International Journal for Parasitology 39 (2009) 607–614

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Haemonchus contortus and *Trichostrongylus colubriformis* did not adapt to long-term exposure to sheep that were genetically resistant or susceptible to nematode infections

K.E. Kemper [a,c,*], R.L. Elwin [b], S.C. Bishop [c], M.E. Goddard [a], R.R. Woolaston [b]

[a] Victorian Department of Primary Industries, Victorian AgriBiosciences Centre, LaTrobe R&D Park, Bundoora, Vic. 3083, Australia
[b] CSIRO Livestock Industries, Locked Bag 1, Armidale, NSW 2350, Australia
[c] The Roslin Institute & Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin Biocentre, Midlothian, EH25 9PS, UK

ARTICLE INFO

Article history:
Received 12 June 2008
Received in revised form 13 August 2008
Accepted 18 August 2008

Keywords:
Haemonchus contortus
Trichostrongylus colubriformis
Host–parasite interaction
Faecal worm egg count
Sheep
Genetic resistance
Serial passage
Coevolution

ABSTRACT

We tested the hypothesis that *Haemonchus contortus* and *Trichostrongylus colubriformis* would adapt to long-term exposure to sheep that were either genetically resistant or susceptible to *H. contortus.* Sheep genotypes were from lines with 10 years prior selection for low (resistant, R) or high (susceptible, S) faecal worm egg count (WEC) following *H. contortus* infection. Long-term exposure of *H. contortus* and *T. colubriformis* to R or S genotypes was achieved using serial passage for up to 30 nematode generations. Thus, we generated four nematode strains; one strain of each species solely exposed to R sheep and one strain of each species solely exposed to S sheep. Considerable host genotype differences in mean WEC during serial passage confirmed adequate nematode selection pressure for both *H. contortus* (R 4900 eggs per gram (epg), S 19,900 epg) and *T. colubriformis* (R 5300 epg, S 13,500 epg). Adaptation of nematode strain to host genotype was tested using seven cross-classified tests for *H. contortus,* and two cross-classified and one outbred genotype test for *T. colubriformis*. In the cross-classified design, where each strain infects groups of R, S or randomly bred control sheep, parasite adaptation would be indicated by a significant host genotype by nematode strain interaction for traits indicating parasite reproductive success; specifically WEC and, for *H. contortus* strains, packed cell volume. We found no significant evidence of parasite adaptation to host genotype ($P > 0.05$) for either the *H. contortus* or *T. colubriformis* strains. Therefore, we argue that nematodes will not adapt quickly to sheep bred for nematode resistance, where selection is based on low WEC, although selecting sheep using a subset of immune functions may increase adaptation risk. Our results support the hypothesis that nematode resistance is determined by many genes each with relatively small effect. In conclusion, selection of sheep for nematode resistance using WEC should be sustainable in the medium to long-term.

1. Introduction

Adaptation of disease-causing pathogens or parasites to chemical control is widespread in many important parasites of humans and livestock. Parasite adaptation is a predictable evolutionary consequence of chemotherapy; intense selection pressure gives a selective advantage to tolerant parasites, allows them to multiply in successive generations and diminishes the overall effectiveness of the chemical (Gilleard and Beech, 2007). Adaptation of sheep gastrointestinal nematodes to anthelmintics follows this general pattern and is problematic because control of infections, for both animal health and production, relies almost exclusively on chemicals. Alternative control measures, including breeding strategies, are now being scrutinised as components of sustainable parasite management systems (Sayers and Sweeney, 2005).

Interest in breeding parasite-resistant sheep began in the 1970s and since then many studies have shown a genetic basis for parasite resistance in sheep. However, the physiological and underlying genetic mechanisms conferring resistance to gastrointestinal nematodes are complex and not fully understood. The most reliable indicator of resistance has been faecal worm egg count (WEC), where the relative resistance of each animal to a nematode challenge is assessed. Using WEC the heritability of nematode resistance generally lies between 0.2 and 0.4 across a range of sheep breeds, nematode species and infection protocols (Bishop and Morris, 2007). The complexity of the physiological response assessed by WEC is reinforced by recent research that has aimed to detect molecular markers for nematode resistance. Although many studies have identified significant quantitative trait loci (QTL) for

* Corresponding author. Address: Victorian Department of Primary Industries, Victorian AgriBiosciences Centre, LaTrobe R&D Park, Bundoora, Vic. 3083, Australia. Tel.: +61 3 9479 5391; fax: +61 3 9479 3618.
E-mail address: kathryn.kemper@dpi.vic.gov.au (K.E. Kemper).

0020-7519/$34.00
doi:10.1016/j.ijpara.2008.08.013

nematode resistance, when compared, these studies tend to identify different chromosomal regions (Dominik, 2005; Crawford et al., 2006; Davies et al., 2006; Beraldi et al., 2007). This suggests that either (i) different experimental protocols were invoking slightly different responses (Dominik 2005), (ii) different genes are heterozygous in different populations, or (iii) many genes each with small effect determine nematode resistance so each study lacked power and could only detect a subset of the genes involved. It appears that the intimate host–parasite relationship assessed by WEC is under the control of many different genes each with variable importance depending on the infection and host conditions.

There is concern that nematodes will adapt to hosts bred for increased nematode resistance. Co-evolutionary theory supports the notion of reciprocal adaptation (Clayton and Lee, 1999), although observing this experimentally is difficult. Webster et al. (2004) provided one of the few experimental examples of reciprocal adaptation in an animal–parasite system using the trematode *Schistosoma mansoni*. However, several studies in sheep have failed to detect any adaptive changes in *Haemonchus contortus* due to host resistance for up to 10 parasite generations of serial passage (Adams, 1988; Albers and Burgess, 1988; Saulai et al., 2001). Generally, these experiments compared nematode strains produced from immunological extremes; where the extremes were obtained by using a combination of either highly resistant breeds, repeated infections or deliberate immunosuppression. One study referred to by Windon (1990) suggested that *Trichostrongylus colubriformis* can adapt to Merino sheep bred for low WEC following vaccination with irradiated *T. colubriformis* larvae. This study found a significant WEC increase in vaccinated Border Leicester × Merino ewes infected with larvae from vaccinated high responder lambs compared with similar ewes infected with larvae from low-responder lambs. However, no study has investigated whether or not the nematodes *H. contortus* or *T. colubriformis* can adapt to hosts selectively bred using WEC for susceptibility or resistance to nematodes.

In this study, we hypothesised that *H. contortus* and *T. colubriformis* would adapt to long-term exposure to host sheep with either a resistant (R) or susceptible (S) genotype. We generated four nematode strains, one strain from each species solely exposed to R sheep and one strain from each species solely exposed to S sheep for up to 30 nematode generations of serial passage. Our hypothesis was tested using a cross-classified design where cohorts of R, S or randomly bred control sheep at pasture were infected with each nematode strain and differences assessed in WEC and, for *H. contortus* strains, packed cell volume (PCV). If the nematodes strains had adapted to their host genotype then the WECs from the R strain nematodes would be high in R sheep and the WECs from S strain nematodes would be high in S sheep. However, our strain tests found no evidence for adaptation in the nematodes, that is, the differences in WEC between R and S genotype sheep was maintained independent of the nematode strain with which they were infected.

2. Materials and methods

2.1. Experimental design

This procedure aimed to test for adaptation of *H. contortus* and *T. colubriformis* in response to long-term exposure to R or S genotype hosts. The experimental design had two components, firstly the generation of the nematode strains exposed solely to either R or S genotypes for up to 30 nematode generations and secondly, testing of these strains for adaptation by infecting either R, S or control genotype sheep.

Although the experiment was designed to be replicated in both nematode species, in the case of *T. colubriformis,* contamination with larvae of *Teladorsagia circumcincta* in the S strain led to the original design being compromised. Following several failed attempts to remove the *T. circumcincta* contaminants, the S strain was restarted from stored larvae. The restarted S strain was delayed seven generations behind the R strain. The contaminants were traced to the foundation *T. colubriformis* population and contamination was controlled by discarding any samples where foreign nematodes were detected. Thus, the experimental timeframe restricted the total number of nematode generations to 23 for *T. colubriformis*, rather than the planned 30 generations, and the number of tests for adaptation was reduced. The original design was followed for *H. contortus* strains.

2.2. Generating nematode strains exposed solely to R or S genotype sheep

We took a number of steps to ensure that nematodes had ample opportunity to adapt to the sheep genotype. These steps included commencing serial passage with an outbred nematode population, selecting representative sheep of the R or S genotype and restricting the average number of sheep per nematode generation to one or two.

Serial passage commenced with a foundation population of each nematode species, i.e. *H. contortus* (F_{Hc}) or *T. colubriformis* (F_{Tc}). In both species, the foundation populations were created by mixing equal proportions of numerous pure strains from different isolates taken from several states of Australia and a single passage through two immunosuppressed (dexamethazone, 0.5 mg kg^{-1} twice weekly) worm-free wethers. The foundation populations aimed to reflect the genetic diversity found in different regions and ensured that these populations contained considerable genetic variation, the primary determinant of response in selection experiments (Barton and Keightly, 2002). Details of the component strains for F_{Hc} can be found in Woolaston et al. (1992).

Serial passage was used as a controlled method to generate strains of nematodes with long-term exposure to either the R or S genotype sheep. The R and S sheep used for serial passage were young rams, sampled from lines previously selected for 10 years (or approximately four sheep generations) for low (R) or high (S) WECs following artificial challenge at 5–6 months of age with *H. contortus*. These genotypes had a seven- and 3-fold difference in WECs following challenge with either *H. contortus* or *T. colubriformis* (Woolaston et al., 1990). The animals used for serial passage were chosen from within one phenotypic standard deviation of their genotype mean for WEC. The procedure for each nematode generation generally involved oral infection of two sheep per strain with L_3 from the previous generation (initially F_{Hc} or F_{Tc}), pooling equal proportions of faecal cultures from faeces collected at 21 and/or 25 days p.i. from both sheep and sampling the pooled larvae for re-infection of the next pair of sheep. This procedure was repeated 30 times for *H. contortus* and 23 times for *T. colubriformis.* WECs were determined at the time of faecal collection using a modified McMaster method with a sensitivity of 100 eggs per gram (epg). Individual collections were cultured to L_3 stage, and approximately 300 *H. contortus* larvae or approximately 100 *T. colubriformis* larvae were checked in each collection to ensure strain purity. No cross-contamination occurred between the sheep genotypes. Throughout serial passage, sheep were housed on slatted floors and fed a pelleted ration ad libitum. Any existing nematode burden was removed by a single dose of Ivermectin at twice the manufacturer's recommended rate 1 week prior to infection (Ivomec® Oral Drench for Sheep, 0.4 mg kg^{-1} liveweight, Merial Australia). Sheep used for passage were considered to be parasite-free prior to infection as the efficacy of Ivermectin was known to be very high. The infective L_3 dose for the passage host was varied slightly according to animal's age and most rams were used for two successive nem-