Comparative Biochemistry and Physiology, Part A 156 (2010) 46–49

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Relationships between circulating leucocytes and *Leucocytozoon simondi* in mallard, *Anas platyrhynchos*, ducklings

Dave Shutler *, Amy G. Lowe, Stephen R. Robinson

Department of Biology, Acadia University, Wolfville, Nova Scotia, Canada B4P 2R6

ARTICLE INFO

Article history:
Received 24 June 2009
Received in revised form 17 December 2009
Accepted 20 December 2009
Available online 31 December 2009

Keywords:
Anas platyrhynchos
Apicomplexan
Blood smear
Duckling
Leucocyte profiles
Leucocytozoon simondi
Immunity
Mallard
Neutrophil:lymphocyte
Parasitism

ABSTRACT

Leucocytozoon simondi is an apicomplexan blood parasite of waterfowl that frequently causes significant mortality, and thus is expected to provoke a significant immune response in hosts. Using blood smears collected in consecutive weeks from 30 wild-stock mallard, *Anas platyrhynchos*, ducklings, we tested with repeated measures analyses for associations between leucocyte profiles and *L. simondi* infection intensities. With each of the five weeks of leucocyte profiles as response variables, we found evidence of fewer circulating heterophils and more circulating lymphocytes in the third week of infection associated with more intense *L. simondi* infections from the second week, but no significant relationships between leucocytes and the other four weeks of *L. simondi* infection. With each of the five weeks of *L. simondi* infection intensities as response variables, we found no associations with leucocyte profiles. Collectively, our results did not reveal tight links between leucocyte profiles and parasitism by *L. simondi*. Our data suggest that *L. simondi* was relatively benign to our wild ducks.

1. Introduction

Immune systems have evolved to contend with various kinds of foreign invaders, including viruses, unicellular microparasites, and multicellular macroparasites. The vertebrate immune system has humoral and cellular branches. Within the cellular branch, the majority of leucocytes are either heterophils (phagocytising cells that are part of the innate immune system) or lymphocytes (the backbone of the acquired immune system) (Sturkie, 1986). Leucocyte profiles are commonly used to assess host capacity to prepare for and fight infections (immunocompetence) (Norris and Evans, 2000; Salvante, 2006; Davis et al., 2008) (although interpretation can be problematic). Optimal allocation to immune function is an expected outcome of host–parasite co-evolution; hosts should invest heavily against serious pathogens, and make lesser investments against benign pathogens (Sheldon and Verhulst, 1996). *Leucocytozoon simondi* is an apicomplexan (Order Haemosporida) parasite of several species of waterfowl, and often causes significant mortality, particularly in young birds (Atkinson and van Riper, 1991; Desser and Bennett, 1993). Thus, we might expect significant investment in immunity by young host waterfowl against this parasite. Herein, we study the relationship between leucocyte profiles of host mallard, *Anas platyrhynchos*, ducklings obtained from wild stocks, and *L. simondi* parasitism.

Leucocytozoon simondi undergoes sexual reproduction in a black fly that has taken a blood meal from an infected avian host (Atkinson and van Riper, 1991; Desser and Bennett, 1993). Shortly after parasites reproduce sexually, they produce asexual stages that make their way to the salivary glands of the fly. From here, they can reach the blood stream of an avian host when the black fly takes a subsequent blood meal. These asexual stages travel through the blood to the liver and other organs, and begin to return to the blood 10 days post-infection, occupying avian erythrocytes as gametocytes. The erythrocytic stage of *L. simondi* is often associated with the most intense signs of infection in host waterfowl (Atkinson and van Riper, 1991; Desser and Bennett, 1993).

There is a range of possible associations between leucocytes and parasites. First, hosts' leucocyte responses could be reactive to parasites, leading to a lag (of an undetermined interval) in leucocyte versus parasite intensities. Second, some hosts could be pre-emptive, producing high densities of circulating leucocytes before parasite exposure that would be associated with subsequent lower intensity infections than in individuals with initially low densities of circulating leucocytes. Third, immune responses may be ineffective, only partially effective, or always effective (also after various intervals), so that no associations would be detected. The best way to assess these

* Corresponding author. Tel.: +1 902 585 1354.
E-mail address: dave.shutler@acadiau.ca (D. Shutler).

1095-6433/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2009.12.015

alternatives is to have leucocyte and parasite data from several successive intervals. Although this is easily achieved under laboratory conditions (e.g., Rose et al., 1979; Garvin et al., 2003), it has seldom been attempted with wild organisms; most studies on wild birds obtain only a single sample. Where only single blood samples have been obtained, some studies observe no association between blood parasite infection intensity and leucocytes (Dufva and Allander, 1995; Booth and Elliott, 2002), and some observe higher densities of total leucocytes, although in some cases of more heterophils (Ots and Hõrak, 1996; Apanius et al., 2000; Davis et al., 2004) and in others of more lymphocytes (Massey et al., 1996; Ots and Hõrak, 1998; Figuerola et al., 1999). Without data from before and after single samples, relationships between leucocytes and blood parasites are difficult to interpret. This study experimentally examined consecutive weeks of leucocyte profiles and assessments of *L. simondi* infection intensities of mallard ducklings.

2. Methods

Details on study locations and sampling methods are provided elsewhere (Shutler et al., 1996, 1999). Briefly, in 1992, eggs from wild mallard nests were collected from Saskatchewan and from in and around Algonquin Park, Ontario, and artificially incubated. Once they were large enough, ducklings were placed in large outdoor cages with water and ad libitum food. In both spring 1993 and 1994, resulting adults were randomly paired in separate cages at Lake St. Clair, Ontario (42°23′ N, 82°24′ W). When ducklings arising from these pairings were between two and nine days old, a set of randomly-chosen "experimentals" from each brood was transported to three different locations in and around Algonquin Park, Ontario (centered at 45°30′ N, 78°30′ W) and housed in outdoor cages that exposed them to the black fly vectors of *L. simondi*. If brood sizes were >4, we retained up to 2 randomly-chosen control ducklings at Lake St. Clair, where no *Leucocytozoon* was detected (Shutler et al., 1996). In total, 11 different pairings produced the ducklings in these experiments. Beginning at 10 days post-exposure, blood smears (Bennett, 1970) were taken from each duckling's tarsal vein (i.e. when ducklings were between 12 and 19 days of age). We took smears for at least three consecutive weeks from controls ($N=7$ duckling, 21 smears), and for at least five consecutive weeks for experimentals ($N=23$ ducklings, 115 smears).

Under oil immersion and at 1000× magnification, AGL and SRR counted 200 leucocytes from each of the smears taken in consecutive weeks from each duckling. AGL and SRR also counted the number of fields of view examined, estimated the number of erythrocytes per field, and the number of *L. simondi* gametocytes, and conducted recounts of 10 randomly-chosen slides to test repeatability. We computed gametocytes per 200 leucocytes, gametocytes per 10,000 erythrocytes, and proportions of each of the leucocytes in 200 leucocytes. Note that Shutler et al. (1996) found no relationship between hematocrit and *L. simondi* infection intensity. We used log (*L. simondi* + 1) transformation to better satisfy expectations of normality in our analyses. *Haemoproteus* (Kruse, 1890) spp. were detected in only 10% of smears, and at most twice in any of the seven ducklings in which they were detected. Because of their low prevalence, *Haemoproteus* spp. were not considered further.

All analyses were run in SAS (Statistical Analysis Systems, Cary, North Carolina). To minimize the number of analyses we ran, we used repeated measures ANOVA, which controls for experiment-wise error rates. We first compared leucocyte profiles of controls and experimentals for each of the three weeks for which we had data. We next restricted analyses to experimentals using five weeks of data. In the latter analyses, we first had leucocytes from each of the five weeks as concomitant response variables, and *Leucocytozoon* data from an individual week as the explanatory variable. Because we could not be sure whether leucocyte profiles were reactive or pre-emptive, we then reversed response and explanatory variables, so that each of the weeks of *Leucocytozoon* data were response variables, and one week of leucocyte data was the explanatory variable.

3. Results

3.1. General observations

Dual counts of blood smears indicated significant repeatability for both observers in lymphocytes ($r=0.84$ and 0.94, $P\leq0.002$), heterophils ($r=0.93$ and 0.94, $P<0.0001$), and *L. simondi* ($r=0.70$ and 0.99, $P\leq0.03$), but not for the less common eosinophils, monocytes, or basophils (at least one $P\geq0.09$). Thus eosinophils, monocytes, and basophils were not analyzed further. Lymphocytes made up an average of 67% and heterophils an average of 24% of leucocytes, which is comparable to values reported elsewhere for mallards between 5 and 42 days of age (Driver, 1981; Sturkie, 1986; Fairbrother and O'Loughlin, 1990).

The proportion of heterophils was significantly higher in control than in experimental ducklings in week 1; there were no differences in the remaining five comparisons (Table 1). No blood parasites were detected in controls (Shutler et al., 1996).

3.2. Relationships between parasites and leucocytes

Among experimentals, *Leucocytozoon simondi* was detected in 111 of 115 (97%) smears, at least 4 times in each of the 23 ducklings sampled, and varied significantly in intensity (0 to 116 parasites per 200 leucocytes, 0 to 41.6 parasites per 10,000 erythrocytes).

There were significant differences among weeks in proportions of lymphocytes ($F_{4,110}=4.0$, $P=0.005$) and heterophils ($F_{4,110}=2.8$, $P=0.03$), and in intensities of *L. simondi* ($F_{4,110}=2.9$, $P=0.03$) (Fig. 1). Lymphocytes made up a significantly smaller proportion of leucocytes in weeks 3 and 4 than they did in week 1 (Tukey tests, $Ps<0.04$), whereas heterophils made up a significantly larger proportion of leucocytes in week 3 than they did in week 1 (Tukey test, $P=0.03$). There were no significant differences in individual pair-wise comparisons of *L. simondi* intensities among weeks (Fig. 1; the smallest Tukey P was 0.08 from a comparison of weeks 1 and 3).

We did not find significant associations between leucocyte profiles over time and *L. simondi* (repeated measures ANOVAs; Table 2). In contrast, we found significant associations between *L. simondi* infection intensities over time and leucocyte profiles in week 2 (Table 2). To identify the source of this significant association, we explored individual correlations between *L. simondi* intensities in each week with each week of leucocyte profiles. These results suggested that more intense parasitism in week 2 of duckling exposure was associated with a less of a reduction in week 3 in the proportion of lymphocytes and less of an increase in the proportion of heterophils (Fig. 2); remaining associations in weeks 1, 3, 4, and 5 were not significant (all $Ps>0.11$).

Table 1
Comparison of proportions of heterophils and lymphocytes in control ($N=7$) and experimental ($N=23$) ducklings.

		Controls		Experimentals			
Leucocyte type	Week	Mean	SE	Mean	SE	$F_{1,28}$	P
Heterophils	1	27.9	3.9	17.7	2.1	5.3	0.03
Heterophils	2	24.5	5.2	21.8	2.9	0.2	0.65
Heterophils	3	26.0	5.6	28.7	3.1	0.2	0.68
Lymphocytes	1	66.6	4.0	73.9	2.2	2.6	0.12
Lymphocytes	2	70.9	5.2	68.6	2.9	0.2	0.70
Lymphocytes	3	70.0	5.3	60.4	2.9	2.5	0.13

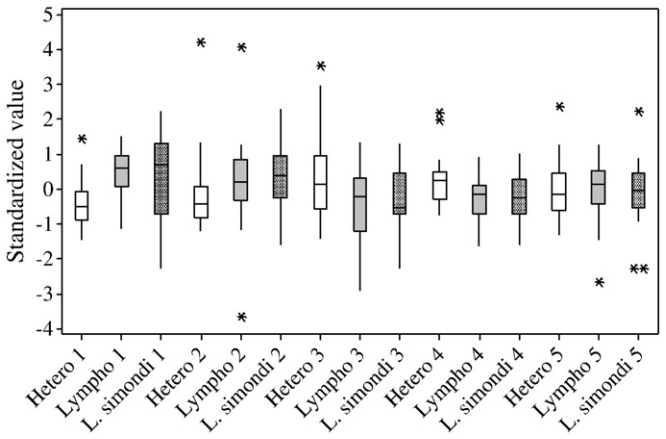

Fig. 1. Box plots by week of the standardized (with mean of 0 and standard deviation of 1) proportion of mallard (*Anas platyrhynchos*) duckling leucocytes that were heterophils (hetero), the standardized proportion of leucocytes that were lymphocytes (lympho), and the standardized log (*Leucocytozoon simondi* + 1) per leucocyte in 23 mallard ducklings. Numbers following *x* variable names indicate the week of sampling. The box encloses the middle 50% of observations, lines indicate the range of values, and asterisks indicate outliers.

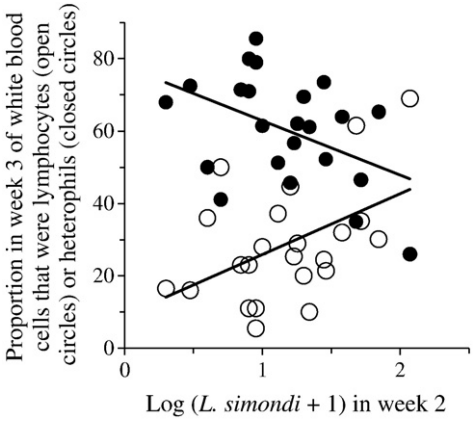

Fig. 2. The proportion of leucocytes that were lymphocytes (open circles) and that were heterophils (closed circles) relative to the log (*Leucocytozoon simondi* parasites + 1) per 200 leucocytes in 23 mallard (*Anas platyrhynchos*) ducklings two weeks after they were exposed to black fly vectors.

4. Discussion

In one of six comparisons, controls and experimentals differed in proportions of leucocytes in their blood, but the cause of this difference is difficult to explain. Higher heterophil proportions are associated with stress (Gross and Siegel, 1983) and if anything, we would have expected heterophil proportions to be higher in experimentals that were stressed by being transported ~575 km to Algonquin Park where they were exposed to novel microclimates, and in particular, *L. simondi*. Possibly, because all ducklings came from grandparents that had laid eggs in and around Algonquin Park (Shutler et al., 1996), experimentals may actually have experienced microclimates to which they were better adapted, or perhaps their cages were deployed in habitats that better matched their instinctive preferences. However, the sample of control ducklings was small, so caution is warranted in interpretation of this result.

We found only limited evidence for relationships between leucocytes and infection intensity. A number of factors could have prevented us from detecting more leucocyte–*L. simondi* associations (Ochs and Dawson, 2008). First, there was significant weekly variation in lymphocytes, heterophils, and *L. simondi* intensities in the mallard ducklings that were experimentally exposed to black fly vectors. Some of the weekly variation in leucocyte profiles may have arisen from age differences among ducklings, but there did not appear to be a clear pattern. Second, the ducklings in these experiments were undergoing maturation of their immune systems, which can influence relative proportions of leucocytes (Sturkie, 1986). This may have introduced more variation in leucocyte profiles than did parasitism by *L. simondi*. Third, although all ducklings did become infected, they did not necessarily acquire infections on the same day, nor did they reach the same infection intensities. However, most experimental ducklings had detectable parasitism by day 10 post-exposure (Shutler et al., 1996) and Desser (1967) reported a range of 6–10 days post-exposure before blood stages of *L. simondi* appeared. Thus, most of our ducklings probably became infected within the first day of exposure. Fourth, some leucocyte responses could have been to other unmeasured parasites, or to black fly bites. However, all of the ducklings were exposed to *L. simondi* at approximately the same age, and were all exposed in a similar manner to other parasites and black fly vectors. In any case, if *L. simondi* has an important influence on leucocyte responses, the former factors should not have prevented us from detecting associations. The lack of associations suggests that *L. simondi* is benign, that it is successful at eluding immune detection, or that immune responses are futile at bringing the parasite under control so that hosts rein in their investments. The only exception to the latter interpretation was that lymphocytes made up comparatively lower and heterophils comparatively higher proportions of leucocytes in the third week in ducklings that in the second week had more intense infections of *L. simondi*.

The importance of *L. simondi* to waterfowl has become entrenched in the literature (Atkinson and van Riper, 1991; Desser and Bennett, 1993; Forrester and Greiner, 2009). However, as has been argued elsewhere (Shutler et al., 1996, 1999), much of this evidence has come from experiments on domestic birds, or from experiments involving artificial infections (Chernin, 1952; Anderson et al., 1962; Kocan and Clark, 1966; note that the seminal work of Khan and Fallis (1968) used game farm ducks that had experienced several generations in captivity). There is less evidence of effects of blood parasites on health of free-living wild birds (but see Merino et al., 2000; Marzal et al., 2005; Tomas et al., 2007). This is not to say that *L. simondi* has no effects on its hosts, only that effects may be subtle. Low parasite virulence may be favoured if debilitation of hosts affects parasites' fitness by preventing them from being transmitted to new hosts. Population densities of adult anatid ducks are often estimated at less than 1/km^2 (Dennis and North, 1984; Boyd, 1984; Maisonneuve et al., 2006) in areas in which *Leucocytozoon* occurs. The majority of black fly vectors travel much shorter distances (Bennett and Fallis, 1971) than would lead to encounters with multiple families of ducks. In other words, a significant majority of transmission probably occurs from mother to ducklings, and among ducklings in the same family; this would select for low virulence. Thus, we believe that the principal reason we failed to find significant associations between leucocytes and *L. simondi* is that this host–parasite association was largely benign in our ducklings.

Table 2
Results of repeated measures ANOVAs exploring whether variation in leucocyte profiles (five weeks combined) were associated with variation in *Leucocytozoon simondi* intensities (top) and whether variation in *L. simondi* intensities (five weeks combined) were associated with variation in leucocyte profiles (bottom).

Explanatory variable	$F_{1,21}$	P
L. simondi intensities in week 1	<0.1	0.94
L. simondi intensities in week 2	5.8	0.03
L. simondi intensities in week 3	0.1	0.76
L. simondi intensities in week 4	0.3	0.58
L. simondi intensities in week 5	3.0	0.10
Leucocyte profiles in week 1	3.4	0.08
Leucocyte profiles in week 2	1.7	0.21
Leucocyte profiles in week 3	1.2	0.29
Leucocyte profiles in week 4	<0.1	0.96
Leucocyte profiles in week 5	1.8	0.20

Acknowledgments

We thank Team Shutler (Cate Little, Andrew Russell, Geoff Williams, and Kate Dalley), Dave Ankney, and five anonymous reviewers for feedback, the numerous agencies and people thanked in Shutler et al. (1996), and NSERC and Acadia University for funding. This manuscript is based on a Special Topics course by AGL and B.Sc. Honours research by SRR.

References

Anderson, J.R., Trainer, D.O., Defoliart, G.R., 1962. Natural and experimental transmission of the waterfowl parasite, *Leucocytozoon simondi* Mathis and Leger, in Wisconsin. Zoo. Res. 1, 155–164.

Apanius, V., Yorinks, N., Bermingham, E., Ricklefs, R.E., 2000. Island and taxon effects in parasitism and resistance of Lesser Antillean birds. Ecology 81, 1959–1969.

Atkinson, C.T., van Riper, C.I.I.I., 1991. Pathogenicity and epizootiology of avian haematozoa: *Plasmodium*, *Leucocytozoon*, and *Haemoproteus*. In: Loye, J.E., Zuk, M. (Eds.), Bird–Parasite Interactions. Oxford University Press, Oxford, pp. 19–48.

Bennett, G.F., 1970. Simple techniques for making avian blood smears. Can. J. Zool. 48, 585–586.

Bennett, G.F., Fallis, A.M., 1971. Flight range, longevity, and habitat preference of female *Simulium euryadminiculum* Davies (Diptera: Simuliidae). Can. J. Zool. 49, 1203–1207.

Booth, C.E., Elliott, P.E., 2002. Hematological responses to hematozoa in North American and neotropical songbirds. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 133, 451–467.

Boyd, H., 1984. US Fish and Wildlife Service estimates of duck numbers in northwestern Ontario, 1955–73. In: Curtis, S.G., Dennis, D.G., Boyd, H. (Eds.), Waterfowl studies in Ontario, 1973–81: Can. Wildl. Serv. Occ. Paper, vol. 54, pp. 10–13.

Chernin, E., 1952. The epizootiology of *Leucocytozoon simondi* infections in domestic ducks in northern Michigan. Am. J. Hyg. 56, 39–57.

Davis, A.K., Cook, K.C., Altizer, S., 2004. Leukocyte profiles in wild house finches with and without mycoplasmal conjunctivitis, a recently emerged bacterial disease. EcoHealth 1, 362–373.

Davis, A.K., Maney, D.L., Maerz, J.C., 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct. Ecol. 22, 760–772.

Dennis, D.G., North, N.R., 1984. Waterfowl densities in northwestern Ontario during the 1979 breeding season. In: Curtis, S.G., Dennis, D.G., Boyd, H. (Eds.), Waterfowl studies in Ontario, 1973–81: Can. Wildl. Serv. Occ. Paper, vol. 54, pp. 6–9.

Desser, S.S., 1967. Schizogony and gametogony of Leucocytozoon simondi and associated reactions in its avian host. J. Protozool. 14, 244–254.

Desser, S.S., Bennett, G.F., 1993. The genera *Leucocytozoon*, *Haemoproteus*, and *Hepatocystis*. In: Krier, J.P. (Ed.), Parasitic protozoa, vol. 4. Academic Press, New York, pp. 273–307.

Driver, E.A., 1981. Hematological and blood chemical values of mallard, *Anas p. platyrhynchos*, drakes before, during and after remige moult. J. Wildl. Dis. 17, 413–421.

Dufva, R., Allander, K., 1995. Intraspecific variation in plumage coloration reflects immune response in Great Tit (*Parus major*) males. Funct. Ecol. 9, 785–789.

Fairbrother, A., O'Loughlin, D., 1990. Differential white blood cell values of the mallard (*Anas platyrhynchos*) across different ages and reproductive states. J. Wildl. Dis. 26, 78–82.

Figuerola, J., Munoz, E., Gutierrez, R., Ferrer, D., 1999. Blood parasites, leucocytes and plumage brightness in the cirl bunting, *Emberiza cirlus*. Funct. Ecol. 13, 594–601.

Forrester, D.J., Greiner, E.C., 2009. Leucocytozoonosis. In: Atkinson, C.T., Thomas, N.J., Hunter, D.B. (Eds.), Parasitic Diseases of Wild Birds. Wiley-Blackwell, Ames, Iowa, pp. 54–107.

Garvin, M.C., Homer, B.L., Greiner, E.C., 2003. Pathogenicity of Haemoproteus danilewskyi, Kruse, 1890, in blue jays (*Cyanocitta cristata*). J. Wildl. Dis. 39, 161–169.

Gross, W.B., Siegel, H.S., 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. 27, 972–979.

Khan, R.A., Fallis, A.M., 1968. Comparison of infections with *Leucocytozoon simondi* in black ducks (*Anas rubripes*), mallards (*Anas platyrhynchos*), and white Pekins (*Anas bochas*). Can. J. Zool. 46, 773–780.

Kocan, R.M., Clark, D.T., 1966. Prepatent period and parasitemia in *Leucocytozoon simondi* infections resulting from short exposures to sporozoites. J. Parasitol. 52, 962–966.

Maisonneuve, C., Belanger, L., Bordage, D., Jobin, B., Grenier, M., Beaulieu, J., Gabor, S., Filion, B., 2006. American black duck and mallard breeding distribution and habitat relationships along a forest-agriculture gradient in southern Quebec. J. Wildl. Manage. 70, 450–459.

Marzal, A., de Lope, F., Navarro, C., Møller, A.P., 2005. Malarial parasites decrease reproductive success: an experimental study in a passerine bird. Oecologia 142, 541–545.

Massey, J.G., Graczyk, T.K., Cranfield, M.R., 1996. Characteristics of naturally acquired *Plasmodium relictum capistranoae* infections in naïve Hawaiian crows (*Corvus hawaiiensis*) in Hawaii. J. Parasitol. 82, 182–185.

Merino, S., Moreno, J., Sanz, J.J., Arriero, E., 2000. Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). Proc. R. Soc. Lond., B Biol. Sci. 267, 2507–2510.

Norris, K., Evans, M.R., 2000. Ecological immunology: life history trade-offs and immune defense in birds. Behav. Ecol. 11, 19–26.

Ochs, C.L., Dawson, R.D., 2008. Patterns of variation in leucocyte counts of female tree swallows, *Tachycineta bicolor*: Repeatability over time and relationships with condition and costs of reproduction. Comp. Biochem. Physiol. A 150, 326–331.

Ots, I., Hõrak, P., 1996. Great tits *Parus major* trade health for reproduction. Proc. R. Soc. Lond. B 263, 1443–1447.

Ots, I., Hõrak, P., 1998. Health impact of blood parasites in breeding great tits. Oecologia 116, 441–448.

Rose, M.E., Hesketh, P., Ogilvie, B.M., 1979. Peripheral blood leucocyte response to coccidial infection: a comparison of the response in rats and chickens and its correlation with resistance to reinfection. Immunology 36, 71–79.

Salvante, K.G., 2006. Techniques for studying integrated immune function in birds. Auk 123, 575–586.

Sheldon, B.C., Verhulst, S., 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Ecol. Evol. 11, 317–321.

Shutler, D., Ankney, C.D., Dennis, D.G., 1996. Could the blood parasite *Leucocytozoon* deter mallard range expansion? J. Wildl. Manage. 60, 569–580.

Shutler, D., Ankney, C.D., Mullie, A., 1999. Effects of the blood parasite *Leucocytozoon simondi* on growth rates of anatid ducklings. Can. J. Zool. 77, 1573–1578.

Sturkie, P.D., 1986. Avian Physiology. Springer-Verlag, New York.

Tomas, G., Merino, S., Moreno, J., Morales, J., Martinez-de la Puente, J., 2007. Impact of blood parasites on immunoglobulin level and parental effort: a medication field experiment on a wild passerine. Funct. Ecol. 21, 125–133.