

Investigating the presence of mycobacterial pathogens in New World primates

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Background

Increased human encroachment of wildlife habitats has resulted in humans living in close contact with wild animals such as nonhuman primates¹. 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 reservoirs⁸⁻¹³.

Objective

To screen wild nonhuman primate populations from 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.

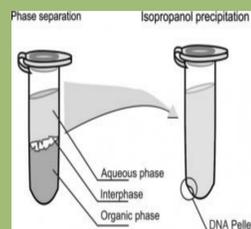


Figure 1: The New World primates screened in this study include marmosets (left) and tamarins (right).

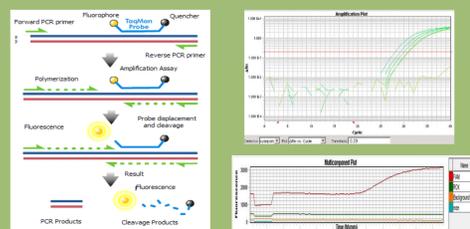
Methods



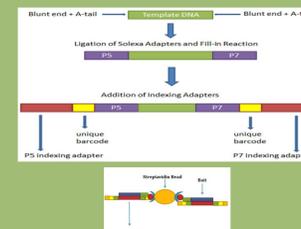
Collection of buccal swabs from nonhuman primates



DNA extraction using phenol-chloroform¹³



Testing for mycobacterial genes using quantitative polymerase chain reaction (qPCR) assays¹⁵⁻¹⁷



Library preparation and targeted enrichment for MTBC genes^{18,19}



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ATGGCATTGCAA
AGATGGATTG
GATGGCATTGCAA
GCATTGCAATTTGAC
ATGGCATTGCAATTT
AGATGGATTGCAATTTG
Consensus Sequence
AGATGGCATTGCAATTTGAC
    
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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				
			<i>rpoB1</i>	<i>rpoB2</i>	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 element¹⁵. 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 World 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 Caribbean²². 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

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