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Short communication

Comparative experimental *Haemonchus contortus* infection of two sheep breeds native to the Canary Islands

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Abstract

This study compares the susceptibility to *Haemonchus contortus* infection in two breeds of sheep endemic to the Canary Islands, the Canaria Hair Breed sheep and the Canaria sheep. Sheep were experimentally infected with 20,000 larvae of *H. contortus* and animals killed on days 7 and 28 post-infection. No difference between sheep breeds were detected in immature worm counts at days 7 or 28 post-infection. However, in comparison to the Canaria sheep breed, the Canaria Hair Breed sheep showed lower mean faecal egg counts, lower adult worm counts, lower number of eggs *in utero* and female worm stunting. Overall, these data suggest that the Canaria Hair Breed sheep has a greater resistance to *H. contortus* infection than Canaria sheep, and that this resistance may act at the level of the adult parasite.

Keywords: Sheep-nematoda; Resistance; Canaria; *Haemonchus contortus*

1. Introduction

Infection with the nematode parasite, *Haemonchus contortus*, is one of the major constraints in small ruminant production worldwide (Gauly et al., 2002). Control is largely based on regular anthelmintic treatment, however, the emergence of increasing drug resistance makes this method of production unsustainable over the short to long term and new strategies of control are needed. Identification and selective breeding of animals with higher genetic resistance to gastrointestinal nematodes is an attractive alternative (Matika et al., 2003; Raadsma and Tammen, 2005). The use of genetically resistant animals may also optimize the efficacy of anthelmintic use by delaying the development of parasite resistant populations and extending the useful life of anthelmintics. Several sheep breeds have shown a natural resistance to gastrointestinal nematodes and many are currently being studied to develop their selective breeding and potential commercial production traits (Gamble and Zajac, 1992; Goossens et al., 1997; Li et al., 2001; Raadsma et al., 2002; Aumont et al., 2003;

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Matika et al., 2003; Amarante et al., 2004; Bricarello et al., 2004; Mugambi et al., 2005).

The topographical and climatic characteristics of the Canary Islands have been an obstacle for the rearing of highly productive sheep breeds leading to almost exclusive exploitation of indigenous sheep breeds by local farmers for centuries. The two major Canarian sheep breeds are the Canaria Hair Breed (CHB) sheep and the Canaria sheep. The CHB sheep are a short haired breed sheep predominately reared for meat production, while the Canaria sheep are predominately utilised for milk production (Muñoz, 2003). Differences in trichostrongylid egg counts in faeces between the breeds have been consistently observed, even though animals of both breeds often co-habit the same grazing areas (Gonzalez et al., unpublished observations). In this study, a direct comparative experiment was carried out to determine whether these sheep breeds demonstrate differential resistance to *H. contortus* infection under standardised conditions.

2. Material and methods

Eight-month-old CHB sheep ($n = 13$) and Canaria sheep ($n = 14$) were purchased from local farms on Gran Canaria. Animals were housed in pens at the Faculty of Veterinary Science, University of Las Palmas de Gran Canaria (Spain). Animals were fed with a commercial pelleted sheep ration and water *ad libitum* throughout the total experimental period. Animals were drenched on arrival and 2 weeks before infection with levamisole (Cyver®, Fort Dodge, Spain) with the recommended dosage (1 ml/10 kg b.w.). Absence of eggs in faeces following drenching of animals was confirmed by coprological examination.

H. contortus parasites used in the experiment were kindly supplied as third-stage larvae by Dr. David Knox and Dr. David Bartley from the Moredun Research Institute, Edinburgh, Scotland, UK (Redmond and Knox, 2004). Animals were inoculated with 20,000 L3 *H. contortus*. Five CHB and five Canaria sheep were slaughtered on day 7 post-infection (p.i.). All other animals (8 CHB and 9 Canaria sheep) were killed on day 28 p.i.

Commencing on day 13 p.i., eggs per gram (EPG) were determined using a McMaster technique (MAFF, 1989). At days 7 and 28 p.i., animals were killed and their abomasums were removed. The abomasums were opened along the greater curvature and the contents placed in graduated test tubes. Each abomasum was then thoroughly washed and the washings added to the respective animal's abomasal contents. Samples were preserved in 5% formalin for total adult worm counts. Subsequently, the mucosa of each abomasum was collected (mucosal scrapings) and stored at −20 °C for the determination of larval counts. Adult parasites were recovered from abomasal washings and thirty adult female worms from each sheep were measured with a calibrated ocular scale. Each female was then crushed on a slide for *in utero* egg determination. Larval numbers from the mucosal scrapings were determined by digestion of mucosa with pepsin-HCl according to a publication by the Ministry of Agriculture Fisheries and Food (MAFF, 1989). Digestion was stopped with formalin and 10 ml aliquots were examined for immature worms.

All animals were bled weekly from the jugular vein commencing 3 weeks before parasite challenge using EDTA (7.5%) as an anticoagulant. Packed cell volume (PCV) was determined using a microhaematocrit, and plasma protein (PP) levels were estimated with a refractometer (FG-301, Protein, Comecta S.A.). Total leucocyte counts were performed using a Neubauer haemocytometer after red blood cells were lysed with Turks solution (1:20). Differential leucocyte counts of blood smears were determined using Fast Staining (Panreac®, Spain) solution.

All statistical analyses were conducted using SPSS Version 13.0 (SPSS Inc., 2000). The data relative to EPG and eosinophil counts in blood were subjected to square root transformation prior to statistical studies to normalize the residual variances. Parameters measured multiple times during the trial (EPG, cell blood counts, total PP and PCV) were analysed by general linear models using the repeated measures option. These data were analysed considering the effects of breed and day p.i. and the interaction between these effects. Significant differences between breeds in parameters taken at a single day such as adult and immature worm counts, female worm lengths and number of eggs *in utero* were determined using the non-parametric Mann–Whitney *U*-test. Probabilities of $p < 0.05$ were considered significant. A multivariate analysis of variance was adopted for analysing the parasitological traits of worm burden, faecal egg count, female worm fecundity and worm development (length) since these traits had shown interdependency and collectively best described host resistance to parasite challenge.

3. Results

CHB animals showed lower EPG counts than Canaria sheep ($p < 0.001$) throughout the experiment (Fig. 1). The mean EPG observed in Canaria sheep (24,778) was almost 5-fold higher compared to the