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Hemoparasites in a wild primate: Infection patterns suggest interaction of *Plasmodium* and *Babesia* in a lemur species[☆]

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ABSTRACT

Hemoparasites can cause serious morbidity in humans and animals and often involve wildlife reservoirs. Understanding patterns of hemoparasite infections in natural populations can therefore inform about emerging disease risks, especially in the light of climate change and human disruption of natural ecosystems. We investigated the effects of host age, sex, host group size and season on infection patterns of *Plasmodium* sp., *Babesia* sp. and filarial nematodes in a population of wild Malagasy primates, Verreaux's sifakas (*Propithecus verreauxi*), as well as the effects of these infections on hematological variables. We tested 45 blood samples from 36 individuals and identified two species of *Plasmodium*, one species of *Babesia* and two species of filarial nematodes. *Plasmodium* spp. and *Babesia* sp. infections showed opposite patterns of age-dependency, with babesiosis being prevalent among young animals, while older animals were infected with *Plasmodium* sp. In addition, *Babesia* sp. infection was a statistically significant negative predictor of *Plasmodium* sp. infection. These results suggest that *Plasmodium* and *Babesia* parasites may interact within the host, either through cross-immunity or via resource competition, so that *Plasmodium* infections can only establish after babesiosis has resolved. We found no effects of host sex, host group size and season on hemoparasite infections. Infections showed high prevalences and did not influence hematological variables. This preliminary evidence supports the impression that the hosts and parasites considered in this study appear to be well-adapted to each other, resulting in persistent infections with low pathogenic and probably low zoonotic potential. Our results illustrate the crucial role of biodiversity in host-parasite relationships, specifically how within-host pathogen diversity may regulate the abundance of parasites.

1. Introduction

Vector-borne hemoparasites, including the apicomplexan protozoa *Babesia* sp. and *Plasmodium* sp., trypanosomes and filarial nematodes, are important pathogens in humans and domestic animals, causing babesiosis, malaria, sleeping sickness, lymphatic filariasis and canine heartworm disease. Endemic as well as introduced hemoparasites may also impact health and fitness of wildlife (e.g. Custer and Pence, 1981; Atkinson et al., 2000; Garvin et al., 2003; Donahoe et al., 2015). Furthermore, babesiosis is an emerging zoonosis worldwide, with wildlife reservoirs playing a particular role in its epidemiology (Gray et al., 2010; Yabsley and Shock, 2013). Thus, characterizing hemoparasite infections in wildlife and understanding patterns of prevalence can potentially reveal emerging disease risks, especially in the light of ecological instabilities as a result of human encroachment into wildlife habitats (Daszak et al., 2001; Keesing et al., 2010) and climate change (Daszak et al., 2000; Barrett et al., 2013; Kronefeld et al., 2014). Wild primate populations are of particular interest in this context, because nonhuman primates have played a major role in the emergence of human diseases, including malaria, in the past (Wolfe et al., 2007; Pedersen and Davies, 2009; Pacheco et al., 2011).

Age, sex or seasonal effects may have an influence on exposure

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and susceptibility to infection with many parasites. Age effects have been demonstrated for *Plasmodium* sp. infections in chimpanzees and humans (Doolan et al., 2009; De Nys et al., 2013) and for *Babesia* sp. infections in dogs and cattle (Boozer and Macintire, 2003; Bock et al., 2004), with decreasing prevalence in older animals being most likely due to the development of an effective adaptive immune response (Frolich et al., 2012). Additionally, the innate immune system is an important controlling factor of apicomplexan parasite infectivity (Frolich et al., 2012) and variation in immune responsiveness may thus influence infection patterns.

Sex differences in hemoparasite infections have been found in several vertebrates, including higher prevalences in male penguins (Merkel et al., 2007), lizards (Schall et al., 2000) and lions (Sherman, 2010). These sex differences could either be due to physiological differences, e.g. immunosuppression caused by higher testosterone levels in males (Klein, 2004; Roberts et al., 2004), or due to differences in parasite exposure. For example, a sex-bias in vector feeding rates towards males was recently demonstrated in birds (Burkett-Cadena et al., 2014). Although hemoparasite infections have been investigated in several wild primate species (de Thoisy et al., 2001; Maamun et al., 2011; De Nys et al., 2013; Thurber et al., 2013), no sex-differences in prevalence have been reported so far.

Furthermore, it has been proposed that group-living may decrease the risk of infection with vector-borne pathogens by means of an encounter-dilution effect, analogous to a decrease of predation risk (Freeland, 1976; Mooring and Hart, 1992; Kappeler et al., 2015). Empirical evidence for this effect is controversial, however. Krebs et al. (2014) recently demonstrated that sentinel hosts caged inside large roosts of American robins seroconverted to West Nile Virus more slowly than those held outside of roosts, suggesting that exposure of individual hosts can indeed be reduced through group-formation. However, larger groups may also be more conspicuous, and thus attract more vectors. In a comparative study, colonially-breeding bird species were shown to experience both higher prevalences and higher species diversity of blood parasites than solitarily breeding species (Tella, 2002). Two studies on Neotropical primates found that prevalence of *Plasmodium* sp. increases with group size (Davies et al., 1991; Nunn and Heymann, 2005), but studies on the effect of within-species variability of group size on hemoparasite infections in primates are lacking.

Furthermore, infections with vector-borne parasites are likely to covary with environmental conditions (Altizer et al., 2006). Rainfall and temperature affect mosquito abundance, biting rates and parasite development within mosquitoes (Altizer et al., 2006; Galardo et al., 2009; Mohammed and Chadee, 2011). Additionally, dry, cold conditions as well as frequent temperature fluctuations can reduce the abundance of host-seeking ticks (Sutherst and Bourne, 2006; Swai et al., 2006; Herrmann and Gern, 2013). Despite this seasonality in vector biology, empirical evidence regarding seasonal variation in host infection rates is contradictory and mainly limited to malaria in humans (Smith et al., 1993; Koram et al., 2003; Mabaso et al., 2007). If transmission results in persistent infections, little seasonal variation in prevalence can be expected (Govender et al., 2011).

Finally, in natural systems co-infections with multiple parasites are common, potentially resulting in complex interspecific interactions. It has been shown that parasite community interactions may explain more variation in infection risk than the effects associated with host and environmental factors. For example, in field voles (*Microtus agrestis*) chronic infection with *Babesia microti* reduces susceptibility to *Bartonella* spp. bacteria by 85% as compared to uninfected individuals (Telfer et al., 2010). Parasites may directly affect the fitness of co-infecting species through interference competition, they may compete for the same resources within a host, or affect each other's abundance via interaction with the host's immune system (Pedersen and Fenton, 2006). Cross-immunity between co-infecting parasite species might limit prevalence at the population level, whereas parasite-induced immunosuppression may lead to synergistic effects (Cox, 2001; Telfer et al., 2008).

We present the first study which systematically characterizes infections with several co-occurring hemoparasites in a population of Malagasy primates, Verreaux's sifakas (*Propithecus verreauxi*). Madagascar faces acute risks of species extinctions as well as disease emergence in humans and wildlife due to intense human disruption of natural ecosystems (Harper et al., 2007; Junge, 2007; Barrett et al., 2013; Ratsimbazafy et al., 2013; Schwitzer et al., 2014). Verreaux's sifakas are diurnal, sexually monomorphic lemurs which live in multi-male multi-female groups of varying size in seasonal habitats, in which mosquitoes are virtually absent for several months of the year, in western and southern Madagascar (Kappeler and Fichtel, 2012). The life expectancy of sifakas in the wild can exceed 20 years (Richard et al., 2002; Kappeler and Fichtel, 2012). We used samples collected from members of all age classes from 10 adjacent groups ranging in size from 2 to 7 individuals during annual captures to detect infections with *Plasmodium* sp., *Babesia* sp. and filarial nematodes using a combination of a PCR-based approach and microscopical examination of blood smears. These parasites have previously been reported to occur in blood samples of sifakas (Uilenberg et al., 1972; Junge and Louis, 2005; Duval et al., 2010; Pacheco et al., 2011; Rasambainarivo et al., 2014), but systematic investigations of infection patterns are lacking. The potential vectors for *Plasmodium* spp. and *Babesia* spp., anopheline mosquitos and haemaphysaline ticks, are present in the study region (Davidson, 1966; Rodriguez et al., 2012), whereas the vectors for filarial parasites of lemurs are unknown (Irwin and Raharison, 2009).

To illuminate natural drivers of infection patterns, we tested the influence of host age and sex, host group size and seasonality on individual infection status and hemoparasite species richness in our study population. To assess possible health consequences, we also tested the influence of these infections on packed cell volume, to assess possible anemia, and total plasma protein as well as the neutrophil-lymphocyte ratio, which are usually increased during inflammatory processes (Thrall et al., 2006). Based on results of previous studies with other vertebrates, we predicted that infection probabilities would decrease with age and that males would harbor more hemoparasites than females. Furthermore, we predicted that group size would negatively affect the probability of testing positive for each hemoparasite, as well as individual hemoparasite species richness, in case of an encounter-dilution effect. Alternatively, we predicted that group size would have a positive effect on these measures if larger groups attract more vectors. We also expected to find lower prevalences of hemoparasite infections during the dry season than during the hot, wet season.

2. Materials and methods

2.1. Sample collection

The study was carried out in Kirindy Forest, Western Madagascar, located at approximately 44°39′E, 20°03′S. The study area is part of a field site operated by the German Primate Center (DPZ) since 1993 and is situated within a forestry concession managed by the Centre National de Formation, d'Etudes et de Recherche en Environnement et Foresterie (CNFEREF). Kirindy Forest is a dry deciduous forest and subject to pronounced seasonality, with a dry season from April to October and a hot, wet