Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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

Experimental infection by microparasites affects the oxidative balance in their avian reservoir host the blackbird *Turdus merula*

Ana Cláudia Norte[a,b,*], David Costantini[c,d], Pedro Miguel Araújo[a], Marcel Eens[c], Jaime Albino Ramos[a], Dieter Heylen[e]

[a] Marine and Environmental Sciences Centre, Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[b] Center for Vector and Infectious Diseases Research, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal
[c] Behavioural Ecology and Ecophysiology Group, Department of Biology, University of Antwerp, Universiteitsplein 1, 2610, Wilrijk, Belgium
[d] UMR 7221 CNRS/MNHN, Muséum National d'Histoire Naturelle, ComEU Sorbonnes Universités, 7 rue Cuvier, 75005, Paris, France
[e] Evolutionary Ecology Group, Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

ARTICLE INFO

Keywords:
Oxidative stress
Reservoir
Host-parasite interactions
Zoonosis
Tick
Borrelia burgdorferi s.l

ABSTRACT

By draining resources, microparasites can negatively affect the host fitness, which in turn can result in reduced transmission when virulence leads to reductions in host population size. Therefore, for a microparasite to persist in nature, the level of harm it can do to its host is expected to be limited. We tested this hypothesis for tick-borne *Borrelia burgdorferi* sensu lato (s.l.) infections in the blackbird *Turdus merula*, one of the most important avian reservoir hosts in Europe. Experimental and observational data were combined to examine the physiological effects caused by *B. burgdorferi* s.l. infection in blackbirds. Pathogen-free blackbirds were exposed to *B. burgdorferi* s.l.-infected *Ixodes ricinus* and *I. frontalis* nymphs, and compared with a control group (exposed to naïve laboratory-derived *I. ricinus* nymphs). Their physiological status was evaluated before and after infection with *B. burgdorferi* s.l., through a set of immunological (erythrocyte sedimentation rate, haptoglobin, white blood cell count and heterophil/lymphocyte ratio), oxidative stress (glutathione peroxidase activity, protein carbonyls and nitric oxide) and general body condition variables (body condition, glucose and haematocrit). Infected males showed higher levels of oxidative damage to proteins (increased levels of protein carbonyls), decreased glutathione peroxidase activity and increased body mass. Infected females had higher levels of glutathione peroxidase activity after infection by *B. burgdorferi* s.l. than the control group. No significant effects of *B. burgdorferi* s.l. infection were detected on erythrocyte sedimentation rate, haptoglobin, heterophil/lymphocyte ratio, nitric oxide, glucose and haematocrit. The first experimental study on the effects of *B. burgdorferi* s.l. on its avian reservoir hosts shows that these bacteria may inflict non-negligible physiological costs. We speculate that during energetically demanding periods, these physiological costs may reduce host fitness and affect pathogen transmission.

1. Introduction

Parasites can modulate the physiology, behaviour, and life-history traits of their hosts. Parasites are costly to their hosts because they consume their hosts' metabolites and tissues, and redirect energy from other vital functions and activities of the host to the host immune system (Sheldon and Verhulst, 1996). Host immunocompetence and ability to fight parasites, on the other hand, is dependent on available resources and life-history decisions, but its relationship with fitness in wild populations needs further study (Norris and Evans, 2000). Different stages of infection, in which parasites may colonize different organs and tissues, may cause different immune reactions on the host, increasing the complexity of the immunology of parasitic infections (Cornet and Sorci, 2014). From an evolutionary, ecological and conservational point of view it is relevant to understand the pathogenicity of a microparasite, and the physiological harm it may impose on its host and the consequent impacts on the host population. The magnitude of the impacts of a parasite on its host may depend on the degree of co-evolution between them (Loye and Zuk, 1991), but also on environmental factors such as stressors, multiple effects of different co-occurring parasites (co-infections), phylogenetic group of the parasite and its degree of specialization and mode of transmission, among others

* Corresponding author at: MARE – Marine and Environmental Sciences Centre, Department of Life Sciences, Largo Marquês de Pombal, Faculty of Sciences and Technology, University of Coimbra, 3004-517, Coimbra, Portugal.
E-mail address: acgnorte@ci.uc.pt (A.C. Norte).

https://doi.org/10.1016/j.ttbdis.2018.02.009
Received 2 October 2017; Received in revised form 8 February 2018; Accepted 8 February 2018
1877-959X/

(Hawley and Altizer, 2011). For a microparasite to persist and be successfully transmitted in nature, limitations are expected in the level of harm it can inflict on its host, and the mode of transmission is one important factor that affects virulence (i.e. extent of damage, pathology and consequent fitness costs to the hosts). It was believed that vector-borne parasites would be more virulent than directly transmitted parasites, because mobile arthropod vectors can transmit the former effectively among immobilized hosts, although there are other factors in vector–borne transmission that also affect virulence (Ewald, 1993; Ewald, 2004). In tick species that use an ambushing strategy to find hosts, the tick-borne parasites depend on the host movement for efficient transmission (Randolph, 1998). Other tick species remain on the host between life stages (from larvae to nymphs; Hillyard, 1996). Thus, tick-borne parasites that are less virulent may benefit from enhanced host-to-tick transmission success.

Nevertheless, tick-borne parasites (e.g. tick-borne encephalitis virus, *Babesia* spp., *Borrelia burgdorferi* sensu lato (s.l.) can have important effects on their vertebrate hosts including cellular and humoral immune responses (Chauvin et al., 2009; Mansfield et al., 2009; Norte et al., 2013a; Heylen et al., 2015), oxidative stress (Esmaeilnejad et al., 2012) and altered body mass (Heylen et al., 2015). *B. burgdorferi* s.l., a bacterial complex in which some members cause Lyme borreliosis, is a tick-borne zoonotic parasite, comprising over 20 genospecies with different host-specificities (Stanek and Reiter, 2011), whose main vector in Europe is the tick *Ixodes ricinus*. Members of this bacterial complex infect several vertebrate species of woodlands and gardens (Gern and Humair, 2002; Piesman and Gern, 2004) but in some species of birds (e.g. *Turdus* spp.), small and medium-sized mammals (e.g. *Apodemus* spp., *Sciurus* spp.), and lizards (e.g. *Psammodromus algirus*), certain *B. burgdorferi* s.l. genospecies (*B. garinii* and *B. valaisiana* in birds, *B. afzelii* in mammals and *B. lusitaniae* in lizards) are able to establish a widespread (systemic) infection (Gern et al., 1998; Margos et al., 2011). These often abundant vertebrate species are significantly involved in the maintenance and transmission of these particular *Borrelia* genospecies in nature because they are highly infective to the vector ticks they are often infested with – those are considered reservoir hosts (Gern and Humair, 2002; Kurtenbach et al., 2002; Ashford, 2003; Mannelli et al., 2012). At present, little is known about the consequences of infection by *B. burgdorferi* s.l. in its avian reservoir hosts. It is expected that avian-associated genospecies of this tick-borne microparasite induce limited virulence in its avian reservoir hosts (Olsen, 2007), given the negative consequences high virulence may have on its transmission success and persistence in nature.

Recently, Heylen et al. (2015) reported that great tits (*Parus major*) infected with *B. burgdorferi* s.l., through exposure to infected *I. ricinus* collected in an enzootic area, increased their nitric oxide production from immune cells and their body mass with increasing levels of *B. burgdorferi* s.l. infection. This study showed that some immune components are activated in response to *B. burgdorferi* s.l. infection, but there was no uninfected control group in the study design. A previous field study showed that one-year old *B. burgdorferi* s.l.-infected blackbirds (*Turdus merula*) had higher levels of circulating immunoglobulins (Norte et al., 2013a). Infected blackbirds also had higher white blood cell counts, and one infected-individual had an exceptionally high value of blood glucose, which is indicative of hyperglycaemia in acute stress situations. Studies on the white-footed mouse (*Peromyscus leucopus*), an important reservoir host, found no effects of *B. burgdorferi* s.l. infection apart from immunological responses (e.g. antibody production (Schwanz et al., 2011). Despite these reported physiological impacts, no fitness effects on its avian and rodent reservoir hosts have been found (Moody et al., 1994; Hofmeister et al., 1999; Schwanz et al., 2011; Chambert et al., 2012; Voordouw et al., 2015). Eco-immunological studies are challenging, especially in field conditions, due to the complex nature of the immune system, biotic and abiotic factors impacting it, and cooperation among its different components in response to parasitic infections. But the evaluation of immunological interactions between vector-host-parasite is fundamental for the understanding of the coevolution of this relationship and maintenance and spread of parasites. The use of standardised physiological and immune-related parameters, for which biological variation is well studied and which can be related with host fitness, will help to overcome this lack of knowledge. In this study we experimentally investigated for the first time the effects of *B. burgdorferi* s.l. on the health status of a passerine bird using a variety of physiological variables. We used the blackbird, which is one of the most abundant and important avian reservoirs for *B. burgdorferi* s.l. in Europe (Humair et al., 1998; Taragel'ová et al., 2008; Norte et al., 2013b). We exposed juvenile pathogen-free blackbirds to *I. ricinus* and *I. frontalis* nymphs, which had previously fed as larvae on *B. burgdorferi* s.l.-infected wild blackbirds. Our study also contained a control group, where juvenile pathogen-free blackbirds were exposed to uninfected nymphs. The effects of *B. burgdorferi* s.l. infection were evaluated using a set of physiological variables reflecting different but interrelated biological systems and functions, often used in ecological studies due to their utility in assessing wildlife health and fitness and functional interactions among the components of life-history traits (Albano, 2012; Costantini, 2008).

We tested whether *B. burgdorferi* s.l. infection reduced the health status of blackbirds, through increased levels of inflammation indicators (erythrocyte sedimentation rate, haptoglobin, white blood cell counts and heterophil/lymphocyte ratio) and increased oxidative stress compared to controls. Inflammatory responses can lead to an imbalance in the oxidative status of the organism (Costantini, 2014), and, therefore, we measured metrics of oxidative stress and damage in the blood (glutathione peroxidase activity, nitric oxide, protein carbonyls), which we expect to increase in the infected group. Oxidative stress is a biochemical mechanism that has been shown to modulate some life-history traits, including reproduction and ageing (Finkel and Holbrook, 2000; Costantini, 2014), therefore, it may indicate potential impacts of *B. burgdorferi* s.l. infection at higher ecological processes involving this reservoir host species (e.g. population dynamics). Finally, we measured physiological variables related to the body and nutritional condition of the birds – body condition, glucose and haematocrit. Due to potential sex differences in endocrine-immune interactions, and consequent susceptibility to infection and eventually parasite load, we evaluated if the effects of *B. burgdorferi* s.l. infection differed between sexes (Zuk and McKean, 1996; Klein, 2000; Oliver-Guimerá et al., 2017). Therefore, we expect that by using such a panel of sensitive physiological and clinical biochemistry assays we will be able to detect even subtle detrimental physiological effects of *B. burgdorferi* s.l. on its reservoir hosts, which may be expected to be of higher magnitude for non-reservoirs hosts. Furthermore, the assessment of host-parasite relationships and the factors that promote and limit the amplification of *B. burgdorferi* s.l. in its reservoir hosts are central to the understanding of the maintenance and emergence of the causative agents of Lyme borreliosis and the impact of this zoonosis on wildlife, and may help to establish mitigating actions to prevent increases in its incidence.

2. Materials and methods

2.1. Host-parasite system characterization

Blackbirds are ubiquitous birds occurring in forests and urban areas. They are often infested by hard ticks of the genus *Ixodes*, especially immature stages, present one of the highest tick infestation prevalence and intensity among Passerines, and are important for tick population maintenance (Norte et al., 2012). Both field and experimental studies, including the use of xenodiagnosis, have proved their reservoir competence for some *B. burgdorferi* s.l. genospecies (e.g. *B. valaisiana*, *B. garinii* and *B. turdi*), revealing their importance in enzootic *B. burgdorferi* s.l. cycles (Norte et al., 2013b, Humair et al., 1998).