Article:
Froy, H, Sparks, AM orcid.org/0000-0002-7697-4632, Watt, K et al. (6 more authors) (2019) Senescence in immunity against helminth parasites predicts adult mortality in a wild mammal. Science, 365 (6459). pp. 1296-1298. ISSN 0036-8075

https://doi.org/10.1126/science.aaw5822

Title: Senescence in immunity against helminth parasites predicts adult mortality in a wild mammal

Authors: H. Froy[1,2]*, A.M. Sparks[1,3], K. Watt[1], R. Sinclair[1], F. Bach[4], J.G. Pilkington[1], J.M. Pemberton[1], T.N. McNeilly[5] & D.H. Nussey[1].

Affiliations:

[1]Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, UK.

[2]Centre for Biodiversity Dynamics, Norwegian University of Science and Technology, Trondheim, Norway.

[3]School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK.

[4]Institute of Immunology and Infection Research, University of Edinburgh, Edinburgh, UK.

[5]Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, UK.

*Correspondence to: hannah.froy@ntnu.no.

Abstract:

Our understanding of the deterioration in immune function in old age – immunosenescence – derives principally from studies of modern human populations and laboratory animals. The generality and significance of this process for systems experiencing complex, natural infections and environmental challenges is unknown. Here, we show that late-life declines in an important immune marker of resistance to helminth parasites in wild Soay sheep predict overwinter mortality. We found senescence in circulating antibody levels against a highly prevalent nematode

worm, which was associated with reduced adult survival probability, independent of changes in body weight. These findings establish a role for immunosenescence in the ecology and evolution of natural populations.

One Sentence Summary:

Declining immune resistance to helminth parasites predicts host mortality in wild Soay sheep.

Main Text:

Demographic senescence, the decline in survival prospects and fertility with age, is well documented in wild vertebrates and is known to play an important role in the dynamics of natural populations (*1-3*). Bio-gerontologists have made great strides towards understanding the genetic and physiological processes underpinning senescence in laboratory organisms (*4-6*), but we do not yet know whether similar mechanisms drive demographic ageing under natural conditions (*1, 3*). Declining immune function in old age, or immunosenescence, is widely observed and associated with age-related increases in morbidity and mortality in laboratory rodents and humans (*7-9*). Parasites represent a major selective force in natural populations, and the ability to mount effective immune defences against them represents a critical determinant of fitness in wild animals (*10, 11*). A growing body of evidence suggests that immunosenescence also occurs in natural populations: several recent, largely cross-sectional, vertebrate field studies have documented age-related variation in immune markers across adulthood (*12*). However, the absence of large-scale longitudinal studies simultaneously measuring parasites, immunity, health and demography in the wild has limited our ability to test how declines in immune function impact ecological and

evolutionary processes (*12*). There is a similar scarcity of human studies linking within-individual changes in immune phenotype in old age with clinical outcomes (*9*).

Gastrointestinal nematode parasites are important drivers of selection in wild vertebrate systems (*13, 14*). Despite the global impact of helminth parasites (*15, 16*), the potential for senescent declines in immune-mediated resistance to helminth infection and the consequences for human, livestock and wildlife health have rarely been considered. Studies of lab mice, humans and domestic ruminants show that resistance to such worm infections is dependent on T helper type 2 (Th2) immune responses (*17*), with a key contribution from serum antibodies (*18, 19*). There is evidence from wild mammals and humans that the intensity of worm burdens may increase in later adulthood (*20, 21*), and cross-sectional studies in laboratory mice suggest Th2 function and anti-worm antibody production may be compromised in old age (*22-24*). In particular, two studies found that elderly mice had a reduced or delayed immunoglobulin-G (IgG) antibody response to worm infection (*22, 24*), suggesting this could be an important marker of immunosenescence. Deterioration in Th2-mediated immunity to worms may therefore be responsible for increasing burdens and negative health outcomes in later life, but currently longitudinal studies testing for associations among immunity, worm burden and components of fitness are completely lacking.

We used an unmanaged population of Soay sheep on the remote St Kilda archipelago in Scotland, which has been the subject of detailed study since 1985 (*25*), to test for fitness consequences of senescent declines in immunity in a wild population. First winter lamb mortality is often high in this population (*25*). Amongst individuals that survive to adulthood, mean longevity is 5.5 years for females (maximum 16 years) and 2.7 for males (maximum 10). Demographic senescence is

well-documented in this population, with female fecundity and survival of both sexes declining progressively from around 5 years (*26*). The sheep are host to a diverse community of gastrointestinal nematode parasites, including several highly prevalent strongyle species, which have been linked to gut pathology and overwinter mortality (*27*). Counts of nematode eggs from faecal samples provide an important proxy of parasite burden (*27*). Faecal egg counts (FEC) increase with advanced age in adult Soay sheep, which could reflect a loss of immune-mediated host control of parasite infection in later life (*26*).

Our previous work has established levels of plasma immunoglobulin-G binding antigen from *Teladorsagia circumcincta* (IgG-Tc), a highly prevalent worm in both wild Soay and domestic sheep, as an important marker of immunity to helminths in this system. In adult Soay sheep, plasma levels of IgG-Tc in summer are weakly correlated with other immune measures, including other antibody isotypes (IgA, IgM and IgE) binding the same Tc antigens (*28-30*). IgG-Tc levels are also negatively associated with FEC and positively associated with subsequent winter survival, independently of other humoral and cellular immune measures (*28-30*). Further analysis showed that IgG-Tc correlates strongly ($r > 0.9$) with levels of IgG binding antigen from a range of strongyle species (Methods; Fig. S1), only some of which are present on St Kilda, suggesting a high degree of cross-reactivity. We also showed that, regardless of which strongyle species is used, levels of binding by this IgG predict subsequent survival (Table S1). This implicates IgG-Tc as a potentially powerful marker of specific immunity to helminths in this study system, and motivated an in-depth study of the causes of its association with adult mortality. Using measurements of IgG-Tc by ELISA from 2215 longitudinal blood samples collected from 797 adult sheep aged ≥ 3 years over 26 years (1990 – 2015) on St Kilda (Methods; Table S2), we tested whether IgG-Tc showed

within-individual declines in later life consistent with immunosenescence, and whether such declines were associated with parasite burden, body weight and subsequent mortality.

We found senescence in our marker of resistance to nematode infection in wild Soay sheep. Levels of IgG-Tc declined with age (β = -0.006, 95%CI = -0.010–-0.002; Table S3a), but since individuals may senesce at different rates, the number of years an individual is away from death may be a better reflection of their biological ageing patterns than chronological age itself (*31*). Accordingly, we found that years before death explained more variation in IgG-Tc than chronological age (Table 1a). Levels of IgG-Tc declined as adults approached death, and the relationship was best described by a threshold function with the decline accelerating over the final year of life (Fig. 1A; Table S3b). Males had lower average levels of IgG-Tc than females (Table 1b), but there was no detectable interaction between years before death and sex, indicating that the pattern of within-individual changes in IgG-Tc was consistent between the sexes. IgG-Tc levels were highly repeatable across the adult lifetimes of sheep, with 58% of the variance in our dataset explained by individual identity (repeatability = 0.576, 95%CI = 0.542–0.609). These results show that despite consistent among-individual differences in IgG-Tc across their lifetimes, average antibody levels declined within individuals as they approached death.

Senescent declines in immunity were associated with increased subsequent mortality risk. We used a bivariate mixed-effects model to estimate the covariance between IgG-Tc measured in summer and the probability of survival over the subsequent winter at three different levels: among-individual, among-year and within-individual (see Methods; 2202 observations of 796 individuals over 26 years). Among-individual covariance captures the association between an individual's

average adult antibody level and its overall lifespan, while within-individual (or residual) covariance represents the association between the deviation in IgG-Tc from an individual's mean value and its prospects of surviving the following winter. Among-year covariance reflects associations between the population's average antibody levels and mortality rates across years. We found that covariance between IgG-Tc and survival was statistically significant only at the within-individual level, and not at either among-individual or among-year levels (Fig. 1; Table S4). The absence of any among-individual covariance reveals that consistent differences in immunity across adulthood, potentially associated with genotype or early-life environment, did not predict lifespan (Fig. 1B). However, the positive within-individual covariance indicates that individuals showing a within-individual decline in IgG-Tc had a reduced survival probability the following winter (Fig. 1C). These data show that longitudinal changes in a marker of immune resistance in later adulthood predict mortality risk in the wild, and indicate immunosenescence may play an important role in age-related declines in demographic rates in natural populations.

The within-individual association between IgG-Tc and survival remained when associations with FEC, our index of parasite burden, and body weight were accounted for (Fig. 2; Table S5). We ran a multivariate mixed model that included all four measures as response variables, and again estimated covariance among the terms at the within-individual, among-individual and among-year levels (see Methods). As expected for a marker of resistance to worm infection, FEC and IgG-Tc were negatively associated at the within-individual level (Table S5). Higher levels of IgG-Tc at both among- and within-individual levels were associated with increased body weight, and weight covaried positively with survival at all three levels (Table S5; accounting for variation in structural size (hindleg length) in models of body weight did not change our results, Table S6). We used this

multivariate approach to test for independent effects of immunity, parasite burden and weight on subsequent survival, while accounting for the inter-dependencies among these terms (analogous to a multiple regression; see Methods). We found that within-individual deviation in IgG-Tc was still a predictor of overwinter survival (Fig. 2). The independence of the immunity–survival relationship from body weight suggests that it was not mediated by variance in individual body condition or resource availability, and that late-life declines in body weight and immunity reflect separate physiological senescence pathways. This highlights the complex, multi-faceted nature of physiological senescence in wild animals, and the need for large-scale multivariate studies to understand which processes are most important for late-life fitness across taxa and ecological contexts (*32*).

Few studies to date have investigated how the immune system changes in later adulthood in response to pathogenic, chronically infective helminth parasites. Our analyses show that associations among adult infection, immunity and survival are not driven by constitutive among-individual differences, determined by genetics or early-life conditions, but rather by within-individual variation late in life linked to senescence. Studies in laboratory mice suggest the Th2 response to worm infection becomes compromised in old age, and that the host's ability to resist infection declines as a result (*22-24*). While the observed within-individual negative correlation between FEC and IgG-Tc is consistent with a resistance function for this immune marker, our multivariate models show longitudinal declines in IgG-Tc predict mortality independently of FEC, suggesting this relationship with mortality is not solely mediated by reduced worm burden. This may reflect the indirect and therefore inherently noisy relationship between FEC and actual worm burden. However, changes in host tolerance of worm infection, a process we have previously

linked to variation in host fitness in our study system (*33*) or density-independent alterations in parasite behavior in response to host physiological deterioration (e.g. helminth suppression of the immune response, (*18*)) could also explain the relationship between IgG-Tc and survival. Our results suggest that changes in the interactions between host immunity and helminth infection during adulthood could have implications for host ecological dynamics, helminth epidemiology and host-parasite co-evolution in wild vertebrates. The focus on the development of immunity to helminths in early life in humans and livestock is understandable, but our data suggest changes in host immune responses to worm infection occur in mammals during later life.

References and Notes:

1. D. H. Nussey, H. Froy, J.-F. Lemaitre, J.-M. Gaillard, S. N. Austad, Senescence in natural populations of animals: Widespread evidence and its implications for bio-gerontology. *Ageing Research Reviews* **12**, 214-225 (2013).
2. D. A. Roach, J. R. Carey, Population biology of aging in the wild. *Annual Review of Ecology, Evolution, and Systematics* **45**, 421-443 (2014).
3. T. Flatt, L. Partridge, Horizons in the evolution of aging. *BMC biology* **16**, 93 (2018).
4. C. J. Kenyon, The genetics of ageing. *Nature* **464**, 504 (2010).
5. C. López-Otín, M. A. Blasco, L. Partridge, M. Serrano, G. Kroemer, The hallmarks of aging. *Cell* **153**, 1194-1217 (2013).
6. L. Partridge, J. Deelen, P. E. Slagboom, Facing up to the global challenges of ageing. *Nature* **561**, 45 (2018).
7. A. Larbi *et al.*, Aging of the immune system as a prognostic factor for human longevity. *Physiology* **23**, 64-74 (2008).
8. J. Nikolich-Žugich, The twilight of immunity: emerging concepts in aging of the immune system. *Nature immunology* **19**, 10 (2018).
9. G. Pawelec, Age and immunity: What is “immunosenescence”? *Experimental gerontology* **105**, 4-9 (2018).
10. B. C. Sheldon, S. Verhulst, Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in ecology & evolution* **11**, 317-321 (1996).

11. P. Schmid-Hempel, *Evolutionary parasitology: The integrated study of infections, immunology, ecology, and genetics*. (Oxford University Press, New York, 2011).

12. A. Peters, K. Delhey, S. Nakagawa, A. Aulsebrook, S. Verhulst, Immunosenescence in wild animals: meta-analysis and outlook. *Ecology Letters* (online early, 2019).

13. E. R. Morgan, E. J. Milner-Gulland, P. R. Torgerson, G. F. Medley, Ruminating on complexity: macroparasites of wildlife and livestock. *Trends in ecology & evolution* **19**, 181-188 (2004).

14. I. M. Cattadori, D. T. Haydon, P. J. Hudson, Parasites and climate synchronize red grouse populations. *Nature* **433**, 737 (2005).

15. J. Charlier, M. van der Voort, F. Kenyon, P. Skuce, J. Vercruysse, Chasing helminths and their economic impact on farmed ruminants. *Trends in parasitology* **30**, 361-367 (2014).

16. N. J. Kassebaum *et al.*, Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet* **388**, 1603-1658 (2016).

17. R. M. Maizels, M. Yazdanbakhsh, Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nature Reviews Immunology* **3**, 733 (2003).

18. N. Harris, W. C. Gause, To B or not to B: B cells and the Th2-type immune response to helminths. *Trends in immunology* **32**, 80-88 (2011).

19. K. M. McRae, M. J. Stear, B. Good, O. M. Keane, The host immune response to gastrointestinal nematode infection in sheep. *Parasite immunology* **37**, 605-613 (2015).

20. L. Cheynel *et al.*, Immunosenescence patterns differ between populations but not between sexes in a long-lived mammal. *Scientific reports* **7**, 13700 (2017).

21. J. Bethony *et al.*, Emerging patterns of hookworm infection: influence of aging on the intensity of Necator infection in Hainan Province, People's Republic of China. *Clinical Infectious Diseases* **35**, 1336-1344 (2002).

22. P. Smith, D. W. Dunne, P. G. Fallon, Defective in vivo induction of functional type 2 cytokine responses in aged mice. *European journal of immunology* **31**, 1495-1502 (2001).

23. N. E. Humphreys, R. K. Grencis, Effects of ageing on the immunoregulation of parasitic infection. *Infection and immunity* **70**, 5148-5157 (2002).

24. S. A. Babayan, A. Sinclair, J. S. Duprez, C. Selman, Chronic helminth infection burden differentially affects haematopoietic cell development while ageing selectively impairs adaptive responses to infection. *Scientific reports* **8**, 3802 (2018).

25. T. H. Clutton-Brock, J. M. Pemberton, *Soay sheep: dynamics and selection in an island population*. (Cambridge University Press, Cambridge, 2004).

26. A. D. Hayward *et al.*, Asynchrony of senescence among phenotypic traits in a wild mammal population. *Experimental gerontology* **71**, 56-68 (2015).

27. K. Wilson, B. T. Grenfell, J. G. Pilkington, H. E. G. Boyd, F. M. Gulland, in *Soay sheep: dynamics and selection in an island population,* T. H. Clutton-Brock, J. M. Pemberton, Eds. (Cambridge University Press, Cambridge, 2004), pp. 17–51.

28. D. H. Nussey *et al.*, 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 (2014).

29. R. L. Watson *et al.*, Cellular and humoral immunity in a wild mammal: Variation with age & sex and association with overwinter survival. *Ecology and evolution* **6**, 8695-8705 (2016).

30. A. M. Sparks *et al.*, Natural selection on antihelminth antibodies in a wild mammal population. *The American Naturalist* **192**, 745-760 (2018).

31. J. G. A. Martin, M. Festa-Bianchet, Age-independent and age-dependent decreases in reproduction of females. *Ecology Letters* **14**, 576-581 (2011).

32. O. R. Jones *et al.*, Diversity of ageing across the tree of life. *Nature* **505**, 169-173 (2014).

33. A. D. Hayward *et al.*, Natural selection on individual variation in tolerance of gastrointestinal nematode infection. *PLoS biology* **12**, e1001917 (2014).

34. F. M. D. Gulland, The role of nematode parasites in Soay sheep (Ovis aries L.) mortality during a population crash. *Parasitology* **105**, 493-503 (1992).

35. T. Coulson *et al.*, Age, sex, density, winter weather, and population crashes in Soay Sheep. *Science* **292**, 1528-1531 (2001).

36. B. H. Craig, J. G. Pilkington, J. M. Pemberton, Gastrointestinal nematode species burdens and host mortality in a feral sheep population. *Parasitology* **133**, 485-496 (2006).

37. A. D. Hayward *et al.*, 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 (2011).

38. E. A. Catchpole, B. J. T. Morgan, T. N. Coulson, S. N. Freeman, S. D. Albon, Factors influencing Soay sheep survival. *Journal of the Royal Statistical Society: Series C (Applied Statistics)* **49**, 453-472 (2000).

39. A. D. Hayward *et al.*, Reproductive senescence in female Soay sheep: variation across traits and contributions of individual ageing and selective disappearance. *Functional Ecology* **27**, 184-195 (2013).

40. D. H. Nussey *et al.*, Patterns of body mass senescence and selective disappearance differ among three species of free-living ungulates. *Ecology* **92**, 1936-1947 (2011).

41. F. M. D. Gulland, S. D. Albon, J. M. Pemberton, P. R. Moorcroft, T. H. Clutton-Brock, Parasite-associated polymorphism in a cyclic ungulate population. *Proc. R. Soc. Lond. B* **254**, 7-13 (1993).

42. B. H. Craig, O. R. Jones, J. G. Pilkington, J. M. Pemberton, Re-establishment of nematode infra-community and host survivorship in wild Soay sheep following anthelmintic treatment. *Veterinary parasitology* **161**, 47-52 (2009).

43. F. M. D. Gulland, M. Fox, Epidemiology of nematode infections of Soay sheep (Ovis aries L.) on St Kilda. *Parasitology* **105**, 481-492 (1992).

44. A. D. Hayward *et al.*, Heritable, heterogeneous, and costly resistance of sheep against nematodes and potential feedbacks to epidemiological dynamics. *The American Naturalist* **184**, S58-S76 (2014).

45. R Core Team, *R: a language and environment for statistical computing*. (R Foundation for Statistical Computing, Vienna, Austria, 2019).

46. D. Bates, M. Maechler, B. Bolker, S. Walker, Fitting linear mixed-effects models using *lme4*. *Journal of Statistical Software* **67**, 1-48 (2015).

47. J. D. Hadfield, MCMC methods for multi-response generalized linear mixed models: the *MCMCglmm* R package. *Journal of Statistical Software* **33**, 1-22 (2010).

48. K. P. Burnham, D. R. Anderson, *Model selection and multimodel inference: a practical information-theoretic approach, 2nd edition* (Springer, New York, 2002)

49. A. Kuznetsova, P. B. Brockhoff, R. H. B. Christensen, *lmerTest* package: tests in linear mixed effects models. *Journal of Statistical Software* **82**, 1-26 (2017).

50. A. B. Phillimore, J. D. Hadfield, O. R. Jones, R. J. Smithers, Differences in spawning date between populations of common frog reveal local adaptation. *Proceedings of the National Academy of Sciences*, 200913792 (2010).

51. A. B. Phillimore, S. Stålhandske, R. J. Smithers, R. Bernard, Dissecting the contributions of plasticity and local adaptation to the phenology of a butterfly and its host plants. *The American Naturalist* **180**, 655-670 (2012).

52. S. Nakagawa, R. P. Freckleton, Missing inaction: the dangers of ignoring missing data. *Trends in Ecology & Evolution* **23**, 592-596 (2008).

Acknowledgments:

We are grateful to all those involved in the long-term study of Soay sheep on St Kilda; Jarrod Hadfield, Ally Phillimore and Joel Pick for statistical advice and discussions; and Andrea Graham, Adam Hayward, Loeske Kruuk and Rick Maizels for constructive comments on the manuscript. Thanks to the National Trust for Scotland for permission to work on St Kilda; QinetiQ and Kilda Cruises for logistical support in the field; and Dave Bartley, Alison Morrison and Rick Maizels for provision of nematode larvae. **Funding:** UK Natural Environment Research Council, Biological and Biomedical Sciences Research Council, Medical Research Council, The Wellcome Trust (204052/Z/16/Z), and Rural & Environment Science & Analytical Services Division of the Scottish Government. **Author contributions:** HF, TNM & DHN designed the study; JGP & JMP collected the samples, life-history data and managed the long-term study; AMS, KW & RS conducted the antibody assays in the laboratory; HF & FB analysed the data; HF & DHN wrote the manuscript with input from all co-authors. **Competing interests:** The authors declare no competing interests. **Data availability:** The data supporting the results are available in the Supplementary Materials.

Supplementary Materials:

Materials and Methods

Figure S1

Tables S1-S6

References (34-52)

Data supporting the results

Table 1. Models of anti-*T. circumcincta* IgG levels measured in plasma samples collected from adult Soay sheep in summer 1990–2015. a) Comparing the explanatory power of linear mixed effects models with different fixed effects structures. All models included individual, year and ELISA plate as random intercept terms. Sex was included as a two-level factor, age as a linear covariate, and years before death (YBD) as a threshold function with a break-point at 1 year (see Table S3). df indicates the number of parameters, and ΔAIC is the difference in AIC value compared to the best model (highlighted in bold). b) Fixed and random effects estimates from the best model, with 95% confidence intervals from 1000 bootstrap replicates.

a) Model selection — n = 1869 observations of 651 individuals

Fixed effects structure	**df**	**AIC**	**ΔAIC**
Null	5	-1662.46	44.63
Sex	6	-1671.53	35.56
Age	6	-1670.06	37.02
YBD threshold	7	-1703.29	3.80
Age + Sex	7	-1680.56	26.53
YBD threshold + Sex	**8**	**-1707.09**	**0.00**
YBD threshold + Age	8	-1702.79	4.30
Age * Sex	8	-1678.72	28.37
YBD threshold * Sex	10	-1703.68	3.40
YBD threshold + Age + Sex	9	-1705.38	1.71
YBD threshold + Age * Sex	10	-1704.31	2.78
YBD threshold * Sex + Age	11	-1702.01	5.08
YBD threshold * Sex + Age * Sex	12	-1700.42	6.67

b) Best model

Random effects	**Estimate**	**Lower 95% CI**	**Upper 95% CI**		
Individual	0.023	0.020	0.026		
Year	0.001	<0.001	0.003		
ELISA Plate	0.004	0.002	0.006		
Residual	0.012	0.011	0.013		
Fixed effects				**P value**	
Intercept	0.541	0.512	0.571	<0.001	***
YBD slope (final year)	0.047	0.028	0.067	<0.001	***
YBD slope (before final year)	0.006	0.002	0.010	0.004	**
Sex (males)	-0.045	-0.081	-0.008	0.016	*

Figure Legends:

Figure 1. A. Levels of circulating anti-*T. circumcincta* IgG declined as Soay sheep approached death. Points and error bars show raw data medians and standard errors (females black circles, males grey triangles), and lines show predictions from a linear mixed effects model (Table 1b) with 95% confidence intervals (grey shading). **B.** Annual overwinter survival probability was not related to an individual's mean levels of IgG-Tc measured over adulthood, as indicated by the regression slope for the among-individual effect of IgG-Tc on survival estimated from a bivariate model (Table S4). **C.** Individuals with relatively low levels of IgG-Tc compared to their average were less likely to survival the winter, as indicated by the within-individual effect of IgG-Tc on survival estimates from a bivariate model (Table S4). Points show raw data, and black lines show regression slope with 95% credible intervals shaded in grey.

Figure 2. Independent effects of anti-*T. circumcincta* IgG, body weight and faecal egg count (FEC) measured in August on overwinter survival probability in adult Soay sheep, accounting for covariance among the traits. Regression coefficients (median of the posterior distribution with 95% credible intervals) are given for the within-individual (green circles), among-individual (black triangles) and annual effects (grey squares). Within-individual deviation in IgG-Tc was predictive of survival probability, independent of the within- and among-individual variance in body weight and FEC. Effects were estimated from a multivariate model of IgG-Tc, weight (both Gaussian), FEC (Poisson) and survival probability (threshold) (see Methods; Table S5). IgG-Tc and weight were standardised prior to inclusion in the model (mean = 0, SD = 1).

Figure 1.

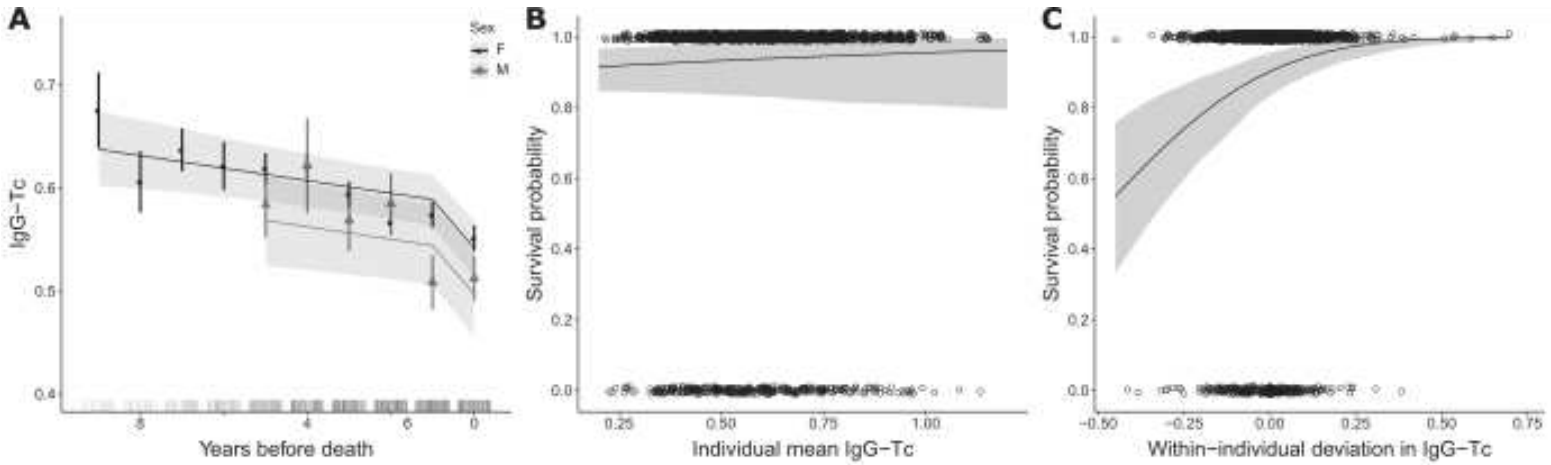
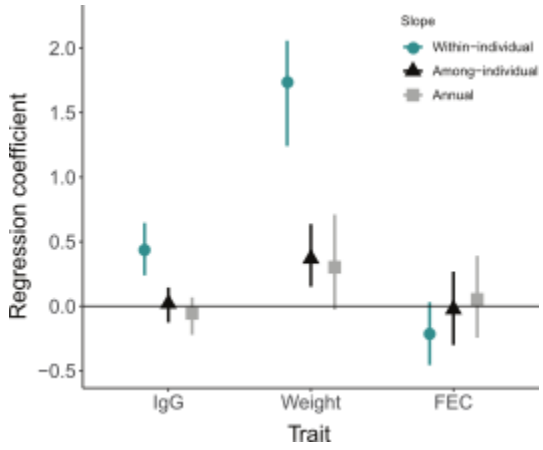


Figure 2.



Science
AAAS

Supplementary Materials for

Senescence in immunity against helminth parasites predicts adult mortality in a wild mammal

H. Froy, A.M. Sparks, K. Watt, R. Sinclair, F. Bach, J.G. Pilkington, J.M. Pemberton, T.N. McNeilly & D.H. Nussey.

Correspondence to: hannah.froy@ntnu.no

This PDF file includes:

Materials and Methods

The study system

The Soay sheep (*Ovis aries*) is a primitive breed of domestic sheep that has been living in an unmanaged and unpredated state on the remote St Kilda archipelago (57849°N, 08834°W), off the west coast of Scotland, for at least the last two millennia. Since 1985, the population resident in the Village Bay area of the main island of Hirta has been subject to detailed individual-based study (*25*). The majority of lambs are born in April, and ~95% of those born in the study area are caught and individually marked within a week of birth. Ten censuses are conducted on each of three annual field seasons, during spring (March – April), summer (July – August) and autumn (October – November), meaning the fate of individuals is known with a high degree of accuracy (*25*). The vast majority of sheep mortality occurs in late winter (85% of adult deaths occur January – April), and daily carcass searches during this period mean that death dates are known to the nearest month for most individuals.

The study population is characterised by large fluctuations in population density. Previous work has revealed that a combination of harsh winter weather, high population density and parasitic nematodes all contribute to increasing levels of malnutrition, and can result in extremely high overwinter mortality (*27, 34-37*). Mortality rates can be over 50% in these periodic 'crash' years. Senescent sheep are particularly vulnerable to the combination of high population density and adverse winter conditions, and they play an important role in determining the overall dynamics of the population (*35*). The sheep show clear signs of demographic senescence from around 5 years of age, with both sexes experiencing increased mortality rates (*26, 38*), and females showing signs of decline in a number of reproductive traits (*26, 39*). Adult sheep also lose body weight in the final years before death, regardless of their longevity (*40*). In this study, we focus on male and female adult sheep aged ≥3 years (females: mean age = 7.6 ± 2.7SD, max = 16 years; males: mean age = 4.9 ± 1.7SD, max = 10 years), encompassing the period from prime age into senescence.

Gastrointestinal nematodes represent an important selective force in the Soay sheep, with *Teladorsagia circumcincta, Trichostrongylus axei* and *Trichostrongylus vitrinus* being highly prevalent and abundant in the gastrointestinal tracts of dead sheep (*34, 36*). In adult sheep, the highest average burdens are of *T. circumcincta*, and they constitute ~50% of the total nematode burden (*36*). As part of previous experimental work, some sheep were treated with anthelmintics (*34, 41, 42*). We excluded any sheep that received a treatment within the previous 12 months from this study (n = 47).

Our previous work on immunity in this population has identified levels of circulating immunoglobulin-G binding antigen from *T. circumcincta* (IgG-Tc) as an important and specific marker of immunity to worm infection. In smaller, cross-sectional studies on subsets of adult sheep, IgG-Tc was only weakly associated with a range of other immune measures, including total antibodies, anti-KLH, ANA and anti-Tc antibodies of other isotypes (*28*), as well as circulating lymphocyte cells and different T cell subsets (*29*). Furthermore, IgG-Tc predicted overwinter survival independently of all other immune measures (*28, 29*). More recently, we found weak associations among anti-Tc antibodies of IgA, IgE and IgG isotypes ($r < 0.11$) in a much larger, longitudinal dataset, and again identified an independent association between IgG-Tc and adult survival (*30*).

Data collection

Sample collection

Each August, as many sheep as possible (usually 50–60% of the resident population) were captured and weighed (to the nearest 0.1 kg), and blood and faecal samples were collected (*25*). Faecal samples were stored at 4°C prior to examination. Blood samples were collected into lithium-heparin vacuettes and stored at 4°C. Within 24 hours, they were centrifuged at 3000 rpm for 10 minutes and the plasma collected and stored at -20°C. In this study, we used samples collected during the 1990 – 2015 August catches (2279 samples from 821 sheep). Data collection was approved by the Animal Experiments Committee, and carried out under UK Home Office Project License (most recently PPL 60/3547) granted under the Animal (Scientific Procedures) Act 1986, in accordance with all relevant guidelines.

Nematode faecal egg counts

The density of strongyle nematode eggs present in faecal samples was determined using a modified McMaster technique (*43, 44*). Briefly, 3g of faecal sample was homogenized in 87ml of saturated salt solution using a pestle and strainer. Residual fluid was strained, and the solids mixed and pipetted into both wells of a 0.30ml McMaster slide. Strongyle nematode eggs were counted at x10 magnification, and counts were multiplied by 100 to give faecal egg count per gram of sample (FEC). There is a positive linear correlation between faecal egg count and post-mortem counts of adult nematode burden in this population (*27, 34*), suggesting that FEC can be used as a proxy for gastro-intestinal parasite burden.

Antibody measurement

Anti-Teladorsagia circumcincta IgG

We used direct ELISAs to quantify plasma concentrations of IgG against antigens of the third larval stage (L3) of *T. circumcincta (30). T. circumcincta* L3 somatic antigen, was prepared by re-suspending *T. circumcincta* L3 in PBS (~5 x 10^5 larvae per ml) in Lysing Matrix D tubes (MP Biomedicals) and homogenising in a Precellys® 24 tissue homogeniser. Following centrifugation to remove debris, total protein concentration of the L3 antigen preparation was estimated using a Pierce™ BCA Protein Assay Kit (Thermo Scientific). L3 antigen was diluted to 2µg/ml in 0.06M Carbonate buffer at pH 9.6, and 50µl added to each well of a Nunc-immuno 96-microwell plate. Plates were covered and incubated at 4°C overnight, and then washed three times in Tris-buffered saline-Tween (TBST) using a plate washer. Soay sheep plasma samples were diluted to 1:12800 (determined following an optimisation protocol (*42*)), and 50µl of sample added to each well. Plates were covered and incubated at 37°C for 1 hour, and then washed five times with TBST. Rabbit anti-sheep IgG detection antibody conjugated to horseradish peroxidase (AbD Serotec 5184-2104) was diluted 1:16000, and 50µl added to each well. Plates were again covered and incubated at 37°C for 1 hour, and washed five times with TBST. 100µl of SureBlue TMB 1-Component microwell peroxidase substrate (KPL) was added to each well, and plates were incubated at 37°C for 5 minutes in the dark. 100µl of 1M hydrochloric acid was then added to each well to stop the reaction, and optical densities (ODs) were read immediately 450nm using a Thermo Scientific Multiskan GO Spectrophotometer.

Each plate included samples from two collection years paired at random, and different age groups and years were run on each plate (*30*). All plates were run in duplicate, and samples were excluded if the absolute difference between the duplicates was >0.2 OD units, or the coefficient of variation between duplicates was >0.2 (n = 13 samples). The correlation between duplicate plates was >0.8 in all cases. Each plate included two blank wells (50µl TBST added instead of sheep plasma) and two positive controls (50µl plasma from a healthy, non-immunised domestic sheep). To control for variation among plates, subsequent analyses used the mean optical density ratio of each sample, calculated as:

$$\text{OD} = \frac{(\text{sample OD - blank OD})}{(\text{positive control OD - blank OD})}$$

To avoid negative ODs, the numerator was set to zero if the sample OD was lower than the blank OD. We took the average score over the duplicate plates for each individual sample.

Cross-reactivity of IgG antibodies

We also used direct ELISAs to measure IgG activity against antigen preparations from the following gastro-intestinal nematode parasites: *Trichostrongylus axei*, *T. vitrinus, Haemonchus contortus* and *Heligmosomoides polygyrus*. *T. axei* and *T. vitrinus* are prevalent strongyle parasites infecting the Soay sheep on St Kilda, whilst *H. contortus* is a common and pathogenic parasite in domestic sheep in some parts of the UK but is not present on St Kilda (*34*). *H. polygyrus* is a strongyle parasite of rodents that is widely used in laboratory studies of nematode infection. *H. polygyrus* adult-worm antigen was prepared by homogenisation in a ground-glass hand-held homogeniser (VWR-Jencons, UK) in ice-cold PBS, followed by centrifugation at 13000g for 30 minutes, from which the supernatant was collected and stored at −80 °C until use. *T. axei*, *T. vitrines and H. contortus* L3 antigens were prepared in an identical manner to that described for *T. circumcincta* antigen. ELISAs were then performed as described above for *T. circumcincta* but using 2µg/ml of each nematode antigen preparation. The cross-reactivity assays were conducted on samples collected from individuals from across the age range (0 – 12 years) in August 2011 (n = 281 samples: 187 females; 96 males) (*29*).

Data analysis

All analyses were conducted in the program R version 3.5.1 (*45*). Univariate mixed effects models were used to characterize the ageing patterns in anti-*T. circumcincta* IgG (IgG-Tc) using *lme4* version 1.1.21 (*46*). Multivariate mixed effects models were the used to examine associations between IgG-Tc and annual survival probability using *MCMCglmm* version 2.26 (*47*).

Characterising ageing patterns in IgG-Tc

We examined how levels of IgG-Tc varied with age using linear mixed effects models, which included individual, year and ELISA plate as random intercept terms to account for repeated measures. We considered both chronological age (in years) and the number of years an individual was from death, which may reflect aspects of their physiological state that are not captured by chronological age (sometimes termed 'biological age'). First, we determined the functions which best described the ageing patterns by comparing a set of candidate models for both chronological age and years before death. We tested linear, quadratic and cubic polynomial functions, as well as threshold models where slopes were allowed to vary independently either side of a break-point (*20*). We tested thresholds between 3 and 12 years of age, and between 1 and 8 years before death. Once we had separately established the best function for chronological age and years before death, we tested their independent explanatory power by including both best fitting functions as covariates in the same model. We compared candidate models that included the best age and years before death functions, sex as a two-level fixed factor, and two-way interactions between sex and the age and years before death terms (to test whether the ageing patterns differed between the sexes).

We assessed the relative support for the different candidate models using Akaike's Information Criterion (AIC), where the model with the lowest AIC value explains the most variation in the data (*48*). Since models with an AIC difference of less than 2 are not considered to be meaningfully different, we took the simplest model within 2 AIC units of the model with the lowest AIC to be the best model (*48*). Models comparing different fixed effect structures were estimated using maximum likelihood, and the final model was estimated using restricted maximum likelihood. The *lmerTest* package was used to generate p-values using the Satterthwaite approximation for the degrees of freedom (*49*), and bootstrapping was used to generate confidence intervals. The repeatability of IgG-Tc was calculated as the variance explained by the random effect of individual over the total variance.

Chronological age at sampling and years until death were only moderately negatively correlated in our dataset ($r = -0.408$, $p<0.001$). Years before death was scored as zero in August year t if the sheep died before 1 May year $t+1$ (i.e. it did not survive the winter following measurement). This analysis only included individuals for which death year was known (n = 1869 observations of 651 individuals). Since 85% of deaths occur January – April, we assumed that if a sheep died in the year following measurement, but its death month was unknown, its death occurred before 1st May.

Associations between IgG-Tc and annual survival probability

We used a bivariate mixed modelling approach to examine the association between antibody levels and overwinter survival at different hierarchical levels using *MCMCglmm* (*47, 50, 51*). IgG-Tc (measured in August year t) and annual survival (to 1 May year $t+1$) were our response variables, with Gaussian and threshold distributions (corresponding to identity and probit link functions), respectively. Unstructured variance–covariance matrices were estimated for the random effects of individual, year and residual variance, allowing us to estimate the covariance between IgG-Tc and survival at multiple levels (*50, 51*). For each of these random effects, we obtained a posterior distribution for the variance and covariance between IgG-Tc and survival:

$$\begin{bmatrix} \sigma^2_{IgG} & \sigma_{IgG,Surv} \\ \sigma_{IgG,Surv} & \sigma^2_{Surv} \end{bmatrix}$$

The quantity $\sigma^2_{IgG,Surv} / \sigma^2_{IgG}$ estimates the slope of survival on IgG-Tc for a particular random effect. The random effect of individual describes the variance and covariance among individuals, and gives the slope estimate for the among-individual effect of IgG-Tc on survival. A positive among-individual slope would suggest that individuals with higher mean levels of IgG-Tc across their lifetimes have a higher probability of overwinter survival. The random effect of year describes the variance and covariance among years, and gives the slope estimate for the annual effect of IgG-Tc on survival. A positive year slope would suggest that increases in mean IgG-Tc across the population are associated with higher annual survival probabilities, and would be indicative of environmentally driven variation. The residual variance–covariance matrix describes the variance and covariance in IgG-Tc and survival within individuals, and gives the slope estimate for the within-individual effect of IgG-Tc on survival. A positive within-individual slope would indicate that individuals with relatively high levels of IgG-Tc compared to their mean during adulthood have a higher probability of surviving the winter than when their levels of IgG-Tc are relatively low.

The model was run for 4.5×10^5 iterations, with 5×10^4 burn-in and thinning interval of 200. We obtained the posterior distribution for each slope estimate and report the 95% highest posterior density (HPD). We also estimated the 95% HPD for the slope difference (Δb), to test whether the within- and among-individual slopes were significantly different. In addition to the random effects of individual and year, the IgG-Tc model included a random intercept term for ELISA plate. The survival model included a linear effect of age (credible intervals overlapped zero for the quadratic effect), and an additive effect of sex (two-level factor, credible intervals overlapped zero for the interaction between sex and age) as fixed effects. Age was standardised (mean=0, standard deviation=1) prior to inclusion in the model. This analysis included all observations of IgG-Tc where individual survival was known (n = 2202 observations of 796 individuals; individuals that were still alive could be included in this analysis, resulting in larger sample sizes than the analysis of ageing patterns).

Associations between IgG-Tc and annual survival probability, accounting for covariance with faecal egg count and body weight

We used a multivariate approach to test whether IgG-Tc, faecal egg count and body weight independently predicted survival probability, while accounting for the covariance among these traits at different hierarchical levels (following (*51*)). Our multivariate model included IgG-Tc, body weight, faecal egg count (FEC) and annual survival as response variables. Faecal egg count was divided by 100, rounded to the nearest whole value and treated as a Poisson response, IgG-Tc and weight were Gaussian, and survival was modelled as a threshold trait. We estimated the posterior distributions of the fully unstructured 4 x 4 variance–covariance matrices for the random effects of individual, year and residual variance. This enabled us to estimate the covariance among all four traits for each random effect:

$$\begin{bmatrix} \sigma^2_{IgG} & \sigma_{IgG,Wt} & \sigma_{IgG,FEC} & \sigma_{IgG,Surv} \\ \sigma_{IgG,Wt} & \sigma^2_{Wt} & \sigma_{Wt,FEC} & \sigma_{Wt,Surv} \\ \sigma_{IgG,FEC} & \sigma_{Wt,FEC} & \sigma^2_{FEC} & \sigma_{FEC,Surv} \\ \sigma_{IgG,Surv} & \sigma_{Wt,Surv} & \sigma_{FEC,Surv} & \sigma^2_{Surv} \end{bmatrix}$$

To assess whether variation in IgG-Tc predicted survival once the covariance with FEC and weight were accounted for, we employed a multiple regression approach (*51*). We estimated **A**, the 3 x 3 variance–covariance matrix among predictors (IgG-Tc, weight and FEC), and **B**, the covariance between the response (survival) and the predictors, for the random effects and the residual:

$$\boldsymbol{A}_R = \begin{bmatrix} \sigma^2_{IgG} & \sigma_{IgG,Wt} & \sigma_{IgG,FEC} \\ \sigma_{IgG,Wt} & \sigma^2_{Wt} & \sigma_{Wt,FEC} \\ \sigma_{IgG,FEC} & \sigma_{Wt,FEC} & \sigma^2_{FEC} \end{bmatrix}$$

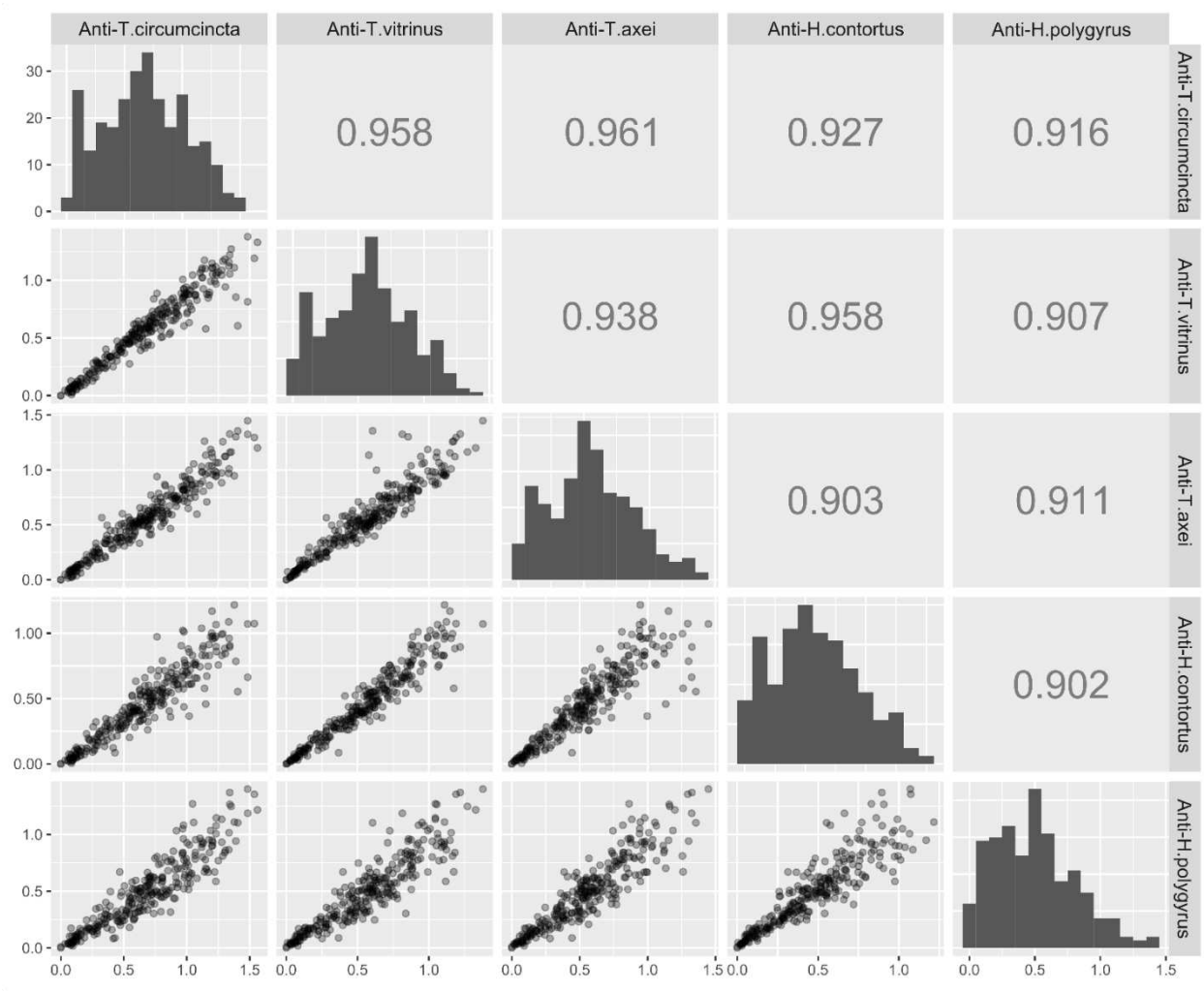
$$\boldsymbol{B_R} = \begin{bmatrix} \sigma_{IgG,Surv} & \sigma_{Wt,Surv} & \sigma_{FEC,Surv} \end{bmatrix}$$

Where $\mathbf{A}_R^{-1}\mathbf{B}_R$ gives the multiple regression coefficients treating IgG-Tc, weight and FEC as predictors of survival. As with the bivariate analysis, the random effect of individual gives the slopes for the among-individual effects of the three predictors on survival, the random effect of year gives the slopes for the annual effects, and the residual variance–covariance matrix gives the slopes for the within-individual effects. The calculation was repeated across the posterior distribution of $\mathbf{A}_R$ and $\mathbf{B}_R$ to obtain estimates of the 95% HPD interval for each coefficient.

The model was run for 1.1×10^6 iterations, with 1×10^5 burn-in and thinning interval of 500. IgG-Tc and weight were standardised to mean = 0 and standard deviation = 1 to make the effect sizes comparable (this was not possible for FEC because of the Poisson distribution). As for the bivariate model above, ELISA plate was included as a random intercept term in the IgG-Tc model. The survival model included fixed effects for age (linear term) and sex (two-level factor). For body weight, we included fixed effects for age (linear and quadratic terms), a two-level factor for sex, and two-way interactions between sex and the age terms. Additionally, we re-ran our model including hindleg length as a linear covariate to account for variation in body size, but our results remained unchanged (Table S6). The FEC model included additive fixed effects of age (linear and quadratic terms) and a two-level factor for sex (credible intervals overlapped zero for the interaction between sex and the age terms). Again, age was standardised prior to inclusion in the models. This analysis included all observations for which survival was known (n = 2215 observations of 797 individuals; missing data were treated as missing at random (*52*)).

Parameter estimates are presented as the posterior mean with 95% credible intervals of 2000 samples of the MCMC chain with minimal autocorrelation (<0.1). All models were run using parameter expanded priors to improve the mixing properties of the MCMC chain and aid model convergence (*47*), but our results remained unchanged when models were re-run using inverse-Wishart priors. The residual variance was fixed at 1 for the survival models (as is the standard recommendation for threshold models), and the latent variables were constrained to be between ±7 to avoid numerical difficulties as the probabilities approached 0 and 1. Effects were considered to be statistically significant when the credible intervals did not overlap zero. pMCMC values are provided to aid interpretation (estimated as twice the posterior probability that the estimate is negative or positive, whichever probability is smallest).

Fig. S1.



Pairwise comparisons of levels of IgG against *T. circumcincta*, *T. vitrinus*, *T. axei*, *H. contortus* and *H. polygyrus*, measured in plasma samples collected from 281 Soay sheep in August 2011 using ELISAs. The lower panels show scatterplots of the pairwise correlations, the upper panels give the Pearson's correlation coefficient for each comparison (p-value < 0.001 in all cases), and the diagonal panels show the distributions of ODs for each antigen. Samples were taken from sheep of both sexes across the age range (0 – 12 years).

Table S1.

Teladorsagia circumcincta (n = 279)	**Estimate**	**Standard Error**	**Z score**	**P value**	
Intercept	-1.491	0.344	-4.333	<0.001	***
Age class (adult)	1.680	0.512	3.278	0.001	**
Age in years	-0.200	0.069	-2.902	0.004	**
Sex (male)	-0.626	0.303	-2.068	0.039	*
Anti-*T. circumcincta* IgG	1.877	0.560	3.352	0.001	***
Trichostrongylus vitrinus (n = 279)					
Intercept	-1.604	0.349	-4.596	<0.001	***
Age class (adult)	1.516	0.512	2.960	0.003	**
Age in years	-0.194	0.069	-2.797	0.005	**
Sex (male)	-0.586	0.306	-1.916	0.055	.
Anti-*T. vitrinus* IgG	2.556	0.671	3.807	<0.001	***
Trichostrongylus axei (n = 281)					
Intercept	-1.416	0.333	-4.260	<0.001	***
Age class (adult)	1.885	0.502	3.752	<0.001	***
Age in years	-0.204	0.069	-2.965	0.003	**
Sex (male)	-0.612	0.301	-2.036	0.042	*
Anti-*T. axei* IgG	1.872	0.629	2.975	0.003	**
Haemonchus contortus (n = 276)					
Intercept	-1.555	0.335	-4.642	<0.001	***
Age class (adult)	1.660	0.483	3.436	0.001	***
Age in years	-0.187	0.070	-2.676	0.007	**
Sex (male)	-0.548	0.305	-1.796	0.072	.
Anti-*H. contortus* IgG	2.753	0.713	3.860	<0.001	***
Heligmosomoides polygyrus (n = 279)					
Intercept	-1.452	0.324	-4.476	<0.001	***
Age class (adult)	1.742	0.493	3.534	<0.001	***
Age in years	-0.179	0.070	-2.566	0.010	*
Sex (male)	-0.576	0.303	-1.899	0.058	.
Anti-*H. polygyrus* IgG	2.219	0.636	3.488	<0.001	***

Estimates from generalised linear models of overwinter survival of Soay sheep. IgG antibody reactivity against *T. circumcincta*, *T. vitrinus*, *T. axei*, *H. contortus* and *H. polygyrus* was measured in plasma samples collected in August 2011. We included levels of IgG against each different antigen as covariates in separate binomial models of survival, to test whether they were predictive of survival over the 2011/2012 high mortality winter. Sex and age class were included as two-level factors to account for differences in survival between males and females, and between lambs and adults (reference levels: age class (lamb); sex (female)). Age in years was included as a covariate to account for age-related variation in survival within the adult age class. IgG antibody reactivity against each of the five antigens was predictive of overwinter survival.

Table S2.

Number of observations	Number of individuals
1	257
2	178
3	143
4	95
5	53
6	25
7	21
8	10
9	4
10	6
11	5

Sample sizes. We measured IgG-Tc in 2215 samples collected from 797 adult sheep aged ≥ 3 years over 26 years (1990 – 2015). Sample sizes exclude missing values for IgG-Tc, body weight and FEC (n = 13, 3, and 169, respectively).

Table S3.

a) Age function

	k	AIC	ΔAIC
Age	**6**	**-1670.06**	**0.00**
Age^2	7	-1668.25	1.81
Age^3	8	-1667.43	2.63
Age threshold (4 years)	7	-1669.29	0.78
Age threshold (5 years)	7	-1668.40	1.66
Age threshold (6 years)	7	-1668.34	1.72
Age threshold (7 years)	7	-1668.07	2.00
Age threshold (8 years)	7	-1668.61	1.46
Age threshold (9 years)	7	-1669.24	0.82
Age threshold (10 years)	7	-1669.21	0.85
Age threshold (11 years)	7	-1669.08	0.99
Age threshold (12 years)	7	-1668.55	1.51

b) Years before death function

	k	AIC	ΔAIC
YBD	6	-1689.47	13.82
YBD^2	7	-1700.44	2.85
YBD^3	8	-1699.28	4.00
YBD threshold (1 years)	**7**	**-1703.29**	**0.00**
YBD threshold (2 years)	7	-1700.08	3.21
YBD threshold (3 years)	7	-1697.27	6.02
YBD threshold (4 years)	7	-1698.08	5.21
YBD threshold (5 years)	7	-1696.51	6.78
YBD threshold (6 years)	7	-1694.83	8.46
YBD threshold (7 years)	7	-1691.69	11.60
YBD threshold (8 years)	7	-1690.65	12.64

Model selection to determine the best function describing the relationship between IgG-Tc levels and a) chronological age (in years) and b) years before death (YBD) in Soay sheep. These linear mixed effects models included individual, year and ELISA plate as random intercept terms. Linear, quadratic and cubic polynomial functions were compared, in addition to threshold functions with a range of different break-points. The function that explained the most variation in the data was chosen in each case (shown in bold).

Table S4.

n = 2202 observations of 796 individuals

Fixed effects	Posterior mean	Lower 95% CI	Upper 95% CI	pMCMC	
Intercept IgG	0.583	0.558	0.605	<0.001	***
Intercept Survival	1.722	1.363	2.128	<0.001	***
Survival : Age	-0.614	-0.797	-0.440	<0.001	***
Survival : Sex (males)	-0.834	-1.136	-0.557	<0.001	***
Random effects					
IgG : ELISA Plate	0.004	0.002	0.006		

Variance–covariance matrices

		IgG	Survival
Individual	IgG	*0.023 (0.020–0.026)*	0.132 (-0.164–0.544)
	Survival	0.010 (-0.007–0.028)	*0.296 (0.000–0.672)*
Year	IgG	*0.001 (0.000–0.003)*	-0.250 (-0.828–0.422)
	Survival	-0.005 (-0.022–0.013)	*0.702 (0.278–1.229)*
Residual	IgG	*0.012 (0.011–0.013)*	**0.289 (0.176–0.398)**
	Survival	**0.032 (0.020–0.044)**	*1.000 (1.000–1.000)*

Posterior mean and 95% credible intervals (CI) for the fixed and random effects from a bivariate MCMC model of IgG-Tc and annual survival in adult Soay sheep (Gaussian and threshold models). Variance–covariance (VCV) matrices were unstructured for individual, year and residual variance. Variances are shown on the diagonal (in italics), covariances below the diagonal (bottom left) and correlations above the diagonal (top right). Covariances and correlations were deemed to be significant when the credible intervals did not overlap zero (shown in bold).

Table S5.

n = 2215 observations of 797 individuals

Fixed effects	Posterior mean	Lower 95% CI	Upper 95% CI	pMCMC	
Intercept IgG	-0.104	-0.228	0.008	0.074	.
Intercept Weight	-0.278	-0.358	-0.192	<0.001	***
Intercept FEC	-0.605	-0.762	-0.466	<0.001	***
Intercept Survival	1.697	1.319	2.075	<0.001	***
Weight : Age	0.111	0.090	0.131	<0.001	***
Weight : Sex (males)	2.653	2.538	2.749	<0.001	***
Weight : Age^2	-0.058	-0.068	-0.047	<0.001	***
Weight : Age : Sex (males)	-0.091	-0.162	-0.011	<0.001	***
Weight : Age^2 : Sex (males)	0.110	0.066	0.158	<0.001	***
FEC : Age	1.525	1.333	1.711	0.016	*
FEC : Age^2	-0.614	-0.772	-0.468	<0.001	***
FEC : Sex (males)	-0.809	-1.080	-0.539	<0.001	***
Survival : Age	0.125	0.071	0.184	<0.001	***
Survival : Sex (males)	-0.249	-0.309	-0.182	<0.001	***
Random effects					
IgG : ELISA Plate	0.109	0.058	0.161		

Variance–covariance matrices

Individual	IgG	Weight	FEC	Survival
IgG	*0.585 (0.517–0.659)*	**0.132 (0.051–0.218)**	-0.003 (-0.138–0.133)	0.088 (-0.146–0.359)
Weight	**0.047 (0.016–0.077)**	*0.221 (0.197–0.246)*	**-0.247 (-0.372–-0.124)**	**0.399 (0.173–0.753)**
FEC	-0.001 (-0.062–0.066)	**-0.071 (-0.111–-0.035)**	*0.380 (0.267–0.486)*	-0.131 (-0.495–0.238)
Survival	0.030 (-0.045–0.111)	**0.086 (0.037–0.142)**	-0.038 (-0.150–0.058)	*0.255 (0.006–0.586)*

Year	IgG	Weight	FEC	Survival
IgG	*0.025 (0.000–0.064)*	-0.230 (-0.793–0.354)	0.313 (-0.266–0.844)	-0.195 (-0.716–0.440)
Weight	-0.006 (-0.025–0.010)	*0.035 (0.014–0.059)*	-0.201 (-0.593–0.286)	**0.425 (0.040–0.733)**
FEC	0.011 (-0.011–0.041)	-0.009 (-0.035–0.015)	*0.068 (0.018–0.134)*	-0.013 (-0.473–0.416)
Survival	-0.023 (-0.107–0.051)	0.065 (-0.007–0.140)	-0.003 (-0.111–0.111)	*0.723 (0.302–1.249)*

Residual	IgG	Weight	FEC	Survival
IgG	*0.311 (0.287–0.335)*	**0.055 (0.001–0.103)**	**-0.148(-0.237–-0.047)**	**0.289 (0.176–0.400)**
Weight	**0.008 (0.000–0.015)**	*0.064 (0.059–0.069)*	**-0.221 (-0.303–-0.122)**	**0.464 (0.360–0.559)**
FEC	**-0.059 (-0.095–-0.019)**	**-0.040 (-0.058–-0.024)**	*0.526 (0.415–0.640)*	**-0.283 (-0.459–-0.119)**
Survival	**0.161 (0.097–0.223)**	**0.117 (0.089–0.142)**	**-0.204 (-0.327–-0.077)**	*1.000 (1.000–1.000)*

Posterior mean and 95% credible intervals (CI) for the fixed and random effects from a multivariate MCMC model of IgG-Tc (Gaussian), weight (Gaussian), faecal egg count (FEC; Poisson) and survival (threshold). Variance–covariance (VCV) matrices were unstructured for individual, year and residual variance. Variances are shown on the diagonal (in italics), covariances below the diagonal (bottom left) and correlations above the diagonal (top right). The fully unstructured VCV matrix for the random effect of individual gives the among-individual effects, the VCV matrix for the effect of year gives the annual effects, and the residual VCV matrix gives the within-individual effects. Covariances and correlations were deemed to be significant when the credible intervals did not overlap zero (shown in bold).

Table S6.

n = 2212 observations of 797 individuals

Fixed effects	Posterior mean	Lower 95% CI	Upper 95% CI	pMCMC	
Intercept IgG	-0.104	-0.230	0.011	0.081	.
Intercept Weight	-0.251	-0.326	-0.177	<0.001	***
Intercept FEC	-0.604	-0.762	-0.459	<0.001	***
Intercept Survival	1.677	1.311	2.050	<0.001	***
Weight : Age	0.103	0.085	0.122	<0.001	***
Weight : Sex (males)	2.429	2.326	2.533	<0.001	***
Weight : Age^2	-0.059	-0.069	-0.049	<0.001	***
Weight : Hindleg	**0.187**	**0.162**	**0.215**	**<0.001**	***
Weight : Age : Sex (males)	0.133	0.075	0.190	<0.001	***
Weight : Age^2 : Sex (males)	-0.236	-0.299	-0.171	<0.001	***
FEC : Age	-0.093	-0.172	-0.019	0.018	*
FEC : Age^2	0.109	0.062	0.161	<0.001	***
FEC : Sex (males)	1.524	1.345	1.711	<0.001	***
Survival : Age	-0.607	-0.755	-0.467	<0.001	***
Survival : Sex (males)	-0.811	-1.118	-0.555	<0.001	***
Random effects					
IgG : ELISA Plate	0.109	0.061	0.164		

Variance–covariance matrices

Individual	IgG	Weight	FEC	Survival
IgG	*0.587 (0.522–0.667)*	**0.128 (0.034–0.205)**	-0.003 (-0.136–0.139)	0.081 (-0.135–0.380)
Weight	**0.039 (0.011–0.066)**	*0.162 (0.144–0.182)*	**-0.290 (-0.397–-0.157)**	**0.471 (0.224–0.820)**
FEC	-0.001 (-0.065–0.063)	**-0.071 (-0.101–-0.037)**	*0.374 (0.269–0.488)*	-0.139 (-0.511–0.234)
Survival	0.027 (-0.057–0.099)	**0.082 (0.035–0.130)**	-0.038 (-0.140–0.062)	*0.227 (0.014–0.541)*

Year	IgG	Weight	FEC	Survival
IgG	*0.026 (0.000–0.067)*	-0.259 (-0.778–0.394)	0.330 (-0.305–0.868)	-0.217 (-0.767–0.361)
Weight	-0.006 (-0.025–0.010)	*0.028 (0.011–0.047)*	-0.157 (-0.599–0.267)	**0.419 (0.080–0.765)**
FEC	0.012 (-0.011–0.044)	-0.007 (-0.030–0.015)	*0.068 (0.018–0.139)*	-0.026 (-0.433–0.48)
Survival	-0.025 (-0.113–0.062)	0.058 (-0.007–0.125)	-0.004 (-0.113–0.108)	*0.719 (0.331–1.293)*

Residual	IgG	Weight	FEC	Survival
IgG	*0.311 (0.286–0.334)*	**0.067 (0.017–0.126)**	**-0.157(-0.247–-0.060)**	**0.290 (0.182–0.393)**
Weight	**0.010 (0.002–0.018)**	*0.065 (0.060–0.070)*	**-0.211 (-0.301–-0.123)**	**0.433 (0.336–0.531)**
FEC	**-0.063 (-0.100–-0.022)**	**-0.039 (-0.056–-0.022)**	*0.527 (0.417–0.650)*	**-0.293 (-0.467–-0.121)**
Survival	**0.162 (0.102–0.222)**	**0.110 (0.084–0.137)**	**-0.214 (-0.348–-0.093)**	*1.000 (1.000–1.000)*

Posterior mean and 95% credible intervals (CI) for the fixed and random effects from a multivariate MCMC model of IgG-Tc (Gaussian), weight (Gaussian), faecal egg count (FEC; Poisson) and survival (threshold). As Table S5, but additionally accounting for the effect of hindleg length in the model of body weight (shown in bold).