

Serological Evidence of Infection with Endemic Human Pathogens among Free-Ranging Old World Monkeys in Puerto Rico

Ryan R. Hemme,* Ricardo Lopez-Ortiz, Brenda Rivera Garcia, Tyler M. Sharp, Renee L. Galloway, Mindy G. Elrod, and Elizabeth A. Hunsperger

Dengue Branch, Centers for Disease Control and Prevention, San Juan, Puerto Rico; Fish and Wildlife Bureau, Puerto Rico Department of Natural and Environmental Resources, San Juan, Puerto Rico; Epidemiology and Research Office, Puerto Rico Department of Health, San Juan, Puerto Rico; Zoonoses and Select Agent Laboratory, Bacterial Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract. Serum specimens from free-ranging but nonnative patas monkeys (*Erythrocebus patas*) and rhesus macaques (*Macaca mulatta*) in southwestern Puerto Rico (PR) were tested for antibodies to infection with dengue viruses (DENVs), West Nile virus (WNV), *Leptospira* species, and *Burkholderia pseudomallei* by microneutralization, plaque reduction neutralization, microscopic agglutination, and indirect hemagglutination, respectively. Of 23 animals (21 *E. patas* and two *M. mulatta*) tested, all had evidence of prior DENV infection, and of 17 animals tested for WNV, nine (53%) had evidence of prior infection. Of 24 (22 *E. patas*, two *M. mulatta*) tested for *Leptospira* spp., 10 (42%) had evidence of prior exposure, and one patas monkey had antibodies against *B. pseudomallei*. The acquisition of pathogens endemic among humans in PR by resident nonhuman primates merits further study to define modes of acquisition.

INTRODUCTION

Nonhuman primates are not native to the Caribbean island of Puerto Rico (PR). Old World patas monkeys (*Erythrocebus patas*) and rhesus macaques (*Macaca mulatta*) were imported from India and Nigeria in the 1960s and 1970s to establish La Parguera Primate Breeding Colony (LPPBC) on the islets of Guayacán and Cueva in southwestern PR.¹ Before LPPBC was closed in 1982, an unknown number of monkeys of both species escaped the facility to the main island of PR. Censuses conducted in 2010 and 2012 estimated the main island population of patas monkeys at 1,460 and rhesus macaques at 1,086, respectively (Department of Natural and Environmental Resources, unpublished data). These free-ranging monkeys frequently raid local farms to forage for food, causing extensive crop damage and economic loss among farmers in southwestern PR.² Primarily for this reason, in 2008, the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services in cooperation with the PR Departments of Natural and Environmental Resources and Department of Agriculture, and the U.S. Fish and Wildlife Services decided to reduce this free-ranging nonhuman primate population and leave only solitary males on mainland PR by 2019.²

In PR, dengue and leptospirosis are endemic human diseases and common causes of acute febrile illness.^{3–5} Dengue virus (DENV) is thought to be exclusively transmitted in a human–mosquito–human cycle.^{6,7} However, nonhuman primates are susceptible to DENV infection, and sylvatic nonhuman primate transmission cycles have been described in Africa and Malaysia.^{6,8} West Nile virus (WNV) is a mosquito-borne virus that is amplified in an enzootic cycle in birds and incidentally infects humans, a dead-end host.⁹ Leptospirosis, a zoonotic disease of global importance, occurs following direct or indirect contact with infected animal urine, usually that of

rodents. However, virtually all mammals are susceptible to infection with *Leptospira* spp.^{10,11} *Burkholderia pseudomallei*, a saprophytic, gram-negative bacillus causes melioidosis, was recently shown to be in PR.^{12,13} Excluding WNV, where mammals are dead-end hosts, it is unknown if free-ranging primates in PR are capable of being infected with any of these endemic pathogens or whether they might contribute to their transmission cycles in PR.

Several government agencies have programs established to remove these free-ranging nonhuman primates. These programs provided the opportunity to obtain specimens to test for pathogens affecting humans. Serum specimens obtained during the removal program were tested for evidence of DENV, WNV, *Leptospira* spp., and *B. pseudomallei* infection.

MATERIALS AND METHODS

In 2010 and 2012, blood from 24 nonhuman primates from southwestern PR was obtained from participating agencies as part of the wildlife damage control program by cardiac puncture, performed soon after death. Blood was transferred to a sterile collection tube, allowed to clot for 15 minutes, centrifuged at 1,500 rpm for 10 minutes, and serum was stored at -20°C until diagnostic testing was performed.

Serum specimens were heat-treated for 30 minutes at 56°C to inactivate any other possible adventitious virus in the specimen.¹⁴ Specimens were tested for the presence of anti-DENV antibodies by microneutralization test (MNT), which is an adaptation of the plaque-reduction neutralization test (PRNT).¹⁵ Primary DENV infection was defined by a single reciprocal titer ≥ 40 ; samples with a reciprocal titer ≥ 40 to more than one DENV type were characterized as having evidence of second or greater DENV infection or having DENV cross-reactive antibodies. Specimens were also tested for evidence of WNV neutralizing antibodies by PRNT₉₀. WNV neutralizing PRNT₉₀ reciprocal titers of ≥ 20 indicated previous exposure to WNV. Evidence of *Leptospira* spp. exposure was determined by microscopic agglutination test (MAT). Individual animals with a reciprocal MAT titer ≥ 100 were considered positive for *Leptospira* antibody.¹⁶ Indirect

*Address correspondence to Ryan R. Hemme, Entomology and Ecology Activity, Dengue Branch, Centers for Disease Control and Prevention, 1324 Calle Cañada, San Juan, Puerto Rico 00920. E-mail: rhemme@cdc.gov

hemagglutination assay was used to detect anti-*B. pseudomallei* antibodies, and seropositivity was defined by a reciprocal titer ≥ 40 .¹⁷

RESULTS

Out of 24 nonhuman primates from four clans that were captured in southwestern PR, 22 were patas monkeys and two were rhesus macaques (Table 1). Sex and approximate age was determined for 11 patas monkeys, of which nine (82%) were female and eight (73%) were adults. Of the two rhesus macaques, one was female and both were adults.

All 23 of the monkeys tested had evidence of prior DENV infection by MNT (one specimen could not be tested). Seven (30%) monkeys had evidence of a primary infection with DENV-4, which included six patas monkeys and one rhesus macaque (Table 1). In the remaining animals, two (9%) had elevated anti-DENV MNT titers against both DENV-1 and DENV-4 (both patas), and 14 (61%) had elevated MNT titers against DENV-3 and DENV-4 (Table 1). Infection with DENV-4 was not associated with any particular clan or geographic area; however, the two monkeys positive for both anti-DENV-1 and anti-DENV-4 neutralizing antibodies came from Cabo Rojo region in the southwest of the island (Figure 1). None of the animals had anti-DENV-2 neutralizing antibodies.

Nine of the 17 (53%) monkeys tested had neutralizing antibodies against WNV. Of these nine monkeys, two had high PRNT₉₀ reciprocal titers of 320 and 640, respectively.

Ten (42%) of 24 monkeys had antibodies to *Leptospira* spp. MAT titers were highest for serogroups Bataviae (4), Ballum (4), Icterohaemorrhagiae (1), and one specimen in

which antibody was equally reactive to serogroups Icterohaemorrhagiae, Autumnalis, and Australis. Dominant serogroups were not associated with geographic regions from which the monkeys were captured.

One patas monkey of unknown age and sex had evidence of previous exposure to *B. pseudomallei*.

DISCUSSION

In this study, antibodies against DENVs were detected in 23 free-ranging nonhuman primates in southwestern PR. As there is no evidence of sylvatic DENV transmission in the Americas, the most likely explanation for these findings is spillback of human DENV into this small, nonhuman primate population, as has been observed in French Guiana and the Philippines.^{18,19} Our findings suggest that DENV spillback may be common in PR. Anti-DENV antibodies were detected in four geographically disparate clans of monkeys that have limited interaction with one another, suggesting that transmission from humans to nonhuman primates has occurred on more than one occasion. During 1992 to 2012, all four DENV types have circulated in humans in southwestern PR, where the dominant types were DENV-1, DENV-2, DENV-4, followed by DENV-3. It is interesting that all of the monkeys that were tested had either a primary DENV-4 infection or had cross-reactive antibodies to DENV-4. Surprisingly, no monkeys in PR had evidence of past infection with DENV-2.^{5,20}

We were not able to conclusively determine if the DENV serologic results found in this study were a result of regular virus spillback from humans to monkeys or if a stable enzootic DENV transmission cycle exists in the absence of

TABLE 1

Characteristics and DENV, WNV, *Leptospira*, and *Burkholderia pseudomallei* serologic diagnostic test results of nonhuman primates from Puerto Rico

Species	Location sample collected	Date sample collected	Sex	Age	DENV type microneutralization titers				WNV plaque reduction neutralization	<i>Leptospira</i> serogroup (highest titer)	<i>Leptospira</i> highest titer	<i>B. pseudomallei</i> titer
					DENV-1	DENV-2	DENV-3	DENV-4				
<i>Erythrocebus patas</i>	A	March 2010	Female	Adult	< 40	< 40	80	40	40	Neg.	< 100	< 40
	A	March 2010	ND	ND	< 40	< 40	40	40	< 20	Neg.	< 100	< 40
	A	March 2010	ND	ND	< 40	< 40	< 40	320	160	Neg.	< 100	< 40
	A	March 2010	Female	Adult	< 40	< 40	80	80	20	Neg.	< 100	< 40
	B	June 2010	Female	Adult	< 40	< 40	< 40	160	NT	Ballum	100	< 40
	B	June 2010	ND	ND	< 40	< 40	80	40	20	Neg.	< 100	< 40
	C	June 2010	Male	Adult	< 40	< 40	< 40	320	NT	Bataviae	800	< 40
	C	June 2010	ND	ND	< 40	< 40	< 40	40	NT	Neg.	< 100	80
	C	June 2010	ND	ND	< 40	< 40	< 40	160	< 20	Neg.	< 100	< 40
	D	June 2010	Female	Juvenile	< 40	< 40	< 40	80	NT	Neg.	< 100	< 40
	D	June 2010	Male	Adult	< 40	< 40	40	80	< 20	Neg.	< 100	< 40
	D	June 2010	ND	ND	< 40	< 40	40	80	80	Bataviae	800	< 40
	D	June 2010	ND	ND	< 40	< 40	160	320	640	Neg.	< 100	< 40
	E	June 2010	Female	Adult	< 40	< 40	160	160	< 20	Bataviae	800	< 40
	E	June 2010	Female	Juvenile	80	< 40	< 40	40	< 20	Neg.	< 100	< 40
	E	June 2010	ND	ND	< 40	< 40	160	80	< 20	Ballum	100	< 40
	E	June 2010	ND	ND	< 40	< 40	320	320	NT	Neg.	< 100	< 40
	E	June 2010	Female	Adult	< 40	< 40	320	80	40	Icterohaemorrhagiae	100	< 40
E	June 2010	Female	Juvenile	80	< 40	< 40	40	NT	Neg.	< 100	< 40	
E	June 2010	ND	ND	< 40	< 40	40	80	< 20	Ballum	100	< 40	
E	June 2010	ND	ND	< 40	< 40	40	320	< 20	Ballum	800	< 40	
F	April 2012	Female	Adult	NT	NT	NT	NT	20	Icterohaemorrhagiae/ Autumnalis/ Australis	400	< 40	
<i>Macaca mulatta</i>	G	April 2012	Male	Adult	< 40	< 40	40	80	NT	Neg.	< 100	< 40
	H	April 2012	Female	Adult	< 40	< 40	< 40	40	320	Bataviae	400	< 40

DENV = dengue virus; WNV = West Nile virus. ND indicates that variable characteristic was not determined (sex, age); NT indicates that the specimen was not tested (DENV, WNV); and Neg. indicates that the specimen sample was negative (*Leptospira* serogroup).

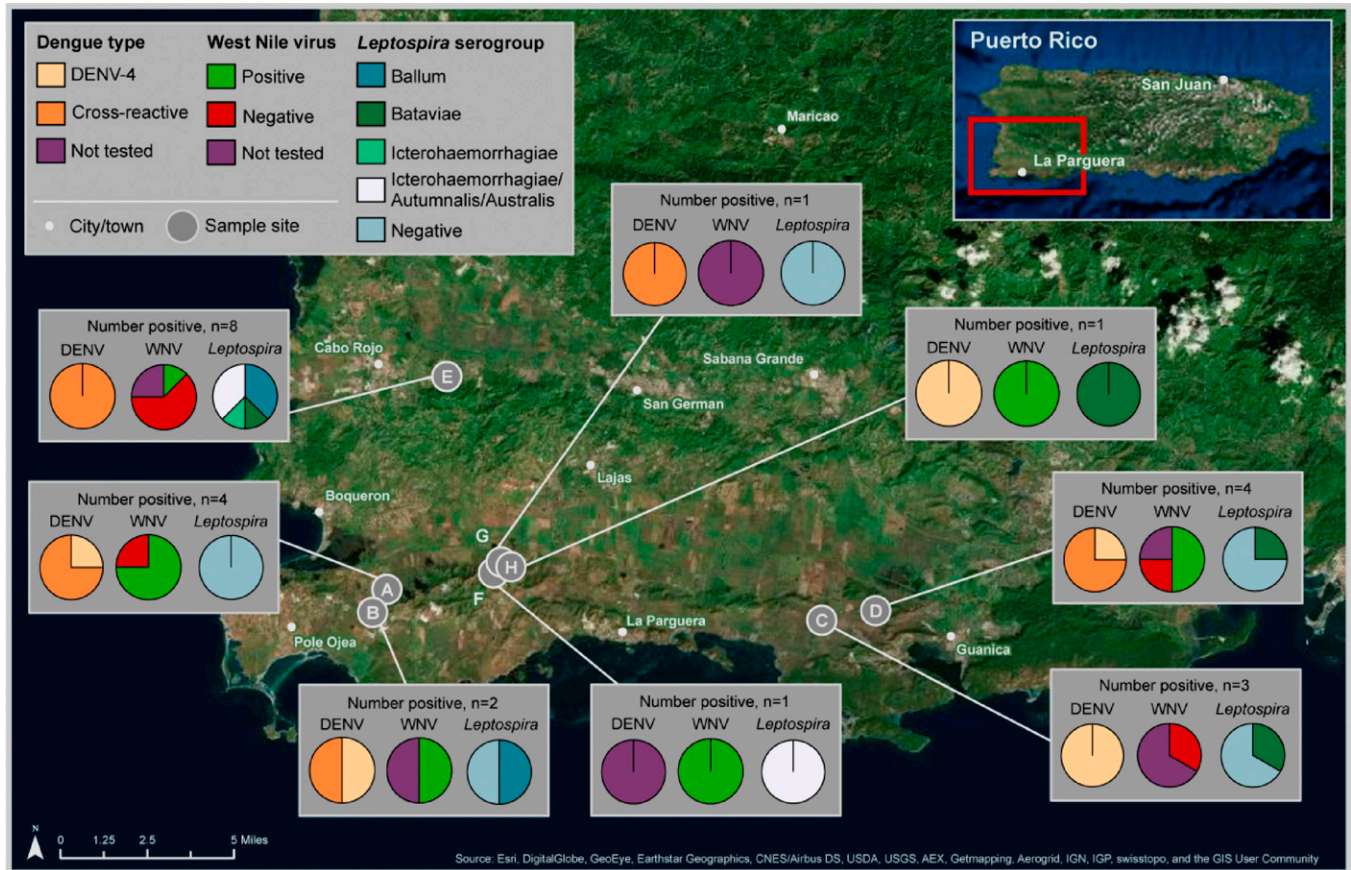


FIGURE 1. Geographic location and serologic test results for dengue virus and *Leptospira* spp. in nonhuman primates from Puerto Rico.

humans. Transmission of DENV from humans to nonhuman primates should occur in a similar ecological setting as sylvatic DENV emergence, a “zone of emergence” as described in Vasilakis and others,⁸ where environmental conditions enable overlap between vectors, humans, and nonhuman primates. Conditions around southwestern PR seem favorable for an enzootic cycle of human DENV transmission due to: 1) patas monkeys, a known host of sylvatic DENV in West Africa and 2) the presence of competent mosquito vectors of DENV in PR.^{21–24}

In addition to the presence of neutralizing antibodies to DENV, the monkey serum also had neutralizing antibodies against WNV. In 2007, WNV was first isolated in PR following the seroconversion of sentinel chickens in municipality of Ceiba.²⁵ This chicken surveillance was dismantled at the end of 2007 leaving PR with no method of identifying active WNV transmission. Since 2007, there has been sporadic evidence of WNV transmission identified in blood donors; however, no locally acquired human cases have been found in PR.²⁵ Previous research in 2007 did not find evidence of WNV-infected rhesus macaques on the island of Cayo Santiago, PR.²⁶ Differences in the local environment and availability of competent vectors on the island may explain this finding.

Nearly half of the nonhuman primates had been previously exposed to *Leptospira* spp. bacteria. Patas monkeys are terrestrial, and rhesus macaques are both terrestrial and arboreal,

making it possible for both to come into contact with contaminated soil or water while foraging for food on the ground. Moreover, because patas monkeys have been observed to venture into areas occupied by humans and consume crops grown in agricultural fields, humans (e.g., agricultural workers, consumers) could potentially come into contact with soil or agricultural crops that have been contaminated with monkey urine. Nonetheless, the public health importance of infection with *Leptospira* spp. bacteria in nonhuman primates is unknown since it is not known whether monkeys shed the bacteria once infected and serve as reservoirs.

Though also transmitted through contact with contaminated soil and water, just one monkey had evidence of *B. pseudomallei* exposure. This finding may be due to either infrequent presence of the bacteria in soil, decreased susceptibility of nonhuman primates to infection, and/or false-positive serologic test results.^{17,27}

Because patas monkeys and rhesus macaques are invasive species in PR, their importance in the local disease ecology has not been previously studied and not well understood. Feral monkeys often come in close proximity to humans; with a home range that often exceeds 20 km² and the ability to travel more than 2 km a day, infected nonhuman primates could be both reservoirs and mechanisms for disease dispersal.¹ Further study is required to better understand the mosquito species and conditions leading to DENV exposure of monkeys in PR, and how frequently and close nonhuman

primates and humans come into contact with each other in natural settings. If a stable enzootic cycle exists between mosquitoes and nonhuman primates, the possibility for DENV elimination in PR may be difficult, lacking the complete elimination of nonhuman primates.⁶ Additional efforts should be made to isolate DENV from arboreal mosquitoes and nonhuman primates, as well as elucidating why DENV-4 was the predominant type of DENV infecting nonhuman primates. Furthermore, the potential of nonhuman primates as sentinels for WNV in PR and the role of nonhuman primates as a reservoir for *Leptospira* spp. and *B. pseudomallei* in PR merits further investigation.

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Authors' addresses: Ryan R. Hemme, Entomology and Ecology Activity, Dengue Branch, Centers for Disease Control and Prevention, San Juan, Puerto Rico, E-mail: rhemme@cdc.gov. Ricardo Lopez-Ortiz, Fish and Wildlife Bureau, Puerto Rico Department of Natural and Environmental Resources, San Juan, Puerto Rico, E-mail: rlopez@drna.gobierno.pr. Brenda Rivera Garcia, Epidemiology and Research Office, Puerto Rico Department of Health, San Juan, Puerto Rico, E-mail: brendarivera@salud.pr.gov. Tyler M. Sharp, Epidemiology Activity, Dengue Branch, Centers for Disease Control and Prevention, San Juan, Puerto Rico, E-mail: tsharp@cdc.gov. Renee L. Galloway and Mindy G. Elrod, Zoonoses and Select Agent Laboratory, Bacterial Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, GA, E-mails: zul0@cdc.gov and wgz0@cdc.gov. Elizabeth A. Hunsperger, Immunodiagnostic, Development, and Research Laboratory, Centers for Disease Control and Prevention, San Juan, Puerto Rico, E-mail: enh4@cdc.gov.

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