



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

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

Citation for published version:

Craig, BH, Pilkington, JG & Pemberton, JM 2006, 'Gastrointestinal nematode species burdens and host mortality in a feral sheep population', *Parasitology*, vol. 133, no. 4, pp. 485-496.
<https://doi.org/10.1017/S0031182006000618>

Digital Object Identifier (DOI):

[10.1017/S0031182006000618](https://doi.org/10.1017/S0031182006000618)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Parasitology

Publisher Rights Statement:

RoMEO green

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



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

B. H. CRAIG*, J. G. PILKINGTON and J. M. PEMBERTON

Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Scotland

(Received 28 February 2006; revised 24 April and 8 May 2006; accepted 8 May 2006; first published online 4 July 2006)

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, 1975*a,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

* Corresponding author. Tel: +0131 650 5516. Fax: +0131 650 6564. E-mail: Barbara.Craig@ed.ac.uk

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,

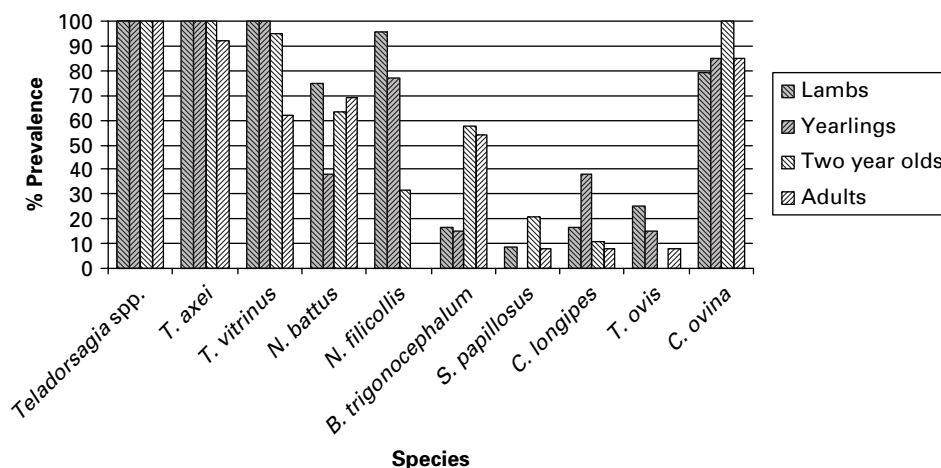


Fig. 1. Prevalence of gastrointestinal nematode species by host age in the 2002 sample. For illustration, age is shown as 4 categories: lambs, yearlings, 2-year-olds and individuals older than 2 years (adults).

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)

$$k = x^2 / (s^2 - x) \quad \text{where } x = \text{mean}; s^2 = \text{variance.}$$

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 ($\Delta\text{deviance} = 10.74$, coefficient (S.E.) = -0.193 (0.055), D.F. = 1, $P = 0.001$) and differed between sexes, with juvenile males having higher burdens ($\Delta\text{deviance} = 3.99$, 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 davtiani* and *Teladorsagia trifurcata*. In this study these adult male morphs were found to make up $80.17 \pm 0.1\%$, $15.03 \pm 0.1\%$ and $4.8 \pm 0.06\%$ 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 ($\Delta\text{deviance} = 16.58$, D.F. = 1,

Table 1. Mean burden ($\pm 95\%$ 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 estimates of the mean were calculated for small sample sizes according to Fowler and Cohen (1990).)

Site	Nematode species	Mean burden ($\pm 95\%$ C.I.) of nematode species of the gastrointestinal tract of the sheep by age class			
		Lambs	Yearlings	2-year-olds	Adults
Abomasum	<i>Teladorsagia</i> spp. (2002)	4179 \pm 735	4782 \pm 1442	9863 \pm 2899	6723 \pm 2985
	<i>Teladorsagia</i> spp. (1999)	3700 \pm 793	8609 \pm 3543	10 712 \pm 6350	6458 \pm 2254
	<i>Trichostrongylus axei</i> (2002)	10 042 \pm 2171	9709 \pm 4848	3724 \pm 1315	1285 \pm 793
	<i>Trichostrongylus axei</i> (1999)	8692 \pm 1733	6900 \pm 2944	6238 \pm 6934	2217 \pm 1425
Small intestine	<i>Trichostrongylus vitrinus</i>	8917 \pm 2205	6615 \pm 4682	4361 \pm 1749	5285 \pm 5841
	<i>Nematodirus battus</i>	175 \pm 77	46 \pm 40	355 \pm 233	100 \pm 55
	<i>Nematodirus filicollis</i>	1571 \pm 778	615 \pm 427	89 \pm 127	0
	<i>Bunostomum trigonocephalum</i>	17 \pm 16	31 \pm 45	79 \pm 50	138 \pm 115
	<i>Strongyloides papillosus</i>	13 \pm 19	0	16 \pm 18	8 \pm 17
	<i>Capillaria longipes</i>	21 \pm 21	62 \pm 53	11 \pm 17	8 \pm 17
	Large intestine	<i>Trichuris ovis</i>	42 \pm 35	15 \pm 23	0
<i>Chabertia ovina</i>		163 \pm 57	292 \pm 220	429 \pm 151	215 \pm 120
GI tract	All species	25 138 \pm 4035	22 168 \pm 8703	18 926 \pm 4360	13 769 \pm 7248

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.)

Species	Mean	Variance	Negative binomial parameter (k)
<i>Teladorsagia</i> spp.* (2002)	6337	21 517 974	1.87
<i>Teladorsagia</i> spp.* (1999)	6303	24 295 544	1.63
<i>T. axei</i> * (2002)	6589	36 308 651	1.20
<i>T. axei</i> * (1999)	6564	27 541 619	1.56
<i>T. vitrinus</i>	6544	43 395 038	0.99
<i>N. battus</i>	186	88 374	0.39
<i>N. filicollis</i>	687	1 721 003	0.27
<i>B. trigonocephalum</i>	59	12 741	0.28
<i>S. papillosus</i>	10	1219	0.09
<i>C. longipes</i>	23	3057	0.18
<i>T. ovis</i>	19	3022	0.12
<i>C. ovina</i>	270	74 435	0.99
Gastrointestinal total*	20 726	131 937 615	3.26

$P < 0.001$; the final model explained 21.10% of the deviance) while *T. axei* decreased significantly with host age (Δ deviance = 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. vitrinus*. Figures 2C and 3C show the intensity data for this species plotted by host age and sex. There was a significant negative

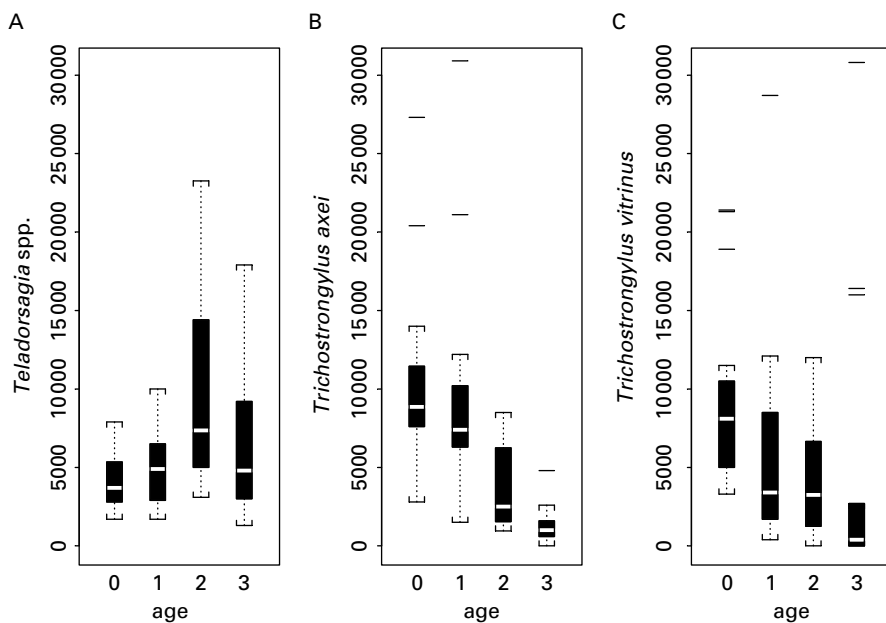


Fig. 2. Box and whisker plots showing burden of (A) *Teladorsagia* spp. (B) *T. axei* and (C) *T. vitrinus* by host age in the 2002 sample. The white line in the middle of each box shows the median of the distribution, the middle 50% of intensity values fall within the range defined by the box and the dotted lines bounded by parentheses show 1.5 times the interquartile range of the data. Horizontal lines out with the parentheses are outliers. No whisker is drawn for *T. vitrinus* burden in adults as no data points lie between the top of the box and the bracket. For illustration, age is shown as 4 categories: lambs (0), yearlings (1), 2-year-olds (2) and adults (3).

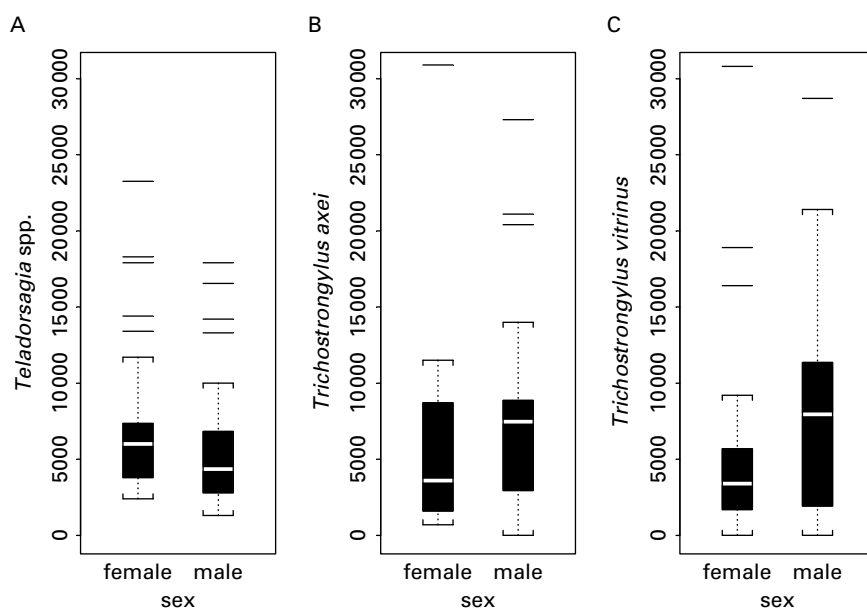


Fig. 3. Box and whisker plots showing burden of (A) *Teladorsagia* spp. (B) *T. axei* and (C) *T. vitrinus* by host sex in the 2002 sample. See Fig. 2 legend for notation.

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 flicollis* showed a significant decrease in prevalence (Δ 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*

Table 3. Results of a GLM, with negative binomial error structure, testing for an association between age, age², 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. Δ 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.)

Retained term	Δ Deviance	Coefficient (S.E.)	D.F.	Pr (Chi)	Association
Age	27.770	0.931 (0.160)	1	0.0000001	Increasing
Age ²	18.785	-0.247 (0.054)	1	0.00001	Implies increasing to peak value then decreasing
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. 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. Δ 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.)

Retained terms	Δ Deviance	Coefficient (S.E.)	D.F.	Pr (Chi)	Association
Age	49.761	-0.531 (0.067)	1	0.000	Decreasing
Sex	3.572	0.146 (0.077)	1	0.05	More worms in males
Removed term					
Year	0.043	-0.011 (0.052)	1	0.836	—

trigonocephalum a significant increase in prevalence (Δ 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. circumcincta*, *T. daviani* and *T. trifurcata*), *T. axei*, *T. vitrinus*, *C. ovina* and *B. trigonocephalum* (Wilson *et al.* 2004). Proportions of males to females for each species were: *Teladorsagia* spp.: 46:54; *T. axei*: 42:58; *T. vitrinus*: 45:55; *B. trigonocephalum*: 44:56 and *Chabertia ovina*: 43:57. For the other nematode species producing more distinctive egg types the proportions of males to females were: *Nematodirus battus* 51:49; *Nematodirus flicollis* 45:55; *Capillaria*

longipes 41:59 and *Trichuris ovis* 38:62. In each case, except for *N. 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 (*Teladorsagia* spp. = 14; *T. axei* = 5 and *T. vitrinus* = 9). Michel (1969) used a similar method to estimate *Ostertagia ostertagi* population fecundity and Stear *et al.* (1995) demonstrated an association between faecal egg count, variation in burden and variation in the number of eggs *in utero* for *T. 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. axei* are roughly equivalent in lambs, despite their disparity in abundance. Male lambs can potentially shed higher levels of *T. vitrinus*

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. Δ deviance values are equivalent to $-2 \times$ the log-likelihood. The sex coefficient indicates the effect of males relative to females.)

(A)

Retained terms	Δ Deviance	Coefficient (S.E.)	D.F.	Pr (Chi)	Association
Age	17.708	-6.517 (21.072)	1	0.00002	Decreasing
Sex	2.796	15.744 (63.198)	1	0.094	—
Age : sex	6.267	-5.971 (21.072)	1	0.012	Juvenile males more often had worms

(B)

Retained terms	Mean sq	Coefficient (S.E.)	D.F.	Pr (Chi)	Association
Age	10.626	-0.332 (0.124)	1	0.001	Decreasing
Sex	5.371	0.208 (0.191)	1	0.020	More worms in males
Removed term					
Age : sex	0.394	0.077 (0.124)	1	0.529	—

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.)

Age class	Host sex	<i>Teladorsagia</i> spp. ♀ (%)	Potential egg output	<i>Trichostrongylus axei</i> ♀ (%)	Potential egg output	<i>Trichostrongylus vitrinus</i> ♀ (%)	Potential egg output	Other strongyle species ♀ (%)
Lambs	♂	13.91	29 400	43.95	33 165	41.30	56 097	0.84
	♀	22.08	32 662	43.93	23 210	33.12	31 500	0.87
Yearlings	♂	18.48	33 754	44.19	28 835	35.79	42 039	1.54
	♀	25.94	38 738	52.03	27 750	20.46	19 647	1.57
2-year-olds	♂	40.74	65 100	24.93	14 225	31.70	32 562	2.63
	♀	70.40	90 566	14.35	6595	11.97	9900	3.28
Adults	♂	51.93	37 562	14.19	3665	28.71	13 347	5.17
	♀	45.91	63 994	10.18	5070	41.89	19 647	2.02

than 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).

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 (Tetzlaff 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 r^2 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

a method that provides quantitative species-specific information.

One promising avenue of research utilizes molecular diagnostic tools. Wimmer *et al.* (2004) have already developed a method that provides species-specific differentiation of helminth eggs concentrated from faecal samples using the ITS-2 rDNA region and yielded prevalence data for 9 helminth species in 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 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.

We thank The National Trust for Scotland and Scottish Natural Heritage for permission to work on St Kilda. Logistical support was provided by the MOD, QinetiQ and Eurest staff on St Kilda and Benbecula. A. Donald, H. Lloyd and A. Mayo helped with sample collection on St Kilda. M. Crawley and L. Kruuk gave advice on statistical analyses and comments from A. Graham improved the manuscript considerably. We thank T. Clutton-Brock and B. Grenfell for the long-term data collection on St Kilda and the Natural Environment Research Council for supporting the project. We thank F. Gulland and K. Wilson for their previous parasitological research in the population on which this work builds. This study was funded by a Wellcome Trust Biodiversity Studentship.

REFERENCES

- Balic, A., Bowles, V. M. and Meeusen, E. N. T.** (2000). The immunobiology of gastrointestinal nematode infections in ruminants. *Advances in Parasitology* **45**, 181–241.
- Bartley, D. J., Jackson, E., Johnston, K., Coop, R. L., Mitchell, G. B. B., Sales, J. and Jackson, F.** (2003). A survey of anthelmintic resistant nematode parasites in Scottish sheep flocks. *Veterinary Parasitology* **117**, 61–71.
- Bishop, S. C., Bairden, K., McKeller, Q. A., Park, M. and Stear, M. J.** (1996). Genetic parameters for faecal egg count following mixed, natural, predominantly *Ostertagia circumcincta* infection and relationships with live weight in young lambs. *Animal Science* **63**, 423–428.
- Bishop, S. C. and Stear, M. J.** (2000). The use of a gamma-type function to assess the relationship between the number of adult *Teladorsagia circumcincta* and total egg output. *Parasitology* **121**, 435–440.
- Boag, B. and Thomas, R. J.** (1971). Epidemiological studies on gastrointestinal nematode parasites of sheep. *Research in Veterinary Science* **12**, 132–139.
- Boag, B. and Thomas, R. J.** (1977). Epidemiological studies on gastrointestinal nematode parasites of sheep: the seasonal number of generations and succession of species. *Research in Veterinary Science* **22**, 62–67.
- Bowman, D. D.** (1995). *Parasitology for Veterinarians*, W.B. Saunders, Philadelphia, USA.
- Braisher, T. L.** (1999). Genetic variation in trichostrongylid parasites of the Soay sheep of Hirta, St Kilda. Ph.D. thesis, University of Cambridge.
- Buchanan, M.** (1995). Introduction. In *St Kilda: The Continuing Story of the Islands* (ed. Buchanan, M.), pp. 11–24. HMSO, Edinburgh.
- Cabaret, J., Mage, C. and Bouilhol, M.** (2002). Helminth intensity and diversity in organic meat sheep farms in centre of France. *Veterinary Parasitology* **105**, 33–47.
- Christensen, N. O., Nansen, P. and Fagbemi, B. O.** (1987). Heterologous antagonistic and synergistic interactions between helminths and between helminths and protozoans in concurrent experimental infection of mammalian hosts. *Parasitology Research* **73**, 387–410.
- Clutton-Brock, T. H., Grenfell, B. T., Coulson, T., MacColl, A. D. C., Illius, A. W., Forchhammer, M. C., Wilson, K., Lindstrom, J., Crawley, M. J. and Albon, S. D.** (2004). Population dynamics in Soay sheep. In *Soay Sheep: Dynamics and Selection in an Island Population* (ed. Clutton-Brock, T. H. and Pemberton, J. M.), pp. 52–112. Cambridge University Press, Cambridge.
- Clutton-Brock, T. H. and Pemberton, J. M.** (2004). *Soay Sheep: Dynamics and Selection in an Island Population*. Cambridge University Press, Cambridge.
- Clutton-Brock, T. H., Wilson, K. and Stevenson, I. R.** (1997). Density-dependent selection on horn phenotype in Soay sheep. *Philosophical Transactions of the Royal Society of London, B* **352**, 839–850.
- Coltman, D. W., Pilkington, J. G., Smith, J. A. and Pemberton, J. M.** (1999). Parasite-mediated selection against inbred Soay sheep in a free-living island population. *Evolution* **53**, 1259–1267.
- Coltman, D. W., Wilson, K., Pilkington, J. G., Stear, M. J. and Pemberton, J. M.** (2001). A microsatellite polymorphism in the gamma interferon gene is associated with resistance to gastrointestinal nematodes in a naturally-parasitized population of soay sheep. *Parasitology* **122**, 571–582.
- Cox, F. E. G.** (2001). Concomitant infections, parasites and immune responses. *Parasitology* **122** (Suppl.), S23–S38.
- Dunsmore, J. D.** (1963). Effect of the removal of an adult population of *Ostertagia* from sheep on concurrently existing arrested larvae. *Australian Veterinary Journal* **39**, 459–463.
- Dybdahl, M. F. and Lively, C. M.** (1998). Host-parasite co-evolution: evidence for rare advantage and time lagged selection in a natural population. *Evolution* **52**, 1057–1066.
- Fowler, J. and Cohen, L.** (1990). *Practical Statistics for Field Biology*. John Wiley and Sons Ltd., Chichester, UK.
- Gibson, T. E.** (1952). The development of acquired resistance by sheep to infestation with the nematode *Trichostrongylus axei*. *Journal of Helminthology* **26**, 43–53.
- Graham, A. L.** (2002). When T-helper cells don't help: immunopathology during concomitant infection. *The Quarterly Review of Biology* **77**, 409–434.
- Gulland, F. M. D.** (1991). The role of parasites in the population dynamics of Soay sheep on St. Kilda, Ph.D. thesis, University of Cambridge.
- Gulland, F. M. D.** (1992). The role of nematode parasites in Soay sheep (*Ovis aries* L.) mortality during a population crash. *Parasitology* **105**, 493–503.
- Gulland, F. M. D., Albon, S. D., Pemberton, J. M., Moorcroft, P. and Clutton-Brock, T. H.** (1993). Parasite-associated polymorphism in a cyclic ungulate population. *Proceedings of the Royal Society of London, B* **254**, 7–13.

- Illius, A. W., Albon, S. D., Pemberton, J. M., Gordon, I. J. and Clutton-Brock, T. H.** (1995). Selection for foraging efficiency during a population crash in Soay sheep. *Journal of Animal Ecology* **64**, 481–492.
- Jewell, P.** (1995). Soay sheep. In *St. Kilda: The Continuing Story of the Islands* (ed. Buchanan, M.), pp. 73–93. HMSO, Edinburgh.
- Kennedy, M. S. and Todd, A. C.** (1975). Age group interactions of *Haemonchus contortus* in pre-munized lambs. *American Journal of Veterinary Research* **36**, 1195–1198.
- Kloosterman, A., Permentier, H. K. and Ploeger, H. W.** (1992). Breeding cattle and sheep for resistance to gastrointestinal nematodes. *Parasitology Today* **8**, 330–335.
- Leignel, V., Cabaret, J. and Humbert, J. F.** (2002). New molecular evidence that *Teladorsagia circumcincta* (nematoda: Trichostrongylidae) is a species complex. *Journal of Parasitology* **88**, 135–140.
- Lello, J., Boag, B., Fenton, A., Stevenson, I. R. and Hudson, P. J.** (2004). Competition and mutualism among the gut helminths of a mammalian host. *Nature, London* **428**, 840–844.
- M.A.F.F.** (1986). *Manual of Veterinary Parasitological Laboratory Techniques*. HMSO, London.
- Michel, J. F.** (1969). The regulation of egg output by *Ostertagia ostertagi* in calves infected once only. *Parasitology* **59**, 767–774.
- Parnell, I. W., Rayski, C., Dunn, A. M. and Mackintosh, G. M.** (1954). A survey of the helminths of Scottish hill sheep. *Journal of Helminthology* **28**, 53–110.
- Polak, M.** (2003). Heritability of resistance against ectoparasitism in the *Drosophila-Macrocheles* system. *Journal of Evolutionary Biology* **16**, 74–82.
- Poulin, R.** (2001). Interactions between species and the structure of helminth communities. *Parasitology* **122** (Suppl.), S3–S11.
- Read, A. F. and Taylor, L. H.** (2001). The ecology of genetically diverse infections. *Science* **292**, 1099–1102.
- Reid, J. F. S. and Armour, J.** (1975a). Studies in Scottish hill sheep: I. Changes in the susceptibility of the breeding ewe to *Ostertagia* spp. *Journal of Comparative Pathology* **85**, 163–170.
- Reid, J. F. S. and Armour, J.** (1975b). Studies in Scottish hill sheep: II. Changes in the susceptibility of the lamb to *Ostertagia* spp. *Journal of Comparative Pathology* **85**, 269–275.
- Roberts, M. G., Dobson, A. P., Arneberg, P., de Leo, G. A., Krecek, R. C., Manfredi, M. T., Lanfranchi, P. and Zaffaroni, E.** (2002). Parasite community ecology and biodiversity. In *The Ecology of Wildlife Diseases* (ed. Hudson, P. J., Rizzoli, A., Grenfell, B. T., Heesterbeek, H. and Dobson, A. P.), pp. 63–82. Oxford University Press, Oxford, UK.
- Ross, J. G.** (1970). Acquired immunity to *Trichostrongylus axei* in lambs: investigations of the development of immune competence. *Journal of Helminthology* **44**, 199–210.
- Samuel, W. M., Pybus, M. J. and Kocan, A. A.** (2001). *Parasitic Diseases of Wild Mammals*. Iowa State University Press, Iowa, USA.
- Smith, W. D., Jackson, F., Jackson, E. and Williams, J.** (1985). Age immunity to *Ostertagia circumcincta*: comparison of the local immune responses of 4 1/2 and 10 month-old lambs. *Journal of Comparative Pathology* **95**, 235–245.
- Soulsby, E. J. L.** (1986). *Helminths, Arthropods and Protozoa of Domesticated Animals*. Balliere Tindall, London, UK.
- Stear, M. J., Abuagob, O., Benothman, M., Bishop, S. C., Innocent, G., Kerr, A. and Mitchell, S.** (2006). Variation among faecal egg counts following natural nematode infection in Scottish blackface lambs. *Parasitology* **132**, 1–6.
- Stear, M. J., Bairden, K., Bishop, S. C., Gettinby, G., McKellar, Q. A., Park, M., Strain, S. and Wallace, D. S.** (1998). The processes influencing the distribution of parasite nematodes among naturally infected lambs. *Parasitology* **117**, 165–171.
- Stear, M. J., Bairden, K., Bishop, S. C., Suitkamp, J., Duncan, J. L., Gettinby, G., McKellar, Q. A., Park, M., Parkins, J. J., Reid, S. W. J., Strain, S. and Murray, M.** (1997). The genetic basis of resistance to *Ostertagia circumcincta* in lambs. *The Veterinary Journal* **154**, 111–119.
- Stear, M. J., Bishop, S. C., Doligalska, M., Duncan, J. L., Holmes, P. H., Irvine, J., McCririe, L., McKellar, Q. A., Sinski, E. and Murray, M.** (1995). Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. *Parasite Immunology* **17**, 643–652.
- Steel, J. W., Jones, W. O. and Symons, L. E. A.** (1982). Effects of a concurrent infection of *Trichostrongylus colubriformis* on the productivity and physiological and metabolic responses of lambs infected with *Ostertagia circumcincta*. *Australian Journal of Agricultural Research* **33**, 131–140.
- Stevenson, L. A., Gasser, R. B. and Chilton, N. B.** (1996). The ITS-2 rDNA of *Teladorsagia circumcincta*, *T. trifurcata* and *T. davtiani* (nematoda: Trichostrongylidae) indicates that these taxa are one species. *International Journal for Parasitology* **26**, 1123–1126.
- Sykes, A. R., Poppi, D. P. and Elliot, D. C.** (1988). Effect of concurrent infection with *Ostertagia circumcincta* and *Trichostrongylus colubriformis* on the performance of growing lambs consuming fresh herbage. *Journal of Agricultural Science* **110**, 531–541.
- Tetzlaff, R. D. and Todd, A. C.** (1973). Protective effects of pre-munition and age group interaction of *Haemonchus contortus* in sheep. *American Journal of Veterinary Research* **34**, 1549–1554.
- Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M. and Jennings, F. W.** (1999). *Veterinary Parasitology*, Blackwell Science Ltd., Oxford, UK.
- Wakelin, D. and Blackwell, J.** (1988). *Genetics of Resistance to Bacterial and Parasitic Infection*. Taylor and Francis, London, UK.
- Waller, P. J. and Thomas, R. J.** (1978). Nematode parasitism in sheep in north-east England: the epidemiology of *Ostertagia* spp. *International Journal for Parasitology* **8**, 275–283.
- Waller, P. J. and Thomas, R. J.** (1981). The natural regulation of *Trichostrongylus* spp. populations in young grazing sheep. *Veterinary Parasitology* **9**, 47–55.

Wilson, K. and Grenfell, B. T. (1997). Generalized linear modelling for parasitologists. *Parasitology Today* **13**, 33–38.

Wilson, K., Grenfell, B. T., Pilkington, J. G., Boyd, H. E. G. and Gulland, F. M. D. (2004). Parasites and their impact. In *Soay Sheep: Dynamics and Selection in an Island Population* (ed. Clutton-Brock, T. H. and Pemberton, J. M.),

pp. 113–165. Cambridge University Press, Cambridge, UK.

Wimmer, B., Craig, B. H., Pilkington, J. G. and Pemberton, J. M. (2004). Non-invasive assessment of parasitic nematode species diversity in wild Soay sheep using molecular markers. *International Journal for Parasitology* **34**, 625–631.