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Hantavirus circulation in *Oligoryzomys Flavescens* rodents of Buenos Aires City, Argentina

Circulación de hantavirus en *Oligoryzomys Flavescens* en Ciudad de Buenos Aires, Argentina

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RESUMEN

El síndrome pulmonar por hantavirus (SPH) es una enfermedad zoonótica. La principal causa en Argentina es el virus Andes. *Oligoryzomys flavescens* (Sigmodontinae) uno de los reservorios de SPH. El objetivo de esta investigación fue estimar la seroprevalencia de hantavirus en *O. flavescens* en la Ciudad de Buenos Aires. Se colocaron trampas de captura viva en tres parques y una reserva ecológica de la Ciudad para detectar individuos con diagnóstico positivo para virus Andes. Se capturaron un total de 286 roedores, *O. flavescens* fue la especie más capturada (49,65%). Se encontró serología positiva para virus Andes (genotipo Lechiguana) en ejemplares capturados en uno de los sitios en estudio (seroprevalencia = 6,62%) si bien encontramos al hospedador en otros dos parques de la Ciudad. El presente estudio confirma la presencia de roedores infectados con el virus Andes en la Ciudad de Buenos Aires, lo que implica un riesgo de transmisión en un ambiente urbano.

Palabras clave: (hantavirus), (*Oligoryzomys flavescens*), (Ciudad de Buenos Aires)

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SUMMARY

Hantavirus pulmonary syndrome (HPS) is a zoonotic disease. The main cause in Argentina is Andes virus. *Oligoryzomys flavescens* (Sigmodontinae) was identify as one of the HPS reservoir. The objective of this research was to estimate the seroprevalence of hantavirus in *O. flavescens* in Buenos Aires City. We set rodents live trapping in three parklands and one ecological reserve in Buenos Aires City in order to screen hantavirus Andes infected rodents. A total of 286 rodents were captured, O. *flavescens* was the most frequently captured species (49.65%). Positive serology for Andes virus (Lechiguanas genotype) was found in *O. flavescens* in one site studied (seroprevalence = 6.62%) and we found the host in other two parks within the City. The present study confirms the presence of rodents infected with Andes virus in Buenos Aires City, which implies transmission risk in an urban environment.

Key words: (hantavirus), (*Oligoryzomys flavescens*), (Buenos Aires City)

INTRODUCTION

Hantavirus pulmonary syndrome (HPS) is a zoonotic disease caused by viruses belonging to the Hantaviridae family, and mainly harbored by some rodent species. These viruses are transmitted to humans by inhalation of aerosols generated by infected rodents[27], although there is also evidence for person to person in the argentine southern region and Buenos Aires Province [15, 21]. The main cause of HPS in Argentina and neighboring countries is Andes virus (ANDV). Seven different ANDV genotypes have been characterized from HPS cases in Argentina [22, 24].

There are 4 geographically and ecologically distinct HPS endemic areas [16]. The Central region, the second in number of cases, comprises Buenos Aires City [16, 14] where one HPS case with no history of travel outside the City was recorded and Lechiguanas genotype was characterized [11].

Several species of Sigmodontinae rodents were identified as HPS reservoirs in the central region: *Akodon azarae, Necromys obscurus* and *Oligoryzomys flavescens* [13]. Particularly in Buenos Aires City few of these species were reported: *A. azarae,* and *O. flavescens* [2, 4, 17, 25]. Recent studies have communicated the presence of *O. flavescens* in Costanera Sur Ecological Reserve [2, 4] and in Presidente Roca Park [4].

The distribution of *O. flavescens* [31] showed a wide extention from the north to the center of Argentina [7], southeast Brazil [32] and Uruguay [12]. This species is a good colonizer of disturbed ecosystems [26], inhabits wild environments such as savannas, marshes and not very dense forests, grass and scrubland, often near water or damp zones [9], and although it is associated to wild microhabitats it has been captured in peridomiciliary urban and periurban areas [5].

Knowing the presence of *O. flavescens* in Buenos Aires City it becomes necessary to study if there is hantavirus circulation between them. Our aim was to estimate the seroprevalence of hantavirus in *Oligoryzomys flavescens* in Buenos Aires City.

MATERIALS AND METHODS

Rodents were captured in four green open sites of the City during the period 2011-2014: De los Niños Park (NP: 32 hectares, 34º31'43.40"S, 58º27'33.99"O), Ribera Sur Park (RSP: 50 hectares, 34º41'57.73"S, 58º28'04.93"O), Presidente Roca Park (PRP: 154 hectares, 34º40'29.76"S, 58º26'29.79"O) and Costanera Sur Ecological Reserve (CSER: 353 hectares, 34º36'29.18"S, 58º21'03.32"O) (Figure 1). According to the characterization of environmental landscape units by Cavia et al. (*11*), the CSER is a natural reserve and NP and PRP are parklands. We considered RSP as parkland because it shares environmental characteristics with the other two. The CSER is occupied by riparian habitats similar to

those developed along the Paraná and de la Plata rivers, like woodlands, riparian thickets, fresh water marshes and flooded grasslands. Parklands are sites of recreation, where areas of spontaneous vegetation and woodlots with planted species are included in a matrix of grass or ornamental lawn [3].

Rodents were captured with Sherman live traps in linear transects actively set for four consecutive nights for each sampling (Table I).

The individuals captured were identified according bibliography [9] and reference material deposited at the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia". All *O. flavescens* specimens captured were anesthetized with isoflurane and euthanized by cervical dislocation. Body measurements, sex and reproductive status were registered. Blood and lung samples were collected for hantavirus infection analysis, following Mills et al. [19]. Animals were treated in accordance with the guidelines of the Sociedad Argentina para el Estudio de los Mamíferos [8].

The relative abundance of each species per study area was estimated with the Trap Success Index (TS) [18]. TS = (number of individuals / number of traps * nights) * 100. The correlation between the number of *O. flavescens* captured and number of positive individuals was calculated with Spearman Correlation Coefficient.

In order to screen ANDV antibody-positive individuals, we utilized an enzyme-linked immunosorbent assay based on the test described for human antibodies detection [22]. Briefly, to determine IgG antibodies against ANDV, rodent blood samples diluted 1:100 were added to 96 wells plate coated with AND recombinant nucleoprotein antigen. To determine ANDV genotype, total RNA extraction was performed on available lung tissues from seropositive rodents (5/9 individuals) using Trizol (Invitrogen) and purified by the RNAid kit (Bio 101). When it was possible (3/5 samples), viral RNA was amplified by RT-PCR and a second round of nested PCR following Ciancaglini et al. [6]. Briefly, RT-PCR was performed by One Step RT-PCR kit (QIAGEN) and Taq DNA Polymerase (Invitrogen) following each manufacturer instructions. For the 952 nt S-Segment fragment was used an inner primer to obtain the 530-nt fragments for S-segment (position 22–550, referred to AH1 AND strain, Gene Bank No AF324902) position. For M-segment was amplified the 461nt fragment (position 6 to 467). Amplification products were analyzed on agarose gels and sequenced. Multiple sequence alignment and comparison of nucleotide sequences were conducted using MEGA version 5 [29].

RESULTS

A total of 286 rodents were captured with a total trapping effort of 13012 trap-nights. *O. flavescens* was the most frequently captured species (49.65%) followed by *Mus musculus* (36.71%), *Deltamys kempi* (10.14%) and *Scapteromys aquaticus* (3.50%). Rodents were captured in all sites but NP. In CSER we captured four species of which the trap success of *O. flavescens* and *M. musculus* were the

Table I. Number of traps night per season and year for each studied site

Site	Season - year	Traps night
CSER	spring 2011; summer 2012; autumn 2012; winter 2012; spring 2012; summer 2013; autumn 2013; winter 2013; spring 2013; summer 2014; autumn 2014; winter 2014; spring 2014	11 624
NP	winter 2014	452
RSP	summer 2015	512
PRP	winter 2014	424

Table II. Rodents captured and trap success per studied site

Site	Species	Trap success (%)	Nº of individuals captured
CSER	*Oligoryzomys flavescens*	1.17	136
	Deltamys kempi	0.25	29
	Mus musculus	0.90	105
	Scapteromys aquaticus	0.09	10
NP	without capture	0	0
RSP	*Oligoryzomys flavescens*	0.39	2
PRP	*Oligoryzomys flavescens*	0.94	4

Table III. Number of captured, positives and seroprevalence of *Oligoryzomys flavescens* in CSER 2011-2014

Period	# *O. flavescens*	# *O. flavescens* positive	seroprevalence *O. flavescens*
spring 2011	7	0	0.00
summer 2012	0	0	0.00
autumn 2012	4	0	0.00
winter 2012	8	1	0.13
spring 2012	3	0	0.00
summer 2013	0	0	0.00
autumn 2013	2	0	0.00
winter 2013	37	2	0.05
spring 2013	33	2	0.06
summer 2014	5	2	0.40
autumn 2014	20	1	0.05
winter 2014	4	0	0.00
spring 2014	13	1	0.08

highest. On the contrary, in RSP and PRP we only captured *O. flavescens* (Table II).

Positive serology for ANDV was found in *O. flavescens* captured in CSER where the seroprevalence was 6.62% (95% c.i.: 2.07% - 11.16%). The nine positive rodents corresponded to male individuals, seven of them were sexually active (with testicles in the scrotal position). These positive individuals were caught in different seasons: 3 in winter, 3 in spring, 2 in summer and 1 in autumn (Table III). They were found in a variety of floristic habitats, six of them were captured on the river side and the rest in different habitats of CSER. The Spearman Correlation Coefficient between number of *O. flavescens* captured and number of positive individuals resulted 0.81 ($p < 0.001$, $N = 13$), showing a positive relationship between both variables throughout the seasons.

O. flavescens did not show any seasonal pattern in CSER and was captured in all seasons but summer 2012 and summer 2013.

Although, we observed an increase of trap success since winter 2013 with a peak in spring at the same year. On the other hand, after the peak of *O. flavescens,* the trap success of *M. musculus* decreased and we also observed a slight increase of *D. kempi* trap success and *S. aquaticus* was found for the first time (Figure 2).

ANDV, Lechiguanas genotype, was identified in 3 IgG positive-rodent, showing a 99.8% nucleotide identity between them in the amplified fragment. The 3 individuals were captured in winter 2012, spring 2013 and summer 2014.

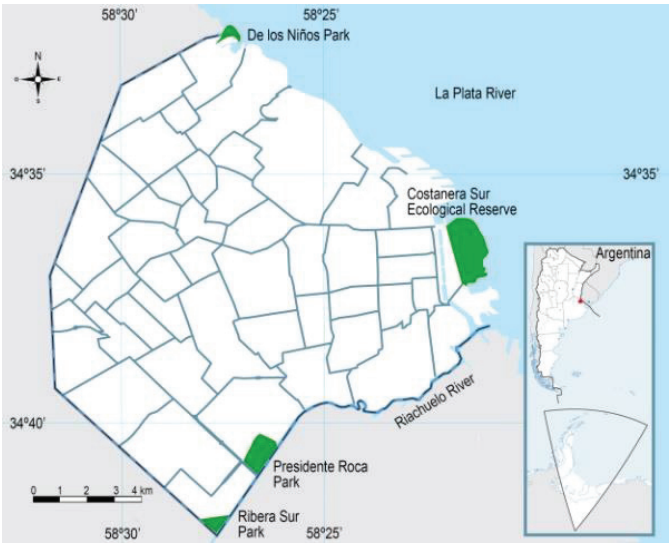

Figure 1. Studied sites in Buenos Aires City.

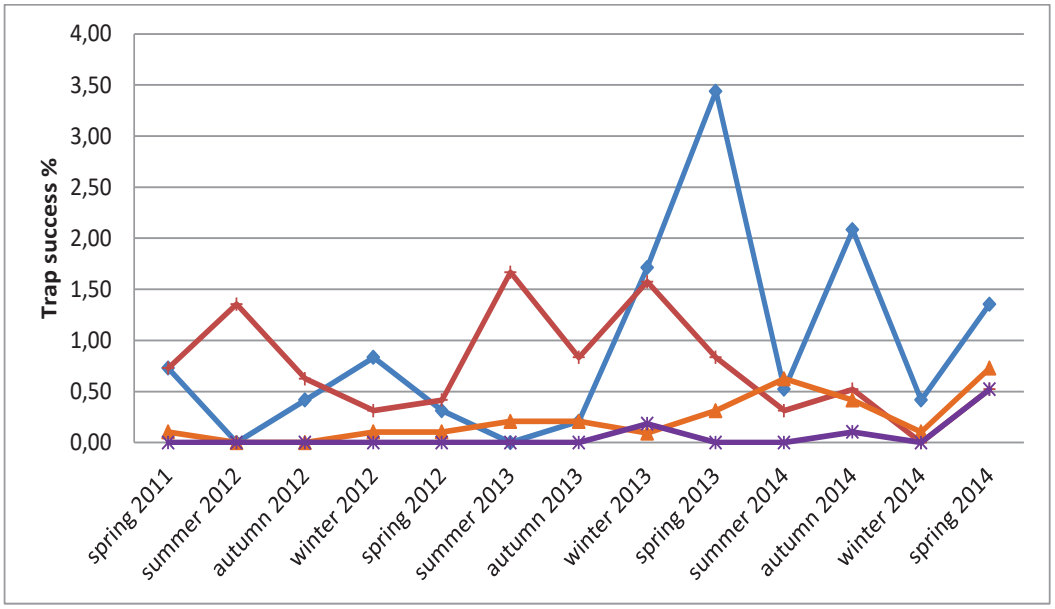

Figure 2. Trap success of *Oligoryzomys flavescens* (), *Mus musculus* (), *Deltamys kempi* () and *Scapteromys aquaticus* () per period in CSER 2011-2014.

DISCUSSION

The present study confirms the presence of rodents infected with Andes virus in Buenos Aires City. This finding is not unexpected because this City is geographically placed in the Central region HPS endemic area.

Positive rodents were captured only in a natural reserve, CSER, which was the most intensely studied site. Since 2014, an exploratory study has been started in parklands in the City. In two of them, RSP and PRP, we found the host. The presence of this hantavirus reservoir species in these two places should be considered as a potential risk *per se*.

The seroprevalence found in CSER was 6.62%. In similar studies the seroprevalence found in *O. flavescens* was 7.51% in Pre Delta National Park [30] and 13.51% in Exaltación de la Cruz [28]. All the positive individuals were males, according to other authors that found that males are more likely to be infected [23, 28, 30]. The presence of *O. flavescens* with positive serology for ANDV in all seasons and in different habitats located in CSER, could implies transmission risk for visitants and workers along all year.

Altought it was not part of the objective of this work, we studied the first 15 *D. kempi* captured for hantavirus infection analysis. All of them were negative for ANDV (data not shown). These results supports, as mention in Vadell et al. [30] that *D. kempi* is an uncommon host species for hantavirus and constitutes a spillover host.

We found rodent community changes along the study period in CSER. These changes can be associated with the dredging works and filling in the lagoons with water since 2013 [2]. Interestingly, we found the highest peak of *O. flavescens* in 2013/2014, period in which an outbreak of HPS cases was registered in Buenos Aires Province [11]. On the other hand, Cavia et al. [4] found in CSER a richness of 6 species, and *O. flavescens* and *D. kempi* were the dominant species; in our study richness was lower (R = 4) and *O. flavescens* and *M. musculus* were the dominant species, which could indicate that rodent community in CSER is not stable.

O. flavescens is an r strategist species, consequently its captured and reproductive activity are strongly conditioned by the density of the rest of the rodent species [26]. If we take this into account and that we found a positive correlation between number of *O. flavescens* positive and individuals captured, it becomes necessary to continue evaluating the *O. flavescens* dynamics in the assemblages of rodent species. There are several variables in an infection system [10]. We found that an increasing population density of *O. flavescens* resulted in an increasing horizontal virus transmission, according to Mills et al. [20], Hussein et al. [10] and Adler et al [1].

CONCLUSIONS

Results presented in this work, can be used to support the adoption of preventive measures and optimize the allocation of resources to avoid disease propagation. Hantavirus´s epidemiological monitoring in rodents must be continued and new sites should be studied in the City.

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