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# Reproduction has different costs for immunity and parasitism in a wild mammal

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Data Accessibility: The data supporting this work are available at <https://github.com/gfalbery/ReproductiveCosts>.

Author contributions: GFA collected the samples, conducted labwork, analysed the data, and drafted the manuscript; KW designed and helped to carry out the ELISAs; RK carried out some antibody extractions and ELISAs; SM and AM helped with sample collection; FK, DN, JP offered comments on methodology and theory throughout and helped draft the manuscript.

Keywords: disease ecology, ecoimmunology, helminths, life history, parasites, reproduction, tradeoff, wild mammal

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## 39 Abstract

- 40 1. Life history theory predicts that reproductive allocation draws resources away from  
41 immunity, resulting in increased parasitism. However, studies of reproductive tradeoffs  
42 rarely examine multiple measures of reproduction, immunity, and parasitism. It is  
43 therefore unclear whether the immune costs of reproductive traits correlate with their  
44 resource costs, and whether increased parasitism emerges from weaker immunity.
- 45 2. We examined these relationships in wild female red deer (*Cervus elaphus*) with  
46 variable reproductive allocation and longitudinal data on mucosal antibody levels and  
47 helminth parasitism. We noninvasively collected faecal samples, counting propagules  
48 of strongyle nematodes (order: Strongylida), the common liver fluke *Fasciola hepatica*  
49 and the red deer tissue nematode *Elaphostrongylus cervi*. We also quantified both total  
50 and anti-strongyle mucosal IgA to measure general and specific immune allocation .
- 51 3. Contrary to our predictions, we found that gestation was associated with decreased  
52 total IgA but with no increase in parasitism. Meanwhile, the considerable resource  
53 demand of lactation had no further immune cost but was associated with higher counts  
54 of strongyle nematodes and *Elaphostrongylus cervi*. These contrasting costs arose  
55 despite a negative correlation between antibodies and strongyle count, which implied  
56 that IgA was indicative of protective immunity.
- 57 4. Our findings suggest that processes other than classical resource allocation tradeoffs  
58 are involved in mediating observed relationships between reproduction, immunity, and  
59 parasitism in wild mammals. In particular, reproduction-immunity tradeoffs may result  
60 from hormonal regulation or maternal antibody transfer, with parasitism increasing as  
61 a result of increased exposure arising from resource acquisition constraints. We  
62 advocate careful consideration of resource-independent mechanistic links and  
63 measurement of both immunity and parasitism when investigating reproductive costs.

## 64 Introduction

65 Costly traits are central to the fields of life history theory and ecoimmunology. Tradeoffs arising  
66 between reproductive allocation and other aspects of life history are a fundamental prediction  
67 of the former (Harshman & Zera, 2007; Stearns, 1989; Williams, 1966), while the latter  
68 examines the ecology of costly immune responses (Graham et al., 2011; Sheldon & Verhulst,  
69 1996). Because reproduction and immunity compete for host resources, in resource-limited  
70 environments, animals that reproduce should have fewer resources to allocate to immune  
71 defences (Deerenberg, Arpanius, Daan, & Bos, 1997; French, Denardo, & Moore, 2007;  
72 Sheldon & Verhulst, 1996). If immunity is protective, these individuals will experience higher  
73 parasitism as a result. Intuitively, traits with higher resource demands should result in the  
74 diversion of more resources away from immunity, leading to higher parasite burdens.  
75 However, recent advances have demonstrated that the interrelationships between host  
76 resources, immunity, and parasitism can be unexpectedly complex (Cressler, Nelson, Day, &  
77 Mccauley, 2014). In addition, reproduction may alter allocation to different immune  
78 components (Becker et al., 2018; Rödel, Zapka, Stefanski, & von Holst, 2016), yet the reasons  
79 for this differential allocation are poorly understood. Few studies in wild mammals have  
80 examined tradeoffs with multiple reproductive traits, so it is unclear whether different  
81 components of reproduction have different costs for immune defence, and whether their costs  
82 are proportional to their resource demand. Furthermore, studies of reproductive tradeoffs  
83 rarely quantify both immunity and parasitism to examine the importance of susceptibility  
84 versus exposure in driving higher parasite intensities in reproductive females (Bradley &  
85 Jackson, 2008; Knowles, Nakagawa, & Sheldon, 2009). Here, we examine the partitioning of  
86 reproductive costs for multiple measures of immunity and parasitism to investigate the  
87 possible mechanisms governing a reproduction-immunity-parasitism tradeoff in a wild  
88 mammal.

89 Mammalian reproduction is a complex, multi-stage process, featuring extensive maternal  
90 allocation which varies in intensity through the reproductive period (Langer, 2008; Maestriperi

91 & Mateo, 2009). As such, different components of reproduction vary substantially in their  
92 resource and fitness costs. In particular, lactation is a highly energetically demanding process  
93 which carries costs for immunity, parasitism or fitness in a range of mammals (Beasley, Kahn,  
94 & Windon, 2010; Christe, Arlettaz, & Vogel, 2000; Clutton-Brock, Albon, & Guinness, 1989;  
95 Froy, Walling, Pemberton, Clutton-brock, & Kruuk, 2016; Jones, Sakkas, Houdijk, Knox, &  
96 Kyriazakis, 2012; Rödel et al., 2016; Woodroffe & Macdonald, 1995). Meanwhile, only one of  
97 these studies uncovered an immunological cost of gestation (Christe et al., 2000), which  
98 requires fewer resources than does lactation (Clutton-Brock et al., 1989). However, although  
99 experimentally modifying resource availability can affect the severity of reproduction-immunity  
100 tradeoffs (French et al., 2007; Jones et al., 2012), this is not always the case (Stahlschmidt,  
101 Rollinson, Acker, & Adamo, 2013). Similarly, studies in birds have questioned whether the  
102 energetic costs of immunity are sufficient to drive tradeoffs (Eraud, Duriez, Chastel, & Faivre,  
103 2005; Svensson, Råberg, Koch, & Hasselquist, 1998). Such findings imply that reproduction-  
104 immunity tradeoffs can be linked mechanistically as well as through resource reallocation.  
105 Potential such links include production of reactive oxygen species, reduction in  
106 immunologically active fat stores, or resource-independent hormonal regulation (Speakman,  
107 2008; Svensson et al., 1998).

108 Different components of mammalian reproduction can have qualitatively different effects on  
109 host immunity as well as varying quantitatively in terms of their resource demand. For  
110 example, pregnancy necessitates modulation of the immune system to avoid mounting an  
111 immune response to the developing foetus, which will directly affect anti-parasite defence  
112 (Weinberg, 1984, 1987). Similarly, lactation draws immune molecules away from the mother  
113 for transfer to offspring, reducing their availability for the mother's own defence (Grindstaff,  
114 Brodie, & Ketterson, 2003; Hasselquist & Nilsson, 2009). Reproduction also induces a suite  
115 of physiological and behavioural changes which will affect susceptibility and exposure to  
116 parasites indirectly: for example, it has been suggested that bats compensate for the energetic  
117 demand of lactation by reducing costly grooming behaviour, with ectoparasite burden

118 increasing as a result (Speakman, 2008). It is unclear how such mechanistic links between  
119 components of reproduction and immunity interact with resource allocation to influence  
120 immune defence and parasite intensity in wild mammals.

121 The wild red deer (*Cervus elaphus*) in the North block of the Isle of Rum exhibit a well-studied  
122 life history tradeoff, in which reproduction substantially decreases the mother's probability of  
123 overwinter survival and reproduction the following year (Clutton-Brock et al., 1989; Froy et al.,  
124 2016). However, not all components of reproduction are equally costly: gestation has a  
125 negligible detectable fitness cost compared to that of lactation (Clutton-Brock et al., 1989).  
126 Moreover, while giving birth late and caring for a male calf compared to a female calf are  
127 associated with decreased maternal fitness, their effects are small compared to the cost of  
128 lactation itself (Froy et al., 2016). The study population has a high prevalence of  
129 gastrointestinal helminths, and parasite burdens can be quantified noninvasively through  
130 faecal propagule counts (Albery et al., 2018). A previous investigation into the parasite  
131 community of Rum deer living outside the study area identified multiple genera of strongyle  
132 nematodes, including *Trichostrongylus*, *Oesophagostomum*, *Cooperia*, a group of ostertagiids  
133 and the red deer-specific nematode *Elaphostrongylus cervi* (Irvine, Corbishley, Pilkington, &  
134 Albon, 2006). Mucosal antibodies, and especially the IgA isotype, are important effectors of  
135 ruminant adaptive immunity to gut helminths (Butler, 1969; McRae, Stear, Good, & Keane,  
136 2015). Mucosal IgA can be quantified in wild ruminant faeces, correlating positively with the  
137 same isotype measured in plasma or serum and negatively with helminth faecal egg counts  
138 (Watt, Nussey, Maclellan, Pilkington, & McNeilly, 2016).

139 In this study, we measured both total and helminth-specific mucosal IgA and propagule counts  
140 of multiple helminth species, using faecal samples collected from the Isle of Rum study  
141 population. We quantified the associations between several reproductive traits of known  
142 fitness cost and subsequent measures of immunity and parasitism. We also examined  
143 covariance between IgA and parasites to discern whether increased IgA was associated with  
144 decreased parasite intensity independently of shared reproductive and seasonal effects,

145 implicating IgA as an indicator of protective immunity. We predicted that reproductive  
146 allocation would be associated with reduced antibody levels and increased parasite counts,  
147 and that aspects of reproduction previously found to be more costly for fitness, especially  
148 lactation, should likewise be more costly in terms of both immunity and parasitism.  
149 Furthermore, providing parasitism is mediated by immune susceptibility, aspects of  
150 reproduction that are costly for immunity should have similar costs in terms of parasitism.

## 151 **Methods**

### 152 **Study system, sampling and parasitology**

153 The study population is located in the North block of the Isle of Rum National Nature Reserve  
154 in the Inner Hebrides, Scotland (57°N 6°20'W). The resident population comprises ~350  
155 animals at any one time, and is regularly censused to keep track of each individual and its life  
156 history. See Clutton-Brock *et al.* (1982) for a full summary of the project and the deer  
157 reproductive cycle. Briefly, the deer mate in September and October and give birth in May-  
158 June, after an approximately 235 day gestation. Females do not reproduce every year, and  
159 produce a maximum of one calf per year. During the calving season, daily monitoring of  
160 pregnant females enables the recording of precise birth dates. Neonates are caught, sexed,  
161 weighed and individually marked, enabling life-long individual identification. Calves are  
162 dependent on their mothers for much of their first year. Regular population censusing  
163 throughout the year and winter mortality searches allow dates of death to be reliably assigned  
164 to the nearest month for the vast majority of individuals. Most calf deaths occur either within  
165 the first few weeks of life, or in the following winter ~6-9 months later. Females that  
166 successfully raise a calf to the age of one, or that lose the calf in its first winter, have lower  
167 rates of overwinter survival and reproduction the following year compared to those that do not  
168 reproduce that year or that lose their calf in the summer (Clutton-Brock *et al.*, 1989; Froy *et al.*  
169 *et al.*, 2016). Many calves die over the winter, but the mothers of these calves have paid the cost  
170 of lactation associated with feeding them until the winter, whether or not the calf survive.

171 Therefore these females are treated as a single category here (Clutton-Brock et al., 1989;  
172 Froy et al., 2016).

173 We collected faecal samples from female deer across the annual reproductive cycle. As a  
174 “deer year” runs from May to April, this study examines the effects of reproduction over a year,  
175 beginning in May, on egg counts and antibody levels until the following April. A description of  
176 the sample collection procedure can be found in Albery *et al.* (2018). Sampling occurred over  
177 seven two-week sampling trips spanning April 2016-April 2018, in August (“summer”),  
178 November (“autumn”) and April (“spring”). Note that our dataset included an April sampling  
179 trip from the deer reproductive cycle starting May 2015, without an accompanying August and  
180 November trip from this reproductive cycle. Figure 1 illustrates how sampling relates to  
181 different aspects of reproductive allocation by female deer across the annual cycle. We  
182 classify a female’s reproductive status for a given year as “No Calf”, “Calf Died” and “Calf  
183 Survived” (see Figure 1). “No Calf” samples were collected from females that did not  
184 reproduce in the calving season preceding the sampling trip; “Calf Died” samples were  
185 collected from females that gave birth to a calf in the preceding calving season which died  
186 before October 1<sup>st</sup> of that year; and “Calf Survived” samples were collected from females that  
187 gave birth to a calf in the preceding calving season which survived past October 1<sup>st</sup> of that  
188 year. We excluded females that were reproducing for the first time from our analyses, as their  
189 reproductive success is heavily confounded with their young age (mean age 4.21 years). In  
190 addition, females may or may not become pregnant during the autumn rut. Samples were  
191 therefore assigned a pregnancy status, beginning in November, based on whether or not the  
192 female gave birth to a calf in the following spring (Figure 1). It is possible that some females  
193 that did not produce a calf conceived but lost the pregnancy. This is most likely to occur very  
194 early in gestation, in which case the female has not borne much of the cost of pregnancy.  
195 Pregnancy becomes obvious in spring from body shape, and udder size and such females  
196 always produce a calf; we therefore do not believe that cryptic pregnancies would introduce  
197 substantial variation into our analysis.



198 In total 837 faecal samples were collected noninvasively from 140 mature females. All samples  
199 were collected by observing known females from a distance, marking the spot in which  
200 defecation happened, and promptly collecting the pellets. In the evening after collection,  
201 samples were homogenised by hand and subsampled, with 1-15g frozen at -20°C for antibody  
202 quantification and the remainder refrigerated at 4°C for parasitological analysis. Subsamples  
203 were transferred to Edinburgh at these temperatures. Parasite propagule counts were carried  
204 out as previously described, without correcting for dry weight, and included counts of strongyle  
205 nematodes (order: Strongylida; counted using a sedimentation-salt flotation method), the  
206 common liver fluke *Fasciola hepatica* (counted using a sedimentation method) and the red  
207 deer-specific tissue nematode *Elaphostrongylus cervi* (isolated and counted using a  
208 baermannisation method; see Albery et al., 2018 for detailed methods). Because of the  
209 difficulty identifying strongyle nematodes from egg morphology, we group them together here  
210 at the order level. Final sample sizes for each variable are displayed in Table SI1. All samples  
211 were counted as a subsample and divided by the weight of the subsample, providing an eggs  
212 per gram (EPG) or larvae per gram (LPG) value.

### 213 Antibody extraction and quantification

214 Faecal antibodies were quantified using a protocol modified from Watt *et al.* (2016). Faecal  
215 matter was stored at -20°C until extraction. Extractions occurred either in January-March 2017  
216 (session “A”, samples collected April-November 2016; N=132), January 2018 (“B”, samples  
217 collected April-November 2016; N=212) or within the sampling trip (“C”, samples collected  
218 April 2017-April 2018, N=460). 0.6g (+/- 0.005g) of the homogenate was weighed out into an  
219 Eppendorf tube and mixed thoroughly with 0.9ml of protease inhibitor solution (cOmplete™  
220 Mini Protease Inhibitor Cocktail tablets, Roche, Basel, Switzerland; 1 tablet mixed with 7ml  
221 Phosphate Buffered Saline). The mixture was left to stand for a minimum of 5 minutes to allow  
222 the protease to act and then centrifuged at 10,000g for 5 minutes. The supernatant was  
223 removed using a micropipette and stored in a separate Eppendorf tube at -20°C until ELISA.

224 We measured two antibodies by faecal ELISA: total IgA and anti-*Teladorsagia circumcincta*  
225 third larval stage IgA (anti-Tc IgA), using a method developed in sheep (Watt et al., 2016). *T.*  
226 *circumcincta* is an abundant and important sheep strongyle, and is also present in the Rum  
227 deer (unpublished data). This method for detecting anti-*T. circumcincta* antibodies shows high  
228 cross-reactivity with other strongyle species (Froy et al., in review). Anti-Tc IgA correlates  
229 therefore negatively with order-level strongyle faecal egg count and with species-level counts  
230 of other strongyle species in wild Soay sheep (Watt et al., 2016; Froy et al., in review). We  
231 therefore interpret this assay as representing a general anti-strongyle response rather than a  
232 response to *T. circumcincta* specifically. ELISA plates were coated the night before using  
233 sheep-derived capture antibodies (Bethyl Laboratories, Montgomery, TX) for total IgA and with  
234 third larval stage antigen for anti-Tc IgA (Moredun Research Institute, Penicuik, Scotland). For  
235 total IgA the samples were diluted in the ratio 1:64; due to lower antibody concentrations  
236 undiluted supernatant was used for the anti-Tc IgA assay. After this stage, the ELISA protocol  
237 was carried out as described in Watt *et al.* (2016). The total IgA dilution was selected by  
238 carrying out serial dilutions on a set of samples and selecting the dilution at which different  
239 concentrations of antibodies were deemed to have the widest spread of optical densities.  
240 ELISA readings diluted linearly as expected. Samples were corrected using controls according  
241 to the calculation: Final OD=(sample OD-mean plate negative OD)/(mean plate positive OD-  
242 mean plate negative OD). All samples were run on duplicate plates, which were highly  
243 correlated (R=0.98 across all duplicates). The mean value for the two duplicates was taken  
244 for each sample and used for analysis.

## 245 **Statistical analysis**

246 We used four sets of Generalised Linear Mixed Models (GLMMs) to test how reproductive  
247 traits were associated with antibody levels and parasite intensity. Analyses were carried out  
248 in R version 3.5.0 (R Core Team 2018) with the package MCMCglmm (Hadfield, 2010). All  
249 models were run for  $2.6 \times 10^6$  iterations with a 2000 iteration thinning interval and a  $6 \times 10^5$   
250 iteration burn in period. Models were run on 5 chains, and convergence of the chains was

251 assessed using the Gelman-Rubin criterion. Posterior prediction was used to confirm that the  
252 model estimates recapitulated the data distribution and between-group differences.  $P_{MCMC}$   
253 values for differences between factor categories were calculated using the proportional  
254 overlap of estimates' posterior distributions, divided by half the number of iterations.

## 255 Full models

256 We first constructed five univariate GLMMs using the full dataset (837 samples from 140  
257 individuals). Three models used an overdispersed Poisson distribution in MCMCglmm, which  
258 accounts for overdispersion in the data in order to approximate a negative binomial  
259 distribution, with strongyle, *F. hepatica* and *E. cervi* intensity as response variables (Albery et  
260 al., 2018). Models initially included the following fixed effects, without interactions: Year (factor  
261 with three levels representative of the deer reproductive cycle beginning in 2015, 2016 and  
262 2017); Season (factor with three levels: Summer, Autumn and Spring); Age in years  
263 (continuous); and Reproductive Status (factor with three levels: No Calf, Calf Died and Calf  
264 Survived). Individual identity was fitted as a random effect. All continuous variables except  
265 parasite counts were scaled to have a mean of 0 and a standard deviation of 1 before analysis.

266 The two remaining models examined antibodies as response variables. As mucosal antibodies  
267 are vulnerable to degradation by temperature-dependent faecal proteases, we had to account  
268 for the extraction session and time to freezing and extraction (Figure SI5-6). There was an  
269 uneven distribution of year, season, and status categories across different extraction sessions,  
270 so that these variables could not all be fitted in the same model. Therefore, to control for  
271 collection factors and quantify reproductive status effects conservatively (risking losing some  
272 information) we first transformed antibody levels to approximate normality (square-root  
273 transform for total IgA and cube-root transform for anti-Tc IgA), and fitted a linear model with  
274 fixed effects including extraction sessions performed at different times (factor with three  
275 levels); day of collection within a sampling trip (continuous integers, range 0-11); time elapsed  
276 from sample collection until freezing (continuous, in hours). The scaled residual values from

277 these models (mean=0, SD=1) were used as the response variables in two Gaussian GLMMs  
278 with the same fixed and random effects as the parasite GLMMs.

279 Previous work on the Rum deer revealed extensive seasonal fluctuations in parasite count  
280 (Albery et al., 2018). We therefore followed up the above five models by fitting a season by  
281 reproductive status interaction in order to investigate whether the effects of reproductive status  
282 varied by season. Each model was compared with and without this interaction to investigate  
283 whether it affected Deviation Information Criterion (DIC) values as a measure of model fit  
284 (threshold values for distinguishing between models  $\Delta$ DIC=2) or changed model estimates.

### 285 **Pregnancy models**

286 Pregnancy may directly affect immunity, and effects attributed to reproductive status could be  
287 due to correlated variation in pregnancy status over the sampling year. To check this we ran  
288 a second set of models investigating the role of pregnancy status. This used a subset of  
289 samples collected in November and April (518 samples from 122 individuals), as mating  
290 occurs in the early autumn and females could not be pregnant in August. These five models  
291 featured the same explanatory variables as the full status models, with only two levels in the  
292 season category (Autumn and Spring), and with Pregnancy included as a binary variable. We  
293 compared these models with and without the pregnancy term as a fixed effect to investigate  
294 whether its inclusion changed reproductive status effect sizes or affected model fit.

### 295 **Calving trait models**

296 We next used a restricted dataset consisting of individuals that had given birth in the year of  
297 sampling (571 samples from 116 individuals) to investigate whether specific traits associated  
298 with a calving event influenced antibody levels and parasitism. We fitted the same fixed and  
299 random effects as the full model set, but with only two factor levels in the reproductive status  
300 category (Calf Died and Calf Survived), and including several variables relating to each birth:  
301 Parturition Date (continuous, centred around median birth date that year); Birth Weight

302 (continuous, in kilograms, calculated from a projection using capture weight and age in hours,  
303 slope 0.01696 kg/h); Calf Sex (Female or Male).

### 304 Multivariate model

305 Multivariate mixed-effects models can be used to investigate covariance between measures  
306 of immunity and parasitism, while accounting for fixed effects. To test whether antibodies and  
307 parasites were correlated we fitted a model with strongyles, *E. cervi*, total IgA and anti-Tc IgA  
308 as response variables, with the same fixed effects as the full univariate models. We specified  
309 Poisson and Gaussian distributions for the parasites and antibodies respectively, as in the  
310 univariate models. Unstructured variance/covariance matrices were fitted for random and error  
311 terms, allowing estimation of the phenotypic correlations between the response variables.  
312 Phenotypic covariance between two response variables A and B ( $\text{Cov}_{\text{phenotypicA,B}}$ ) is calculated  
313 using the random (G) and residual (R) variance structure of the model, with the formula  
314  $\text{Cov}_{\text{phenotypicA,B}} = \text{Cov}_{\text{IndividualA,B}} + \text{Cov}_{\text{ResidualA,B}}$ . Phenotypic correlation between two response  
315 variables ( $r_{\text{phenotypicA,B}}$ ) was calculated by dividing the phenotypic covariance by the square root  
316 of the sum of the variance in both response variables:  
317  $r_{\text{phenotypicA,B}} = \text{Cov}_{\text{phenotypicA,B}} / (\text{V}_{\text{phenotypeA}} + \text{V}_{\text{phenotypeB}})^{0.5}$ .

## 318 Results

319 Reproduction was associated with both lower antibody levels and increased parasite counts,  
320 but patterns differed considerably between different response variables (Figure 2, SI1).  
321 Compared to “No Calf” individuals, “Calf Survived” status was associated with higher intensity  
322 strongyle ( $P_{\text{MCMC}} < 0.001$ ) and *E. cervi* infection ( $P_{\text{MCMC}} = 0.01$ ), and with lower total IgA  
323 ( $P_{\text{MCMC}} = 0.016$ ) and anti-Tc IgA levels ( $P_{\text{MCMC}} < 0.001$ ). “Calf Survived” females also had higher  
324 parasite counts than “Calf Died” individuals ( $P_{\text{MCMC}} < 0.001$  for strongyles and *E. cervi*), but  
325 these reproductive status categories did not differ in total IgA ( $P_{\text{MCMC}} = 0.502$ ) or anti-Tc IgA  
326 ( $P_{\text{MCMC}} = 0.336$ ; Figure 2-3). “Calf Died” individuals did not differ from “No Calf” females in  
327 strongyle, *E. cervi* or anti-Tc IgA levels (Figure 2) but had lower total IgA levels ( $P_{\text{MCMC}} = 0.018$ ).

328 That is, “Calf Died” individuals had slightly lower total IgA than “No Calf” females, but with  
329 similar parasite intensities, while “Calf Survived” individuals had the same antibody levels as  
330 “Calf Died” individuals, but with increased parasite intensities. *F. hepatica* was not associated  
331 with reproductive status, but decreased with age ( $P_{\text{MCMC}}=0.004$ ) as did *E. cervi* ( $P_{\text{MCMC}}<0.001$ ;  
332 Figure SI1, SI7).

333 Strongyles and both antibodies all exhibited the same seasonality, peaking in the spring and  
334 being lowest in the autumn, with the summer intermediate (Figure 3, all differences  
335  $P_{\text{MCMC}}<0.001$ ). *F. hepatica* was higher in the spring than in the summer or autumn  
336 ( $P_{\text{MCMC}}<0.034$ ), and *E. cervi* was lowest in the summer, with the autumn intermediate  
337 ( $P_{\text{MCMC}}<0.001$ ). There was also some between-year variation: strongyle levels increased  
338 between 2015 and 2016, and again in 2017 (all  $P_{\text{MCMC}}<0.001$ ), while total IgA levels decreased  
339 in 2017 compared to 2015 and 2016 ( $P_{\text{MCMC}}<0.024$ ). Anti-Tc IgA was also lower in 2017 than  
340 2016 ( $P_{\text{MCMC}}<0.001$ ). Inclusion of season-by-status interactions improved strongyle model fit  
341 ( $\Delta\text{DIC}=-3.79$ ), but did not improve the fit of any other models ( $\Delta\text{DIC}<2$ ). Fixed status effects  
342 remained largely unchanged in magnitude or significance, suggesting that the observed  
343 associations with reproductive status were consistent across seasons (Figure 3). All  
344 interaction terms implied an attenuation of reproductive status effects from summer through  
345 winter to spring, rather than any major qualitative change in this association (Figure 3). Both  
346 “Calf Died” and “Calf Survived” females had increased antibody levels and decreased parasite  
347 intensities relative to “No Calf” females over this period. See Figure SI2 for a comparison of  
348 the full model estimates and DIC changes when a season-by-status interaction was included.

349 Pregnancy models examining April and November samples revealed marginally lower total  
350 IgA in pregnant females ( $P_{\text{MCMC}}=0.034$ , Figure 2, SI1, SI3). Including pregnancy status in our  
351 models did not alter the direction or significance of reproductive status effects; in fact, in the  
352 case of total IgA and anti-Tc IgA it increased the significance of the “Calf Survived” category’s  
353 effect (Figure SI3). It also slightly improved the fit of the total IgA model ( $\Delta\text{DIC}=-3.00$ ). No  
354 other models were impacted notably by the inclusion of the pregnancy term, although it slightly

355 reduced the effect size of the “Calf Survived” category in influencing strongyle count (Figure  
356 SI3). Although the “Calf Died” category was not statistically significant in the total IgA  
357 pregnancy model as it was in the full model, the fact that adding and removing pregnancy as  
358 a variable did not change the model estimate (Figure SI3) implies that this did not arise from  
359 confounding effects of pregnancy.

360 None of the calving traits modelled (parturition date, calf birth weight or calf sex) were  
361 associated with maternal parasite or antibody levels (Figure 2, SI1).

362 The fixed effects of the multivariate model were very similar to those of the full models (Figure  
363 SI4). Phenotypic correlations ( $R_p$ ) derived from the variance structure of the multivariate model  
364 are as follows. There were positive correlations between strongyles and *E. cervi* ( $R_p=0.26$ ,  
365  $P_{MCMC}<0.001$ ) and between total and anti-Tc IgA ( $R_p=0.424$ ,  $P_{MCMC}<0.001$ ). Strongyle count  
366 was also weakly negatively correlated with total IgA ( $R_p=-0.074$ ,  $P_{MCMC}=0.016$ ) and slightly  
367 more strongly with anti-Tc IgA ( $R_p=-0.142$ ,  $P_{MCMC}<0.001$ ).

## 368 Discussion

369 Lactation is associated with weaker immunity or increased parasitism in a range of mammals  
370 (Festa-Bianchet, 1989; Jones et al., 2012; Rödel et al., 2016; Woodroffe & Macdonald, 1995).  
371 In accordance with these studies, we found that lactating females had both decreased  
372 antibody levels and increased parasite counts relative to non-reproductive females. In  
373 contrast, gestation is rarely found to be costly for immunity or parasitism in mammals (Irvine  
374 et al., 2006; Rödel et al., 2016; Woodroffe & Macdonald, 1995), and carries no detectable  
375 fitness cost in the Rum red deer (Clutton-Brock et al., 1989). Here, deer that gave birth to a  
376 calf that died within six months, thereby incurring a limited lactation cost, had lower total IgA  
377 levels than non-reproducing females. Gestation therefore carried an immune cost in this study.  
378 We predicted that resource depletion incurred through allocation to a given reproductive trait  
379 would lead to reduced antibody levels, and that this would lead to increased parasite intensity  
380 (Knowles et al., 2009; Sheldon & Verhulst, 1996). Our results deviated from our expectations

381 in two ways: first, gestation's long-lasting immune cost was not accompanied by increased  
382 parasite intensities. Second, the considerable additional resource allocation of prolonged  
383 lactation was not associated with additional immune costs relative to gestation, but was  
384 instead associated with an increase in parasite intensity. These results have two implications:  
385 reproduction-immunity tradeoffs were unlikely to be mediated by simple resource reallocation,  
386 and reproduction-parasitism tradeoffs were unlikely to be mediated solely by immunity –  
387 despite our observation that higher immune allocation was associated with lower parasite  
388 counts between individuals.

389 If gestation's lack of detectable fitness cost in our study population (Clutton-Brock et al., 1989)  
390 demonstrates a small resource cost, why was gestation associated with reduced total IgA  
391 levels, and why did the additional resource cost of lactation not decrease antibody levels  
392 further? First, it is possible that reproductive hormones suppress the immune system without  
393 being sensitive to resource availability (Foo, Nakagawa, Rhodes, & Simmons, 2017;  
394 Svensson et al., 1998). Similarly, gestation may lead to alterations in immune allocation and  
395 antibody production, so that lower IgA resulted from selective allocation to alternative immune  
396 cells or functions rather than from lower absolute resource allocation to immunity.  
397 Reproductive mammals are commonly found to exhibit different (rather than weaker)  
398 immunity, but specific patterns of immune prioritisation are unpredictable. For example,  
399 reproductive vampire bats (*Desmodus rotundus*) prioritise innate over adaptive immunity  
400 (Becker et al., 2018), while reproductive rabbits (*Oryctolagus cuniculus*) exhibit reduced white  
401 blood cell counts but stronger humoral immunity (Rödel et al., 2016). Assessing whether  
402 reproductive deer allocate resources preferentially to aspects of immunity other than mucosal  
403 antibodies would therefore necessitate examining numerous additional immune measures –  
404 however, in this study we were restricted to quantifying mucosal antibodies using noninvasive  
405 faecal samples as the deer are rarely handled as adults (Clutton-Brock et al., 1982).

406 Alternatively, gestation and early lactation may necessitate export of IgA from the gut to the  
407 blood for transfer to offspring (Jeffcoate et al., 1992; Sheldrake, Husband, Watson, & Cripps,



408 1984). In ungulates a substantial proportion of maternal antibody transfer occurs via the  
409 colostrum in the first few days of life (Hurley & Theil, 2011). It is feasible that this diversion of  
410 IgA from the gut occurs around parturition and is detectable for an extended period of time  
411 without an underlying resource allocation tradeoff, creating lower IgA levels in all reproductive  
412 females regardless of their calf's survival. The necessity of transferring immune effectors to  
413 offspring may therefore be an important obligate mechanism contributing to reduced antibody  
414 levels in reproductive wild mammals (Rödel et al., 2016). In a proposed mechanism for the  
415 periparturient rise in helminth egg count in domestic sheep, exportation of IgA from the gut  
416 around parturition releases helminths from immune control (Jeffcoate et al., 1992). However,  
417 in this study, the lower total IgA and intermediate anti-Tc IgA levels in female deer that only  
418 paid the cost of gestation were not accompanied by any change in parasitism. This is  
419 surprising, given that the results of our multivariate model implied that both IgA measures are  
420 representative of increased resistance to strongyles. However, the phenotypic correlations of  
421 strongyles with total IgA and with anti-Tc IgA are relatively weak ( $R=0.07$  and  $0.14$   
422 respectively); this is unsurprising, given the messy nature of ecoimmunological data, yet it also  
423 implies the need for other mechanisms of resistance and exposure contributing to parasitism.  
424 As such, additional immune measures would be desirable. Our application of a veterinary  
425 reagent to a non-model species may have introduced some variation; however,  
426 ecoimmunological tools for non-model systems are thin on the ground (Garnier & Graham,  
427 2014), and we believe that our findings of reproduction-immunity tradeoffs and a significant  
428 negative correlation with parasitism implies that this measure is immunologically relevant.

429 If antibody levels were indicative of allocation to protective immunity, how were the deer that  
430 paid the immune cost of gestation able to maintain low strongyle and *E. cervi* intensities? Or,  
431 what produced the higher parasite counts in lactating individuals? Lactating females' anti-Tc  
432 IgA levels were lower than nonreproductive females', which could explain their increased  
433 parasitism in the absence of a contrast with any other reproductive categories. However,  
434 levels of total and anti-Tc IgA in lactating females were not detectably lower than those

435 exhibited by females that paid the cost of gestation (Figure 2). This disparity suggests that  
436 additional processes such as exposure were important in driving the high parasite intensities  
437 in lactating females (Knowles et al., 2009; Sheldon & Verhulst, 1996). The energetic and  
438 resource demand of milk production necessitates substantially increased forage intake and  
439 grazing time (Hamel & Côté, 2008, 2009), and may reduce feeding selectivity or the ability to  
440 exhibit parasite avoidance behaviours (Hutchings, Judge, Gordon, Athanasiadou, &  
441 Kyriazakis, 2006; Speakman, 2008). In addition, hinds inevitably share space with their calves  
442 and those of other females, which exhibit very high parasite intensities (Albery et al., 2018).  
443 Thus, lactating females may suffer increased exposure to infective larvae, resulting in higher  
444 parasite burdens. This mechanism offers an explanation for our observation that lactation was  
445 associated with increased parasite counts, while gestation was not, as individuals that lost  
446 their calf as a neonate were not then saddled with a necessity for such high resource  
447 acquisition. Given that we observed some inter-annual variation in parasitism, it is possible  
448 that annual variation in density and resource availability may alter the severity of this  
449 reproduction-parasitism tradeoff. Based on our results, we suggest that severe effects of  
450 mammalian reproduction on parasite infection are partly mediated by exposure as a result of  
451 constraints on resource acquisition, foraging selectivity, and antiparasite behaviours, in  
452 addition to increased immune susceptibility represented by antibody levels or additional  
453 unmeasured parameters.

454 Effects of foraging on exposure can profoundly affect epidemiological dynamics: for example,  
455 in the water flea *Daphnia dentifera*, temperature-induced increases in food intake can increase  
456 the magnitude of fungal pathogen epidemics (Shocket et al., 2018). Similar processes may  
457 act in the deer, if spatiotemporal variation in climatic conditions, deer density, or food  
458 abundance modify feeding behaviour or the threat of exposure. In particular, strongyle and *E.*  
459 *cervi* parasitism will be further exacerbated in years and areas of the study system where deer  
460 density is high and food availability is low (Wilson, Grenfell, Pilkington, Boyd, & Gulland, 2004).  
461 It is possible that higher parasitism in reproductive individuals will reduce their fitness, thereby

462 producing lactation's fitness cost – and, by extension, gestation's lack of fitness cost – in this  
463 system (Clutton-Brock et al., 1989; Froy et al., 2016; Harshman & Zera, 2007; Williams, 1966).  
464 If exposure is determining parasitism and parasitism is reducing fitness, we would expect that  
465 parasite-mediated life history tradeoffs would be exacerbated in years and areas of high deer  
466 density, as more deer will translate to higher levels of pasture contamination (Wilson et al.,  
467 2004). However, a previous study in this population determined that accounting for strong  
468 spatial autocorrelation in immunity and parasitism, fixed effects were not affected when this  
469 spatial dependence was accounted for (Albery, Becker, Kenyon, Nussey, & Pemberton,  
470 2019). Therefore we assert that these exposure effects are more likely to be mediated by  
471 individual drivers of variation such as individuals' forage intake rather than by environmental  
472 variation or density.

473 Reproductive tradeoffs are a potential driver of seasonal dynamics of immunity and parasitism,  
474 in which periodic reproduction-associated relaxation of immunity leads to increased parasitism  
475 (Martin, Weil, & Nelson, 2008). Our results do not support this mechanism for several reasons:  
476 all status categories exhibited seasonality of antibodies, strongyles, and *E. cervi* rather than  
477 only reproductive individuals; increased parasitism in reproductive females were not linked to  
478 lower immunity; and immunity did not correlate with resource availability, being highest in April,  
479 when the deer are in poor condition, having just survived the winter. In fact, antibody levels  
480 and strongyle counts correlated positively across seasons despite their negative correlation  
481 among individuals. This suggests that seasonality of propagule output is adaptive for  
482 helminths, facilitating highest transmission when environmental conditions are favourable and  
483 immunologically naïve calves are present, and leading to seasonal upregulation of immunity  
484 in warmer months to combat increased exposure (Møller, Erritzøe, & Saino, 2003; Wilson et  
485 al., 2004). Our models revealed substantial inter-annual variation in all examined variables;  
486 although we did not have enough annual replicates to test the causal factors behind this  
487 variation, further data collection in this population may allow testing of whether e.g. density-

488 related competition effects or climatic variation are producing variation in immunity and  
489 parasitism.

490 This study describes unexpected and complex interrelationships between different  
491 components of mammalian reproduction, immunity, and parasitism in the wild. We suggest  
492 that classical resource allocation mechanisms which are often hypothesised to underlie  
493 tradeoffs with immunity (e.g. Sheldon & Verhulst 1996; Deerenberg *et al.* 1997; French *et al.*  
494 2007) are insufficient to explain many of the patterns seen in wild mammals, corroborating  
495 findings in other taxa (Stahlschmidt *et al.*, 2013; Svensson *et al.*, 1998). As such, studies  
496 examining such tradeoffs in mammals should consider mechanistic links between  
497 reproduction and immunity, resource acquisition limitations, and exposure components of  
498 parasitism, particularly by quantifying both immunity and parasitism simultaneously (Bradley  
499 & Jackson, 2008; Graham *et al.*, 2011). The potential complexity of such interrelationships  
500 may contribute to the relative rarity of conclusive evidence for reproduction-immunity-  
501 parasitism tradeoffs in mammals.

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