}}$$

Enzyme-linked immunosorbent assay (ELISA)

ELISA plates were coated with 1 µg/well of WGH, diluted in carbonate coating buffer and incubated at 4°C overnight. After two washes with PBS, containing 0.05% Tween 20 (PBS-T), plates were blocked by bovine serum albumine (0.1% in coating buffer) for 1 h at room temperature. Following two washes, 100 µl of each serum, diluted 1/100 in PBS-T, was added to a well and incubation was performed for 1 h. Wells were washed three times and then 100 µl of peroxidase conjugated anti-sheep IgG (sigma), diluted 1/1500 in PBS-T, was added to each well. After 1 h incubation, wells were washed three times more, peroxidase substrate chromogen (ABTs, Roche) was added and optical densities (OD) were read at 405 nm on a plate reader. The data were analysed by

SPSS (version 11) software. The t-test was used for the comparison of EPG, number of worms, OD of sera and fecundity ratio (FCR) between the groups. Significant differences were defined as $p \leq 0.05$.

Results

The WGH of *Haemonchus contortus* was prepared and analysed by SDS-PAGE. The WGH protein bands were in a wide range of molecular weights, but the protein bands of 20, 29, 32 and 35 kD seem to be the predominant proteins (Fig. 1). The results of ELISA on the sera are presented in Fig. 2. As indicated, the level of WGH specific antibodies in vaccinated lambs was significantly increased from day zero (the first immunization) until the fourth bleeding (day 31), then it reached the plateau. Comparison of OD of sera between vaccinated and control groups also indicated a significantly ($P < 0.05$) higher OD in vaccinated group, after the third bleeding until the end of the study.

First appearance of eggs in faeces was noticed in one of the five vaccinated and one

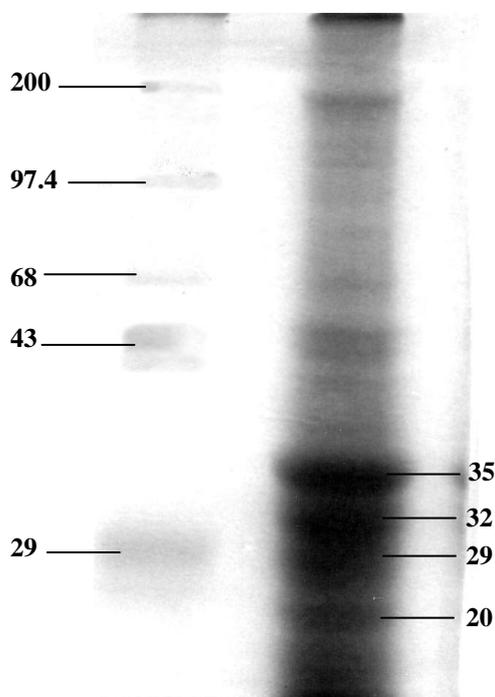


Fig. 1: Protein profile of WGH in SDS-PAGE. WGH was electrophoresed in a 7.5% polyacrylamide gel and stained by Coomasi blue. Lanes 1 and 2 represent the standard molecular weight and WGH, respectively

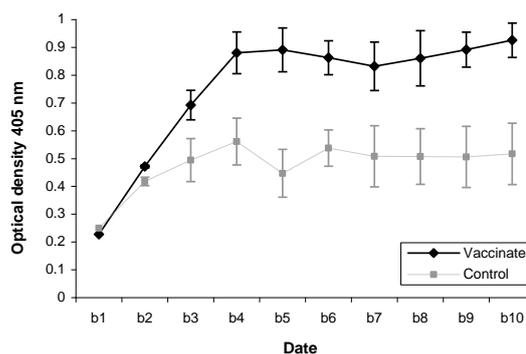


Fig. 2: Optical densities of sera in ELISA during the study (Mean ± SE), b1 = The first bleeding and b10 = The last bleeding

of the five control lambs on day 19 post challenge. On 26th day post challenge, all the lambs (control and vaccinated) had eggs in their faeces. The data of worm burden and EPG of the individual lambs for each group, on day 42 post challenge, are indicated in Table 1. Average number of worms showed a significant reduction of 78% in total worms recovered from the vaccinated group ($P < 0.05$). Animals in this group showed an average significant reduction of 80% ($P < 0.05$) and 74% ($P < 0.05$) in the number of female and male worms, respectively. Mean EPG of vaccinated group was 77%, significantly lower than that of the controls ($P < 0.05$).

Calculation of FCR revealed that vaccination has reduced the fertility of female nematodes, however, difference was not statistically significant ($P > 0.05$).

Discussion

The results indicated that immunization of Arabian sheep with WGH of *Haemonchus contortus* could significantly reduce the number of worms and EPG of the remaining female worms. These findings are in accordance with the results of Siefker and Rickard (2000).

In this study, following vaccination, the number of female and male worms were reduced up to 80 and 74%, respectively. it could be related to the fact that female worms are larger than males and presumably ingest more blood; alternatively, they may possess different gut membrane proteins which somehow make them more vulnerable to this type of immunization (Smith, 1993).

Table 1: Numbers of male and female *Haemonchus contortus* at autopsy and EPG on day 42 post challenge

Group	Sheep no.	Total worms	Male worms	Female worms	EPG	FCR
Control	1271	8	2	6	155	25.83
Control	1284	721	312	409	7082	17.31
Control	1286	802	352	450	3889	8.64
Control	1294	156	63	93	1353	15.54
Control	1304	387	133	254	1851	7.28
Mean values \pm SE		414.8 \pm 154/4	172.4 \pm 86/4	242.4 \pm 68/6	2866 \pm 1214.3	14.92 \pm 3.33
vaccinated	1280	5	0	5	42	8.40
vaccinated	1288	65	29	36	500	13.88
vaccinated	1291	2	0	2	23	11.50
vaccinated	1300	382	185	197	2690	13.65
vaccinated	1285	2	0	2	36	18
Mean values \pm SE		91.2 \pm 37/7	42.8 \pm 36	48.4 \pm 73/7	658.2 \pm 515.9	13.08 \pm 1.57

However, vaccination did not completely eliminate nematodes from vaccinated animals, but it can be efficient to reduce pasture contamination and consequently the level of reinfection. In fact, the greater value of vaccines may be in controlling the infective pasture rather than eliminating the nematodes in the host (Siefker and Rickard, 2000).

The reduction of total worms and EPG in vaccinated animals showed a similar trend with the ELISA optical densities of sera. This is in agreement with the findings of Munn *et al.* (1993), Smith (1993) and Smith and Smith (1996) who showed a correlation between reduction in number of nematodes and EPG with the serum antibody levels related to animals vaccinated with H11 and H-gal-GP. Therefore, the choice of adjuvant (Siefker and Rickard, 2000), antigen quantity (Munn *et al.*, 1993) and immunization protocol can affect the development of the resultant immune response and the level of protection. East *et al.* (1993) found that reduction in faecal egg count (FEC) was greater with dextran sulfate than the Freund's complete adjuvant. Smith *et al.* (1999) found that Quil A with H-gal-GP resulted in levels of protection in sheep that are consistent with initial immunizations and boosters with Freund's complete adjuvant.

The breed, age and individual characteristics of animals can also influence the immune responses to vaccination or infection. In a study carried out by Jasmer and McGiure (1991) on Pygmy and Saanen goats infected with 10,000 infective larvae, the number of worms in the abomasum and

eggs in the faeces were lower for control Pygmy goats compared with control Saanen kids. The variability in reduction of faecal egg counts and worms among the immunized goats most likely reflects differences in the immune responses of individual goats. In a study performed by kabagambe *et al.* (2000), large variations in susceptibility to infection existed between individual ewes, regardless of whether they had been immunized with antigen or not. For example, individual faecal EPG count in 10 sheep of the 25 control sheep never exceeded 250, despite FEC of several thousands being recorded in some of their cohorts. The presence of this high proportion of relatively resistant individuals in the control group could have prevented detection of some of the beneficial effects of vaccination. In conclusion, the use of WGH of *H. contortus* may have significant impact on the sheep protection against haemonchosis, particularly in areas where haemonchosis is prevalent.

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