Microhabitat characteristics of *Akodon montensis,* a reservoir for hantavirus, and hantaviral seroprevalence in an Atlantic forest site in eastern Paraguay

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ABSTRACT: Hantaviruses may cause serious disease when transmitted to humans by their rodent hosts. Since their emergence in the Americas in 1993, there have been extensive efforts to understand the role of environmental factors on the presence of these viruses in their host rodent populations. HPS outbreaks have been linked to precipitation, but climatic factors alone have not been sufficient to predict the spatial-temporal dynamics of the environment-reservoir-virus system. Using a series of mark-recapture sampling sites located at the Mbaracayú Biosphere Reserve, an Atlantic Forest site in eastern Paraguay, we investigated the hypothesis that microhabitat might also influence the prevalence of Jaborá hantavirus within populations of its reservoir species, *Akodon montensis.* Seven trapping sessions were conducted during 2005-2006 at four sites chosen to capture variable microhabitat conditions within the study site. Analysis of microhabitat preferences showed that *A. montensis* preferred areas with little forest overstory and denser vegetation cover on and near the ground. Moreover, there was a significant difference in the microhabitat occupied by antibody-positive vs antibody-negative rodents, indicating that microhabitats with greater overstory cover may promote transmission and maintenance of hantavirus in *A. montensis*. ***Journal of Vector Ecology*** **34 (1): 104-113. 2009.**

Keyword Index: Hantavirus, *Akodon montensis,* microhabitat, Atlantic Forest, Paraguay.

INTRODUCTION

Hantaviruses are zoonotic, RNA viruses that are harbored and vectored by muroid rodents of the families Muridae and Cricetidae (Schmaljohn and Hjelle 1997). Hantaviruses cause two serious diseases in humans, hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia, and hantavirus pulmonary syndrome (HPS) in the Americas (Butler et al. 1996). HPS was first recognized in 1993 during an outbreak in the southwestern United States (Hjelle et al. 1994). Since that initial outbreak, hantaviruses associated with HPS have been shown to be endemic throughout the Americas (Bi et al. 2008). Despite the wide geographic distribution of reservoir species that harbor hantaviruses and the consistent presence of the hantavirus within host populations, outbreaks of HPS are sporadic and unpredictable. This situation has resulted in intensive efforts to understand and predict the relationship between environmental factors, prevalence of hantaviruses in reservoir rodent populations, and occurrence of disease caused by hantavirus. In North America, outbreaks of HPS have been correlated with weather and climatic events, especially precipitation (Yates et al. 2002). Precipitation is presumed to increase reservoir species populations, and therefore prevalence of viral infection, by enhancing resource availability -- an extension of the trophic cascade hypothesis originally proposed to explain outbreaks of plague (Parmenter et al. 1999). Remotely-sensed indices of vegetation greenness, a surface property closely related to interannual climatic variation, have also been associated with HPS occurrence, as have topography (elevation and aspect) and host macrohabitat (Calisher et al. 2002, Glass et al. 2000, Glass et al. 2002, Mills et al. 1997).

Although the broad influence of climate on the prevalence of hantaviruses in their reservoir species is well-known, it has become apparent that occurrence of HPS cases within areas of reservoir endemicity is complex and may result from a combination of environmental and landscape factors. Increases in host rodent population density driven by climate-induced resource availability do not necessarily translate into an increase in viral infection rates within the host rodent population, or to increased HPS occurrence (Davis et al. 2005). Recent work suggests that habitat composition and structure might also promote or constrain the prevalence of hantavirus in the host population (Goodin et al. 2006, Langlois et al. 2001, McIntyre et al. 2005). Anthropogenic landscape disturbances have been linked to the presence of hantaviruses, especially in tropical or subtropical forests (Suzan et al. 2006). In other environments, human disturbance is negatively correlated with hantavirus presence (Lehmer et al. 2008). In South America, Palma et al. (2009) have shown that hantaviruses do not always occur across the full geographic range of their primary reservoir, suggesting that "hotspots" of viral occurrence may be associated with localized environmental/landscape factors. Comprehensive understanding of the hantavirus-rodent system, therefore, depends both on understanding the habitat preferences of reservoir species (Mills and Childs 1998) and on how these habitat characteristics might act to promote or inhibit viral dynamics within the reservoir population. We hypothesize that both the presence of hantavirus reservoir species and increased hantavirus prevalence within the reservoir population are associated with identifiable microhabitat characteristics as defined by Brown and Lomolino (1998). We present results that address these hypotheses for *Akodon montensis,* a reservoir for Jaborá hantavirus in Paraguay. To evaluate our hypotheses, we addressed two questions: 1) what are the microhabitat characteristics associated with *A. montensis* at an Atlantic Forest site in eastern Paraguay, and 2) do patterns of microhabitat occupation differ for individual *Akodon* that harbor hantavirus vs those that do not?

MATERIALS AND METHODS

Study area

This research was conducted within the Reserva Natural del Bosque Mbaracayú (RNBM), a World Biosphere Reserve located within Departmento Canindeyú in northeastern Paraguay. Although no confirmed HPS cases have been reported in Canindeyú, several cases have been reported from similar areas in eastern Paraguay. Moreover, the region is significant for research because of the presence of a unique hantavirus harbored by *Akodon montensis* (Chu et al. 2006). Identification of this hantavirus as Jaborá was confirmed as part of this research effort (Chu and Jonsson unpublished data).

RNBM is located within the Paraná-Paraíba subtype of the Interior Atlantic Forest (Figure 1) and is one of several privately managed protected areas in Paraguay. RNBM covers about 590 km^2, and is divided into an inner conservation core, dominated by one large forest fragment (the largest extant Interior Atlantic Forest fragment in Paraguay, see Figure 1), and an outer "buffer" area. Access to the inner core area is limited, and forest conversion does not occur in this area due to its protected status. Within the "buffer zone" of the reserve are a number of inhabited areas, including indigenous (Ache, Guarani) and rural Paraguayan communities, minifundias (small, subsistence farms), latifundias (larger commercial farms), and estancias (ranches). Although prohibited by law since 2004, some land-use/land-cover conversion continues to occur in the outer buffer zone of the reserve, typically near the inhabited areas.

Management of the area as a biosphere reserve also provides some relative "control" to compare areas surrounding the Reserve that are undergoing rapid land-cover conversion. This conversion is primarily of forested land to agriculture (crop or pasture), interspersed by smaller forest remnants (Koch et al. 2007). The intensive mosaic of surface cover characteristic of this area provides a variety of potential rodent habitat, thus providing an excellent opportunity to investigate the habitat characteristics of a predominant reservoir for hantavirus, *Akodon montensis. Akodon montensis* preferentially inhabits disturbed woodlands, also venturing into oldfields and grasslands which include forbs and brushy growth (Redford and Eisenberg 1992) and has been found to be abundant in habitats of intermediate levels of anthropogenic disturbance. The little information available on life history of this species indicates that they may breed year-round, when resource conditions permit.

Field data collection

Data collection for this study consisted of two components: 1) mark-recapture sampling of rodents for ecological (population and community) and seroprevalence analysis, and 2) vegetation sampling to determine the composition and structural characteristics of the rodents' habitat. We selected four sites on which to establish rodent trapping grids (Table 1, Figure 1). These sites were chosen to represent a cross-section of potential microhabitat types representing a continuum of human disturbance. Two of the sites, Jejui Mi A (JJA) and Jejui Mi B (JJB), were native forest sites chosen for their relatively low levels of human disturbance. Both of these sites show some evidence of selective logging, although indicators of human disturbance (stumps, etc.) are much more evident on JJA than JJB. On both sites, the observable human disturbance is more than 15 years old, and the general history of land management in this area would suggest that this selective logging probably occurred c. 1960-1990 (Cartes 2003). Rama III B (R3B), another native forest site along the edge of the reserve, was included due to its more recent anthropogenic disturbance, which probably occurred within the past ten years. Rama III A (R3A) is a deforested grassland/pasture with scattered woody shrubs and trees, chosen to represent a high level of human disturbance. No peridomestic or synanthropic sites were included in this analysis.

A mark-recapture grid was established at each of the four field sites to collect demographic and ecological information, as well as blood and other samples from rodents. These mark-recapture grids were monitored on a rotating basis between February 2005 and December 2006 (Table 2). To account for the sensitivity of rodent populations to seasonal variability, the trapping sessions were spaced throughout the year. Each of the four mark-recapture grids consisted of an 11x11 array of trap stations spaced 10 m apart over an area of ≈1 ha. Except for the initial session in 2005, each trapping campaign consisted of eight consecutive nights to enable robust estimates of population sizes. Although our primary focus in this paper is on *A. montensis*, our trapping protocol was designed to sample the total small-mammal community in each grid. Each trapping station had one standard Sherman live-trap placed on the ground, and, where vegetation structure permitted, another placed in branches or vines 2-3 m above ground to capture species that are primarily arboreal. Traps were left closed during the

Table 1. Summary of the vegetation characteristics of the four mark-recapture sites.

Trapping Site	Dominant Vegetation	General Description
Jejui Mi A (JJA)	*Chusquea ramosissima* *Sorocea bonplandii* *Merostachys* sp.	Native forest. Lowest level of human disturbance among all sites. Evidence of selective logging visible.
Jejui Mi B (JJB)	*Sorocea bonplandii* *Merostachys* sp. *Heliconia* sp.	Native forest. Lowest level of human disturbance among all sites. Evidence of selective logging visible.
Rama III A (R3A)	*Andropogon* sp. *Xyris* sp. *Merostachys* sp.	Highest level of human disturbance. Actively managed pasture land. Frequent burning. Grazing animals present.
Rama III B (R3B)	*Sorocea bonplandii* *Bromelia* sp. *Merostachys* sp.	Native forest cover, but evidence of more recent human disturbance by selective logging and road building. Characterized by numerous internal edges and canopy openings resulting in a dense understory.

day and opened and baited in late afternoon. Bait consisted of a mixture of rolled oats and peanut butter.

Traps were checked each morning, and animals were processed and released at the site of capture. For each capture, the species, date, grid, trap station number, and trap placement (ground or above ground) were noted. Upon first capture, a Passive Integrated Transponder (PIT) tag was implanted subdermally in each animal, and a small (1-2 mm) snip was taken from the tip of the tail, to provide material for DNA sequencing for confirmation of specific identity, if needed. The PIT tag number was recorded upon each recapture, along with the animal's specific identity, sex, age class, reproductive condition, weight, general health, and the presence of any lesions or scars (evidence of agonistic encounters, possibly pertaining to horizontal hantavirus transmission). If the capture was the first for the animal during each sampling session, a blood sample was obtained by retroorbital bleeding and preserved in a 2 ml cryotube in liquid nitrogen. These samples were tested for the presence of viral antibodies using Immunofluorescence Assay (IFA) with Andes virus infected Vero E6 cells as the antigen (Chu et al. 2003). Throughout the study, the presence of viral antibodies was used as an indicator of current or previous infection by a hantavirus.

Vegetation measurements were collected at each trap station to quantify microhabitat characteristics within the mark-recapture grids. From these measurements, a number of microhabitat metrics were derived. Microhabitat data was collected in June, 2006. Microhabitat variables were formed from structural and compositional elements of vegetation at the trap sites that could be expected to remain consistent throughout the study period. See Table 3 for a full list of variables including methods used, and associated references.

Analysis methods

Microhabitat suitability of the area surrounding a particular trap station was quantified by the number of *A. montensis* captured during a trapping session (seven or eight nights; see Table 2), divided by the number of trap nights. Seropositivity rate at each station was estimated by the number of seropositive rodents divided by the number of rodents captured, using only those individuals for which serosurvey results were available. We used multiple logistic regression to identify microhabitat factors associated with the presence or absence of the reservoir species and with serostatus. We chose this technique (rather than other approaches such as ordination) because of binary response variables (presence/absence and seropositive/seronegative). Two analyses were conducted, one to determine microhabitat

Table 2. Dates of data collection. See Figure 1 for location of trapping sites. Seasons, based on 30-year precipitation averages, are Dry (D: June-September), Wet (W: October-January), Transitional (T: February-May).

Trapping Site	Dates (2005)	Season	Dates (2006)	Season
JJA and JJB	15 MAR – 22 MAR	T	15 FEB – 22 FEB	T
	7 AUG – 14 AUG	D	9 JUL – 16 JUL	D
	1 OCT – 8 OCT	W	22 NOV – 29 NOV	W
R3A and R3B	12 FEB – 18 FEB	T	27 FEB – 6 MAR	T
	14 JUN – 21 JUN	D	19 MAY – 26 MAY	T
	12 SEP – 19 SEP	D	27 JUL – 3 AUG	D
	10 NOV – 17 NOV	W	3 DEC – 10 DEC	W

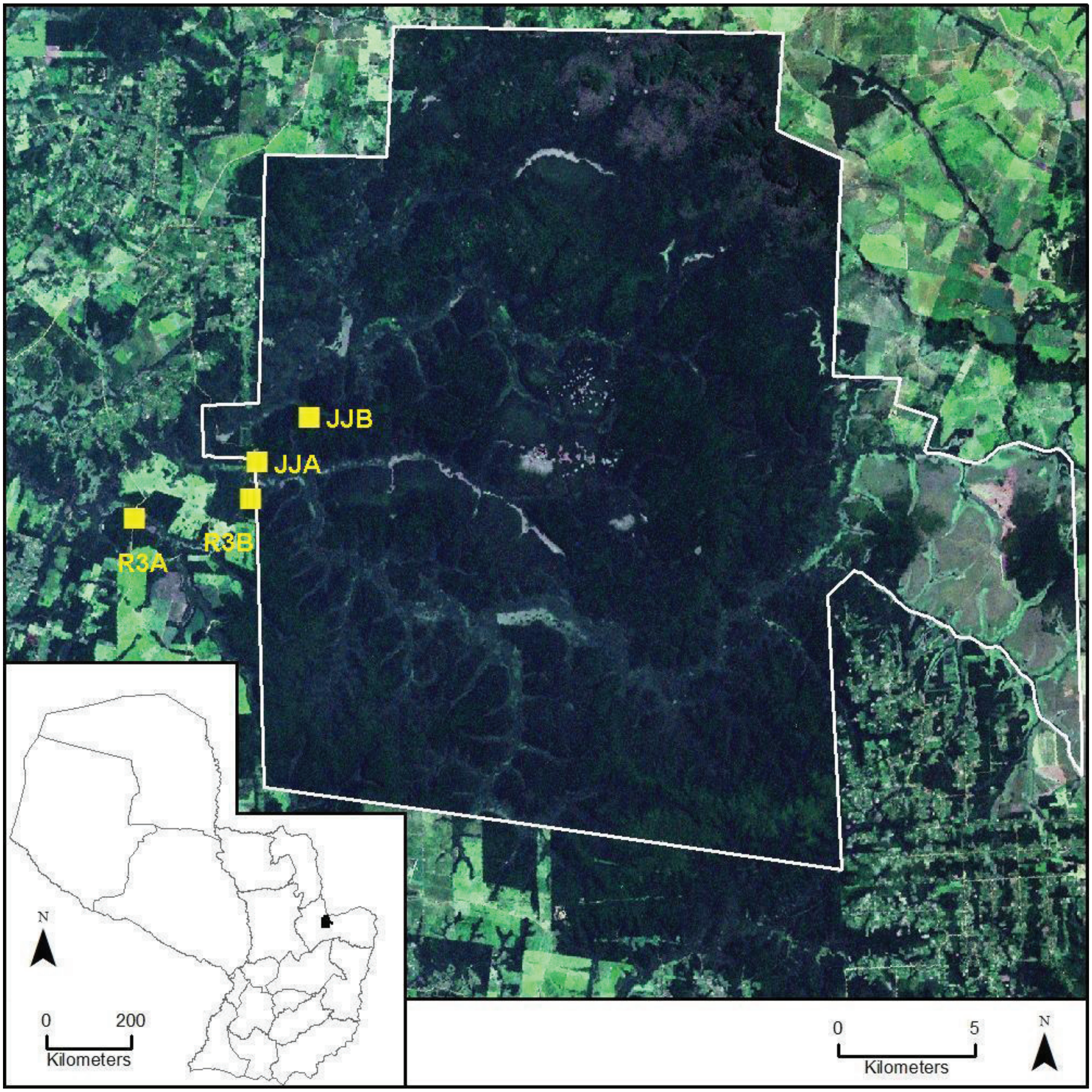

Figure 1. Location and layout of the study area. The Landsat ETM+ image (acquired February, 2003) shows the inner conservation area of the RNBM, consisting of a single large forest fragment. Yellow markers indicate the locations of the four mark-recapture grids. The inset map shows the location of the study site within Paraguay.

Table 3. Summary of vegetation analysis techniques.

Description of Variable	Symbol(s)	Technique	Reference	Notes
Horizontal Density at Ground Level, 25cm, 50cm and 100 cm	HDEN000 HDEN025 HDEN050 HDEN100	White board	(MacArthur and MacArthur 1961)	10cm x 20cm white board was placed at trap site. Measurement data type is continuous, based on normalized distance at which half of board was visible.
Vertical Canopy Density	VDEN1 VDEN2 VDEN3	Optical site (GRS Densitometer)	(Cartes 2003)	Categorical data, based on observed presence or absence of primary (> 10m), secondary (5-10m), and tertiary (1-5 m) canopy layers at each trap site.
Ground Cover	LITT BARE GRAM FORB WOOD FERN BROM BAMB	Daubenmeier	(Daubenmeier 1959)	Categorical data based on percentage cover within a 10 x 50 cm sampling frame. Measured at two random locations within a 2 x 2m area around each trap site. Cover categories were: Litter (LITT) Bare Ground (BARE) Graminoid (GRAM) Herbaceous (FORB) Woody Shrub (WOOD) Fern (FERN) Bromeliad (BROM) Bamboo (BAMB)
Total Woody Stems	TOT_STEM	Point Centered Quarter (PCQ)	(Bonham 1989)	Continuous variable. Count of all woody stems within two randomly chosen 2m x 2m areas adjacent to each trap.
Total Woody Stems >2m tall	TOT_ STEM_2m	Point Centered Quarter (PCQ)	(Bonham 1989)	Continuous variable. Count of all woody stems > 2m tall within two randomly chosen 2m x 2m areas adjacent to each trap.
Diameter at Breast Height (DBH) of all Trees >2m tall. Min, max, median, 1st and 3rd quartile values, and interquartile range determined for each site.	DBHMIN DBHQ1 DBHMED DBHQ3 DBHMAX DBHIQR	PCQ; DBH using diameter tape or caliper.	(Bonham 1989)	Continuous variable. Measurements made within two randomly chosen 2m x 2m areas adjacent to each trap. Distribution variables were derived from counts recorded at each selected site.

Table 4. Summary of trapping data and serostatus for Akodon montensis on the mark-recapture grids. Numbers in parentheses represent seropositive rodent captures in each trapping session.

	GRID			
TRAPPING SESSION[1]	R3A	R3B	JJA	JJB
FEB 2005	14(3)	110(15)	-	-
MAR 2005	-	-	80(3)	76(4)
JUN 2005	0(0)	101(15)	-	--
AUG 2005	-	-	13(0)	37(9)
SEP 2005	4(2)	60(4)	-	-
OCT 2005	-	-	39(0)	35(6)
NOV 2005	4(2)	26(12)	-	-
FEB 2006	0(0)	41(15)	19(0)	13(0)
MAY 2006	7(0)	52(14)	-	-
JUL 2006	0(0)	68(6)	8(0)	10(5)
NOV 2006	-	-	3(1)	4(0)
DEC 2006	1(0)	63(4)	-	-
TOTAL CAPTURES	30	521	162	175
SEROPOSTIVE CAPTURES	7	85	4	24
SEROPREVALENCE[2] (Percentage)	23.3	16.3	2.5	14.0

[1] See Table 2 for dates of each trapping session.

[2] Seroprevalence values were calculated using the total number of *Akodon montensis* captures only from those trapping sessions for which immunoflourescence assay results were available.

metrics associated with the presence of all *A. montensis* and the other to determine differences in microhabitat associations between *A. montensis* that were seropositive vs seronegative for hantaviral antibodies. This second analysis, evaluating viral associations with microhabitat characteristics, was necessarily restricted to only those stations where *A. montensis* was captured at least one time.

Multiple logistic regression models were fit using PROC GENMOD in SAS version 9.1.3 (SAS Institute, Inc. Cary, NC). We first used univariate analyses to identify potentially important variables. Inputs to the model included all of the microhabitat variables summarized in Table 3. To control for potential confounding factors, we also inserted several control variables including season of capture (dry, wet, or transitional, see Table 2), year (2005 vs 2006), sex, and age class (juvenile, sub-adult, adult) of captured rodents. Nonparametric smoothing was applied to plots of empirical logits to check linearity assumptions. We then fit a multiple logistic model to those microhabitat variables which were at least moderately associated with the response variables (i.e., those with p-value < 0.25; a fairly typical "cut-off" value for variable inclusion in model selection). Using an iterative process, we eliminated microhabitat variables that did not appear to be significant (when the others were present), and fit a new model. If the new model was significantly different from the old model, the deleted microhabitat variable was deemed important and was put back into the model. Multi-collinearity in the model was evaluated by inspection of parameter estimate standard errors, inspection of the correlation matrix of parameter estimates, and computation of variance inflation factors. Variables with the largest variance inflation factors in a collinear set were removed from the model, one at a time, until the variance inflation factors of the remaining predictors were all less than 2. The final model consisted of all variables remaining once the insignificant microhabitat variables had been removed. Likelihood ratio tests were used to determine the significance of variables. In addition, we included several potentially confounding non-habitat variables in order to evaluate and control for their effects on model fit. These included year, season, sex, and age class. The Hosmer-Lemeshow goodness-of-fit statistic was used as a diagnostic test to ensure that the models fit the data well (Hosmer and Lemeshow 2000).

RESULTS

Data from all trapping sessions and grids are summarized in Table 4. The large number of seropositive *Akodon* captured support the conclusions of Chu et al. (2003) that this species is the primary reservoir host for hantaviruses in the study area. All grid and trapping dates were pooled for analysis.

Table 5. Logistic regression results of *Akodon montensis* microhabitat associations. Only those microhabitat variables used in the final models are included in this table. Positive estimate values indicate that a variable is significantly correlated with the presence of *A. montensis*. Negative estimate values indicate a significant negative correlation with *A. montensis* presence. See Methods section for description of model development and Table 3 for description of microhabitat variables.

Microhabitat Variable	Estimate	X^2 (LRT)	$P > X^2$
VDEN2=0	0.175	5.42	0.0199
VDEN3=1	-0.252	11.37	0.0029
BARE	-3.100	71.94	0.0007
FORB	0.364	6.46	0.0110
TOT_STEM	-0.007	5.28	0.0216
BAMB	1.328	154.45	<0.0001
BROM	0.834	12.19	0.0005
GRAM	-1.047	9.03	0.0027
DBHIQR	0.004	6.33	0.0119
WOOD	0.543	5.28	0.0009

Hosmer-Lemeshow P-value = 0.3220.

Akodon microhabitat associations

Results of the logistic analysis of microhabitat association for all *A. montensis* are shown in Table 5. The presence of *A. montensis* was associated with the absence of both intermediate and upper canopy layers (VDEN2=0, indicating absence of the upper layer) but the presence of a tertiary (1-5m) canopy layer (VDEN=1). Larger numbers of *Akodon* were collected from areas with fewer tree stems (i.e., negative association with TOT_STEM) but greater occurrence of bamboo (BAMB). Greater interquartile range of tree diameter (DBHIQR) was also positively associated with *Akodon* presence. Among the ground-level canopy constituents, *A. montensis* was positively associated with the presence of forbs, bromeliads (BROM), and woody shrubs (WOOD), and negatively correlated with graminoids (GRAM).

The microhabitat associations revealed by the logistic model can be explained generally by the relative density of the forest canopy. In areas where a taller, more dense forest canopy was present (i.e., areas where the upper two canopy layers are present), light penetration to the forest floor was attenuated, suppressing the growth of understory vegetation such as bamboo, bromeliads, and flowering forbs. These areas of dense overstory and bare or lightly vegetated ground surface were least likely to be associated with the presence of *A. montensis*. In areas where the upper forest canopies were not present, whether due to natural tree fall or human disturbance, there was greater light penetration to the ground. These “light gaps” in the canopy were associated with the presence of woody shrubs and herbaceous plants, and also with the presence of *Akodon*. The negative association between graminoids (an herbaceous vegetation requiring higher light levels to grow) and *A. montensis* might at first seem inconsistent, but grasses in the study area are most closely associated with completely deforested sites such as R3A, where few *Akodon* were collected. The association of *A. montensis* with bamboo observed here also differs somewhat from other results for the Atlantic Forest (Püttker et al. 2008). However, the association can be explained by using the same reasoning as with the other low canopy woody species; that is, bamboo grows in areas where sunlight can penetrate due to a thin or absent upper canopy, and it provides some cover for the ground-dwelling *Akodon*.

Association of seroprevalence with microhabitat

Logistic regression was also used to test for any relationship between antibody status and microhabitat

Table 6. Logistic regression results for seropositivity, controlled by sex, season, age group, and year. Only those sites for which IFA results were available were used in this analysis. Positive estimate values indicate that a variable is significantly correlated with the presence of seropostive A. montensis. Negative estimate values indicate a significant negative correlation with seropositive A. montensis. See Methods section for description of model development and Table 3 for description of variables.

Microhabitat Variable	Estimate	X^2 (LRT)	**$P > X^2$**
GRAM	1.72	7.27	0.0070
TOT_STEM	-0.03	8.98	0.0027
DBHMED	0.01	5.59	0.0180

Hosmer-Lemeshow P-value = 0.24.

(Table 6). As with the total *Akodon montensis* population, we observed significant relationships between microhabitat characteristics and seropositive status in *Akodon*. Seropositive individuals were more likely than seronegatives to be associated with the presence of herbaceous graminoids, a significant microhabitat predictor for all *Akodon*. However, compared to the total population, seropositive *Akodon* were significantly more likely to be positively associated with trees with larger median values of DBH (i.e., higher values of DBHMED), and negatively associated with total number of woody stems (TOT_STEM). From this, we can infer that rodents that were positive for hantavirus antibodies were more likely to be found in microhabitats characterized by a denser upper forest canopy composed of a smaller number of larger diameter trees, with sparser vegetation cover at the canopy floor.

DISCUSSION

Within the Atlantic Forest, *A. montensis* is known to be a habitat generalist, but shows a preference for semi-deciduous wooded areas characterized by less dense upper canopy layers (Püttker et al. 2008) and denser vegetation cover nearer the ground (Dalmagro and Vieira 2005, Lozada et al. 2000). Our observations of microhabitat occupation by *A.* *montensis* generally agreed with these findings. Our results showed the presence of *A. montensis* in all of our trapping grids (showing its adaptability to a variety of locations), but significant patterns of microhabitat preference emerged that are relevant to understanding the role of *A. montensis* as a reservoir for hantaviruses. The greatest numbers of *A. montensis* occurred in those microhabitats that showed some evidence of anthropogenic disturbance, but where native forest was still substantially intact. In the two JeJui Mi grids (JJA and JJB), evidence of past disturbance in the form of selective logging (i.e., presence of stumps, snags, and downed trees) could be seen, along with the presence of smaller diameter secondary growth trees. Larger gaps in the upper forest layer were still present, and those areas were associated with the greatest concentrations of *A. montensis.* In the forested Rama III B grid (R3B), evidence of disturbance was more recent, but the net effect was still similar: gaps in the forest canopy were associated with denser vegetation on the forest floor and thus with preferred *A. montensis* microhabitat. In contrast, the mostly deforested grid at Rama III A (R3A) showed the fewest *Akodon* captures, most of which occurred along the side of the grid containing some brushy growth, and nearest to the forest edge.

Our results also showed a significant difference in microhabitat association between seropositive and seronegative *A. montensis.* Seropositive individuals were more likely to be found where the upper forest canopy was thicker and the vegetation at the forest floor sparser, relative to sites associated with seronegative *Akodon.* Interpreting the relationships suggests that seropositive *A. montensis* are slightly more prevalent in microhabitat that is less suitable for the species, based on the microhabitat associations indicated by the results presented in the previous section. These less suitable microhabitats can be characterized in two ways: 1) by a thicker forest overstory composed of a smaller number of larger trees (i.e., less disturbed forest), and 2) absence of dense growth on the forest floor.

The association between serostatus and microhabitat has potential importance for understanding the interaction of microhabitat with hantavirus. Our finding that microhabitat association of seropositive individuals can differ from that of seronegatives is similar to that of McIntyre et al. (2005) for Bayou hantavirus in *Oryzomys palustris*, and suggests that the prevalence of hantavirus within the reservoir population depends on more than just population size or density. Our results also show that specific microhabitats may harbor disproportionally large numbers of infected hosts, and that these microhabitat effects operate in addition to increases in reservoir population associated with trophic cascades (Yates et al. 2002). Thus, the dynamics of viral prevalence in the wild is controlled by more than just the influence of antecedent climatic conditions on the carrying capacity of the environment. Our current data do not allow any conclusions as to why microhabitat factors may influence viral dynamics, but some reasonable hypotheses can be advanced. For example, it is possible that viral infection represents a physiological burden on the infected rodents that compromises their ability to compete for more preferable microhabitats. Alternatively, rodents already occupying less preferable habitats might undergo stress-related behavioral changes (e.g., increased aggression) that increase the likelihood of infection by the virus (Lafferty and Holt 2003). Some combination of these factors might also be at work, or some other mechanism might be responsible. Regardless, the results of our analyses show a significant association between microhabitat characteristics and viral seroprevalence.

In this study, we showed that *Akodon montensis*, a hantavirus reservoir, displays definite patterns of microhabitat occupancy across a spectrum of slightly to highly disturbed Atlantic Forest sites in eastern Paraguay. Further, our analysis showed that microhabitat occupation of *A. montensis* depended to some degree on the viral serostatus of the host rodents, although with our current data it is not possible to say why this association occurred.

In addition to elucidating the relationships between the viral reservoir and its microhabitat, the results here also shed light on the problem of understanding spatial patterns of hantavirus seroprevalence. Understanding the transmission and maintenance of hantavirus in rodent populations is largely a matter of understanding the host-pathogen system within its environment. We know that inter-annual climate variation affects this system, although the proximate cause of these variations (precipitation increases) are a regional phenomenon in most places. Our results show that microhabitat composition also affects reservoir populations at scales much finer than the regional scale variability of climate.

Although hantaviral dynamics are best understood within the context of the environment-vector-pathogen system, determinations of the risk of HPS occurrence must also consider the human element. Once again, the information presented here can provide insight into predicting human risk. Within our Paraguayan study area, we know that certain land covers are more likely to be associated with human presence (Koch et al. 2007). The results reported here show that land cover characteristics (and their associated microhabitats) are also likely to be associated with reservoir rodents. Fine scale mapping schemes showing the proximity or intersection of these cover types could therefore identify areas of greater potential risk of the virus occurring within the host population and being transmitted from host to humans (Mertz and Kitron 2007). Of course, the actual process of transmission requires more than just proximity between the host organism and potential human victims; nevertheless, it is clear that the risk of transmission would be greater in places where favorable conditions for the reservoir rodents and the virus coincide with human presence. Coupling landscape-based risk assessment based on an analysis like that presented here to existing climate-based schemes (e.g., Glass et al. 2000) is a promising avenue for refining environmental risk models for HPS occurrence.

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