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 efforts2,3. Nonhuman primates may also serve as a reservoir for pathogens, leading to diseases in humans4,5.

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 primates6,7. 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 for 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.

Methods
Collection of buccal swabs from nonhuman primates
DNA extraction using phenol-chloroform
Testing for mycobacterial genes using quantitative polymerase chain reaction (qPCR) assays
Library preparation and targeted enrichment for MTBC genes
Illumina sequencing and data analysis

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.

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. leprae16,17.

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
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, FPS 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.