



Strategic vaccination of hair sheep against *Haemonchus contortus*

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Abstract

In this study, we evaluated in two trials a protocol designed to protect hair sheep using Barbervax®, a vaccine containing *Haemonchus contortus* gut membrane glycoprotein antigens. Results indicated that naturally infected vaccinated sheep had significant egg count reductions ($90.2 \pm 4.03\%$) compared with controls, although blood parameters remained relatively unchanged probably because the level of challenge was low. Vaccination prevented the periparturient rise in egg shedding of ewes, as well as egg shedding in lambs (37.1%). In the second trial, sheep which were experimentally exposed to higher artificial challenge also showed an efficient response to the vaccine as confirmed by high antibody levels and reduced egg counts and worm burdens ($87 \pm 5.4\%$ and 79%) respectively. Thus, we believe that the vaccine should be integrated with other management practices for meat hair sheep as it has the advantages of adequate efficacy, reducing anthelmintic utilization and avoiding milk and environmental contamination with chemical residues.

Keywords Nematode · Infection · Control · *Haemonchus*

Introduction

Haemonchus contortus is one of the most important small ruminant parasites in the tropics. The problem becomes worse when it is associated with anthelmintic resistance thus increasing economic losses to prohibitive levels (de Oliveira et al. 2017). The low efficacy of current control methods has directed attention to non-chemical alternatives, which are in high demand from Brazilian livestock farmers.

The gut-antigen approach to vaccination was discovered approximately 20 years ago, and interest in it became stronger once the production of specific antibodies capable of neutralizing the enzymes involved in the adult parasite's digestive processes were confirmed (Tavernor et al. 1992a, b; Munn et al. 1993a, b; Smith et al. 1993; Smith and Smith 1993; Smith 1993). Since these antigens are located in the gut membrane within the parasite, they do not come in contact with host's immune system under natural conditions. Therefore, these molecules are commonly referred as hidden antigens (Smith and Zarlenga 2006).

The studied vaccine has been trialed around the world to evaluate its efficacy in herds of sheep (Bassetto et al. 2014) and goats (Matos et al. 2017) and is now marketed exclusively for sheep in Australia and South Africa where it is named Barbervax® and Wirevax®, respectively. Vaccine production could be scaled up for export to further countries where haemonchosis is important such as several in South America, but more efficacy studies are necessary to see how useful the vaccine is in these locations.

Previous studies have shown the efficacy of the vaccine in cattle and sheep in São Paulo State (Bassetto et al. 2011, 2014; Bassetto and Amarante 2015), Rio Grande do Sul State (Souza et al. 2011), and Uruguay (Castells et al. 2013). In these trials, the vaccine was evaluated in wool sheep located in a temperate humid climate where the gastrointestinal

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parasite challenge is stronger. In North East Brazil, hair meat sheeps which are adapted to the local semi-arid climate predominate (Amarante et al. 2009; Souza et al. 2013; Bastos et al. 2017). This region has long annual drought periods ranging from 7 to 9 months interspersed with 3–5 months of rain when *Haemonchus* infection becomes a serious problem. This is a great situation to test the vaccine strategically since it should only be required during the rainy season. Therefore, the aim of this research was to evaluate the vaccine as an alternative for managing *Haemonchus* infections in hair sheep.

Materials and methods

The Barbervax® vaccine used in this work was made from gut antigens of adult *H. contortus* (Smith et al. 2000a) and contained 5 µg/ml of integral glycoprotein complexes (H11 and H-gal-GP), 1 mg saponin (QuilA) in 1 ml of Tris-buffered saline pH 7.4 and 0.1% Triton X-100.

Two trials were conducted in Ceara State: a field trial on a private farm and a pen trial in the facilities of the National Center of Research on Goats and Sheep—Embrapa Caprinos e Ovinos. Both trials were performed using crossbred Santa Ines hair sheep, the most common breed in the North East region of Brazil. Due to the semi-arid climate, these flocks are usually managed, so they lamb three times in 2 years; one lambing can occur inside (early January to late May) or outside the rainy season some years. In both trials, sheep diets met the nutritional requirements for maintenance and growth (NRC 2007).

The field trial contained 90 adult ewes split into two equal groups, vaccinated or non-vaccinated, grazing the same bushland area during the day and brought indoors at night where they had access to feed. Sheep were balanced for body weight (40.2 kg ± 0.71) and previous fecal egg count (254 ± 47) in two equal groups, vaccinated or not. The vaccination protocol, which attempted to prevent the parasite from establishing within the host, was designed with the production system and the time of greatest *Haemonchus* challenge (rainy season) in mind. Three priming doses were given subcutaneously 21 days apart before the rainy season and boosts were administered every 6 weeks until the end of the rains. The ewes were monitored by fecal egg counts and blood parameters every 15 days during the rainy period, and a single fecal sample was also collected from 67 lambs aged 6 weeks. Specific *H. contortus* fecal egg counts (FEC) and fecal cultures were performed with standard methods (Roberts and O’Sullivan 1950; Ueno and Gonçalves 1988). Eosinophils were stained and counted using a Neubauer chamber (Dawkins et al. 1989). Packed cell volume (PCV) was determined by centrifugation in capillary tubes, and total plasma protein (TPP) measured with an ocular refractometer.

The degree of natural challenge by gastrointestinal parasites in the field trial just described was low, so a second trial with housed sheep receiving food and water ad libitum was performed. Thus, sheep aged 1 year were allocated to two equal groups ($n = 12$), vaccinated or not, each one balanced for body weight (38.7 kg ± 1.02) and fecal egg count (745 ± 130) and previously drenched with a single dose of Closantel (10 mg/kg body weight). Each sheep was challenged with 3000 L3 from a local strain of *H. contortus* per week for 4 weeks and sampled as in the first trial. Adult mature worms were counted in the abomasal contents (Ueno and Gonçalves 1988). Since the most important effect of the *Haemonchus* vaccine is the reduction of the egg shedding, the average efficacy was calculated using the same equation used for anthelmintics (Coles et al. 2006) as follows:

$$\text{Efficacy} = \frac{\text{Mean EPG of controls} - \text{Mean EPG of vaccinates} \times 100}{\text{Mean EPG of controls}}$$

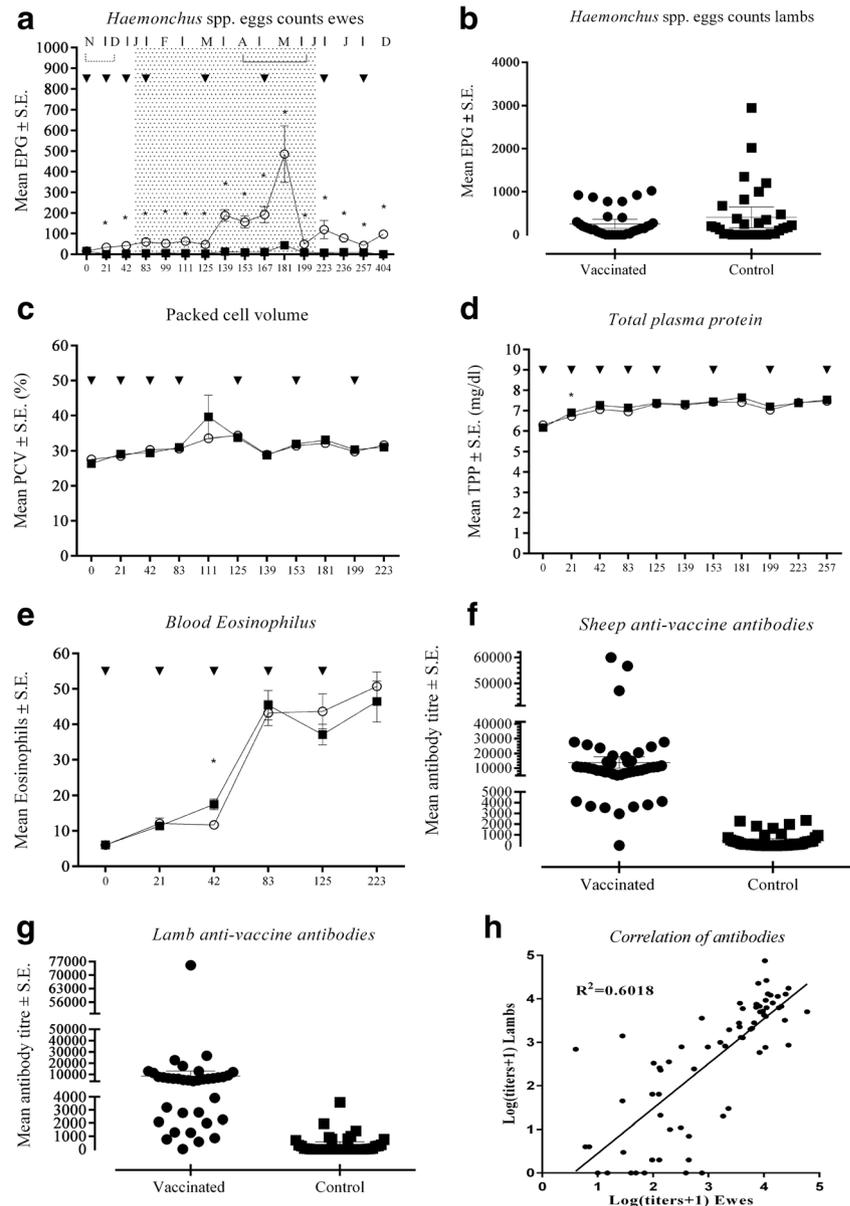
Antibody titers were measured according to Bassetto et al. (2011) using the horseradish peroxidase (HRP)-conjugated secondary antibody anti-Sheep IgG (Sigma-Aldrich, St. Louis, U.S.A.) following serial dilutions in 96 well plates.

Significant differences between parasitological and serological parameters were obtained by analysis of variance using SAS (version 9.2). Data from FEC was transformed into log ($x + 1$) before analysis. Significant differences between the groups were tested with Tukey’s test at 5%. Results are expressed as arithmetic means and standard error of back-transformed data. Titer data was tested for normality using the Shapiro-Wilk test (raw and log-transformed data), Spearman correlation was calculated for log-transformed data considering 4 groups (vaccinated lambs, vaccinated ewes, unvaccinated lambs, unvaccinated ewes) and 2 groups (ewes and lambs) maintaining the association of individual mothers and their respective offspring. We also used linear regression analysis to model the log-transformed data (GraphPad Prism v. 6.05).

Results

Overall, naturally infected vaccinated sheep and their lambs had significantly reduced egg counts compared with non-vaccinated controls. The mean ewe efficacy was 90.2% (± 4.03) (Fig. 1a) while that of lambs born from vaccinated ewes was a 37.1% reduction in egg counts when aged 6 weeks (Fig. 1b). Blood parameters showed little variation during the trial, probably because of the low field challenge (Albers et al. 1990). Total plasma proteins and eosinophils from vaccinates differed at 21 and 42 days after V1 respectively ($P < 0.05$, Fig. 1c–e). Immunized ewes and their lambs showed increased production of specific antibodies ($P < 0.05$, Fig. 1f, g). Titer data did not pass the normality test, and Spearman correlation

Fig. 1 **a** *Haemonchus contortus* fecal egg counts of vaccinated (black square) and control (circle) sheep naturally infected (inverted black triangle) vaccinations; (dots) rainy season; (bracket) Between N and D: sheep breeding period; (bracket) between A and J: peripartum; **b** fecal egg counts their lambs, **c** packed cell volume values, **d** total protein values, and **e** eosinophils counts. Antibody response from **f** sheep and their **g** lambs. **h** Titer correlation between ewes and lambs ($r = 0.8158$, $p < 0.0001$) and linear regression of log-transformed data. Values are individual means \pm S.E.M. *Statistically significant difference of group vaccinated compared with controls ($P < 0.05$)



values were low but significant between vaccinated ewes and lambs ($r = 0.365$, $p = 0.031$) and unvaccinated ewes and lambs ($r = 0.375$, $p = 0.026$). A higher correlation was observed when animals were grouped as ewes and lambs ($r = 0.8158$, $p < 0.0001$), and linear regression results of log-transformed data are in Fig. 1h.

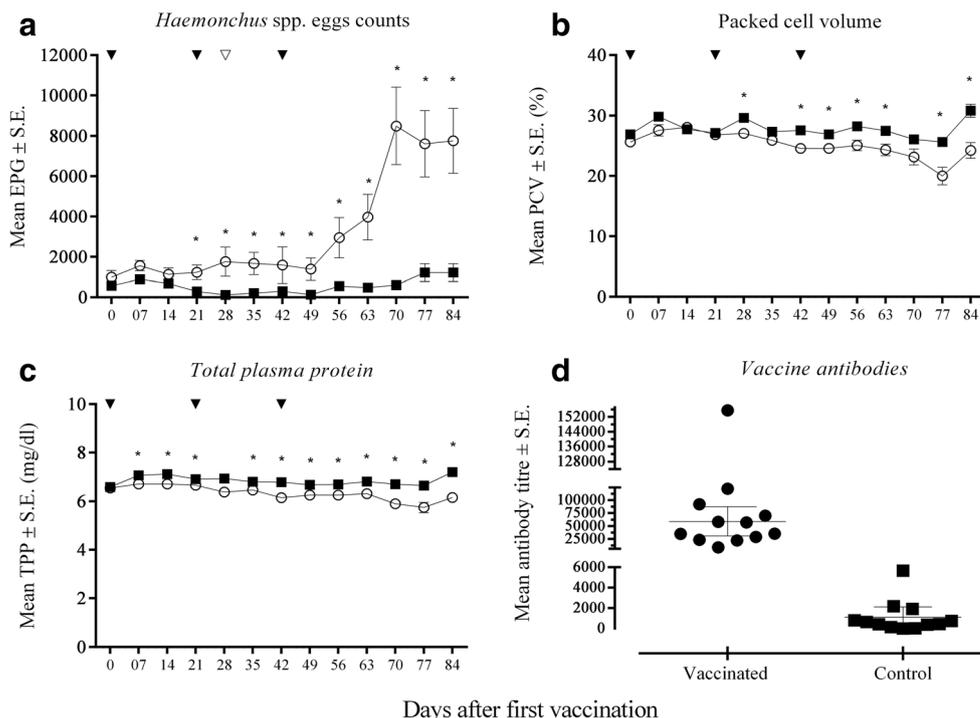
Results from the pen trial confirmed the efficacy observed in the field trial. Vaccinated sheep had reduced egg counts from the third week after the first vaccination. This effect was maintained over the course of the pen trial, but it was most obvious from the 8th week of infection onwards. The average efficacy of the vaccine in the pen trial was 87% (± 5.4) (Fig. 2a). Adult worms were also reduced significantly (79%) in the immunized sheep compared to controls ($P < 0.05$) (Table 1). In this trial, blood parameters of vaccinates were

positively affected as shown by PCV and TPP values (Fig. 2b, c). This was borne out to some extent by the ELISA data (Fig. 2d).

Discussion

Small ruminant farming in Brazil is especially important in the North East because local breeds are well adapted to the semi-arid climate and are productive at reasonably low costs for the smallholder farmers. *Haemonchus contortus* has been reported as the main health issue for goats and sheep (Vieira 2008) usually controlled with anthelmintic drugs, mostly benzimidazoles. Consequently, anthelmintic resistance within this chemical group is widespread in Ceara State (Santos et al.

Fig. 2 **a** *Haemonchus contortus* fecal eggs counts of vaccinated (black square) and control (circle) sheep artificially infected (inverted triangle) 28 days. (inverted black triangle) vaccinations. **b** Packed cell volume values. **c** Total protein values. **d** Anti-vaccine antibodies. Values are means ± S.E.M. *Statistically significant difference of group vaccinated compared with controls ($P < 0.05$)



2014). Because this semi-arid zone has a well-defined rainy season, there is an opportunity to control *Haemonchus* infection by focusing on the period of highest challenge. The main effect of the *Haemonchus* vaccine is to reduce egg shedding thus decreasing pasture contamination (Barnes et al. 1995), and so the vaccination protocol used above was

designed to prevent female *Haemonchus* from producing eggs during the rainy season.

The results indicated that when vaccine priming doses were started in November, vaccinated ewes shed 90% fewer worm eggs during the challenge period. Four additional injections were sufficient to protect the sheep until the end of the

Table 1 Comparison of adult worm counts (AWC), terminal fecal eggs counts (EPG), terminal packed cell volume (PCV), and terminal total plasma protein (TPP) in sheep vaccinated or non-vaccinated controls following experimental *Haemonchus contortus* challenge

Groups	Adult <i>Haemonchus contortus</i> counts and terminal parameters							
	Vaccinated				Control			
Animal	AWC	EPG	PCV (%)	TPP (mg/dl)	AWC	EPG	PCV (%)	TPP (mg/dl)
1	740	5133	27	7.1	810	4406	27	6.2
2	662	3306	35	6.4	455	4406	26	6.3
3	871	1588	28	7.6	7525	11,726	18	5.4
4	22	87	30	7.9	4818	16,309	23	5.7
5	27	1218	29	6.9	709	1847	32	6.8
6	384	1175	33	6.6	5745	17,644	19	5.1
7	40	196	39	6.5	734	5029	31	6.6
8	678	827	30	7.6	3013	8032	20	5.4
9	23	44	31	7.7	543	1758	25	6.7
10	2687	805	25	7.3	3001	12,549	22	6.3
11	14	174	30	6.9	646	2092	21	6.4
12	33	152	33	8	1286	7365	27	7.1
Mean ± S.E	515 ± 220	1225 ± 444	31 ± 1	7.2 ± 0.16	2440 ± 695	7763 ± 1611	24 ± 1.3	6.2 ± 0.18

AWC Adult worm count

challenge in late May when the risk of infection declined. Furthermore, if lambs born from vaccinated ewes have adequate colostrum antibodies, they would only need to be vaccinated in the next season. So, the protocol used here is efficient and potentially cheaper than protocols designed for temperate regions of Brazil (Bassetto et al. 2014), since sheep will be vaccinated only in the most challenging period of the year (rainy season of around 6 months).

This is the first attempt to control *Haemonchus* infection using a vaccine against the parasite in hair meat sheep, and the efficacy results obtained were equivalent or better than previous Brazilian studies done in other regions and with different breeds (Bassetto and Amarante 2015). It is important to mention that protection was maintained during pregnancy and prevented the periparturient egg count rise (Fig. 1a). Blood parameters were not consistently affected during the field trial probably because of low challenge (Albers et al. 1990). Like previous studies (Bassetto et al. 2011, 2014), the vaccination did not affect circulating eosinophils, and so further investigations should focus on tissue samples (Robinson et al. 2010). Furthermore, the vaccinated animals in the field trial grazed the same bushland area as the non-vaccinated controls and could not enjoy the epidemiological benefit of grazing separately. Thus it is likely that the impact of the vaccination was underestimated.

The efficacy of the studied vaccine was confirmed in the pen trial where reductions in the egg count became more evident starting the third week after the challenge infection. Vaccinated sheep had 79% fewer worms, similar to sheep naturally infected under strong challenge conditions (Cachat et al. 2010; Smith 2007; Smith et al. 2000a, b) but less substantial than the previously reported 93% reduction (Smith et al. 1993).

High levels of anti-vaccine antibodies and blood proteins were also observed. However, the production of vaccine antibodies by non-vaccinates indicates low natural exposure of the host's immune system to the gut antigens of *H. contortus*. It is likely that small concentrations of the *Haemonchus* gut membrane antigens are exposed to the host immune system after parasite death (LeJambre et al. 2008). Our results also contribute to the idea that gut antigens are conserved among geographic isolates of the nematode (Emery et al. 2016). Notwithstanding, it remains unknown if populations of *H. contortus* could genetically change under selective pressure of vaccine-induced antibodies.

Extensive field trials with the studied vaccine performed in sheep and lambs in Australia indicated 70–80% reductions in the egg counts, and modeling studies indicated that this effect would be as efficient or more than a conventional anthelmintic program (Besier et al. 2015). Furthermore, vaccination has other advantages over anthelmintic drugs, for example, no withdrawal period. Now that the vaccine has been shown to be effective against *H. contortus* in hair sheep, it can be tested

in combination with other control measures to improve its control in Brazilian sheep.

Conclusion

As determined by reduced fecal egg counts, worm burdens, and hematology, Barbervax® succeeded in controlling *H. contortus* in hair sheep farmed under typical field conditions in North East Brazil. The vaccine does not have a withdrawal period, and so it could be a valuable tool for the control of this nematode in meat sheep farms in Brazil.

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Compliance with ethical standards

This study was approved by the Ethics Committee for the Use of Animals of the Universidade Estadual Vale do Acaraú (CEUA protocol number 029.12), and all of procedures were based on Resolution Number 1000 of the Federal Board of Veterinary Medicine (Brazil, 2012).

Conflict of interest Dr. Smith is employed by Wormvax Australia which is a subsidiary of the Moredun Research Institute, a not for profit organization which manufactures and sells Barbervax®. He supplied the vaccine for the trial and advised on the trial designs, but was not involved in obtaining any of the results.

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