

Squirrel Monkey Cytomegalovirus Antibodies in Free-ranging Black Howler Monkeys (*Alouatta caraya*), Misiones, Argentina

Hebe Ferreyra,^{1,3} Hernan Argibay,^{1,2} Miguel A. Rinas,² and Marcela Uhart¹ ¹ Global Health Program, Wildlife Conservation Society, Amenabar 1595 (1426), Ciudad Autónoma de Buenos Aires, Argentina; ² Parque Ecológico El Puma, Ministerio de Ecología y Recursos Naturales Renovables, Ruta 12 km 1355, CP 3308, Candelaria, Provincia de Misiones, Argentina; ³ Corresponding author (email: hferreyra@wcs.org)

ABSTRACT: Serum from four black howler monkeys (*Alouatta caraya*) was screened for antibodies to seven viruses by dot immunoassay. Cytomegalovirus antibodies were detected in three of four individuals and provide the first evidence of exposure by black howler monkeys to this virus.

Two species of howler monkeys occur in Argentina, the black howler (*Alouatta caraya*) and the southern brown howler (*A. guariba clamitans*). Brown howlers live only in the Atlantic Forest ecoregion of Brazil and Argentina (Kinzey, 1982). Black howlers live in the Chaco and Pantanal ecoregions of Brazil, Paraguay, Bolivia, and north-northeastern Argentina (Rumiz, 1990) and a small portion of the Atlantic Forest in Misiones, Argentina, and Rio Grande do Sul, Brazil (Leiroz Codenotti et al., 2002; Di Bitetti et al., 2003). Both species are threatened as a result of habitat loss and fragmentation, hunting pressure (Di Bitetti et al., 1994), susceptibility to epidemic diseases (Holzmann et al., 2010), and parasite infestation (Milton, 1982). Identifying infectious agents that might influence wildlife populations is critical for the management and conservation of endangered species.

Although the information provided by serologic techniques is limited, these tools are still useful to detect exposure of animals to pathogens (Dohoo et al., 2003), particularly for free-ranging wildlife in which access to diagnostic samples is often difficult and opportunistic. A yellow fever virus outbreak affected southern black and brown howlers in Misiones, Argentina, and Paraná, Brazil, from 2007 to 2008 (Cardoso et al., 2010; Holzmann et al., 2010).

Four male black howler monkeys (three adults and one juvenile) were found dead

from yellow fever at three sites in Misiones province (27°44'47"S, 55°46'52"W; 27°31'39"S, 55°52'02"W; 27°45'57"S, 55°50'13"W) in January 2009. Whole blood was collected directly from the heart of each animal and centrifuged at 1,000 × G (Moblispin, Model 128, Grandview, Missouri, USA); serum was stored in liquid nitrogen for later analysis. Antibody testing for selected viral pathogens was performed in VRL Laboratories (San Antonio, Texas, USA), using dot immunoassays for herpes platyrhinae virus, herpes saimiri saimiri virus, measles virus, squirrel monkey cytomegalovirus, human herpesvirus 1 and 2, influenza A virus, and encephalomyocarditis virus. Based on protocols established by Heberling and Kalter (1986), antibody titers less than 5 were considered negative for all tests.

All tests were negative except for the finding of antibody to squirrel monkey cytomegalovirus in three of four individuals tested. To the best of our knowledge, exposure to squirrel monkey cytomegalovirus has not been previously documented for howler monkeys.

As a member of the herpesvirus family, cytomegalovirus persists as a latent infection, and viral shedding can occur for extended periods in body fluids (saliva, blood, milk, semen; King, 2000). Cytomegalovirus infections are common in humans (Kinney et al., 1985) and nonhuman primates (Jones-Engel et al., 2006). However, infection is mostly asymptomatic or presents clinical relevance only for weak and immunocompromised individuals, who can develop fatal disease (Baskin, 1987). Although cytomegaloviruses are host specific, horizontal transmission to other species can occur (King, 2000). In our

study, the source of infection for this virus is unknown. Cross-reactivity among cytomegaloviruses from different monkey species is possible (Swack and Hsiung, 1982). Thus, isolation and molecular characterization of viruses from body fluids of infected animals should be performed to determine whether the humoral response detected was caused by a new cytomegalovirus specific to howler monkeys. Despite the limited number of animals tested, our results contribute to the currently scant knowledge on South American wild primate population health.

We value support from the Wildlife Conservation Society, Parque Ecológico El Puma staff, Santa Ines Ranch owners, and the Wildlife Agency of Argentina (Secretaría de Ambiente y Desarrollo Sustentable de la Nación) for providing permits to conduct this study. Special thanks to P. Beldomenico, D. McAloose, B. Raphael, and three anonymous reviewers for their contributions to the improvement of this manuscript.

LITERATURE CITED

- BASKIN, G. B. 1987. Disseminated cytomegalovirus infection in immunodeficient rhesus monkeys. *American Journal of Pathology* 129: 345–352.
- CARDOSO, J. C., M. A. B. DE ALAMEIDA, E. DOS SANTOS, D. F. DA FONSECA, M. A. M. SALLUM, C. A. NOLL, H. A. DE O. MONTEIRO, A. C. R. CRUZ, V. L. CARVALHO, E. V. PINTO, F. C. CASTRO, J. P. NUNES NETO, M. N. O. SEGURA, AND P. F. C. VASCONCELOS. 2010. Yellow fever virus in *Haemagogus leucocelaenus* and *Aedes serratus* mosquitoes, southern Brazil, 2008. *Emerging Infectious Diseases* 16: 1918–1924.
- DI BITETTI, M. S., G. PLACCI, A. D. BROWN, AND D. I. RODE. 1994. Conservation and population status of the brown howling monkey (*Alouatta fusca clamitans*) in Argentina. *Neotropical Primates* 2: 1–4.
- , ———, AND L. A. DIETZ. 2003. A biodiversity vision for the upper Paraná Atlantic Forest ecoregion: Designing a biodiversity conservation landscape and setting priorities for conservation action. World Wildlife Fund, Washington, D.C., 154 pp.
- DOHOO, I., W. MARTIN, AND H. STRYHN. 2003. Veterinary epidemiologic research. AVC Inc., Prince Edward Island, Canada, 706 pp.
- HEBERLING, R. L., AND S. S. KALTER. 1986. Rapid dot immunobinding assay on nitrocellulose for viral antibodies. *Journal of Clinical Microbiology* 23: 109–113.
- HOLZMANN, I., I. AGOSTINI, J. I. ARETA, H. FERREYRA, P. BELDOMENICO, AND M. S. DI BITETTI. 2010. Impact of yellow fever outbreaks on two howler monkey species (*Alouatta guariba clamitans* and *A. caraya*) in Misiones, Argentina. *American Journal of Primatology* 71: 1–6.
- JONES-ENGEL, L., G. A. ENGEL, J. HEIDRICH, M. CHALISE, N. POUDEL, R. VISCIDI, P. BARRY, J. S. ALLAN, R. GRANT, AND R. KYES. 2006. Temple monkeys and health implications of commensalism, Kathmandu, Nepal. *Emerging Infectious Diseases* 12: 900–906.
- KING, W. N. 2000. Herpesvirus infections. In *Infectious diseases of wild mammals*, 3rd Edition. E. S. Williams and I. K. Barker (eds.). Iowa State University Press, Ames, Iowa, pp. 147–157.
- KINNEY, J. S., I. M. ONORATO, J. A. STEWART, R. F. PASS, S. STAGNO, S. H. CHEESEMAN, J. CHIN, M. L. KUMAR, A. S. YAEGER, K. L. HERRMANN, E. S. HURWITZ, AND L. B. SCHONBERGER. 1985. Cytomegaloviral infection and disease. *Journal of Infectious Disease* 151: 772–774.
- KINZEY, W. G. 1982. Distribution of primates and forest refuges. In *Biological diversification in the tropics*, G. T. Prance (ed.). Columbia University Press, New York, New York, pp. 455–482.
- LEIROZ CODENOTTI, T., V. MARTINS DA SILVA, V. J. DE ALBUQUERQUE, E. V. CAMARGO, AND R. M. MARTINS SILVEIRA. 2002. Distribuição e situação actual de conservação de *Alouatta caraya* (Humboldt, 1812) no Rio Grande do Sul, Brazil. *Neotropical Primates* 10: 132–141. Portuguese.
- MILTON, K. 1982. Dietary quality and demographic regulation in a howler monkey population. In *The ecology of tropical forest: seasonal rhythms and long-term changes*, E. G. Leigh, A. Stanley Rand and D. M. Windsor (eds.). Smithsonian Institution Press, Washington, D.C., pp. 273–289.
- RUMIZ, D. I. 1990. *Alouatta caraya*: Population density and demography in northern Argentina. *American Journal of Primatology* 21: 279–294.
- SWACK, N. S., AND G. D. HSIUNG. 1982. Natural and experimental simian cytomegalovirus infections at a primate center. *Journal of Medical Primatology* 11: 169–177.

Submitted for publication 11 April 2011.

Accepted 1 October 2011.