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Ecological study of hantavirus infection in wild rodents in an endemic area in Brazil

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ABSTRACT

A 3-year ecological study of small mammals was carried out in an endemic area for hantavirus pulmonary syndrome in the state of Santa Catarina in Southern Brazil. A total of 994 rodents of 14 different species corresponding to the subfamilies of Sigmodontinae, Murinae, Eumysopinae, and Caviinae were captured during 2004–2006. *Oligoryzomys nigripes* and *Akodon montensis* were the most abundant species and showed a clear seasonal pattern with higher population sizes during the winter. Rodent population outbreaks, associated within bamboo mast seeding events, were detected predominantly in areas where hantavirus pulmonary syndrome cases were notified in the state. Antibody reactivity to *Hantavirus* was detected in five sigmodontine species: *O. nigripes* (39/435), *A. montensis* (15/318), *Akodon paranaensis* (4/37), *Thaptomys nigrita* (1/86) and *Sooretamys angouya* (1/12). The highest hantavirus antibody prevalence occurred during the period of highest population size in *A. montensis*. For *O. nigripes*, hantavirus prevalence was higher in late spring, when reproduction was more frequent. Co-circulation of Juquitiba (JUQV) and Jabora (JABV) viruses was observed – JABV in *A. paranaensis* and *A. montensis*; JUQV in *O. nigripes* and *T. nigrita*. JABV occurrence was associated to gender and population size of the rodent while JUQV was related to gender, season, temperature, and locality.

1. Introduction

The genus *Hantavirus* (family Bunyaviridae), a group of rodent/insectivore-borne RNA viruses, is widely distributed in the world and includes a variety of strains recognized as human pathogens (Hjelle and Torres-Pérez, 2010; Jonsson et al., 2010; Schmaljohn and Hjelle, 1997). Rodents of the families Muridae and Cricetidae were described as the primary zoonotic reservoirs of these viruses, but distinct hantaviruses have also been discovered in several species of shrews and moles (Arai et al., 2007; Kang et al., 2009; Klempa et al., 2007; Yadav et al., 2007). Transmission is assumed to occur through human inhalation of aerosolized virus from rodents' urine and/or feces, direct (agonistic encounters), or indirect (contaminated food or environment) contact among rodents (Sauvage et al., 2003).

Hantavirus cardiopulmonary syndrome (HCPS) is considered as one of the major emerging diseases in Brazil, mainly owing to its high mortality rate (~40%). Since 1993, over 1600 cases of HCPS have been reported and at present, there are eight hantavirus described in Brazil related to Sigmodontinae rodents: Juquitiba/Araucaria, Araraquara, Castelo dos Sonhos, Anajatuba, Laguna Negra, Rio Mearim, Jabora, and Rio Mamore viruses carried by *Oligoryzomys nigripes*, *Necromys lasiurus*, *Oligoryzomys utiaritensis*, *Oligoryzomys fornesi*, *Calomys callidus*, *Holochilus sciureus*, *Akodon montensis*, and *Oligoryzomys microtis*, respectively (Oliveira et al., 2011; Firth et al., 2012; Johnson et al., 1999; Raboni et al., 2009, 2005; Rosa et al., 2005; Suzuki et al., 2004; Travassos Da Rosa et al., 2012; Travassos da Rosa et al., 2011, 2010), the latest three were not described as causing human HCPS.

Outbreaks of different genotypes of *Hantavirus* in Brazil have often been related to periods of high population sizes of sigmodontine rodents that are commonly associated to

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agricultural and peridomestic rural environments (Mills and Childs, 1998; Suzuki et al., 2004). These rodents have predominantly granivorous feeding habits and opportunistic reproductive strategies (Gentile et al., 2000). These features allow them to reach high population sizes in certain periods, mainly in productive cropland areas responding quickly to environmental changes. Both the abundance of hantavirus reservoir species and the serostatus within the reservoir population are associated with a suite of biotic and abiotic environmental variables, such as precipitation, habitat quality, and food availability (Mills, 2005).

Ecological studies of hantaviruses are still lacking in Brazil. Little is known about the ecology and temporal dynamics of hantavirus infection in host populations, since most of the studies were punctual in space and time (Oliveira et al., 2009; Raboni et al., 2009; Suzuki et al., 2004; Travassos da Rosa et al., 2010). In the Municipality of Jaborá, an endemic area of HCPS in Southern Brazil, we observed the co-circulation of Juquitiba and Jabora viruses in *O. nigripes* and *A. montensis* and recently described the genetic characterization of these two hantaviruses in this region (Oliveira et al., 2011). The present paper reports a population dynamic study of the hantavirus rodents' reservoirs in this area, investigating the factors related to the hantavirus infection and transmission in the rodent populations during three years.

2. Materials and methods

2.1. Study area and sampling

An ecological study of small mammals was carried out in the municipality of Jaborá located in the mid-western region of Santa Catarina State, in Southern Brazil (Fig. 1). Jaborá has 4041 inhabitants (IBGE – Brazilian Institute of Geography and Statistics) and most of them (60.28%) live in rural areas. The economic development of this region is based on farming and cattle raising (agricultural activities). The study area presents a mixed ombrophilous forest that is a vegetation type in the Brazilian Atlantic Rainforest (Rizzini, 1997). Nowadays, native vegetation is being replaced by cultivated areas (mainly cornfields), secondary forests, and shrublands. The climate is subtropical humid mesothermal (Cfb, Köppen) with an annual temperature average of around 18.7 °C, and a total accumulated rainfall of 1930 mm (mainly in summer). The mean altitude is about 689 m (Nimer, 1989).

The National Institute of Meteorology of Brazil (INMET) provided the monthly data on air temperature (average) and rainfall (accumulated) obtained from the Campos Novos Station in Santa Catarina for 2004–2006. This was the closest and most representative weather station around Jaborá municipality. During the study period (March 2004 to December 2006), the average temperature in the warm months (November to April) ranged from 17.5 °C to 22.1 °C, and in the cold months (May to October) the corresponding range was 11.2–18.2 °C. The mean monthly rainfall was 143.1 mm (range: 32.1–381.4 mm) and 165.6 mm (29.4–323.1 mm) in the warm and cold months, respectively (Fig. 2).

Captures were conducted in four small rural properties formed by patches of secondary vegetation intermixed with subsistence plantations under the same climatic conditions and the same vegetation domain (mixed ombrophilous forest). (1) Avelino Vieira Farm (AV): small forest fragment with disturbed understory, sparse trees and shrubs, grasslands, surrounded by pastureland and cornfields. (2) Avelino Cumerlato Farm (AC): shrub vegetation with brushwood, grass, and understory of bamboo thickets along a stream near dwellings and cornfields. (3) Domingos Vieira Farm (DV): small forest fragments with sparse high trees and dense bamboo tickets surrounded by grasslands and dirt paths. (4) Linha Castelhano (LC): pasturelands mixed with short trees along a dirt road. On each rural property, transects of traplines were placed in peridomestic environments, cornfields, shrub, bamboo thickets, and forest borders.

The rodents were captured during three consecutive years, in fall, winter and spring, totaling ten capture sessions between 2004 and 2006. In 2006, two capture sessions were carried out during the winter owing to bamboo mast-seeding, which occurred in November and December of 2005 (approximately 6 months before). Each capture station was sampled with Sherman® (7.62 cm × 9.53 cm × 30.48 cm) and Tomahawk® (40.64 cm × 12.70 cm × 12.70 cm) live traps, placed 10 m apart, in linear ground transects of 20 capture stations. The bait was a mixture of bacon, peanut butter, banana, and oatmeal. The traps were set in the late afternoon and inspected in the early morning on five consecutive days.

Each animal was anesthetized and euthanized in accordance with the Guidelines for the Care and Use of Laboratory Animals of Oswaldo Cruz Foundation, Brazil. Blood was collected by heart puncture from each anesthetized rodent. Tissue samples of liver, spleen, kidney, lung, and heart were collected and stored immediately in liquid nitrogen for further processing. All the animals collected were deposited as voucher specimens at the National Museum of the Federal University of Rio de Janeiro.

Capture of small mammals were authorized by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA)/Chico Mendes Institute for Biodiversity and Conservation, and it was carried out and handled according to recommended safety procedures (Gannon and Sikes, 2007; Mills et al., 1995).

2.2. Small mammal taxonomy

Rodents and marsupial species were identified by external morphology and cranial characteristics. Taxonomy of cryptic rodent species was confirmed by karyotypical analysis according to Bonvicino et al. (1996). Analyses of DNA sequence data of the mitochondrial cytochrome b gene were also carried out to confirm species identification for specimens without cell material for karyotypical analysis and to estimate the phylogenetic relationship of hantavirus-positive specimens (specimens with and without karyotyping data) following the procedures previously described in Oliveira et al. (2011).

2.3. Rodent hantavirus infection diagnostics

2.3.1. Serology and molecular analysis

Rodent serum samples were examined for IgG antibodies against the recombinant Andes nucleocapsid (N-ANDV) protein used as the specific antigen by enzyme-linked immunosorbent assay (Padula et al., 2000) as described by Oliveira et al. (2011).

Total RNA was extracted from lung and kidney tissues of antibody positive rodents by using the PureLink Micro-to-Midi Total RNA Purification System kit (Invitrogen, San Diego, CA) following the manufacturer's protocol. Nested reverse transcription-PCR (RT-PCR) of genome partial S-segment was performed as described previously (Oliveira et al., 2011). For RNA purification, the Wizard®SV Gel and PCR Clean-Up System kit (Promega, Corp., Madison, WI, USA) was used according to the manufacturer's recommendations and the strands were directly sequenced. Direct nucleotide sequencing of amplicons was performed using BigDye®Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's recommendations, and the reaction was run in an ABI Prism 3130x (Applied Biosystems). Nucleotide sequences were analyzed by using MEGA5 software,