DOI: 10.7589/2015-09-252

Journal of Wildlife Diseases, 52(3), 2016, pp. 766–769

Serologic Evidence of Mammarenaviruses among Wild Rodents in Brazil

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ABSTRACT: We screened blood samples from 560 wild rodents collected in southeastern Brazil for antibodies to a recombinant nucleoprotein (rN) of Junín virus. Six rodents were antibody positive (1.1%), demonstrating evidence of infection with mammarenaviruses in several species of Brazilian rodents.

Until recently, viruses of the family *Arenaviridae* were thought to only infect rodents, and in one case, possibly phyllostomid bats (Radoshitzky et al. 2015). However, with the identification of arenaviruses in reptiles, the family *Arenaviridae* now comprises two genera, *Mammarenavirus* and *Reptarenavirus* (Stenglein et al. 2012). The mammarenaviruses are canonically classified into two serocomplexes, the Tacaribe serocomplex, which includes those viruses indigenous to the New World, and the Lassa-Lymphocytic choriomeningitis serocomplex, which includes those viruses within the Old World (Cajimat et al. 2007; Charrel et al. 2010). These two groups are reflected in the distribution of their mammal reservoir species. The Old World mammarenaviruses are harbored by the Old World murid rodents and the New World mammarenaviruses are found predominantly in the family Cricetidae, subfamilies Sigmodontinae and Neotominae (Salazar-Bravo et al. 2002; Charrel et al. 2010). However, there is a notable exception, Tacaribe virus, which was isolated from fruit-eating bats of the genus *Artibeus* spp. and from host-seeking ticks *Amblyomma americanum* (Sayler et al. 2014).

In Brazil, there are six mammarenaviruses recognized: Amapari, Cupixi, Flexal, Latino, Oliveros, and Sabiá. Among them, Sabiá is thus far the only arenavirus known to cause a fatal hemorrhagic fever, and its natural reservoir is not conclusively known (Coimbra et al. 1994; Salazar-Bravo et al. 2002; Fernandes et al. 2015). To further understand the ecology of mammarenaviruses in Brazil, we captured and screened wild small mammals by enzyme-linked immunosorbent assay (ELISA) for arenavirus antibodies. Rodents were captured at three ecologically distinct sites in the northeastern region of São Paulo State, southeastern Brazil (Fig. 1). Site 1 is the Jataí Ecological Station, located in Luis Antonio County, which encompasses an area of 9,000 ha and currently is the largest protected area in the state, with continuous cerrado vegetation in addition to small enclaves of semideciduous forest patches. Site 2 is located in Cajuru County, with most of the original cerrado converted into monospecific cultivars (e.g., *Brachiaria decumbens*). Site 3, located in Batatais County, is characterized by large sugarcane plantations, with small patches of other types of vegetation (e.g., cerrado intermixed with various patches of secondary vegetation). We visited our field sites four times from June 2008 to July 2009. During each visit, two grids, each of 100 (8×9×23–cm) Sherman live-traps (10 columns by 10 rows set at 10-m intervals) were set 800 m apart for mark-recapture studies of rodents. At the same time, a linear transect of 100 Sherman live-traps, set at 10-m intervals, was

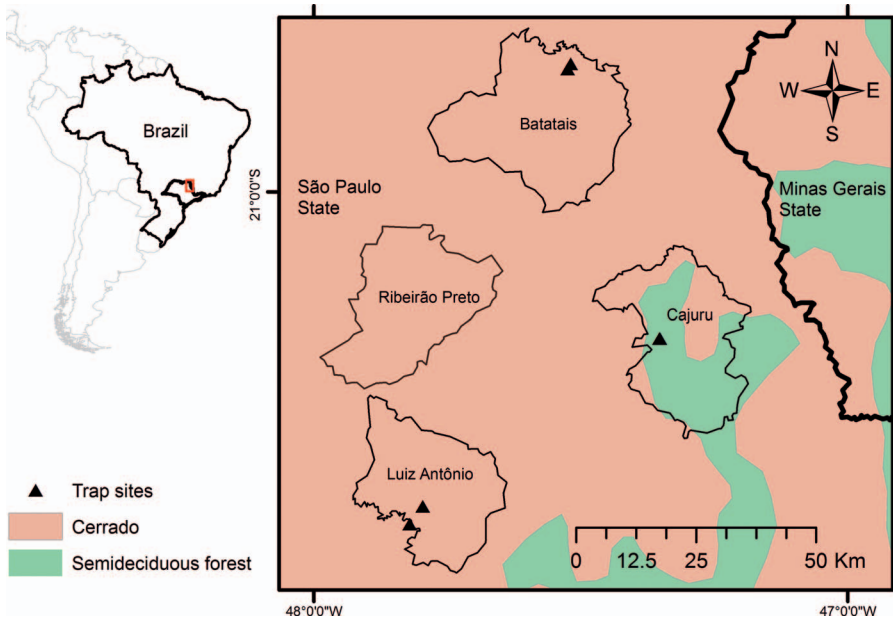

FIGURE 1. Study area. Map highlighting the northeastern region of São Paulo State, southeastern Brazil. Triangles show trap sites where wild rodents were captured.

deployed at least 1 km from the grids. Traps were baited with a mixture of crunchy peanut butter and oatmeal and the grids and transect were set for at least two nights. Animals were anesthetized using Halothane™ (Sigma-Aldrich, St. Louis, Missouri, USA), and blood was obtained from the retro-orbital sinus using heparinized capillary tubes. Small mammals were identified by external morphologic characters with taxonomic keys (Bonvicino et al. 2008). When needed, identification to species level was based on a fragment of the *cyt-b* gene following standard protocols (Salazar-Bravo et al. 2013).

Captured animals were handled and sampled according to recommendations of Mills et al. (1995), and under protocols approved by the Chico Mendes Institute of Biodiversity Conservation (Ministry of Environment, Brasília, Distrito Federal, Brazil) and the Ethics Committee for Animal Research of the School of Medicine in Ribeirão Preto, University of São Paulo (0115-07 and 115/2008, respectively). Our sampling effort resulted in 11,700 trap-nights, during which 560 animals were trapped. Average trap success was 4.8 individuals per 100 trap-nights. All these animals belong to two rodent families, Cricetidae and Muridae.

Rodent blood samples were screened using an indirect ELISA for detection of immunoglobulin G (IgG) antibodies against a recombinant nucleocapsid protein (rNP) of Junín virus (JUNV) as described by Machado et al. (2010). The ELISA detected six rodents with IgG antibodies reactive to the JUNV rNP antigen for an overall prevalence of 1.07%. When analyzed by species we observed a differential spectrum of prevalence as shown in Table 1. The murid rodent, *Mus musculus*, had the highest IgG titers. *Necromys lasiurus* had the same titer as one of the three specimens of *Calomys tener*, but it was only one dilution greater than *Riphidomys mastacalis*. Although *M. musculus* is the natural reservoir of the ubiquitous lymphocytic choriomeningitis virus (LCMV), studies in our laboratory and others suggest that JUNV rNP does not react, or only weakly reacts, with the LCMV nucleoprotein (Machado et al. 2010; Fukushi et al. 2012). In follow-up studies, we intend to use an alternative, specific test against LCMV to reject the hypothesis that cross-reaction against this virus biased our

TABLE 1. Distribution of rodents captured according to species and prevalence of antibody to Junín virus recombinant nucleocapsid protein by immunoglobulin G (IgG) enzyme-linked immunosorbent assay test.

Species	Captured animals	Antibody-positive animals	Antibody prevalence (%)	IgG titer[a]
Necromys lasiurus	212	1	0.47	1:400
Akodon montensis	151	0	0	0
Calomys tener	103	3	2.91	1:200/1:200/1:400[b]
Oligoryzomys nigripes	45	0	0	0
Mus musculus	27	1	3.70	1:6400
Euryoryzomys russatus	13	0	0	0
Rhipidomys mastacalis	5	1	20.0	1:200
Oxymycterus dasytrichus	4	0	0	0

[a] Average of four independent experiments.

[b] IgG titer from the three specimens of *C. tener* that were antibody positive.

preliminary results. The phyllotine rodent *Calomys tener* was reported as the possible natural host for Pinhal virus, a new Clade C mammarenavirus closely related to Oliveros mammarenavirus (OLVV; Bisordi et al. 2015). *Necromys lasiurus* should be considered the reservoir species of OLVV. This statement is based on recent changes to *Necromys* systematics and taxonomy whereby the species formerly known as *Necromys benefactus*—historically considered the natural reservoir of OLVV—was relegated to the synonymy of *N. lasiurus* (D'Elía et al. 2008). In addition, a recent report documented the cocirculation of OLVV and Latino virus at two localities in Mato Grosso do Sul State, in the midwestern Brazil (Fernandes et al. 2015). However, antibodies to mammarenavirus antigens had not been previously reported in *R. mastacalis*; rodents of this broadly distributed species may serve as potential natural reservoirs for mammarenaviruses, but further studies are needed. Our understanding of the biology of all these viruses will be improved by understanding the ecology of their natural hosts and potential alternate hosts. Results reported here are as part of an ongoing study on mammarenavirus ecology among wild small mammals in Brazil. Future manuscripts will report ongoing attempts to amplify and further characterize the viral genomes that appear to be circulating in the study region.

We thank the São Paulo State Research Foundation for support of this research under grants 2008/06411-4 to G.S.-S.Jr. and 2008/50617-6 to L.T.M.F. This research was also supported by NIH grant NIH 1 R03 TW007813 to C.B.J., L.T.M.F., D.G.G., and J.S.B. We thank Mario Figueiredo, Felipe Morais, Jaqueline Silva, Paulo Paulosso, and Dercílio Pavanelli for help with fieldwork. We are grateful to Edison Montilha, the Jataí Ecological Station manager for his attention and Armando Nascimento who provided access to his farm Santa Gabriela in Batatais County. We appreciate the help of José Teotônio and the Secretary of Health of Cajuru County. Thanks to Renata Muylaert for the map construction.

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Submitted for publication 27 September 2015.
Accepted 12 March 2016.