Early-life glucocorticoids accelerate lymphocyte count senescence in roe deer

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Abstract

Immunosenescence corresponds to the progressive decline of immune functions with increasing age. Although it is critical to understand what modulates such a decline, the ecological and physiological drivers of immunosenescence remain poorly understood in the wild. Among them, the level of glucocorticoids (GCs) during early life are good candidates to modulate immunosenescence patterns because these hormones can have long-term consequences on individual physiology. Indeed, GCs act as regulators of energy allocation to ensure allostasis, are part of the stress response triggered by unpredictable events and have immunosuppressive effects when chronically elevated. We used longitudinal data collected over two decades in two populations of roe deer (*Capreolus capreolus*) to test whether higher baseline GC levels measured within the first year of life were associated with a more pronounced immunosenescence and parasite susceptibility. We first assessed immunosenescence trajectories in these populations facing contrasting environmental conditions. We found no evidence that juvenile GC levels can modulate these trajectories except for lymphocytes for which depletion was accelerated late in life when FGMs were elevated early in life. Although the exact mechanism remains unknown, this result suggests a role of GCs in promoting T cells apoptosis and thymic involution. Adrenal GCs can also promote the production of thymic GCs, specifically amplifying the effects of GCs on lymphocyte compared to other immune traits. In addition, elevated GC levels in juveniles were associated with a higher abundance of lung parasites during adulthood for individuals born during bad years, suggesting short-term negative effects of GCs on juvenile immunity, having in turn long-lasting consequences on adult parasite load, depending on juvenile environmental conditions. These findings offer promising research directions in assessing the carry-over consequences of GCs on life-history traits in the wild.

Keywords: Faecal glucocorticoid metabolites, parasite, ageing, immunity, stress, allostatic load

1. Introduction

The immune system is a key component of health and viability (Schmid-Hempel 2003). It includes two interacting arms: the innate immunity that provides a fast and generalist response to pathogens and includes the inflammatory response, and the adaptive immunity that is characterised by a slower but more specific long-term protection against pathogens (Klasing 2004). The development, maintenance, and activation of the immune system is energy-demanding and can damage tissues if activation is excessive or prolonged (Klasing 2004). Consequently, when energy acquisition is limiting, the allocation to each arm of the immune system should be traded for growth, reproduction or other maintenance-related functions (Sheldon and Verhulst 1996, Lee 2006, Martin 2009, Maizels and Nussey 2013, McDade et al. 2016). Inevitably, these costs and the subsequent trade-offs can explain, at least partly, why the efficiency of the immune system cannot be maximised and maintained throughout life, and why it declines with increasing age, a process defined as 'immunosenescence' (Makinodan 1980). Coming mostly from human and laboratory mammal studies (Pawelec et al. 2020, but see Peters et al. 2019 for a review in free-ranging and captive animals), accumulated empirical evidence shows consistent declines of the adaptive arm and no clear changes in the innate arm, even if its inflammatory component shows consistent increase (leading to a chronic low-grade inflammatory state, named '*inflammageing*', which constitutes a hallmark of the ageing process; Franceschi et al. 2000, 2007, López-Otín et al. 2023). These age-associated changes in immune functioning are generally the underlying causes of an increased susceptibility to pathogens (such as parasites) with age in mammals in the wild (Hayward et al. 2009, 2015, Froy et al. 2019, but see Hämäläinen et al. 2015), ultimately leading to higher morbidity and mortality risks and lower reproductive success (Sheldon and Verhulst 1996, Bauer and Fuente 2016, Froy et al. 2019).

Accordingly, these consequences on populations make the identification of the ecological and physiological drivers of immunosenescence in the wild an important research topic.

The resource-based allocation trade-off between immunity and other biological functions (Sheldon and Verhulst 1996, McDade et al. 2016) is expected to be mediated, at least partially, by glucocorticoids (GCs). GCs are steroid hormones with pleiotropic effects. Although they are mainly known for their involvement in the stress response (Wingfield 2013, Hau et al. 2016), their primary role is to regulate the organism energy balance, and as a result, they modulate energy allocation among growth, reproduction, and somatic maintenance (Hau et al. 2016). Therefore, GCs contribute to allostasis (*i.e.* achieving stability through change) by ensuring energy homeostasis (McEwen and Wingfield 2003, 2010, Romero et al. 2009), according to predictable and unpredictable events. Unpredictable environmental perturbations elicit a stress response involving partly the activation of the hypothalamic-pituitary-adrenal (HPA) axis resulting in the release of GCs. Short-term (*i.e.* acute; within minutes to hours) GC elevation promotes reallocation of stored energy to processes enhancing survival (Sapolsky et al. 2000), after what GCs quickly return to baseline levels to support daily maintenance of energy homeostasis. Following exposure to repeated or long-lasting stressors, however, GC concentrations can remain elevated for longer periods of time (*i.e.* days to weeks). Long-term GC elevation can lead to 'allostatic overload', which occurs when the energy required to ensure daily and seasonal activities and to cope with the perturbations is higher than the energy intake (McEwen and Wingfield 2010). Allostatic overload can be deleterious through hypermetabolism and increased rate of telomere attrition, epigenetic age acceleration and accelerated cellular ageing (Seeman et al. 2001, Casagrande et al. 2020, Lee et al. 2021, Bobba-Alves et al. 2023), or through body growth inhibition and body condition weakening (Reeder and Kramer 2005). The detrimental consequences of allostatic overload also include the disruption of immune responses due to the

immunosuppressive effects of GCs (Lee 2006, Martin 2009, Dhabhar 2014, Hau et al. 2016). Indeed, chronic GC elevations decrease the production, activity and functioning of immunoprotective cells and lead to inflammageing and immune dysregulation (Dhabhar 2014).

While chronic GC elevations have been reported to suppress immunity in laboratory studies, the carry-over consequences of GCs on immunosenescence have never been investigated in the wild. Baseline GC levels and immune parameters have effectively been experimentally shown to covary in roe deer (*Capreolus capreolus*, Carbillet et al. 2022), but longer term relationships between GCs and immunity have not yet been assessed. Similarly, GCs and parasite burden are generally positively linked on the short term in mammals (Defolie et al. 2020). The underlying mechanisms of these associations could involve the impairing effects of GCs on host immunity and their supporting role in parasite development (Herbert et al. 2022), but are not fully elucidated. While the immunosuppressive effects of GCs in chronically stressed individuals (Lee 2006, Martin 2009, Dhabhar 2014) and the consequences of acute stress-induced GC concentrations on individual performance (leading to the concept of '*emergency life-history stage*', Wingfield et al. 1998) have been investigated quite intensively using laboratory animal models, the consequences of elevated baseline GC levels (resulting from repeated or chronic stress exposure) on life-history traits are more equivocal (*e.g.* Pride 2005, Pauli and Buskirk 2007, Cabezas et al. 2007, Wey et al. 2015). Similarly, the literature on stress exposure or chronically elevated GC levels and immunosenescence suggests the predominance of negative relationships (Bauer 2005, 2008, Garrido et al. 2022), but most studies so far focused on humans or laboratory rodents, and none has been performed in the wild. Importantly, these studies generally investigate covariation patterns between chronic stress exposure or chronically elevated GC levels and

changes in immune parameters in elderly, rather than to the study of early-life stress and carry-over consequences in immune functioning.

Here, we took advantage of an exceptionally detailed long-term individual-based monitoring based on a capture-mark-recapture (CMR) program in two roe deer populations in the wild. We aimed at exploring the long-term effects of juvenile GC levels on immunosenescence patterns. Previous studies in these populations showed that immunosenescence patterns were mainly driven by population-level characteristics (Cheynel et al. 2017). However, whether and how physiological markers, and more particularly GCs, modulate those patterns have not been investigated. We first assessed and updated mean individual immunosenescence patterns for 12 immune traits encompassing both adaptive and innate immune components, and for susceptibility to 4 parasites frequently occurring in roe deer. We accounted for confounding factors that are known to modulate mammalian senescence patterns such as current environmental context (*e.g.* Tidière et al. 2016, Cheynel et al. 2017), sex (*e.g.* Brooks and Garratt 2017, Lemaître et al. 2020) or natal environmental conditions (*e.g.* Nussey et al. 2007, Cooper and Kruuk 2018). Then, we used baseline GC levels (estimated through faecal glucocorticoid metabolites, FGMs) measured during the first year of life to investigate whether and how juvenile GCs influenced immunosenescence patterns. The juvenile stage is a major determinant of future individual performance in this income breeder (Gaillard et al. 2003) and early-life stress is expected to have detrimental consequences on adult performance (Monaghan and Haussmann 2015). We thus expected individuals exhibiting higher levels of GCs during the juvenile stage to display earlier and/or accelerated immunosenescence due to the role of GCs in resource allocation. High GCs in juveniles were also expected to result in impaired immunity and enhanced parasite development during late life (Herbert et al. 2022).

2. Material and methods

2.1. Study populations

Two forest roe deer populations at Trois-Fontaines (TF - 1360 ha) in north-eastern France (48°43'N, 4°55'E) and at Chizé (CH - 2614 ha) in western France (46°05'N, 0°25'W) have been monitored through an intensive CMR program every winter since 1975 and 1977, respectively. At TF, forest productivity is high due to rich soils and the habitat is homogeneous at a large spatial scale and of good quality. By contrast, at CH, poor soils and frequent summer droughts result in overall low forest productivity and heterogeneous habitat quality with three main tree stands (Pettorelli et al. 2006), which makes this forest less suitable for roe deer. In both populations, quality of natal environmental conditions can be measured through the cohort-specific average mass of weaned offspring at the onset of winter (*i.e.* at about 8 months of age) corrected for the Julian date of capture (Gaillard et al. 1996). This metric of environmental quality allows accounting for the influence of environmental conditions early in life on roe deer performance, which predominate over current environmental conditions in roe deer (Pettorelli et al. 2002), and captures more reliably the effect of 'cohort quality' (*sensu* Gaillard et al. 2003 and see Lalande et al. 2023 and Cambreling et al. 2023 for case studies for GCs-body mass relationships and senescence of antler size, respectively). Such an integrative metric of natal environmental conditions encompasses variations in early environmental conditions (*e.g.* climate, resource availability and quality, roe deer density) as well as information about individual quality (*e.g.* mother condition and/or maternal care, themselves partly dependent on environmental quality; Toïgo et al. 2006). Cohort quality was included as a categorical variable with two levels (poor *v.* good cohorts) split around the median cohort quality in each population. Captures take place each year during 10-12 days, spread from December (at TF) or January (at CH) to early March (Gaillard et al. 1993). Yearly population size estimated from CMR analyses indicate that both populations were much lower during the present study period (2010-2022, Table S1)

than the maximum density recorded throughout the forty year-long monitoring. Since 2010, faecal matter is collected rectally and blood sampled from the jugular vein (1 mL/kg to a maximum of 20 mL) on weighed (to the nearest 0.1 kg and 0.05 kg in TF and CH, respectively) individuals of known sex and age (*i.e.* captured within their first year of life, either as newborn in spring, or as 8 months of age in winter).

2.2. Faecal Glucocorticoid Metabolites

In the present study, baseline HPA activity was estimated through faecal glucocorticoid metabolites (FGMs, Palme 2019) on juvenile roe deer (*i.e.* 8-9 months old). FGMs represent an integrative measure of GC concentrations and thus adrenocortical activity several hours before sampling (*i.e.* baseline stress; Palme, 2019), with a delay of 12 hours on average in roe deer (ranging from 6 to 23 hours, Dehnhard et al. 2001). Thus FGMs are widely used as a proxy for evaluating the baseline activity of the HPA axis of an individual (Palme 2019), notably in roe deer (Dehnhard et al. 2001, Möstl et al. 2002, Zbyryt et al. 2018).

Extraction of FGMs consisted in 0.5 g (± 0.005) of fresh faeces vortexed in 5 mL of 80% methanol and centrifuged for 15 minutes at 2500 *g* (Palme et al. 2013). The amount of FGMs was determined in an aliquot of the supernatant after a 1+9 dilution with assay buffer and was performed using a group-specific 11-oxoaetiocholanolone enzyme immunoassay (EIA), according to a method previously described in Möstl et al. (2002) and validated for roe deer (Zbyryt et al. 2018). Measurements were done in duplicate and intra- and inter-assay coefficients of variation were lower than 10% and 15%, respectively. Results of FGMs are expressed as nanograms per gram of wet faeces (ng/g).

Faeces collection in the wild is subject to several issues that might impact FGM levels. In both populations, faeces were collected rectally and immediately frozen at -80 °C after

collection, except prior to 2017 in TF where faeces were stored at 4 °C and frozen at -20 °C within 24 hours. The time from faecal collection to freezing can impact FGM levels due to different bacterial activity (Lexen et al. 2008, Hadinger et al. 2015, Carbillet et al. 2023b). Secondly, FGMs are likely to show variation throughout the capture period due to seasonal variation in HPA axis activity (Bubenik et al. 1998, Huber et al. 2003). Finally, animals are usually manipulated and sampled from 1 to 4 hours following the capture, but this delay can overpass 6 hours in exceptional cases so that the variation in the time delay between capture and sampling can also impact FGM levels. We first tested whether these three protocol issues affected the FGM levels measured in 162 individuals during their first winter. A linear mixed effect model (LMM) accounting for *i)* the Julian date of capture (linear and quadratic), *ii)* the time delay (min) between capture and sampling, and *iii)* whether faeces were immediately frozen at -80 °C or frozen at -20 °C within 24 hours, showed no effect of these confounds on log-transformed values of FGMs (Table S2). During the capture period, roe deer's diet is mostly composed of brambles (*Rubus* sp.) and ivy (*Hedera helix*) and similar in both study sites (Tixier and Duncan 1996), so we do not expect any bias in the measurement of FGM between populations that could be associated with diet.

2.3. Immune traits

To assess immunosenescence in both the innate and adaptive components of the immune function, we monitored 12 immune traits of both the humoral and cellular immune activity (Cheynel et al. 2017) measured between 2010 and 2021 (as well as 2022 for haemagglutination (HA) and haemolysis (HL) scores). In the field, blood samples are partitioned upon collection into *i*) EDTA tubes for white blood cells (WBCs) count and *ii*) serum collection tubes which were centrifuged 10 minutes at 3000 *g* before the serum is collected and frozen for subsequent HA-HL and globulin measurements.

2.3.1. Cellular innate immunity

Cellular innate immunity was assessed by describing the composition of the WBC population. We counted the first hundred WBCs in Wright-Giemsa-stained blood smears to evaluate the proportion of the different types of WBCs and brought back proportions to concentrations (10^3 cells/mL) (Houwen 2001). WBC population is composed of five different cell types: neutrophils are the most numerous WBCs and, together with monocytes, are key components of the innate arm of the immune system through their antimicrobial/biotoxic and phagocytic activity. Moreover, neutrophils and monocytes have recently been identified as having a role in auto-immune and inflammatory diseases when their activation is dysregulated (Auffray et al. 2009, Burn et al. 2021). Basophils, the least abundant WBCs, have a specific role in the inflammatory response as they are particularly active at sites of ectoparasite infections such as ticks, and communicate with the adaptive arm of the immune system (Siracusa et al. 2010, Karasuyama et al. 2011). Eosinophils are also involved in the inflammatory responses and reaction to allergies alongside basophils and mast cells. They also play a role in the defence against internal parasites such as helminths, and in the modulation of both components of immunity. If dysregulated, their cytotoxic action can have detrimental consequences on the organism (Rothenberg and Hogan 2006). Finally, lymphocytes are involved in the cellular adaptive immunity, as described below.

2.3.2. Humoral innate immunity

To evaluate the humoral part of the innate immune system, we performed HA-HL assays, as described in Matson et al. (2005) and previously used on roe deer blood samples (Gilot-Fromont et al. 2012, Cheynel et al. 2017, Carbillet et al. 2022, 2023a). Briefly, haemagglutination [HA, 10^{-2} log(dilution)] measures the plasmatic ability of an individual to agglutinate exogeneous cells, as a proxy of the concentration of circulating natural antibodies (NAbs). Similarly, haemolysis [HL, 10^{-2} log(dilution)] measures the plasmatic activity of the complement, a group of proteins acting in chain reactions to provoke the lysis of exogeneous

cells in the presence of antigen-antibody complexes (Matson et al. 2005). Secondly, we assessed the concentrations (mg/mL) of alpha1-globulins, alpha2-globulins and beta-globulins to characterise the level of inflammatory proteins involved in the acute phase response, a part of the early innate immune system (Cray et al. 2009). It was done using refractometry and automatic agarose gel electrophoresis (HYDRASYS, Sebia, Evry, France) that separates blood proteins into five fractions: albumin, alpha1-, alpha2-, beta- and gamma-globulin fractions. More specifically, we also assayed haptoglobin concentration, a protein from the alpha2-globulin fraction produced in case of chronic inflammation and infection. Haptoglobin concentration (mg/mL) was measured with a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France) using phase haptoglobin assay chromogenic kit (Tridelta Development LTD, County Kildare, Ireland).

2.3.3. Cellular adaptive immunity

Cellular adaptive immunity was characterised by measuring the concentration of lymphocytes (10^3 cells/mL). Lymphocytes include both B and T cells. T lymphocytes have many functions, among which the recognition of exogeneous or infected cells and can provoke cell death. B lymphocytes are an essential part of the humoral adaptive immunity, being the main antibody-producing cells. Lymphocytes also include natural killer (NK) cells, as part of the innate immunity. However, as NK cells are a minority among lymphocytes (*e.g.* 0.5-10 % in cattle; Kulberg et al. 2004), they have been neglected here.

2.3.4. Humoral adaptive immunity

Humoral adaptive immunity was measured through the concentration (mg/mL) of gamma-globulins (or immunoglobulins) which represent the majority of circulating antibodies. Gamma-globulins levels were also determined from electrophoresis as described above.

2.4. Parasite abundance

We counted the number of faecal propagules of 4 parasites commonly occurring in roe deer as it is a reliable estimator of the parasitic load in roe deer in these populations during the capture period (Body et al. 2011). Investigated parasites encompassed pulmonary nematodes (protostrongylids), gastro-intestinal (GI) nematodes (GI strongyles and *Trichuris* sp.) and GI protozoan (coccidia, *Eimeria* sp.). For GI nematodes and protozoan, we obtained the counts of eggs per gram (EPG) and oocysts per gram (OPG) of faeces, respectively, using a modified McMaster protocol (Raynaud 1970) with a solution of zinc sulfate ($ZnSO_4$, s.g. = 1.36) and by counting in the whole chambers of the McMaster slide (*i.e.* quantitative examination with a theoretical sensitivity of 15 EPG/OPG). We also centrifuged a 14 mL tube with the remaining solution, covered with a coverslip, before seeking for the presence of parasite propagules not detected on the McMaster slide (*i.e.* control slide, qualitative examination). For pulmonary nematodes, we obtained the count of stage 1 larvae per gram of faeces (LPG) using the Baermann faecal technique (Baermann 1917).

2.5. Statistical analyses

All analyses were performed using R version 4.3.0 (R Core Team 2023).

2.5.1. Immunosenescence and age-specific changes in parasite abundance

For the 12 immune traits and the 4 parasites, we tested different ageing trajectories using linear mixed models (LMMs): no age effect, a linear effect of age, a quadratic effect, and threshold trajectories. We tested three threshold models differing by whether pre- and post-threshold trajectories were set as constant or not: the first model corresponded to no age-related variation until a threshold age and a linear change of traits beyond that threshold, the second corresponded to a linear change of traits before the threshold age and no age-related variation beyond, and the third corresponded to two different linear changes of traits before

and beyond the threshold age. Threshold models were implemented following an approach previously described and commonly used in ageing research (*e.g.* Douhard et al. 2017, Briga et al. 2019). Briefly, thresholds varied between 3 and 10 years old with one-year increments and we identified the best-fitting threshold as described in subsection 2.5.2 below. Thresholds were not tested above 10 years old because it is the last age for which we have observations of at least one individual for each population-sex-cohort group.

Immunosenescence and parasite abundance trajectory models included each immune or parasitic trait as the response variable. Parasite abundances were log-transformed as $ln(n+1)$ to reach a normal distribution. For HL scores, we used the residuals of the regression of the score on the serum coloration as a response variable, to correct for the ability of the complement to cause haemolysis according to the level of haemolysis of the serum.

We expected immunosenescence patterns to differ between populations and between sexes (Cheynel et al. 2017). Early-life environmental conditions may also influence sex-specific immunosenescence because cohort-specific environmental quality has been shown to drive differential variation in adult phenotypes between males and females in the two populations (Douhard et al. 2013, Garratt et al. 2015). Models included the additive fixed effects of age (linear, quadratic or threshold function), population, sex and cohort quality, and the two-way interactions between age terms and the three other factors. Models also included four possible confounding variables as fixed effects: 1) the body mass (hereafter named ‘mass’: standardised for the Julian date of capture for juveniles; Douhard et al. 2017) as body condition affects immune phenotype (Gilot-Fromont et al. 2012) and as parasitism is negatively linked to body mass in roe deer (Body et al. 2011); 2) the age at last observation to account for the selective disappearance of individuals with low immune performance, which could ultimately lead to an under-estimation of the intensity of senescence (hereafter named 'age-last'; van de Pol and Verhulst 2006, van de Pol and Wright 2009); 3) the Julian date of

capture to account for potential temporal variations in immune parameters and parasite exposure (hereafter named 'julian'); and 4) the time delay between capture and blood sampling, for immune but not parasitic traits, to account for changes in immune parameters in response to hormonal changes triggered by the stress of capture (hereafter named 'delay'). We refer to these four additional fixed effects as 'confounding effects' hereafter. Finally, all models included random effects of the individual identity ('ID') to account for repeated measurements on the same individuals, as well as the year of capture nested within the population, to account for year-to-year variation in demographic and environmental conditions in both populations. At the end, we thus compared six models (*i.e.* no age effect, linear, quadratic, and three threshold age functions) per immune or parasitic trait (see Table S3 for the full list of models and their selection). We did not include random slopes of age terms as they resulted in perfect correlations between the random intercepts and slopes (Harrison et al. 2018).

2.5.2. Model selection for senescence trajectories

To select for the most relevant ageing trajectories, we performed model selection to compare the constant, linear, quadratic and threshold models based on the second-order Akaike Information Criterion corrected for small sample size (AICc) as implemented in the package 'MuMIn' (Bartoń 2021). Briefly, among the set of models within 2 ΔAICc, we considered the simplest model (*i.e.* with the lowest number of parameters) as the supported model to satisfy parsimony rules (Burnham and Anderson 2002, see also Arnold 2010; Table S3). Note that for all threshold models, for comparison with other model types, we added one parameter and 2 to their AICc values to penalise these models for the extra-parameter of the threshold, but which is not explicitly included in the models.

2.5.3. Juvenile-stage FGMs and immunosenescence

We then tested whether FGMs levels measured in the juvenile period modulated the retained immunosenescence patterns by testing the effect of juvenile-stage FGMs on the rates of senescence of each immune trait. Because having individuals with both FGMs measured during their first year of life and adult immune/parasite traits substantially decreased sample sizes, we kept the structure of the previously found immunosenescence patterns and added the additive effect of FGMs. We also added the two-way interactions between FGMs and *i*) the different retained age terms and *ii*) population, sex and/or cohort quality when they were initially retained. If no detectable age change occurred in the previously retained trajectories, we only added the additive effect of early-life FGMs on the immune/parasitic traits, in addition to the confounding variables (*i.e.* 'mass', 'age-last', 'julian', 'delay') that were previously retained. Model selection tables for each trait are presented in Supplementary Information (SI, Table S4). Note that for some variables, confidence intervals of estimates might overlap 0. This is because these variables have previously been retained when describing immunosenescence patterns on the full dataset, and this structure was considered as fixed when testing FGM effects on immunosenescence rates with the reduced dataset. The random effect structure was also kept as described above, except for alpha1- and alpha2-globulin for which the inclusion of ID resulted in singularities. Therefore, when testing for the effect of FGMs during the juvenile stage on the immunosenescence rates of these two traits, we included only one observation per individual, keeping the observation for which the age at measurement was the closest to the mean age of all individuals that were sampled only once.

Once the trajectory had been identified for the 12 immune and the 4 parasitic traits, we used the same model selection approach to retain or not the additive and interacting effects of juvenile FGMs with the age function terms, and population, sex, and cohort quality factors (Table S4).

3. Results

3.1. Immune traits

Immunosenescence patterns were determined on 1021-1201 observations obtained from 483-556 individuals, depending on the investigated trait (see Table 1). Immunosenescence patterns are displayed in Figure 1, whereas immune traits for which no age variation was detected are displayed in Supplementary Information (Figure S1). For innate traits, neutrophil levels were higher at TF than at CH. The retained model evidenced a similar increase with age in both populations, without any detectable between-sex or among-cohort differences (Table 1, Figure 1A). Monocytes decreased from 2 to 5 years of age. Afterwards our approach could not detect any age-related change until the end of life (Table 1, Figure 1B). Basophils, eosinophils, and HA and HL scores did not display any detectable age-related variation, although eosinophil levels were higher in females than in males at all ages, without any evidence of between-population or among-cohort differences (Table 1, Figure S1A-D). For the inflammatory response, alpha1-globulins, beta-globulins and haptoglobins showed an overall increase in their concentrations throughout lifespan (Table 1, Figure1C-E, Figure S1E), except in males for alpha1-globulins that were overall independent of age (Table 1, Figure 1C). Note that for alpha1-globulins, concentration in females was lower than those of males at the beginning of life but reached that of males at around 10 years of age before keeping increasing until the end of life. Beta-globulin concentration increased linearly with age and similarly in both sexes, with higher levels in males than in females, and without any between-population or among-cohort differences (Table 1, Figure 1D). Haptoglobin concentration increased quadratically across life similarly in both sexes but with higher concentration for males compared to females (Table 1, Figure 1E). We detected no age-related variation in alpha2-globulin concentration throughout life but the concentration was slightly higher in females compared to males (Table 1, Figure S1E). Models for adaptive immunity showed that gamma-globulin concentration increased linearly throughout life and

similarly in both populations, with a higher concentration in CH than TF (Table 1, Figure 1F). Lymphocyte concentration decreased in the first part of life (until 4 years of age) in both populations. Afterwards, lymphocyte count kept slightly decreasing in CH until the end of life, whereas they increased in TF. Lymphocyte count was consistently higher at TF than at CH, and higher in males than in females (Table 1, Figure 1G). Note that the retained variables explained more variation in the adaptive immune parameters than they did for innate or inflammatory traits (see R^2 in Table 1). Immunosenescence onsets and rates for each trait are summed up in Table 3.

Our analyses did not suggest any sign of selective disappearance, as the age at last observation was never retained in the selected models, except for alpha1-globulins ($\beta \pm$ standard-error (SE) = -0.02 ± 0.01; Table 1). Some immune trait values were impacted by the delay between capture and sampling (neutrophils: $\beta \pm SE = 0.04.10^{-1} \pm 0.06.10^{-2}$; basophils: $\beta \pm SE = 0.08.10^{-3} \pm 0.03.10^{-3}$; eosinophils: $\beta \pm SE = -0.04.10^{-2} \pm 0.05.10^{-3}$; HL: $\beta \pm SE = 0.08.10^{-2} \pm 0.03.10^{-2}$; lymphocytes: $\beta \pm SE = -0.01.10^{-1} \pm 0.03.10^{-2}$; Table 1). Body mass was negatively associated to globulin concentrations mostly, suggesting that individuals of poorest condition had lowest protein resources (alpha1-globulins: $\beta \pm SE = -0.04 \pm 0.01$; beta-globulins: $\beta \pm SE = -0.10 \pm 0.03$; gamma-globulins: $\beta \pm SE = -0.19 \pm 0.06$; Table 1). Only alpha1-globulins showed temporal variation throughout the capture season, with a trend to decrease across the season ($\beta \pm SE = -0.03.10^{-1} \pm 0.01.10^{-1}$; Table 1).

The relationship between juvenile FGMs and immunosenescence rates was evaluated on 249-332 observations from 136-159 individuals, depending on the investigated trait (see Table S4). The slope of the lymphocyte count after the onset of senescence (*i.e.* 4 years of age) decreased as juvenile FGMs increased ($\beta_{FGM} \pm SE = 0.19 \pm 0.11$; $\beta_{age.2} \pm SE = 1.77 \pm 0.62$; $\beta_{FGM:age.2} \pm SE = -0.24 \pm 0.10$; Figure 3A, Table S4). For all other traits, we failed to detect a link between juvenile FGMs and immunosenescence rates (Table S4). Indeed, models

including either the additive or interacting effect of juvenile FGMs with age did not improve the model fit in any case (Table S4).

3.2. Parasite abundance

Age-specific patterns of parasite abundance were determined based on 915-976 observations from 449-469 individuals, depending on the parasite investigated. The abundance of GI strongyles increased with age similarly in both sexes and in both populations until 10 years of age. Afterwards, male parasitic abundance increased even more. GI strongyles abundance was consistently higher in males than in females (Table 2, Figure 2A). At CH, the abundance of *Trichuris* sp. increased in both sexes, although more strongly in males than in females up to 10 years of age, after what parasitic abundance tended to decrease in females and to increase even more in males until the end of life. At TF, parasitic abundance in males increased up to 10 years of age and accelerated afterwards, whereas it remained constant in females up to that age and increased slightly less than for males afterwards. *Trichuris* sp. abundance was always higher in males compared to females, and in CH compared to TF (Table 2, Figure 2B). Protostrongylid abundance was higher in males than in females and increased with age in both sexes, although faster in males compared to females. Parasitic abundance increased from *ca.* 5 and 6 years of age in males and females, respectively, whereas it increased from *ca.* 2 years of age in both sexes for individuals born during years of good quality, although faster in males compared to females. Protostrongylid abundance was lower at CH than at TF, and was higher and increased more rapidly for individuals born during years of good quality compared to those born during years of poor quality (Table 2, Figure 2C). We did not detect any change in abundance of coccidia in either population or sex (Table 2, Figure 2C). Onsets and rates of change in parasite abundance with age are summed up in Table 3.

All parasite abundances, except for coccidia, were negatively related to individual body mass, with lighter individuals being more parasitised than heavier ones (GI strongyles: $\beta \pm SE = -0.07 \pm 0.02$; *Trichuris* sp.: $\beta \pm SE = -0.16 \pm 0.02$; Protostrongylids: $\beta \pm SE = -0.06 \pm 0.01$; Table 2). We detected signs of selective disappearance for GI strongyles ($\beta \pm SE = -0.15 \pm 0.03$; Table 2) and protostrongylids ($\beta \pm SE = -0.02 \pm 0.01$; Table 2).

The effect of juvenile FGMs on retained trajectories was evaluated on 252-274 observations from 129-140 individuals, depending on the investigated parasite (see Table S4). We found no evidence for a link between juvenile FGMs and GI nematodes or protozoan abundance trajectories (Table S4). For protostrongylids, however, we retained the additive effect of juvenile FGMs on adult abundance interacting with the cohort quality ($\beta_{FGM} \pm SE = 0.04 \pm 0.12$, $\beta_{Cohort\ quality(Poor)} \pm SE = -3.24 \pm 1.21$; $\beta_{FGM:Cohort_quality(Poor)} \pm SE = 0.50 \pm 0.18$; Table S4, Figure 3B). This suggests that among individuals born during years of poor quality, those with higher juvenile FGMs display higher protostrongylid loads as adult, whereas no particular trend was detected for individuals born during years of good quality (Figure 3B). Note that when removing the high residual value on the top right of Figure 3B, only a positive additive effect of juvenile FGMs on adult abundance was retained ($\beta \pm SE = 0.15 \pm 0.08$).

Table 1. Selected linear mixed effect models for the 12 immune traits according to age (linear, quadratic, threshold), population ('Pop', reference level is Trois-Fontaines; CH: Chizé) and sex ('Sex', reference level is female; M: male). Models accounted for the following potential confounding effects: body mass ('Mass'), age at last observation ('Age-last'), time delay between capture and sampling ('Delay') and Julian day of capture ('Julian'). All models included the random effects of the individual identity and the year of capture nested within the population. SE: standard error, R^2m – R^2c: marginal – conditional model variance, respectively.

Immune trait	Model	Fixed effects	Estimate	SE	R^2m	R^2c
INNATE IMMUNE SYSTEM						
Neutrophil (N = 1021 obs., n = 484 ind.)	Quadratic	Intercept	4.81	0.26	0.09	0.52
		Age²	$0.07.10^{-1}$	$0.02.10^{-1}$		
		Pop(CH)	-0.79	0.26		
		Delay	$0.04.10^{-1}$	$0.06.10^{-2}$		
Monocyte (N = 1021 obs., n = 484 ind.)	Threshold (5 years of age)	Intercept	0.25	0.04	0.01	0.41
		Age.1	-0.02	$0.07.10^{-2}$		
Basophil (N = 1021 obs., n = 484 ind.)	-	Intercept	0.08	0.01	0.01	0.30
		Delay	$0.08.10^{-3}$	$0.03.10^{-3}$		
Eosinophil (N = 1021 obs., n = 484 ind.)	Sex	Intercept	0.26	0.02	0.09	0.19
		Sex(M)	-0.04	0.01		
		Delay	$-0.04.10^{-2}$	$0.05.10^{-3}$		
Haemagglutination (N = 1201 obs., n = 556 ind.)	-	Intercept	3.90	0.15	-	0.38
Haemolysis (N = 1191 obs., n = 555 ind.)	-	Intercept	-0.16	0.18	$0.04.10^{-1}$	0.49
		Delay	$0.08.10^{-2}$	$0.03.10^{-2}$		
INFLAMMATORY SYSTEM						
Alpha1-globulin (N = 1029 obs., n = 485 ind.)	Quadratic	Intercept	4.07	0.19	0.06	0.46
		Age²	$0.04.10^{-1}$	$0.07.10^{-2}$		
		Sex(M)	0.31	0.05		
		Age²:sex(M)	$-0.02.10^{-1}$	$0.01.10^{-1}$		
		Mass	-0.04	0.01		
		Julian	$-0.03.10^{-1}$	$0.01.10^{-1}$		
		Age-last	-0.02	0.01		
Alpha2-globulin (N = 1029 obs., n = 485 ind.)	Sex	Intercept	5.83	0.15	0.01	0.23
		Sex(M)	-0.28	0.10		
Beta-globulin (N = 1029 obs., n = 485 ind.)	Linear	Intercept	7.70	0.55	0.12	0.41
		Age	0.23	0.02		
		Sex(M)	0.69	0.13		
		Mass	-0.10	0.03		
Haptoglobin (N = 1034 obs., n = 483 ind.)	Quadratic	Intercept	0.25	0.08	0.03	0.20
		Age²	$0.04.10^{-1}$	$0.01.10^{-1}$		
		Sex(M)	0.32	0.08		
ADAPTIVE IMMUNE SYSTEM						
Gamma-globulin (N = 1029 obs., n = 485 ind.)	Linear	Intercept	17.66	1.49	0.23	0.63
		Age	0.42	0.05		
		Pop(CH)	3.70	1.04		
		Mass	-0.19	0.06		
Lymphocyte (N = 1021 obs., n = 484 ind.)	Threshold (4 years of age)	Intercept	3.42	0.20	0.14	0.48
		Age.1	-0.19	0.04		
		Age.2	0.06	0.02		
		Pop(CH)	-0.55	0.19		
		Sex(M)	-0.18	0.07		
		Age.2:pop(CH)	-0.08	0.03		
		Delay	$-0.01.10^{-1}$	$0.03.10^{-2}$		

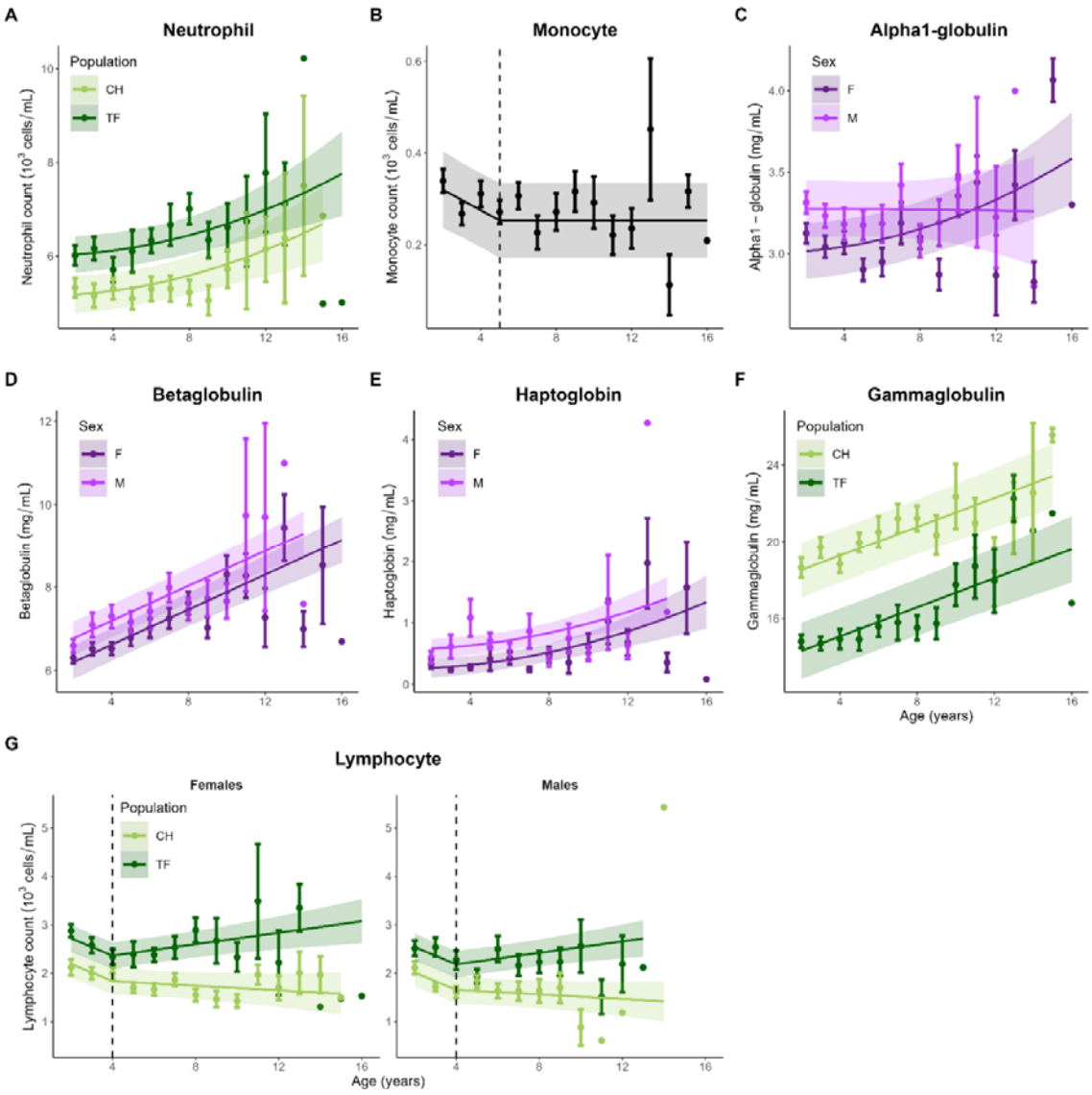

Figure 1. Immunosenescence patterns in roe deer. Lines are retained model predictions for each immune trait (excluding retained confounding variables for graphical representation) and shaded areas are 95% CIs. Points are age-specific average trait values ± standard errors.

Table 2. Selected linear mixed effect models for the 4 parasitic traits according to age (linear, quadratic, threshold), population ('Pop', reference level is Trois-Fontaines; CH: Chizé), sex ('Sex', reference level is females; M: males), and cohort quality ('Cohort', reference level is 'good cohort quality'; P: poor cohort quality). Models accounted for the following potential confounding effects: body mass ('Mass'), age at last observation ('Age-last') and Julian day of capture ('Julian'). All models included the random effects of the individual identity and the year of capture

Parasite trait	Model	Fixed effects	Estimate	SE	R^2m	R^2c
Gastro-intestinal strongyles (N = 976 obs., n = 469 ind.)	Threshold (10 years of age)	Intercept	3.39	0.49	0.09	0.36
		Age.1	0.18	0.03		
		Age.2	0.25	0.12		
		Sex(M)	0.35	0.12		
		Age.2:sex(M)	0.79	0.24		
		Mass	-0.07	0.02		
		Age-last	-0.15	0.03		
***Trichuris* sp. (N = 938 obs., n = 450 ind.)**	Threshold (10 years of age)	Intercept	3.38	0.56	0.32	0.51
		Age.1	0.10	0.04		
		Age.2	0.48	0.15		
		Pop(CH)	0.54	0.30		
		Sex(M)	0.17	0.24		
		Age.1:pop(CH)	0.14	0.04		
		Age.1:sex(M)	0.12	0.04		
		Age.2:pop(CH)	-0.84	0.22		
		Age.2:sex(M)	0.81	0.26		
		Mass	-0.16	0.02		

nested within the population. SE: standard error, R^2m – R^2c: marginal – conditional model variance, respectively.

Protostrongylids (N = 915 obs., n = 449 ind.)	Quadratic	Intercept	1.81	0.26	0.17	0.39
		Age	-0.15	0.05		
		Age²	0.02	$0.03.10^{-1}$		
		Pop(CH)	-0.17	0.08		
		Sex(M)	0.08	0.07		
		Cohort(P)	-0.47	0.18		
		Age:cohort(P)	0.21	0.06		
		Age²:sex(M)	0.01	$0.01.10^{-1}$		
		Age²:cohort(P)	-0.02	$0.05.10^{-1}$		
		Mass	-0.06	0.01		
		Age-last	-0.02	0.01		
Coccidia (N = 974 obs., n = 469 ind.)	-	Intercept	1.17	0.07	-	0.11

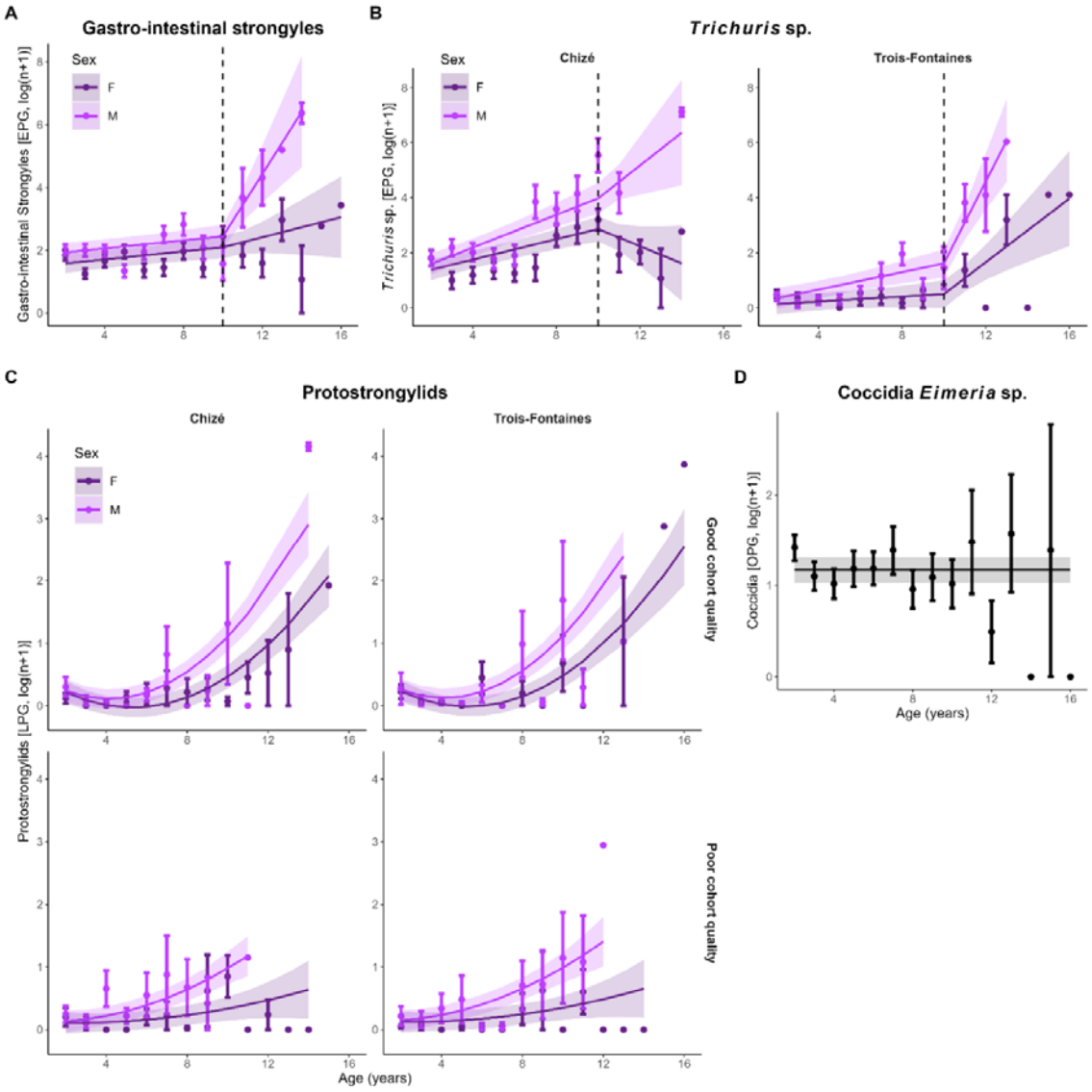

Figure 2. Parasite abundance age trajectories in roe deer. Lines are retained model predictions for each parasitic trait (excluding retained confounding variables for graphical representation) and shaded areas are 95% CIs. EPG: eggs per gram, LPG: larvae per gram, OPG: oocysts per gram. Points are age-specific average trait values ± standard errors.

Table 3. Summary of evidence for immunosenescence and change in parasite abundances with age in roe deer. 12 immune traits and four parasites were investigated. Estimations of onsets and rates of changes with age are provided based on retained model predictions. F: females; M: males, CH: Chizé, TF: Trois-Fontaines, EPG: eggs per gram of faeces, OPG: oocysts per gram of faeces. For quadratic trajectories, onsets were estimated as the age for which the derivative was null. Rates were estimated using the derivative of the function for the onset and for the last age available to represent the range of change with age from the onset to the end of life. When the onset was <2, rates were estimated at 2 years of age.

Trait	Immunosenescence / Change in parasite abundance with age	Onset (years)	Rate
INNATE IMMUNITY			
Neutrophil	Yes	<2	+28–224 cells/year
Monocyte	Yes	2	-20 cells/year
Basophil	No	-	-
Eosinophil	No	-	-
Haemagglutination	No	-	-
Haemolysis	No	-	-
INFLAMMATORY RESPONSE			
Alpha1-globulin	Yes	<2	F: +0.02–0.13 $mg.mL^{-1}$/year; M: +0–0.04 $mg.mL^{-1}$/year
Alpha2-globulin	No	-	-
Beta-globulin	Yes	2	+0.23 $mg.mL^{-1}$/year
Haptoglobin	Yes	<2	+0.02–0.13 $mg.mL^{-1}$/year
ADAPTIVE IMMUNITY			
Gamma-globulin	Yes	2	+0.42 $mg.mL^{-1}$/year
Lymphocyte	Yes	4	CH: -20 cells/year; TF: +60 cells/year
PARASITES			
Gastro-intestinal strongyles	Yes	10	F: +0.28 EPG/year M: +1.86 EPG/year
***Trichuris* sp.**	Yes	10	F-CH: -0.30 EPG/year; F-TF: +0.61 EPG/year; M-CH: +0.58 EPG/year; M-TF: +2.63 EPG/year
Protostrongylids	Yes	Good cohort: F = 4.2; M = 3.2 Poor cohort: <2	Good cohort-F: +0.00–0.53 LPG/year; Good cohort-M: +0.00–0.60 LPG/year; Poor cohort-F: +0.06-0.07 LPG/year; Poor cohort-M: +0.09–0.23 LPG/year
Coccidia	No	-	-

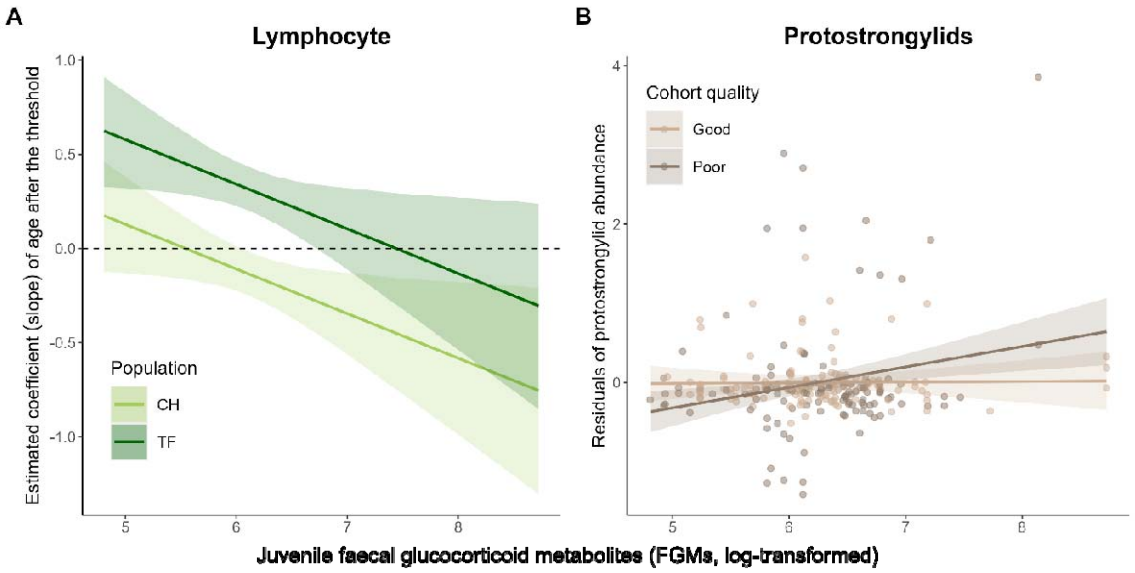

Figure 3. (A) Predicted slope of lymphocyte concentration as a function of age after the threshold (*i.e.* 4 years of age, Figure 1G) according to faecal glucocorticoid metabolites (FGMs, log-transformed) measured during the first year of life and the population (CH: Chizé; TF: Trois-Fontaines). (B) Predicted roe deer protostrongylid abundance accounting for age, population, sex, cohort, body mass and age at last observation (residuals of the retained parasite trajectory, Figure 2C) according to FGMs (log-transformed) measured during the first year of life and cohort quality. Note that when removing the particularly high parasite abundance value (*i.e.* top right corner), no cohort effect was retained. Lines are the model predictions and shaded areas are 95% CIs.

4. Discussion

There is a gap in the literature on the putative relationship between early-life GC levels and immunosenescence in mammals. Studies that demonstrated negative relationships between GCs and immunosenescence focused mostly on humans (Bauer 2005, 2008, Garrido et al. 2022), and these studies did not explore the carry-over effects of high GCs early in life on immunosenescence. Only few studies have assessed the effect of stress exposure or GC levels during the juvenile period on late-life performance, evidencing negative relationships, but they were all conducted on birds (Spencer et al. 2009, Monaghan et al. 2011, Herborn et al. 2014, Monaghan and Haussmann 2015, Casagrande et al. 2020, but see also Spencer and Verhulst 2008). Here, we explored whether GCs, measured as FGMs during the juvenile period, can be linked to patterns of immunosenescence in roe deer in the wild. Using a remarkably large dataset and controlling for several confounding factors, we found that, overall, our patterns of immunosenescence supported those previously reported by Cheynel et al. (2017) in roe deer, although few differences and novelties arose (*e.g.* sex effects, different shape trajectories). Most importantly, our results do not support our prediction that FGM levels in juveniles modulate immunosenescence rates, except for lymphocytes for which we detected an accelerated depletion of the pool after 4 years of age with increasing juvenile FGMs. Also, FGM levels in juveniles were positively associated, although weakly, with the abundance of pulmonary parasites (*i.e.* protostrongylids), measured during adulthood for individuals born during years of poor quality, but without modulating the ageing trajectory of these parasite loads.

Lymphocyte count was higher at TF compared to CH, and higher in females compared to males. For both sexes and populations, lymphocyte count decreased linearly from to 2 to 4 years of age, after what count trajectories diverged between populations, with a decrease at CH and an increase at TF. A decrease was expected because of the thymic involution

occurring with advancing age resulting in a decreased production of naïve B and T lymphocyte and finally changes in the lymphocyte pool population with a greater proportion of mature memory cells and a smaller repertoire diversity compared to younger ages (Hakim and Gress 2007). By contrast, the increase observed at TF rather suggests that the expected decline in lymphocyte count can be dampened or prevented. The more favourable environmental conditions encountered at TF could explain this trend (McDade et al. 2016). Gamma-globulin concentration increased with age in both populations. While it was higher at CH than at TF, lymphocyte count was lower at CH than at TF. Thus, B lymphocytes from individuals of CH could produce more antibodies relative to their number compared to TF, potentially resulting in a stronger auto-immunity at CH than at TF. Indeed, immunosenescence has been found to be accompanied by a decrease in the production of high-affinity antibodies (Frasca et al. 2011), as well as by an increase in the production of auto-antibodies which can be in some cases associated to autoimmune diseases (Hakim and Gress 2007, Kovaiou et al. 2007, Lleo et al. 2010, Bachi et al. 2013). This suggests a dysregulation of the adaptive immunity, rather than a mere decline in adaptive immunity cell counts with increasing age. Nevertheless, we do not have sufficient information about the lymphocyte pool composition to draw strong conclusions here, since we do not discriminate between B and T or between naïve and mature lymphocytes for instance and especially regarding the relative proportions of NK cells and B and T lymphocytes. Moreover, additional characterisation of the immunoglobulins (isotypes, affinity, glycolisation, …) would be necessary to evaluate their potential efficiency against certain parasites or ability to induce an autoimmune response. Still, altogether, our results suggest that the adaptive immunity functioning could be altered, even if in different ways, between populations and this could be in line with a decline in adaptive immunity with increasing age (Peters et al. 2019).

Age-related variation in lymphocyte count beyond 4 years of age was in addition modulated by FGM exposure during the juvenile stage. Indeed, the slope of the change in lymphocyte count with age diminished as juvenile FGMs increased. Lymphocytes are especially sensitive to chronically elevated GCs, which promote thymocyte (*i.e.* immature lymphocyte within the thymus) apoptosis and thymic involution (Gruber et al. 1994, Gomez-Sanchez 2009, Taves et al. 2017), whereas GCs below stress-induced levels seem to rather enhance survival of lymphocytes and to delay thymic involution (Taves et al. 2017). As the thymus is the primary lymphoid organ responsible for the maturation and selection of T cells (Palmer 2013), this suggests that the age-related depletion of the lymphocyte pool can be accelerated by GCs. Although GCs are primarily produced by the adrenal glands, they are also locally produced, notably by the thymus. Chronic elevation of adrenal GCs, such as during adverse environmental conditions, has been suggested to result in an increased thymic GC production, which can amplify the adverse effect of GCs on thymus functioning (Taves et al. 2017). This increased sensitivity of the thymus to elevated GC levels can partly explain why we observed an effect of juvenile GCs particularly on lymphocytes but not on other immune traits. Investigating how adrenal and thymic GC production covary in these populations would be of interest to better understand how environmental conditions can relate to changes in lymphocyte count.

FGMs during the juvenile period were weakly but positively related to the abundance of lung parasites during adulthood. Indeed, once accounting for age, sex, population, cohort quality, body mass and selective disappearance, adult abundance of lung parasites increased as juvenile FGMs increased. This result was nevertheless modulated by cohort quality: juveniles with higher FGM levels exhibited a higher abundance of protostrongylids during adulthood compared to juveniles with lower FGM levels, only for individuals born during years of poor quality. Protostrongylids have a long period of patency (> 2 years) and do not

enable the final host to develop acquired immunity (Adem 2016). Thus, innate immunity might be the only line of defence against such lung parasites. As young individuals are expected to rely more heavily on innate immunity than on adaptive immunity (McDade et al. 2016), and because adaptive immunity does not seem to defend the host against protostrongylids, high juvenile FGMs could disrupt early innate immunity (Carbillet et al. 2023a) and favour infection by protostrongylids, resulting in individuals more heavily infected during adulthood. Our results suggest that this is even more the case for individuals born during years of poor quality for which innate immunity might be more sensitive to glucocorticoids (Carbillet et al. 2023a). Note, however, that the cohort effect appeared to be mainly driven by one heavily parasitised individual from a poor cohort.

Protostrongylids are weakly pathogenic parasites, except when individuals are heavily infected (Adem 2016). The retained model suggests that selective disappearance occurred (β = -0.02, se = 0.01, Table 2), with surviving individuals at advanced age tending to be the least parasitised. This can explain that individuals born during years of poor quality displayed lower parasitic loads compared to individuals born during years of good quality. Individuals from poor quality years might not survive heavy parasite loads, whereas individuals from good cohorts may better cope with the costs of being more heavily parasitised. Similarly, our results showed that selective disappearance occurred for GI strongyles (β = -0.13, se = 0.03, Table 2) and that this effect was even stronger than for protostrongylids. Thus, individuals heavily parasitised by GI strongyles are likely not represented in our dataset, what can mask a potential effect of juvenile GCs on adult abundance for this parasite. GI strongyle species are diverse in both populations, and certain of these species are highly pathogenic (*e.g. Bunostomum trigonocephalum*, *Haemonchus contortus*; Beaumelle et al. 2021). Hence, the selective pressure of parasites on individual capacity to maintain efficient immunity, regardless of GC levels, can potentially be higher for GI parasites than for protostrongylids,

Trichuris sp. or coccidia. Interestingly the highly pathogenic GI nematodes (*B. trigonocephalum* and *H. contortus*) were only found in TF.

Overall, except for lymphocyte count, we observed a lack of relationship between juvenile FGMs and immunosenescence, and this could be due to a certain number of limitations when dealing with GCs. First, the stress response does not only involve the HPA axis and GCs but also other hormones, cytokines and neurotransmitters (Sapolsky et al. 2000). Similarly, GCs are not the sole contributors to allostasis, and estimating allostatic load should ideally be done using several markers to summarise the level of physiological activity (including markers of neuroendocrine, immune, metabolic, and cardiovascular functions, Seeman et al. 1997, 2001, Romero et al. 2009, Juster et al. 2010). Secondly, it is admitted that acute/short-term GCs elevations can stimulate immune functions, whereas high baseline GC levels or chronic GC elevations often result in immunosuppression (Martin 2009, Dhabhar 2014). However, in some instances, chronic/long-term GC elevations have also been reported to decrease the responsiveness of macrophages to GCs, resulting in an up-regulation of immune activity (Martin et al. 2005, Martin 2009). Such a pattern may be expected for individuals living at risk of disease or infection threats to ensure competent immune system at all times (Martin et al. 2005). Thirdly, the consequences of GCs on certain traits, such as immune ones, could be restricted to immediate or relatively short-term consequences (*i.e.* days-weeks), but could be lifelong on others, such as telomere dynamics, as evidenced in the same roe deer populations (Lemaître et al. 2021). Also, the timing of FGM measurement, at 8 months of age, could be too late to assess carry-over consequences of GCs on physiology as such consequences could be restricted to very early-life stages during the maturation of the HPA axis. Finally, it is important to note that juvenile FGMs are measured on individuals that have survived up to that age after a period of strong viability selection (Garratt et al. 2015).

A recent study on the same two roe deer populations has shown that baseline GC levels were negatively associated with individual body mass, but GCs measured during the first year of life showed no delayed relationship with adult body mass (Lalande et al. 2023). Similarly, baseline GC levels have been shown to be associated with immunity over time in this species (Carbillet et al. 2022), but we were not able to detect any global carry-over effects of GCs on roe deer immunity and immunosenescence in either sex or population, except for lymphocytes. One possible explanation for the observed overall absence of relationship can be the time window considered, as many events can happen between the juvenile and the senescence stage. Among these events, most juvenile natal dispersal occurs about three months after juvenile FGMs are measured (Hewison et al. 2021). Dispersal can influence both current (Maag et al. 2019) and future FGM levels depending on the local environmental conditions dispersers settle in, especially in the heterogeneous forest of CH.

By considering a larger dataset and more confounding factors, we expected to refine the immunosenescence patterns previously reported in the focal roe deer populations by Cheynel et al. (2017). Overall, our patterns of immunosenescence supported those previously found, although some traits exhibit slightly different age trajectories (*i.e.* beta-globulins, haptoglobins, gamma-globulins, lymphocytes GI strongyles, *Trichuris* sp. and protostrongylids). However, some differences, likely due to an increase in sample size (by 66-95% depending on the trait), deserve to be noted. First, monocytes that previously showed no changes with age displayed senescence here (Table 3). Secondly, between-population differences are not the main modulator of immunosenescence patterns anymore. While Cheynel et al. (2017) showed population-specific trajectories for six traits, only lymphocyte count and *Trichuris* sp. abundance displayed between-population differences in their age trajectories here. Thirdly, we found no effect of natal environmental conditions on the value of immune traits during adulthood, nor on immunosenescence patterns except for

protostrongylids. Yet, environmental conditions at the time of birth likely affect resource availability and quality, which can interfere with lifetime trajectories of life-history traits (Nussey et al. 2007, Cooper and Kruuk 2018), and notably changes in the immune system with age (Lochmiller and Deerenberg 2000, McDade 2005, Rauw 2012, McDade et al. 2016).

Otherwise, our findings confirm that population, not sex, is the main modulator immunosenescence patterns as previously shown in roe deer (Cheynel et al. 2017). Immune traits displayed no sex-specific age trajectories, except for alpha1-globulins. In females, alpha1-globulins displayed lower concentration at young age but increased later in life until catching up and going beyond male values, although this increase was weak due to marked age-related variation. The previously documented sex-specific trajectory for neutrophils is no longer observed in the current study, while it remained similar for *Trichuris* sp. and protostrongylids, and even extended to GI strongyles. Our results therefore suggest that population is the main driver of patterns of immunosenescence and that sex rather modulates age-related variations in parasite load. The existence of sex differences in immunocompetence have for long been accepted. According to the immunocompetence handicap hypothesis, males should display a weaker immunity due to allocation trade-offs between immunity and reproduction (Zuk 1990) and the detrimental effects of male sex hormones on immunity (Foo et al. 2017). However, empirical evidence for a stronger female immunocompetence is mixed, and a recent meta-analysis actually showed no detectable bias towards females (Kelly et al. 2018). Similarly, although sex-specific patterns of senescence are expected from sex-specific life-histories (Brooks and Garratt 2017), a meta-analysis showed no bias towards an earlier or accelerated immunosenescence in males compared to females (Peters et al. 2019). In line with this, we showed that, throughout life, immune trait values were higher (*i.e.* alpha1-globulins, beta-globulins, haptoglobins), similar (*i.e.* neutrophils, monocytes, basophils, HA and HL scores, gamma-globulins and coccidia), or lower (*i.e.* eosinophils, alpha2-globulins,

lymphocytes) in males compared to females in this weakly sexually dimorphic species (Andersen et al. 1998). However, except for coccidia, parasite load was constantly greater in males than in females, and even increased more rapidly with age for males (*i.e. Trichuris* sp. and protostrongylids). This is unlikely due to sex-specific behaviours that could result in different parasite exposure between males and females, as both sexes do not segregate spatially throughout the year (Bonenfant et al. 2007). This rather suggests that although immune trait values do not strongly differ between sexes, resistance to parasite could actually be stronger in females than in males, as it has previously been suggested in other species (Poulin 1996, Zuk and McKean 1996, Tidière et al. 2020).

Finally, immunosenescence patterns for innate and inflammatory traits are consistent with the trajectories described in the literature on wildlife, humans or laboratory organisms (Peters et al. 2019), with innate traits showing no particular ageing directions, and inflammatory markers increasing with advancing age (Table 3). Also, neutrophil count was higher at TF than at CH, suggesting a poorer phagocytic capacity in the latter population, and potentially a poorer immunocompetence, hence supporting the fact that low resource quality and availability at CH can impact some immune traits (McDade et al. 2016). Similarly, we evidenced that parasite load was greater in CH than TF at all ages for *Trichuris* sp. and protostrongylids, but not for coccidia and GI strongyles (showing no population-specific differences), a pattern recently reported in those two populations (Bariod et al. 2023). Whether this is due to different pathogen exposure between populations or whether it is a consequence of a poorer immunocompetence in CH compared to TF remains to be assessed.

Declarations of interest

None.

Fundings

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Ethics

The research presented in the current article was done according to all institutional and/or national guidelines. For both populations (*i.e.* Trois-Fontaines and Chizé), the protocol of capture and blood sampling of roe deer is under the authority of the *Office Français de la Biodiversité* (OFB) and was approved by the Director of Food, Agriculture and Forest (Prefectoral order 2009-14 from Paris). All procedures were approved by the Ethical Committee of Lyon 1 University (project DR2014-09, June 5th, 2014 / MESRI #40903-2023021016306261, March 7th, 2023).

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5. References

Adem, J. 2016. Lungworm infection of small ruminant in Ethiopia: A review. *World Journal of Pharmaceutical and Life Sciences* **2**: 22–43.

Andersen, R., Duncan, P. and Linnell, J. D. C. 1998. The European roe deer: The biology of success. - Scandinavian University Press.

Arnold, T. W. 2010. Uninformative parameters and model selection using Akaike's Information Criterion. *Journal of Wildlife Management* **74**: 1175–1178.

Auffray, C., Sieweke, M. H. and Geissmann, F. 2009. Blood monocytes: Development, heterogeneity, and relationship with dendritic cells. *Annual Review of Immunology* **27**: 669–692.

Bachi, A. L. L., Suguri, V. M., Ramos, L. R., Mariano, M., Vaisberg, M. and Lopes, J. D. 2013. Increased production of autoantibodies and specific antibodies in response to influenza virus vaccination in physically active older individuals. *Results in Immunology* **3**: 10–16.

Baermann, G. 1917. A simple method for the detection of Ankylostomum (nematode) larvae in soil tests. *A simple method for the detection of Ankylostomum (nematode) larvae in soil tests.*: 41–47.

Bariod, L., Saïd, S., Calenge, C., Scheifler, R., Fritsch, C., Peroz, C., Benabed, S., Débias, F., Duhayer, J., Pardonnet, S., Poirel, M.-T., Vuarin, P. and Bourgoin, G. 2023. Essential trace elements in roe deer: Associations with parasite

burden and immune phenotype in two contrasted populations. in press.

Bartoń, K. 2021. MuMIn: Multi-Model Inference.

Bauer, M. E. 2005. Stress, glucocorticoids and ageing of the immune system. *Stress* **8**: 69–83.

Bauer, M. E. 2008. Chronic stress and immunosenescence: A review. *Neuroimmunomodulation* **15**: 241–250.

Bauer, M. E. and Fuente, M. D. la 2016. The role of oxidative and inflammatory stress and persistent viral infections in immunosenescence. *Mechanisms of Ageing and Development* **158**: 27–37.

Beaumelle, C., Redman, E. M., de Rijke, J., Wit, J., Benabed, S., Debias, F., Duhayer, J., Pardonnet, S., Poirel, M.-T., Capron, G., Chabot, S., Rey, B., Yannic, G., Gilleard, J. S. and Bourgoin, G. 2021. Metabarcoding in two isolated populations of wild roe deer (*Capreolus capreolus*) reveals variation in gastrointestinal nematode community composition between regions and among age classes. *Parasites & Vectors* **14**: 594.

Bobba-Alves, N., Sturm, G., Lin, J., Ware, S. A., Karan, K. R., Monzel, A. S., Bris, C., Procaccio, V., Lenaers, G., Higgins-Chen, A., Levine, M., Horvath, S., Santhanam, B. S., Kaufman, B. A., Hirano, M., Epel, E. S. and Picard, M. 2023. Cellular Allostatic Load is linked to Increased Energy Expenditure and Accelerated Biological Aging.: 2022.02.22.481548.

Body, G., Ferté, H., Gaillard, J.-M., Delorme, D., Klein, F. and Gilot-Fromont, E. 2011. Population density and phenotypic attributes influence the level of nematode parasitism in roe deer. *Oecologia* **167**: 635–646.

Bonenfant, C., Gaillard, J.-M., Dray, S., Loison, A., Royer, M. and Chessel, D. 2007. Testing sexual segregation and aggregation: Old ways are best. *Ecology* **88**: 3202–3208.

Briga, M., Jimeno, B. and Verhulst, S. 2019. Coupling lifespan and aging? The age at onset of body mass decline associates positively with sex-specific lifespan but negatively with environment-specific lifespan. *Experimental Gerontology* **119**: 111–119.

Brooks, R. C. and Garratt, M. G. 2017. Life history evolution, reproduction, and the origins of sex-dependent aging and longevity. *Annals of the New York Academy of Sciences* **1389**: 92–107.

Bubenik, G. A., Schams, D., White, R. G., Rowell, J., Blake, J. and Bartos, L. 1998. Seasonal levels of metabolic hormones and substrates in male and female reindeer (*Rangifer tarandus*). *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology* **120**: 307–315.

Burn, G. L., Foti, A., Marsman, G., Patel, D. F. and Zychlinsky, A. 2021. The neutrophil. *Immunity* **54**: 1377–1391.

Burnham, K. P. and Anderson, D. R. 2002. Model selection and multimodel inference: A practical information-theoretic approach. - Springer.

Cabezas, S., Blas, J., Marchant, T. A. and Moreno, S. 2007. Physiological stress levels predict survival probabilities in wild rabbits. *Hormones and Behavior* **51**: 313–320.

Cambreling, S., Gaillard, J.-M., Pellerin, M., Vanpé, C., Débias, F., Delorme, D., Garcia, R., Hewison, A. J. M. and Lemaître, J.-F. 2023. Natal environmental conditions modulate senescence of antler length in roe

deer. *Frontiers in Ecology and Evolution* **11**: 1139235.

Carbillet, J., Rey, B., Palme, R., Monestier, C., Börger, L., Lavabre, T., Maublanc, M.-L., Cebe, N., Rames, J.-L., Le Loc'h, G., Wasniewski, M., Rannou, B., Gilot-Fromont, E. and Verheyden, H. 2022. Covariation between glucocorticoids, behaviour and immunity supports the pace-of-life syndrome hypothesis: An experimental approach. *Proceedings of the Royal Society B: Biological Sciences* **289**: 20220464.

Carbillet, J., Hollain, M., Rey, B., Palme, R., Pellerin, M., Regis, C., Geffré, A., Duhayer, J., Pardonnet, S., Debias, F., Merlet, J., Lemaître, J.-F., Verheyden, H. and Gilot-Fromont, E. 2023a. Age and spatio-temporal variations in food resources modulate stress-immunity relationships in three populations of wild roe deer. *General and Comparative Endocrinology* **330**: 114141.

Carbillet, J., Palme, R., Maublanc, M.-L., Cebe, N., Gilot-Fromont, E., Verheyden, H. and Rey, B. 2023b. Instability of fecal glucocorticoid metabolites at 4°C: Time to freeze matters. *Journal of Experimental Zoology. Part A, Ecological and Integrative Physiology* in press.

Casagrande, S., Stier, A., Monaghan, P., Loveland, J. L., Boner, W., Lupi, S., Trevisi, R. and Hau, M. 2020. Increased glucocorticoid concentrations in early life cause mitochondrial inefficiency and short telomeres. *Journal of Experimental Biology* **223**: jeb222513.

Cheynel, L., Lemaître, J.-F., Gaillard, J.-M., Rey, B., Bourgoin, G., Ferté, H., Jégo, M., Débias, F., Pellerin, M., Jacob, L. and Gilot-Fromont, E. 2017. Immunosenescence patterns differ between populations but not between sexes in a long-lived mammal. *Scientific Reports* **7**: 1–11.

Cooper, E. B. and Kruuk, L. E. B. 2018. Ageing with a silver-spoon: A meta-analysis of the effect of developmental environment on senescence. *Evolution Letters* **2**: 460–471.

Cray, C., Zaias, J. and Altman, N. H. 2009. Acute phase response in animals: A review. *Comparative Medicine* **59**: 517–526.

Defolie, C., Merkling, T. and Fichtel, C. 2020. Patterns and variation in the mammal parasite–glucocorticoid relationship. *Biological Reviews* **95**: 74–93.

Dehnhard, M., Clauss, M., Lechner-Doll, M., Meyer, H. H. D. and Palme, R. 2001. Noninvasive monitoring of adrenocortical activity in roe deer (*Capreolus capreolus*) by measurement of fecal cortisol metabolites. *General and Comparative Endocrinology* **123**: 111–120.

Dhabhar, F. S. 2014. Effects of stress on immune function: The good, the bad, and the beautiful. *Immunologic Research* **58**: 193–210.

Douhard, M., Gaillard, J.-M., Delorme, D., Capron, G., Duncan, P., Klein, F. and Bonenfant, C. 2013. Variation in adult body mass of roe deer: Early environmental conditions influence early and late body growth of females. *Ecology* **94**: 1805–1814.

Douhard, F., Gaillard, J.-M., Pellerin, M., Jacob, L. and Lemaître, J.-F. 2017. The cost of growing large: Costs of post-weaning growth on body mass senescence in a wild mammal. *Oikos* **126**: 1329–1338.

Foo, Y. Z., Nakagawa, S., Rhodes, G. and Simmons, L. W. 2017. The effects of sex hormones on immune function: A meta-analysis. *Biological Reviews* **92**: 551–571.

Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E. and De Benedictis, G. 2000. Inflamm-aging: An evolutionary perspective on immunosenescence. *Annals of the New York Academy of Sciences* **908**: 244–254.

Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., Panourgia, M. P., Invidia, L., Celani, L., Scurti, M., Cevenini, E., Castellani, G. C. and Salvioli, S. 2007. Inflammaging and anti-inflammaging: A systemic perspective on aging and longevity emerged from studies in humans. *Mechanisms of Ageing and Development* **128**: 92–105.

Frasca, D., Diaz, A., Romero, M., Landin, A. M. and Blomberg, B. B. 2011. Age effects on B cells and humoral immunity in humans. *Ageing Research Reviews* **10**: 330–335.

Froy, H., Sparks, A. M., Watt, K., Sinclair, R., Bach, F., Pilkington, J. G., Pemberton, J. M., McNeilly, T. N. and Nussey, D. H. 2019. Senescence in immunity against helminth parasites predicts adult mortality in a wild mammal. *Science* **365**: 1296–1298.

Gaillard, J.-M., Delorme, D., Boutin, J.-M., Van Laere, G., Boisaubert, B. and Pradel, R. 1993. Roe deer survival patterns: A comparative analysis of contrasting populations. *Journal of Animal Ecology* **62**: 778–791.

Gaillard, J.-M., Delorme, D., Boutin, J.-M., Van Laere, G. and Boisaubert, B. 1996. Body mass of roe deer fawns during winter in 2 contrasting populations. *Journal of Wildlife Management* **60**: 29–36.

Gaillard, J.-M., Loison, A., Toïgo, C., Delorme, D. and Van Laere, G. 2003. Cohort effects and deer population dynamics. *Écoscience* **10**: 412–420.

Garratt, M., Lemaître, J.-F., Douhard, M., Bonenfant, C., Capron, G., Warnant, C., Klein, F., Brooks, R. C. and Gaillard, J.-M. 2015. High juvenile mortality is associated with sex-specific adult survival and lifespan in wild roe deer. *Current Biology* **25**: 759–763.

Garrido, A., Toda, I. M. de, Cerro, E. D. del, Félix, J., Ceprián, N., González-Sánchez, M. and Fuente, M. D. la 2022. Social environment as a modulator of immunosenescence. *Expert Reviews in Molecular Medicine* **24**: e29.

Gilot-Fromont, E., Jégo, M., Bonenfant, C., Gibert, P., Rannou, B., Klein, F. and Gaillard, J.-M. 2012. Immune phenotype and body condition in roe deer: Individuals with high body condition have different, not stronger immunity. *PLoS ONE* **7**: e45576.

Gomez-Sanchez, C. E. 2009. Glucocorticoid production and regulation in thymus: Of mice and birds. *Endocrinology* **150**: 3977–3979.

Gruber, J., Sgonc, R., Hu, Y. H., Beug, H. and Wick, G. 1994. Thymocyte apoptosis induced by elevated endogenous corticosterone levels. *European Journal of Immunology* **24**: 1115–1121.

Hadinger, U., Haymerle, A., Knauer, F., Schwarzenberger, F. and Walzer, C. 2015. Faecal cortisol metabolites to assess stress in wildlife: Evaluation of a field method in free-ranging chamois. *Methods in Ecology and Evolution* **6**: 1349–1357.

Hakim, F. T. and Gress, R. E. 2007. Immunosenescence: Deficits in adaptive immunity in the elderly. *Tissue Antigens* **70**: 179–189.

Hämäläinen, A., Raharivololona, B., Ravoniarimbinina, P. and Kraus, C. 2015. Host sex and age influence

endoparasite burdens in the gray mouse lemur. *Frontiers in Zoology* **12**: 25.

Harrison, X. A., Donaldson, L., Correa-Cano, M. E., Evans, J., Fisher, D. N., Goodwin, C. E. D., Robinson, B. S., Hodgson, D. J. and Inger, R. 2018. A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ* **6**: e4794.

Hau, M., Casagrande, S., Ouyang, J. Q. and Baugh, A. T. 2016. Glucocorticoid-mediated phenotypes in vertebrates: Multilevel variation and evolution. - In: Advances in the Study of Behavior. Elsevier Science Publishing Co Inc, pp. 41–115.

Hayward, A. D., Wilson, A. J., Pilkington, J. G., Pemberton, J. M. and Kruuk, L. E. B. 2009. Ageing in a variable habitat: environmental stress affects senescence in parasite resistance in St Kilda Soay sheep. *Proceedings of the Royal Society B: Biological Sciences* **276**: 3477–3485.

Hayward, A. D., Moorad, J., Regan, C. E., Berenos, C., Pilkington, J. G., Pemberton, J. M. and Nussey, D. H. 2015. Asynchrony of senescence among phenotypic traits in a wild mammal population. *Experimental Gerontology* **71**: 56–68.

Herbert, D. R., Stoltzfus, J. D. C., Rossi, H. L. and Abraham, D. 2022. Is *Strongyloides stercoralis* hyperinfection induced by glucocorticoids a result of both suppressed host immunity and altered parasite genetics? *Molecular and Biochemical Parasitology* **251**: 111511.

Herborn, K. A., Heidinger, B. J., Boner, W., Noguera, J. C., Adam, A., Daunt, F. and Monaghan, P. 2014. Stress exposure in early post-natal life reduces telomere length: An experimental demonstration in a long-lived seabird. *Proceedings of the Royal Society B: Biological Sciences* **281**: 20133151.

Hewison, A. J. M., Gaillard, J.-M., Morellet, N., Cagnacci, F., Debeffe, L., Cargnelutti, B., Gehr, B., Kröschel, M., Heurich, M., Coulon, A., Kjellander, P., Börger, L. and Focardi, S. 2021. Sex differences in condition dependence of natal dispersal in a large herbivore: Dispersal propensity and distance are decoupled. *Proceedings of the Royal Society B: Biological Sciences* **288**: 20202947.

Houwen, B. 2001. The differential count. *Laboratory Hematology* **7**: 89–100.

Huber, S., Palme, R. and Arnold, W. 2003. Effects of season, sex, and sample collection on concentrations of fecal cortisol metabolites in red deer (*Cervus elaphus*). *General and Comparative Endocrinology* **130**: 48–54.

Juster, R.-P., McEwen, B. S. and Lupien, S. J. 2010. Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neuroscience and Biobehavioral Reviews* **35**: 2–16.

Karasuyama, H., Mukai, K., Obata, K., Tsujimura, Y. and Wada, T. 2011. Nonredundant roles of basophils in immunity. *Annual Review of Immunology* **29**: 45–69.

Kelly, C. D., Stoehr, A. M., Nunn, C., Smyth, K. N. and Prokop, Z. M. 2018. Sexual dimorphism in immunity across animals: A meta-analysis. *Ecology Letters* **21**: 1885–1894.

Klasing, K. C. 2004. The costs of immunity. *Acta Zoologica Sinica* **50**: 961–969.

Kovaiou, R. D., Herndler-Brandstetter, D. and Grubeck-Loebenstein, B. 2007. Age-related changes in immunity: Implications for vaccination in the

elderly. *Expert Reviews in Molecular Medicine* **9**: 1–17.

Kulberg, S., Boysen, P. and Storset, A. K. 2004. Reference values for relative numbers of natural killer cells in cattle blood. *Developmental & Comparative Immunology* **28**: 941–948.

Lalande, L. D., Gilot-Fromont, E., Carbillet, J., Débias, F., Duhayer, J., Gaillard, J.-M., Lemaître, J.-F., Palme, R., Pardonnet, S., Pellerin, M., Rey, B. and Vuarin, P. 2023. Glucocorticoids negatively relate to body mass on the short-term in a free-ranging ungulate. *Oikos* **2023**: e09769.

Lee, K. A. 2006. Linking immune defenses and life history at the levels of the individual and the species. *Integrative and Comparative Biology* **46**: 1000–1015.

Lee, R. S., Zandi, P. P., Santos, A., Aulinas, A., Carey, J. L., Webb, S. M., McCaul, M. E., Resmini, E. and Wand, G. S. 2021. Cross-species association between telomere length and glucocorticoid exposure. *The Journal of Clinical Endocrinology and Metabolism* **106**: e5124–e5135.

Lemaître, J.-F., Ronget, V., Tidière, M., Allainé, D., Berger, V., Cohas, A., Colchero, F., Conde, D. A., Garratt, M., Liker, A., Marais, G. A. B., Scheuerlein, A., Székely, T. and Gaillard, J.-M. 2020. Sex differences in adult lifespan and aging rates of mortality across wild mammals. *Proceedings of the National Academy of Sciences* **117**: 8546–8553.

Lemaître, J.-F., Carbillet, J., Rey, B., Palme, R., Froy, H., Wilbourn, R. V., Underwood, S. L., Cheynel, L., Gaillard, J.-M., Hewison, A. J. M., Verheyden, H., Débias, F., Duhayer, J., Régis, C., Pardonnet, S., Pellerin, M., Nussey, D. H. and Gilot-Fromont, E. 2021. Short-term telomere dynamics is associated with glucocorticoid levels in wild populations of roe deer. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **252**: 110836.

Lexen, E., El-Bahr, S., Sommerfeld-Stur, I., Palme, R. and Möstl, E. 2008. Monitoring the adrenocortical response to disturbances in sheep by measuring glucocorticoid metabolites in the faeces. *Wiener tierärztliche Monatsschrift* **95**: 64–71.

Lleo, A., Invernizzi, P., Gao, B., Podda, M. and Gershwin, M. E. 2010. Definition of human autoimmunity - Autoantibodies *versus* autoimmune disease. *Autoimmunity Reviews* **9**: A259–A266.

Lochmiller, R. L. and Deerenberg, C. 2000. Trade-offs in evolutionary immunology: Just what is the cost of immunity? *Oikos* **88**: 87–98.

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. and Kroemer, G. 2023. Hallmarks of aging: An expanding universe. *Cell* **186**: 243–278.

Maag, N., Cozzi, G., Bateman, A., Heistermann, M., Ganswindt, A., Manser, M., Clutton-Brock, T. and Ozgul, A. 2019. Cost of dispersal in a social mammal: Body mass loss and increased stress. *Proceedings of the Royal Society B: Biological Sciences* **286**: 20190033.

Maizels, R. M. and Nussey, D. H. 2013. Into the wild: Digging at immunology's evolutionary roots. *Nature Immunology* **14**: 879–883.

Makinodan, T. 1980. Nature of the decline in antigen-induced humoral immunity with age. *Mechanisms of Ageing and Development* **14**: 165–172.

Martin, L. B. 2009. Stress and immunity in wild vertebrates: Timing is everything. *General and Comparative Endocrinology* **163**: 70–76.

Martin, L. B., Gilliam, J., Han, P., Lee, K. and Wikelski, M. 2005. Corticosterone suppresses cutaneous immune function in temperate but not tropical house sparrows, *Passer domesticus*. *General and Comparative Endocrinology* **140**: 126–135.

Matson, K. D., Ricklefs, R. E. and Klasing, K. C. 2005. A hemolysis–hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Developmental & Comparative Immunology* **29**: 275–286.

McDade, T. W. 2005. Life history, maintenance, and the early origins of immune function. *American Journal of Human Biology: The Official Journal of the Human Biology Council* **17**: 81–94.

McDade, T. W., Georgiev, A. V. and Kuzawa, C. W. 2016. Trade-offs between acquired and innate immune defenses in humans. *Evolution, Medicine, and Public Health* **2016**: 1–16.

McEwen, B. S. and Wingfield, J. C. 2003. The concept of allostasis in biology and biomedicine. *Hormones and Behavior* **43**: 2–15.

McEwen, B. S. and Wingfield, J. C. 2010. What is in a name? Integrating homeostasis, allostasis and stress. *Hormones and Behavior* **57**: 105–111.

Monaghan, P. and Haussmann, M. F. 2015. The positive and negative consequences of stressors during early life. *Early Human Development* **91**: 643–647.

Monaghan, P., Heidinger, B. J., D'Alba, L., Evans, N. P. and Spencer, K. A. 2011. For better or worse: Reduced adult lifespan following early-life stress is transmitted to breeding partners. *Proceedings of the Royal Society B: Biological Sciences* **279**: 709–714.

Möstl, E., Maggs, J. L., Schrötter, G., Besenfelder, U. and Palme, R. 2002. Measurement of cortisol metabolites in faeces of ruminants. *Veterinary Research Communications* **26**: 127–139.

Nussey, D. H., Kruuk, L. E. B., Morris, A. and Clutton-Brock, T. H. 2007. Environmental conditions in early life influence ageing rates in a wild population of red deer. *Current Biology* **17**: R1000–R1001.

Palme, R. 2019. Non-invasive measurement of glucocorticoids: Advances and problems. *Physiology & Behavior* **199**: 229–243.

Palme, R., Touma, C., Arias, N., Dominchin, M. F. and Lepschy, M. 2013. Steroid extraction: Get the best out of faecal samples. *Wiener Tierärztliche Monatsschrift* **100**: 238–246.

Palmer, D. B. 2013. The effect of age on thymic function. *Frontiers in Immunology* in press.

Pauli, J. N. and Buskirk, S. W. 2007. Risk-disturbance overrides density dependence in a hunted colonial rodent, the black-tailed prairie dog *Cynomys ludovicianus*. *Journal of Applied Ecology* **44**: 1219–1230.

Pawelec, G., Bronikowski, A., Cunnane, S. C., Ferrucci, L., Franceschi, C., Fülöp, T., Gaudreau, P., Gladyshev, V. N., Gonos, E. S., Gorbunova, V., Kennedy, B. K., Larbi, A., Lemaître, J.-F., Liu, G.-H., Maier, A. B., Morais, J. A., Nóbrega, O. T., Moskalev, A., Rikkert, M. O., Seluanov, A., Senior, A. M., Ukraintseva, S., Vanhaelen, Q., Witkowski, J. and Cohen, A. A. 2020. The conundrum of human immune system "senescence." *Mechanisms of Ageing and Development* **192**: 111357.

Pazirandeh, A., Jondal, M. and Okret, S. 2004. Glucocorticoids delay age-associated thymic involution through directly affecting the thymocytes. *Endocrinology* **145**: 2392–2401.

Peters, A., Delhey, K., Nakagawa, S., Aulsebrook, A. and Verhulst, S. 2019. Immunosenescence in wild animals: Meta-analysis and outlook. *Ecology Letters* **22**: 1709–1722.

Pettorelli, N., Gaillard, J.-M., Van Laere, G., Duncan, P., Kjellander, P., Liberg, O., Delorme, D. and Maillard, D. 2002. Variations in adult body mass in roe deer: The effects of population density at birth and of habitat quality. *Proceedings. Biological Sciences* **269**: 747–753.

Pettorelli, N., Gaillard, J.-M., Mysterud, A., Duncan, P., Stenseth, N. C., Delorme, D., Laere, G. V., Toïgo, C. and Klein, F. 2006. Using a proxy of plant productivity (NDVI) to find key periods for animal performance: The case of roe deer. *Oikos* **112**: 565–572.

Poulin, R. 1996. Sexual inequalities in helminth infections: A cost of being a male? *The American Naturalist* **147**: 287–295.

Pride, R. E. 2005. High faecal glucocorticoid levels predict mortality in ring-tailed lemurs (*Lemur catta*). *Biology Letters* **1**: 60–63.

R Core Team 2023. R: A language and environment for statistical computing.

Rauw, W. 2012. Immune response from a resource allocation perspective. *Frontiers in Genetics* in press.

Raynaud, J. P. 1970. Etude de l'efficacité d'une technique de coproscopie quantitative pour le diagnostic de routine et le contrôle des infestations parasitaires des bovins, ovins, équins et porcins. *Annales de parasitologie humaine et comparée* **45**: 321–42.

Reeder, D. M. and Kramer, K. M. 2005. Stress in free-ranging mammals: Integrating physiology, ecology, and natural history. *Journal of Mammalogy* **86**: 225–235.

Romero, L. M., Dickens, M. J. and Cyr, N. E. 2009. The reactive scope model - A new model integrating homeostasis, allostasis, and stress. *Hormones and Behavior* **55**: 375–389.

Rothenberg, M. E. and Hogan, S. P. 2006. The eosinophil. *Annual Review of Immunology* **24**: 147–174.

Sapolsky, R. M., Romero, L. M. and Munck, A. U. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* **21**: 55–89.

Schmid-Hempel, P. 2003. Variation in immune defence as a question of evolutionary ecology. *Proceedings of the Royal Society B: Biological Sciences* **270**: 357–366.

Seeman, T. E., Singer, B. H., Rowe, J. W., Horwitz, R. I. and McEwen, B. S. 1997. Price of adaptation - Allostatic load and its health consequences. MacArthur studies of successful aging. *Archives of Internal Medicine* **157**: 2259–2268.

Seeman, T. E., McEwen, B. S., Rowe, J. W. and Singer, B. H. 2001. Allostatic load as a marker of cumulative biological risk: MacArthur studies of successful aging. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 4770–4775.

Sheldon, B. C. and Verhulst, S. 1996. Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution* **11**: 317–321.

Siracusa, M. C., Perrigoue, J. G., Comeau, M. R. and Artis, D. 2010. New paradigms in basophil development, regulation and function. *Immunology & Cell Biology* **88**: 275–284.

Spencer, K. A. and Verhulst, S. 2008. Post-natal exposure to corticosterone affects standard metabolic rate in the zebra finch (*Taeniopygia guttata*). *General and Comparative Endocrinology* **159**: 250–256.

Spencer, K. A., Evans, N. P. and Monaghan, P. 2009. Postnatal stress in birds: A novel model of glucocorticoid programming of the hypothalamic-pituitary-adrenal axis. *Endocrinology* **150**: 1931–1934.

Taves, M. D., Hamden, J. E. and Soma, K. K. 2017. Local glucocorticoid production in lymphoid organs of mice and birds: Functions in lymphocyte development. *Hormones and Behavior* **88**: 4–14.

Tidière, M., Gaillard, J.-M., Berger, V., Müller, D. W. H., Bingaman Lackey, L., Gimenez, O., Clauss, M. and Lemaître, J.-F. 2016. Comparative analyses of longevity and senescence reveal variable survival benefits of living in zoos across mammals. *Scientific Reports* **6**: 36361.

Tidière, M., Badruna, A., Fouchet, D., Gaillard, J.-M., Lemaître, J.-F. and Pontier, D. 2020. Pathogens shape sex differences in mammalian aging. *Trends in Parasitology* **36**: 668–676.

Tixier, H. and Duncan, P. 1996. Are European roe deer browsers? A review of variations in the composition of their diets. *Revue d'Ecologie (Terre et Vie)* **51**: 3–17.

Toïgo, C., Gaillard, J.-M., Laere, G. V., Hewison, M. and Morellet, N. 2006. How does environmental variation influence body mass, body size, and body condition? Roe deer as a case study. *Ecography* **29**: 301–308.

van de Pol, M. and Verhulst, S. 2006. Age-Dependent Traits: A New Statistical Model to Separate Within- and Between-Individual Effects. *The American Naturalist* **167**: 766–773.

van de Pol, M. and Wright, J. 2009. A simple method for distinguishing within- versus between-subject effects using mixed models. *Animal Behaviour* **77**: 753–758.

Wey, T. W., Lin, L., Patton, M. L. and Blumstein, D. T. 2015. Stress hormone metabolites predict overwinter survival in yellow-bellied marmots. *Acta Ethologica* **18**: 181–185.

Wiegers, J. G. and Tischner, D. 2011. A role for glucocorticoids in thymic involution? *Current Immunology Reviews* **7**: 133–134.

Wingfield, J. C. 2013. Ecological processes and the ecology of stress: The impacts of abiotic environmental factors. *Functional Ecology* **27**: 37–44.

Wingfield, J. C., Maney, D. L., Breuner, C. W., Jacobs, J. D., Lynn, S., Ramenofsky, M. and Richardson, R. D. 1998. Ecological bases of hormone-behavior interactions: The "emergency life history stage." *American Zoologist* **38**: 191–206.

Zbyryt, A., Bubnicki, J. W., Kuijper, D. P. J., Dehnhard, M., Churski, M., Schmidt, K. and Editor, B. W. 2018. Do wild ungulates experience higher stress with humans than with large carnivores? *Behavioral Ecology* **29**: 19–30.

Zuk, M. 1990. Reproductive strategies and disease susceptibility: An evolutionary viewpoint. *Parasitology Today (Personal Ed.)* **6**: 231–233.

Zuk, M. and McKean, K. A. 1996. Sex differences in parasite infections: Patterns and processes. *International Journal for Parasitology* **26**: 1009–1024.