SHORT COMMUNICATIONS

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Optimizing a Noninvasive Oral Sampling Technique for Semicaptive Neotropical Primates in Peru

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ABSTRACT: Disease surveillance in Neotropical primates (NP) is limited by the difficulties associated with anesthetizing NP for sample collection in remote settings. Our objective was to optimize a noninvasive method of oral sampling from semicaptive NP in Peru. We offered 40 NP at Taricaya Rescue Centre in Madre de Dios, Peru ropes coated in various attractants and measured variables (acceptance of the rope, chewing time, and volume of fluid eluted from ropes) that may affect sample acquisition and quality. We preserved samples by direct freezing in liquid nitrogen or by storing samples in RNA stabilization reagent at room temperature. Sample integrity was measured by testing for mammalian cytochrome b with the use of conventional PCR. The NP successfully chewed on a rope in 82% (125/152) of trials. Overall sample integrity was high, with 96% (44/46) of samples (both directly frozen and stored in stabilization reagent) testing positive for cytochrome b. The number of times that an individual NP was exposed to the rope procedure and NP age were associated with higher acceptance rates and the NP successfully chewing on the rope. We conclude that ropes serve as a feasible noninvasive method of obtaining oral samples from NP at rescue centers and could be used in future studies to evaluate population genetics and for pathogen surveillance for population health monitoring.

Key words: Neotropical primate, noninvasive sampling method, oral samples, Peru.

The Amazon Basin in Peru is home to 51 species of Neotropical primates (NP) (International Union for Conservation of Nature 2006). Anthropogenic pressures threaten NP populations while also contributing to increased potential for zoonotic disease transmission between NP and humans (Michaud et al. 2003; Gillespie et al. 2008; Shanee 2011). Confiscated or abandoned NP are housed in rehabilitation centers, where programs exist to reintroduce NP into the wild. Zoonotic transmission of parasites, bacteria, and viruses have been detected in trafficked Peruvian NP (Michaud et al. 2003; Ghersi et al. 2015; Rosenbaum et al. 2015); however, disease screening at rehabilitation facilities is limited by scarce resources and expertise for invasive sampling procedures and diagnostics. Blood and direct oral swab collection require anesthesia, which carries inherent risks for the NP and requires highly trained personnel, who are not always available in resource-limited settings (Evans et al. 2015). A noninvasive method using sterile ropes covered in jam was developed to collect samples from Old World primates (Inoue et al. 2007; Smiley et al. 2010; Evans et al. 2015). A similar technique using dental rope flavored with juice was used to obtain samples for tuberculosis surveillance in free-ranging macaques in Bali (Wilbur et al. 2012). However, this technique has not been used in NP, which are smaller, forage at different canopy levels, and have distinct diets compared to Old World primates (Dew 2005; Terborgh 2014; Whiten et al. 1991). Our objective was to optimize noninvasive oral sampling from NP to facilitate sample acquisition and testing without requiring anesthesia or highly trained personnel.

Field research was performed at the Taricaya Rescue Center in Madre de Dios, Peru (12°32′11.92″S, 69°00′14.81″W), in June 2017. Research was conducted under research and collection permits issued by the Peruvian Government (0173-2016-SERFOR-DGGSPFFS; 213-2016-SERFOR-DGGSFFS) and in accor-

FIGURE 1. Red howler monkey (*Alouatta peruensis*) chewing on flavored ropes used for noninvasive collection of oral samples.

dance with a Tufts University Institutional Animal Care and Use Committee approved protocol (G2017-42). SalivaBio Children's Swabs (Salimetrics, State College, Pennsylvania, USA) were attached to nylon string, coated in approximately 5–10 mL of guava paste, strawberry jam, or mashed banana, and offered to the NP (Fig. 1). A total of 152 trials were performed with a population of 40 NP over the course of 8 d. Each attractant was offered two to three times to NP. For each trial, the following information was recorded: number of previous exposures to the rope, total chewing time, and whether rope sharing occurred. Rope samples were centrifuged for 5 min at 1,350 × G and the volume of fluid eluted from each rope was recorded. The oral samples obtained likely contained both mucosal cells and saliva because of the sampling method used.

Eluted samples from 46 of the 152 trials were preserved for molecular detection of cytochrome b to assess the integrity of DNA in the sample (De Assis et al. 2016). Eluted samples were stored in either 0.5 mL of RNALater (Thermo-Fisher Scientific, Waltham, Massachusetts, USA) at room temperature (n=15) or without preservation medium and frozen in liquid nitrogen (n=31). Sampling strategies were designed to cover all species, age groups, and attractant types, and thus repeat sampling of some individuals was required.

Laboratory work was performed at the Centro de Investigaciones Tecnológicas, Biomédicas y Medioambientales (Instituto de Medicina Tropical, Universidad Nacional Mayor de San Marcos, Lima, Peru), in July 2017. We extracted DNA with the use of QIAamp DNA Investigator kits (Qiagen, Germantown, Maryland, USA) according to the manufacturer's instructions and tested by conventional PCR for the presence of a 350–base-pair sequence of mammalian cytochrome b (Kocher et al. 1989; De Assis et al. 2016).

Statistical analysis was performed with Stata/IC 15.0 (StataCorp LLC, College Station, Texas). The Shapiro–Wilks test was used to test for normality of the data and a generalized estimating equation model was used to determine whether age, sex, attractant type, and number of exposures to rope affected the NP willingness to chew on the

TABLE 1. Demographic information for the Neoptropical primates (n=40), stratified by taxonomic genus, used in a study of the use of chew ropes as a noninvasive technique for collection of oral samples.

Variable	Percent (number) of the study population belonging to demographic variables stratified by Neotropical primate genus				
	Ateles	Lagothrix	Cebus or Sapajus[a]	Alouatta	Other[b]
Age					
Infant	5 (1/20)	0 (0/4)	0 (0/5)	13 (1/8)	0 (0/3)
Juvenile	50 (10/20)	75 (3/4)	40 (2/5)	37 (3/8)	33 (1/3)
Adult	45 (9/20)	25 (1/4)	60 (3/5)	50 (4/8)	67 (2/3)
Sex					
Male	30 (6/20)	25 (1/4)	40 (2/5)	50 (4/8)	100 (3/3)
Female	70 (14/20)	75 (3/4)	60 (3/5)	37 (3/8)	0 (0/3)
Not recorded				13 (1/8)	
Origin					
Born at center	0 (0/20)	0 (0/4)	40 (2/5)	37 (3/8)	0 (0/3)
Confiscated from trade	35 (7/20)	50 (2/4)	20 (1/5)	0 (0/8)	67 (2/3)
Transferred or donated	40 (8/20)	50 (2/4)	40 (2/5)	50 (4/8)	0 (0/3)
Other or unknown	25 (5/20)	0 (0/4)	0 (0/5)	13 (1/8)	33 (1/3)

[a] Cebus and Sapajus were recently divided taxonomically, but these genera were grouped together in our study because they lived in the same enclosure and have Cebus/Sapajus hybrid offspring.

[b] Other category includes genera Leontecebus and Saimiri.

rope. We used the Kruskal–Wallis equality-of-populations rank test to compare chew times and volume eluted by different attractants. We considered P values <0.05 to indicate statistical significance.

In total, across 40 NP (Table 1), 82% (125/152) of trials resulted in a NP successfully chewing on a rope. Time spent chewing on ropes by NP ranged from <1 to 304 s. Adult NP were 4.0 times more likely to chew on a rope compared to juvenile NP (P=0.011). The NP exposed to the rope methods four or more times were 7.6 times more likely to chew on rope compared to NP who were exposed to rope methods for the first time (P=0.044; Table 2). There was no statistically significant association between attractant type and rope acceptance, indicating that all three attractant types are suitable for use (Table 2). Rope sharing between NP (chewing or sucking) occurred nine times. Rope handling between NP (contact with hand or tail) was more frequent, occurring greater than 30% of the time.

A total of 94% (29/31) of samples stored in liquid nitrogen tested positive for mammalian cytochrome b, indicating that the sample contained NP DNA. The two samples that did not test positive were both from the Lagothrix genus. All 15 samples stored in RNALater at room temperature tested positive for mammalian cytochrome b.

We found that offering ropes to NP was an effective, noninvasive, and behaviorally accepted technique to collect oral samples. Overall, 82% of trials at the Taricaya Rescue Centre resulted in NP chewing on the ropes. Most of the samples collected (96%) tested positive for mammalian cytochrome b, indicating that mammalian cellular material was present. On further review for possible reasons for the two negative results of the Lagothrix samples, it was found that the left primer used had no target in the Lagothrix cytochrome B gene. In future studies with Lagothrix sampling, a different left primer should be considered to test for cytochrome b. Most of the NP in this study were habituated to humans and would receive ropes directly from people outside the enclosure.

Three out of 40 NP masticated and consumed the rope; however, following close observation by sanctuary veterinarians, none of these individuals had complications from

TABLE 2. Results of generalized estimating equation model showing the willingness of Neotropical primates to chew on flavored ropes used to obtain noninvasive oral samples, based on sex, age, number of exposures to rope, and attractant type.

	Odds ratio	95% CI[a]	*P* value
Age			
Juvenile	Reference	–	–
Adult	4.0	1.4–11.8	0.011
Exposures to rope			
1	Reference	–	–
2	1.8	0.4–8.7	0.439
3	1.9	0.4–10.3	0.436
≥4	7.6	1.1–54.0	0.044

[a] Dash (–) = the reference for comparison.

consuming the ropes. Subsequent careful observation and rapid retrieval resulted in no additional incidents of rope consumption, highlighting a need for vigilance when employing this technique with captive NP. In previous research using similar techniques with Old World primates, rope consumption did not occur, even when the ropes were concealed in a plantain (Evans et al. 2015), suggesting potential behavioral differences in the likelihood of rope consumption between captive and free-living individuals as well as between species.

Retrieval strings reduced but did not completely eliminate rope sharing. There were no aggressive events caused by rope distribution or when rope sharing occurred during the course of our study, but the potential for aggressive behaviors should be considered. A limitation to this study was that obtaining an oral sample at the individual level was challenging, because NP frequently shared ropes with other NP. Although sampling at the individual level was not always possible, this method is still valuable for collecting information at the population level.

In conclusion, this study demonstrates that we were able to detect NP DNA from rope samples, both directly frozen and stored in RNAlater, indicating that this method is a feasible way to obtain oral samples noninvasively from NP housed at rescue centers. This noninvasive technique is more feasible in remote, resource-limited settings than anesthetizing NP to collect blood and oral swabs and holds potential for future use in infectious disease surveillance of captive and free-living NP.

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