# Epidemiology of parasitic protozoan infections in Soay sheep (*Ovis aries* L.) on St Kilda

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SUMMARY

The feral Soay sheep (*Ovis aries* L.) population on Hirta, St Kilda, is host to a diverse component parasite community, but previous parasitological studies of the population have only focussed on the metazoan species. This paper reports the first epidemiological study of the protozoan species comprising *Cryptosporidium parvum*, *Giardia duodenalis* and 11 species of *Eimeria* in Soay sheep across 3 years of varying host population density. Prevalence and intensity of almost all species of protozoa significantly decreased with host age, with the exception of *E. granulosa*, which increased in prevalence with host age. The prevalence of *C. parvum* appeared to vary positively with host population density but that of *G. duodenalis* did not vary significantly with density. Most species of *Eimeria* showed a distinct lag in infection level following the host population crash of 2002, taking up to 2 years to decrease. Mixed *Eimeria* species intensity and diversity were highest in 2002, a year of low host density. Parasite diversity decreased with host age and was higher in males. There were 5 positive pair-wise associations between protozoa species in terms of prevalence. The results of this study highlight the potential for protozoal infection to shape the evolution of parasite resistance in wild host populations harbouring diverse parasite species.

Key words: Soay sheep, St Kilda, *Cryptosporidium parvum*, *Giardia duodenalis*, *Eimeria* spp.

## INTRODUCTION

Enteric protozoan parasites are ubiquitous in domestic mammal populations (Taylor, 2000). Many species have intracellular life-cycle phases in the intestinal epithelia and have the potential to cause disease. Their widespread occurrence, economic importance coupled with limited options for treatment (Haberkorn, 1996) and sometimes zoonotic potential have meant most investigations of protozoan infections in ruminants have come from outbreaks on farms or from experiments involving production animals (e.g. Catchpole *et al.* 1976; Berriatua *et al.* 1994; Xiao, 1994; De Graaf *et al.* 1999).

Few parallel epidemiological studies have monitored intracellular protozoan infections in wild ungulate populations, where they often present as chronic infections which intensify or abate in response to environmental conditions and host susceptibility (Duszynski and Upton, 2001) and more commonly co-occur with extracellular helminth parasites in the absence of widespread anthelmintic treatment. Studying co-infections in the wild is of interest for two main reasons. First, there is evidence for interactions between parasite species in mammals (Christensen *et al.* 1987; Cox, 2001; Poulin, 2001),

highlighting the importance of understanding these relationships before devising control strategies against specific species in livestock systems (Lello *et al.* 2004). Secondly, from an evolutionary perspective, there is the possibility of balancing selection for host resistance to co-infecting protozoa and helminths in natural populations since intracellular and extracellular parasite infections are controlled via a compromise between the mutually inhibitory Th1 and Th2 immune responses (Graham, 2002).

The feral Soay sheep population of Hirta, St Kilda, provides a unique opportunity to investigate the ecological and evolutionary consequences of co-infection. Monitored intensively since 1985, it is host to a diverse array of metazoan and protozoan parasites (Wilson *et al.* 2004). Every few years a high proportion of the population dies due to food shortage. Previous research has shown that in these high-density years, although the proximate cause of death is malnutrition, high helminth burdens also contribute to host mortality (Gulland, 1992; Illius *et al.* 1995; Coltman *et al.* 1999). Furthermore, nematode resistance is a heritable trait in the population (Smith, 1999; Coltman *et al.* 2001) with evidence of advantageous alleles at specific loci such as the MHC (Paterson *et al.* 1998) and $\gamma$-interferon (Coltman *et al.* 2001). Considering the apparently strong selection that helminth parasites exert on the Soay sheep it is unclear why beneficial alleles do not rise to fixation in the population and why genetic variation is

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Table 1. *Total Soay sheep population size recorded on the island of Hirta and sample size ranges used from August of each sampling year*

|  | Host sex | 2001 (High host density) | 2002 (Low host density) | 2003 (Intermediate host density) |
|---|---|---|---|---|
| Island population |  | 1889 | 907 | 1568 |
| Total lambs |  | 687 | 284 | 615 |
| Total adults |  | 1202 | 623 | 953 |
| Sample size range | Host sex |  |  |  |
| Lambs | ♂ | 27–39 | 28–36 | 32–38 |
|  | ♀ | 29–44 | 22–28 | 38–55 |
| Yearlings | ♂ | 21–28 | * | 20–27 |
|  | ♀ | 17–27 | * | 15–20 |
| Two-year-olds | ♂ | 11–20 | 8–9 | * |
|  | ♀ | 11–20 | 17–27 | * |
| Adults | ♂ | 1–6 | 6–7 | 8–9 |
|  | ♀ | 27–61 | 61–92 | 51–70 |

* No data were available from the 2001 cohort as yearlings or 2-year-olds as they virtually all died in their first winter.

maintained. One possibility is that parasite diversity both within parasite groups such as helminths and protozoa, and between groups such as parasites with extracellular versus intracellular life-stages, may explain why host genetic diversity is maintained.

As a step towards testing this hypothesis we report the first epidemiological survey of gastrointestinal protozoan species in the population. Thirteen species of protozoa, also known from domestic sheep (M.A.F.F., 1986), were identified. In this paper, we present point prevalence and/or intensity and diversity of infections in sheep caught in summer, document epidemiology in relation to host age, host sex and year of sample, where years varied in host density, and report an investigation of associations between species.

MATERIALS AND METHODS

*Population dynamics and sample collection*

The feral Soay sheep population of Hirta, St Kilda experiences persistent fluctuations in density regulated primarily by food availability. The population dynamics and study area are described in detail elsewhere (Clutton-Brock and Pemberton, 2004; Craig *et al.* 2006). Parasitological sampling reported in this paper took place in 3 consecutive years of varying host population density. Between the Augusts of 2001 and 2002, the Hirta population of sheep decreased by 52%. Between the Augusts of 2002 and 2003 the population increased by 74% (Table 1). The population crash early in 2002 demonstrated that the August 2001 population was above carrying capacity for the following winter. Despite the dramatic increase in population size that took place in 2003, sheep numbers did not exceed the carrying capacity as the population did not decrease early in 2004. Therefore, the 3 years can be

consecutively termed high, low and intermediate population density years.

Results derived for this paper originate from representative and random subsets of sheep caught for sampling in the Augusts of 2001–2003. At the time of sampling, lambs, yearlings, 2-year-olds and adults were 4, 16, 28 and $\geqslant 40$ months old respectively. The 2001 cohort suffered 95% mortality in the population crash of 2002 and so no yearling or 2-year-old sheep were available for sampling in August 2002 and 2003 respectively. Sample sizes of hosts examined for each protozoan genus varied slightly due to the different diagnostic tests used and are given in Table 1.

*Parasitology*

Diagnosis of parasite species infection was by coprological examination. Helminth eggs and *Eimeria* oocysts were quantified per gram of faeces using a modification of the McMaster technique (M.A.F.F., 1971). A further 3 g wet weight of faecal sample was homogenized in 42 ml of distilled water and filtered though a tea-strainer. The filtrate was then used to fill two 15 ml test tubes that were centrifuged at around 800 *g* for 10 min. The resulting pellets were retained; one was used to make a smear slide which was stained using a modification of the Ziehl-Neelsen technique (J. McGoldrick, personal communication) to test for the presence or absence of *Cryptosporidium parvum*. The other pellet was incubated in 2·5% potassium dichromate solution at room temperature for 2 weeks for sporulation of *Eimeria* oocysts (Duszynski and Wilber, 1997) and then stored at 4 °C until analysed. Following removal of the potassium dichromate solution the pellet was mixed with 33% zinc sulphate solution (specific gravity = 1·25), subjected to centrifugal flotation onto a coverslip and examined under oil at 1000×
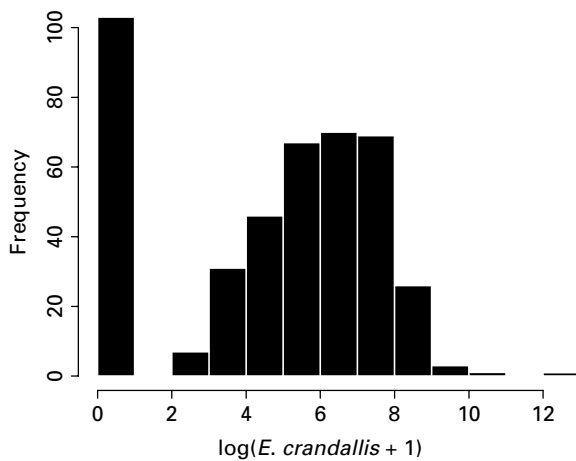
Fig. 1. Frequency distribution of log transformed *Eimeria crandallis* count (+1) 2001–2003.

magnification to identify *Eimeria* species and check for the presence or absence of *Giardia duodenalis* cysts. Up to 100 *Eimeria* oocysts on each coverslip were identified to species level based on morphological traits (Levine, 1973; M.A.F.F., 1986; Soulsby, 1986). An estimate of intensity of each *Eimeria* species was achieved by relating their proportions to the McMaster counts. No intensity data were collected for *C. parvum* or *G. duodenalis* as the tests were only qualitative.

*Statistical analysis*

The epidemiology of each protozoan species, and of total mixed *Eimeria* species, in terms of associations between their prevalence and/or intensity and host age, sex and year was investigated. Prevalence of each species was analysed using a generalized linear model (GLM) with binomial error structure and logit link function on binary data (zero versus non-zero counts) testing for associations between presence/absence of infection and the possible explanatory variables of age, sex and year. The *Eimeria* species counts were analysed first by considering the total count of all species, and secondly by considering individual species separately. The abundance of total mixed *Eimeria* species was aggregated in the host population ($k = 0.12$) in all years, and was best described by a negative binomial distribution and analysed using a GLM with negative binomial error structure and log link function. However, some of the individual *Eimeria* species showed distributions that were more awkwardly distributed and hence failed to conform to a negative binomial distribution.

In these cases, the highest count of zero was not followed by a second highest count of 1, and so on, as the negative binomial distribution assumes. By way of example, Fig. 1 shows the distribution of *E. crandallis*; similar distributions were observed for all the other *Eimeria* species. Therefore, intensity of these species was modelled using a linear model

(LM) on log-transformed count data, excluding the zero counts. All models specified sex (D.F. = 1) and year (D.F. = 2) as categorical variables and age as a continuous variable (D.F. = 1) and these main effects were tested along with all possible interactions. Where year was found to explain significant variation in prevalence or intensity, model coefficients were used to interpret patterns.

Two analyses were used to investigate different aspects of protozoan diversity. First, diversity of *Eimeria* species was measured across all years using the Shannon-Weiner index, a heterogeneity measure that combines species richness and evenness components (Magurran, 2004). Indices were tested for associations with age, sex and year using a linear model with normal error structure. Second, we investigated whether there were systematic relationships between protozoan species that might indicate competition or mutualism between species. Associations between prevalence of protozoan species were tested for using a GLM with a binomial error structure and logit link function with the presence/absence of each species (in turn) as the response variable and the possible explanatory variables of age, sex, year and presence/absence of all other species.

Statistical significance in the GLMs was assessed by the change in deviance associated with a term, distributed as $\chi^2$ on the respective degrees of freedom, and using F-statistics for models with normal errors. Non-significant terms were dropped from a full model containing all possible main effects and interactions to give a final model containing only significant terms. In order to limit the chances of making type 2 errors in the analyses presented in this paper, a sequential Bonferroni correction (Rice, 1989) was applied to the 39 statistical tests yielding a threshold value of $P \leqslant 0.001$. All statistical analyses were conducted in S-Plus® professional edition version 6.2.1 for Microsoft windows (Insightful Corp.). In the figures, for illustration purposes, age is presented as up to 4 categories: lambs (4 months), yearlings (16 months), 2-year-olds (28 months) and adults ($\geqslant$40 months).

RESULTS

*Prevalence of* Cryptosporidium parvum

The prevalence of *C. parvum* in lambs and adults and in both sexes by year is illustrated in Fig. 2 with overall herd prevalence given in Table 4. In the GLM of *C. parvum* prevalence there was no main association with host age or sex, but there was significant variation among years ($\chi^2_2 = 28.94$, $P < 0.001$) and an interaction between host age and year ($\chi^2_2 = 14.27$, $P < 0.001$; final model explained $7.27\%$ of the deviance). The year of highest host density, 2001, was also the year of highest prevalence (Table 4) and
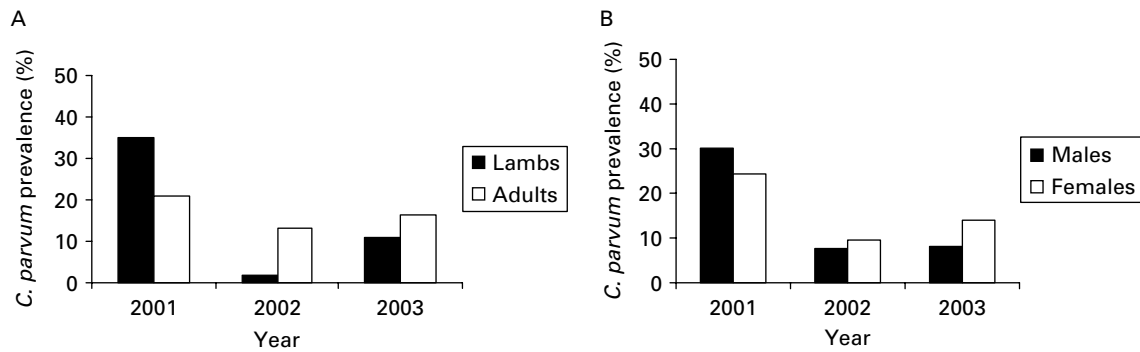
Fig. 2. Prevalence of *Cryptosporidium parvum* by (A) host age and (B) host sex in each year of study. Only prevalence in lambs and adults are shown here but statistical analyses were done on the full range of ages (see text).
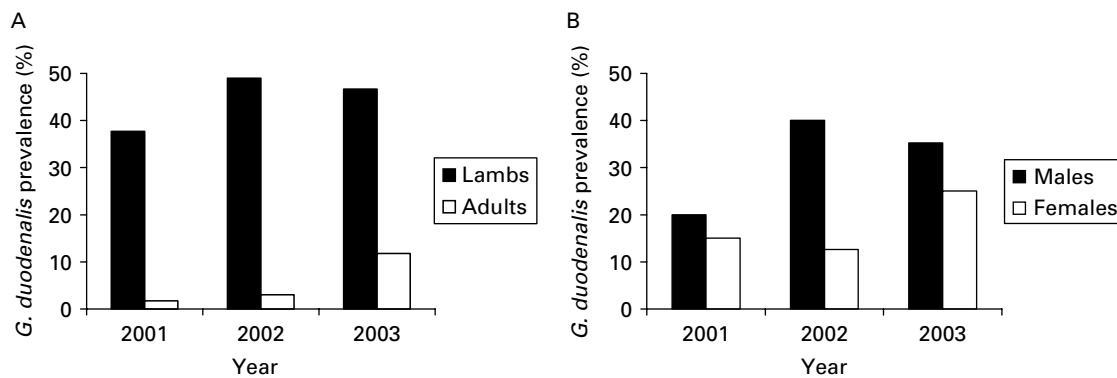


Fig. 3. Prevalence of *Giardia duodenalis* spp. by (A) host age and (B) host sex in each year of study. Only prevalence in lambs and adults are shown here but statistical analyses were done on the full range of ages (see text).

lambs that year had higher prevalence than any other age class in any of the 3 years (Fig. 2A).

### Prevalence of *Giardia duodenalis*

The prevalence of *G. duodenalis* in lambs and adults and in each sex by year is illustrated in Fig. 3 with overall herd prevalence given in Table 4. In a GLM of *G. duodenalis* prevalence there was a significant association with host age, with young animals, especially lambs, having higher prevalence than older age classes ($\chi^2_1 = 94 \cdot 67$, $P < 0 \cdot 001$; final model explained $17 \cdot 55 \%$ of the deviance) but there was no association with host sex, year or any interactions.

### Prevalence, intensity and diversity of *Eimeria* spp.

*Eimeria* was the most speciose genus of protozoan in the Soay sheep, with 11 species identified. Overall herd prevalence and abundance values are given in Table 4. The prevalence of each species by year in lambs and adults is shown in Fig. 4 and significant associations are shown in Table 2. There was a strong negative association between host age and prevalence of all the *Eimeria* species, except *E. granulosa* for which prevalence increased with host age. Four species, namely *E. ahsata*, *E. bakuensis*, *E. marsica* and *E. parva* were significantly more prevalent in

males than females; other species showed no difference between host sexes.

All except 3 species (*E. crandallis*, *E. pallida* and *E. weybridgensis*) showed significant prevalence variation among years, but different species showed different patterns (Fig. 4). One group, comprising *E. ahsata*, *E. bakuensis*, *E. faurei* and *E. intricata*, increased in prevalence from 2001 to 2002 before decreasing in 2003. *E. marsica* varied positively with host population density (declining from 2001 to 2002 then increasing from 2002 to 2003). *E. parva* prevalence increased with each successive year of sample while *E. ovinoidalis* and *E. granulosa* decreased with each sample year. Finally, the prevalence of 2 species, *E. marsica* and *E. parva*, was associated with interaction terms, in both cases indicating an interaction between host age and year in which lambs had highest prevalence in 2003 (Table 2).

The overall intensity of total mixed *Eimeria* species decreased with age and reached a maximum in 2002. There was also an age-year interaction so that the highest counts were in lambs sampled in 2002 (Fig. 5 and Table 3), Intensities of the individual *Eimeria* species (Fig. 6) showed similarities with the associations found for prevalence, although for individual species there were fewer significant associations and some differences were also found.
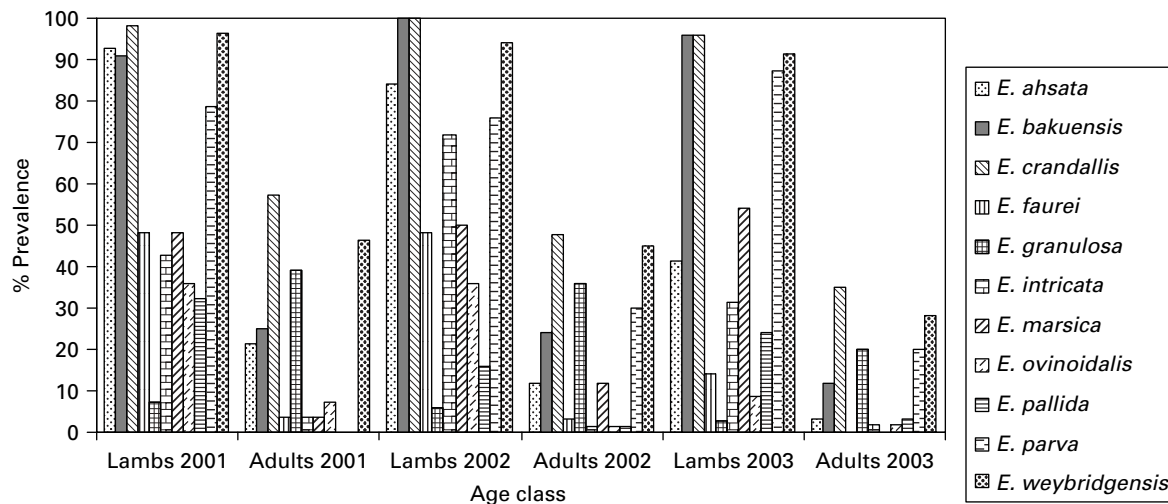
Fig. 4. Prevalence of each species of *Eimeria* by host age in each year of study. Only prevalence in lambs and adults are shown here but statistical analyses were done on the full range of ages (see text).

In individual species intensity tests, all except 2 (*E. granulosa* and *E. ovinoidalis*) showed a significant association with host age, in which intensity declined with host age. There were no main order associations between intensity and host sex. Three species showed associations between intensity and year of sample of which *E. ahsata* decreased in each year of sample, while *E. crandallis* and *E. weybridgensis* increased from 2001 to 2002 before decreasing from 2002 to 2003. Interaction terms explained further variation in the intensity of two species: in *E. ahsata* there was a host age-year of sample interaction in which juveniles in 2001 had the highest intensity; in *E. bakuensis* there was a host age-host sex interaction in which juvenile females had the highest intensity and a host age-year of sample interaction in which juveniles in 2002 had the highest intensity.

Diversity of *Eimeria* species was measured for each host using the Shannon-Weiner diversity index and illustrated in Fig. 7. Diversity significantly decreased with age ($F_{1,384} = 153 \cdot 99$, $P < 0 \cdot 001$) was higher in males than females ($F_{1,384} = 40 \cdot 26$, $P < 0 \cdot 001$) and showed significant differences between years ($F_{2,384} = 7 \cdot 77$, $P < 0 \cdot 001$; final model explained $35 \cdot 33\%$ of the variance).

*Associations between protozoan species prevalence 2001–2003*

Some *Eimeria* species consistently co-occurred. Taking into account the effects of host age, sex, year, and the multiple tests performed (i.e. a threshold of $P \leqslant 0 \cdot 001$), 5 positive associations were found across the 3 years. These were between (i) *G. duodenalis* and *E. crandallis*; (ii) *E. ahsata* and *E. crandallis*; (iii) *E. ahsata* and *E. bakuensis*; (iv) *E. crandallis* and *E. weybridgensis* and (v) *E. granulosa* and *E. weybridgensis*. No negative associations were found.

DISCUSSION

Elucidating the diversity of parasite species in a host population is a prerequisite to understanding how parasites and their hosts co-exist and how the relationship can ultimately steer the direction of natural selection. Prior to this research, the greatest gap in our knowledge of the St Kilda Soay sheep parasites was among the protozoa. The discovery of 13 species, most of which have intracellular phases in their life-cycles and which co-exist with a diverse array of helminth parasites in the intestines of the sheep (Wilson *et al.* 2004; Craig *et al.* 2006), highlights the potential for them to be exerting additional and conflicting demands on the host immune system. Examining their epidemiology across 3 years of varying host population density has revealed several points for discussion.

*Associations with host age and sex*

In Soay sheep, the prevalence as well as intensity and diversity (where measured) of *C. parvum*, *G. duodenalis* and mixed *Eimeria* spp. declined with host age. Prevalence of *C. parvum* infection decreased with host age in 2001 and this has been found in other unmanaged mammal populations such as feral pigs in Western California (Atwill *et al.* 1997) and rodents in Poland (Bajer *et al.* 2002) and also in numerous livestock systems (Olson *et al.* 1997; Majewska *et al.* 2000; Huetink *et al.* 2001). Due to the highly immunogenic nature of *C. parvum*, acute primary infection results in a good level of resistance to any subsequent challenge (Okhuysen *et al.* 1998; Chappell *et al.* 1999). Although acquired resistance is likely to contribute to the negative association of infection with host age, Ortega-Mora and Wright (1994) demonstrated an independent age-related resistance to infection in which a significant extension of the pre-patent period and decrease in intensity

Table 2. Summary of significant results of GLM analyses, with binomial error structure, testing for associations between age, sex and year and prevalence of individual *Eimeria* species

(Dashes denote associations were not significant.)

| | Age | | | | Sex | | | | Year | | | | Interactions | | | | % deviance explained by final model |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\chi^2$ | D.F. | $P$ | Association | $\chi^2$ | D.F. | $P$ | Association | $\chi^2$ | D.F. | $P$ | Association | $\chi^2$ | D.F. | $P$ | Association | |
| *E. ahsata* | 94·24 | 1 | <0·001 | Decreasing | 16·79 | 1 | <0·001 | Higher in males | 45·79 | 2 | <0·001 | Lowest in 2003 | — | — | — | — | 27·50 |
| *E. bakuensis* | 154·72 | 1 | <0·001 | Decreasing | 17·71 | 1 | <0·001 | Higher in males | 23·40 | 2 | <0·001 | Highest in 2002 | — | — | — | — | 33·74 |
| *E. crandallis* | 93·87 | 1 | <0·001 | Decreasing | — | — | — | — | — | — | — | — | — | — | — | — | 22·08 |
| *E. faurei* | 60·98 | 1 | <0·001 | Decreasing | — | — | — | — | 31·49 | 2 | <0·001 | Lowest in 2003 | — | — | — | — | 24·00 |
| *E. granulosa* | 42·56 | 1 | <0·001 | Increasing | — | — | — | — | 17·14 | 2 | <0·001 | Lowest in 2003 | — | — | — | — | 13·3 |
| *E. intricata* | 97·59 | 1 | <0·001 | Decreasing | — | — | — | — | 29·80 | 2 | <0·001 | Highest in 2002 | — | — | — | — | 28·24 |
| *E. marsica* | 68·79 | 1 | <0·001 | Decreasing | 14·57 | 1 | <0·001 | Higher in males | — | — | — | — | 14·71 | 2 | <0·001 | Highest in juveniles in 2003 | 19·87 |
| *E. ovinoidalis* | 34·79 | 1 | <0·001 | Decreasing | — | — | — | — | 21·76 | 2 | <0·001 | Lowest in 2003 | — | — | — | — | 19·79 |
| *E. pallida* | 30·75 | 1 | <0·001 | Decreasing | — | — | — | — | — | — | — | — | — | — | — | — | 9·5 |
| *E. parva* | 101·47 | 1 | <0·001 | Decreasing | 15·04 | 1 | <0·001 | Higher in males | 27·21 | 2 | <0·001 | Highest in 2003 | 15·02 | 2 | <0·001 | Highest in juveniles in 2003 | 27·08 |
| *E. weybridgensis* | 87·75 | 1 | <0·001 | Decreasing | — | — | — | — | — | — | — | — | — | — | — | — | 18·69 |
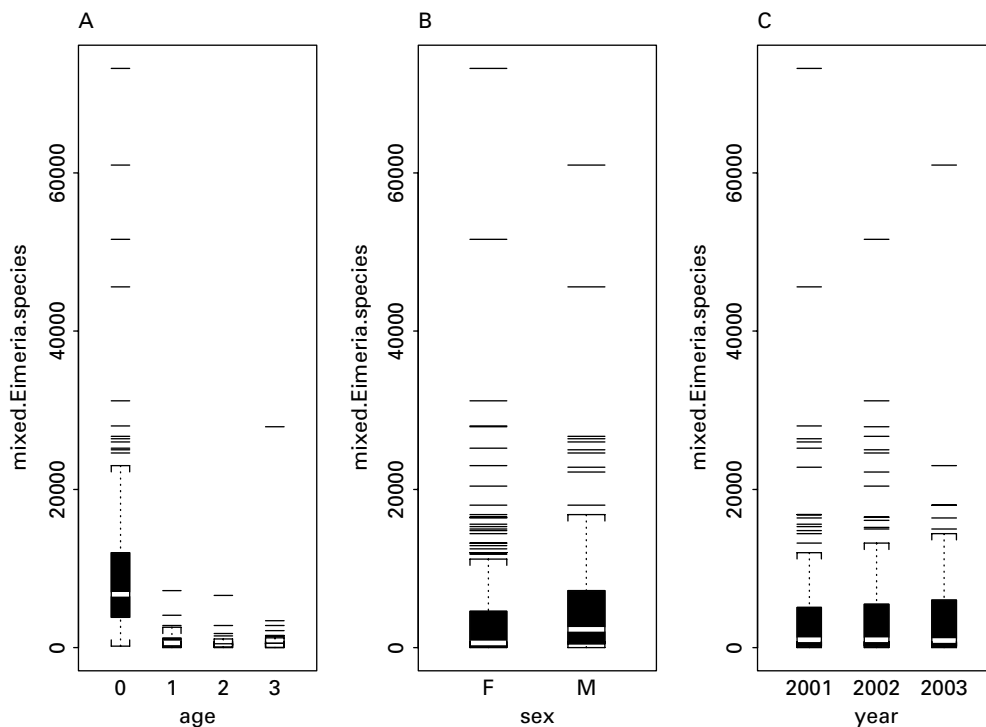
Fig. 5. Box and whisker plots of mixed *Eimeria* species intensity, plotted by (A) host age, (B) host sex and (C) year. The white line in the middle of each box shows the median of the distribution, the middle 50% of intensity values fall within the range defined by the box and the dotted lines bounded by brackets show 1·5 times the interquartile range of the data. Horizontal lines outwith the brackets are outliers. One extreme outlier (a female lamb from 2002 with 250 800 oocysts) was removed for illustration purposes. Age is shown as 4 categories: lambs (0), yearlings (1), 2-year-olds (2) and adults (3).

occurred in lambs experiencing their first infection of *C. parvum* with increasing time since birth.

*G. duodenalis* is the only protozoan species studied here with an entirely extra-cellular life-cycle in the small intestine. Prevalence decreased with host age and this association has also been found in wild (Olson and Buret, 2001) and domestic animals (Olson *et al.* 1997; Wade *et al.* 2000). The decrease in infection levels was probably due to the effect of acquired immunity to the protozoan (Faubert, 2000).

Virtually all measures of *Eimeria* species declined markedly with host age. All species except, *E. granulosa*, were more prevalent in juveniles and all species except *E. granulosa* and *E. ovinoidalis* were more intense in juveniles. Both mixed *Eimeria* species intensity and species diversity showed the same pattern. This general juvenile bias in infection of *Eimeria* spp. has also been observed in badger populations in England (Anwar *et al.* 2000; Newman *et al.* 2001) and is well documented in domestic sheep throughout the world (O'Callaghan *et al.* 1987; Maingi and Munyua, 1994; Arslan *et al.* 1999). Acquired immunity has been shown to cause a decrease in infection of various *Eimeria* species with host age (Chapman, 1974*a,b*; Gregory and Catchpole, 1989; Yun *et al.* 2000). It is not clear why *E. granulosa* prevalence increased significantly with host age although this tendency for the parasite to

have an apparently longer pre-patent period in lambs was also observed by Jorg Reeg *et al.* (2005).

Where host sex differences occurred, males usually had higher rates of infection. *C. parvum* showed no difference in prevalence between the host sexes which is similar to a study on wild rodents in Spain (Torres *et al.* 2000). Similarly, there was no significant difference in the prevalence of *G. duodenalis* between the host sexes despite a tendency for higher levels in male hosts. Diversity of *Eimeria* species was higher in males and in terms of prevalence, 4 *Eimeria* species were commoner in males than females, but this was not reflected in the intensity data (where the only effect of sex appeared as an interaction suggesting juvenile females had the highest intensity of *E. bakuensis*). This apparent male bias in infection with parasitic protozoa is consistent with the findings for helminth parasites in the population where males have significantly higher burdens (Craig *et al.* 2006) causing greater parasite-induced mortality and higher reproductive fitness costs in males than females (Wilson *et al.* 2004). It is therefore possible that parasitic protozoa have comparable detrimental effects in male Soay sheep.

*Associations with year*

Host density is an important factor in dictating host nutritional status and rate of transmission of

Table 3. Summary of significant results of LM analyses, testing for associations between age, sex and year and log transformed counts (excluding zero counts) of individual *Eimeria* species

(A GLM with negative binomial error structure and log function was applied to the analysis of total mixed *Eimeria* species count. Dashes denote associations were not significant.)

| | Age | | | | Sex | | | | Year | | | | Interactions | | | | % deviance explained by final model |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\chi^2$ | D.F. | $P$ | Association | $\chi^2$ | D.F. | $P$ | Association | $\chi^2$ | D.F. | $P$ | Association | $\chi^2$ | D.F. | $P$ | Association | |
| *E. ahsata* | 106·88 | 1 | <0·001 | Decreasing | — | — | — | — | 37·26 | 2 | <0·001 | Lowest in 2003 | 19·27 | 2 | <0·001 | Highest in juveniles in 2001 | 43·82 |
| *E. bakuensis* | 312·37 | 1 | <0·001 | Decreasing | — | — | — | — | — | — | — | — | 26·13 | 1 | <0·001 | Higher in juvenile females | 46·23 |
| | | | | | | | | | | | | | 12·66 | 2 | 0·001 | Higher in juveniles in 2002 | |
| *E. crandallis* | 316·76 | 1 | <0·001 | Decreasing | — | — | — | — | 23·66 | 2 | <0·001 | Lowest in 2003 | — | — | — | — | 43·82 |
| *E. faurei* | 33·30 | 1 | <0·001 | Decreasing | — | — | — | — | — | — | — | — | — | — | — | — | 27·89 |
| *E. granulosa* | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| *E. intricata* | 42·79 | 1 | <0·001 | Decreasing | — | — | — | — | — | — | — | — | — | — | — | — | 25·71 |
| *E. marsica* | 25·07 | 1 | <0·001 | Decreasing | — | — | — | — | — | — | — | — | — | — | — | — | 26·47 |
| *E. ovinoidalis* | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| *E. pallida* | 72·07 | 1 | <0·001 | Decreasing | — | — | — | — | — | — | — | — | — | — | — | — | 35·76 |
| *E. parva* | 147·18 | 1 | <0·001 | Decreasing | — | — | — | — | — | — | — | — | — | — | — | — | 30·52 |
| *E. weybridgensis* | 227·40 | 1 | <0·001 | Decreasing | — | — | — | — | 17·92 | 2 | <0·001 | Lowest in 2003 | — | — | — | — | 39·95 |
| *Total mixed Eimeria count* | 315·76 | 1 | <0·001 | Decreasing | — | — | — | — | 60·88 | 1 | <0·001 | Highest in 2002 | 12·51 | 2 | 0·001 | Highest in lambs in 2002 | 42·50 |

Table 4. *Overall prevalence (%) of species and mean* Eimeria *oocyst count per gram of faeces $\pm 95$ % C.I. for all sheep sampled in each year*

(See Table 1 for the composition of host ages and sex in each sample set. Dashes denote no quantitative data was obtained.)

| Species | 2001 | | 2002 | | 2003 | |
|---|---|---|---|---|---|---|
| | % Prevalence | Abundance | % Prevalence | Abundance | % Prevalence | Abundance |
| *C. parvum* | $28\cdot6 \pm 5\cdot6$ | — | $9\cdot0 \pm 4\cdot2$ | — | $11\cdot9 \pm 4\cdot3$ | — |
| *G. duodenalis* | $16\cdot9 \pm 5\cdot0$ | — | $20\cdot3 \pm 6\cdot6$ | — | $27\cdot8 \pm 6\cdot8$ | — |
| *E. ahsata* | $50\cdot7 \pm 8\cdot1$ | $787 \pm 351$ | $39\cdot4 \pm 8\cdot1$ | $424 \pm 379$ | $22\cdot4 \pm 6\cdot4$ | $61 \pm 24$ |
| *E. bakuensis* | $52\cdot1 \pm 8\cdot2$ | $1221 \pm 474$ | $53\cdot5 \pm 8\cdot2$ | $2036 \pm 820$ | $52\cdot7 \pm 7\cdot6$ | $1532 \pm 595$ |
| *E. crandallis* | $79\cdot9 \pm 6\cdot6$ | $890 \pm 442$ | $69\cdot7 \pm 7\cdot6$ | $2238 \pm 2732$ | $66\cdot1 \pm 7\cdot2$ | $824 \pm 234$ |
| *E . faurei* | $22\cdot9 \pm 6\cdot9$ | $92 \pm 52$ | $21\cdot8 \pm 6\cdot8$ | $136 \pm 164$ | $6\cdot7 \pm 7\cdot1$ | $41 \pm 48$ |
| *E. granulosa* | $29\cdot2 \pm 7\cdot4$ | $73 \pm 28$ | $29\cdot6 \pm 7\cdot5$ | $124 \pm 73$ | $12\cdot1 \pm 5\cdot0$ | $20 \pm 11$ |
| *E. intricata* | $20\cdot1 \pm 6\cdot5$ | $233 \pm 167$ | $28\cdot9 \pm 7\cdot5$ | $179 \pm 86$ | $14\cdot5 \pm 5\cdot4$ | $87 \pm 53$ |
| *E. marsica* | $29\cdot9 \pm 7\cdot5$ | $92 \pm 49$ | $24\cdot6 \pm 7\cdot1$ | $57 \pm 21$ | $25\cdot4 \pm 6\cdot7$ | $133 \pm 53$ |
| *E. ovinoidalis* | $20\cdot8 \pm 6\cdot6$ | $52 \pm 27$ | $14\cdot1 \pm 5\cdot7$ | $46 \pm 27$ | $4\cdot8 \pm 7\cdot6$ | $18 \pm 17$ |
| *E. pallida* | $22\cdot2 \pm 6\cdot8$ | $140 \pm 84$ | $8\cdot4 \pm 6\cdot0$ | $136 \pm 125$ | $16\cdot9 \pm 5\cdot7$ | $262 \pm 209$ |
| *E. parva* | $41\cdot7 \pm 8\cdot1$ | $365 \pm 216$ | $50\cdot0 \pm 8\cdot3$ | $365 \pm 211$ | $58\cdot8 \pm 7\cdot5$ | $548 \pm 146$ |
| *E. weybridgensis* | $72\cdot9 \pm 7\cdot3$ | $520 \pm 171$ | $64\cdot8 \pm 7\cdot9$ | $813 \pm 325$ | $61\cdot2 \pm 7\cdot4$ | $492 \pm 130$ |
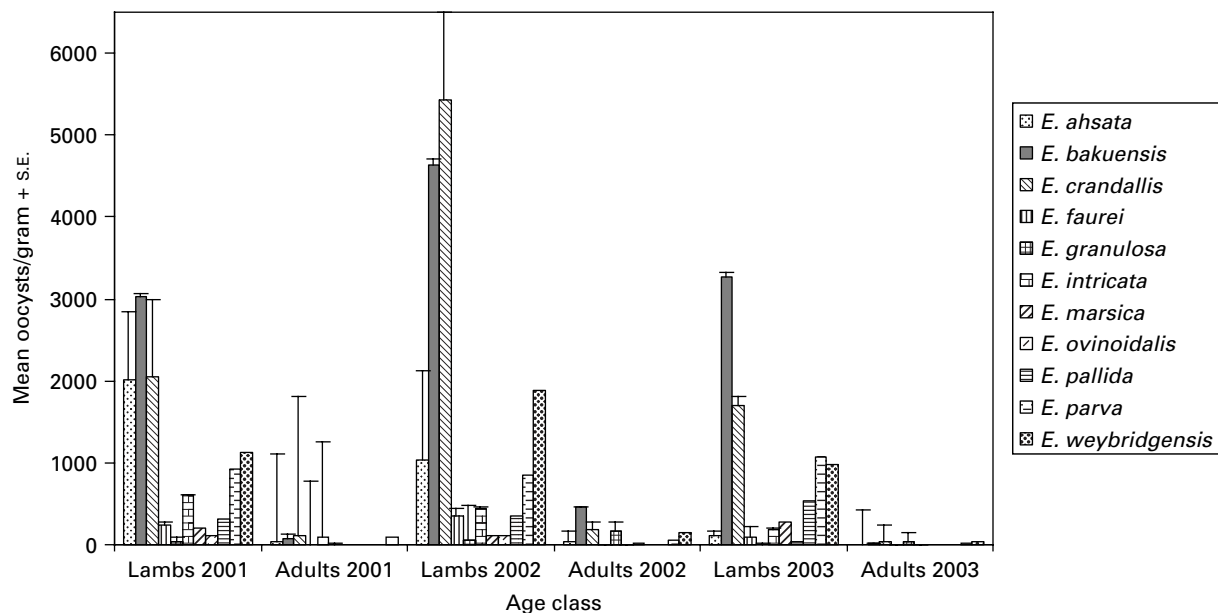


Fig. 6. Intensity (and S.E.) of each species of *Eimeria* by host age in each year of study. The standard error bar for *E. crandallis* ($\pm 6942$) has been truncated for illustrative purposes. Note that these data include all individuals including those that were negative for the species in question. Only intensity in lambs and adults are shown here but statistical analyses were done on the full range of ages (see text).

infection (Anderson, 1998), and the Soay sheep population displays extreme variation in density from year to year. In the Soay sheep, prevalence and intensity of the protozoan parasites generally decrease with host age but low levels of infection still persist in the older age classes. Therefore, when population density increases, a persisting reservoir allows recrudescence of infection within the population to occur.

*C. parvum* prevalence appeared to vary positively with population density. This finding is similar to that of Atwill *et al*. (1997) who found that above a certain threshold density of feral pigs, infection rates

of *C. parvum* increased dramatically. In the high-density year of 2001, lambs had significantly higher prevalence than any other age class across the years. In August 2002, following a population crash, intensity levels in all cohorts had dropped and prevalence in lambs was $1\cdot6$% compared with $34\cdot9$% the previous year. One explanation for the observed results is that in the high density year lambs were less well nourished and with high levels of *C. parvum* oocysts in the environment, more susceptible to acquiring a chronic infection which was still patent around 4 months after initial exposure. In 2002, neonates born in the wake of the population crash
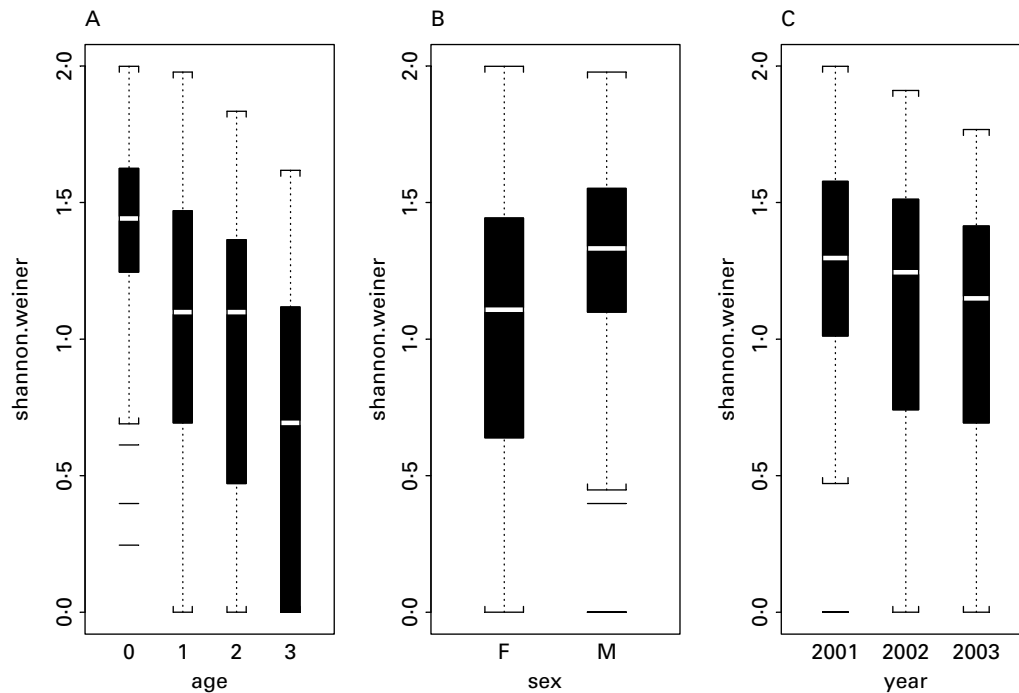
Fig. 7. Box and whisker plots of Shannon-Weiner diversity index, calculated for all species of *Eimeria* for all hosts sampled in all years and plotted by (A) host age, (B) host sex and (C) year. See Fig. 5 for notation.

would have been in poor condition (Clutton-Brock, 2004) and would have quickly acquired infection. Despite initial infection, this cohort would have experienced a marked improvement in nutrition in the following months so that infection acquired soon after birth had probably dissipated by August. This implies that hosts experiencing malnutrition, especially lambs, in the high-density year were more prone to infection. Similarly, studies involving human populations have also shown that malnutrition contributes significantly to the predisposition of infection of the parasite in children (Macfarlane and Horner-Bryce, 1987; Allam *et al.* 2002; Kirkpatrick *et al.* 2002; Gendrel *et al.* 2003).

In contrast, of the *Eimeria* species only 1, *E. marsica*, seemed to vary positively with host population density in terms of prevalence. Four species were at highest prevalence in 2002 and 6 at lowest prevalence in 2003. This was also the pattern shown by mixed *Eimeria* intensity and species diversity. It seemed, therefore, that there was a lag in infection rate following the host population crash, in which levels of most *Eimeria* species peaked and then decreased. This is strikingly different to the infection pattern with *C. parvum*. The different infection patterns of the two intracellular parasites may reflect the response of the host immune system to co-infection. As *C. parvum* tends to be highly immunogenic and prolific (2 auto-infective stages), high levels of infection in lambs in 2001 may have suppressed levels of *Eimeria* species oocyst shedding either due to immuno-cross-reactivity (Lorenzo *et al.* 1998) or as a result of the so called 'crowding effect' as demonstrated in *Eimeria* infection in chickens

where very high levels of infection result in a decrease in oocyst output due to a combination of competition between and within species and sloughing off of epithelial cells (Brackett and Bliznick, 1952; Williams, 1973).

After the epidemic of *C. parvum* had allayed in 2002, *Eimeria* species may have taken advantage, fulfilling their reproductive potential in the intestines at a time of poor host condition and high environmental contamination of oocysts. Also it is possible that increased availability of herbage in the months following the population crash may have promoted *Eimeria* species proliferation. Abo-Shehada and Muwalla (1989) found that in naturally infected sheep fed 3 different nutritional planes, prevalence of some species such as *E. bakuensis* was significantly higher in higher plane fed animals. Also, a pilot study by Pout and Catchpole (1974) indicated that sheep on a low plane of nutrition may suffer less clinical coccidiosis than those on a higher nutritional plane.

Regardless of population density, prevalence of *G. duodenalis* remained at comparable levels across the years. Similarly, Atwill *et al.* (1997) found that population density was not significantly associated with the prevalence of *Giardia* spp. infection in feral pigs.

### Associations between protozoan species

There are 2 types of explanation for the 5 positive pair-wise correlations between protozoan species. In natural populations, parasites are usually over-dispersed, that is most individuals harbour few parasites and a few harbour many. The cause of

aggregation may be variation in the exposure or susceptibility to infection within the host population. On St Kilda various factors influence susceptibility in the sheep such as host age, sex, previous exposure to infection and nutritional status. Variation in any of these factors, causing aggregation both within and across parasite taxa, could therefore explain the positive associations observed between protozoan species. In addition, or alternatively, to this main effect, it is possible that concurrent parasite infection exerts a synergistic effect. Although not easy to detect (Behnke *et al.* 2005) such interactions have recently been documented for protozoa (Bajer *et al.* 2002) and helminths (Lello *et al.* 2004) in wild mammal populations. That is, infection with one species enhances proliferation of another species. For instance, Catchpole *et al.* (1976) found that pure inoculations of 4 species of *Eimeria* in lambs produced shorter patent infections than when all 4 species were administered at once. The immunogenicity of the different parasite species and the extent to which hosts develop cross-immunity will play a role in this phenomenon.

*Implications for the study of evolution of parasite resistance in wild populations*

In nature, infections with multiple and taxonomically diverse parasite species are usual, but few studies of wild animal populations have attempted to examine the epidemiology of all the metazoan and protozoan species present. In order to understand how resistance to certain parasite species evolves in a population it is crucial to consider the potential effect of other co-infecting species. In the St Kilda Soay sheep, despite evidence that strongyle nematode parasites have the potential to promote nematode resistance in the population, heritable variation in resistance to this group is maintained. There is a possibility that the maintenance of host genetic variation in the Soay sheep is an optimal response to co-infection with diverse parasites and balancing selection could be in operation for resistance to helminths and protozoa due to the Th1/Th2 trade-off response against these groups. This study highlights the importance of considering the potential influence of microparasites as well as macroparasites in long-term evolutionary studies of host-parasite relationships.

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