Evolutionary biology

Immunocompetence and high metabolic rates enhance overwinter survival in the root vole, Microtus oeconomus

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Despite its presumed significance, the association between immune defence, energy expenditures and overwinter survival is rarely studied. We analysed individual variation in immunocompetence quantified as neutrophil-to-lymphocyte ratio (N/L), total white blood cells (WBC) and natural antibody levels, along with resting (RMR) and peak metabolic rates (PMR) and mortality during three consecutive winter seasons in a natural population of the root vole, Microtus oeconomus. In early winter, WBC count was negatively correlated with RMR, whereas N/L ratio was negatively correlated with swim-elicited PMR. We suggest that while the first correlation reflected the trade-off between energy allocation in immunocompetence and other metabolically demanding processes, the latter correlation stemmed from stress-induced immunosuppression elicited by the necessity to cope with swimming in frequently flooded habitat. In addition, the analysis carried out during the first year of study characterized by a high population density and prevalence of infestation with a blood parasite—Babesia spp., showed that its intensity was inversely correlated with the N/L ratio. In summary, our results suggest that elevated N/L ratio increases the winter survival of free-ranging rodents by increasing their ability to cope with parasitic infections.

1. Introduction

Survival is one of the most important parameters driving animals’ life history under natural conditions, and its probability hinges on the effectiveness of the immune system in combating infections [1]. An immune response, in turn, is a costly mechanism mediated through metabolic trade-offs [2–4] and/or hormonal signalling that may not be directly related to energy expenditures [5–7]. Surprisingly, the associations between immunocompetence, energy expenditure and survival have not been extensively studied and are typically researched separately. We therefore analysed individual variation in immunocompetence and metabolic rates, and examined their effect on energy-demanding winter survival in a natural population of the root vole (Microtus oeconomus). Voles are small mammalian herbivores well characterized with respect to prevalent pathogens [8,9] and the effect of energy expenditure on individual survival [10,11].

We focused on the constitutive arm of innate and adaptive immunity, which contributes to energy expenditures through increased cell turnover and fever [12,13]. Here, we characterized immunocompetence as neutrophil-to-lymphocyte ratio (N/L), total white blood cell (WBC) and natural antibody levels (NAbs), evaluated at the beginning of three consecutive winter seasons. We chose these parameters because they characterize the first line of defence against pathogens through leucocyte-generated rapid recognition and production of molecules that signal to other components of the immune system [12,13]. We analysed their...
correlations with the intensity of infestation of the tick-borne parasite *Babesia* spp. to validate their association with parasite infections in a year of high *Babesia* spp. prevalence [8].

We tested the winter-elicited trade-offs between immunocompetence and energy expenditures at the level of resting metabolic rate (RMR). We also measured the swim-elicited peak metabolic rate (PMR), the level of which may influence the survival probability of root voles living in periodically flooded sedge meadows [10]. By definition, PMR does not constitute a significant part of energy expenditures. Nevertheless, it may correlate with immunocompetence through swim-induced elevation of the level of corticosteroids rather than through energetic trade-offs [5].

2. Material and methods

The studied population inhabited a fenced plot in Biebrza National Park in northeast Poland. In November, January and March of the winters from 2008 to 2010, live box traps were used to capture voles and monitor individual overwinter survival. RMRs and PMRs were measured on animals every November using a positive-pressure, open-circuit respirometry system (Sable Systems TR-1 set-up, Las Vegas, NV). Blood sampling followed metabolic measurements. For further details, see the electronic supplementary material and reference [10].

We used a general linear-mixed model to estimate the effects of body mass, metabolic rates, sex and year of study (as a random effect) on blood indices. We applied logistic regression to determine the effect of variation in blood indices and physiological traits on winter survival of voles. We analysed those effects for early (November–January) and late (January–March) winter. We used two sets of the above models—with and without body mass as a covariate—to estimate the effect of whole-body (RMR and PMR) and body mass-corrected (thereafter referred to as cRMR and cPMR) metabolic rates. In all analyses, we applied a stepwise approach and retained only significant factors (at \(a = 0.05\); details on the full (tables S1–S4) and reduced models (tables S1a–S4a) in electronic supplementary material).

3. Results

The N/L ratio \((r = -0.60; p = 0.0003; n = 31; \text{figure 1})\), but not WBC \((r = -0.43; p = 0.09; n = 16)\) and NAbs \((r = -0.04; p = 0.80; n = 30)\) was inversely correlated with the intensity of infestation with *Babesia* spp. The intensity of infestation was also inversely correlated with body mass \((r = -0.35; p = 0.04; n = 32)\).

The N/L ratio was higher in larger individuals (electronic supplementary material, table S1a and figure S1), which were also less likely to survive early winter (November–January; electronic supplementary material, table S3a and figure S2). None of the blood indices was associated with survival during this period, whereas survival was positively associated with cPMR (electronic supplementary material, table S3a and figure 2a). During late winter (January–March), the survival of voles was positively associated with N/L ratio, but not with WBC count or NAbs (electronic supplementary material, table S4a and figure 2b). Body mass, metabolic rates and sex did not significantly affect survival of voles during this period (electronic supplementary material, tables S3a–S4a).

cPMR was inversely related to the N/L ratio (electronic supplementary material, table S1a and figure S3c), whereas PMR was not associated with blood indices (electronic supplementary material, table S2a). RMR was positively associated with N/L ratio, but negatively with WBC (electronic supplementary material, table S2a and figure S3a,b).

![Figure 1. Correlation between neutrophil-to-lymphocyte (N/L) ratio and the intensity of infection with blood parasite (expressed as the log-transformed number of infected erythrocytes per 100 fields of vision) in a random subset of voles captured in 2008—a year of high prevalence of the tick-borne protozoan *Babesia* spp. (4).](http://rsbl.royalsocietypublishing.org/)
4. Discussion

Root voles characterized by a low N/L ratio were highly infested with *Babesia* spp. (figure 1) and suffered from higher overwinter mortality, particularly late in the season (figure 2b). This agrees with the results of a concurrent study on the same vole population, which reported a 56% prevalence of infection with blood parasites, chiefly *Babesia* spp. [8], a tick-borne protozoan causing haemolytic anaemia [14]. In 2008 (the study year of high population density [8], for which we analysed the correlation depicted in figure 1), infection with *Babesia* spp. decreased overwinter survival almost twofold, with its effect especially apparent in late winter [8]. Neutrophils provide the first line of defence against protozoans [15,16]. We therefore relate increased survival of individuals that have high N/L ratios to their ability to effectively control *Babesia* spp. infection. Our study is the first, to the best of our knowledge, to demonstrate that the N/L ratio can be a predictor of winter survival in rodents.

We expected that costs of immunocompetence would compete with other winter-elicted energy demands. We found a negative correlation between WBC count and RMR (electronic supplementary material, figure S3a), which in turn enhanced overwinter survival in the studied population [10]. Above-mentioned physiological traits are likely to be repeatable over long periods of time [17,18], so one can therefore expect that the sign of correlation between them will remain consistent throughout the whole winter. We therefore suggest that wintering voles may be confronted with trade-offs between the allocation of energy to immunocompetence and other energy-demanding activities (e.g. thermoregulation) reflected in RMR.

Our results concur with Zub et al.’s study [10] in demonstrating that cPMR in voles is positively associated with winter survival (figure 2a), most likely through enhancing swim-elicited aerobic performance in a frequently flooded habitat. Our findings from early winter also agree with Downs et al. [5], who demonstrated an inverse association between induced (not constitutive) innate immunity and cPMR (elicited by forced exercise) in laboratory mice. Swimming in both laboratory and wild rodents has been shown to elicit stress, which impairs aerobic performance [19] and elevates serum corticosteroid levels [20]. The latter, however, increases the N/L ratio [16], which enhances late-winter survival (figure 2b), presumably through the control of *Babesia* spp. infection (figure 1). We therefore suggest that a positive association between early winter survival and high cPMR (figure 2a) selects individual voles characterized by above average tolerance to swim-induced stress, but also low corticosteroid levels resulting in their poor ability to combat *Babesia* spp. infection. Later in the winter season, however, when water freezes and swimming no longer poses a challenge, the parasitic infection becomes a significant factor enhancing survival of individuals having high corticosteroid levels and thus high N/L ratios.

Our results also suggest that the association between N/L ratio, body mass and winter survival is complex and indirect. Smaller individuals were characterized by lower mortality during early winter (electronic supplementary material, figure S2), whereas body mass was positively associated with survival later in the season [10]. However, in early winter, the correlation between N/L ratio and body mass was positive (electronic supplementary material, figure S1), whereas body mass and the intensity of *Babesia* spp. infection were inversely correlated. Kloch et al. [8] noted that heavier animals were more often infected with *Babesia* spp. and *Apostatandria* spp., which negatively affected survival. This contradicts our supposition about positive associations between N/L ratio, body mass and survival. Body mass of the root voles is strongly positively correlated with age [21]. The age effect, however, may simply reflect the duration of exposure to infection, and therefore increase the probability of contraction. If positive associations between N/L ratio, body mass and survival hold, they indicate that the high N/L ratio manifests in the ability to combat already developing infection, rather than its contraction. This is in agreement with the analyses of Zub et al. [10], which show that survival was not significantly affected by age of individual root voles in either early or late winter.

In conclusion, our study, though correlative in nature, is the first demonstration of the reliability of the N/L ratio as an indicator of the ability to combat infections in a wild rodent, as well as its importance for predicting winter survival. We also suggest that a positive association between winter survival and both N/L ratio and cPMR is mediated through the ability to avoid swim-stress eliciting immunosuppression.

**Ethics statement.** This research was approved by the Local Ethical Committee on Animal Testing at the Medical University of Białystok (permit nos. 2008/26 and 1/2009).

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References