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Short communication

The experimental establishment of ruminant nematodes in European hares (*Lepus europaeus*)

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ABSTRACT

The factors that control the demography of European hare *Lepus europaeus* populations are poorly understood, but it has been recognized that the decline of hares in Europe is associated with an increasing intensity of agricultural activity. Many mechanisms have been suggested. We propose another mechanism; a negative impact arising from ingestion of the infective larvae of ruminant livestock. We dosed juvenile hares from a worm-free colony with a conservative dose from a mixed culture of infective larvae of the nematode parasites of sheep and cattle. We examined the hares post-mortem for the establishment of those ruminant nematodes, differences in weight changes, and the shedding of eggs.

We found that under the circumstances of our trial, *Trichostrongylus colubriformis* and to a lesser extent *T. rugatus*, *T. vitrinus*, and *Teladorsagia circumcincta* were able to establish as adults in the dosed animals. We found strongyle eggs in the faeces of the dosed hares, and were able to culture larvae from those eggs. However, the ecological significance of our findings, if any, remains to be elucidated. Because of their mobility, hares may transmit resistant strains of parasites between grazing properties.

1. Introduction

In recent years there has been concern about the decline of the European hare (*Lepus europaeus*) in Europe, where it is an important game animal (Langbein et al., 1999). Australian populations are also at low densities, despite reaching plague densities on occasions (Douglas, 1972) since its introduction from England in the 1860s (Rolls, 1969). In Australia, there is high spatial overlap between hares and sheep (Stott, 2003a,b), sheep are universally infested with nematode parasites including at least one ovine *Trichostrongylus* spp. (Beveridge and Ford, 1982), and infective larvae of ovine parasites are available on the pastures (Martin et al., 1990). Hence, hares must be ingesting large numbers of the infective larvae of ovine parasites, or of other ruminant species where they occur.

The sheep industry is a major contributor to the Australian economy, but nematode parasites cause considerable economic loss (McLeod, 1995), and anthelmintic resistance is a major problem (Besier and Love, 2003). A method of minimizing the transfer of anthelmintic resistance is quarantine on a property basis (Dobson et al., 2001). The success of quarantine assumes that the parasites are unable to cross the property boundary, but the potential for free-ranging animals (deer) to breach a quarantine barrier has been recognized (Nilsson, 1971). Stott (2003a,b) showed that hares range over areas approaching 200 ha and in so doing freely cross property boundaries, and as hares are more common than free-ranging deer, there is greater potential for inter-property carriage of nematodes by hares. This potential role was dismissed by Saulai and Cabaret (1998), but they sampled

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only five hares, finding only one carrying a ruminant nematode (*Trichostrongylus capricola*).

Broekhuizen and Kemmers (1976) have suggested that hare populations in Europe are adversely affected by nematode parasites sourced from rabbits. It may be that heterologous infestation of European hares with a nematode normally parasitic in ruminants would affect the demographics of hare populations. Because the establishment of ruminant nematodes in European hares, if it occurs, would have ramifications for the hare populations themselves, and because it would also have ramifications for the management of the spread of anthelmintic resistance in livestock (Dobson et al., 2001), this paper examines the ability of ruminant nematodes prevalent in south-eastern South Australia to establish in hares.

2. Materials and methods

Twelve captive-reared helminth-free European hares *L. europaeus* c. 8 weeks old were used. Sulphaquinoxaline 400 mg/l was used in the water supply as a coccidiostat, and the substrate of the test arena was concrete. The hares were divided randomly into four groups; a control group and three test groups, and initial weights were obtained.

The intention of the present trial was to mimic a moderate challenge in a field situation, and so the hares were dosed by stomach tube with c. 10,000 L_3 strongyle larvae obtained from a culture of sheep and cattle faeces, a dose between the 2500 used by Bailey (1967) and the 25,000 used by Barker and Ford (1975) in rabbits. The composition of the culture is given in Table 2.

The control-group animals were sacrificed and weighed at 21 days and the test animals at 15, 21, and 42 days post-dosing. The stomach, small intestines, and large intestines were removed, and sectors were preserved for histopathological examination. Faecal samples were retained for egg counts and coproculture. The contents of each organ were collected and fixed in 5% formalin, and each sample was sieved using a 75 μm mesh sieve to remove debris before all nematodes were identified and enumerated microscopically, including 222 mature male *Trichostrongylus* spp. that were identified to species using spicule morphology.

Body weights were compared by analysis of variance. The use of a Poisson generalized linear model (McCullagh and Nelder, 1999) in the first instance is appropriate for the count data. Over-dispersion was assessed using the residual deviance. This led to the use of a negative binominal generalized linear model, fitted using the glm.nb function in MASS library of the VR bundle in the R software environment (R Development Core Team, 2006). The factors assessed were treatment (dosed vs. control) and the interaction between treatment and time after treatment.

Table 1
Growth rate of hares during the trial ($F_{0.05(2)3,8} = 5.42$).

	Growth rate (g/day)
Control	18.4 ± 1.0
15 day span since infection	17.6 ± 1.2
21 day span since infection	15.2 ± 4.7
42 day span since infection	11.1 ± 4.8
F statistic and significance	1.14 n.s.

Table 2
Species composition (mean %) of the larval culture used to dose the hares.

	Mean
Teladorsagia circumcincta	54
Ostertagia ostertagi	18
Haemonchus contortus	0.5
Trichostrongylus spp.	12
Nematodirus spp.	ND
Cooperia oncophora	14
Oesophagostomum venulosum	1.5

ND: not detected

3. Results

All of the hares gained weight during the trial, and there were no significant differences in weight gain between treatments ($F = 1.63$; $F_{0.05(2)3,8} = 5.42$). No nematodes were found in the control animals. Adult *Teladorsagia circumcincta* (mean 99.0; range 2–666) were found in the stomachs, and adult *Trichostrongylus* spp. (mean 353.6; range 9–1200) were found in the small intestines, of all dosed hares. Small numbers of adult *Trichostrongylus* spp. were found in some large intestines, and small numbers (<20) of L_4 larvae of *Teladorsagia circumcincta* and *Trichostrongylus* spp. were found in some hares. The distribution of the *Trichostrongylus* spp. was *Trichostrongylus colubriformis* 57%, *T. rugatus* 37%, and *T. vitrinus* 6%. Small numbers of *Nematodirus* spp. and *Cooperia* sp. were found (Table 1).

The data for the dosed hares were clearly over-dispersed. The residual deviance was 2675 with 8 degrees of freedom. On fitting a negative binomial generalized linear model, the residual deviance was reduced to 10.53 again on 8 degrees of freedom. Removing the treatment by time (day) interaction term resulted in a residual deviance of 10.57 on 10 degrees of freedom; the likelihood ratio statistic for testing no treatment by time interaction was 0.33, and $P = 0.56$. Hence there was no evidence of a change in the level of infestation over time. However, the difference between the treatment and the control was highly significant, with the residual deviance for the model under the null hypothesis of no difference being 14.23 on 11 degrees of freedom. The likelihood ratio statistic for testing no treatment effect was 17.29 and $P < 0.0001$. Hence it is highly unlikely that an undetected pre-existing infestation with *Trichostrongylus* spp. was present in the test population.

Strongyle eggs were recovered from all but one of the dosed hares, but generally in low numbers. Coprocultures from two hares successfully yielded larvae identified as 96% *Trichostrongylus* spp., 3% *T. rugatus* (see O'Callaghan, 2004), and 1% *Teladorsagia circumcincta* ($n = 106$), and coccidial infestation was ubiquitous. Only mild histopathological changes were observed.

4. Discussion

Nematode parasites tend to be host-specific. Susceptibility to cross-infection is low (Rehbein and Haupt, 1994),