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Co-infection patterns of intestinal parasites in arboreal primates (proboscis monkeys, *Nasalis larvatus*) in Borneo

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ABSTRACT

Non-human primates of South-East Asia remain under-studied concerning parasite epidemiology and co-infection patterns. Simultaneously, efforts in conservation demand knowledge of parasite abundance and biodiversity in threatened species. The Endangered proboscis monkey, *Nasalis larvatus*, a primate flagship species for conservation in Borneo, was investigated in the present study. Habitat loss and fragmentation are among the greatest threats to bachelor and harem groups of this folivorous colobine. Designed as a follow-up study, prevalence and co-infection status of intestinal parasites from *N. larvatus* in a protected area in Malaysian Borneo were analyzed from fecal samples using a flotation method. For the first time, the intestinal parasite co-infection patterns were examined using quantitative analyses. Overall, 92.3% of fecal samples ($N = 652$) were positive for helminth eggs. Five helminth groups were detected: (1) trichurids (82.7% prevalence) including *Trichuris* spp. (82.1%) and *Anatrichosoma* spp. (1.4%), (2) strongyles (58.9%) including *Trichostrongylus* spp. (48.5%) and *Oesophagostomum/Ternidens* spp. (22.8%), (3) *Strongyloides fuelleborni* (32.7%), (4) *Ascaris lumbricoides* (8.6%), and (5) *Enterobius* spp. (5.5%). On average, an individual was co-infected with two different groups. Significant positive associations were found for co-infections of trichurids with strongyles and *S. fuelleborni* as well as *S. fuelleborni* with *A. lumbricoides* and strongyles. This study shows a high prevalence of various gastrointestinal helminths with potential transmission pathways primarily related to soil and with zoonotic relevance in wild proboscis monkeys in their remaining natural habitats. Observed positive associations of trichurids with strongyles and *Strongyloides* spp. may result from the high prevalence of trichurids. Similarly, positive associations between *Strongyloides* and *Ascaris* were found, both of which typically occur predominantly in juvenile hosts. These findings should be considered when proposing conservation actions in altered habitats nearby human settlements and when managing captive populations.

1. Introduction

The study of wildlife parasites plays a crucial role for conservation efforts of threatened species worldwide (Daszak et al., 2000). To understand the impact of parasitic infections on wildlife endangerment, comprehensive datasets on parasite abundance, co-infection status, and transmission pathways of potential pathogens in natural systems are required (Thompson et al., 2010). Parasitic infections are among the most common diseases found in non-human primates (hereafter referred to as ‘primates’) (Strait et al., 2012). Specifically, intestinal parasitic infections are the focus of a large proportion of studies, but their role in influencing ecosystems and population dynamics remains controversial (Marcogliese, 2005; Gillespie and Chapman, 2008; Nguyen et al.,

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2015). In addition, the zoonotic potential of pathogens in wild primates has received considerable attention as contact between domestic communities and local primate species has increased over the past decades.

Mechanisms of interactions among parasitic species were examined for nematodes as well as between helminths and protozoa, but results remain ambiguous (Petney and Andrews, 1998; Murphy et al., 2013). Several mechanisms of direct and indirect interaction between co-infecting parasites have been proposed. Parasites may compete for host resources (e.g. space and food), or they may benefit from immunosuppression by one species favoring host infection with another species (Pedersen and Fenton, 2007). However, few data are available on co-infection patterns in primates. To date, most is known about intestinal helminths and protozoa in wild African ape populations or New World monkey species (e.g. Nizeyi et al., 2002; Michaud et al., 2003; Eckert et al., 2006; Gillespie et al., 2010). In contrast, parasites in wild primate populations of South-East Asia have been little studied, especially in relation to their total population size, geographical distribution and conservation status (Hopkins and Nunn, 2007).

The island of Borneo, South-East Asia, is one of the world's 34 biodiversity hotspots (De Bruyn et al., 2014), suffering from the highest deforestation rate in the tropics (Sodhi et al., 2010) as a result of the growing economy of local human populations, particularly resource extraction. As Borneo also attracts many tourists who visit its endemic wildlife (King et al., 2013), it is important to study the prevalence and diversity of zoonotic parasites in local primate species, that may act as reservoirs for human infections.

The folivorous proboscis monkey, *Nasalis larvatus*, is a colobine Old World monkey endemic to Borneo. Classified as Endangered EN A2cd since 2000 (EN: considered to be facing a very high risk of extinction in the wild, A: through a reduction in population size, 2: of ≥50% over the last 10 years or three generations, based on c: a decline in area of occupancy, extent of occurrence and/or quality of habitat and d: actual or potential levels of exploitation) (IUCN, 2016), it acts as a primate flagship species for conservation in Sabah, Malaysian Borneo (Goossens and Ambu, 2012) attracting many tourists (Leasor and Macgregor, 2014). Priority areas of proboscis monkey populations exist near the coast up to the headwaters of major rivers in all provinces of Borneo. Groups have also been recorded in disturbed habitats of secondary forest near human settlements and remnant tidal forest close to agricultural land use (Sha et al., 2008).

To date, data are available from necropsy of a single individual (Hasegawa et al., 2003) and from fecal examinations of captured animals (Hernasari, 2011) and wild proboscis monkeys (Ranglack and Yeager, 1986; Salgado Lynn, 2010). Overall, infection rates were high (62–96% overall prevalence) and infections with soil-transmitted helminth species were most common (Ranglack and Yeager, 1986; Salgado Lynn, 2010; Hernasari, 2011). Salgado Lynn (2010) pointed out that almost 90% of proboscis monkeys from different forest lots of the Lower Kinabatangan Wildlife Sanctuary (LKWS) in Sabah, Malaysian Borneo, were co-infected with multiple parasite species. Analyses of these concomitant parasite infections are not available but are urgently required for drawing conclusions regarding transmission dynamics, as well as for identifying whether this host may act as a potential super-spreader within primate populations.

The present study aimed to examine gastrointestinal parasite co-infection patterns via quantitative analyses in wild proboscis monkeys for the first time. Based on previous findings in 2007/2008 (Salgado Lynn, 2010), in 2012 we conducted a follow-up study to record the infection status of wild proboscis monkey groups after five years. Special attention was given to potentially zoonotic parasite species. We predicted that 1.) infection rates in wild proboscis monkeys would remain high, and 2.) interactions among intestinal parasite species do not exist, but co-infection patterns in wild proboscis monkeys were shaped by the frequency of the occurrence of intestinal parasite species. Used as a baseline for further investigations in different proboscis monkey habitats, this study could support conservation actions for subpopulations of this tropical flagship species in Borneo. Furthermore, it contributes to assessing the poly-parasite infection risk originating from these primate populations to nearby villagers by identifying potential connections to human disease in the region.

2. Material and methods

2.1. Study site

The Malaysian state of Sabah is located at the North-Eastern tip of Borneo (Fig. 1). The climate throughout the island is tropical with small variations in temperature, usually between 21° and 34° Celsius with annual precipitation being around 3000 mm (Ancrenaz et al., 2004). Over the past century most parts of the dry lowland forests have suffered from human agricultural activities and have been logged for oil palm plantations. In 2005, the State Government declared 27,000 ha of highly disturbed forests along the floodplain of the Lower Kinabatangan River (5°10′–5°50′N; 117°40′–118°30′E) a wildlife sanctuary (Goossens et al., 2005). Divided into 10 riparian forest blocks, called ‘Lots’, the sanctuary forms a corridor of different forest types including mangrove and riverine forest surrounded by oil palm plantations. Eco-tourism and wildlife tours are popular along the Kinabatangan (Leasor and Macgregor, 2014). Furthermore, Sabah includes many fishing communities along the coastline and river mouths. The majority of the human settlements in rural areas are closely associated with river systems and coincide with major concentrations of proboscis monkeys (Sha et al., 2008).

Lot 6 of the LKWS was the specific sampling area for the present study. Starting from the Danau Girang Field Centre located in Lot 6, sample collection took place 6.1 km upstream (3.7 km linear distance) and 4.7 km downstream (3.0 km linear distance) along the southern bank of the river (Fig. 1).

2.2. Study species

River surveys along the majority of Lots of the LKWS counted 1454 proboscis monkeys in 101 groups in 2008 (Sha et al., 2008). In 2010, 818 individuals in 113 groups with group encounter rates about 1 group/km on average were observed along 30 km of the Kinabatangan River and a nearby tributary of Lot 6 (Stark et al., 2012), which represents around one-fifth of the estimated population in Sabah.

Proboscis monkeys are sexually dimorphic and the largest foregut-fermenting colobines, with males being about twice as heavy (20 kg body mass on average) as females (10 kg body mass on average) (Bennett and Sebastian, 1988). Their social organization consists of either harems (one male-multi female-groups with their offspring) or bachelor (all male) groups of up to 30 individuals, which are closely associated with waterways and forage about 1 km each day before returning to their sleeping sites at the riverbank in the evenings (Boonratana, 2000). Home range areas of groups were estimated to be 80 ha on average (Stark et al., 2017) and can extensively overlap each other (Yeager, 1989).

2.3. Sample collection

A total of 652 fecal samples were collected from June to