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Attempts to vaccinate ewes and their lambs against natural infection with *Haemonchus contortus* in a tropical environment

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ABSTRACT

A vaccine containing integral membrane glycoproteins from the intestine of *Haemonchus contortus* was evaluated in three groups of grazing sheep each containing 13 ewes and their 16 lambs naturally infected with gastrointestinal nematodes. Two groups were vaccinated with either 5 or 50 μg of the antigen per immunisation, while the third, the control group, received adjuvant alone. The sheep were immunised six times at 3 week intervals, partly because the vaccine antigens are hidden and thus no immunological boost would be delivered by subsequent infection and partly because the level of *Haemonchus* spp. challenge was expected to be high. The vaccinated ewes, first immunised approximately 1 month before lambing, showed a circulating antibody response but no signs of reduced anaemia or *Haemonchus* spp. egg counts, compared with control ewes. Several ewes with severe haemonchosis in all three groups had to be given precautionary treatment with anthelmintic drugs. In contrast, vaccinating their lambs with either 5 or 50 μg of the antigen per immunisation resulted in 10 fold higher antibody titres. In the case of the lower antigen dose this was associated with significantly less anaemia, 72% reduction in the overall number of *Haemonchus* spp. eggs produced and significantly fewer worms compared with control lambs. It is hypothesised that the heavily pregnant or lactating ewes did not have sufficient physiological reserves to mount a protective response following vaccination in the tropical weather and high challenge conditions that prevailed. Nevertheless, the vaccine could afford useful protection for lambs against *H. contortus*.

1. Introduction

Worldwide, blood sucking *Haemonchus contortus* is probably the most economically important nematode parasite of small ruminants, mainly in warm damp climates, and is therefore particularly problematic in the tropics and subtropics. It is a problem in most of South America (reviewed by Amarante, 2014), including the farm where the present trial was conducted (Wilmsen et al., 2014). Normally *Haemonchus* spp. and other gastrointestinal parasites are controlled with anthelmintic drugs but resistance to these drugs is a problem in Sao Paulo State (Almeida et al., 2010; Veríssimo et al., 2012; Nunes et al., 2013), other parts of Brazil (Sczesny-Moraes et al., 2010; Santos et al., 2014), and across many other countries (reviewed by Kaplan and Vidyashankar, 2012; reviewed by Torres-Acosta et al., 2012).

One potential option to control *H. contortus* in sheep is by means of a vaccine which has been showing promising results in Australia (Smith, W.D., personal communication). This vaccine, which is currently being reviewed by the regulatory authorities in Australia and South Africa, contains native gut membrane proteins including the well characterised protective antigens H11 (Smith et al., 1993, 1997) and H-gal-GP (Smith et al., 1999). These vaccine antigens are "hidden", meaning that the vaccine response is not boosted by infection, and thus repeated immunisation is needed to maintain the protective response (Le Jambre et al., 2008).

The present paper describes a trial to evaluate this vaccine in lactating ewes and their lambs subjected to a natural *Haemonchus*

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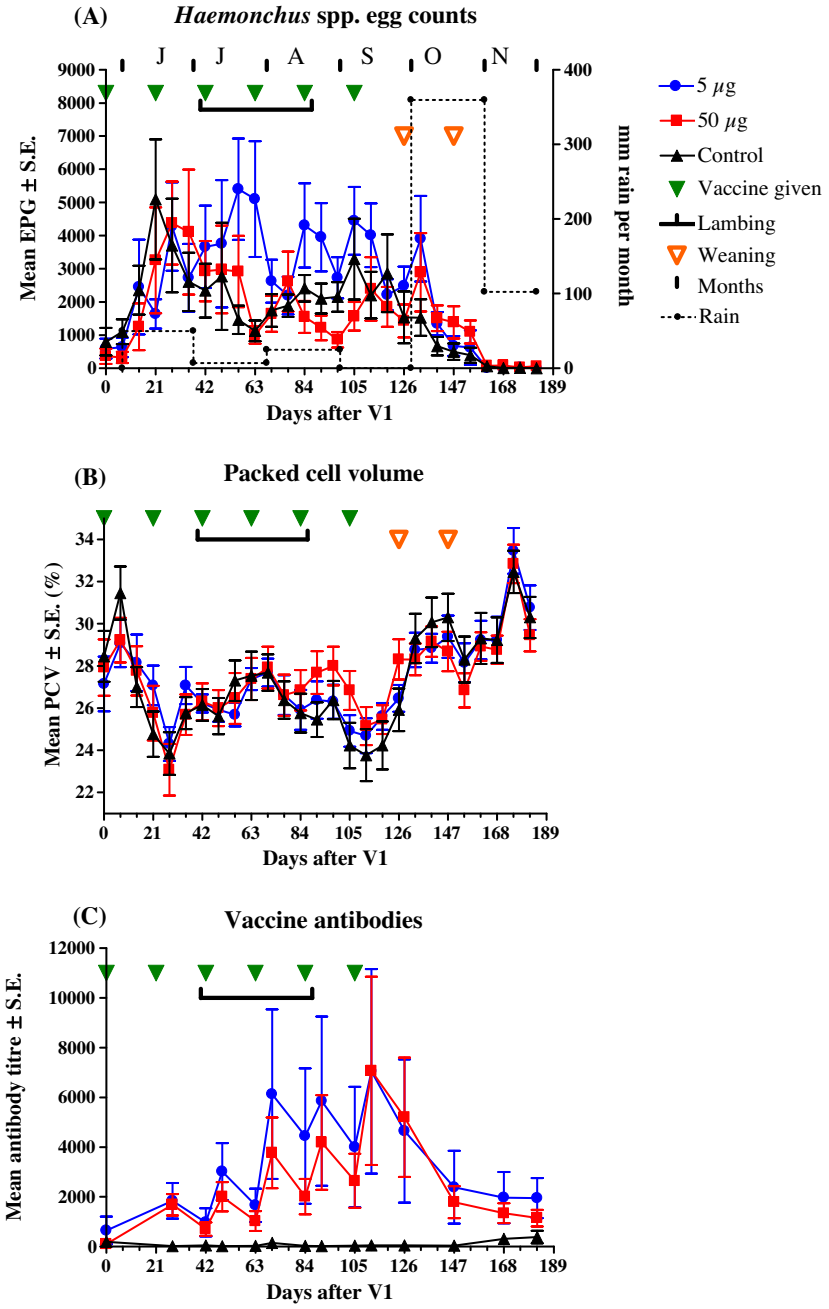

Fig. 1. Kinetics of *Haemonchus* spp. (A) eggs per gram of faeces (EPG), (B) packed cell volume (PCV) and (C) anti-vaccine antibody response of the ewes immunised with 5 μg or 50 μg of *Haemonchus contortus* gut membrane antigen or vaccinated with adjuvant only (control). V1, first immunisation – day 0. The upper case letters indicate the months of development of the trial (J, June; J, July; A, August; S, September; O, October; N, November).

spp. challenge on tropical pasture in Sao Paulo State, Brazil. It was anticipated that the level of challenge would be high for both ewes and lambs, and thus a severe test of the vaccine was expected. For this reason, the vaccine was trialled at 5 μg, the normal dose of antigen per injection, but also at 50 μg per dose. In addition, the vaccine was administered repeatedly at 3 weeks intervals and lambs were given their first dose when only 4 weeks old, as it is known that patent *H. contortus* infections can be observed as early as 6 weeks of age in this environment. Finally, the three groups, 5 or 50 μg vaccine and the controls, grazed on separate but adjacent paddocks, so that if the vaccinated animals' egg counts were suppressed, they might enjoy the subsequent epidemiological benefit.

The overall rationale was that if the vaccine did work under this rather extreme regime, further work to identify a more practical immunisation protocol could be undertaken. On the other hand, if the result was negative, the vaccine could probably be dismissed as useless for sheep in the tropics.

2. Materials and methods

2.1. Preparation and administration of the vaccine

The vaccine antigen was prepared at Moredun Research Institute, Edinburgh, UK from gut membranes of adult *H. contortus* as described before (Smith et al., 2000). Briefly, Triton X-100 extracts of the parasite membranes were prepared (Smith et al., 1999), diluted four-fold with 10 mM Tris–HCl, 0.5 M NaCl, 0.02% NaN_3, pH 7.4 and were added to 100 μM Ca++ and 10 μM Mg++. The