Investigating the presence of mycobacterial pathogens in New World primates

TP Honap[1], G Housman[2], G Erkenswick[3], J Malukiewicz[2,4], V Boere[4], LC Machado-Pereira[5], AD Grativol[6], CR Ruiz-Miranda[6], I Silva[7], M Watsa[8], and AC Stone[2]

[1]School of Life Sciences, Arizona State University | [2]School of Human Evolution and Social Change, Arizona State University | [3]Department of Biology, University of Missouri St. Louis | [4]Departamento de Bioquimica e Biologia Molecular, Universidade Federal de Viçosa, Brazil | [5]Centro de Conservação e Manejo de Fauna da Caatinga, Universidade Federal do Vale do São Francisco, Brazil | [6]Laboratório de Ciências Ambientais, Universidade Estadual do Norte Fluminense, Brazil | [7]Departamento de Biologia Animal, Universidade de Viçosa, Brazil | [8]Department of Anthropology, Washington University in St. Louis

Background

Increased human encroachment of wildlife habitats has resulted in humans living in close contact with wild animals such as nonhuman primates[1]. The close evolutionary relationships among different primate species increases the ease of disease transmission. Disease transmission from humans to nonhuman primates can lead to a decline in nonhuman primate populations and hamper conservation efforts[2,3]. Nonhuman primates may also serve as a reservoir for pathogens, leading to diseases in humans[4,5].

Tuberculosis and leprosy are caused by the *Mycobacterium tuberculosis* complex (MTBC) and *M. leprae*, respectively. Members of the MTBC can be transmitted bidirectionally among humans and several other mammalian species including nonhuman primates. In tuberculosis-endemic countries such as Peru and Brazil, MTBC infection as well as tuberculosis disease has been reported in captive nonhuman primates[6,7]. In contrast, *M. leprae* primarily infects humans and nine-banded armadillos. Natural leprosy has been observed in certain nonhuman primates including cynomolgus macaques, chimpanzees, and sooty mangabeys, suggesting that *M. leprae* may have nonhuman primate reservoirs[8-13].

Objective

To screen wild nonhuman primate populations form tuberculosis and leprosy-endemic countries for the presence of pathogens such as the *Mycobacterium tuberculosis* complex (MTBC), *M. leprae*, and closely related bacteria. In this study, we present our results for a screening of wild *Callithrix* populations (marmosets) from Brazil and *Saguinus* populations (tamarins) from the Peruvian Amazon.

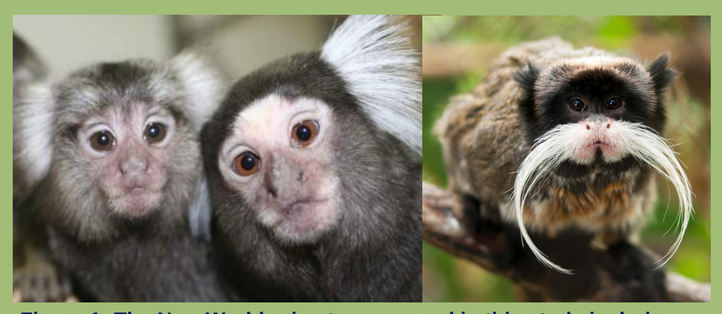
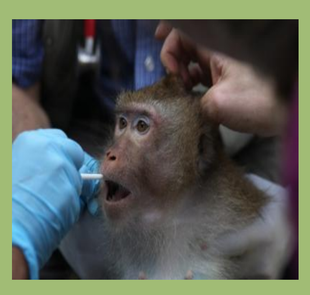
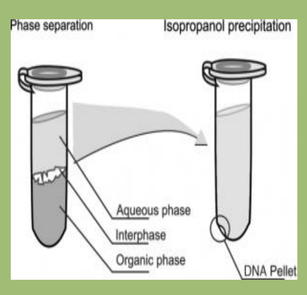


Figure 1: The New World primates screened in this study include marmosets (left) and tamarins (right).

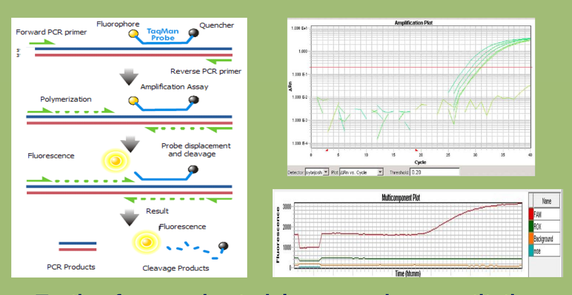
Methods



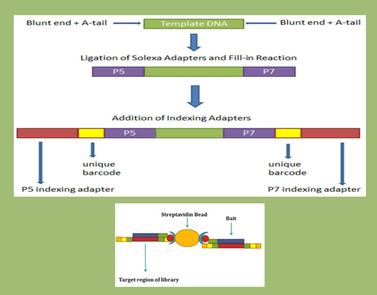
Collection of buccal swabs from nonhuman primates



DNA extraction using phenol-chloroform[13]



Testing for mycobacterial genes using quantitative polymerase chain reaction (qPCR) assays[15-17]



Library preparation and targeted enrichment for MTBC genes[18,19]

Illumina sequencing and data analyses[20,21]

Results

qPCR: 14 marmoset and 8 tamarin samples tested positive for the rpoB1 qPCR assay. None of the samples were positive for the MTBC-specific assays rpoB2 and IS6110 as well as the *M. leprae*-specific assays 85B and RLEP.

Targeted Enrichment: 8 out of 14 positive marmoset samples were target-enriched and sequenced for the mycobacterial genes *rpoB*, *gyrA*, *gyrB*, *katG*, and *mtp40*. None of the samples contained reads mapping to the *M. tuberculosis* H37Rv genome, but several reads were assigned to the genus *Mycobacterium.*

Nonhuman primates	Location	Samples tested	No. of samples positive for qPCRs targeting mycobacterial genes				
			rpoB1	rpoB2	IS6110	85B	RLEP
Marmosets	Brazil	98	14	0	0	0	0
Tamarins	Peru	50	8	0	0	0	Not tested

Discussion

A total of 14 marmoset and 8 tamarin samples were positive for the rpoB1 qPCR assay, which targets the mycobacterial *rpoB* gene. However, all samples were negative for the rpoB2 assay, which targets a region of the *rpoB* gene specific to the MTBC, as well as for the IS6110 assay targeting the MTBC-specific multi-copy insertion element[15]. In addition, all of the samples were negative for the 85B and RLEP assays, which target genes specific to *M. leprae*[16,17].

Absence of the MTBC in a subset of the qPCR-positive samples was confirmed by targeted enrichment and sequencing, suggesting that the marmosets may contain a member of the genus *Mycobacterium* distantly related to the MTBC. Due to low sequence coverage obtained, the mycobacterial species present could not be identified. Overall, our study suggests that wild New Word primates in tuberculosis- and leprosy-endemic countries may harbor mycobacterial species.

Future Work

M. lepromatosis is a newly-recognized bacterial species that causes a severe form of leprosy and is endemic in Mexico and the Caribbean[22]. We plan to design a qPCR assay that targets the *hsp65* gene of this bacterium, and subsequently, test the New World primate populations using this assay as well.

Acknowledgements

We would like to thank Katherine Skerry for carrying out the tamarin DNA extractions. The marmoset work was funded by NSF BCS-1063939, Fulbright Fellowship, NSF DDRIG, IPS Grant, and the Graduate and Professional Student Association (GPSA) Research Grant to J. Malukiewicz. The tamarin work is funded by a GPSA Research Grant to T. Honap. We also thank GPSA for providing funding to attend this conference. References for this poster are available upon request.