Veterinary Parasitology 205 (2014) 717–720

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Short Communication

A comparison of the physiological status in parasitized roe deer (*Capreolus capreolus*) from two different populations

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ARTICLE INFO

Article history:
Received 21 May 2014
Received in revised form
12 September 2014
Accepted 13 September 2014

Keywords:
Coccidia
Hematology
Nematode
Plasma biochemistry
Roe deer
Wildlife

ABSTRACT

Studies of the impact of parasites on host performance have mainly focused on body mass, a phenotypic trait that responds relatively slowly to the presence of parasites, and the expectedly faster response of physiological parameters has been mostly overlooked. We filled the gap by measuring the impact of endoparasites on four hematological/biochemical parameters (hematocrit, albumin, creatinine and fructosamine) in two contrasting free-living populations of roe deer. We generally found negative relationships between parasites and physiological parameters. Our findings also indicate little role of host sex on parasite impact and strongest parasite effects on young and senescent hosts.

1. Introduction

Although parasites play a key role in wildlife population processes (Grenfell and Gulland, 1995), there is still limited evidence of negative effects of parasites on host individual performance. Parasites often colonize blood, mucus or tissues, leading to protein loss and nutrient deficiency or anemia in their hosts (Parkins and Holmes, 1989). Helminths and lungworms can also alter protein metabolism and depress appetite, leading to reduced food intake and decreased mass gain (Corrigall et al., 1982).

Most studies describing the effects of parasites on body condition focused on body mass (Irvine et al., 2006), a relevant integrative measure of phenotypic quality (Toïgo et al., 2006). However, body mass is a non-specific indicator of performance that responds relatively slowly to the presence of parasites. In contrast, hematological and biochemical parameters reflect single well defined aspects of host physiology, which respond faster to parasites and allow identifying aspects of host physiology altered by parasites (Beldomenico and Begon, 2010).

The impact of parasites on physiological performance depends on the species and abundance of parasites, and on host initial condition and resilience (Beldomenico and Begon, 2010). Both progressive establishment of immunity in young hosts and immune senescence should lead

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http://dx.doi.org/10.1016/j.vetpar.2014.09.016
0304-4017/

to age-specific variation in infestation, with old and young individuals being more sensitive to parasites than adults (Turner and Getz, 2010). Moreover, males often have lower levels of immunity than females (Forbes, 2007). Finally, environmental conditions should determine the energy and resources available to hosts for controlling parasites, likely causing individuals to allocate less energy against parasites in poor environments than in rich ones.

A negative effect of parasites should thus occur on host body condition (H1), especially in males compared to females (H2), in young and senescent individuals compared to adults (H3), and when resources are scarce (H4).

2. Material and methods

2.1. Data collection

Data were collected in two contrasted areas: Trois Fontaines (1360 ha, 48°43′N, 4°55′E, France), with a continental climate and high quality soils; and Chizé (2614 ha, 46°05′N, 0°25′W, France), with a temperate oceanic climate and poor quality soils. Data were collected every winter from December 2009 to March 2013 on roe deer captured using drive netting (Gaillard et al., 1993). Roe deer were sexed and weighed and blood samples were collected (EDTA and serum tube) at the jugular vein and fecal matter (5–10 g) *per rectum*.

2.2. Body condition

Body condition was measured by body mass (in kg) and four hematological and biochemical parameters, obtained from classical biochemical measurements: hematocrit (HCT in %), to reflect changes in an animal's oxygen-carrying capacity; albumin (ALB in g/L), measured using total protein content of the serum (obtained with a refractometer) and electrophoresis (processor HYDRASIS, Sebia, Evry, France), to access the level of protein resources; creatinine (CREA in μmol/L), to access the turnover of muscular proteins, and fructosamine (FRU in μmol/L), measured using Thermo scientific reagents and ABX Pentrafructosamine reagents (Horiba, Montpellier, France), respectively, on a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France), to get a measure of the level of carbohydrates independent of capture stress (Stockham and Scott, 2008) (see the Supplementary material for details).

2.3. Parasites

Fecal propagule counts of four frequent groups of species were investigated: gastrointestinal strongyles (GI strongyles), including Trichostrongyloidea and Strongyloidea, *Trichuris* sp., coccidia and Protostrongylids. Each fecal sample was analyzed by two different techniques at the laboratory of the faculty of pharmacy (University of Reims Champagne-Ardenne, EA4688 Vecpar): the McMaster protocol (Raynaud, 1970) for gastrointestinal nematode eggs and coccidia, and the Baermann fecal technique (Baermann, 1917) for pulmonary nematode larvae (Protostrongylids). Parasite abundance was estimated by using two metrics: the fecal propagule count (on a log ($n+1$) scale) of each individual, infected or not (Bush et al., 1997) and the diversity of the parasitic community (IPC) measured as the Shannon index (Shannon, 1948).

2.4. Statistical analysis

Only known-aged individuals (i.e. captured during their first year of life) were included, split into three age classes (young: <1 year of age; adult: between 2 and 8 years of age; senescent: ≥8 years of age, see Gaillard et al., 1993). As young body mass provides a reliable indicator of resource availability during summer–autumn in temperate ungulates (Hamel et al., 2009), the average mass of young (AMY) was used in a given population and for a given year to measure resource availability.

A linear mixed model was fitted for each physiological parameter by including individual as a random effect, and age, sex, AMY, body mass and parasite abundance as independent variables to allow testing hypotheses (using Likelihood Ratio Tests with Maximum Likelihood estimation method applied by deleting each variable in turn). A *p*-value <0.05 was considered statistically significant. All analyses were run using R (version 2.15.3, 2013, R Foundation for Statistical Computing, ISBN 3-900051-07-0).

2.5. Ethical consideration

This research was conducted with the approval of the Director of Food, Agriculture and Forest (Prefectoral order 2009–14 from Paris) and of the Office National des Forêts (ONF), and received the approval of the ethical committee of University Lyon 1 (project DR2014-09).

3. Results

After accounting for possible confounding effects of age and sex, body mass in Trois Fontaines decreased with increasing counts of GI strongyles ($\beta = -0.15 \pm 0.07$, $p = 0.043$), *Trichuris* sp. ($\beta = -0.17 \pm 0.08$, $p = 0.030$), and Protostrongylids ($\beta = -0.44 \pm 0.20$, $p = 0.027$), and with increasing IPC ($\beta = -0.87 \pm 0.38$, $p = 0.025$). At Chizé, no statistically significant association between parasite abundance and body mass was found. Moreover, in both populations, there was no statistically significant association between any parasite abundance and ALB.

At Trois Fontaines, HCT decreased with increasing counts of coccidia ($\beta = -0.30 \pm 0.15$, $p = 0.034$), CREA decreased with increasing counts of GI strongyles ($\beta = -2.93 \pm 0.80$, $p < 0.001$) and Protostrongylids ($\beta = -7.54 \pm 2.18$, $p < 0.001$) and with increasing IPC ($\beta = -13.90 \pm 4.03$, $p < 0.001$). At Chizé, HCT decreased with increasing counts of Protostrongylids ($\beta = -2.07 \pm 0.57$, $p < 0.001$), and CREA decreased with increasing counts of GI strongyles ($\beta = -1.80 \pm 0.87$, $p = 0.036$).

Moreover, at Trois Fontaines, there were interactions between sex and parasites (involving GI strongyles and IPC, Table 1) for HCT and between age and parasites (involving *Trichuris* sp. and IPC for HCT, and coccidia and IPC for FRU, Table 1). At Chizé, there were interactions between age and parasites (GI strongyles, Table 2) for HCT and between AMY

Table 1
Effect of parasites in interaction with age, sex and AMY on hematological parameters of roe deer in Trois Fontaines (France).

Parasites	HCT p estimate ± SE	CREA p estimate ± SE	FRU p estimate ± SE
GI		$p = 0.035$ (M × GI) 3.31 ± 1.59	
T	$p < 0.001$ (Y × T) −0.93 ± 0.42 (S × T) −2.03 ± 0.50		
C			$p = 0.015$ (Y × C) −1.70 ± 1.83 (S × C) 6.28 ± 2.76
IPC	$p = 0.030$ (Y × IPC) −2.40 ± 1.98 (S × IPC) −6.08 ± 2.33	$p = 0.011$ (M × IPC) 18.99 ± 7.54	$p = 0.025$ (Y × IPC) −31.72 ± 11.94 (S × IPC) −10.76 ± 14.04

GI: Gastrointestinal strongyles; T: *Trichuris* sp.; C: coccidia; IPC: index of diversity of parasitic community; HCT: hematocrit; CREA: creatinine; FRU: fructosamine; Y: young; S: senescent; M: males; AMY: average mass of young; ×: interaction between parasite and body condition; p: p-value of the Likelihood Ratio Test; estimate ± SE: coefficients estimated from the linear mixed model with standard error using females and adults as references.

Table 2
Effect of parasites in interaction with age, sex and average mass of young AMY on hematological parameters of roe deer in Chizé (France).

Parasites	HCT p estimate ± SE	CREA p estimate ± SE	FRU p estimate ± SE
GI	$p = 0.032$ (Y × GI) 0.67 ± 0.58 (S × GI) −1.12 ± 0.54		
T		$p = 0.032$ (AMY × T) 4.66 ± 2.18	
C			$p = 0.038$ (AMY × C) 5.04 ± 2.45
IPC			

GI: gastrointestinal strongyles; T: *Trichuris* sp.; C: coccidia; IPC: index of diversity of parasitic community; HCT: hematocrit; CREA: creatinine; FRU: fructosamine; Y: young; S: senescent; M: males; AMY: average mass of young; ×: interaction between parasite and body condition; p: p-value of the Likelihood Ratio Test; estimate ± SE: coefficients estimated from the linear mixed model with standard error using females and adults as references.

and parasites (involving *Trichuris* sp. for CREA and coccidia for FRU, Table 2).

For Protostrongylids, no interaction with any variable investigated was statistically significant.

4. Discussion

All physiological parameters except ALB were negatively correlated with parasite abundance, at least for one parasite group, in accordance with the H1 hypothesis. As previously reported (Stien et al., 2002), negative associations were found between body mass and most parasites investigated at Trois Fontaines. HCT, CREA and FRU were also often negatively correlated with parasite abundance, suggesting either losses of blood and proteins in infested individuals (Baker et al., 2003) or a negative energy balance leading to a decrease of blood/protein production (Coop and Holmes, 1996). However, in this cross-sectional study, causal relationships could not be demonstrated. Our finding that parasite abundance negatively impacts body condition could also be interpreted as highest susceptibility of individuals with poorest body condition (Ezenwa, 2004).

The susceptibility of host and its ability to respond to parasite infestations could impact on fecal egg counts (Beldomenico and Begon, 2010). However, based on the high correlation reported in roe deer between fecal egg counts and number of adult parasites in the digestive tract (Appendix 1 in Body et al., 2011), fecal egg counts can be assumed to be reliable markers for parasite infestation.

Host sex (H2) only had a minor role on parasitism patterns, but age (H3) had a determinant role, with stronger negative correlations in young and senescent individuals. Young animals had lower resilience than adults because they have been less exposed to pathogens and acquired immunity to GI nematode infestation at a slower rate (e.g. Coop and Holmes, 1996 for sheep). The higher susceptibility of old animals to parasites matches with increasingly reported evidence of senescence in performance-related traits like reproduction or survival in the wild (Nussey et al., 2013). Accordingly, recent research in wild mammals has demonstrated a decrease of immune system efficiency with increasing age (Nussey et al., 2012). A decreasing food intake with increasing age leading to decrease physiological and immune parameters could also be involved. The loss of immune capacity may itself increase parasitism, leading to a vicious circle (Beldomenico and Begon, 2010).

Lastly, contrary to the H4 hypothesis, environmental conditions only had a negligible role on parasite-body condition relationship maybe because of limited variation in environmental conditions during this 4-year study. Alternatively, highly condition-dependent mortality in the worse situations could lead the most heavily infested individuals to die before capture following parasite infestation. However, the decrease in some physiological parameters with increasing parasite infestation was more pronounced at Chizé when the average mass of young animals was low (Table 2). We suggest that such pattern might be detected only under the strong limiting resource conditions of Chizé.

At Trois Fontaines, even years of low performance would not be limiting enough to lower body condition and physiological parameters of roe deer.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

We thank all the ONCFS staff volunteers who organized the roe deer captures, Céline Dussart for her help in field and laboratory analyses, Damien Jouet for his help in coprological techniques, and two anonymous reviewers for insightful comments on a previous draft. This study was financially supported by ONCFS (grant nos. 2011/18/6171 and 2010/03/6171).

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