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Metazoan/protozoan parasite co-infections and host body weight in St Kilda Soay sheep


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SUMMARY

For hundreds of years, the unmanaged Soay sheep population on St Kilda has survived despite enduring presumably deleterious co-infections of helmhnt, protozoan and arthropod parasites and intermittent periods of starvation. Important parasite taxa in young Soay sheep are strongyles (Trichostrongylus axei, Trichostrongylus vitrinus and Teladorsagia circumcincta), coccidia (11 Eimeria species) and keds (Melophagus ovinus) and in older animals, Teladorsagia circumcincta. In this research, associations between the intensity of different parasite taxa were investigated. Secondly, the intensities of different parasite taxa were tested for associations with variation in host weight, which is itself a determinant of over-winter survival in the host population. In lambs, the intensity of strongyle eggs was positively correlated with that of Nematodirus spp. eggs, while in yearlings and adults strongyole eggs and coccidia oocysts were positively correlated. In lambs and yearlings, of the parasite taxa tested, only strongyle eggs were significantly and negatively associated with host weight. However, in adult hosts, strongyoles and coccidia were independently and negatively associated with host weight. These results are consistent with the idea that strongyles and coccidia are exerting independent selection on Soay sheep.

Key words: Soay sheep, St Kilda, co-infection, body weight, Trichostrongylus spp., Teladorsagia spp., Eimeria spp., Melophagus ovinus.

INTRODUCTION

In natural host populations, component parasite communities are composed of a diversity of metazoan and protozoan parasite species so that mixed infections in individual hosts are normal. Many investigations on the nature of co-infections in wild mammals have been described (e.g. Montgomery and Montgomery, 1990; Haukisalmi and Henttonen, 1993; Behnke et al. 2005) but despite the fact that interactions between co-infecting species are commonly discovered wherever studied (Christensen et al. 1987) and that different parasite taxa exert different demands on the host immune system, few studies have attempted to elucidate the nature and evolutionary consequences of complex co-infections in the wild (Cox, 2001). Given that extracellular and intracellular parasites provoke different (Th1 and Th2) and mutually inhibitory host immune responses (Graham, 2002) and that appreciable heritable variation has been detected in resistance to parasites in the face of strong selection for resistance (Wakelin and Blackwell, 1988; Bishop et al. 1996; Coltman et al. 2001b), it seems probable that the evolution of resistance to any particular co-infecting species is subject to host genetic constraints and immunological compromise.

The feral Soay sheep population of Hirta, St Kilda represents a naturally parasitized population infected with taxonomically diverse parasites (Wilson et al. 2004; Craig et al. 2006, 2007). Previous research, largely focussing on the nematode parasites, has shown that high nematode burdens contribute to host mortality in high host density years (Gulland, 1992) making them a likely agent for natural selection. However, despite the deleterious impacts of parasites the population harbours heritable variation for nematode resistance (Smith et al. 1999; Coltman et al. 2001a), genetic variation associated with nematode resistance at the MHC (Paterson et al. 1998) and in the region of the γ-interferon gene (Coltman et al. 2001b) and potential QTL for coccidia resistance (Beraldi et al. 2007). This maintained genetic variation in parasite resistance could be explained if balancing selection for both extracellular (helminths and arthropods) and intracellular (e.g. protozoan) parasites was in operation.

This study represents the first attempt to investigate the potential role of co-infection as a selective force within the Soay sheep population. First, we provide a basic description of associations between helmhnt, protozoa and arthropod species across hosts. Second, we investigate whether the intensities of different parasite taxa have independent
associations with host weight, which is itself a predictor of over-winter survival and reproductive success in the study population (Clutton-Brock et al. 1992, 1997; Illius et al. 1995; Forchhammer et al. 2001).


to the counts in adults. The epidemiology of the 11 species of *Eimeria* in the population across 3 representative years of varying host density is detailed by Craig et al. (2007). Generally, intensity of all species tends to decrease with host age and varies with host population density.

**Parasitology**

Annual parasitological sampling and weighing takes place each August at a catch-up of individually-marked sheep that live in Village Bay. At the time of sampling, lambs and yearlings were 4 and 16 months old, respectively, and adults were 28 months and older. Assessment of *M. ovinus* (ked) infestation was made by a 1-min search for live keds in the sheep underbelly (area of white wool in wild type sheep). Diagnosis of endoparasite species infection was by coprological examination. Helminth eggs and coccidia (comprising a mixture of 11 *Eimeria* spp.; Craig et al. 2007) were quantified per gram of faeces using a modification of the McMaster technique (M.A.F.F., 1971). Helminth egg counts distinguished strongyle type egg (encompassing *Teladorsagia* spp., *Trichostrongylus axei*, *Trichostrongylus vitrinus*, *Bunostomum trigonocephalum* and *Chabertia ovina*), *Nematodirus* spp. (encompassing *N. battus* and *N. filicollis*), *Capillaria longipes* and *Trichuris ovis* and gave presence or absence of *Moniezia expansa*.

**Statistical analysis**

Separate analyses were conducted on lambs \(n=778\); one observation per individual), yearlings \(n=417\); one observation per individual) and adults \(2\) years and older; \(n=1332\) observations on 561 individuals). Separate analyses were conducted within each age class because previous research indicated substantial differences in the predominant helminth species present at different host ages (Craig et al. 2006; see Discussion) and because different non-parasite covariates are relevant for August weight in different age classes; for example birth date, weight and litter size are important determinants of August body weight in lambs but not in older age classes.

(i) **Prevalence and intensity.** For each age class, the prevalence (%) and mean count for each parasite taxa was calculated for the period 1993–2005 in order to illustrate the variation in prevalence and intensity of the different taxa.

(ii) **Testing for associations between parasite taxa.** Our aim was to provide a basic description of the data rather than a detailed study of the possible ecological interactions between species. In common with much parasitological data, the distributions of intensity estimates for most species were not normal, so we used a non-parametric approach. Associations in the ranked intensity between species within each host age class because previous research indicated substantial differences in the predominant helminth species present at different host ages (Craig et al. 2006; see Discussion) and because different non-parasite covariates are relevant for August weight in different age classes; for example birth date, weight and litter size are important determinants of August body weight in lambs but not in older age classes.

(iii) **Testing for associations between parasite taxa and August body weight in lambs.** A general linear model
Table 1. Overall prevalence (%) and mean count (±95% C.I.) of helminth and coccidia eggs/oocysts
detected per gram of faeces and of keds detected from the underbelly of the sheep across all hosts and years
(1993–2005) by age

<table>
<thead>
<tr>
<th></th>
<th>Lambs n = 778</th>
<th>Yearlings n = 417</th>
<th>Adults n = 1332</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevalence (%)</td>
<td>Mean count ±95% C.I.</td>
<td>Prevalence (%)</td>
</tr>
<tr>
<td>Strongyles</td>
<td>95</td>
<td>719 ± 50</td>
<td>86</td>
</tr>
<tr>
<td>Coccidia</td>
<td>99</td>
<td>5788 ± 678</td>
<td>96</td>
</tr>
<tr>
<td>Keds</td>
<td>82</td>
<td>3·36 ± 0·24</td>
<td>28</td>
</tr>
<tr>
<td>Nematodirus</td>
<td>32</td>
<td>86·82 ± 11·52</td>
<td>0·5</td>
</tr>
<tr>
<td>Moniezia</td>
<td>15</td>
<td>NA</td>
<td>13</td>
</tr>
<tr>
<td>Trichuris</td>
<td>2</td>
<td>3·21 ± 1·99</td>
<td>0·7</td>
</tr>
<tr>
<td>Capillaria</td>
<td>0·4</td>
<td>0·38 ± 0·43</td>
<td>3</td>
</tr>
</tbody>
</table>

Results

Parasite taxa prevalence and intensity

The parasite taxa with the highest prevalence and mean intensity in all age classes were strongyle eggs and
coccidia oocysts (Table 1). Strongyles, coccidia, keds, Nematodirus and Moniezia had highest prevalence
in lambs and decreased with age in terms of both prevalence and intensity. Trichuris and Capillaria had
negligible prevalence and intensity in all age classes (Table 1).

Associations between parasite taxa

Spearman’s rank correlation tests were applied for all possible pairings of parasite taxa in each age class
(Table 2). Only 3 associations were significant after Bonferroni correction. In lambs, there was a positive
correlation between strongyle eggs and Nematodirus eggs ($r_s = 0·25, n = 778, P < 0·0001$). In yearlings there
was a positive correlation between strongyle eggs and coccidia oocysts ($r_s = 0·18, n = 417, P < 0·001$). This
positive association between strongyle eggs and coccidia oocysts was also found in adult hosts
($r_s = 0·299, n = 1332, P < 0·0001$). These 3 associations were not driven by the presence of hosts with
decreasing with host age), sex, coat type, horn type (fitted as factors), strongyle egg count, presence/absence
of Moniezia (as a factor), coccidia oocyst count and ked count were fitted as explanatory variables (there were not enough adult hosts infected with Nematodirus spp., Capillaria or Trichuris to justify the inclusion of these taxa). All two-way interactions between potential explanatory variables were also tested for. Body weight has a normal error structure and the model was checked for non-normality of errors by assessment of the appropriate diagnostic plots.

All statistical analyses were conducted in S-plus® professional edition version 6.2.1 for Microsoft windows (Insightful Corp.).
neither parasite. When double negative hosts were removed, the correlation between strongyle and Nematodirus eggs in lambs survived \( r_s = 0.22, n = 778, P < 0.0001 \), as did the correlation between strongyle eggs and coccidia oocysts in yearlings \( r_s = 0.16, n = 413, P < 0.001 \) and between strongyle eggs and coccidia oocysts in adults \( r_s = 0.131, n = 1151, P < 0.0001 \).

**Table 3.** Results of a GLM of August body weight for lambs

(Of the parasite taxa tested, only strongyle egg count had a significant and negative association with host weight. Rejected terms were coat type, Moniezia, Keds, Coccidia, Nematodirus, Trichuris and Capillaria. Sample size was 778, no two-way interactions were significant, the final model had a normal error structure and explained 64\%–12\% of the variance.)

<table>
<thead>
<tr>
<th>Terms</th>
<th>D.F.</th>
<th>F</th>
<th>P-value</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth year</td>
<td>11,760</td>
<td>11.74</td>
<td>&lt;0.0001</td>
<td>Varies between years</td>
</tr>
<tr>
<td>Sex</td>
<td>1,760</td>
<td>119.15</td>
<td>&lt;0.0001</td>
<td>Males weigh more</td>
</tr>
<tr>
<td>Birth weight</td>
<td>1,760</td>
<td>478.35</td>
<td>&lt;0.0001</td>
<td>Heavier born weigh more</td>
</tr>
<tr>
<td>Birth date</td>
<td>1,760</td>
<td>38.40</td>
<td>&lt;0.0001</td>
<td>Later born weigh less</td>
</tr>
<tr>
<td>Litter size</td>
<td>1,760</td>
<td>32.35</td>
<td>&lt;0.0001</td>
<td>Twins weigh less</td>
</tr>
<tr>
<td>Capture day</td>
<td>1,760</td>
<td>45.10</td>
<td>&lt;0.0001</td>
<td>Later caught weigh more</td>
</tr>
<tr>
<td>Strongyles</td>
<td>1,760</td>
<td>37.70</td>
<td>&lt;0.0001</td>
<td>Hosts with higher egg counts weigh less</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Until recently the Soay sheep host-parasite system had been considered to be a relatively simple one. However, we now know that the situation is complex.
with no one nematode species achieving a monopoly throughout the lifetime of the host (Craig et al. 2006) and with a significant component of the parasite community comprised of a diverse array of protozoa (Craig et al. 2007). The potential role of metazoan-protozoan parasite co-infection as a selective force in the population has never been investigated. This study, therefore, attempted to initiate a debate on whether one parasite measure can capture the force of parasitism in the Soay sheep population simply because different species tend to be positively correlated or whether different taxa can exert independent effects on host weight and by inference, fitness.

**Associations between metazoan and protozoan parasites**

According to Lotz and Font (1991) an over-riding observation in studies of parasite infra-community structure and assembly is that generally the number of positive associations between species far out-numbers the negative associations. In this study there were several positive correlations of which 3 survived Bonferroni correction and re-analysis after dropping double negative cases. These comprise a correlation in ranked intensity between strongyle and *Nematodirus* eggs in lambs and a correlation between ranked strongyle eggs and coccidian oocysts in yearlings and adults. Although positive associations between parasite taxa may reflect synergistic effects of one taxon on another (Christensen et al. 1987) they may simply be a reflection of a similar response to host condition or co-variation in intake in parasites.

**Associations between parasite taxa and host weight**

The weight of Soay sheep in August has been shown to be important in virtually all analyses of their potential over-winter survival and reproductive success (Clutton-Brock et al. 1992, 1997; Illius et al. 1995; Forchhammer et al. 2001). In lambs and yearlings, of the various parasite taxa, only strongyle egg count explained variation in August weight. At this stage we can only speculate on the main nematode species contributing to egg count in young Soay sheep in August but from previous post-mortem studies performed on sheep in the winter months, high numbers of *Trichostrongylus axei* and *Trichostrongylus vitrinus* species (as occurs in Soay lambs and yearlings) cause...
even more severe pathology (Steel et al. 1982; Sykes et al. 1988). As sheep with higher August weight are more likely to survive over winter and strongyle egg count is negatively associated with over-winter survival in the population (Illius et al. 1995; Wilson et al. 2004) it seems possible that a synergistic effect of Trichostrongylus and Teladorsagia species infection could be exerting selection on Soay lambs and yearlings.

Table 5. Results of a GLMM of August body weight in adults
(Of the parasite taxa tested, strongyle egg count and coccidian oocyst count had independent, negative associations with host weight. Rejected terms were coat type, horn type, Moniezia, Keds and Nematodirus. Sample size was 1332 observations on 561 individuals, no two-way interactions were significant, the final model had a normal error structure and explained 62.2% of the variance.)

<table>
<thead>
<tr>
<th>Terms</th>
<th>D.F.</th>
<th>F</th>
<th>P-value</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth year</td>
<td>24, 535</td>
<td>4.55</td>
<td>&lt;0.0001</td>
<td>Varies between years</td>
</tr>
<tr>
<td>Age</td>
<td>1, 767</td>
<td>193.83</td>
<td>&lt;0.0001</td>
<td>Increases</td>
</tr>
<tr>
<td>Age²</td>
<td>1, 767</td>
<td>155.06</td>
<td>&lt;0.0001</td>
<td>Increases up to age 5–6, then declines</td>
</tr>
<tr>
<td>Sex</td>
<td>1, 535</td>
<td>116.34</td>
<td>&lt;0.0001</td>
<td>Males weigh more</td>
</tr>
<tr>
<td>Strongyles</td>
<td>1, 767</td>
<td>28.09</td>
<td>&lt;0.0001</td>
<td>Hosts with higher egg counts weigh less</td>
</tr>
<tr>
<td>Coccidia</td>
<td>1, 767</td>
<td>9.69</td>
<td>0.0019</td>
<td>Hosts with higher oocyst counts weigh less</td>
</tr>
</tbody>
</table>

In adults it was found that both strongyle eggs and coccidian oocysts were independently negatively associated with host weight measured in August. Again, we can only speculate on the likely species involved. From winter post-mortem worm counts it is known that with increasing age, the once predominant Trichostrongylus genus becomes trivial in terms of intensity and Teladorsagia circumcincta monopolizes, therefore probably contributing almost all the eggs to the strongyle egg counts (Craig et al. 2006). In terms of species of Eimeria, an epidemiological study has shown that the only species to increase with host age in the sheep is Eimeria granulosa which was found to significantly co-occur with E. weybridgensis and E. crandallis (Craig et al. 2007). It can therefore be inferred that Teladorsagia circumcincta, parasitizing the abomasum, and Eimeria spp. (probably a combination of E. granulosa, E. weybridgensis and E. crandallis), parasitizing the small intestine, have the potential to exert selection on older Soay sheep independently of each other.

The observation that Eimeria spp. intensity is associated with depressed body weight in adult Soay sheep is of interest for two reasons. Firstly, we know of several studies that have demonstrated a deleterious effect of Eimeria spp. infection on host weight in domestic lambs (e.g. Chapman, 1974; Pout and Catchpole, 1974; Gauly et al. 2004; Platzer et al. 2005; Jorg Reeg et al. 2005) but the present study is the first we are aware of to suggest that there may be a detrimental effect on body weight in adult sheep. Secondly, from an evolutionary perspective, metazoan-protozoan co-infections have the potential to exert complex demands on the host immune system which should evolve an optimal response (Graham, 2001). Regulated by the activity of cytokines, metazoan (extracellular) and protozoan (intracellular) parasites elicit a broadly dichotomous immune response (Graham, 2002). It is generally observed that helminth parasites provoke a Th2 response predominantly activated by the cytokine IL-4 which simultaneously inhibits Th1 responses.
Intracellular protozoa, such as *Eimeria*, are targeted by a Th1 response, activated by the cytokine interferon gamma, which in turn inhibits Th2 responses (Lydyard et al. 2001). As a consequence of the Th1 and Th2 dichotomy it is possible that in the Soay sheep on St Kilda there exists an immunological trade-off between resistance to metazoan and susceptibility to protozoan parasites which might explain why advantageous alleles conferring resistance to nematodes do not rise to fixation in the population as would be expected.

If a genetic trade-off does exist, we might have anticipated a negative phenotypic correlation between the strongyle and coccidia parasites instead of the positive correlation actually observed in yearlings and adults. There are several possible explanations for this observation. Apart from simply reflecting general host condition (van Noordwijk and de Jong, 1986) or parasite facilitation, there may also be more specific reasons for positive correlation between the two taxa. Firstly, there may be density-dependent effects in which intense infections of either *Teladorsagia circumcincta* or *Eimeria* spp. may cause fewer eggs or oocysts to be produced (Brackett and Bliznick, 1952; Williams, 1973; Bishop and Stear, 2000). Secondly, the mixed coccidia count, which is known to generally decrease with host age, may be masking an increase in intensity of an individual *Eimeria* species with a detrimental effect on host condition (see Craig et al. 2007). If this is the case then a balancing selection for resistance to the *Eimeria* spp. and *Teladorsagia circumcincta* could be in process (Craig et al. 2007).

**Conclusion**

Taken together, these results suggest a role for multiple parasite species in shaping the fitness and evolutionary trajectory of Soay sheep. In young Soay sheep, there is some evidence that the worm genus *Trichostrongylus* is a significant pathogen and that there may be synergistic effects of an emerging *Teladorsagia* infection with age, and these species are likely to be responsible for the detrimental association with body weight. In older sheep, strongyle eggs are likely to be produced predominantly by *Teladorsagia circumcincta*, and both this species and coccidia had independent negative associations with host weight. Since August weight is associated with the probability of over-winter survival, these results imply that at least 2 genera of parasite are likely to be exerting selection in the population, and for the first time suggest that intracellular (protozoa) as well as extracellular (strongyle) parasites are involved.

This work has, therefore, highlighted the need for further research in 2 main areas. Firstly there is a need to determine which nematode species are contributing to egg counts in the different age classes, not only in August, but throughout the year. Temporal variation in the abundance of the different parasite taxa is likely and strongyle and coccidia infections at other time-points in the year may be more important. Following this, there is a need to explore whether there are genetic trade-offs in resistance and susceptibility to infection with *Trichostrongylus* and *Teladorsagia* spp. and for infection with *Teladorsagia* and *Eimeria* spp. This would require further development of a molecular method developed by Wimmer et al. (2004) to provide quantitative species-specific data for strongyle eggs recovered from host faeces. If similar quantitative molecular methods could be developed to monitor protozoan species oocyst output, then this information, coupled with host pedigree information, could be used to estimate heritability for, and genetic correlations between, resistance to specific taxa, allowing hypotheses regarding associations with host genetic variation to be tested, including the possibility of balancing selection.

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