Population Ecology of Hantavirus Rodent Hosts in Southern Brazil

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Abstract. In this study we analyze population dynamics of hantavirus rodent hosts and prevalence of infection over a 2-year period in Southern Brazil, a region with a high incidence of hantavirus pulmonary syndrome. The 14 small mammal species captured were composed of 10 rodents and four marsupials, the six most abundant species being *Akodon serrensis*, *Oxymycterus judex*, *Akodon montensis*, *Akodon paranaensis*, *Oligoryzomys nigripes*, and *Thaptomys nigrita*. These species displayed a similar pattern with increasing population sizes in fall/winter caused by recruitment and both, increase in reproductive activity and higher hantavirus prevalence in spring/summer. Specific associations between *A. montensis*/Jaborá Virus (JABV) and *O. nigripes*/Juquitiba-like Virus (JUQV-like) and spillover infections between *A. paranaensis*/JABV, *A. serrensis*/JABV, and *A. paranaensis*/JUQV-like were observed. Spillover infection in secondary hosts seems to play an important role in maintaining JABV and JUQV-like in the hantavirus sylvatic cycle mainly during periods of low prevalence in primary hosts.

INTRODUCTION

Hantavirus pulmonary syndrome (HPS) in Brazil is a zoonosis usually associated with rural activities in disturbed environments or in sylvatic interface areas.^{1,2} Since the first HPS case in 1993, more than 1,600 cases have been confirmed in Brazil, distributed among all regions, mainly in the southern states of Paraná, Santa Catarina, and Rio Grande do Sul.³ The State of Paraná is the fourth in HPS occurrence with two hantavirus genotypes described, Araucaria Virus (ARAV) and Jaborá Virus (JABV).^{4–6} Because Araucaria Virus is recognized as a Juquitiba-like Virus (JUQV-like), we adopt this nomenclature herein to report this hantavirus genotype.

So far, five hantavirus genotypes associated with HPS cases have been described in rodent hosts and humans in many Brazilian regions: 1) Juquitiba Virus (JUQV) distributed in the Atlantic Forest in the South and Southeastern regions, the host being *Oligoryzomys nigripes*,^{4,7} with seroprevalence of 2.5–18.3%^{6–9}; 2) Araraquara Virus (ARAV) with a wide distribution in the Cerrado Biome (Central plateau and Southeastern), transmitted by *Necromys lasiurus*,⁷ with seroprevalence of 1.2–11%^{7,10–12}; 3) Laguna Negra-like Virus (LNV) from a transition area of Cerrado and Amazon, restricted until now to Mato Grosso State in Northwestern Brazil, associated with *Calomys callidus*,^{13,14} with a seroprevalence of 17.4%¹³; 4) Castelo dos Sonhos Virus (CASV) from North and Northwestern Brazil in the Amazonian State of Pará, and in a transition area with Cerrado in the State of Mato Grosso, harbored by *Oligoryzomys utiariensis*,^{15–17} with seroprevalence of 4.5%¹⁶; and 5) Anajatuba Virus (ANAJV) from lowland areas of the Amazon Forest Biome in the

northeastern state of Maranhão, transmitted by *Oligoryzomys fomesi* with seroprevalence of 41.7%.¹⁸ Another four genotypes were described only in rodents without any human infection reported: 1) Seoul Virus (SEOV) associated with the sinanthropic species *Rattus norvegicus*^{19,20} in the Amazonian State of Pará in Northern Brazil; 2) Rio Mearim Virus (RIMEV) from the lowlands of the Amazon Forest, sympatric to ANAJV in Northeastern Brazil, whose host is *Holochilus sciureus*, with seroprevalence of 29.4%¹⁸; 3) Rio Mamore Virus (RMV) from North Brazil in the Amazon Forest, whose host is *Oligoryzomys microtis*²¹; and 4) Jaborá Virus (JABV) distributed in the Atlantic Forest from the Southern region harbored by *Akodon montensis* with seroprevalence of 14.5%.⁹ However, SEOV and RMV have also been associated with human infection in other countries.^{22,23}

Five of these nine genotypes are also present in rodent hosts from other South American regions: 1) JUQV in Paraguay,²⁴ Argentina,²⁵ and Uruguay²⁶ with seroprevalence of 3.2–11.9% in *O. nigripes*^{25,26} and 6% in *Oxymycterus* sp.²⁶; 2) LNV in Paraguay,^{24,27} Bolivia,²⁸ and Argentina,²⁹ with seroprevalence of 5.1–8.1% in *C. callosus*^{28,29} and 23% in *C. laucha*³⁰; 3) RMV in Bolivia^{28,31,32} and Peru³³ with seroprevalence of 7.7% in *O. microtis*²⁸; 4) JABV in Paraguay²⁴; and 5) SEOV distributed worldwide.

Despite the general rule of specificity between hantavirus genotypes and rodent host species, an increasing number of studies have reported hantavirus spillover into secondary hosts.³⁴ However, little information about natural history of spillover infection on the host population dynamics is available. South American hantavirus genotypes do not correspond with the same level of co-evolution in studies with hantaviruses in Europe and Asia, thus affording great potential for host switching and adaptation.³⁵ In Brazil, spillover has been witnessed in many hantavirus genotypes and host species, like ARAV harbored by secondary hosts, such as *A. montensis*, *O. nigripes*, *Thaptomys nigrita*, and *Juliomys* sp.,^{36,37} and species of marsupials and bats.³⁸ Furthermore, JUQV-like and JABV have been reported in secondary

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FIGURE 1. General Carneiro municipality, State of Paraná, southern Brazil.

hosts in Southern Brazil, JUQV-like associated with *Akodon paranaensis*,⁶ *A. montensis*, and *Oxymycterus judex*,⁵ and JABV with *Akodon serrensis*⁶ and *A. paranaensis*.³⁹

Studies of small mammals have clarified many aspects on population ecology of Brazilian rodents, mainly in the Atlantic Forest.^{40–49} Notwithstanding the great diversity of rodents in Brazil (about 240 species),^{50,51} consequently providing a high potential for many new hantavirus hosts, no studies have considered the infection dynamics. Population ecology studies are crucial for understanding the temporal patterns of infection in host populations and furnishing data on epidemiology, outbreak prediction, and risk of human infection.^{52–60}

In this study, we analyze population dynamics of hantaviruses in wild rodent hosts and prevalence of infection over a 2-year period from December 2009 to December 2011 in General Carneiro municipality, State of Paraná, Southern Brazil. This municipality has a high incidence of HPS, mainly affecting rural workers of pine tree management. This region

also is inhabited by infected rodents with spillover infection of JUQV-like and JABV genotypes.^{5,6}

MATERIALS AND METHODS

Field data: study area and small mammal sampling. The fieldwork was conducted in General Carneiro municipality, State of Paraná, Southern Brazil (Figure 1), located in a Mixed Ombrophilous Forest region. We studied different vegetation types, including native forests of *Araucaria angustifolia* (Bertol.) Kuntze 1898, native forest with anthropic disturbance, and exotic pine tree reforestation areas (*Pinus elliotti* and *Pinus taeda*) for timber trade.

The climate is humid subtropical (Cfa), according to the Köppen climate classification, with mild summers and cold winters, and without dry seasons.⁶¹ The Technological Institute SIMEPAR provided monthly data of average temperature and accumulated rainfall from December 2009 to December 2011 obtained from Palmas Station, Paraná (Figure 2), the

FIGURE 2. Climatic diagram of the study area obtained from December 2009 to December 2011. Rainfall (mm) = dark bars; Average temperature (°C) = Dark line.

closest and most representative weather station in proximity to General Carneiro municipality.

Rodent sampling was conducted every 3 months from December 2009 to December 2011, during five consecutive nights for each of the nine capture sessions. All capture sessions took place at the end of each season as follows: December in late spring, March in late summer, June in late fall, and September in late winter. The capture effort was constant in all capture sessions. We established 12 transects with 20 capture stations setting Tomahawk (Tomahawk Live Trap, Hazelhurst, WI) (40.64 × 12.70 × 12.70 cm) and Sherman traps (HB Sherman Traps, Tallahassee, FL) (7.62 × 9.53 × 30.48 cm), 300 traps in each capture night, baited with a mixture of bacon, peanut butter, banana, and oatmeal.

Specimens were captured, anesthetized, and euthanized according to recommended safety procedures⁶² and under the Guidelines for the Care and Use of Laboratory Animals, Oswaldo Cruz Foundation, Brazil (FIOCRUZ, License number L-049/08). Animals were captured with authorization of the Chico Mendes Institute for Biodiversity Conservation (ICMBIO Authorization 13373). Specimens were then measured, weighted, sexed, and identified by both karyotyping^{63,64} and cranial morphology/morphometry. Hantavirus antibody-positive specimens were confirmed by molecular analysis (amplification of the cytochrome *b* gene).

Hantavirus infection analysis. Serum and tissue samples were obtained from all small mammals captured and submitted to immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) for detection of hantavirus antibodies with the N-Araraquara hantavirus recombinant nucleocapsid protein (USP, Ribeirão Preto, Brazil).⁶⁵ Samples of December 2009 and March 2010 captures were also tested with the Hantec Kit (FIOCRUZ-ICC, Curitiba, Brazil).⁶⁶ Total RNA was extracted from lung, liver, or kidney tissue samples of hantavirus antibody-positive rodent species with the PureLink Micro-to-Midi total RNA Purification Kit (Invitrogen, San Diego, CA). The rodent samples were analyzed using the polymerase chain reaction with reverse transcription (750 base pair [bp]) and nested reactions (478 bp) of the S segment, with primers AG04-25F (5' TAGTAGACTCCTTGAKAAGCT 3'), AG733-752R (5' GAYTGGATGGAAAGGATWGA 3'), and AG274-291F (5' CCCTGTTGGATCAACTGG 3').^{39,67} For purification we adopted WizardSV Gel and the PCR Clean-Up System kit (Promega, Corp., Madison, WI) and for sequencing the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Grand Island, NY). Virus sequences were analyzed with MEGA5 software, and hantavirus genotypes were identified with BLAST (<http://blast.ncbi.nlm.nih.gov>) through comparison with other hantavirus sequences.^{68,69}

Data analysis and seasonal patterns. Population size, reproductive status of females, and age structure were analyzed by species and year/season. Population size was estimated as the absolute number of captured specimens over a constant trapping effort. Lactating and pregnant females were considered as reproductively active. Females were classified as mated or unmated by uterus analysis, thus females with tumescence and marks of previous pregnancy were classified as non-virgins. Frequency of reproductively active females of the total number of mated females in each capture session was calculated to determine the reproductive season. Total sex ratio of each species was tested in the expected 1:1, with a χ^2 analysis. Age structures were based on body weight (grams),

dividing individuals into three classes with approximately the same number of animals.⁵⁴

We recorded the presence of wounds and scars on the tail or ear as indicative of antagonistic encounters, the main form of hantavirus transmission among rodents.⁷⁰ Prevalence of infection was calculated for each species, regarding sex, age class, number of specimens, and capture season. The χ^2 test with Yates correction was adopted to test the association with these data.

Influence of monthly rainfall, average monthly temperature, and reproducing females in population sizes was analyzed by multiple linear regression considering backward criterion for a 1-, 2-, and 3-month time lag. Population sizes were also included in this analysis to verify the influence of these variables on prevalence.

RESULTS

Population analysis and seasonal patterns. The 14 small mammal species consisted of 10 rodents (Rodentia, Sigmodontinae: *Akodon serrensis* [*N* = 401]; *Oxymycterus judex* [*N* = 294]; *Akodon montensis* [*N* = 253]; *Akodon paranaensis* [*N* = 87]; *Oligoryzomys nigripes* [*N* = 73]; *Thaptomys nigrita* [*N* = 70]; *Sooretamys angouya* [*N* = 13]; *Delomys dorsalis* [*N* = 2]; *Nectomys squamipes* [*N* = 2]; and *Brucepattersonius iheringi* [*N* = 2]); and four marsupials (Didelphimorphia, Didelphidae: *Monodelphis dimidiata* [*N* = 34]; *Philander frenatus* [*N* = 9]; *Gracilinanus* sp. [*N* = 5]; and *Lutreolina crassicaudata* [*N* = 1]).

Population dynamics and prevalence were analyzed for *A. serrensis*, *O. judex*, *A. montensis*, *A. paranaensis*, *O. nigripes*, and *T. nigrita*, the six most abundant species. All species displayed similar seasonal patterns of population sizes, with higher values during the cooler months (from June to September) (Figure 3). This pattern was emphasized by *A. serrensis*, *A. montensis*, *O. nigripes*, *T. nigrita*, and *A. paranaensis*, this latter species still maintaining higher population values until December 2010. *Oxymycterus judex* presented a quite different pattern, with more homogeneous population sizes and less marked peaks (Figure 3).

All six species exhibited a reproductive pattern with higher reproductive frequency during the warmer months (from December to March) (Figure 3). *Akodon serrensis* and *A. montensis* also reproduced in June and September, although at lower rates. *Oxymycterus judex* presented a reproduction activity with similar rates throughout the year in accordance with population size dynamics data.

Age classes were based on weight. *Akodon serrensis* males (♂) and females (♀) were divided into class I (11–22 g), class II (23–26 g), and class III (27–40 g for ♂ and 27–45 g for ♀), *A. montensis* class I (7–25 g ♂ and 8–21 g ♀), class II (26–31 g ♂ and 22–28 g ♀), and class III (32–49 g ♂ and 29–44 g ♀), *A. paranaensis* class I (14–31 g ♂ and 17–25 g ♀), class II (32–41 g ♂ and 26–34 g ♀), and class III (44–61 g ♂ and 35–48 g ♀), *O. judex* class I (22–82 g ♂ and 34–69 g ♀), class II (83–110 g ♂ and 70–87 g ♀), and class III (111–160 g ♂ and 88–140 g ♀), *O. nigripes* class I (10–20 g ♂ and 9–15 g ♀), class II (21–25 g ♂ and 16–19 g ♀), and class III (26–37 g ♂ and 20–31 g ♀), and *T. nigrita* class I (11–17 g ♂ and 11–15 g ♀), class II (18–20 g ♂ and 16–20 g ♀), and class III (21–27 g ♂ and 21–24 g ♀).

FIGURE 3. Population sizes, proportion of reproducing females, and prevalence of hantavirus infection for: (A) *Akodon serrensis*, (B) *Akodon montensis*, (C) *Akodon paranaensis*, (D) *Oligoryzomys nigripes*, (E) *Oxymycterus judex*, and (F) *Thaptomys nigrita*, in General Carneiro, between December 2009 and December 2011. (Dark bars = proportion of reproducing females; Dotted line = hantavirus prevalence; Continuous line = population size).

A high frequency of young individuals (class I and II) was observed during the cooler months (from June to September). This pattern was conspicuous mainly in *A. serrensis* and *A. montensis* (Figure 4). Adults (class III) presented a more homogeneous distribution throughout the year as expected because of population aging. However, peaks of higher adult abundance were evident during the breeding season (December to March).

Five of the six most abundant species presented male sex bias: *A. serrensis* ($\chi^2 = 4.611$, degrees of freedom [df] = 1, $P = 0.0360$), *A. montensis* ($\chi^2 = 4.271$, df = 1, $P = 0.0451$), *A. paranaensis* ($\chi^2 = 4.545$, df = 1, $P = 0.0428$), *O. judex* ($\chi^2 = 9.918$, df = 1, $P = 0.002$), and *T. nigrita* ($\chi^2 = 4.629$, df = 1, $P = 0.0422$).

We observed an inverse relationship between temperature and population sizes in *A. serrensis*, *A. montensis*, *O. nigripes*, and *O. judex* with a 1-month time lag ($R^2 = 0.552$, $\beta = -0.743$, $P = 0.022$; $R^2 = 0.580$, $\beta = -0.762$, $P = 0.017$; $R^2 = 0.748$, $\beta = -0.865$, $P = 0.003$; $R^2 = 0.532$, $\beta = -0.729$, $P = 0.026$, respectively), in *A. paranaensis* with a 1- and 2-month time lag ($R^2 = 0.630$, $\beta = -0.793$, $P = 0.011$; $R^2 = 0.708$, $\beta = -0.841$, $P = 0.004$, respectively), and in *T. nigrita* with a 2- and 3-month time lag ($R^2 = 0.829$, $\beta = -0.910$, $P = 0.001$; $R^2 = 0.874$, $\beta =$

-0.768 , $P = 0.002$, respectively). There was also an inverse relationship between rainfall and *T. nigrita* population size with a 3-month time lag ($R^2 = 0.874$, $\beta = -0.378$, $P = 0.045$). There was no significant influence of reproductive activity on population sizes whatsoever.

Hantavirus infection. We screened 26 hantavirus-infected rodents (ELISA and PCR confirmed) (Table 1), proving statistically biased to males ($\chi^2 = 7.3$; gL = 1; $P = 0.0121$). Two hantavirus genotypes were confirmed, establishing between a 95% and 99% identity with deposited sequences: i) JABV in *A. montensis*, *A. serrensis*, and *A. paranaensis* and ii) JUQV-like in *O. nigripes* and *A. paranaensis*. The other species, *O. judex*, *T. nigrita*, *S. angouya*, *D. dorsalis*, *N. squamipes*, and *B. iheringi* were not diagnosed seropositive.

Infection by JABV was apparent throughout the year with peaks during the warmer months (December–March), especially in *A. montensis*, which rendered infected specimens in eight of the nine trap sessions. Only four animals presented JUQV-like infection (Table 1) in four different capture sessions; consequently, the prevalence of JUQV-like species corresponded always to only one infected animal in each capture session (Figure 3). Likewise, JUQV-like also had a similar pattern, with infection in warmer months (Figure 3E).

FIGURE 4. Age structure of (A) *Akodon serrensis*, (B) *Akodon montensis*, (C) *Akodon paranaensis*, (D) *Oligoryzomys nigripes*, (E) *Oxymycterus judex*, and (F) *Thaptomys nigrita*, in General Carneiro, between December 2009 and December 2011 (Black bar = class I; Dark Gray bar = class II; Light Gray bar = class III).

Akodon paranaensis was the only species that harbored two hantavirus genotypes, JUQV-like (March 2010) and JABV (December 2010 and June 2011).

The majority of the infected animals belonged to class III ($N = 23$; $\chi^2 = 32.462$, degrees of freedom [df] = 2, $P < 0.0001$), two to class II (one *A. montensis* and one *A. paranaensis*), and only one to class I (one *O. nigripes*). The scar analysis revealed that 60% of all infected *A. montensis* had ear/tail scars, expressing a significant relationship between infection and scars ($\chi^2 = 9.192$; df = 1; $P = 0.0063$). No significant influence of temperature, precipitation, population

size, and reproductive activity on prevalence of *A. montensis* (the only species with sufficient data for this analysis) was evident.

DISCUSSION

The six most abundant species displayed similar patterns of population dynamics, with increasing population sizes in fall/winter (June to September) and increasing reproductive activity in spring/summer (December to March), although reproductive activity throughout the year was confirmed for

TABLE 1

Species prevalence, sex, and hantavirus genotype of infected specimens (ELISA and PCR confirmed)*

Species	No. of infected rodents/total of captured rodents	Gender	Hantavirus genotype
<i>Akodon montensis</i>	16/253 (5.91%)	16 M	JABV
<i>Akodon paranaensis</i>	5/87 (5.68%)	2 M/2 F	JABV
		1 F	JUQV-like
<i>Akodon serrensis</i>	2/401 (0.74%)	1 M/1 F	JABV
<i>Oligoryzomys nigripes</i>	3/73 (4.17%)	3 M	JUQV-like
TOTAL	26/814 (3.19%)	22 M	JABV
		4 F	JUQV-like

*PCR = polymerase chain reaction; M = males; F = females; JABV = Jaborá Virus; JUQV-like = Juititaba-like Virus.

A. serrensis, *A. montensis*, and *O. judex*. Climate analysis showed the influence of the low temperature periods on population sizes, with population increase in cooler months probably related to food availability, mainly seed production of the Brazilian Pine, *A. angustifolia*. Some authors reported the availability of mature seeds on the forest floor during fall and winter in other areas of Mixed Ombrophilous forest⁴⁶ and also associated this high seed production to rodent population increases.⁴⁵ Other studies have also reported *A. angustifolia* seeds as an important food source in the diet of these rodent species.^{71–73} In fact, we found a great amount of *A. angustifolia* seeds on the forest floor of the studied areas during late fall, many with rodent gnaw marks.

During the spring/summer more individuals of age class III were apparent, which is in agreement with the reproductive results, because reproductive activity occurs mainly in this age class. Likewise, in the fall/winter, we observed a predominance of individuals of age class I, indicating that the population increase is a result of the younger individual recruitment. These population dynamics patterns agree with other studies on Sigmodontinae ecology in both Atlantic Forest landscape types, Mixed Ombrophilous Forest,^{45,48,49} and Dense Ombrophilous Forest.^{44,47,74,75}

Hantavirus exhibited a seasonal pattern with higher prevalence in spring/summer, when population sizes were lower and both reproductive activity and numbers of adults were higher. This pattern was also witnessed for other hantavirus genotypes in North^{52,56} and South America.^{54,55,59}

Hantavirus transmission among rodents is largely accepted as a consequence of bites and inoculation of the virus from the saliva, which contains higher levels of viral RNA than urine.⁷⁶ This transmission occurs in antagonistic encounters and many authors have determined the presence of scars as indicative of this behavior.^{10,70,77,78} Aggressive encounters occur mainly in the breeding season⁷⁹ and are more frequent in adult males.^{52,54,56,58,59} Our results corroborated these postulations, with a higher hantavirus prevalence found in spring/summer, seasons with higher reproductive activity, and a higher prevalence of infection in males of age class III (adults), with correlation between the presence of scars and hantavirus infection.

In addition to antagonistic encounters, inhalation of contaminated aerosols and ingestion of a contaminated shared food source might be important modes of transmission among hosts.⁷⁶ Although *A. paranaensis* is not so dependent as *O. nigripes* on *A. angustifolia* seeds, this is an important alternative food source in cooler months, when others decrease. Both species also possessed a trophic niche overlap in the

seasons without the *A. angustifolia* seeds (Perini AA, unpublished data). In addition to the common consumption of *A. angustifolia* seeds, other food sources such as fruits, seeds, and invertebrates are also shared by *A. montensis* and *O. nigripes*.⁸⁰ Thus, the oral route by mutual food source intake may also be contributing to both intraspecific and spillover transmissions in the study area.

In addition to the already established host–parasite-specific interactions between *A. montensis*/JABV⁹ and *O. nigripes*/JUQV,⁷ we also observed spillover associations between *A. paranaensis*/JABV, *A. serrensis*/JABV, and *A. paranaensis*/JUQV-like. In previous studies in General Carneiro municipality, we first presented these two latter associations⁶ and spillover infections in *A. montensis* and *O. judex*, both infected by JUQV-like⁵; during this 2-year study, only one in five *A. paranaensis* was infected by JUQV-like. Furthermore, no spillover was observed in *A. montensis* and *O. judex*. Considering the low frequency, we cannot confirm the importance of spillover on JUQV-like maintenance in the wild. However, the occurrence of JUQV-like infection in three different genera of secondary hosts suggested the adaptation of this genotype to genetically distinct rodent species. This scenario could be a determinant to establish both hantavirus genetic diversity⁸¹ and the evolution of new hantavirus genotypes.² Furthermore, in low prevalence times in the primary host, spillover infection can be very important in maintaining the virus in the wild until spillback infection,² as was likely the case for JUQV-like spillover in *A. paranaensis*, which took place in a month without *O. nigripes* (primary host) infection.

We also attested to low relative abundance and disruptions in the population dynamics of *O. nigripes*, with the absence of animals on the December 2009 and December 2011 capture sessions, a pattern observed in other Atlantic Forest areas.^{47,75} This low abundance and instability on *O. nigripes* populations may be playing an important role to keep JUQV-like at low frequency.

Spillover infections of JABV seem to be restricted to the three *Akodon* species. We can speculate that JABV was harbored by the common ancestor of these species and remained associated with them, a fact also discussed for spillover infections between *Calomys laucha* and *Calomys callosus*, hosts of LNV in Paraguay.²⁸ However, the geographic distribution of these *Calomys* species does not overlap, unlike *A. montensis*, *A. serrensis*, and *A. paranaensis*, which occurred in sympatry in our studied area, a pattern also reported for three *Reithrodontomys* species hosts of Rio Segundo Virus (RSV) in Panama.⁸² Thus, infection of *A. serrensis* and *A. paranaensis* by JABV may be related to overlap in habitat and food sharing.

Despite the low frequency of spillover infection in *A. serrensis*, there were JABV infections in all sampling months (regardless of host species) and a high prevalence of infection in the secondary host, *A. paranaensis*. Moreover, the spillover *A. paranaensis*/JABV was also reported in another area of the Mixed Ombrophilous Forest³⁹ emphasizing the importance of this species in maintaining JABV. As in JUQV-like, JABV spillover infections seem to be important during periods of low viral prevalence in the primary host, because all spillover infections occurred in months of low *A. montensis* infection.

The JUQV-like is the genotype related to HPS cases in South and Southeastern Brazil.^{7,83,84} Until now, no studies

have related JABV to HPS, even in endemic areas. The HPS cases in General Carneiro municipality appeared every year from 1999 to 2006, but no cases were reported from 2007 to 2011, probably as a result of epidemiological vigilance by the Health Division of the State of Paraná (SESA-PR). The main labor activity related to human infection in General Carneiro municipality was Pine Tree (*Pinus elliotti* and *Pinus taeda*) management.^{83,85} Pine tree workers usually occupied precarious, temporary structures near management areas, storing food, and thus, attracting wild rodents, mainly the habitat generalist species *O. nigripes*,⁸⁵ thereby increasing probability of exposure to JUQV-like. From 2001, these precarious structures were modified to prevent wild rodent invasion. This resulted in a gradual decrease of HPS cases in the General Carneiro municipality (Health Division of State of Paraná, unpublished data).

Another explanation for the absence of HPS cases may be related to a possible change in hantavirus transmission patterns among rodents, because in the current study (2009–2011) we detected a higher prevalence of JABV (84.6% JABV and 15.4% JUQV-like), whereas in the previous study (2006), where HPS cases were reported, the prevalence of JUQV-like was higher (66.6%).⁵ Therefore, we point out that epidemiological vigilance and the low frequency of JUQV-like infection in rodents in the current study may be correlated to the absence of HPS cases in this period.

The results described here reinforce the importance of pluri-annual studies on hantavirus infection dynamics in rodent hosts. We observed a clear pattern of higher hantavirus prevalence in seasons with higher reproductive activity, greater numbers of adults, and lower population size. Increases in population occurred in cooler months with higher numbers of younger animals in accordance with resource availability.

Spillover infection reported in this study and in our previous study⁵ allows us to speculate that this host–virus association is common in this area. Therefore, we can infer that the spillover infection on secondary hosts plays an important role in maintaining JABV and JUQV-like in the hantavirus sylvatic cycle until the spillback infection for primary hosts.

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