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Protection studies with a globin-enriched protein fraction of *Ostertagia ostertagi*

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Abstract

The protective capacity of an adult stage *Ostertagia ostertagi* globin antigen was tested in four vaccination experiments in cattle. In a preliminary experiment, calves were vaccinated three times intraperitoneally with 250 μg globin in Freund's adjuvant and challenged with a trickled infection of 25,000 infective larvae. In three subsequent field studies, calves were vaccinated twice or three times intramuscularly with 80–100 μg globin in Quil A and challenged with a natural gastrointestinal nematode infection on pasture. Higher globin-specific antibody levels were detected in the vaccinated calves than in the control animals in all vaccine trials. In the preliminary experiment, geometric mean cumulative egg counts in the globin group were reduced by 52% and total worm burdens were reduced by 28%, compared to the controls. In the first field trial cumulative faecal egg counts were reduced by 63% in the vaccinated calves. However, the reduction in faecal egg output in these two experiments was not statistically significant and no reduction in faecal egg counts was observed in the vaccinated animals in the two last field trials. In conclusion, vaccination of calves with *O. ostertagi* globin resulted in highly variable protection levels after challenge infection.

Keywords: *Ostertagia ostertagi*; Nematode; Cattle; Vaccine; Globin

1. Introduction

Ostertagia ostertagi is one of the most important gastrointestinal (GI) nematodes in cattle. Control of GI nematodes in cattle relies heavily on the preventive use of anthelmintics. However, consumer concerns about chemical residues in the environment and in animal products, and the threat of anthelmintic resistance warrant the search for alternative control measures, such as vaccination.

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Recently, protection against *O. ostertagi* challenge infection was obtained by immunising calves with gut membrane glycoproteins (Smith et al., 2000) or with protein fractions enriched for cysteine proteases (Geldhof et al., 2002, 2004) or *Ostertagia* polyprotein allergen (Vercauteren et al., 2004). The most obvious effect of these experimental vaccines on the parasite's biology was a marked suppression of worm egg output. Smith et al. (2000) reported a 30–50% decrease in faecal egg counts, while cumulative faecal egg counts were reduced by 56–60% over a 2-month period in the other vaccine trials (Geldhof et al., 2002, 2004; Vercauteren et al., 2004). It is believed that a similar reduction of faecal egg output under natural infection conditions would be sufficient to substantially protect calves during the entire first grazing season (Vercruysse and Claerebout, 2003). However, despite the fact that in some of these vaccine trials a trickle challenge infection was given to mimic the uptake of an overwintered larval population on a first grazing season pasture (Geldhof et al., 2002, 2004; Vercauteren et al., 2004), these experimental vaccines still need to prove their efficacy in the field.

In the present study an *O. ostertagi* globin was evaluated as a potential protective antigen in calves. *O. ostertagi* globin is present in the L4 and adult life stages of the parasite (de Graaf et al., 1996). Similarly, globins are only found in the L4 and/or adult stages of *Trichostrongylus colubriformis* (Frenkel et al., 1992), *Nippostrongylus brasiliensis* (Blaxter et al., 1994), *Ascaris suum* (Barrett and Brophy, 2000) and *Haemonchus contortus* (Fetterer et al., 1999). The stage-specific expression of nematode globins may represent an adaptation to the parasitic environment. In the micro-aerobic environment of the vertebrate intestine nematode globins may have diverse functions such as oxygen transport, osmotic regulation, iron storage or oxygen detoxification (Blaxter, 1993), which makes them interesting targets for chemotherapy or vaccine development. Vaccination with a globin-like antigen protected guinea pigs against *T. colubriformis* infection (Frenkel et al., 1992).

The objective of the present study was to determine the protective capacity of an adult stage *O. ostertagi* globin antigen for calves. In a preliminary study the protective capacity of globin was tested in calves immunised with globin in Freund's adjuvant, followed by an experimental trickle infection. The protective capacity of globin was further evaluated in three field studies in which calves immunised with globin in Quil A received a continuous natural challenge infection. In the first two field studies vaccinated and control calves grazed together to ensure that all calves were exposed to the same level of nematode challenge. In the final field study vaccinated and non-vaccinated calves were grazed on separate plots, to evaluate the effect of vaccination on pasture infection levels.

2. Materials and methods

2.1. Preparation of antigen

Adult *O. ostertagi* parasites were obtained as described previously (Geldhof et al., 2000). Phosphate-buffered saline soluble (S1) protein extract was prepared from adult parasites as described by Smith et al. (2000). Globin was purified from the S1 protein fraction by gel filtration chromatography followed by anion exchange chromatography and reverse phase chromatography, essentially as described by de Graaf et al. (1996). Extract (2 ml/run) was loaded on a Sephacryl S-200 gel filtration column at 1.2 ml/min in elution buffer A (50 mM ethanolamine + 1 mM EDTA). The purification was monitored spectrophotometrically at both 280 and 405 nm and 5 ml fractions were collected. The 405 nm peaks were pooled and concentrated using centrifugal filtration devices with a 10 kDa molecular weight cut-off to 10 ml. This solution was washed twice with 2 volumes of Drabkin's reagent ($NaHCO_3/K_3FeCN_6/KCN$ at 100/20/5 from Sigma) and concentrated again to 10 ml. Twenty five millilitres of buffer A was added and concentrated again. This product was loaded on a Mono Q HR 5/5 ion exchange column at 1 ml/min. Unbound proteins were eluted in 10 ml of buffer A followed by a gradient from 0 to 100% buffer B (=buffer A + 1 M NaCl) in 10 ml to elute the bound proteins. One millilitre fractions were collected, haemoglobin-enriched fractions were pooled and concentrated to < 10 ml. The sample (maximum 10 ml) was loaded on a Resource ^{TM} Reverse Phase Chromatography column at 2 ml/min in buffer C (50 mM triethylamine pH 8.5). The globin was eluted