ECOHEALTH

Original Contribution

First Evidence of *Akodon*-Borne Orthohantavirus in Northeastern Argentina

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Abstract: Orthohantaviruses (genus *Orthohantavirus*, family *Hantaviridae*) are the etiologic agents of Hantavirus Pulmonary Syndrome in the Americas. In South America, orthohantaviruses are highly diverse and are hosted by sigmodontine rodents (subfamiliy Sigmodontinae, family Cricetidae), an also diverse group of rodents. The aims of this work were to (1) identify orthohantavirus hosts and (2) to study the spatial and temporal variations in the prevalence of infection and their associations with community, environmental and individual characteristics, in different environments of Misiones province, northeastern Argentina. Live-capture sessions were carried out during two years in different land uses, with a trapping effort of 31,653 trap nights. We captured 719 individuals from the species *Akodon montensis, Rattus rattus, Mus musculus, Calomys tener, Thaptomys nigrita, Oligoryzomys nigripes, Euryoryzomys russatus, Oligoryzomys flavescens, Brucepattersonius* sp., and *Juliomys pictipes*. Antibodies against orthohantavirus were detected in *Akodon montensis* in one natural protected and one periurban areas, and it was the most abundant species in almost every study sites. We observed the presence of spatial focality of orthohantavirus infection and a positive association with host abundance suggesting the existence of a threshold density. At the individual level, large, reproductively active, and male individuals were more likely to have antibodies against orthohantavirus. This is the first record of orthohantavirus infection in *A. montensis* in Argentina, which shows the importance of investigations about emerging diseases.

Keywords: emerging infectious diseases, hantavirus pulmonary syndrome, national parks, rodents, sigmodontinae, zoonoses

INTRODUCTION

Orthohantaviruses (genus *Orthohantavirus*, family *Hantaviridae*) are the etiologic agents of Hemorrhagic Fever

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with Renal Syndrome (HFRS) and Hantavirus Pulmonary Syndrome (HPS). The viruses causing HFRS are particularly widespread in Asia and Europe and are hosted by cricetid and murid rodents (families Cricetidae and Muridae) as *Rattus norvegicus, R. rattus* and *Apodemus agrarius*, while those causing HPS in America are hosted by cricetid rodents of the subfamilies Sigmodontinae and Neotominae (de Oliveira et al. 2014; Oliveira et al. 2014; Milholland et al. 2018). Though there have been a few reports of natural infected rodents by Seoul virus (an etiologic agent of HFRS) in South America, mainly in *R. norvegicus* (Seijo et al. 2003; Padula et al. 2010), HFRS has never been reported in this continent (Mull et al. 2020). Regarding HPS, more than 5,000 cases have been reported up to 2016 in South America, where Brazil, Argentina and Chile are the most affected countries (Pan American Health Organization 2020). Risk of acquiring HPS depends on numerous factors such as the spatial distribution of the viruses and hosts, the seasonal and multi-annual fluctuations in their prevalence and abundances, and several human-associated factors such as agricultural work, outdoor recreation and peridomestic activities (Alonso et al. 2019; Kuenzi et al. 2000). Each virus is generally hosted by one or a few closely related species, though spillover and host switching events are frequent (Mull et al. 2020).

Transmission among hosts is horizontal, either through social interactions or through the inhalation of infectious particles released into the environment (Forbes et al. 2018). Shedding of the virus occurs throughout the host's life, though some evidence suggests it is higher shortly after infection (Safronetz et al. 2008; Voutilainen et al. 2015). Infection is thought to be mostly asymptomatic for the hosts (Forbes et al. 2018), but there is some evidence supporting a decrease in growth rate and survival (Douglass et al. 2007; Luis et al 2012). The genus *Orthohantavirus* is still not fully known, and new variants and hosts are described or reclassified almost every year (Mull et al. 2020). This is particularly so in South America where its reservoirs seem to be particularly complex and diverse. To date, more than 20 variants of orthohantaviruses and more than 25 host species have been reported in South America, and approximately half of these genotypes have been associated with human disease (de Oliveira et al. 2014; Oliveira et al. 2014; Mull et al. 2020). However, because hosts and viruses are highly diverse, and studies, particularly in remote areas, have not been sufficient, the number of variants and reservoirs is almost certainly being underestimated. In Argentina, ten viruses and seven main hosts

have been described so far (López et al. 1996; Levis et al. 1998, 2004; Pini et al. 2003; Padula et al. 2007; Martinez et al. 2010). In Misiones province (northeastern Argentina), two genotypes, Juquitiba and Lechiguanas, have been found, both causing HPS. Juquitiba was found to be hosted by *Oligoryzomys nigripes*, while Lechiguanas was only isolated from humans in this province (Padula et al. 2007), though it is known to be hosted by *O. flavescens* in central Argentina (Palma et al. 2012; Vadell et al. 2011). However, other sigmodontine species known to host orthohantaviruses in other regions of Argentina and neighboring Paraguay and Brazil are also present in this province, suggesting that other genotypes may be circulating in rodent populations in northeastern Argentina. This could be the case of *Akodon montensis*, host of Apé-Aimé in Paraguay and Jaborá in Brazil and Paraguay; *A. paranaensis,* host of Jaborá in Brazil; *Necromys lasiurus*, host of Araraquara in Brazil; *Thaptomys nigrita*, host of Juquitiba in Brazil; *Callomys callidus*, host of Laguna Negra in northwest Argentina, Brazil and Paraguay and *Holochilus* sp., host of Alto Paraguay in Paraguay (Mull et al. 2020; de Oliveira et al. 2014; Oliveira et al. 2014).

To date, there is no effective treatment or approved vaccine against HPS (Brocato and Hooper 2019), and therefore, preventive measures are the only means to curtail human disease. Because HPS is the result of a spillover event from a rodent host, a correct understanding of the distribution and ecology of orthohantaviruses in their host populations is crucial in order to design effective preventive measures that could help reduce transmission to humans. To aid in reducing transmission to humans, the aims of this work were to identify orthohantavirus hosts and to study the spatial and temporal variation in the prevalence of infection and their association with community, environmental and individual characteristics in different environments of the Argentinean Atlantic Forest, Misiones, Argentina.

METHODS

Study Area

The study was conducted in Iguazú department (26°01′21.7"S 54°36′45.6"W), Misiones province, Argentina. Misiones borders to the west with Paraguay and to the east with Brazil, with the Paraná and Iguazú rivers as its west and north natural borders (Fig. 1). This area belongs
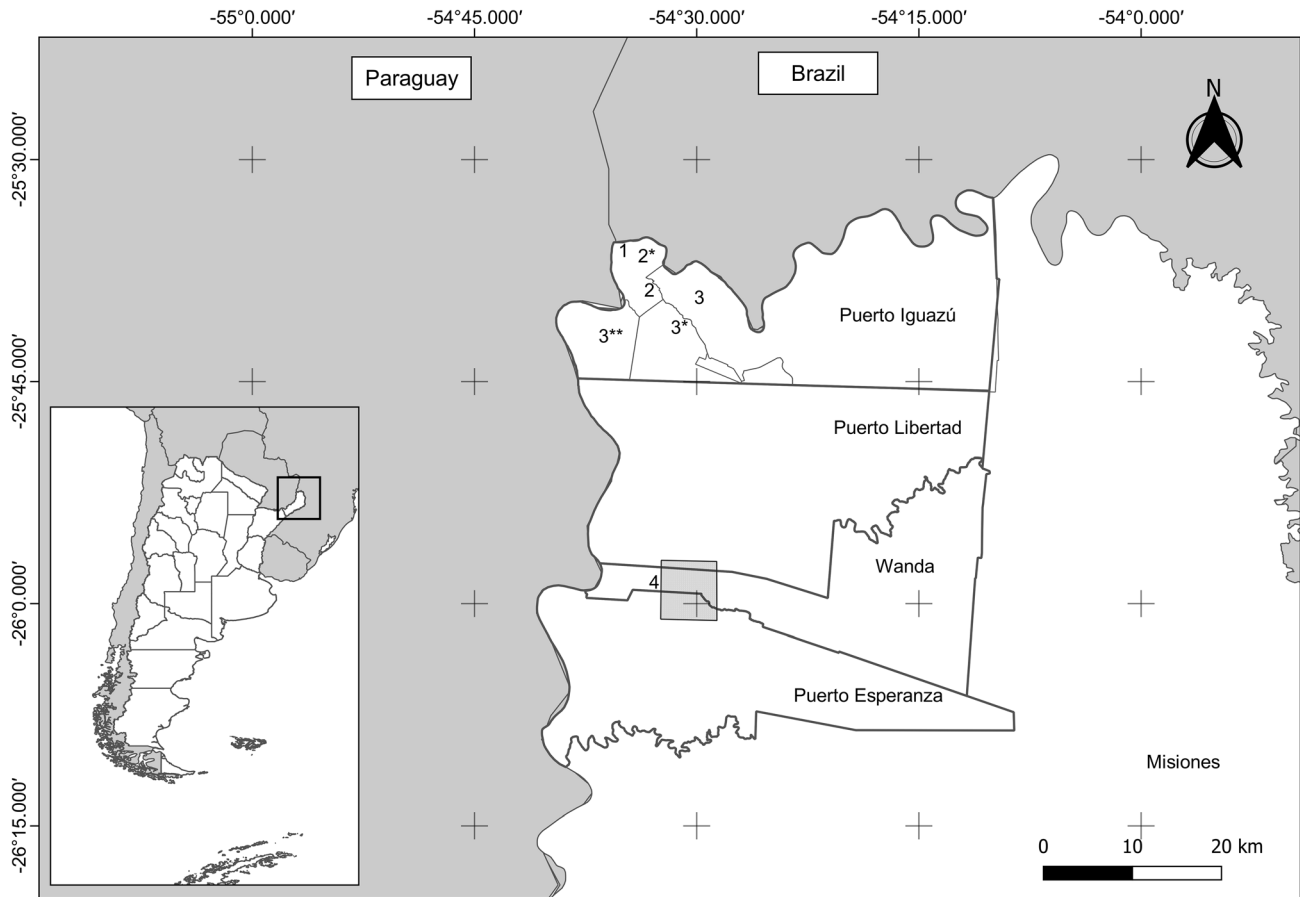
Figure 1. The Study Area in Northwest Misiones, Argentina, Included Puerto Iguazú City (1) as the urban area; the 2000 hectáreas neighborhood (2) and Reserva Indígena Iryapú (2*) as the periurban area; Reserva Nacional Iguazú (3), Parque Provincial Puerto Península (3*) and Reserva Nacional de la Defensa del Puerto Peninsula (3**) as the natural protected areas and, the area of crops and forestations as rural land use (4; Indicated with a gray box).

to the Paranaense Rainforest Ecoregion, the largest one of the Atlantic Forest ecoregion complex (Cabrera 1994). The weather is subtropical with mean minimum and maximum temperatures of 11 °C and 32 °C, respectively. Rainfalls are abundant all around the year with average annual values of 2000 mm (Plací and Di Bitetti 2005).

This study was conducted in urban, periurban, rural and natural protected areas (Fig. 1). Puerto Iguazú city (urban area) has a population around 40,000 people and its main economic activity is tourism, being the hotel and gastronomic services the main sources of employment (Nuñez 2009). The periurban area studied includes an area known as 2000 hectáreas (2,000 hectares, in English) which has low human population and where subsistence farming is the main activity, and the Mbya-Guaraní natural and cultural reserve called Reserva Indígena Iryapú (RII). In both urban and periurban areas, rodent samples were carried out in gardens and yards (in and around houses,

pigpens, henhouses and orchards, when present). The natural protected areas include the reserves Reserva Nacional Iguazú (RNI, 7,675 ha), Parque Provincial Puerto Península (PPPP, 6,900 ha) and Reserva Natural de la Defensa del Puerto Peninsula (RNDPP; 8,800 ha), all of which are part of the green corridor which aims to preserve the Atlantic Forest. The rural area is composed of fields of crops and forestations in the localities of Puerto Libertad, Puerto Esperanza and Wanda (Fig. 1).

Trapping Methods

Rodent trapping was carried out every three months from 2017 to 2020, resulting in ten seasonal trapping sessions in the urban (July 2017 to March 2020; total capture-effort = 4,035 trap nights), periurban (July 2017 to March 2020; total capture-effort = 7,245 trap nights) and rural (July 2017 to March 2020; total capture-effort = 10,935

trap nights) areas; eight trapping sessions in RNI and PPPP (November 2017 to March 2020 total effort-capture = 7,398 trap nights) and four in RNDPP (May 2019 to March 2020 total capture-effort = 2,040 trap nights). Small rodents were live-trapped using Sherman live traps (8 × 9 × 23 cm) baited with a mixture of peanut butter, fat, and rolled oats, and cage traps (15 × 15 × 30 cm) baited with meat and apple. Traps were active for three consecutive nights during each session. Approximately the same amount of Sherman and cage traps (75–90) were set each trapping session in the urban and periurban sites, except for the RII site where less Sherman traps were used (50–70) and where the use of cage traps (75) was interrupted in 2018. In rural areas, 300 Sherman traps and 150 cage traps were used per season. Finally, in natural protected areas only Sherman traps were used (200 per season in RNI and PPPP, and 150 per season in RNDPP). Traps were set in approximately the same locations in successive trapping sessions.

Captured rodents were anaesthetized, and sex, reproductive condition (active: open vagina/descended testes, and inactive: close vagina /abdominal testes), weight, total body length, body–tail length, foot length and ear length were recorded. The species were identified based on external morphology (the metrics recorded were used to double check the identification done on the field, whenever necessary). Individuals were tagged with a uniquely numbered ear tag and were released at the point of capture. Animals were handled according to the 14,346 Argentinean National Law of Animal Care.

Hantavirus Seroprevalence

A small sample of blood was obtained from each rodent through a small cut on the tip of the tail. These samples were serologically screened by IgG ELISA using the ANDV recombinant nucleocapsid protein (Padula et al. 2000a, 2000b). IgG antibodies were detected in rodent blood diluted 1:200 in PBS-Tween buffer, using peroxidase-labeled affinity-purified IgG antibodies anti *Peromyscus leucopus,* and/or anti-rat conjugate for *Rattus rattus,* in conjunction with the ABTS Microwell Peroxidase Substrate System and absorbance was measured at 405 nm. Rodents showing optical density (OD) up 0.3 were consider negatives, while those with OD higher than 0.3 were considered positive. We used antibody presence as an indication of infection.

Total RNA was extracted from lung samples from the IgG-positive rodents, when available, as previously de-scribed (Padula et al. 2000a, b). The RNA was subjected to RT-PCR targeting a conserved region of the orthohantavirus genome. Specific primers were used in order to amplify a 952nts fragment of the S-segment (positions 22 to 974 from AF324902 Gene Bank accession number).

The amplified DNA was sequenced using BigDye Terminator^{TM} v3.1 Cycle Sequencing kit and for purification the BigDye® X-Terminator Purification kit (Applied Biosystems, Foster City, CA, USA), according to the manufacture's recommendations. The nucleotide sequences were analyzed using MEGA10 software (Kumar et al. 2018). Multiple sequence alignment, nucleotide comparison, and deduced amino acid sequences were performed by Mega software.

Rodent and Environmental Variables

Variables thought to affect the dynamics of the orthohantavirus/rodent system were grouped in three: rodent community variables, weather variables, and individual variables. For community variables, we included the proportion of individuals of each rodent species, the proportion of males in the host population, species richness, and diversity of the assemblage (Shannon Index). Weather variables included mean, minimum and maximum temperatures, and accumulated rainfall. These variables were calculated for 30, 60 and 90 days prior to rodent sampling. Weather data were provided by the National Meteorological Service (Servicio Meteorológico Nacional 2020) based on data taken at the meteorological station located 12 km away from Puerto Iguazú. As individual variables we used body weight, body length, sex and reproductive condition, and body condition (calculated as following: body condition = $\sqrt[3]{\text{body}}$ weight/body length). Trap success was expressed as number of captures per 100 trap nights.

Statistical Analyses

We studied which factors modulate orthohantavirus infection using three different types of models, each with a different set of explanatory variables: weather variables, rodent community variables, and individual variables. Reproductive activity was not included in the analyses given that most individuals had the same status. Seroprevalence ((number of individuals with orthohantavirus antibodies/number of individuals tested)*100) was used as response variable in the weather and rodent community models, and the presence/absence of orthohantavirus

antibodies was used as response variable in the individual models. The analyses were conducted using generalized mixed models with forward stepping selection (Bates et al. 2014; R-Core-Team. 2020). The weather and community models were constructed using binomial family distributions of errors and *logit* link functions, and R^2 was calculated to estimate their degree of fit (Barton 2020).The individual models were built using a Bernoulli distribution with *cloglog* link functions (Zuur et al. 2007), and Kappa Cohen´s concordance estimators were calculated to assess the accuracy of the selected models (Landis and Koch 1977) using the package *PresenceAbsence* (Freeman and Moisen 2008). Multiple correlation tests and variance inflation factor (VIF; Burnham and Anderson 2002) were performed among all the explanatory variables in order to avoid redundancy in the models. Model selection was based on Akaike's information criterion corrected for small sample size (AICc) and variables with parameters that differed from zero were considered candidate models. All models with ΔAICc > 2 with respect to the null model, and a ΔAICc < 2 with respect to the best model, with a weight higher than 0.5 were selected as candidate models (Zuur et al. 2007).

RESULTS

During the study period, we captured and sampled 719 individuals (on average, 2.33 rodents per 100 trap nights) from the species *Akodon montensis* (551), *Rattus rattus* (63), *Mus musculus* (49), *Calomys tener* (16), *Thaptomys nigrita* (15), *Oligoryzomys nigripes* (12), *Euryoryzomys russatus* (10), *Oligoryzomys flavescens* (1), *Brucepattersonius* sp. (1) and *Juliomys pictipes* (1; Fig. 2).

Akodon montensis was the dominant species in periurban, rural and natural protected areas, and was not captured in urban areas (Fig. 2). The highest trap success of *A. montensis* in natural protected areas was in RNI with 7.02 rodents per 100 trap nights, while in periurban areas it was in RII with 2.75.

We detected orthohantavirus antibodies in 14 individuals of the species *Akodon montensis* (seroprevalence: 3.3%), 12 of which were captured in the natural reserve RNI (seroprevalence: 3.23%, $n = 371$; Table 1), and two in the periurban area RII (seroprevalence: 3.63%, $n = 55$; Table 2). We did not find antibody-positive individuals in any other site. We did not detect orthohantavirus antibodies in any other captured species.

Despite the restrictions to remove animals in protected areas, we were able to euthanize and collect six individuals of *A. montensis* captured at RNI, two of which were IgG positive to orthohantavirus. Viral genome amplification was successful from one of them (rodent No. 391). The fragment obtained from viral S-segment (72 to 897 nts from AF324902 Gene Bank accession number) was subjected to nucleotide sequencing. The obtained sequence was compared with previously published genomes from orthohantaviruses by BLAST algorithm. The highest nucleotide identity (92.9%) was found with an orthohantavirus previously identified from an *Akodon cursor* in Paraguay (GeneBank Acc. Num. EU373732; Padula et al. 2007), followed by other viruses found in Brazil (Table 3).

Temporal variations in orthohantavirus seroprevalence in RNI were positively associated with *A. montensis* trap success (AICc: 35.85, w_i: 0.52, AICc_null model: 39; R^2: 0.87, Table 4, Fig. 3). No significant association between seroprevalence and weather variables, community richness and diversity were detected.

The presence of orthohantavirus antibodies at individual level was explained by sex and body weight (AICc: 90.29, w_i: 0.77, AICcnull: 104.28; Table 3). Body condition did not significantly explain the occurrence of infection. The reproduction condition was not evaluated because almost all individuals were sexually actives. This model showed almost perfect values of classification and specificity indexes, but a discrete Kappa index (Kappa = 0.2784; PCC = 0.94; Specificity = 0.967; Sensitivity = 0.333). Big individuals and males had the highest probability of infection (higher than smaller individuals and females). The mean weight of infected males was 44.18 g (SE = 2.12) while that of non-infected males was 38.61 g (SE = 0.72). The only infected female weighted 27.50 g, whereas the mean weight of all females was 28.74 g (SE = 0.76).

DISCUSSION

Akodon-borne orthohantaviruses were previously described in Argentina, Brazil and Paraguay (de Oliveira et al. 2014; Oliveira et al. 2014). *Akodon azarae* is the known reservoir host of Pergamino virus, a non-pathogenic virus prevalent in central-east Argentina (Levis et al. 1998; Vadell et al. 2011). In 2007, a distinct virus was identified in an *A. cursor* from the natural reserve Limoy, eastern Paraguay, near the border with Misiones (Padula et al. 2007). In the present study, we reported for the first time evidence of ortho-
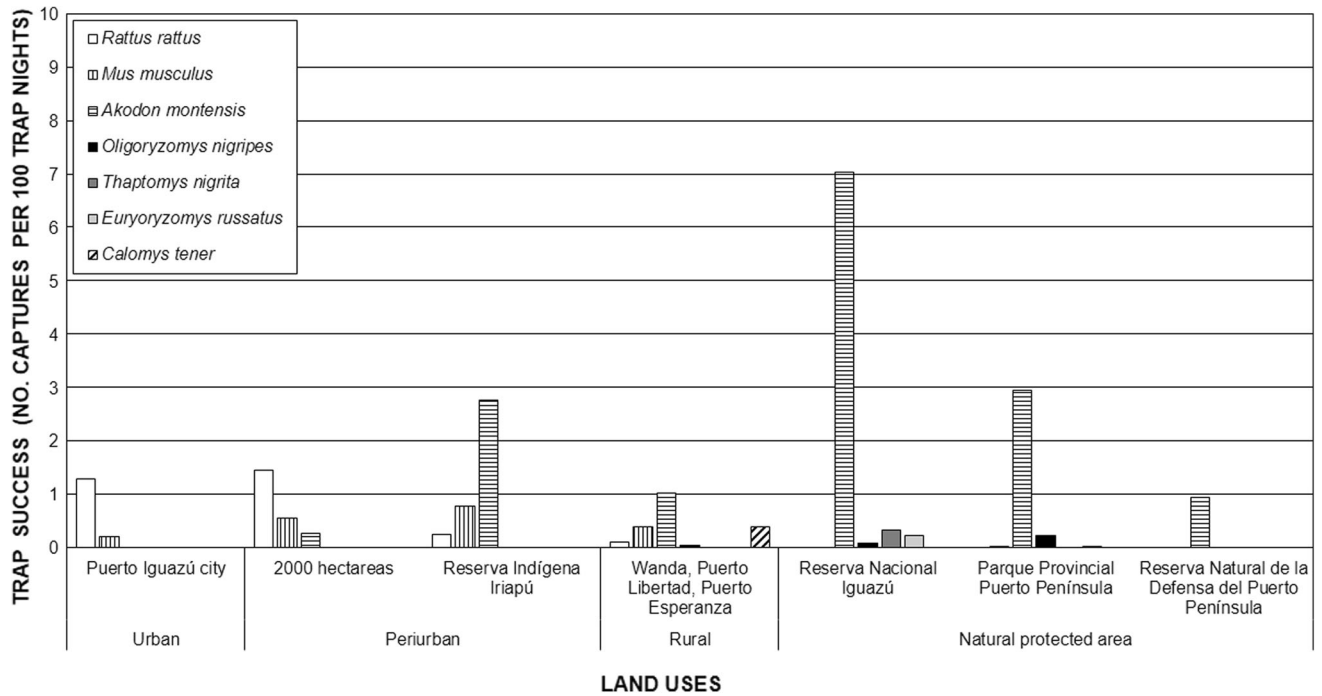
Figure 2. Trap success (No. captures per 100 trap nights) by rodent species in each land use in Iguazú department, Argentina, 2017–2020. *Oligoryzomys flavescens*, *Brucepattersonius* sp. and *Juliomys pictipes* were not included because too few animals were captured.

Table 1. Number of captures, trap success, percentage of males, richness, diversity index, and orthohantavirus seroprevalence (number of seropositive individuals is shown in brackets) of *Akodon montensis* (*A.m.*) in the natural reserve Reserva Nacional Iguazú (RNI), Misiones, Argentina from 2018 to 2020.

Year	Season	No. *A.m.*	TS *A.m.*	Males %	Richness	Shannon index	Seroprevalence		
							Male	Female	Total
2018	Summer	1	0.17	100	1	0	0	0	0
	Autumn	18	3.05	55.60	1	0	10.00 (1)	0	5.55 (1)
	Winter	26	4.95	62.90	3	0.26	0	0	0
	Spring	47	7.83	55.30	4	0.13	3.85 (1)	0	2.13 (1)
2019	Autumn	64	10.72	54.40	2	0.02	9.68 (3)	0	4.68 (3)
	Winter	95	16.33	65.30	4	0.23	9.37 (6)	2.94 (1)	7.37 (7)
	Spring	69	11.50	50.70	3	0.04	0	0	0
2020	Summer	54	9.00	53.70	3	0.37	0	0	0

hantavirus infection (antibodies and viral genome) in *Akodon montensis* populations in Misiones, Argentina, a region for which there are no previous reports of *Akodon*-borne orthohantavirus. The seroprevalence for this species was 3.3%. Further studies are required to obtain complete sequences to identify this strain.

A. montensis has a wide distribution and high abundance throughout most of the Atlantic forest, including its southernmost remnants in Misiones province (Lanzone et al. 2018). This is probably related to certain tolerance to anthropogenic disturbance which allows the species to inhabit not only forests but also cultivated lands and grasslands (de Oliveira et al. 2014; Oliveira et al. 2014). We detected *A. montensis* in periurban, rural and natural protected areas, while we did not detect the species in urban areas. Despite the fact *A. montensis* was present in the three

Table 2. Number of captures, trap success, percentage of males, richness, diversity index, and seroprevalence (number of seropositive individuals is shown in brackets) of *Akodon montensis* (*A.m.*) in periurban area Reserva Indígena Iryapú (RII), Misiones, Argentina, from 2018 to 2020.

Year	Season	No. *A.m.*	TS *A.m.*	Male %	Richness	Shannon index	Seroprevalence		
							Male	Female	Total
2017	Spring	0	0	0	1	0	0	0	0
2018	Summer	0	0	0	1	0	0	0	0
	Autumn	9	3.85	55.55	2	0.65	20.00(1)	0	11.11(1)
	Winter	10	4.44	75.00	2	0.45	0	0	0
	Spring	5	2.22	60.00	1	0	33.33(1)	0	20.00(1)
2019	Autumn	4	1.77	25.00	2	0.68	0	0	0
	Winter	15	10.00	75.00	2	0.36	0	0	0
	Spring	7	4.67	57.10	1	0	0	0	0
2020	Summer	1	0.51	100	1	0	0	0	0

Table 3. Percentage nucleotide sequence identity between rodent No. 391 and the more closely related sequences published in the National Center for Biotechnology Information database. Nucleotide sequence identities based on a fragment of 905 bp amplified from the vial S-segment. Comparison was performed by BLAST program.

Virus	Host species	Country	Year	Accession number	Percentage of identities
Hantavirus AC210PY	*A. cursor*	Paraguay	2007	EU373732	92.9
Hantavirus Strain IP16	*A. montensis*	Paraguay	2006	DQ345764	92.9
Ape Aime-Itapúa	*A. montensis*	Paraguay	2009	GU205340	92.5
Jaborá	*A. montensis*	Paraguay	2014	MG575411	91.3

Table 4. Variation in orthohantavirus seroprevalence and antibody presence in *Akodon montensis* (*A.m.*) as a function of community and individuals features in the natural reserve Reserva Nacional Iguazú (RNI), Misiones, Argentina. Estimates, standard errors and 95% confidence intervals (CI) are shown.

	Estimate	Standard error	CI
Community			
Intercept	− 5.84	1.17	− 7.98; − 4.09
A.m. trap success	0.21	0.09	0.07; 0.36
Individual features			
Intercept	− 8.08	1.79	− 11.85; − 5.68
Weight	0.08	0.02	0.04; 0.12
Sex male	2.33	1.27	0.64; 5.06

areas types specified above, we found orthohantavirus-infected individuals only in one of the three natural protected and one of two periurban areas (patchy distribution of hantavirus). This particularity has also been observed in several other pathogen-host systems, and is considered a relevant factor in determining the risk of disease trans-
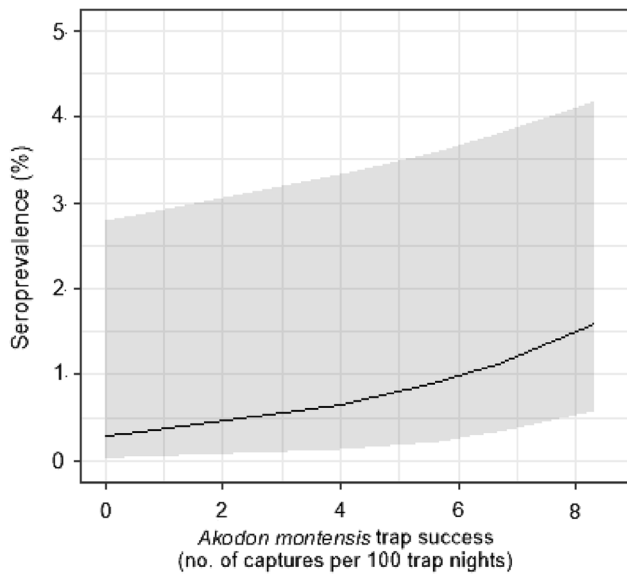
Figure 3. Orthohantavirus seroprevalence predicted values as a function of *Akodon montensis* trap success in the natural protected area Reserva Nacional Iguazú, Misiones, Argentina.

mission (Abbott et al. 1999; Chen et al. 2020). The reason why there are infectious rodents in some but not all sites could be related to a threshold population density below which the virus is prone to experience local extinctions (Tersago et al. 2011; Luis et al. 2015; Maroli et al. 2018). The association between the proportion of infected individuals and *A. montensis´*s abundance in RNI supports this idea. Orthohantavirus circulation in the numerically dominant species of a rodent community has been observed in this research and in other orthohantavirus-rodent systems (Vadell et al. 2011; de Oliveira et al. 2014; Oliveira et al. 2014; Maroli et al. 2018) and may be a quite common characteristic of the ecology of these viruses. Also, low abundance of the reservoir may decrease the chance of detecting infected individuals, resulting in a lack of detection of the virus when it is in fact present.

Orthohantavirus infection in *A. montensis* was most frequent in adult males in agreement with other studies on orthohantavirus-rodent systems in Argentina, Brazil, Paraguay and United States (Douglass et al. 2001; Owen et al. 2010; Polop et al. 2010; Vadell et al. 2011; de Oliveira et al. 2014; Oliveira et al. 2014). Adult individuals may be mainly responsible for the largest contacts in the population, and thus the main intra-population transmitters of the virus (Clay et al. 2009; Dearing et al. 2015). In agreement with other studies in central Argentina, we did not observed any relationship between orthohantavirus infection in *A. montensis* and body condition (Mills et al. 2007;

Vadell et al. 2011) suggesting that infected rodents have no changes in their health conditions (Maroli et al. 2020). However, due to the negative effect produced by another hantavirus, Sin Nombre virus on *Peromyscus maniculatus,* with a 13.4% decrease on survival of infected males compared to uninfected mice (Luis et al. 2012), we have to be cautious, and therefore, we recommend more studies to better understand the effects of hantavirus infection on rodent health.

We did not observe association between orthohantavirus infection and richness or diversity of the rodent community in the natural protected area RNI. Both dilution and amplification effects were proposed to explain the transmission of orthohantavirus in rodents communities by several authors (as, Dizney and Ruedas 2009; Dearing et al. 2015)), but evidence supporting these effects seem insufficient (Salkeld et al. 2013; Vadell et al. 2020). In fact, a meta-analysis examining the dilution effect in orthohantavirus/rodent systems in the Americas found no clear support for this effect, suggesting instead that it is the abundance and the relative proportion of reservoir individuals in the community which have an effect on orthohantavirus infection (Vadell et al. 2020).

Weather conditions have long been proposed to affect infection and transmission of orthohantaviruses in a variety of ways (Mills et al. 1999; Owen et al. 2010; Maroli et al. 2018). For example, some authors proposed that low temperatures and high humidity could increase survival outside the host (Kallio et al. 2006), others observed a positive association between seroprevalence and rainfall (Owen et al. 2010), while other authors proposed that weather conditions during winter may affect body condition of the hosts, making them more susceptible to infection (de Oliveira et al. 2014; Oliveira et al. 2014). However, our results do not support this idea because no significant associations between infection and body condition or weather variables were detected. However, as above said, further long-term studies are necessary to confirm this hypothesis.

In this research we captured two other known HPS rodent host species of Argentina, *O. nigripes* and *O. flavescens,* but we did not detect orthohantavirus antibodies in these species. The lack of detection of seropositive individuals in these species could be due to the low number of individuals captured (particularly in the case of *O. flavescens*, in which only one individual was captured), and not necessarily to the absence of infected individuals, so additional studies are recommended. We also captured *R.*

rattus, host of Seoul virus, one of the etiologic agents of HFRS, mainly in Asia, though reports of infection in other continents have been increasing (Clement et al. 2019; Knust et al. 2020). Though up to date no HFRS case has been confirmed in South America, infected Norway rats (*Rattus norvegicus*) were detected in Argentina and Brazil (Padula et al. 2010; Mull et al. 2020; Pan American Health Organization 2020), and evidence of past infection was found in humans in Brazil (Iversson et al. 1994). Therefore, vigilance of urban rodent populations is important because they are involved in the transmission of several zoonoses.

Despite the fact *A. montensis* has not been associated with HPS cases up to now, high abundance and widespread distribution throughout southern Brazil, eastern Paraguay and Northeast Argentina, together with its generalist habits and relatively high tolerance to anthropogenic disturbance (de Oliveira et al. 2014; Oliveira et al. 2014; Lanzone et al. 2018) suggest a potential risk to humans through viral mutation (becoming a pathogenic strain) or host switching (*A. montensis* becoming host of a pathogenic strain).

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DECLARATIONS

CONFLICT OF INTEREST
All authors have contributed significantly, are in agreement with the content of this manuscript and don't have any conflicts of interests.

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