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Reproductive effort influences intra-seasonal variation in parasite-specific antibody responses in wild Soay sheep

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ABSTRACT

1. A trade-off between reproduction and immune function has been suggested to potentially underpin between-individual and genetic variation in reproductive strategy, immunity, and lifespan, with potential consequences for host and parasite dynamics.
2. Previous studies in wild animal populations have shown that experimentally-induced or natural variation in reproductive effort is negatively associated with general immune markers. Few studies, however, have tackled this question by measuring specific immune responses against prevalent pathogens, and only rarely has variation in immune measures been linked to parasite burden, making it impossible to draw conclusions about the functional relevance of covariation between reproductive effort and immune markers.
3. We collected faecal samples in a longitudinal manner from wild female Soay sheep across late pregnancy and early lactation, and measured gastrointestinal nematode faecal egg count (FEC) and worm-specific antibody responses.
4. FEC was highly repeatable, with ~80% of variation due to between-individual differences, while three isotypes of worm-specific and total antibodies had moderate repeatability (range: 11-43%). Females making a greater reproductive effort (those that reproduced, and especially those with heavier litters), showed a more rapid increase in FEC across the season, while non-reproducing females and those producing lighter litters experienced lower FEC and had higher antibody levels.
5. Associations between antibodies and FEC were complex: worm-specific immunoglobulin (Ig) G was negatively associated with FEC, while total IgM was positively associated, emphasizing the importance of measuring both immune markers and parasite burden in ecological studies.
6. Our results support the predicted trade-off between reproductive effort and parasite-specific immunity: high reproductive effort can limit the ability of individuals to defend themselves against prevalent parasites, with potential downstream consequences for fitness and parasite transmission.

INTRODUCTION

A cornerstone of evolutionary life-history theory is that individuals must allocate resources to traits such as reproduction and self-maintenance in order to maximise their fitness (Stearns 1992). Since resources such as energy, protein and time are limited, individuals face trade-offs: resources allocated to one trait cannot be re-allocated to another (Stearns 1989). It has been well-established in studies of wild animal populations that allocation of resources to current reproduction may come at a cost to future reproduction or survival (Clutton-Brock, Guinness & Albon 1983; Gustafsson & Sutherland 1988; Clutton-Brock *et al.* 1996). One mechanism potentially underpinning the trade-off between current and future reproduction is reduced effectiveness of immune responses during reproduction (Sheldon & Verhulst 1996). In this study, we use data collected from a wild population of Soay sheep to test the hypothesis that greater reproductive effort should be associated with reduced specific antibody responses to, and increased infection burden of, a prevalent and damaging parasite.

Experimental and observational studies in wild animal populations have revealed greater probability of infection and/or greater parasite burdens in reproducing versus non-reproducing individuals (Festa-Bianchet 1989; Luong *et al.* 2010; East *et al.* 2015) and in individuals producing more offspring (Richner, Christe & Oppliger 1995; Nordling *et al.* 1998; Knowles, Nakagawa & Sheldon 2009). In addition, many studies of wild populations have shown that measures of immune responsiveness are weaker in reproductive versus non-reproductive individuals and in individuals spending more time engaged in reproductive behaviours, producing or caring for more offspring, or carrying larger sexual ornaments (Nordling *et al.* 1998; Ardia, Schat & Winkler 2003; Hanssen *et al.* 2005; Neggazi *et al.* 2016; Rödel *et al.* 2016; Palacios & Bronikowski 2017). Limitations of such studies, however, include a reliance on non-specific markers of immune function, which may be uninformative about an individual's response to prevalent parasites (Bradley & Jackson 2008). Further, a failure to quantify associations between immune parameters and parasite burden can limit interpretation of associations between reproduction and immunity (Graham *et al.* 2011). For example, if, as in many studies, positive rather than negative associations between reproductive effort and immune markers are observed, it is difficult to draw meaningful conclusions because such associations could be due to

increased parasite exposure, higher body condition, or alternative defence strategy of reproductive individuals (Xu, Yang & Wang 2012; McCullough & Emlen 2013; Rödel *et al.* 2016). Such limitations arise because of the difficulty of producing reliable parasite-specific immune markers for most non-model organisms. The depth of knowledge garnered from veterinary science about parasites of sheep, cattle and horses, and the immunological tools that have been developed, mean that wild ungulates offer excellent systems for disease ecological research (Jolles & Ezenwa 2015).

Reproduction in domestic sheep is associated with the peri-parturient rise (PPR), an increase in shedding of gastrointestinal nematode eggs in the faeces of pregnant and lactating ewes (Connan 1967; O'Sullivan & Donald 1970). The PPR is largely a consequence of protein deficit due to the heavy nutritional demands of reproduction (Donaldson, Houtert & Sykes 2001), which mean that fewer resources are available to mount parasite-specific antibody responses, which are diminished in pregnant and lactating ewes (Beasley, Kahn & Windon 2010; Beasley, Kahn & Windon 2012). The PPR has also been documented in the wild population of Soay sheep living in the remote St Kilda archipelago (Wilson *et al.* 2004; Tempest 2005), with recent work showing that females that successfully raised a lamb to weaning had a more pronounced PPR than those that failed to wean a lamb and, as a consequence, reduced survival (Leivesley et al, unpublished data). The degree to which changes in strongyle-specific immune responses during reproduction can explain the PPR in Soay sheep has not, however, been established. More broadly, few studies have used markers of parasite-specific immunity when investigating trade-offs between reproduction and immune function.

In this study, we monitored parasite-specific antibody responses and nematode faecal egg counts (FEC) during the peri-parturient period in wild female Soay sheep living in the St Kilda archipelago, Scotland (Figure 1). *Post mortems* of Soay sheep on St Kilda and elsewhere have revealed linear relationships between adult worm numbers and FEC, suggesting that FEC provides a reliable estimate of infection intensity (Gulland 1992; Wilson *et al.* 2004). We measured strongyle-specific antibodies to capture variation in specific responses to highly prevalent parasites and also measured constitutively-produced natural (total) antibodies that are among the first responders to infection,

binding to novel 'non-self' and 'self' antigens, and which can have important roles in defence (Binder 2010). We predicted that increased reproductive effort would be generally associated with increased strongyle FEC and reduced strongyle-specific antibody responses. We used two measures of reproductive effort: first, whether or not a female reproduced; second, how heavy a female's litter was at birth. Our results provide evidence for costs of reproduction, specifically in terms of higher FEC and reduced antibody responses in females producing heavier litters.

MATERIALS AND METHODS

Study population and data collection

Soay sheep are descended from the domesticated sheep present in NW Europe in the Bronze Age, and were taken to the St Kilda archipelago (57°49'N, 08°34'W, 65km NW of the Outer Hebrides, Scotland) several thousand years ago (Clutton-Brock *et al.* 2004). A population has lived on the island of Soay (99 ha) since their arrival in St Kilda; meanwhile, the largest island in the archipelago, Hirta (638 ha), was home to a human population until 1930, when the people were evacuated along with their modern domesticated livestock. In 1932, 107 Soay sheep were moved from Soay to Hirta, from which Hirta's current unmanaged population grew. The sheep population living in the Village Bay (c. 170 ha) area of Hirta has been the subject of an intensive individual-based study since 1985 (Clutton-Brock *et al.* 2004). Females give birth in spring (March-April) and >95% of lambs born in Village Bay are captured within a week of birth, weighed, blood and tissue sampled for genetic analysis, and given a unique identifying ear tag. Most lambs are born in single births, but ~12% of litters (2-21% annually) are twin births. Since lambs are captured at different ages, we calculated 'corrected litter weight' ('litter weight' from hereon) using the formula $CW - ((0.108 \times CD) \times LS)$, where *CW* is capture weight (of a single lamb, or the summed weights of twins) in kg; *CD* is capture date in days since birth (twins are captured on the same day); *LS* is litter size (1 or 2). This formula provides an estimated litter weight at birth, based on known growth rates (Robertson *et al.* 1992).

Faecal sampling

In spring 2016, 266 faecal samples were collected from 54 female Soay sheep living in Village Bay (mean: 4.92 samples per female; range: 1-6; 48 females sampled ≥4 times). Females were aged 1-13 years; six females aged ≥9 were pooled together in the oldest age class for all analyses. The first sample from each female was collected between April 2nd and 9th; thereafter, females were re-sampled at intervals of 1-19 (mean = 5.93) days until May 3rd. These dates coincided with the main lambing period; females included in the study bore lambs between April 7th and May 8th. Females were identified by observing unique ear tags with binoculars. Fresh faeces were collected within a minute of defecation with an inverted zip-lock bag and homogenized. Within an hour of collection, samples were weighed and separated: subsamples of ≥2g each were stored at 4°C for faecal egg count analysis and at -20°C for antibody assays.

Faecal egg counts

Faecal egg counts (FEC) were conducted using a modified salt-flotation method (Jackson 1974). Briefly, 0-4 days after sample collection, 2-10g of faeces were weighed out and placed in a fresh bag with 10ml of water per gram and emulsified. After storage at 4°C for up to 24 hours, the sample was re-suspended and a 10ml subsample was taken and dispensed through a sieve into a beaker, with the retentate washed into the beaker. The filtrate was transferred to a 15ml polyallomer tube and centrifuged for 2 minutes at 1000rpm. The supernatant was removed and the faecal pellet re-suspended with 10ml of saturated NaCl solution before centrifugation for another 2 minutes at 1000rpm. Artery forceps were used to clamp the tube below the meniscus and the fluid in the upper chamber (including strongyle eggs) was poured into a cuvette; 1ml of NaCl solution was used to wash the upper chamber and added to the cuvette. The cuvette was filled with NaCl solution and strongyle eggs (predominantly *T. circumcincta* and *Trichostrongylus* spp., but also including *Chabertia ovina, Bunostomum trigonocephalum* and *Strongyloides papillosus*) were counted to a precision of 1epg (eggs per gram).

Antibody assays

We measured strongyle-specific and total antibodies in faecal samples, using a recently-developed protocol (Watt *et al.* 2016). The advantages of using faecal, rather than circulating, antibodies are twofold: first, the animal does not need to be captured, facilitating longitudinal sampling of known individuals; second, antibodies measured in faeces are likely to reflect antibody responses at the intestinal mucosa where parasitic nematodes meet the host immune system (Gill *et al.* 1993). Faecal samples were analysed for six antibody isotypes: *Teladorsagia circumcincta*-specific and total IgA, IgG and IgM, based on methods described previously (Watt *et al.* 2016). 0.8g of defrosted and homogenized faeces was placed in a 2ml microcentrifuge tube with 1.2ml of chilled protease inhibitor solution (1x cOmplete™ Mini EDTA-free tablet, Roche, Switzerland, dissolved in 7ml PBS solution), mixed thoroughly, allowed to stand for 5 minutes, and centrifuged at 15,000*g* for 5 mins. The supernatant was aliquoted into a fresh 1.5ml Eppendorf and frozen at -20°C.

For total antibody assays, 96-well Nunc-Immuno™ plates were coated overnight at 4°C with 50µl/well of anti-ovine IgA, IgG, and IgM (Bethyl Laboratories Inc, catalogue numbers A130-108A, A130-101A and A130-109A, respectively) diluted to 2µg/ml carbonate buffer at pH 9.6. For *T. circumcincta*-specific assays, plates were coated with 50µl/well of *T. circumcincta* L3 somatic antigen (Watt *et al.* 2016) and diluted to 2µg/ml in 0.06M carbonate buffer at pH 9.6. Plates were washed three times in TBST (Tris-buffered saline containing 0.05% Tween-20), 50µl of diluted faecal sample was added to each well and plates were incubated at 37°C for 1 hour. Dilutions were selected by performing doubling dilutions on a set of test samples until optical densities (ODs) reached background levels for each assay and determining the range of dilutions across which ODs decreased linearly for each assay. For *T.circumcincta*-specific isotypes, we used undiluted supernatant; for total antibodies, we used dilutions of 1:128, 1:256 and 1:32 respectively. Plates were washed five times with TBST before addition of 50µl/well of the appropriate detection antibody conjugated to horseradish peroxidase (HRP). For IgA, IgG and IgM, we used anti-ovine IgA-, IgG- and IgM-HRP (Bethyl Laboratories Inc, catalogue numbers: A130-108P, A130-101P and A130-109P respectively).

All plates were incubated at 37°C for 1 h and, following a final wash with TBST, 100µl of SureBlue TMB 1-component microwell peroxidase substrate (KPL) was added per well and then left to incubate for 5 min in the dark at 37°C. Reactions were then stopped by adding 100µl 1M HCl, and optical densities (OD) were read immediately at 450 nm using a Thermo Scientific Multiskan GO Spectrophotometer.

Each assay was performed twice on separate ELISA plates. On each of the *T. circumcincta* plates, two sample-free wells were included as negative controls, and two wells with 50µl of gastric lymph fluid from a *T. circumcincta*-infected domestic sheep, diluted 1:50 in TBST, were included as positive controls. On the total antibody plates, we included two sample-free wells as negative controls and purified sheep IgA or IgG (Alpha Diagnostic International: Purified Sheep IgA: ADI 20006-3, Purified Sheep IgG: ADI 20006-1, respectively) as positive controls. For subsequent analyses, we took the average OD across the duplicate runs minus the average of the four negative control well ODs across the two plates as our assay measure.

Statistical analysis

All analyses were performed in R ver. 3.5.1 (R Core Team 2018).

Changes in strongyle FEC across the season

We analysed changes in strongyle faecal egg count (FEC) across the lambing season using generalized linear mixed-effects models (GLMMs) in the package 'glmmTMB' (Brooks *et al.* 2017). Specifically, we assessed whether changes in FEC across the season varied with reproductive effort. We characterized 'relative date' as the date on which a sample was collected relative to the day on which a female gave birth (47 females, 238 samples); if a female did not reproduce, 'relative date' was calculated relative to the mean lambing day for the year (7 females, 28 samples). 'Relative date' is referred to as 'date' from hereon. Strongyle FEC was modelled with the 'nbinom2' negative

binomial error structure. We tested a number of error structures in our preliminary analyses, including log-transforming FEC with Gaussian errors, and Poisson and both the 'nbinom1' and 'nbinom2' parameterizations (Brooks *et al.* 2017) of negative binomial models, including zero-inflated versions. The distribution of residuals was assessed using the 'DHARMa' R package (Hartig 2018) and the negative binomial model produced the best diagnostics, with heterogeneous residuals and absence of overdispersion and zero-inflation.

First, we constructed a model (model 0) that included: the location of the individual when the sample was collected (a categorical fixed effect with eight levels; see Figure S1 in Supporting Information); the female's age as a categorical fixed effect (1-9 years); female identity as a random effect. We then fitted: a model that also included a linear effect of date (model 1); linear and quadratic effects of date (model 2); whichever of model 1 or 2 was supported, plus a main effect of reproductive status (whether or not a female reproduced as a two-level factor; model 3); model 3 plus an interaction between date and reproductive status (model 4). Terms were tested using likelihood-ratio tests (LRTs), where the χ^2-distributed test statistic is calculated as $-2*(loglik_{m1} - loglik_{m2})$ where *m1* is the full model and *m2* is a model with the term of interest omitted. The intra-class correlation coefficient (the proportion of variance explained by individual identity in our model) was calculated for the best-fitting model using the 'sjstats' R package (Lüdecke 2018).

Next, we restricted our analyses to those females that reproduced and had known litter weight (220 samples from 43 females). We repeated models 0-4 described above, but replaced reproductive status with a linear effect of litter weight: thus, we assessed the impact of litter weight upon changes in FEC across the season.

Changes in antibody levels across the season

We next assessed changes in the levels of each of the six antibody isotypes across the lambing season, using linear mixed-effects models (LMMs) in 'glmmTMB'. We used untransformed antibody levels as our response variable, since these showed superior model diagnostics when assessed using

'DHARMa'. First, we used a dataset of 248 samples from 54 females (18 samples were omitted from the analyses where one of the antibody readings was unreliable) to test for changes in antibody levels across the season and whether these changes were associated with reproductive status (as models 0-4, described above). Next, we fitted models 0-4 testing for effects of litter weight on changes in antibody levels across the season, restricting our analyses to 205 samples from 43 females that reproduced and had known litter weight.

Associations between antibody isotypes

Previous analyses have revealed strong positive associations between different antibody isotypes measured in Soay sheep plasma (Nussey *et al.* 2014; Watson *et al.* 2016) and faeces (Watt *et al.* 2016) and has shown that *T. circumcincta*-specific pan-isotypic antibody contains substantial among-individual variation (Hayward *et al.* 2014). We aimed to build upon this work by estimating the individual-level variation (repeatability) and individual-level correlations between antibody measures with multivariate mixed-effects models in ASReml-R ver 3.0 (Gilmour *et al.* 2009) with the dataset of 248 samples from 54 females. We fitted all six antibody isotypes as response variables in a six-trait linear mixed-effects model, with age and location as fixed effects. We fitted individual identity as a random effect and estimated the individual and residual 6x6 variance-covariance matrices and calculated the proportion of phenotypic variation due to between-individual variation ('repeatability') for each isotype and the individual and residual correlations between pairs of antibodies. We tested each of the between-individual covariances with LRTs, comparing the full model with models with the covariance of interest constrained to zero.

Associations between antibodies and strongyle FEC

We analysed associations between FEC and antibodies using negative binomial GLMMs of FEC on the data set of 248 samples from 54 females. First, we fitted each antibody isotype in a separate model, including fixed effects of age, location and a linear effect of date, and with identity as a random effect. We tested for both linear and quadratic terms for each isotype in turn. Second, we

fitted all linear antibody terms in the same model and used stepwise deletion, based on LRTs as described above, to simplify the model until no antibody terms could be removed from or added to the model.

RESULTS

Changes in strongyle FEC across the season

Analysis supported a linear increase in FEC across the study period (Figure 2A; Table 1), but did not suggest that the association was non-linear (Table 1). While there was no evidence that reproductive status influenced FEC as a main effect, there was evidence for an interaction between date and reproductive status: the FEC of non-reproducing females remained constant across the season, while females that reproduced experienced an increase in FEC (Figure 3; Table 1). Among the females that did reproduce, there was no evidence that litter weight influenced FEC as a main effect, but there was support for an interaction between date and litter weight: females that produced heavier litters experienced a more rapid increase in FEC across the season (Figure 2B; Table 1). After accounting for variation in individual location and age, between-individual variation explained ~80% of variation in FEC (Tables S1 & S2).

Changes in antibody levels across the season

From hereon, we refer to *T. circumcincta*-specific antibodies as TcIgA, TcIgG and TcIgM, and to total antibodies as TotIgA, TotIgG and TotIgM. There was limited evidence for changes in antibody levels across the lambing season (Table 2) and for effects of reproduction on antibodies. There were trends for linear increases in TcIgA, TotIgA and TotIgG across the lambing season, but these were marginally non-supported (Figures. S2A, C & E). Meanwhile, interactions between date and reproductive status for TotIgG and TotIgM were marginally non-supported, while the interaction between date and reproductive status was marginally supported for TcIgG. In each case, females that

did not reproduce experienced an increase in antibody levels across the season, but those that reproduced did not (Figures. S2B, D & F).

Among females that did reproduce, all of the isotypes were influenced by an interaction between date and litter weight, apart from TcIgG (Table 3): females that produced lighter litters experienced a more rapid increase in antibody levels across the season, while those with the heaviest litters had antibody levels that remained relatively constant or even declined (Figure 4). Meanwhile, there were linear negative associations between TcIgG (Figure 4B) and TotIgG and litter weight: females with heavier litters had lower antibody levels (Table 3).

Associations among antibody isotypes

Our six-trait mixed-effects model of antibody levels converged successfully. Between-individual variation accounted for 11-43% of phenotypic variation in antibody levels, after accounting for fixed effects of location and age (Table 4). Thus, all isotypes were moderately repeatable apart from TotIgG, for which between-individual variation explained <15% of phenotypic variation. Associations between isotypes were uniformly positive (Figure S3), but correlations were generally stronger at the residual level (range 0.56-0.88) than at the individual level (range 0.08-0.89; Table S3). Of the individual-level correlations, only that between TcIgM and TotIgM had a z-ratio of >2, suggestive of statistical support (Table S3). However, when we tested individual-level covariances by fixing them to zero, we experienced model convergence issues. We therefore conducted formal LRTs on bivariate models where we fitted pairs of antibodies in turn; we fitted 15 models with all possible bivariate combinations of the six antibodies (Table S4). Of all the bivariate individual-level covariances, only that between TcIgM and TotIgM was supported (estimate = 0.0223±0.0067SE, X^2 = 27.86, P<0.001).

Associations between antibody isotypes and strongyle FEC

When fitting each of the antibody isotypes in a separate GLMM, there was marginal support for a negative linear association between FEC and TcIgA (estimate = -0.28±0.14, χ^2_1 = 3.91, P = 0.048). There was also stronger support for negative linear associations with TotIgG (estimate = -0.24±0.11, χ^2_1 = 4.62, P = 0.032) and especially TcIgG (estimate = -0.45±0.17, χ^2_1 = 6.64, P = 0.010). None of the other antibody terms were supported. When we fitted all antibodies together (Table S5), we found support for a negative linear association between TcIgG and FEC (Figure 5A) and a positive linear association between TotIgM and FEC (Figure 5B). We also re-ran these analyses on the restricted dataset of females which reproduced and for which all antibodies were measured (N = 205, females = 43), but results were identical (Tables S6 & S7). There was also no evidence that associations between antibodies and FEC varied between reproducing and non-reproducing females (Table S8).

DISCUSSION

In this study, we collected data on strongyle nematode faecal egg count (FEC) and antibody responses during the lambing season in wild female Soay sheep. We found high levels of between-individual variation in strongyle FEC and that FEC increased across the lambing season, particularly in females that reproduced and those that produced heavier litters. We found moderate between-individual variation in both total and strongyle-specific antibodies and all antibodies were mutually positively correlated. Variation in antibody levels across the season was associated with litter weight: antibody levels generally increased across the season in females that produced light litters but remained constant or even decreased in females that produced relatively heavy litters. Finally, *T. circumcincta*-specific (Tc)IgG was negatively associated with strongyle FEC, while total (Tot)IgM was positively associated with strongyle FEC. Our results reveal that, as with general markers of immune function (Nordling *et al.* 1998; Ardia, Schat & Winkler 2003; Hanssen *et al.* 2005; Neggazi *et al.* 2016; Rödel *et al.* 2016; Palacios & Bronikowski 2017), parasite-specific immune responses appear to be compromised by greater reproductive effort and that this is associated with higher parasite burden.

We examined between-individual variation in FEC within a four-week period and found that between-individual effects explained ~80% of variation (Tables S1 & S2): the usually large environmental effect on FEC (Coltman *et al.* 2001) was vastly reduced in our within-season study, emphasizing between-individual differences. Here, we used a cuvette-based method for estimating FEC (accurate to 1epg), rather than the McMaster method used in previous studies of Soay sheep (accurate to 100epg), which may have helped to boost the repeatability of FEC, along with the fact that we assessed repeatability across a short period of time. FEC increased linearly across the season (Figure 2A), with no evidence for the inverse U-shaped dynamic seen in previous studies of Soay (Wilson *et al.* 2004; Tempest 2005) and domestic sheep (Connan 1967; O'Sullivan & Donald 1970). The likely reason for this discrepancy is that our sampling period was April 2nd-May 3rd, with a mean lambing date of April 24th; as a result, only 75/266 (28% of) samples were collected after lamb birth. Thus, we may not have sampled enough of the post-parturition period to capture the post-parturition decline in FEC.

Our analyses revealed that changes in FEC across the season were influenced by reproductive effort: females that reproduced (Figure 3, Table S1) and those that produced heavier litters (Figure 2B, Table S2) exhibited steeper rises in FEC across the season. These observations mirror the reproduction-induced increase in parasite burden exhibited in many studies previously, including in Soays (Leivesley *et al*. unpublished data), bighorn sheep *Ovis canadensis* (Festa-Bianchet 1989), domestic sheep (Beasley, Kahn & Windon 2010) and other wild vertebrates (Nordling *et al.* 1998; Knowles, Nakagawa & Sheldon 2009; East *et al.* 2015). These results could potentially be attributed to two mechanisms: a cost of reproduction, in the form of fewer resources remaining for allocation to immune-mediated parasite resistance (Sheldon & Verhulst 1996), or an increase in exposure across the season as hungry females spend more time foraging in nutrient-rich, but also parasite-rich tussock vegetation (Hutchings *et al.* 2002). While changes in exposure could explain some of the change in FEC across the season, experimental studies in domestic sheep show that variation in exposure has a weaker effect on the PPR compared to variation in nutrition (Kidane *et al.* 2009). Our results add to the evidence from wild populations that females making a large reproductive effort experience a cost

in the form of increased parasite burden (Richner, Christe & Oppliger 1995; Nordling *et al.* 1998; Knowles, Nakagawa & Sheldon 2009).

The effects of reproduction on changes in FEC were mirrored by antibody levels: females producing lighter litters experienced increases in antibody responses across the season, while those producing heavier litters experienced no such increase (Figure 4; Table 3). Moreover, for TcIgG, TotIgG and TotIgM, we found support for negative associations with litter weight as a main effect (Table 3). Thus, the results of our study and previous work on domestic sheep (Beasley, Kahn & Windon 2010) and other wild animals (Nordling *et al.* 1998; Ardia, Schat & Winkler 2003; Neggazi *et al.* 2016; Palacios & Bronikowski 2017) support the prediction that females making a larger reproductive effort experience diminished immune responses. This observation, however, is not universal: reproduction in wild female garter snakes (*Thamnophis elegans*) did not influence bactericidal capacity of plasma (Palacios & Bronikowski 2017) and European rabbits (*Oryctolagus cuniculus*) showed a positive association between litter mass and total IgG levels (Rödel *et al.* 2016). In contrast to the results for FEC, we found that reproductive status was only weakly associated with changes in antibodies across the season, with support for date-by-reproduction associations restricted to TcIgG (Table 2). Our ability to detect this interaction, and effects of reproductive status in general, could have been hampered by the fact that only 7/54 females failed to reproduce.

Our observation of a reduction in faecal antibodies with increased litter weight could arise through two non-mutually exclusive mechanisms. First, increased maternal antibody transfer could leave lower antibody levels in the maternal circulation. In ungulates, maternally-derived antibodies are selectively transported from the maternal circulation to colostrum, boosting the lamb's immune system (Mayer *et al.* 2002). While the selective transfer of antibodies to ruminant colostrum is established (Brandon, Watson & Lascelles 1971), variation between individuals in the amount of antibody transferred is less well-studied. While there is some evidence that ewes with larger litters produce colostrum with higher antibody levels (Gilbert *et al.* 1988; Higaki *et al.* 2013), no associations between antibodies in ewe serum and colostrum are reported (Sawyer *et al.* 1978;

Tabatabaei *et al.* 2013). Second, allocation of resources towards reproduction could leave fewer remaining for antibody responses, as a direct consequence of the trade-off between reproduction and immunity (Sheldon & Verhulst 1996). Experimental studies on domestic sheep support this idea: total antibody titres of Merino ewes increased across the lambing season and were generally higher in ewes that produced a lamb but that did not suckle it but remained stable in ewes that suckled their lambs (Beasley, Kahn & Windon 2010). Similarly, Coopworth ewes that reared single lambs had higher *T. circumcincta*-specific antibody levels than those rearing twins (Sykes *et al.* 2007). Overall, these results suggest that increased reproductive effort is associated with lower antibody levels in sheep. The functional significance of these changes can only be determined by examining associations between immune parameters and relevant measures of parasite infection (Bradley & Jackson 2008; Graham *et al.* 2011).

When fitting models assessing associations between FEC and each antibody in turn, we found support for negative associations with FEC for TcIgA, TcIgG and TotIgG. When we fitted all antibodies together in the same model we found a negative association between FEC and TcIgG and a positive association between FEC and TotIgM. Importantly, TcIgG was associated with FEC both separately and independently of other antibodies; the univariate associations with FEC for both TcIgA and TotIgG are likely to have arisen because of their associations of TcIgG. Whilst IgA is the isotype most commonly associated with protection against sheep nematodes (Strain *et al.* 2002), IgG confers resistance to nematodes including *Heligmosomoides polygyrus* (McCoy *et al.* 2008), *Ascaris suum* (Khoury, Stromberg & Soulsby 1977) and *Stronglyoides ratti* (Murrell 1981), in other host species. Parasite-specific IgG1 controls *H. polygyrus* in mice through antibody-dependent activation of macrophages (Esser-von Bieren *et al.* 2013) and specific binding to parasite excretory-secretory antigens (Hewitson *et al.* 2015). It is possible that similar IgG-dependent mechanisms of anti-nematode immunity occurs in sheep, particularly as IgG1 is prominent at mucosal surfaces in sheep (Cripps, Husband & Lascelles 1974).

The positive association between TotIgM and FEC was somewhat unexpected, but suggests that TotIgM may reflect higher exposure to strongyles. This suggestion is slightly undermined by our finding of no association between FEC and TcIgM: if IgM reflected exposure, one would expect FEC to be associated with strongyle-specific IgM. However, constitutively-produced 'natural antibodies' are produced even in the absence of infection and are largely composed of cross-reactive IgM (Murphy, Travers & Walport 2008); thus, natural IgM may react to strongyle antigens early in infection and result in a high TotIgM level in sheep with high exposure and higher FEC. Studies from other wild populations also find complex associations between measures of infection and antibody responses. Wild wood mice (*Apodemus sylvaticus*) with higher burdens of *H. polygyrus* had higher worm-specific IgG1 levels than those with lower burdens, suggesting greater exposure to *H. polygyrus* (Clerc *et al.* 2018). Meanwhile, *H. polygyrus*-specific IgG1 was negatively associated with pinworm burden, suggesting protective effects and cross-reactivity of antibodies between nematode species (Clerc *et al.* 2018). The results of our study and others like it clearly show the more complex insights that can be gained into host-parasite interactions through measuring multiple immune markers.

CONCLUSIONS

We investigated changes in faecal egg counts of, and antibody responses to, prevalent strongyle nematode parasites in relation to reproductive effort in a wild mammal population. Females producing heavier litters experienced reduced resistance to parasites, with increased strongyle FEC and reduced parasite-specific and total antibody responses across the lambing season. Associations between FEC and different antibody isotypes were complex and suggested that strongyle-specific IgG exerts a protective effect, but that total IgM may reflect exposure to infection. This adds to a growing body of evidence in the Soay sheep linking reproductive effort, strongyle-specific antibodies, strongyle FEC and survival (Gulland 1992; Tempest 2005; Hayward *et al.* 2011; Nussey *et al.* 2014; Watt *et al.* 2016) which suggest that the peri-parturient relaxation of immunity (PPRI) is likely to have downstream consequences for parasite transmission and host fitness. There is profound variation

between individuals in how FEC and immune responses change during reproduction, which is by no means all explained by reproductive effort. Future work could aim to determine both the proximate drivers of variation in the PPRI, and more explicitly determine its downstream consequences for parasite transmission and the fitness of mother and offspring.

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AUTHOR CONTRIBUTIONS

ADH conceived the ideas, collected and analysed data and led writing of the manuscript, with assistance from KW and TNM. JGP and KAW directed methodology and collected data. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.886m8t6, (Hayward et al, 2019).

REFERENCES

Ardia, D.R., Schat, K.A. & Winkler, D.W. (2003) Reproductive effort reduces long-term immune function in breeding tree swallows (*Tachycineta bicolor*). *Proceedings of the Royal Society B,* **270,** 1679-1683.

Beasley, A.M., Kahn, L.P. & Windon, R.G. (2010) The periparturient relaxation of immunity in Merino ewes infected with *Trichostrongylus colubriformis*: parasitological and immunological responses. *Veterinary Parasitology,* **168,** 60-70.

Beasley, A.M., Kahn, L.P. & Windon, R.G. (2012) The influence of reproductive physiology and nutrient supply on the periparturient relaxation of immunity to the gastrointestinal nematode *Trichostrongylus colubriformis* in Merino ewes. *Veterinary Parasitology,* **188,** 306-324.

Binder, C.J. (2010) Natural IgM antibodies against oxidation-specific epitopes. *Journal of Clinical Immunology,* **30,** 56-60.

Bradley, J.E. & Jackson, J.A. (2008) Measuring immune system variation to help understand host-pathogen community dynamics. *Parasitology,* **135,** 807-823.

Brandon, M.R., Watson, D.L. & Lascelles, A.K. (1971) The mechanism of transfer of immunoglobulin into mammary secretion of cows. *Australian Journal Of Experimental Biology And Medical Science,* **49,** 613-623.

Brooks, M.E., Kristensen, K., Benthem, K.J.v., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Mächler, M. & Bolker, B.M. (2017) glmmTMB balances speed and flexibility among packages for zero-inflated Generalized Linear Mixed Modeling. *The R Journal,* **9,** 378-400.

Clerc, M., Devevey, G., Fenton, A. & Pedersen, A.B. (2018) Antibodies and coinfection drive variation in nematode burdens in wild mice. *International Journal for Parasitology*.

Clutton-Brock, T.H., Guinness, F.E. & Albon, S.D. (1983) The costs of reproduction to red deer hinds. *Journal of Animal Ecology,* **52,** 367-383.

Clutton-Brock, T.H., Pemberton, J.M., Coulson, T., Stevenson, I.R. & MacColl, A.D.C. (2004) The sheep of St Kilda. *Soay Sheep: Dynamics and Selection in an Island Population* (eds T.H. Clutton-Brock & J.M. Pemberton), pp. 17-51. Cambridge University Press, Cambridge.

Clutton-Brock, T.H., Stevenson, I.R., Marrow, P., MacColl, A.D.C., Houston, A.I. & McNamara, J.M. (1996) Population fluctuations, reproductive costs and life-history tactics in female Soay sheep. *Journal of Animal Ecology,* **65,** 675-689.

Coltman, D.W., Pilkington, J.G., Kruuk, L.E.B., Wilson, K. & Pemberton, J.M. (2001) Positive genetic correlation between parasite resistance and body size in a free-living ungulate population. *Evolution,* **55,** 2116-2125.

Connan, R.M. (1967) Observations on the post-parturient rise in the faecal nematode egg count of ewes. *Veterinary Record,* **80,** 401-405.

Cripps, A., Husband, A. & Lascelles, A. (1974) The origin of immunoglobulins in intestinal secretion of sheep. *Australian Journal Of Experimental Biology And Medical Science,* **52,** 711-716.

Donaldson, J., Houtert, M.F.J.v. & Sykes, A.R. (2001) The effect of dietary fish-meal supplementation on parasite burdens of periparturient sheep. *Animal Science,* **72,** 149-158.

East, M.L., Otto, E., Helms, J., Thierer, D., Cable, J. & Hofer, H. (2015) Does lactation lead to resource allocation trade-offs in the spotted hyaena? *Behavioral Ecology and Sociobiology,* **69,** 805-814.

Esser-von Bieren, J., Mosconi, I., Guiet, R., Piersgilli, A., Volpe, B., Chen, F., Gause, W.C., Seitz, A., Verbeek, J.S. & Harris, N.L. (2013) Antibodies trap tissue migrating helminth larvae and prevent tissue damage by driving IL-4Rα-independent alternative differentiation of macrophages. *PLOS Pathogens,* **9,** e1003771.

Festa-Bianchet, M. (1989) Individual differences, parasites, and the cost of reproduction for bigorn ewes (*Ovis canadensis*). *Journal of Animal Ecology,* **58,** 785-795.

Gilbert, R.P., Gaskins, C.T., Hillers, J.K., Parker, C.F. & McGuire, T.C. (1988) Genetic and environmental factors affecting immunoglobulin G1 concentrations in ewe colostrum and lamb serum. *Journal of animal science,* **66,** 855-863.

Gill, H.S., Gray, G.D., Watson, D.L. & Husband, A.J. (1993) Isotype-specific antibody responses to Haemonchus contortus in genetically resistant sheep. *Parasite Immunology,* **15,** 61-67.

Gilmour, A.R., Gogel, B.J., Cullis, B.R. & Thompson, R. (2009) ASReml User Guide: Release 3.0.

Graham, A.L., Shuker, D.M., Pollitt, L.C., Auld, S.K.J.R., Wilson, A.J. & Little, T.J. (2011) Fitness consequences of immune responses: strengthening the empirical framework for ecoimmunology. *Functional Ecology,* **25,** 5-17.

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.

Gustafsson, L. & Sutherland, W.J. (1988) The costs of reproduction in the collared flycatcher *Ficedula albicollis*. *Nature,* **335,** 813.

Hanssen, S.A., Hasselquist, D., Folstad, I. & Erikstad, K.E. (2005) Cost of reproduction in a long-lived bird: incubation effort reduces immune function and future reproduction. *Proceedings of the Royal Society of London B,* **272,** 1039-1046.

Hartig, F. (2018) DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.2.0.

Hayward, A.D., Garnier, R., Watt, K.A., Pilkington, J.G., Grenfell, B.T., Matthews, J.B., Pemberton, J.M., Nussey, D.H. & Graham, A.L. (2014) Heritable, heterogeneous, and costly resistance of sheep against nematodes and potential feedbacks to epidemiological dynamics. *American Naturalist,* **184,** S58-S76.

Hayward, A.D., Pilkington, J.G., Wilson, K., McNeilly, T.N. & Watt, K.A. (2019) Data from: Reproductive effort influences intra-seasonal variation in parasite-specific antibody responses in wild Soay sheep. Dryad Digitial Repository. https://doi.org/10.5061/dryad.886m8t6

Hayward, A.D., Wilson, A.J., Pilkington, J.G., Clutton-Brock, T.H., Pemberton, J.M. & Kruuk, L.E.B. (2011) Natural selection on a measure of parasite resistance varies across ages and environmental conditions in a wild mammal. *Journal of Evolutionary Biology,* **24,** 1664-1676.

Hewitson, J.P., Filbey, K.J., Esser-von Bieren, J., Camberis, M., Schwartz, C., Murray, J., Reynolds, L.A., Blair, N., Robertson, E., Harcus, Y., Boon, L., Huang, S.C.-C., Yang, L., Tu, Y., Miller, M.J., Voehringer, D., Le Gros, G., Harris, N. & Maizels, R.M. (2015) Concerted activity of IgG1 antibodies and IL-4/IL-25-dependent effector cells trap helminth larvae in the tissues following vaccination with defined secreted antigens, providing sterile immunity to challenge infection. *PLOS Pathogens,* **11,** e1004676.

Higaki, S., Nagano, M., Katagiri, S. & Takahashi, Y. (2013) Effects of parity and litter size on the energy contents and immunoglobulin G concentrations of Awassi ewe colostrum. *Turkish Journal of Veterinary and Animal Science,* **37,** 109-112.

Hutchings, M.R., Milner, J.M., Gordon, I.J., Kyriazakis, I. & Jackson, F. (2002) Grazing decisions of Soay sheep, *Ovis aries*, on St Kilda: a consequence of parasite distribution? *Oikos,* **96,** 235-244.

Jackson, F. (1974) New technique for obtaining nematode ova from sheep faeces. *Laboratory Practice,* **23,** 65-66.
Jolles, A.E. & Ezenwa, V.O. (2015) Ungulates as model systems for the study of disease processes in natural populations. *Journal of Mammalogy,* **96,** 4-15.
Khoury, P.B., Stromberg, B.E. & Soulsby, E.J. (1977) Immune mechanisms to *Ascaris suum* in inbred guinea-pigs. I. Passive transfer of immunity by cells or serum. *Immunology,* **32,** 405-411.
Kidane, A., Houdijk, J.G.M., Tolkamp, B.J., Athanasiadou, S. & Kyriazakis, I. (2009) Consequences of infection pressure and protein nutrition on periparturient resistance to *Teladorsagia circumcincta* and performance in ewes. *Veterinary Parasitology,* **165,** 78-87.
Knowles, S.C.L., Nakagawa, S. & Sheldon, B.C. (2009) Elevated reproductive effort increases blood parasitaemia and decreases immune function in birds: a meta-regression approach. *Functional Ecology,* **23,** 405-415.
Lüdecke, D. (2018) _sjstats: statistical functions for regression models. Version 0.17.2.
Luong, L.T., Perkins, S.E., Grear, D.A., Rizzoli, A. & Hudson, P.J. (2010) The relative importance of host characteristics and co-infection in generating variation in *Heligmosomoides polygyrus* fecundity. *Parasitology,* **137,** 1003-1012.
Mayer, B., Zolnai, A., Frenyó, L.V., Jancsik, V., Szentirmay, Z., Hammarström, L. & Kacskovics, I. (2002) Redistribution of the sheep neonatal Fc receptor in the mammary gland around the time of parturition in ewes and its localization in the small intestine of neonatal lambs. *Immunology,* **107,** 288-296.
McCoy, K.D., Stoel, M., Stettler, R., Merky, P., Fink, K., Senn, B.M., Schaer, C., Massacand, J., Odermatt, B., Oettgen, H.C., Zinkernagel, R.M., Bos, N.A., Hengartner, H., Macpherson, A.J. & Harris, N.L. (2008) Polyclonal and specific antibodies mediate protective immunity against enteric helminth infection. *Cell Host & Microbe,* **4,** 362-373.
McCullough, E.L. & Emlen, D.J. (2013) Evaluating the costs of a sexually selected weapon: big horns at a small price. *Animal Behaviour,* **86,** 977-985.
Murphy, K., Travers, P. & Walport, M. (2008) Janeway's Immunobiology. 887.
Murrell, K.D. (1981) Protective role of immunoglobulin G in Immunity to *Strongyloides ratti*. *The Journal of Parasitology,* **67,** 167-173.
Neggazi, S.A., Noreikiene, K., Öst, M. & Jaatinen, K. (2016) Reproductive investment is connected to innate immunity in a long-lived animal. *Oecologia,* **182,** 347-356.
Nordling, D., Andersson, M., Zohari, S. & Lars, G. (1998) Reproductive effort reduces specific immune response and parasite resistance. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **265,** 1291-1298.
Nussey, D.H., Watt, K.A., Clark, A., Pilkington, J.G., Pemberton, J.M., Graham, A.L. & McNeilly, T.N. (2014) Multivariate immune defences and fitness in the wild: complex but ecologically important associations among plasma antibodies, health and survival. *Proceedings of the Royal Society B: Biological Sciences,* **281,** 20132931.
O'Sullivan, B.M. & Donald, A.D. (1970) A field study of nematode parasite populations in the lactating ewe. *Parasitology,* **61,** 301-315.
Palacios, M.G. & Bronikowski, A.M. (2017) Immune variation during pregnancy suggests immune component-specific costs of reproduction in a viviparous snake with disparate life-history strategies. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology,* **327,** 513-522.
R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Richner, H., Christe, P. & Oppliger, A. (1995) Paternal investment affects prevalence of malaria. *Proceedings of the National Academy of Sciences,* **92,** 1192-1194.
Robertson, A., Hiraiwa-Hasegawa, M., Albon, S.D. & Clutton-Brock, T.H. (1992) Early growth and sucking behaviour of Soay sheep in a fluctuating population. *Journal of Zoology,* **227,** 661-672.

Rödel, H.G., Zapka, M., Stefanski, V. & von Holst, D. (2016) Reproductive effort alters immune parameters measured post-partum in European rabbits under semi-natural conditions. *Functional Ecology,* **30,** 1800-1809.
Sawyer, M., H Willadsen, C., Osburn, B. & C McGuire, T. (1978) Passive transfer of colostral immunoglobulins from ewe to lamb and its influence on neonatal lamb mortality. *Journal of the American Veterinary Medical Association,* **171,** 1255-1259.
Sheldon, B.C. & Verhulst, S. (1996) Ecological immunity: costly parasite defences and tradeoffs in evolutionary ecology. *Trends in Ecology and Evolution,* **11,** 317-321.
Stearns, S.C. (1989) Trade-offs in life-history evolution. *Functional Ecology,* **3,** 259-268.
Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.
Strain, S.A.J., Bishop, S.C., Henderson, N.G., Kerr, A., McKellar, Q.A., Mitchell, S. & Stear, M.J. (2002) The genetic control of IgA activity against *Teladorsagia circumcincta* and its association with parasite resistance in naturally infected sheep. *Parasitology,* **124,** 545-552.
Sykes, A.R., Xie, H.L., Stankiewicz, M., Huntley, J.F., Mackellar, A., Sedcole, J.R., McAnulty, R.W. & Green, R. (2007) The effect of vaccinating infection during pregnancy and dietary protein supply on the peri-parturient immune response of sheep to infection with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* larvae. *animal,* **1,** 249-260.
Tabatabaei, S., Nikbakht, G., Vatankhah, M., Sharifi, H. & Alidadi, N. (2013) Variation in colostral immunoglobulin G concentration in fat tailed sheep and evaluation of methods for estimation of colostral immunoglobulin content. *Acta Veterinaria Brno,* **82,** 271-275.
Tempest, L.J. (2005) Parasites and the cost of reproduction in Soay sheep. PhD, University of Stirling.
Watson, R.L., McNeilly, T.N., Watt, K.A., Pemberton, J.M., Pilkington, J.G., Waterfall, M., Hopper, P.R.T., Cooney, D., Zamoyska, R. & Nussey, D.H. (2016) Cellular and humoral immunity in a wild mammal: variation with age & sex and association with overwinter survival. *Ecology and Evolution,* **6,** 8695-8705.
Watt, K.A., Nussey, D.H., Maclellan, R., Pilkington, J.G. & McNeilly, T.N. (2016) Fecal antibody levels as a noninvasive method for measuring immunity to gastrointestinal nematodes in ecological studies. *Ecology and Evolution,* **6,** 56-67.
Wilson, K., Grenfell, B.T., Pilkington, J.G., Boyd, H.E.G. & Gulland, F.M.D. (2004) Parasites and their impact. *Soay Sheep: Dynamics and Selection in an Island Population* (eds T.H. Clutton-Brock & J.M. Pemberton), pp. 113-165. Cambridge University Press, Cambridge.
Xu, Y.-C., Yang, D.-B. & Wang, D.-H. (2012) No evidence for a trade-off between reproductive investment and immunity in a rodent. *PLOS ONE,* **7,** e37182.

SUPPORTING INFORMATION: Supporting tables and figures
Additional supporting information may be found in the online version of this article.

Appendix S1: Supporting Tables and figures

Table S1: Results of generalized linear mixed effects model of FEC in all females.
Table S2: Results of generalized linear mixed effects model of FEC in reproducing females.
Table S3: Variance components from a multivariate mixed-effects model of antibody levels.
Table S4: Variance components from fifteen bivariate mixed-effects models of antibody levels.
Table S5: Results of generalized linear mixed-effects models assessing association between FEC and antibodies.
Table S6: Results of generalized linear mixed-effects models assessing association between FEC and separate antibodies in reproducing females only.
Table S7: Results of generalized linear mixed-effects models assessing association between FEC and all antibodies in reproducing females only.

Table S8: Results of generalized linear mixed-effects models of FEC assessing interactions between reproductive status and antibodies.
Figure S1: A map of the study area.
Figure S2: Effects of reproductive status on changes in antibodies across the season.
Figure S3: Associations between the six different antibodies.
Figure S4: Changes in TcIgG across the season in all individuals studied.

TABLES

Table 1. A comparison of negative binomial generalized linear mixed-effects models (GLMMs) testing for associations between faecal egg count and reproductive effort during the lambing season. The estimate, standard error (SE), χ^2_1 statistic and P-value are given for the model parameter denoted under 'Structure'. Each model was compared with the model denoted in 'Structure': e.g. model 4 is simply model 3 plus the interaction term; hence, to test the interaction, we compared models 3 and 4. The superscripts by the P-values denotes the figures where the association is presented graphically.

Model	Structure	Estimate	SE	NDF	χ^2	P
	All females (N = 266; individuals = 54)					
0	Location + Age					
1	0 + Date	0.0258	0.0045	1	31.87	<0.001[1A]
2	1 + Date²	0.0005	0.0004	1	1.50	0.220
3	1 + Reproduced	-0.0508	0.5976	1	0.01	0.932
4	3 + Reproduced:Date	0.0348	0.0141	1	5.80	0.016[2]
	Reproducing females (N = 238; individuals = 47)					
0	Location + Age					
1	0 + Date	0.0315	0.0050	1	36.84	<0.001
2	1 + Date²	0.0004	0.0004	1	0.88	0.349
3	1 + Litter weight	-0.0061	0.3652	1	0.00	0.987
4	3 + Litter weight:Date	0.0257	0.0105	1	5.81	0.016[1B]

Table 2. A comparison of linear mixed-effects models (LMMs) testing for associations between six different antibody isotypes and reproductive effort during the lambing season. The estimate, standard error (SE), χ^2_1 statistic and P-value are given for the model parameters denoted under 'Structure'. The superscript letter by the P-values denotes positions in Figure S2.

Isotype	Model	Structure	Estimate	SE	NDF	χ^2	P
TcIgA	0	Location + Age					
	1	0 + Date	0.0038	0.0020	1	3.58	0.058[A]
	2	1 + Date²	0.0003	0.0002	1	3.17	0.075
	3	1 + Reproduced	0.0313	0.0838	1	0.14	0.709
	4	3 + Reproduced:Date	-0.0093	0.0067	1	1.92	0.165
TcIgG	0	Location + Age					
	1	0 + Date	0.0020	0.0015	1	1.68	0.195
	2	1 + Date²	0.0000	0.0001	1	0.13	0.716
	3	1 + Reproduced	-0.0314	0.0652	1	0.23	0.630
	4	3 + Reproduced:Date	-0.0115	0.0052	1	4.87	0.027[B]
TcIgM	0	Location + Age					
	1	0 + Date	0.0000	0.0008	1	0.00	0.955
	2	1 + Date²	0.0001	0.0001	1	0.76	0.384
	3	1 + Reproduced	-0.0618	0.0390	1	2.40	0.121
	4	3 + Reproduced:Date	-0.0027	0.0025	1	1.15	0.284
TotIgA	0	Location + Age					
	1	0 + Date	0.0052	0.0027	1	3.67	0.055[C]
	2	1 + Date²	0.0002	0.0002	1	0.52	0.469
	3	1 + Reproduced	-0.0223	0.1218	1	0.03	0.855
	4	3 + Reproduced:Date	-0.0133	0.0092	1	2.08	0.149
TotIgG	0	Location + Age					
	1	0 + Date	0.0045	0.0024	1	3.38	0.066[E]
	2	1 + Date²	0.0001	0.0020	1	0.26	0.610
	3	1 + Reproduced	-0.0392	0.1029	1	0.15	0.703
	4	3 + Reproduced:Date	-0.0165	0.0084	1	3.81	0.051[F]
TotIgM	0	Location + Age					
	1	0 + Date	0.0037	0.0027	1	1.98	0.160
	2	1 + Date²	-0.0001	0.0002	1	0.08	0.782
	3	1 + Reproduced	-0.2404	0.1643	1	2.09	0.149
	4	3 + Reproduced:Date	-0.0145	0.0086	1	2.80	0.095[D]

Table 3. A comparison of linear mixed-effects models (LMMs) testing for associations between six different antibody isotypes and litter weight during the lambing season. The estimate, standard error (SE), χ^2_1 statistic and P-value are given for the model parameters denoted under 'Structure'. The superscript letter by the P-values denotes positions in Figure 3.

Isotype	Model	Structure	Estimate	SE	NDF	χ^2	P
TcIgA	0	Location + Age					
	1	0 + Date	0.0034	0.0021	1	2.56	0.110
	2	1 + Date²	0.0002	0.0002	1	1.82	0.178
	3	1 + Litter weight	-0.1088	0.0610	1	2.98	0.084
	4	3 + Litter weight:Date	-0.0096	0.0042	1	5.03	0.025[A]
TcIgG	0	Location + Age					
	1	0 + Date	0.0017	0.0015	1	1.21	0.271
	2	1 + Date²	0.0000	0.0001	1	0.00	0.999
	3	1 + Litter weight	-0.0991	0.0452	1	4.54	0.033[B]
	4	3 + Litter weight:Date	-0.0045	0.0031	1	2.13	0.145
TcIgM	0	Location + Age					
	1	0 + Date	-0.0003	0.0008	1	0.14	0.709
	2	1 + Date²	0.0000	0.0001	1	0.50	0.479
	3	1 + Litter weight	-0.0496	0.0276	1	3.04	0.081
	4	3 + Litter weight:Date	-0.0036	0.0015	1	5.53	0.019[C]
TotIgA	0	Location + Age					
	1	0 + Date	0.0049	0.0028	1	3.02	0.082
	2	1 + Date²	0.0001	0.0002	1	0.17	0.676
	3	1 + Litter weight	-0.0875	0.0879	1	0.97	0.325
	4	3 + Litter weight:Date	-0.0159	0.0056	1	7.86	0.005[D]
TotIgG	0	Location + Age					
	1	0 + Date	0.0036	0.0025	1	2.11	0.146
	2	1 + Date²	0.0001	0.0002	1	0.12	0.725
	3	1 + Litter weight	-0.1713	0.0688	1	5.75	0.016
	4	3 + Litter weight:Date	-0.0166	0.0049	1	11.13	<0.001[E]
TotIgM	0	Location + Age					
	1	0 + Date	0.0026	0.0025	1	1.08	0.299
	2	1 + Date²	-0.0002	0.0002	1	0.91	0.341
	3	1 + Litter weight	-0.2323	0.1151	1	3.84	0.050
	4	3 + Litter weight:Date	-0.0111	0.0050	1	4.74	0.029[F]

Table 4. Individual- and Residual-level variance components from the multivariate mixed-effect model of six antibody isotypes. Estimated variances for each antibody are shown on the diagonal in the shaded boxes, with the proportion of variance explained by that component in parentheses. For example, for TCIRC IgA, the individual variance component was 0.02, explaining 18% of the variation. Below-diagonal terms show covariances between pairs of antibodies, while above-diagonal terms show correlations standardized to between -1 and +1. Full model results are provided in Table S3.

Variance component	Antibody	TcIgA	TcIgG	TcIgM	TotIgA	TotIgG	TotIgM
Individual	TCIRC IgA	0.02 (0.18)	0.2756	0.2199	0.6456	0.0774	0.1607
	TCIRC IgG	0.0042	0.01 (0.16)	-0.1249	0.0761	0.8211	0.1667
	TCIRC IgM	0.0022	-0.0009	<0.00 (0.26)	0.3107	-0.0967	0.8862
	Total IgA	0.0177	0.0015	0.0041	0.04 (0.17)	0.1105	0.2676
	Total IgG	0.0016	0.0122	-0.0009	0.0029	0.02 (0.11)	0.2593
	Total IgM	0.0080	0.0061	0.0212	0.0174	0.0125	0.12 (0.43)
Residual	TCIRC IgA	0.09 (0.82)	0.7862	0.6423	0.8760	0.6678	0.6595
	TCIRC IgG	0.0595	0.06 (0.84)	0.6198	0.7014	0.8694	0.6786
	TCIRC IgM	0.0231	0.0178	0.01 (0.74)	0.5588	0.5807	0.6329
	Total IgA	0.1145	0.0732	0.0277	0.18 (0.83)	0.7167	0.7599
	Total IgG	0.0829	0.0862	0.0274	0.1227	0.16 (0.89)	0.7170
	Total IgM	0.0801	0.0659	0.0292	0.1274	0.1141	0.16 (0.57)

FIGURE LEGENDS

Figure 1. An adult female Soay sheep with new-born lamb. Photo by Jill Pilkington.

Figure 2. (A) Strongyle faecal egg count (FEC) generally increased across the season; points show raw data; line and shaded area show predicted FEC±95% confidence intervals from model 1 in the upper part of Table 1. Among females that reproduced, (B) the increase in FEC across the season was stronger in females that produced heavier litters (darker lines) than those that produced lighter litters (lighter lines); lines show predictions from model 4 in the lower part of Table 1; filled symbols show raw data for females with above-median litter weight and open symbols show raw data for females with equal-to-or-below-median litter weight. Predictions are given for 90% of the range of corrected litter weight (1kg – 2.8kg) and 90% of the range of date (day -24 – day +9).

Figure 3. Strongyle faecal egg count (FEC) did not appear to change across the season in females which did not produce a lamb, but generally increased across the season in females which did reproduce. Points show raw data and line and shaded area show predicted FEC±95% confidence intervals from model 4 in the upper part of Table 1. Predictions are given for 90% of the range of date (day -24 – day +9).

Figure 4. Among reproducing females, all of the antibodies apart from worm-specific IgG showed evidence for changes across the lambing season that were influenced by corrected litter weight. In (A) and (C-F), females with lighter litters (lighter-coloured lines) showed a greater increase in antibody levels across the season than females with heavier litters (darker lines). Points indicate raw data, with closed points showing data for females with corrected litter weights greater than the median of 2.034kg and open points showing data for females with corrected litter weights at or under the median. In (B), females with heavier litters exhibited lower worm-specific IgG levels. Points show the raw data, while the line and shaded area show predicted worm-specific IgG±95%CI. Model predictions are shown for 90% of the distribution of date (day -24 to day +9) and 90% of the distribution of litter weight (1kg – 2.8kg).

Figure 5. Results from a linear-mixed effects model of natural log-transformed FEC, as described in Table 4, showing (A) a negative linear association with *T. circumcincta*-specific IgG and (B) a positive linear association with total IgM. Points show raw data; lines and shaded areas show model predictions ± 95%CI. Predictions are plotted over 90% of the range of IgG (0.05-0.95OD) and IgM (0.1-1.66OD).