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Original Contribution

Detection of *Mycobacterium tuberculosis* Complex in New World Monkeys in Peru

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Abstract: The *Mycobacterium tuberculosis* complex causes tuberculosis in humans and nonhuman primates and is a global public health concern. Standard diagnostics rely upon host immune responses to detect infection in nonhuman primates and lack sensitivity and specificity across the spectrum of mycobacterial infection in these species. We have previously shown that the Oral Swab PCR (OSP) assay, a direct pathogen detection method, can identify the presence of *M. tuberculosis* complex in laboratory and free-ranging Old World monkeys. Addressing the current limitations in tuberculosis diagnostics in primates, including sample acquisition and pathogen detection, this paper furthers our understanding of the presence of the tuberculosis-causing bacteria among New World monkeys in close contact with humans. Here we use the minimally invasive OSP assay, which includes buccal swab collection followed by amplification of the IS*6110* repetitive nucleic acid sequence specific to *M. tuberculosis* complex subspecies, to detect the bacteria in the mouths of Peruvian New World monkeys. A total of 220 buccal swabs from 16 species were obtained and positive amplification of the IS*6110* sequence was observed in 30 (13.6%) of the samples. To our knowledge, this is the first documentation of *M. tuberculosis* complex DNA in a diverse sample of Peruvian Neotropical primates.

Keywords: tuberculosis, *Mycobacterium tuberculosis* complex, new world monkey, nonhuman primate, Peru, direct pathogen detection, oral swab PCR

INTRODUCTION

The *Mycobacterium tuberculosis* complex (MTBC) is made up of the subspecies *M. tuberculosis, M. africanum, M. bovis, M. canettii*, and *M. microti*, among others, and is responsible for causing the infectious disease tuberculosis in humans and

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other mammalian species. Tuberculosis (TB) is one of the leading causes of death globally, with an estimated 8.6 million new cases and 1.3 million deaths in 2012 (WHO 2013). Peru has historically been listed as one of the 22 countries with highest incidence of TB (Dye et al. 1999). Additionally, TB is currently a significant Peruvian public health concern, with 11,200 new cases and over 12,500 documented cases recorded in humans in 2013 (Bonilla Asalde et al. 2006; Ministerio de Salud and Gobierno del Peru 2013).

In both captive and free-ranging nonhuman primates (NHP), TB caused by *M. tuberculosis*, *M. bovis*, and *M. africanum* has been reported for over a century (Michel and Huchzermeyer 1998; Michel et al. 2003; Alfonso et al. 2004; Rocha et al. 2011; Engel et al.; 2012). Presumed contexts of transmission have included exposure to infected humans (Lerche et al. 2008) and/or contact with infected domestic animals (Sapolsky and Else 1987), zoo animals, or wildlife (Parsons et al. 2009; Michel et al. 2013; Stephens et al. 2013). As the pathology of TB in NHP is diverse and still incompletely characterized, it is likely that NHP infection with TB has been underreported (Capuano et al. 2003; Flynn et al. 2003; Gormus et al. 2004; Wilbur et al. 2012a). Recently, Coscolla et al. (2013) identified a chimpanzee infected with a strain of MTBC that clustered within the larger clade that includes ‘‘animal-associated’’ strains (e.g., *M. bovis*), but is more closely related to ‘‘human-associated’’ lineage and may represent a chimpanzee-specific Mycobacterium (Coscolla et al. 2013). The observed distribution of MTBC infection in Primates from all continents suggests that New World monkeys (NWM) in South America may also harbor the mycobacterial infection. However, TB has been thought to be uncommon in South American NWM (Montali et al. 2001), although this is likely due to limited investigation and documentation. Molecular techniques such as Mtp40 PCR (Alfonso et al. 2004) and spoligotyping (Barragán and Brieva 2005) have been used to detect MTBC DNA in zoo and sanctuary NWM in Colombia, and active TB was recently reported in a captive red-faced spider monkey (*Ateles paniscus*) in a Brazilian zoo (Rocha et al. 2011).

The probability-estimates for incidence and prevalence of TB in captive and free-ranging NWM are unknown, largely because methods commonly used to detect MTBC bacteria and diagnose disease, such as the intradermal tuberculin skin test (TST), are inadequate, presenting a major challenge to both laboratory and field research (Engel et al. 2013; Lerche et al. 2008; Wilbur et al. 2012a). The TST requires an intradermal injection in the eyelid of NHP followed by a 3-day period of observation for evidence of an immune response that is logistically cumbersome; in addition, results of this form of testing are unreliable—particularly in NHP that lacks an intact immune system (Vervenne et al. 2004; Lerche et al. 2008; Engel et al. 2012). Serological assays for detecting TB remain insensitive and nonspecific throughout the spectrum of disease and are particularly troublesome when applied to species of NHP that are not consistently used in biomedical research, as available serological assays were designed and tested on only a few laboratory macaques species(Lerche et al. 2008). Identification of infectious organisms in post-mortem specimens of affected tissue is often used to confirm diagnosis of TB, but does not aid in early detection of active disease or latent infections (Lin et al. 2009).

Acknowledging the limitations of commonly employed methodology, direct detection of the mycobacterial pathogen holds promise as an additional diagnostic assay and is gaining attention and application in the zoological setting (Montali et al. 2001; Maas et al. 2013). Biological samples, including gastric lavage, saliva, feces, broncho-alveolar lavage (Flynn et al. 2003), or sputum from elephant trunk washes or swabs (Mikota et al. 2001; Ong et al. 2013) have been used to isolate and detect MTBC DNA in a variety of wildlife including elephants, deer, buffalo, and meerkats. Used in combination with serological assays, molecular tools may aid in differentiating active from latent infections (Mikota et al. 2001; Maas et al. 2013).

Polymerase chain reaction amplification of the IS*6110* repetitive nucleic acid sequence, which is specific to MTBC subspecies, has recently been employed to detect the presence of MTBC DNA in buccal swabs from laboratory pigtailed macaques and free-ranging macaques in Asia (Engel et al. 2012; Wilbur et al. 2012b). Amplification of the IS*6110* sequence is both sensitive and specific for the detection of MTBC DNA (Wood et al. in revision; Eisenach 1994), and buccal swab collection may overcome logistical barriers associated with use and interpretation of TST, radiographs and physical examinations in NHP.

The Peruvian Amazon, one of the richest regions for NHP diversity in the world, supports at least 32 distinct species of NWM, many of which are listed as threatened or endangered (Bennett et al. 2001; Hopkins and Nunn 2007). Human population growth and economic development in Peru is increasing human-NWM interactions through habitat encroachment, hunting, wildlife trade, as well as through access to zoos and sanctuaries (Shanee 2011). Well over 28,000 NWM are trafficked annually across the Peru-Colombia border (Brown 2004; Nijman et al. 2011). Hunting and trapping of rainforest NWM for the purposes

of food sources, medicinal use, as bait for predatory wildlife, for ornamentation or tool-creation, for family pets, for sport, for exportation, and crop-raiding has long been documented by early naturalists (Mittermeier 1987; Smith 1978; Peres 1990; Shanee et al. 2012). In a country with a long history of a rich human-NWM interface and endemic TB in human populations (Dye et al. 1999; Pelly et al. 2004; Smith-Nonini 2005; WHO 2013), characterizing and documenting the prevalence of MTBC in NWM with close contact to humans is the first step toward understanding how this complex social interaction may affect MTBC transmission and primate conservation.

Addressing current shortcomings in TB diagnostics in NHP—including sample acquisition, subjective interpretation of TST reactivity and pathogen detection—our research sought to further our understanding of MTBC presence among NWM in close contact with humans in Peru using a minimally invasive buccal swab collection technique. As MTBC is thought to be predominately a respiratory pathogen, we hypothesized that DNA from MTBC would be present in the oral cavities of NWM and could be detected by amplification of the IS*6110* repetitive nucleic acid sequence using a previously described Oral Swab PCR assay (OSP) (Engel et al. 2012; Wilbur et al. 2012b). To our knowledge, this is the first documentation of MTBC in Peruvian NWM.

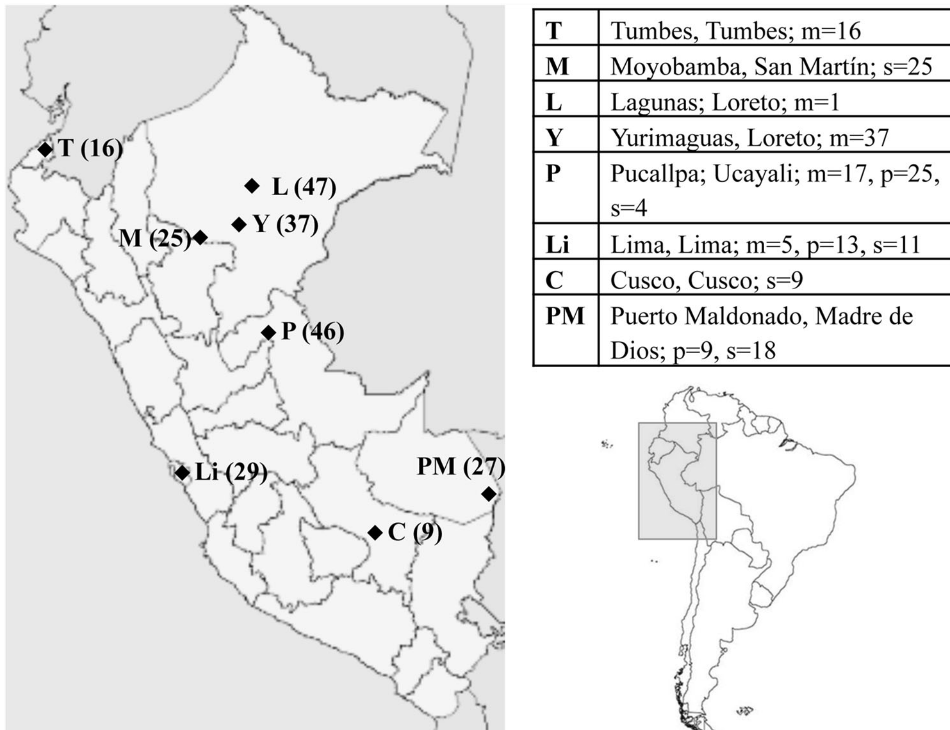
Methods

Study Design, Setting, and Sampling

Between December 2010 and April 2012, this study conducted cross-sectional convenience sampling of NWM. This methodology allowed us to maximize data collection from NWM in close contact with humans.

New World monkeys in the following contexts were sampled: markets, pets, and sanctuaries/zoos throughout Peru (Fig. 1). Market animals represent NWM that were captured from the wild and sold in the city market as pets, tourist attractions, or for export. These animals were sampled in the Pucallpa and Tumbes markets in Peru (Fig. 1).

New World monkeys from the pet context represent animals that were currently or had recently been living in a house with human caregivers. Pet NWM were sampled from Pucallpa, Lima, and Puerto Maldonado (Fig. 1). New World monkeys sampled from the sanctuary/zoo context were living in a sanctuary or zoo at the time of sampling. These animals were typically housed in large, at times multi-species groups, and were often former market, pet, and confiscation animals. We sampled NWM from 7 different sanctuary/zoo facilities located in Lagunas, Moyobamba, Pucallpa, Lima, Puerto Maldonado, and Cusco.



T	Tumbes, Tumbes; m=16
M	Moyobamba, San Martín; s=25
L	Lagunas; Loreto; m=1
Y	Yurimaguas, Loreto; m=37
P	Pucallpa; Ucayali; m=17, p=25, s=4
Li	Lima, Lima; m=5, p=13, s=11
C	Cusco, Cusco; s=9
PM	Puerto Maldonado, Madre de Dios; p=9, s=18

Figure 1. Geographic distribution of New World monkeys sampled and tested for *Mycobacterium tuberculosis* Complex using the direct detection oral swab PCR (OSP) assay in Peru. *Inset table* shows the distribution of NWM sampled from each context (*m* Market, *p* Pet, *s* Sanctuary/Zoo) at each geographic location. The total number of animals sampled at a site is shown in *parentheses*.

Multiple contexts were often present in a geographic sampling area (Fig. 1).

These research protocols were reviewed and approved by the University of Washington Institutional Animal Care and Research Committee (4233-01) and the Navy Medical Research Unit no. 6 Institutional Animal Care and Use Committee (NAMRU6 11-06). This research adhered to the legal requirements of Peru and biological samples were collected and processed in compliance with a Peruvian collection and access to genetic materials permits (Resolución Directoral N° 0016-2014-MINAGRI-DGFFS/DGEFFS), and shipped internationally under a Convention on International Trade in Endangered Species (CITES) permit.

Oral Swab PCR (OSP)

Buccal swabs were obtained from NWM according to a protocol previously described by Jones-Engel et al. (2007). Briefly, the buccal mucosa was gently brushed using a sterile cytology swab (Omniswab no. WB10-004, Whatman) to collect saliva and buccal cells. The swab was then placed in a 2-ml cryo-vial containing 0.5 ml of sterile lysis buffer (50 nM Tris pH 8.0, 50 mM EDTA, 50 mM sucrose, 100 mM NaCL, and 1% SDS) and stored at room temperature.

DNA Extraction

DNA extraction was performed using a standard phenol–chloroform extraction procedure in a pre-PCR laboratory (Sambrook and Russell, 2001). Prevention of cross-contamination and contamination from positive controls was achieved by adhering to the following precautionary measures: (1) Use of pre-PCR and post-PCR laboratories with unidirectional flow from pre- to post-PCR laboratories; (2) reagents were aseptically aliquoted into smaller project-dedicated containers and tested for purity prior to use; (3) hoods were cleaned with bleach solution and RNase followed by UV irradiation between each extraction and PCR-preparation; and (4) a 50 ul aliquot of sterile lysis buffer was used as a negative extraction control for each extraction performed.

Following extraction, DNA quantification for each sample was performed using mass spectrometry, and samples containing <1 ng/ul of DNA were discarded. Samples containing >100 ng/ul of DNA were diluted to a working concentration of 50 ng/ul prior to DNA amplification.

Amplification of NWM Mitochondrial DNA

To ensure DNA was present and that inhibition by plant material or other foreign material was not occurring, a subset of samples were tested for the presence of a 101 base pair fragment of the primate mitochondrial genome present in all members of the Order Primates (Poinar et al. 2001).

Amplification of the IS*6110* Sequence

A 130 base pair fragment from the IS*6110* MTBC-specific repetitive element was amplified by following Inoue et al. (2011). Reactions were set up on 96 well plates in a pre-PCR laboratory. Each sample was run in triplicate, and three blanks lacking DNA were included on each plate to detect low-level contamination of reagents. Buccal swab DNA from a human who tested TST negative was used as a negative control. All wells were capped using an adhesive covering. Positive controls for *M. tuberculosis* H37Rv and *M. bovis* BCG were added to the plate immediately prior to thermal cycling in a post-PCR laboratory by removing only the section of adhesive that was covering the positive wells. Wells containing sample DNA, negative control DNA, and blanks were not opened in the post-PCR laboratory prior to amplification. Amplification using real-time PCR was performed in a post-PCR laboratory as previously described (Engel et al. 2012; Wilbur et al. 2012b).

The following criteria were used to define an IS*6110*-positive sample: (1) amplified in at least 1 of 3 wells, and (2) high resolution melt peak within ± 0.5 °F of the MTBC-positive controls, and (3) amplified ≥ 100 d(RFU)/dt. Amplicons fitting these criteria were direct sequenced and confirmed prior to this study as IS*6110* (Wilbur et al. 2012b). An additional two samples from this study were direct sequenced to ensure the amplicon was indeed IS*6110*.

Statistical Methods

To assess bivariate relationships between predictor variables (context, taxonomic family, age, sex) and primary outcome (OSP status) Fishers exact test was used. Multivariate adjusted logistic regression models assessed the associations with OSP status among a subset of 184 animals. Given that all NHP <13 months of age and belonging to taxonomic families other than Atelidae and Cebidae were OSP negative (n = 36), they were excluded from the model. All analyses were conducted in Stata 12.0 (StataCorp, College

Station, TX), with an alpha level of less than 0.05 being considered significant.

Results

A total of 220 buccal swabs representing 5 taxonomic families, 10 genera, and 16 species were obtained from NWM in Peru, the great majority of which belonged to the taxonomic families Atelidae (n = 97) and Cebidae (n = 113) (Fig. 2). Sampled NWM came from 8 geographic locations representing 3 different contexts (Fig. 1). Sex was equally distributed and the majority (191/220, 86.8%) of the samples were from animals >12 months of age (Table 1).

Three of 61 samples (5%) with mass spectrometry numbers >100 d(RFU)/dt did not amplify using the NHP mitochondrial DNA primers. The purpose of amplifying for NHP mitochondrial DNA was to ensure that the overwhelming majority of samples contained NHP DNA. Since we were only able to amplify a subset of the samples with the NHP mitochondrial primers, we included the samples that were negative for NHP mitochondrial DNA in further laboratory processing. These three samples that did not amplify with the NHP mitochondrial DNA primers were OSP negative.

Positive amplification of the IS*6110* sequence (OSP+) was observed in 30 (30/220, 13.6%) of the NWM sampled in this study (Table 1) indicating that MTBC bacteria were present in the mouths of NWM at the time of sampling. One of the two OSP+ samples that were direct sequenced was identical to the target IS*6110* sequence from the NCBI Reference Sequence NC_000962.2. The second sample had insufficient DNA and could not be sequenced.

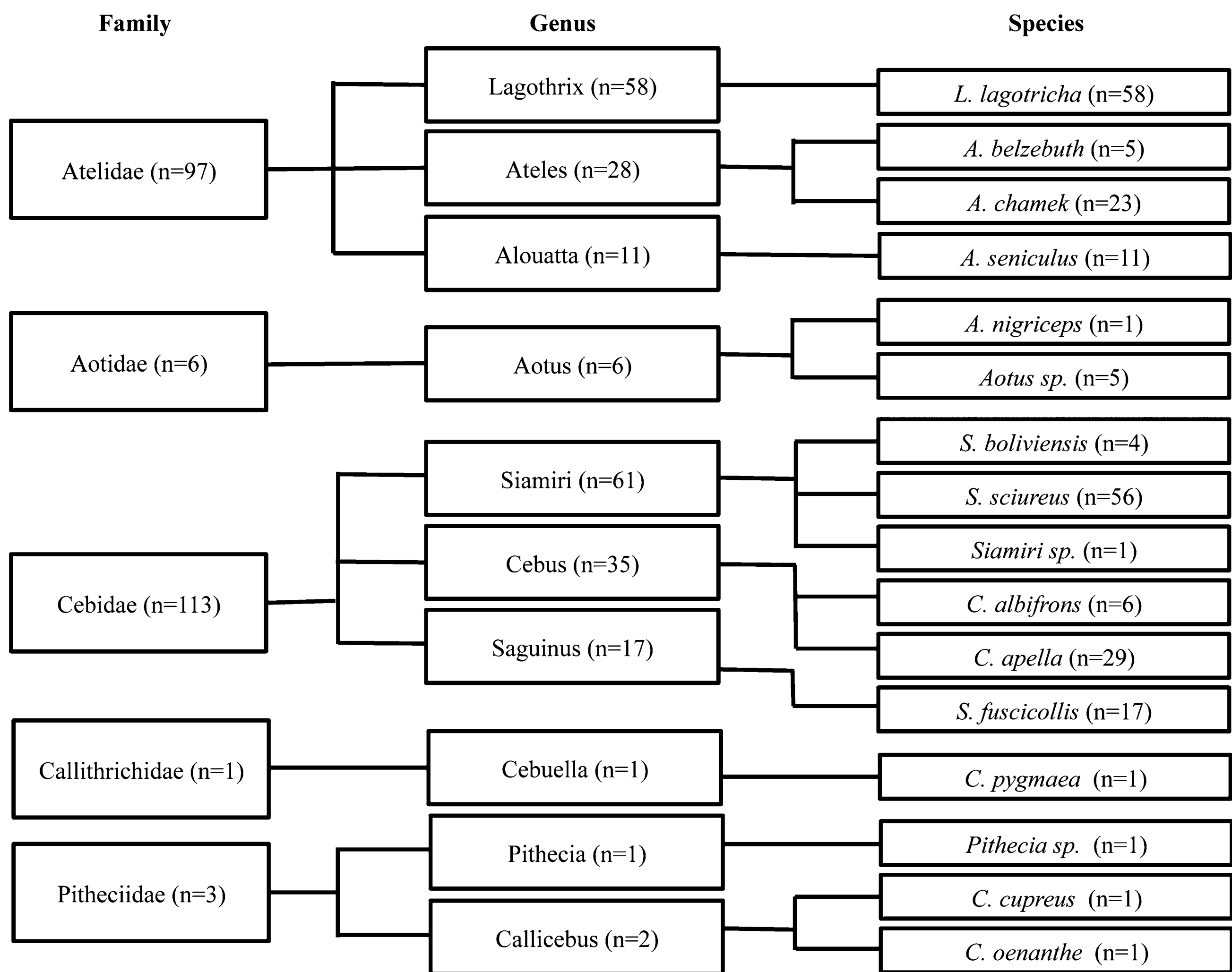


Figure 2. Taxonomic flow chart of New World monkeys sampled in this study in Peru.

Table 1. Oral Swab PCR (OSP) Status Stratified by Age, Sex, Taxonomic Family, and Context.

	OSP status: # (%)		
	Negative (n = 190)	Positive (n = 30)	*P*-values
Sex			**0.048**
Female, n = 115	94 (81.7)	21 (18.3)	
Male, n = 105	96 (91.4)	9 (8.6)	
Age (months)			**0.02**
0–12, n = 29	29 (100)	0 (0.0)	
13–36, n = 88	77 (87.5)	11 (12.5)	
≥37, n = 103	84 (81.5)	19 (18.5)	
Family			**<0.01**
Atelidae, n = 97	75 (77.3)	22 (22.7)	
Cebidae, n = 113	105 (92.9)	8 (7.1)	
Other, n = 10	10 (100)	0 (0.0)	
Context			**<0.01**
Sanc/Zoo, n = 100	78 (78.0)	22 (22.0)	
Market, n = 72	67 (93.1)	5 (6.9)	
Pet, n = 48	45 (93.7)	3 (6.3)	
Total (n = 220)	**190 (86.4)**	**30 (13.6)**	

Univariate *P*-values assessed the crude relationship between predictor variables and the outcome of interest, OSP status. Reported *P*-values under a priori 0.05 threshold are bolded to highlight significance.

Table 1 illustrates that both male and female NWM were OSP + (male = 9/96, 8.6%; female = 21/94, 18.3%), and OSP + NWM were represented in all three contexts (sanctuary/zoo = 22/100, 22%; market = 5/72, 6.9%; and pet = 3/45, 6.3%). Positive samples were not observed in NWM <13 months of age. The greatest prevalence of OSP + NWM (19/103, 18.5%) was reported among animals ≥37 months old. Oral Swab PCR+ monkeys were only detected in the taxonomic families Atelidae and Cebidae (Atelidae = 22/97, 22.7%; Cebidae = 8/113, 7.1%).

New World monkeys housed in sanctuaries/zoos were 3.7 times more likely to be OSP+ than pet NWM ($p < 0.05$, Table 2, Model 1). This association neared significance when adjusting for taxonomic family ($p = 0.06$, Table 2, Model 2) and when adjusting for taxonomic family and sex ($p = 0.07$, Table 2, Model 3). When the model was adjusted for all three predictor variables (taxonomic family, sex, and age), the association lost significance ($p = 0.15$, Table 2, Model 4).

Significant associations between sex and OSP status, and taxonomic family and OSP status lost significance when adjusting for all variables in the model (Table 2, Models 3 and 4). No predictor was significant after applying a Bonferroni correction.

Discussion

This is the first study to document the presence of MTBC DNA in a diverse sample of NWM in Peru. Positive samples were detected in NWM living in sanctuaries/zoos, NWM being sold at wet markets, and from pet NWM. Various species, age groups, and both males and females were OSP+ (Table 1). Few published reports of TB screening and detection in NWM exist outside of the laboratory setting. In Colombia, MTBC was identified via Mtp40 PCR in 11% of NWM studied at the Cali Zoo (Alfonso et al. 2004), and MTBC DNA was detected in 2.4% of NWM sampled at two rescue centers using spoligotyping (Barragán and Brieva 2005). None of the animals in these studies exhibited signs of active TB. Additionally, a 2011 study in Colombia reported that 20 pet NWM tested for TB using the intradermal TST test were negative (Estrada-Cely et al. 2011). The first published report of active TB in the neotropical primate comes from *Ateles paniscus* housed in a zoo in Brazil in 2011 (Rocha et al. 2011). To our knowledge, there are no reports of TB in free-ranging, wild South American NWM.

In Peru, NWM are generally trapped from the wild as infants or young animals and are sold in the city markets, destined as a pet or performing monkey, or placed in a sanctuary/zoo if confiscated by the authorities or abandoned by their caretaker (Shanee et al. 2012). Many sanctuaries housing confiscated or abandoned NWM in Peru lack space and resources to adequately care for increasing numbers of animals (Shanee et al. 2012), and some are beginning reintroduction programs to release NWM into their natural habitats.

The implications of releasing NHP who have been exposed to MTBC and other diseases of human origin has been a concern for years (Schoene and Brend 2002; Mugisha et al. 2011; Schaumburg et al. 2012; Wolf et al. 2014). The larger, more established NWM sanctuary programs in Peru are beginning to employ pre-release screening for TB using TST and gamma-interferon tests; unfortunately these assays lack sensitivity and specificity over the range of host responses to MTBC infection. TST, the most widely used screening test, is notoriously subjective in its interpretation and it is often difficult to access and maintain a steady supply of old mammalian tuberculin (Lerche et al. 2008).

Table 2. Crude and Multivariate Adjusted Logistic Regression Models Assessing Associations Between Oral Swab PCR-positive (OSP+) New World Monkeys (NWM) and the Context in Which they were Sampled in a Subset of 184 Animals.

	MODEL 1: Crude unadjusted association between context and OSP + NWM				MODEL 2: Model adjusting for taxonomic Family			
	Odds ratio	Std. error	95% CI	*P* value	Odds ratio	Std. error	95% CI	*P*-value
Context								
Pet	Ref.	–	–	–	Ref.	–	–	–
Market	1.17	0.89	0.26, 5.20	0.84	2.08	1.73	0.41, 10.63	0.38
Sanctuary/Zoo	3.72	2.42	1.04, 13.29	**0.04**	3.43	2.24	0.95, 12.32	0.06
Family								
Cebidae					Ref.	–	–	–
Atelidae					3.11	1.72	1.06, 9.17	**0.04**

	MODEL 3: Model adjusting for taxonomic family and sex				MODEL 4: Model adjusting for taxonomic family, sex and age			
	Odds ratio	Std. error	95% CI	*P*-value	Odds ratio	Std. error	95% CI	*P*-value
Context								
Pet	Ref.	–	–	–	Ref.	–	–	–
Market	1.86	1.55	0.36, 9.53	0.46	1.92	1.62	0.37, 10.05	0.44
Sanctuary/zoo	3.31	2.17	0.91, 11.98	0.07	2.61	1.76	0.70, 9.78	0.15
Family								
Cebidae	Ref.	–	–	–	Ref.	–	–	–
Atelidae	2.68	1.5	0.89, 8.04	0.07	2.93	1.71	0.93, 9.21	0.07
Age								
13–36 months	Ref.	–	–	–	Ref.	–	–	–
$\geq$37 months	1.94	0.86	0.82, 4.64	0.13	1.74	0.79	0.72, 3.10	0.22
Sex								
Male					Ref.	–	–	–
Female					1.31	0.58	0.55, 3.10	0.55

Given all NWM $<$13 months and belonging to the taxonomic families Atelidae and Cebidae were OSP negative, they were excluded from the model. Reported *P*-values under a priori 0.05 threshold are bolded to highlight significance.

The OSP direct pathogen detection, which we have recently shown to be 100% specific and 90% sensitive (Wood et al. in revision), provides a new method for early detection of TB and screening for subclinical infections which could be critical since undetected TB infections in released animals may pose a threat to naïve free-ranging primate groups and to humans. In addition, the minimally invasive OSP assay is an ideal way to screen animals in a field setting. Once placed in lysis buffer the samples do not require special handling or refrigeration and the DNA extraction and PCR require standard laboratory facilities generally available in the habitat countries.

Sanctuary/zoo animals, in contrast to market animals which tend to transit through the system quickly, generally have a near life-long history of close contact with humans and are housed with other NWM, often in mixed-species enclosures. Sanctuary/zoo animals were more likely to be OSP+ in this study, yet, when controlling for taxonomic family status, age, and sex, potential associations were no longer statistically significant. However, it seems logical that among sanctuary/zoo animals, MTBC transmission would be facilitated by prolonged contact with potential MTBC sources (namely humans and a high density of NWM in the sanctuary/zoo) (Nunn and Hare 2012; Wolf et al. 2014). The age of an animal, as a proxy measure of their total exposure, may also predict their likelihood of being OSP+. Longitudinal OSP sampling of animals throughout their life span could confirm this hypothesis.

Increasingly, research indicates a wider range of TB infection and disease manifestations in NHP than was previously recognized. Although epizootics of TB in primate laboratory colonies suggest a highly virulent disease course, the pathology of TB among Old World primates is now understood to be much more complex (Habel 1947; Ruch 1959). Among the genus *Macaca*, the only primate taxa in which TB has been extensively studied, response to MTBC experimental infection differs greatly among congeneric species (Walsh et al. 1996; Lin et al. 2009; Kaushal et al. 2012).

There have been no experimental studies to characterize the range of TB manifestations in NWM. Interestingly, we found that within a year of sampling, several OSP + NWM from two sanctuaries that showed no signs of illness at the time of sampling, developed fulminate effusive and granulomatous disease consistent with TB and died (Simmons and Gibson 2012). Unfortunately, no post-mortem diagnostics were performed. While fulminant TB has been documented in a captive NWM (Rocha et al. 2011), detection of the microorganisms that cause TB has also been reported in apparently healthy neotropical primates (Alfonso et al. 2004; Barragán and Brieva 2005), similar to what we documented in this study.

One additional question that emerges concerns the origin of the MTBC detected among NWM in this study. MTBC may have been acquired from humans, or NWM may have contracted MTBC from other NWM that previously contracted MTBC through human contact (Michel and Huchzermeyer 1998; Smith et al. 2006). Another possibility is that a naturally occurring strain of *Mycobacterium* that also contains the IS*6110* sequence exists in NWM, similar to the recent report of a novel MTBC isolate in a wild chimpanzee in Africa (Coscolla et al. 2013). Domestic animals and wildlife may also contribute to MTBC transmission to NWM.

There are limitations to this study. The cross-sectional design limited our ability to determine causality and/or temporality of MTBC infection in NWM, and while this dataset represents the largest number to date of NWM in these contexts to be assessed, the convenience sampling strategy may have introduced a sampling bias, potentially under- or over-representing the true prevalence of OSP + NWM in Peru. Lack of additional demographic and historical information such as body condition score, health status at time of sampling, medical history, length of time in captivity and follow-up health status reduce our ability to comment on correlations between OSP status and NWM health status. Future case and matched-control longitudinal studies are warranted.

Taken together, our results provide valuable information regarding the presence of MTBC DNA in captive NWM in Peru and highlight the lack of knowledge regarding pathophysiology of MTBC in NWM, the diagnosis of the disease TB in neotropical primates, and the public health and conservation threat posed by MTBC in NWM in a country that has been long recognized as a biodiversity hotspot facing serious conservation threats (Myers et al. 2000). The data presented here clearly highlight the importance of establishing systematic, long term surveillance of Peru's NWM populations. While the Atelidae and Cebidae, which made up the majority of our dataset, are among the most ubiquitous primates they represent only a fraction of the country's taxonomic richness. The application of molecular direct detection assays such as the OSP can play an important role in the development of comprehensive integrated approaches to NMW management in South America, as well as to better our understanding of the transmission of TB between NWM and humans.

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