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The microbiome of *Haemaphysalis lemuris* (Acari: Ixodidae), a possible vector of pathogens of endangered lemur species in Madagascar

Paula Lado[a,*], Barbara Qurollo[b], Cathy Williams[c], Randall Junge[d], Hans Klompen[a]

[a] Department of Evolution, Ecology and Organismal Biology, The Ohio State University, 1315 Kinnear Rd, Columbus, OH 43210, USA
[b] Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 1060 William Moore Drive, Raleigh, NC 27607, USA
[c] Duke Lemur Center, 3705 Erwin Rd., Durham, NC 27705, USA
[d] Department of Animal Health, 9990 Riverside Drive, Columbus Zoo and Aquarium, Powell, OH 43065, USA

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ABSTRACT

Lemurs are primate species that are endemic to Madagascar. At present, about 90% of lemur species are endangered, and 5 species are among the 25 most endangered primates worldwide. Health status is a major factor impacting the viability of wild populations of many endangered species including lemurs. Given this context, we analyzed the microbiome of 24 specimens of *Haemaphysalis lemuris,* the most common tick parasitizing lemurs in their native habitats. Ticks were collected from 6 lemur species and microbiomes analyzed using next-generation sequencing. Our results show that the *H. lemuris* microbiome is highly diverse, including over 500 taxa, 267 of which were identified to genus level. Analysis of the microbiome also shows that there is a distinct "host" (lemur species) component when explaining the differences among and between microbial communities of *H. lemuris*. This "host" component seems to overwhelm any "locality" (geographic origin of the sample) component. In addition to the microbiome data, targeted PCR was used to test for the presence of three pathogens recently detected in the blood of wild lemurs: *Borrelia* sp., *Candidatus* Neoehrlichia sp., and *Babesia* sp. Overall, the presence of DNA of *Rickettsia* spp., *Bartonella* spp., *Francisella* spp., and a *Babesia* sp., in *H. lemuris*, is consistent with the hypothesis that these ectoparasites may act as vector for these pathogens. Further studies assessing vector competence are needed to confirm this hypothesis.

1. Introduction

Madagascar is a top priority for conservation (Myers et al., 2000) in part because lemurs constitute approximately 15% of global primate species diversity (Wilme et al., 2006; Mittermeier, 2008). About 90% of lemur species are endangered (Bublitz et al., 2015), and 5 of them are included in the list of the 25 most endangered primates worldwide (IUCN, 2014-16; Schwitzer et al., 2015). Lemur survival in Madagascar is threatened by a rapidly growing human population, increased deforestation for agriculture and cattle grazing, hunting for bush meat, and likely, climate change (Allnutt et al., 2008; Barrett et al., 2013; Dufils, 2003; Harper et al., 2007). The ability of lemurs to survive in disturbed areas varies by species. Those that can survive in secondary forests tend to be more susceptible to disease and experience physiological alterations compared to the cohorts occupying more pristine forests (Junge et al., 2011). This is concerning because habitat destruction and natural resource extraction have contributed to a nearly 80% reduction of primary forests in Madagascar (Harper et al., 2007). With respect to climate change, temperatures in Madagascar have risen, and rainfall patterns have changed over recent years (Tadross et al., 2008). These changes are likely to alter spatial patterns of disease, especially those caused by pathogens transmitted by non-permanent parasites −such us ticks- which are impacted by environmental conditions such as temperature and humidity (Brooker et al., 2007; Guernier et al., 2004).

Ectoparasites are widespread across lemur species, and even though they are often not highly pathogenic, they can decrease fitness and cause damage to the skin (Bischoff et al., 2009; Junge et al., 2011; Wall, 2007). *Haemaphysalis lemuris* Hoogstraal is an ixodid tick that commonly parasitizes wild lemurs and can have a negative effect on lemur body mass (Barrett et al., 2013; Koyama et al., 2008; Rodriguez et al., 2015). In addition, ticks can vector a variety of pathogenic bacteria, protozoa, and viruses (Williams et al., 2002; Springer et al., 2015). Larsen et al. (2016) used high-throughput sequencing methods to identify pathogenic microorganisms in lemur blood from wild lemurs, and demonstrated the presence of three pathogens that might be

* Corresponding author.
E-mail addresses: ladohenaise.1@osu.edu (P. Lado), baquroll@ncsu.edu (B. Qurollo), cathy.williams@duke.edu (C. Williams), randy.junge@columbuszoo.org (R. Junge), klompen.1@osu.edu (H. Klompen).

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Table 1
General information of *Haemaphysalis lemuris* samples used in this study. All specimens are from Madagascar. Each row corresponds to an individual tick specimen and the columns to the collection and processing information. (Washed: surface sterilized individuals; DNA extraction: individuals whose DNA yield was enough to proceed with the analyses).

TickID	HostID	Host	Col. Date	Tick sex	Washed	DNA Extraction
		Ambotavy, Toamasina, 18.8298S 48.3123E				
OSAL 0102427	OSAL 0102427	*Avahi laniger*	18-Jan-2013	M	Yes	yes
OSAL 0102396	OSAL 0102396	*A. laniger*	24-Jan-2013	M	Yes	yes
OSAL 0099986	OSAL 0099986	*A. laniger*	16-Aug-2013	M	Yes	yes
OSAL 0099967	OSAL 0099967	*A. laniger*	23-Aug-2013	M	Yes	yes
OSAL 0102415	OSAL 0102415	*Propithecus diadema*	19-Jan-2013	M	No	yes
OSAL 0102416B	OSAL 0102416	*P. diadema*	19-Jan-2013	M	No	no
OSAL 0099775	OSAL 0099775	*P. diadema*	Jan-2013	M	No	yes
OSAL 0099950	OSAL 0099950	*P. diadema*	20-Aug-2013	M	No	yes
OSAL 0099964A	OSAL 0099964	*P. diadema*	20-Aug-2013	M	No	no
OSAL 0099964B	OSAL 0099964	*P. diadema*	20-Aug-2013	M	No	yes
OSAL 0099778	OSAL 0099778	*P. diadema*	1-Sep-2014	M	No	yes
OSAL 0099784A	OSAL 0099784	*P. diadema*	5-Sep-2014	M	No	yes
OSAL 0099785	OSAL 0099785	*P. diadema*	5-Sep-2014	M	No	no
OSAL 0099791	OSAL 0099791	*P. diadema*	9-Sep-2014	M	No	no
OSAL 0099783	OSAL 0099783	*P. diadema*	10-Sep-2014	M	No	yes
OSAL 0102404	OSAL 0102404	*Lepilemur mustelinus*	18-Jan-2013	F	Yes	yes
OSAL 0102409	OSAL 0102409	*L. mustelinus*	21-Jan-2013	M	Yes	yes
OSAL 0099985	OSAL 0099985	*L. mustelinus*	17-Aug-2013	M	Yes	yes
OSAL 0099999	OSAL 0099999	*L. mustelinus*	17-Aug-2013	M	Yes	yes
OSAL 0099954	OSAL 0099954	*L. mustelinus*	23-Aug-2013	M	Yes	yes
		Betampona, Toamasina, 17.9000S 49.2167E				
OSAL 0005974A	OSAL 0005974	*P. diadema*	Jun-2003	M	No	yes
OSAL 0005974B	OSAL 0005974	*P. diadema*	Jun-2003	M	No	yes
OSAL 0005974C	OSAL 0005974	*P. diadema*	Jun-2003	M	No	yes
OSAL 0005974D	OSAL 0005974	*P. diadema*	Jun-2003	M	No	yes
		Kianjavato, 21.3833S 47.8667E				
OSAL 0084988	OSAL 0084988	*Prolemur simus*	25-Nov-2009	F	Yes	yes
OSAL 084989A	OSAL 084989A	*Prolemur simus*	25-Nov-2009	F	Yes	yes
		Analamazoatra Nat. Pk., 18.4869S 48.3481E				
OSAL 0104421	OSAL 0104421	*Indri indri*	2009	M	Yes	yes
		Mahajanga, 16.0000S 45.7167E				
OSAL 0006009	OSAL 0006009	*Propithecus verreauxi*	Oct-2003	N	No	yes

transmitted by ticks: *Borrelia* sp., *Candidatus* Neoehrlichia sp., and *Babesia* sp. However, there is no hard evidence for which species of ticks is/are acting as vectors.

Aiming to bridge the gap in knowledge of how pathogens are transmitted to lemurs, we explored the microbial community of *H. lemuris*. *Haemaphysalis lemuris* has been recorded from at least 9 different lemur species collected across Madagascar (Durden et al., 2010; Hoogstraal and Theiler, 1959; Junge and Louis, 2005; Klompen et al., 2015; Rodriguez et al., 2015; Uilenberg et al., 1979).

The present study aimed to explore the entire microbiome of *H. lemuris* including pathogens. Non-pathogenic organisms in tick microbiomes may influence pathogen susceptibility (Gall et al., 2016) and thus a better understanding of the entire microbiome may ultimately provide a better understanding of the role of *H. lemuris* in lemur disease dynamics. The questions addressed in this study are: 1) to get a first estimate of microbiome structure and composition 2) to what degree is the microbiome of *H. lemuris* influenced by host species or locality (environmental effects), and 3) whether pathogens demonstrated in lemurs are also present in *H. lemuris*. To do so, we analyze the microbiome of *H. lemuris* using next-generation sequencing and PCR.

2. Material and methods

2.1. Tick samples

As part of an ongoing, long-term project monitoring lemur health in Madagascar, a variety of ectoparasites have been opportunistically collected and preserved in 95% ethanol. Lemurs were examined under Research permit #200/12/MEF/SG/DGF/DCB SAP/SCB, issued by the Secretary General, Department of Water and Forests, Republic of Madagascar. All animals underwent medical evaluations following the standard protocol used by the Prosimian Biomedical Survey Project while under anesthesia (see Junge et al., 2011).

For this study, we analyzed 28 ticks collected from different localities and from different lemur species (Table 1; Fig. 1). Notes on tick ecology and host distribution based on this study are presented in Klompen et al. (2015). Ticks were morphologically identified using published taxonomic keys (Hoogstraal, 1953). All ticks used herein were identified as *Haemaphysalis lemuris*. Of the 28 ticks analyzed, 27 were adults (3 females and 24 males), and one was a nymph. We chose the least engorged ticks for this study to increase our chances of detecting microorganisms from the tick itself, instead of from host blood. Ticks were analyzed individually.

2.2. DNA extraction

Before DNA extraction some of the specimens were surface sterilized (to allow comparison between surface sterilized ticks and non-sterilized) using the following protocol twice: 1 min in commercial bleach, washed in distilled water, and 1 min in 95% ethanol (see Table 1 for details). Surface sterilization minimizes “contamination” by microorganisms present on the surface of the ticks. Individual DNA extractions were performed using the QIAgen Blood and Tissue kit following the manufacturers’ instructions, with the following exception: while in ATL buffer a portion of the ticks idiosoma was cut with a scalpel and the entire specimen cuticle was recovered to serve as voucher. Cuticles were individually barcoded and data based. All voucher collection data are available on-line through the Ohio State University Acarology