Faecal avoidance in Romney sheep lines selected for resistance or resilience to gastro-intestinal nematodes

JC Hamie, CM Logan, RW McAnulty and AW Greer

Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln 7647, Christchurch, New Zealand

**Corresponding author:* Email: JosephChakana.Hamie@lincolnuni.ac.nz

Abstract

Faecal avoidance was assessed in Romney lambs from lines selected for resistance or resilience to gastro-intestinal nematode parasites. Ten lambs from each selection line that were maintained in separate farmlets and naturally exposed to parasites were assessed on distance grazed from either a mud-clay or faecal ball at 130, 150, 180 and 210 days-of-age. Resistant line animals maintained lower faecal-egg counts compared with resilient lambs, viz. 25.6 *c.f.* 771.0 eggs per gram of faeces ($P<0.001$) and greater *Trichostrongylus colubriformis*-specific immunoglobulin G absorbance ($P=0.005$) indicating differences between selection lines in parasite loading and immunlogical status. Grazing distance from the object was greatest for faecal ball, *viz*. 11.53 ± 1.14 cm compared with mud-clay ball, *viz*. 4.19 ± 1.00 cm ($P<0.001$) and decreased with time ($P<0.001$). Grazing distance tended to be greater for resilient, *viz*. 8.63 ± 1.14 cm than resistant, *viz*. 7.09 ± 0.99 cm ($P=0.09$) but there was no interaction between selection line and ball type ($P=0.709$) indicating similar levels of faecal-specific aversion between the selection lines. These results suggest that the lesser infection levels in resistant-line animals when grazing is unlikely to be due to reduced exposure to infective larvae as a consequence of greater faecal avoidance.

Keywords: faecal avoidance; sheep; resistance; resilience; parasitism

Introduction

Grazing animals make decisions around faecal avoidance based on existing parasite burden and immune status, thus reducing the risks of further parasite infection (Hutchings et al. 2001; Fox et al. 2013). Pasture swards around faeces are often highly contaminated with infective L3 larvae (Hutchings et al. 2007) and as ruminants cannot detect L3, the presence of faeces has been suggested as a deterent for the potential detrimental health risk associated with particular sward patches (Hutchings et al. 1998; Hutchings et al. 1999; Cooper et al. 2000). Further, the extent of faecal avoidance has been shown to be influenced by both the previous exposure of animals to parasites and also the host immune status, with parasite-naïve and immune animals exhibiting lesser faecal avoidance than their parasitised counterparts (Hutchings et al. 1998; Hutchings et al. 1999).

In lines of Romney lambs that have undergone selection for resistance, resilience or susceptibility to parasite challenge (Bisset et al. 1994; Bisset et al. 1996a; Bisset et al. 1996b; Morris et al. 2010), differences in levels of infection have been reported when grazed on pasture compared with indoor fixed challenges. In field challenges, where animals from these lines are grazed together and are left to selectively graze, resistant animals are able to maintain very low faecal-egg counts (FEC), seldom above 200 eggs per gram of faeces (epg) (Greer et al. 2016) while the faecal-egg count of resilient-line animals is consistently in excess of 1000 epg. This difference has been attributed to the greater immune capacity of resistant-line animals which limits parasite establishment and/or fecundity compared with animals selected for susceptibility to nematode infection (Douch et al. 1994; Douch et al. 1995; Shaw et al. 1999). However, when animals from Romney breeding lines selected for either resistance or resilience were raised indoors and given a constant and equal parasite challenge of 2000 *Trichostrongylus colubriformis* L3 larvae per day, FEC profiles between the lines were more similar, with peak FEC of 1200 and 1700 epg for resistant and resilient, respectively (Greer et al. 2018). This difference in response when given a constant parasite challenge then raises the possibility that the much lower FEC observed in resistant animals when grazing pasture may, at least in part, be a result of greater faecal aversion which reduces their exposure to infective larvae. From this perspective, Perendale sheep from lines selected for resistance have been reported to be less willing to graze pastures which are heavily contaminated with infective larvae (Hutchings et al. 2007) which may, in turn, contribute to their lower infection levels. Therefore, the objective of this study was to determine if a difference in faecal avoidance exists between lines of Romney sheep selected for resistance or resilience.

Materials and methods

Aversion to either a mud-clay or faecal ball was assessed in Romney lambs from lines that had undergone selection for either resistance or resilience to parasite challenge. Animals were part of a larger cohort that were maintained in pasture-based farmlets where resistant and resilient lines grazed separately and were consequently exposed to differing levels of parasite challenge. On four occasions, at a mean of 130, 150, 180 and 210 days-of-age, which spanned the anticipated periods of immune development to gastro-intestinal nematodes, 10 resistant and 10 resilient lambs were removed from their farmlet and housed in individual pens. Animals were fasted overnight with access to fresh water. Each animal was then presented with two swardlets (measuring 39 cm x 29 cm each) in succession, consisting of newly-sown ryegrass that had never been grazed. In the centre of one swardlet a faecal ball made from compressed freshly produced faeces measuring approximately 8 cm in diameter and weighing 50 grams and on the other a mud-clay ball of the same dimension and weight was placed at the center. Animals were allowed access to each swardlet for one hour. The order of presentation of either faecal ball or mud-clay ball was randomly decided for each animal by the flip of a coin. Following the presentation of the second swardlet, the animals were then returned to their original farmlets until the next assessment time. The swardlets were trimmed to ground level after each time and allowed to regrow for use in the subsequent testing time.

Herbage mass and faecal avoidance measurements

Prior to grazing and immediately following grazing the herbage mass was recorded using a miniature rising-plate meter which had been calibrated using herbage cuts from the same pasture, which was then dried in an oven at 70°C until constant weight. Using a string template, each swardlet was divided into 24 sections (6 x 4) and the size of each matched the plate meter area. The height of the plate for each section was then recorded and the herbage mass prior to and after grazing each section calculated. Faecal avoidance (cm) was determined as distance from the faecal or mud-clay ball that was grazed.

Animal measurements

At each sampling time, indicators of the animals' parasite and immunological status were assessed immediately prior to housing. For parasite status, faecal samples were obtained directly from the rectum of each individual for the determination of the concentration of nematode eggs per gram of faeces (faecal-egg counts; FEC, epg) using a modified McMaster method with a sensitivity of 100 epg. An indicator of immune status was determined using *Trichostronglyus colubriformis*-specific L3 antibodies in saliva collected using a mouth swab which was then centrifuged at 12,000 x g and the liquid collected and stored at -20°C until analysis. Immunoglobulin G (IgG) antibody specific to *T. colubriformis* L3 in saliva was measured using an ELISA as described by Greer et al. (2018). Briefly, 100 µl of neat saliva was incubated in duplicate in 96-well plates that had been coated with 0.1 µg *T. colubriformis* L3 antigen per well. Following washing with phosphate buffered saline solution (pH 7.2 + 0.05 % Tween 20), each well was incubated with 100 µl of horseradish peroxide conjugated polyclonal rabbit anti-sheep IgG immunoglobulins (Pierce Immunopure Antibodies, cat #31480, lot #GI959969) at a dilution of 1:4,000. Colour was developed using 100 µl of 0.05 M phosphate-citrate buffer adjusted to pH 5.0 with 0.02 % of 30 % H_2O_2 added and containing 100 µg of tetramethylbenzidine dihydrochloride (Sigma Aldrich, U.S.A) for 25 min before the reaction was stopped with the addition of 1.25 M H_2SO_4. Colour intensity (optical density; OD) was measured using a microplate reader (Multiscan Go, 1510-01462C, Thermofisher Scientific, Finland) at 450 nm. Four wells in each plate contained a positive standard serum sample which were used to correct for differences between plates. For each plate, all values were adjusted by multiplying by the product of the overall mean of all standard serum samples on all plates divided by the mean of the four standard serum samples on each plate. The mean OD of the duplicate wells for saliva samples was used in the analysis.

Statistical analysis

Saliva IgG and faecal egg count, distance grazed from object (faecal ball or mud-clay ball) and herbage consumed data were analysed by GENSTAT statistical package (version 18.1.0.17005, 2015, VSN International Ltd, U.K.) using Restrict Maximum Likelihood (REML) model with selection line and sampling time as factors in repeated measures and with animal included as a random effect. For FEC, data were transformed ($\log_{10}$ (count+100)) prior to analysis and results reported as back transformed means. Three resilient animals did not graze from swardlets containing either a faecal or mud-clay ball, one on each of the testing occasions, one on three testing occasions and one on two testing occasions. Data from these animals were excluded from the analysis.

Results

Parasitological and immunological profiles

Back transformed mean FEC (epg) are given in Fig. 1. Overall, there was an effect of selection line (P<0.001) but not time (P=0.206) and there was no significant interaction between selection line and time (P=0.175) which reflected consistently greater FEC in resilient compared with resistant animals.

Mean optical densities (OD) for L3 *T. colubriformis*-specifc immunoglobulin G (IgG) are given in Fig. 2. Overall, IgG was greater in resistant than in resilient animals (P=0.005) and this increased with time (P<0.001) but there was no interaction between selection line and time (P=0.130).

Herbage mass (g)

Mean herbage dry matter offered to animals reduced with time (P< 0.001) but was similar between lines at each point; day 130, *viz.* (24.75 c.f. 25.55 g), day 150, *viz.* (20.78 c.f. 19.67 g), day 180, *viz.* (20.32 c.f. 20.17 g), and day 210, *viz.* (14.72 c.f. 14.96 g) for resilient and resistant, respectively. Overall, mean herbage dry matter consumed decreased with time (P<0.001) but was similar for both resistant and resilient animals, *viz.* 7.60 ± 0.43 g and 7.03 ± 0.50 g, respectively, (P=0.300).

Grazing avoidance

Mean distance grazed to either a faecal or mud-clay ball is given in Fig. 3. Overall, animals grazed further away from the faecal ball, *viz*. 11.53 ± 1.138 cm compared with mud-clay ball, *viz*. 4.19 ± 0.995 cm (P<0.001) and there

Figure 1 Mean (±95% confidence interval) back transformed mean faecal egg count (FEC) at each sampling time for Romney lambs from lines selected and maintained in their own farmlets for resistance (triangles) or resilience (squares) to gastro-intestinal nematodes.

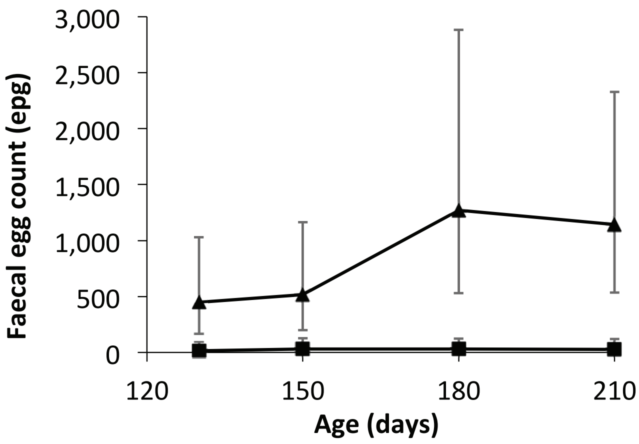
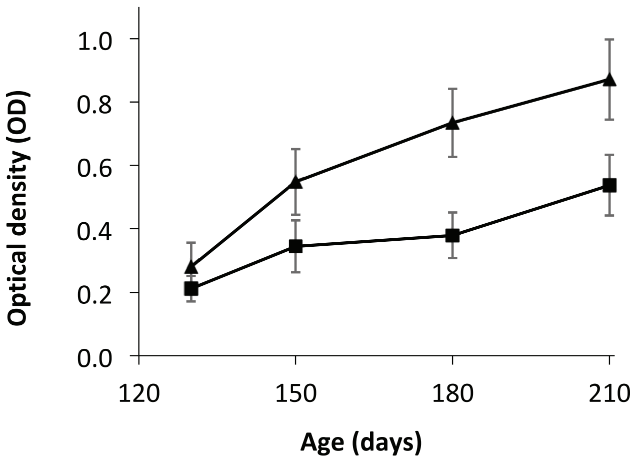
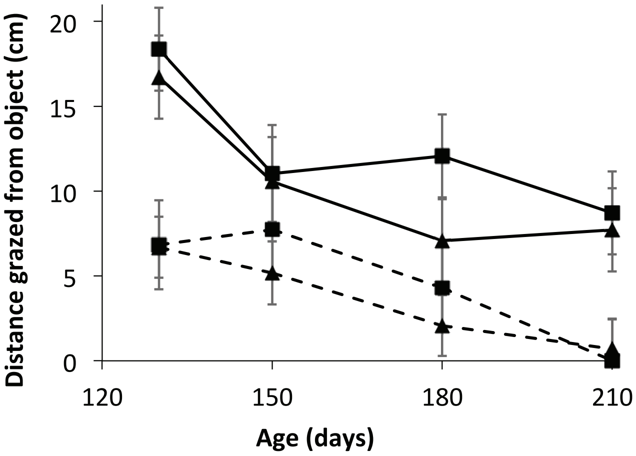


Figure 2 Mean (± s.e.m) optical density (OD) for L3 *Trichostrongylus colubriformis*-specific IgG antibody for Romney lambs from lines selected for resistance (triangles) or resilience (squares) to gastro-intestinal nematodes at each sampling time.



was a tendency for resilient-line animals to graze a greater distance from objects, *viz*. 8.63 ± 1.14 cm compared with resistant-line animals, *viz.* 7.09 ± 0.99 cm (P=0.093). The grazing distance decreased with time (P<0.001) but there were no interactions between selection line and type of object (P=0.709) or selection line with time (P=0.738).

Figure 3 Mean (± s.e.m.) grazing distance from either a faecal ball (solid line) or mud-clay ball (dashed line) when offered in swardlets for Romney lambs at different ages from lines selected for resistance (triangles) or resilience (squares) to gastro-intestinal nematodes.



Discussion

A clear difference in phenotypic indicators of parasite infection existed between the selection lines. Resistant-line lambs had FEC that were consistently below 100 eggs per gram (epg) of faeces, whereas their resilient counterparts had FEC with peak in excess of 1000 epg. Despite the fact that animals may have been exposed to different levels of challenge on their farmlets, these observations are consistent with previous reports from Romney selection lines when grazed together (Bisset et al. 1994; Bisset et al. 1996a; Morris et al. 2010). Similarly, the greater IgG antibody responses in resistant animals are in agreement with previous investigations in lines selected for resistance or susceptibility to infection (Douch et al. 1994; Douch et al. 1995; Shaw et al. 1999). Further, despite maintaining a high FEC the resilient-line animals showed evidence of immune development through increasing parasite-specific IgG antibody, which is in agreement with previous observations of animals from these lines (Greer at al. 2018). Combined, these observations give a clear indication these animals were a fair representation of their respective selection lines and their phenotypic response to field infection is consistent with what was expected.

Both lines showed similar levels of faecal-specific grazing aversion, being consistently willing to graze closer to the mud-clay than the faecal ball. Although the presence of a mud-clay ball provides an artificial stimulus, the grazing distance from this gives an indication of the aversion an animal may have to an object *per se*. The balance of the greater grazing distance consistently recorded for the faecal ball then reflects the faeces-specific aversion, which was similar for both the resistant and resilient lines. Faeces-specific avoidance in parasitised animals has been reported previously to be affected by the animal's immune status and state of parasitism (Hutchings et al. 1998; Hutchings et al. 1999) and has been suggested to be beneficial for the animal to reduce their potential exposure to larval challenge (Fox et al. 2013). Despite a tendency of resilient animals to graze further away from any object, the absence of faecal-specific differences between these Romney selection lines despite their differences in infection levels and immune responses was unexpected. These results contrast with a previous report that genetically resistant Perendale sheep were less willing to graze swards that were heavily contaminated with parasitic larvae (Hutchings et al 2007) and the findings of Seó et al. (2015) that cattle with high levels of parasitism had greater faecal avoidance and grazed further away from dung compared with medium to low level infection animals. The typically low FEC observed in resistant-line animals when grazing has been accepted as being due to a greater immunological capacity to reduce nematode establishment and/or fecundity (Bisset et al. 1996b; Shaw et al. 1999). However, when animals from these lines were raised indoors and given a constant and equal challenge of *T. colubriformis* L3 larvae (Greer et al. 2018), the differences between the lines in FEC were much less evident than when grazing pasture. While the reasons for the differences between grazing and indoor studies remain unclear, the results of the current study suggest that the lower FEC in resistant animals when grazing is not due to a greater faecal aversion which may reduce exposure to infective larvae.

Avoidance behaviour did appear to decrease with time. A reduction in aversion was observed with both selection lines increasingly becoming more willing to graze closer to both the faecal and mud-clay balls. Reduced faecal avoidance has been associated with increases in animals' immune status (Hutchings et al. 1999; Hutchings et al. 2001) although there is limited evidence from the current study to support this. Despite the reduction in object avoidance with time in both lines coinciding with a rise in *T. colubriformis*-specific IgG levels, the consistently lower IgG profiles in resilient animals was not associated with differences in faecal-specific aversion, indicating factors other than immune development may be a primary driver of faecal avoidance. With this in mind, however, in the current study the herbage offered at each time did decline which raises the possibility the reduced grazing distance may have simply reflected the animals becoming less averse to any object in an attempt to satisfy their hunger. Alternatively, animals may have become familiar with the presence of objects which indicates some learning may have occurred. Faecal avoidance has been accepted as a learned behaviour with previous parasitological experience influencing willingness to graze close to faeces (Hutchings et al. 1998; Hutchings et al. 1999). Therefore, despite the resilient animals maintaining a considerably greater FEC, the lack of difference in faeces-specific grazing avoidance between these selection lines that did not change with time may simply reflect a lack of suffering they perceived themselves to be experiencing and the associated learning this may have afforded.

In summary, faecal-specific grazing aversion existed and was similar in magnitude for animals from both the resistant and resilient selection lines and did not appear to be directly associated with either differences in parasite loading or changes in immunological status. These results do not support the suggestion that some of the difference in parasite loading between the selection lines may be a consequence of greater faecal avoidance in resistant animals that reduces their potential exposure to infective larvae whilst grazing.

Acknowledgements

Joseph Hamie is supported by a New Zealand Aid Commonwealth Scholarship. The authors would also like to thank Rebecca Johnson, James Meyer and Sara Lundberg for technical assistance, Simon Hodge for assistance with data analysis and Graham Barrell for comments made to the manuscript.

References

Bisset SA, Morris CA, Squire D, Hickey S 1996a. Genetics of resilience to nematode parasites in young Romney sheep – use of weight gain under challenge to assess individual anthelmintic treatment requirements. New Zealand Journal of Agricultural Research 39: 313-323.

Bisset SA, Vlassoff A, Douch PGC, Jonas WE, West CJ, Green RS 1996b. Nematode burdens and immunological responses following natural challenge in Romney lambs selectively bred for low and high faecal worm egg count. Veterinary Parasitology 61: 249-263.

Bisset SA, Morris CA, Squire D, Hickey S, Wheeler M 1994. Genetics of resilience to nematode parasites in Romney sheep. New Zealand Journal of Agricultural Research 37: 521-534.

Cooper J, Gordon IJ, Pike AW 2000. Strategies for the avoidance of faeces by grazing sheep. Applied Animal Behaviour Science 69: 15-33.

Douch PGC, Green RS, Morris CA, Bisset SA, Vlassoff A, Baker RL, Watson TG, Hurtford AP, Wheeler M 1995. Genetic and phenotypic relationships among anti-*Trichostrongylus colubriformis* antibody level, faecal egg count and body weight traits in grazing Romney sheep. Livestock Production Science 41: 121-132.

Douch PGC, Green RS, Risdon PL 1994. Antibody responses of sheep to challenge with *Trichostrongylus colubriformis* and effect of dexamethasone treatment. International Journal for Parasitology 24: 921-928.

Fox NJ, Marion G, Davidson RS, White PC, Hutchings MR 2013. Modelling parasite transmission in a grazing system: the importance of host behaviour and immunity. PloS one 8: e77996. doi: 10.1371/journal.pone.0077996.

Greer AW, Hamie JC, McAnulty RW 2016. Resistance vs resilience: A solvable dilemma? Proceedings of the Society of Sheep and Beef Cattle Veterinarians of the NZVA Conference. Pp. 65-71.

Greer AW, McKenzie JL, McAnulty RW, Huntley JF, McNeilly TN 2018. Immune development and performance characteristics of Romney sheep selected for either resistance or resilience to gastrointestinal nematodes. Veterinary Parasitology 250: 60-67.

Hutchings MR, Kyriazakis I, Gordon IJ 2001. Herbivore physiological state affects foraging trade-off decisions between nutrient intake and parasite avoidance. Ecology 82: 1138-1150.

Hutchings M, Knowler K, McAnulty RW, McEwan J 2007. Genetically resistant sheep avoid parasites to a greater extent than do susceptible sheep. Proceedings of the Royal Society of London B: Biological Sciences 274: 1839-1844.

Hutchings M, Kyriazakis I, Anderson D, Gordon I, Coop RL 1998. Behavioural strategies used by parasitized and non-parasitized sheep to avoid ingestion of gastro-intestinal nematodes associated with faeces. Animal Science 67: 97-106.

Hutchings M, Kyriazakis I, Gordon I, Jackson F 1999. Trade-offs between nutrient intake and faecal avoidance in herbivore foraging decisions: the effect of animal parasitic status, level of feeding motivation and sward nitrogen content. Journal of Animal Ecology 68: 310-323.

Morris CA, Bisset SA, Vlassoff A, Wheeler M, West CJ, Devantier BP, Mackay AD 2010. Selection for resilience in Romney sheep under nematode parasite challenge, 1994-2007. New Zealand Journal of Agricultural Research 53: 245-261.

Seó HLS, Pinheriro MFLC, Honorato LA, da Silva BF, do Amarante AFT, Bricarello PA 2015. Effect of gastrointestinal nematode infection level on grazing distance from dung. PLoS ONE 6: e0126340. Doi:10.1371/journal.pone.0126340.

Shaw RJ, Morris CA, Green RS, Wheeler M, Bisset SA, Vlassoff A, Douch PGC 1999. Genetic and phenotypic relationships among *Trichostrongylus colubriformis* antibody, immunologlobulin G1, faecal egg count and body weight traits in grazing Romney lambs. Livestock Production Science 58: 25-32.