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Gastrointestinal nematode species burdens and host mortality in a feral sheep population

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SUMMARY

Every few years a large proportion of the feral sheep on Hirta, St Kilda die due to food shortage. The effects of malnutrition are exacerbated by gastrointestinal nematodes. As found in sheep flocks in mainland Britain, *Teladorsagia circumcincta* has long been considered the predominant and most pathogenic nematode species in all age classes of Soay sheep. Previous research indicated that intensity of this species showed a negative association with host age and comprised 75% of the entire gastrointestinal burden. Here we present new data that show *Trichostrongylus axei* and *Trichostrongylus vitrinus* to be the predominant worm pathogens in young Soay sheep. In the present study, *Trichostrongylus* spp. burdens declined with host age whereas *T. circumcincta* actually increased in burden over the first few age classes. Also, male hosts had significantly higher burdens of *Trichostrongylus* spp. than females, with this genus making up a higher proportion of the strongyle egg producing community in male hosts than female hosts. These new findings raise questions concerning our previous interpretation of the main nematode species contributing to strongyle egg count in the population, and the contrasting infection patterns of these nematode species in unmanaged St Kilda Soay sheep compared with domestic sheep in mainland Britain.

Key words: Soay sheep, St Kilda, *Teladorsagia circumcincta, Trichostrongylus axei, Trichostrongylus vitrinus.*

INTRODUCTION

Natural and domestic animal populations commonly experience mixed species parasite burdens (Soulsby, 1986; Samuel et al. 2001). However, in any particular situation, to make the problem tractable, research and therapy tend to concentrate on the one or a few parasite species with the greatest perceived effects (Stear et al. 1997, 1998). There are two general reasons for current increased interest in the problem of co-infection by different parasite taxa (Cox, 2001; Poulin, 2001; Read and Taylor, 2001; Roberts et al. 2002). First, on a phenotypic level, different kinds of parasite make different demands on the immune system, the classic example being the propensity of intracellular versus extracellular infections to induce different, mutually inhibitory responses (Th1 and Th2), so that an individual host’s response to any given infection is likely to be a compromise with its response to other infections (Graham, 2002). Second, on a purely genetic level, scientists have recorded appreciable heritable variation in resistance to specific parasites despite strong selection for resistance (e.g. Wakelin and Blackwell, 1988; Kloosterman et al. 1992; Bishop et al. 1996; Coltman et al. 2001; Polak, 2003). As well as the well-known Red Queen hypothesis, in which hosts are condemned to track intraspecific temporal variation in parasite genotypes (Dybdahl and Lively, 1998) another hypothesis explaining genetic variation for parasite resistance is that maximal response to any one species, even within the helminth community, is genetically constrained by the need to respond to other species (Christensen et al. 1987; Cox, 2001; Lello et al. 2004). As part of an investigation of these questions, we report a study of the helminth species burden in a feral sheep population.

Epidemiological surveys of helminth species infection in hill and lowland sheep flocks in northern Britain consistently identify *Teladorsagia circumcincta* as the most abundant species (Parnell et al. 1954; Boag and Thomas, 1971, 1977; Reid and Armour, 1975a,b; Stear et al. 1997; Bartley et al. 2003). Similarly, *T. circumcincta* has long been considered the most significant species infecting the feral population of Soay sheep on St Kilda. From post mortem nematode counts in 1989 Gulland (1992) estimated that *T. circumcincta* accounted for around 75% of the burden in all age classes of sheep. Lesions observed on the abomasum wall were assumed to be caused by the species (Gulland, 1992; Wilson et al. 2004). Furthermore, Wilson et al. (2004) discussed several lines of evidence suggesting that strongyle egg count is an appropriate measure of burden of *Teladorsagia* spp., with the two variables being correlated in Soay sheep on Hirta. To date, it has therefore been assumed that most of the strongyle

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eggs counted in host faeces on Hirta are generated by *Teladorsagia* spp.

This paper presents new data of *post mortem* nematode species burdens in the Soay sheep population for the first time since the above conclusion was drawn. We report hitherto unknown and unexpected trends in the abundance of the main nematode species with host age, presenting a clear challenge to the notion that *T. circumcincta* has a consistent parasitological monopoly in the St Kilda Soay sheep. We discuss the implications this discovery has for our interpretation of the main species contributing to strongyle egg count in the population and ways in which species-specific counts for strongyle species with morphologically identical eggs could be determined in the future. We also speculate on reasons for the contrast between St Kilda and mainland Britain.

**MATERIALS AND METHODS**

**Study area**

St Kilda (57°49′N, 08°34′W) is comprised of the 4 islands of Hirta, Soay, Boreray and Dun. Its position in the Atlantic gives it a temperate climate, subject to spells of heavy rainfall and strong winds (Buchanan, 1995). Hirta (638 ha) is the largest island of the archipelago and the domain of the Soay sheep study population. The most sheltered part of the island and focus of the field study is Village Bay (~200 ha).

**Host population**

Soay sheep were first brought to St Kilda perhaps as early as the Bronze Age (Jewell, 1995). In this isolated location, further domestication of the breed came to a standstill. The Hirta population is an offshoot of the original stronghold found on the neighbouring island of Soay. In 1932, after all humans and domestic stock were evacuated, the island was repopulated with 107 transferred Soays (Clutton-Brock et al. 1997). Since then the feral population has experienced intermittent winter mortality as numbers inevitably exceed the food supply. Since 1985, individuals occupying the Village Bay area have been tagged, observed and sampled throughout the year. A detailed account has been given by Clutton-Brock and Pemberton (2004).

**Sample collection**

Data presented in this paper were obtained from representative subsets of sheep that died in 1999 and 2002. At the time of sampling in the winter/spring months, lambs, yearlings, 2-year-olds and adults were around 10, 22, 34 and >34 months old respectively. In 1999 only abomasum samples were collected for examination but in 2002 entire gastrointestinal necropsy samples were obtained. Sample sizes in 1999 for male and female lambs, yearlings, 2-year-olds and adults were 12, 12, 5, 6, 6, 2, 6 and 6 respectively (a total of 55 hosts) and the equivalent numbers in 2002 were 12, 12, 7, 6, 11, 8, 6 and 7 respectively (a total of 69 hosts).

**Parasitology**

The St Kilda Soay sheep are host to a diverse component parasite population comprising 14 species of helminth, 13 species of protozoa and 2 species of arthropod (Wilson *et al*. 2004). Only quantitative data for the adult gastrointestinal nematode species are reported in this paper. Recovery of the gastrointestinal nematodes was done using a modification of the method outlined by M.A.F.F. (1986). All material was obtained from freshly dead sheep (≤24 h since death). The abomasum, small intestine, large intestine and caecum were dealt with separately; the contents of each were washed into a receptacle with water and made up to a volume of 10 litres. Following thorough mixing, a 10% aliquot was removed and passed through a 38 μm sieve for the abomasum and small intestine and a 300 μm sieve for the large intestine and caecum and the retained material was stored in 5% formalin. Abomasal mucosa was peeled from the abomasum wall in strips and frozen at −20 °C within a few hours. All gastrointestinal nematodes in a 1% aliquot (or 2% for 2-year-old sheep in 2002) were stained with iodine solution and identified to developmental stage, species and sex and the tallies multiplied by 100 (or 50 for 2-year-old sheep in 2002) to obtain an estimate of total burden. Abomasal mucosae were subject to peptic digestion for the recovery of encapsulated larvae as outlined by M.A.F.F. (1986).

**Statistical analysis**

Except where 100% present, prevalence of each helminth species was analysed using a GLM with log link function and binomial error structure on binary data (zero versus non-zero counts) to test for associations with host age (fitted as a continuous variable in years with one degree of freedom) and sex (fitted as a categorical variable with 2 levels and 1 degree of freedom).

As is usual, gastrointestinal nematode species burdens were aggregated across hosts. That is, the highest burdens were found in only a few individuals. When parasite burdens are thus over-dispersed in a host population, variance greatly exceeds the mean and the data most probably conform to the negative binomial distribution (Wilson and Grenfell, 1997).

The negative binomial parameter (*k*) gives a useful indication of aggregation in the data. Generally,
as aggregation increases the value of $k$ decreases. The exponent $k$ was calculated for each of the gastrointestinal nematode species using the following formula (Fowler and Cohen, 1990)

$$k = x^2/(s^2 - x)$$

where $x = \text{mean}; s^2 = \text{variance}$.

Each of the nematode species burdens was tested for associations with host age and sex fitted as for prevalence (above) and where applicable, year of sample (fitted as a categorical variable with 2 levels and 1 degree of freedom) using a generalized linear model (GLM) with log link function and negative binomial error structure. In order to test for a possible non-linear association of Teladorsagia spp. intensity with host age, age was fitted as a quadratic in the GLM by fitting an additional term, age$^2$, with 1 degree of freedom. During modelling it was discovered that only total gastrointestinal nematode intensity, Teladorsagia spp. and T. axei were best described by the negative binomial error distribution, confirmed by evaluating the normality of the error structures using the qqplot function in S-plus.

Applying a GLM with negative binomial error structure was not the best approach for the highly aggregated intestinal nematode species such as T. vitrinus. When the intestinal nematode species counts (+1) were log transformed, the highest count of zero was not followed by a second highest count of 1 and so on as the negative binomial distribution assumes. Instead, intensity for these species was modelled using a linear model (LM) with log link function on log-transformed count data, excluding the zero counts. This method was also used for investigating differences in the epidemiology of male and female parasites. All statistical analyses were conducted in S-Plus professional edition version 6.2.1 for Microsoft windows (Insightful Corp.).

**RESULTS**

**Combined gastrointestinal nematode species prevalence and burden**

All sheep examined by full gastrointestinal necropsy from 2002 were parasitized by at least 1 nematode species (Fig. 1) and most harboured many thousands of worms (Table 1). Combined nematode species burden was aggregated in the population ($k = 3.26$) (Table 2), decreased significantly with host age ($\Delta$deviance$= 10.74$, coefficient (S.E.)$= 0.193$ (0.055), D.F. = 1, $P = 0.001$) and differed between sexes, with juvenile males having higher burdens ($\Delta$deviance$= 3.99$, coefficient (S.E.)$= 0.113$ (0.055), D.F. = 1, $P = 0.045$). The final model explained 17.36% of the deviance.

**Abomasum nematode species prevalence and burden**

Only 2 species of nematode are found in the abomasum of the St Kilda Soay sheep, Teladorsagia spp. and T. axei. Both species are highly prevalent reaching 100% in all age classes of sheep for Teladorsagia spp. and all but the oldest age class of sheep for T. axei (Fig. 1). Teladorsagia spp. comprises a complex with 3 morphologically distinct, though genetically indistinct (Stevenson et al. 1996; Braisher, 1999; Leignel et al. 2002) adult male types, namely T. circumcincta, Teladorsagia daviani and Teladorsagia trifurcata. In this study these adult male morphs were found to make up 80.17 ± 0.1%, 15.03 ± 0.1% and 4.8 ± 0.06% of the complex respectively. Since most of the complex is comprised of T. circumcincta, this term and Teladorsagia spp. have been used interchangeably in previous publications on St Kilda Soay sheep (Wilson et al. 2004).

In 2002, the 2 abomasum species showed contrasting patterns of burden with host age (Fig. 2). Teladorsagia spp. increased significantly over the first few age classes ($\Delta$deviance$= 16.58$, D.F. = 1,
Table 1. Mean burden (±95% C.I.) of all adult gastrointestinal nematode species from different age classes of Hirta Soay sheep that died in 2002
(Data for *Teladorsagia* spp. and *T. axei* from 1999 are also included for comparison. Standard error means of the raw were calculated for small sample sizes according to Fowler and Cohen (1990).)

<table>
<thead>
<tr>
<th>Site</th>
<th>Nematode species</th>
<th>Lambs</th>
<th>Yearlings</th>
<th>2-year-olds</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abomasum</td>
<td><em>Teladorsagia</em> spp. (2002)</td>
<td>4179±735</td>
<td>4782±1442</td>
<td>9863±2899</td>
<td>6723±2985</td>
</tr>
<tr>
<td></td>
<td><em>Teladorsagia</em> spp. (1999)</td>
<td>3700±793</td>
<td>8609±3543</td>
<td>10712±6350</td>
<td>6458±2254</td>
</tr>
<tr>
<td></td>
<td><em>Trichostrongylus axei</em> (2002)</td>
<td>10042±2171</td>
<td>9709±4848</td>
<td>3724±1315</td>
<td>1285±793</td>
</tr>
<tr>
<td></td>
<td><em>Trichostrongylus axei</em> (1999)</td>
<td>8692±1733</td>
<td>6900±2944</td>
<td>6238±6934</td>
<td>2217±1425</td>
</tr>
<tr>
<td>Small intestine</td>
<td><em>Trichostrongylus vitrinius</em></td>
<td>8917±2205</td>
<td>6615±4682</td>
<td>4361±1749</td>
<td>5285±5841</td>
</tr>
<tr>
<td></td>
<td><em>Nematodirus battus</em></td>
<td>175±77</td>
<td>46±40</td>
<td>355±233</td>
<td>100±55</td>
</tr>
<tr>
<td></td>
<td><em>Nematodirus filicollis</em></td>
<td>1571±778</td>
<td>615±427</td>
<td>89±127</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Bunostomum trigonocephalum</em></td>
<td>17±16</td>
<td>31±45</td>
<td>79±50</td>
<td>138±115</td>
</tr>
<tr>
<td></td>
<td><em>Strongyloides papillosus</em></td>
<td>13±19</td>
<td>0</td>
<td>16±18</td>
<td>8±17</td>
</tr>
<tr>
<td></td>
<td><em>Capillaria longipes</em></td>
<td>21±21</td>
<td>62±53</td>
<td>11±17</td>
<td>8±17</td>
</tr>
<tr>
<td>Large intestine</td>
<td><em>Trichuris ovis</em></td>
<td>42±35</td>
<td>15±23</td>
<td>0</td>
<td>8±17</td>
</tr>
<tr>
<td></td>
<td><em>Chabertia ovisca</em></td>
<td>163±57</td>
<td>292±220</td>
<td>429±151</td>
<td>215±120</td>
</tr>
<tr>
<td>G1 tract</td>
<td>All species</td>
<td>25138±4035</td>
<td>22168±8703</td>
<td>18926±4360</td>
<td>13769±7248</td>
</tr>
</tbody>
</table>

Table 2. The mean, variance and negative binomial parameter (k) of the frequency distributions of the gastrointestinal nematode species across all hosts sampled in 2002
(Data for *Teladorsagia* spp. and *T. axei* from 1999 are also included for comparison. Species marked * were best described by the negative binomial error distribution.)

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean</th>
<th>Variance</th>
<th>Negative binomial parameter (k)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Teladorsagia</em> spp.* (2002)</td>
<td>6337</td>
<td>21517974</td>
<td>1.87</td>
</tr>
<tr>
<td><em>Teladorsagia</em> spp.* (1999)</td>
<td>6303</td>
<td>2429544</td>
<td>1.63</td>
</tr>
<tr>
<td><em>T. axei</em> (2002)</td>
<td>6589</td>
<td>36308651</td>
<td>1.20</td>
</tr>
<tr>
<td><em>T. axei</em> (1999)</td>
<td>6564</td>
<td>27541619</td>
<td>1.56</td>
</tr>
<tr>
<td><em>T. vitrinius</em></td>
<td>6544</td>
<td>43395389</td>
<td>0.99</td>
</tr>
<tr>
<td><em>N. battus</em></td>
<td>186</td>
<td>88374</td>
<td>0.39</td>
</tr>
<tr>
<td><em>N. filicollis</em></td>
<td>687</td>
<td>1721003</td>
<td>0.27</td>
</tr>
<tr>
<td><em>B. trigonocephalum</em></td>
<td>59</td>
<td>12741</td>
<td>0.28</td>
</tr>
<tr>
<td><em>S. papillosus</em></td>
<td>10</td>
<td>1219</td>
<td>0.09</td>
</tr>
<tr>
<td><em>C. longipes</em></td>
<td>30</td>
<td>3087</td>
<td>0.18</td>
</tr>
<tr>
<td><em>T. ovis</em></td>
<td>19</td>
<td>3022</td>
<td>0.12</td>
</tr>
<tr>
<td><em>C. ovisca</em></td>
<td>270</td>
<td>7443</td>
<td>0.99</td>
</tr>
<tr>
<td>Gastrointestinal total*</td>
<td>20726</td>
<td>13937615</td>
<td>3.26</td>
</tr>
</tbody>
</table>

*P < 0.001; the final model explained 21.10% of the deviance* while *T. axei* decreased significantly with host age (Δdeviance = 44.91, D.F. = 1, P < 0.001; the final model explained 37.59% of the deviance). There was no significant difference between the sexes for either species (Fig. 3).

These results were enhanced by similar counts made from the 1999 mortality event. When the 1999 and 2002 data sets were combined (n = 124 sheep) and tested for associations with year, host age and sex, there were no significant differences in burdens of *Teladorsagia* spp. (Table 3), *Teladorsagia* spp. male morphs (not shown), *T. axei* (Table 4) or mixed *Teladorsagia* and *T. axei* larvae (not shown) between the 1999 and 2002 data sets. The opposing patterns in age-specific burdens of the 2 abomasum species described above were maintained. In the model for *Teladorsagia* spp., age was fitted as a quadratic in order to test for a non-linear association of burden with age. The significant associations with both age and age² imply that *Teladorsagia* spp. burden does eventually decrease in the host after peaking at 2 years (Table 3) as suggested by the data for 2002 alone (Fig. 2A). *T. axei* burden again declined linearly with age (Table 4). There was no significant association between burden and host sex or between nematode sex and host sex for *Teladorsagia* spp., but in this larger dataset there was a significantly greater burden of *T. axei* in male hosts than female hosts (Table 4), again as suggested by the data for 2002 alone (Fig. 3B). There were also higher numbers of *T. axei* females in male hosts than female hosts (mean sq = 3.59, D.F. = 1, P = 0.05; the final model explained 52.54% of the deviance).

Intestinal nematode species prevalence and burden

In 2002 samples were taken from the small and large intestines of 69 sheep of different age and sex classes and 8 nematode species were recorded (Table 1; Fig. 1). From the mean nematode counts given in Table 1 it is clear that the most abundant species in the small intestine was *T. vitrinius*. Figures 2C and 3C show the intensity data for this species plotted by host age and sex. There was a significant negative
association between host age and both prevalence (Table 5A) and intensity (Table 5B). Juvenile males had highest prevalence (Table 5A) and there was a significant male bias in intensity of infection (Table 5B). Furthermore, there were higher intensities of both male (mean sq = 4.19, D.F. = 1, \( P = 0.04 \); final model explained 17.55% of the deviance) and female (mean sq = 6.11, D.F. = 1, \( P = 0.01 \); final model explained 20.45% of the deviance) \( T. vitrinus \) in male hosts.

Of the other intestinal species only \( Nematodirus filicollis \) showed a significant decrease in prevalence (\( \Delta \)deviance = 47.31, D.F. = 1, \( P < 0.001 \); final model explained 50.74% of the deviance) and intensity (mean sq = 15.23, D.F. = 1, \( P < 0.001 \); final model explained 23.86% of the deviance) and \( Bunostomum \)
trigonocephalum a significant increase in prevalence ($\text{Deviance} = 10.62$, d.f. = 1; $P = 0.001$; final model explained 18.0% of the deviance) with host age. None of the other species showed any significant associations with host age and sex in either prevalence or intensity.

Proportions of strongyle egg-producing nematodes

In the Soay sheep population, strongyle egg count is used as an indirect measure of burden and it is therefore of interest to know the sex ratio of the adult worm populations documented here. On St Kilda 5 species produce the strongyle egg type, namely, Teladorsagia spp. (comprising $T. \text{circumcincta}$, $T. \text{davtiani}$ and $T. \text{trifurcata}$), $T. \text{axei}$, $T. \text{vitrinus}$, $C. \text{ovina}$ and $B. \text{trigonocephalum}$ (Wilson et al. 2004). Proportions of males to females for each species were: Teladorsagia spp.: 46:54; $T. \text{axei}$: 42:58; $T. \text{vitrinus}$: 45:55; $B. \text{trigonocephalum}$: 44:56 and $Chabertia \text{ovina}$: 43:57. For the other nematode species producing more distinctive egg types the proportions of males to females were: $Nematodirus \text{battus}$ 51:49; $Nematodirus \text{filicollis}$ 45:55; $Capillaria \text{longipes}$ 41:59 and $Trichuris \text{ovis}$ 38:62. In each case, except for $N. \text{battus}$, females predominated over males.

Finally, we estimated the proportion of the main strongyle egg producing female nematode species and their potential egg output in hosts of different sex and age (Table 6). The average number of adult female nematodes of each species in each host sex-age class was multiplied by the approximate number of eggs present in the uterus of each species ($T. \text{circumcincta}$). In this study the estimates do not purport to give an indication of fecundity but simply of the biotic potential of the nematode species to produce eggs. From these hypothetical calculations it seems that the potential egg output of Teladorsagia spp. and $T. \text{axei}$ are roughly equivalent in lambs, despite their disparity in abundance. Male lambs can potentially shed higher levels of $T. \text{vitrinus}$.

Table 3. Results of a GLM, with negative binomial error structure, testing for an association between age, age$^2$, sex and year and burden of Teladorsagia spp. using combined data from 1999 and 2002

(Burden significantly increased over the first few age classes before decreasing. The final model explained 27.23% of the deviance. $\text{Deviance}$ values are equivalent to $-2 \times$ the log-likelihood. The sex and year coefficients indicate effects of males relative to females and 2002 relative to 1999 respectively.)

<table>
<thead>
<tr>
<th>Retained term</th>
<th>$\Delta$Deviance</th>
<th>Coefficient (s.e.)</th>
<th>d.f.</th>
<th>Pr (Chi)</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27.770</td>
<td>0.931 (0.160)</td>
<td>1</td>
<td>0.0000001</td>
<td>Increasing</td>
</tr>
<tr>
<td>Age$^2$</td>
<td>18.785</td>
<td>$-0.247$ (0.054)</td>
<td>1</td>
<td>0.0001</td>
<td>Implies increasing to peak value then decreasing</td>
</tr>
</tbody>
</table>

Removed terms

| Sex           | 2.125             | $-0.078$ (0.052)  | 1    | 0.144    | —           |
| Year          | 0.276             | $-0.018$ (0.035)  | 1    | 0.599    | —           |

Table 4. Results of a GLM, with negative binomial error structure, testing for an association between age, sex and year and burden of $T. \text{axei}$ using combined data from 1999 and 2002

(Burden was significantly higher in males and decreased with age. The final model explained 27.34% of the deviance. $\text{Deviance}$ values are equivalent to $-2 \times$ the log-likelihood. The sex and year coefficients indicate effects of males relative to females and 2002 relative to 1999 respectively.)

<table>
<thead>
<tr>
<th>Retained terms</th>
<th>$\Delta$Deviance</th>
<th>Coefficient (s.e.)</th>
<th>d.f.</th>
<th>Pr (Chi)</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>49.761</td>
<td>$-0.531$ (0.067)</td>
<td>1</td>
<td>0.000</td>
<td>Decreasing</td>
</tr>
<tr>
<td>Sex</td>
<td>3.572</td>
<td>0.146 (0.077)</td>
<td>1</td>
<td>0.05</td>
<td>More worms in males</td>
</tr>
</tbody>
</table>

Removed term

| Year          | 0.043             | $-0.011$ (0.052)  | 1    | 0.836    | —           |
the female lambs. Whereas egg output of both Trichostrongylus species is predicted to fall with increasing host age, Teladorsagia spp. egg output has the potential to rise to a peak in the 2-year-old host.

**DISCUSSION**

**Associations between gastrointestinal nematode infection and host age**

Although overall gastrointestinal nematode burden decreased with host age this masked a significant increase in intensity of Teladorsagia spp., at least until the age of 2 years. Intensity of *T. axei* and *T. vitrinus* decreased with host age. The veterinary literature provides evidence that the decrease in intensity of *T. axei* is due to a sterilizing immune response (Gibson, 1952; Ross, 1970; Waller and Thomas, 1981; Soulsby, 1986) and although acquired immunity is also known to regulate infection with *Teladorsagia* spp. (Smith et al. 1985) the effect is not as strong or as long lasting as for *T. axei*. Within the abomasum, L3 and L4 larvae of *Teladorsagia* spp. develop within the gastric glands while those of *T. axei* tunnel between the epithelial layer and lamina propria (Balic et al. 2000). Furthermore, Waller and Thomas (1981) found that in lambs, an abrupt reduction in accumulated burden of *T. axei* was found to occur only after threshold intensity of the species was reached but for *Teladorsagia* spp., a more continual density-dependent turnover occurred in which adult worms were lost and replaced (Waller and Thomas, 1978).

### Table 5.

(A) Results of a GLM, with binomial error structure, testing for an association between host age and sex and presence/absence of *T. vitrinus*. Prevalence significantly decreased with age. The final model explained 65·60% of the deviance. (B) Results of LM, with normal error structure, testing for an association between age and sex and abundance using log transformed *T. vitrinus* counts > 0

(Burden significantly decreased with age. The final model explained 21·11% of the variance. Deviance values are equivalent to \(-2 \times \text{the log-likelihood. The sex coefficient indicates the effect of males relative to females.}

(A)

<table>
<thead>
<tr>
<th>Retained terms</th>
<th>Deviance</th>
<th>Coefficient (s.e.)</th>
<th>D.F.</th>
<th>Pr (Chi)</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>17·708</td>
<td>-6·517 (21·072)</td>
<td>1</td>
<td>0·00002</td>
<td>Decreasing</td>
</tr>
<tr>
<td>Sex</td>
<td>2·796</td>
<td>15·744 (63·198)</td>
<td>1</td>
<td>0·094</td>
<td></td>
</tr>
<tr>
<td>Age: sex</td>
<td>6·267</td>
<td>-5·971 (21·072)</td>
<td>1</td>
<td>0·012</td>
<td>Juvenile males more often had worms</td>
</tr>
</tbody>
</table>

(B)

<table>
<thead>
<tr>
<th>Retained terms</th>
<th>Mean sq</th>
<th>Coefficient (s.e.)</th>
<th>D.F.</th>
<th>Pr (Chi)</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>10·626</td>
<td>-0·332 (0·124)</td>
<td>1</td>
<td>0·001</td>
<td>Decreasing</td>
</tr>
<tr>
<td>Sex</td>
<td>5·371</td>
<td>0·208 (0·191)</td>
<td>1</td>
<td>0·020</td>
<td>More worms in males</td>
</tr>
<tr>
<td>Age: sex</td>
<td>0·394</td>
<td>0·077 (0·124)</td>
<td>1</td>
<td>0·529</td>
<td></td>
</tr>
</tbody>
</table>

### Table 6.

Proportions (%) of the main strongyle egg-producing female nematodes and their corresponding potential egg outputs

(Calculations were based on nematode counts from of Hirta Soay sheep that died in 2002.)

<table>
<thead>
<tr>
<th>Age class</th>
<th>Host sex</th>
<th><em>Teladorsagia</em> spp. (%</th>
<th>Potential egg output</th>
<th><em>Trichostrongylus axei</em> (%</th>
<th>Potential egg output</th>
<th><em>Trichostrongylus vitrinus</em> (%</th>
<th>Potential egg output</th>
<th>Other strongyle species (%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambs</td>
<td>♂</td>
<td>13·91</td>
<td>29·400</td>
<td>43·95</td>
<td>33·165</td>
<td>41·30</td>
<td>56·097</td>
<td>0·84</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>22·08</td>
<td>32·662</td>
<td>43·93</td>
<td>23·210</td>
<td>33·12</td>
<td>31·500</td>
<td>0·87</td>
</tr>
<tr>
<td>Yearlings</td>
<td>♂</td>
<td>18·48</td>
<td>33·754</td>
<td>44·19</td>
<td>28·835</td>
<td>35·79</td>
<td>42·039</td>
<td>1·54</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>25·94</td>
<td>38·738</td>
<td>52·03</td>
<td>27·750</td>
<td>20·46</td>
<td>19·647</td>
<td>1·57</td>
</tr>
<tr>
<td>2-year-olds</td>
<td>♂</td>
<td>40·74</td>
<td>65·100</td>
<td>24·93</td>
<td>14·225</td>
<td>31·70</td>
<td>32·562</td>
<td>2·63</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>70·40</td>
<td>90·566</td>
<td>14·35</td>
<td>6595</td>
<td>11·97</td>
<td>9900</td>
<td>3·28</td>
</tr>
<tr>
<td>Adults</td>
<td>♂</td>
<td>51·93</td>
<td>37·562</td>
<td>14·19</td>
<td>3665</td>
<td>28·71</td>
<td>13·347</td>
<td>5·17</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>45·91</td>
<td>63·994</td>
<td>10·18</td>
<td>5070</td>
<td>41·89</td>
<td>19·647</td>
<td>2·02</td>
</tr>
</tbody>
</table>
One possible explanation for the opposing trends of the 2 abomasum species observed in this study may be that until sufficient intensity of *T. axei* is reached to stimulate expulsion, *Teladorsagia* spp. remains at low levels in a response consistent with the phenomenon of premunition (Tetztlaff and Todd, 1973; Kennedy and Todd, 1975) whereby a chronic infection of parasites remains at a plateau of adult worms either through rejection or arrest of incoming larvae or as a result of the larvae replacing adults (Bowman, 1995). Hence, the dramatic increase in *Teladorsagia* spp. at 2 years of age may mark the demise of *T. axei* and the beginning of an acute proliferation of *Teladorsagia* spp. as arrested larvae become uninhibited and continue their development in the absence of a predominant adult worm population (Dunsmore, 1963). In this analysis acquired immunity to *Teladorsagia* spp. only really becomes apparent in hosts older than 2 years.

**Associations between gastrointestinal nematode infection and host sex**

Strongyle egg count data from the Soay sheep population has consistently shown that male sheep are prone to more intense nematode infections than females (Wilson et al. 2004). Gulland et al. (1993) demonstrated that, in a year of high mortality, yearling males treated with an anthelmintic bolus suffered significantly less mortality than controls, but there was no such effect in females. In this study, juvenile males had higher mixed gastrointestinal nematode burden than any other demographic group and of the 3 predominant species, males had higher burdens of *T. axei* and *T. vitrinus* although there was no significant difference of intensity levels of *Teladorsagia* spp. between the host sexes. Furthermore, combined *Trichostrongylus* species made up a significantly higher proportion of the strongyle egg producing adult community in male hosts than female hosts. These new data indicate a potentially more important contribution from *Trichostrongylus* species than *Teladorsagia* spp. to higher strongyle egg counts (Wilson et al. 2004) and increased mortality (Clutton-Brock et al. 2004) in juvenile males in high density years than previously believed.

**Implications for strongyle egg count as a measure of species burdens in St Kilda Soay sheep**

In the Soay sheep population, strongyle egg count has been used to reveal detrimental effects of nematode infection. In high host density years a consistent finding has been that juveniles and males have lower survivorship (Clutton-Brock et al. 2004) and strongyle egg count is negatively associated with host survival (Illius et al. 1995; Coltman et al. 1999). Through experiment, Gulland (1992) was able to prove that gastrointestinal nematodes play a significant role in contributing to mortality. Until now, *Teladorsagia* spp. has been assumed to be primarily responsible. Experiments in domestic sheep, however, show that concurrent infections of *Teladorsagia* spp. and *Trichostrongylus* species cause more severe pathology than single infections of those species (Steel et al. 1982; Sykes et al. 1988). Account should, therefore, be taken of the burden of concurrent *Trichostrongylus* species infections in assessing the impact of *Teladorsagia* spp. in Soay sheep.

The differences in the predominant species occurring in juvenile and adult sheep that died in 2002 raises the issue of whether strongyle egg count is a reliable indicator of burden of each of the species of nematodes, especially *Teladorsagia* spp. Wilson et al. (2004) show that strongyle egg count and burden of *Teladorsagia* spp. are correlated in Soay sheep on Hirta but with an r² of only 0.147, much variance in each measure is unexplained by the other. Furthermore, these findings were only based on abomasum counts, excluding the abundant *T. vitrinus* in the small intestine. Stear et al. (2006) found that in Blackface lambs naturally infected with a mixture of nematode species of which *T. circumcincta* predominated, individuals with high mean strongyle egg counts did not necessarily have a greater number of adults but had a greater number of species other than *T. circumcincta*, particularly *T. axei* and *T. vitrinus*. These findings and those of previous studies (Bishop and Stear, 2000) suggest that *T. circumcincta* is prone to density-dependent constraints on fecundity and hence strongyle egg count is not as reliable an indicator of *T. circumcincta* burden as it perhaps is for other contributing species. On St Kilda, strongyle egg count has been shown to be generally higher in males than females and to decrease significantly with age in females and, at least to the yearling stage, in males (Wilson et al. 2004). Given that there are on average 4 times more *Trichostrongylus* species than *Teladorsagia* spp. in the juvenile host and that proportions of female to male adults is higher for *Trichostrongylus* species than for *Teladorsagia* spp. it is possible that *Trichostrongylus* species are contributing considerably towards the higher strongyle counts in juveniles especially juvenile males.

**Towards determining species-specific data from strongyle egg counts**

The data presented in this paper reveal the populations of gastrointestinal nematode species in dead hosts whose immune systems were depressed due to malnutrition. However, we cannot know the burden of species in contemporary hosts that survived or the burden in healthy hosts in a year of negligible mortality. The advantage of strongyle egg counts is that non-invasive, repeated measures over host lifetimes can be obtained, but clearly there is a need to develop
Teladorsagia spp. in juvenile hosts, while Teladorsagia on average, 4 times more abundant than of 1989, 1999 and 2002 burdens in the mortality events.

Comparisons between gastrointestinal nematode different species. approach to monitor intensities of helminth eggs of living St Kilda Soay sheep (B. Wimmer, unpublished data). This method is a precursory step towards development of a quantitative PCR approach to monitor intensities of helminth eggs of different species.

Comparisons between gastrointestinal nematode burdens in the mortality events of 1989, 1999 and 2002

Teladorsagia spp. has been considered the predominant and most significant nematode in St Kilda Soay sheep since Gulland (1992) estimated that it constituted 75% of the entire gastrointestinal nematode burden in all age classes of sheep that died in 1989. However, data presented here from 2002 show that combined Trichostrongylus species were, on average, 4 times more abundant than Teladorsagia spp. in juvenile hosts, while Teladorsagia spp. was around 4 times more abundant than the combined Trichostrongylus species in adult hosts.

It is possible that temporal changes in the epidemiology of nematode species in the Soay sheep population could account for the lack of congruence between the findings of 1989 and 2002. For instance, Stear et al. (1998) conducted an epidemiological study of mixed nematode species infections in Blackface lambs on a Scottish farm and found considerable changes in the prevalence and intensities of different species over 4 years. As a preliminary test for this, in the Soay sheep population, abomasum nematode species burden estimates from 1999 were compared with 2002 but no significant differences were found. In order to properly resolve this issue, however, gastrointestinal nematode burden estimates from future mortality events will be analysed.

It is also important to consider if the different burden estimation techniques used were responsible for the different findings. In 1989 Gulland (1991) made total worm counts based on examination of 20% aliquots of the abomasum. One hundred adult nematodes were then randomly selected for identification to species level, enabling relative proportions of each to be calculated. This method, however, may have produced an inadvertent bias as the much larger Teladorsagia spp. (0.7–1.2 cm) probably has more chance of being observed and identified than the smaller T. axei (0.3–0.8 cm). In this present study, the potential source of bias was eliminated when all nematodes in a 1% aliquot were identified to species level, having been first stained with iodine.

Why do nematode species intensity patterns in St Kilda Soay sheep differ from mainland sheep?

According to Urquhart et al. (1999) Teladorsagia spp. is more important in terms of abundance and pathogenicity in sheep in mainland Britain than Trichostrongylus spp. and is considered the primary pathogen in parasitic gastroenteritis in lambs. An interesting finding in this research, therefore, is that the predominance of Trichostrongylus species over Teladorsagia spp. in juvenile hosts in St Kilda Soay sheep. This contrast may be due in part to the unique circumstances of the population. Specifically, there has never been any artificial selection or large-scale exposure to anthelmintic treatment since the founder flock was imported to the archipelago around 2000 years ago. Two possible explanations could therefore be that there is either some inherent difference in the Soay breed’s ability to tolerate infection with Teladorsagia spp. or Trichostrongylus species or that widespread use of anthelmintics in domestic mainland sheep has altered the natural ecology of the gastrointestinal helminth fauna.

Gulland (1991) provided some empirical evidence for a greater natural resistance to Teladorsagia spp. in Soay than Blackface lambs. In an experiment, groups of 5-month-old sheep of each breed and both sexes were assigned as either controls or infected with T. circumcincta larvae. Establishment of infection was greater, strongyle egg counts higher and live weight gain lower in Blackface than Soay lambs (Gulland, 1991). This finding is consistent with the results presented in this paper that Soay lambs in high-density years have surprisingly low burdens of Teladorsagia spp.

Although Trichostrongylus species monopolize the gastrointestinal tract in Soay lambs and yearlings, Teladorsagia spp. does increase in intensity over the first few age classes and seems to have a more successful long-term strategy, as it is the only species to reach 100% prevalence in the population. This implies an adaptive ability which would be consistent with the emerging trend of anthelmintic resistance in the species in the mainland (Bartley et al. 2003). After decades of anthelmintic use it is plausible that the natural ecology of helminth communities in domestic sheep have been altered (Cabaret et al. 2002) to give more robust species like T. circumcincta a selective advantage in re-colonization of the host.

Conclusion

Prior to this research it was generally believed that within the diverse component parasite community of the St Kilda Soay sheep only Teladorsagia spp. predominated, making it a relatively simple host-parasite system. This recent analysis has revealed a more complex situation in which it appears that no one nematode species predominates throughout the
life of the host. This highlights the desirability of developing molecular techniques that can provide species-specific differentiation of morphologically indistinguishable eggs. Quantifying the eggs from different nematode species could reveal more about how they challenge hosts at different stages of life. This would enable specific theories regarding associations with host genetic variation to be tested, thus providing a unique opportunity to understand the evolutionary consequences of co-infection in the wild.

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REFERENCES


