Original Contribution

Daily Movements and Microhabitat Selection of Hantavirus Reservoirs and Other Sigmodontinae Rodent Species that Inhabit a Protected Natural Area of Argentina

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Abstract: Abundance, distribution, movement patterns, and habitat selection of a reservoir species influence the dispersal of zoonotic pathogens, and hence, the risk for humans. Movements and microhabitat use of rodent species, and their potential role in the transmission of hantavirus were studied in Otamendi Natural Reserve, Buenos Aires, Argentina. Movement estimators and qualitative characteristics of rodent paths were determined by means of a spool and line device method. Sampling was conducted during November and December 2011, and March, April, June, October, and December 2012. Forty-six *Oxymycterus rufus*, 41 *Akodon azarae*, 10 *Scapteromys aquaticus* and 5 *Oligoryzomys flavescens* were captured. Movement patterns and distances varied according to sex, habitat type, reproductive season, and body size among species. *O. flavescens*, reservoir of the etiologic agent of hantavirus pulmonary syndrome in the region, moved short distances, had the most linear paths and did not share paths with other species. *A. azarae* had an intermediate linearity index, its movements were longer in the highland grassland than in the lowland marsh and the salty grassland, and larger individuals traveled longer distances. *O. rufus* had the most tortuous paths and the males moved more during the non-breeding season. *S. aquaticus* movements were associated with habitat type with longer distances traveled in the lowland marsh than in the salty grassland. Hantavirus antibodies were detected in 20% of *A. azarae* and were not detected in any other species. Seropositive individuals were captured during the breeding season and 85% of them were males. *A. azarae* moved randomly and shared paths with all the other species, which could promote hantavirus spillover events.

Keywords: habitat use, hantavirus, movements, protected natural areas, rodents, spool and line

INTRODUCTION

Rodents cause economic losses (Castillo et al. 2003; Lovera et al. 2015) and public health problems (Schmaljohn and

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Hjelle 1997) acting as reservoirs of many diseases that affect humans and domestic animals (Gratz 1994; Tobin and Fall 2004). In recent years, the number of discovered emerging zoonotic diseases transmitted by rodents has steadily increased (Mills and Childs 1998). Hantavirus pulmonary syndrome (HPS) is one of the recently discovered diseases. HPS is a human viral disease caused by hantaviruses of the family Bunyaviridae. Most types of hantaviruses are rodent species reservoir specific, but some exceptions to specificity have emerged probably due to a succession of spillover events resulting in host-switching (i.e., a process in which a species becomes a new host of a given pathogen) that occurred during hantavirus evolution (Levis et al. 1998; Plyusnin 2002). Adult males are more likely to become infected, suggesting a horizontal transmission mechanism (Glass et al. 1998; Abbott et al. 1999; Douglass et al. 2007; Mills et al. 2007; Polop et al. 2010; Vadell et al. 2011) which has been confirmed in experiments carried out in *Oligoryzomys longicaudatus* (Padula et al. 2004). Horizontal transmission may include direct methods such as aggressive interactions and grooming, or indirectly by environmental transmission (Calisher et al. 1999; Douglass et al. 2001; Khalil et al. 2014). Some authors consider transmission to be density dependent with increases in contact rates leading to increases in prevalence. However, the density-dependent relationship is not completely clear due to confounding effects that occur during periods of rapid population growth. During periods of rapid population growth, the lack of vertical transmission leads to a dilution of susceptible rodents and reduced prevalence (Mills et al. 2007; Adler et al. 2008).

Humans can be infected by inhalation of aerosols from saliva, urine, or feces released by infected rodents (Parisi et al. 1996; Delfraro et al. 2003). Consequently, abundance, distribution, movement patterns, and habitat selection of the reservoir species influence the dispersal of the virus, and hence, the risk of spillover to humans.

The abundance and distribution of animal populations vary in space and time according to the availability of environmental factors required by each species (Litvaitis et al. 1994). Habitat structure, distribution of food resources, and intrinsic factors (such as species identity, sex, size, or age), probably, have the greatest influence on local movements inside home ranges (Taylor 1978; Stroud 1982; Litvaitis et al. 1994; Gómez Villafañe et al. 2008; Prevedello et al. 2010). Therefore, animal movement patterns are a product of habitat selection because individuals are faced with multiple choices during their biological activities (foraging, mate

searching, refuge use, and escape from predators) (Johnson 1980; Rosenzweig 1981; Garshelis 2000). Determining the characteristics of rodent movement contributes to the understanding of genetic, evolutionary, and epidemiological aspects of animal populations (Nams 2006).

In Argentina, there are seven species of rodents of the family Cricetidae, subfamily Sigmodontinae that serve as reservoirs of different hantavirus genotypes (Palma et al. 2012): *O. longicaudatus*, *O. flavescens*, *O. nigripes*, *O. chacoensis, Calomys callidus, Akodon azarae*, and *Necromys benefactus*. The hantavirus genotypes carried by the last two rodent species have not been associated with human cases yet (Levis et al. 1998). Several spillover events and possible host-switching events have been documented in Argentina (Vadell et al. 2011; Polop et al. 2010; Piudo et al. 2005). Rural and protected natural areas have rich communities of sylvan Sigmodontinae rodents (Teta et al. 2009a; Teta et al. 2009b; Gómez Villafañe et al. 2012) including hantavirus reservoir species (Suárez et al. 2003; Vadell et al. 2011; Piudo et al. 2012), in contrast to urban areas where there are commensal rodents, which are not hantavirus reservoirs in Argentina (Cavia et al. 2009, Palma et al 2012). Therefore, sylvan and rural areas are places where contact between people (tourists and workers) and sylvan rodents is favored (Marconi and Kravetz 1986).

The study of habitat use by rodents, particularly, differences in habitat characteristics that determine movement patterns or contact rates among rodents or between rodents and human beings, provides helpful information about the relative risk for human infection in different habitats (Mills and Childs 1998; Goodin et al. 2009; Khalil et al. 2014). However, few rodent habitat studies have been conducted in protected natural areas in Argentina (Tabeni and Ojeda 2005; Tabeni et al. 2007; Gómez Villafañe et al. 2012).

The aim of this work was to determine the movements and microhabitat use of several species of Sigmodontinae rodents, and to investigate the potential role of movements and space use in the transmission of hantavirus in Otamendi Natural Reserve, a protected natural area of Buenos Aires province, Argentina. We expected that (1) rodents use space in a selective, non-random fashion; (2) space use is rodent species specific; (3) larger species move farther than smaller species; (4) the largest individuals within a species move the greatest distances; (5) males move farther than females; (6) movements, both characteristics and distance, are different during the breeding than during the non-breeding season; and (7) paths of the different species overlap.

METHODS

Study Area

Otamendi Natural Reserve (ONR) is a protected natural area of 3000 ha located in the floodplain of the Lower Paraná River Basin, Buenos Aires province, Argentina (34°10′S; 58°48′O) (Figure 1).

The reserve was created in 1990 and preserves three eco-regions: Paranaense, Pampeana, and Espinal. The climate is humid temperate, with an annual mean temperature of 16°C and precipitation distributed evenly throughout the year, reaching 1000 mm of annual accumulated rainfall (Haene et al. 2003).

We studied rodent communities in three contiguous habitat types usually visited by tourists: a lowland marsh (1879.2 ha) located in the low river terrace and dominated by Cyperaceae species; a salty grassland (680.7 ha) located in the low river terrace, consisting of an herbaceous matrix dominated by grasses; and a highland grassland (179.4 ha) located in the upper river terrace and composed of grasses, broadleaf herbs, isolated trees, and patches of *Dypsacus fullonum* (Haene et al. 2003; Figure 1). The low and upper river terrace habitats also have many patches of *Cortaderia selloana*, a robust grass that grows to 3 m in height and is wide spread in the Pampean eco-region. Two other habitat types in the reserve, the *Celtis tala* forest and the riparian forest, were not studied because the first produced few captures of rodents during previous studies (Gómez Villafañe et al. 2012). The riparian forest is not contiguous to the others which does not provide opportunities for movements among habitats which we wanted to detect.
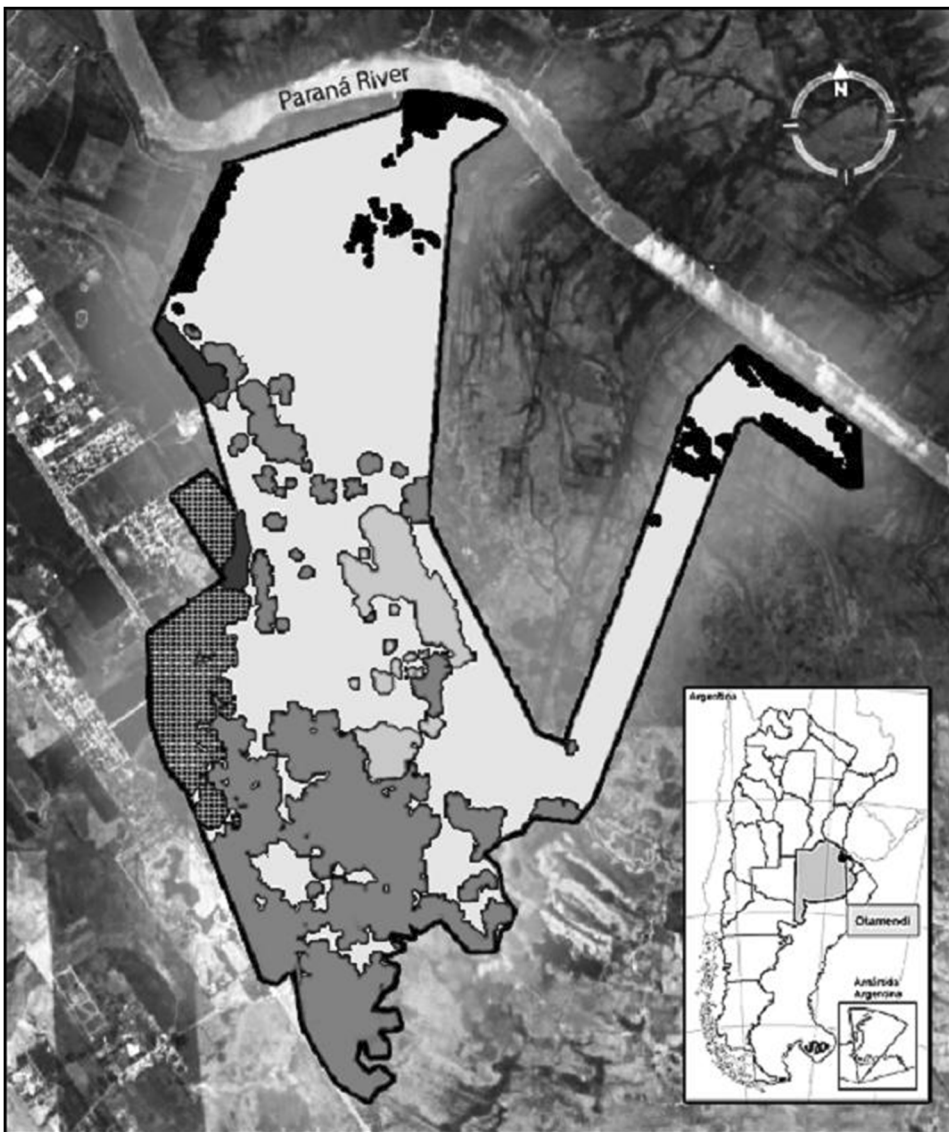

Figure 1. Location of Otamendi Natural Reserve in Buenos Aires province, Argentina and habitat types of the reserve. *White* lowland grassland, *light gray* water bodies, *dark gray* salty marshes, *checkered pattern* highland grassland, and *black* forest.

Rodent Surveys

We sampled during November and December 2011, and March, April, June, October, and December 2012. Rodents were captured with live traps baited with a mixture of fat, oats and peanut butter. We placed two hundred and forty traps at 10 m intervals throughout the three habitat types during each sampling period. Traps were opened during three consecutive nights, except for the last sampling period when we opened traps during four consecutive nights in an attempt to increase the number of captures.

We recorded species, sex, head-body length (HBL), and trap location and marked each individual with an individually numbered ear tag.

Hantavirus Antibody Detection

We collected blood samples from every captured individual and were screened for Andes (AND) virus-specific immunoglobulin G (IgG) antibodies using enzyme-linked immunosorbent assays, as previously described by Padula et al. (2000). This method detects the presence of antibodies but does not discriminate among the different hantaviruses genotypes. We calculated seroprevalence as the proportion of individuals with hantavirus antibodies (seropositives) over the total number of samples analyzed.

Analysis of Rodent Movement

We equipped captured individuals with a spool and line device (Shanahan et al. 2007). This method does not affect survival in small mammals (Boonstra and Craine 1986; Steinwald et al. 2006). We followed threads the following day and each path was tracked using a Global Position System receiver. We followed paths until the spools were finished, were broken or until they come under dense vegetation. For daily movement analysis, data from individuals that traveled less than 10 m were excluded from the analysis. We handled all animals according to the Argentine law for the protection of animal welfare (Penal Code law number 14.346) and followed safety precautions appropriate for handling hantavirus reservoirs (Mills et al. 1995). Animal handling, including blood collection, has been found to not affect the behavior of rodents (Douglass et al. 2000).

We used the spool and line tracking data (MapSource 6.13.7, Garmin Ltd.; Easy GPS 4.58, TopoGrafix 2012) to calculate several movement estimators: total distance (TD, total length of the path), maximum linear distance (MLD, the maximum distance in straight line between any two points of the path), total area (TA), and a linearity index (LI). LI was calculated from the distance between the start and end points of a path divided by TD. LI values equal or near one indicate a linear path; LI values smaller than one indicate non-linear paths (or more tortuous paths) (Shanahan et al. 2007).

We examined the relation between the HBL and the movement estimators by means of a Spearman correlation for all species combined and for each species separately (Zar 1996). Movement and linear estimators were compared among species by means of an Analysis of Variance or a Kruskal–Wallis Test (Zar 1996).

In order to evaluate rodent movements (response variable: TD, TA, and MLD) according to sex, reproductive season (breeding, from October to December, and non-breeding, from March to June), habitat types, and HBL (explanatory variables), a General Linear Model analysis was conducted (R 3.0 and RStudio 0.97.551, 2009–2012; Nicholls 1991).

We used a Linear Model to evaluate the linearity of paths (LI, response variable) according to the same explanatory variables.

Vegetation Surveys

Vegetation was described at a microhabitat scale in sites used by rodents as well as in available sites.

In the paths (i.e, those paths used by individuals and tracked with the spool and line device), vegetation was described every 10 m, resulting in variable numbers of sites depending on the length of the rodent paths.

To determine the available sites, four paths (available paths) of 80 m long were generated by joining eight randomly selected trap stations. We described vegetation at 10 m intervals, resulting in eight available sites per path.

In each site (available or used), the vegetation cover of several life form groups of plants, the percentage of bare soil, and the height of the path were registered in quadrants of 1 m × 1 m. We designated life form categories as grasses, broad leaf herbs, *C. selloana* plants, shrubs, and trees. We evaluated all categories as fresh and dry plant coverage. This was done for each habitat type and each sampling session. In a subsample of 61 paths used by rodents, we recorded qualitative characteristics such as presence of nests and use/no use of tunnels, among others.

Analysis of Microhabitat Selection

We assessed species-specific habitat selection including all paths independent of length with a Generalized Linear Model analysis (R 3.0 and RStudio 0.97.551, 2009–2012; Nicholls (1991)). The response variable was binary and took a value of 1 if the path was used by a rodent and 0 if the path was randomly generated (available path), assuming a binomial error distribution. For every path (used or available), the median value of every explanatory variable was used. Explanatory variables were the ones described in the Vegetation Survey section, plus the habitat type. We analyzed explanatory variables previous to a logistic regression by means of a Multiple Correlation test (Zar 1996) in order to avoid including correlated variables.

RESULTS

A total of 46 individuals of *Oxymycterus rufus*, 41 individuals of *A. azarae*, 10 individuals of *Scapteromys aquaticus*, and 5 individuals of *O. flavescens* ($n = 102$) were captured with a trapping effort of 5235 trap-nights.

Hantavirus antibodies were detected in 20% ($n = 41$) of the captured *A. azarae* individuals. Eighty-eight percent of them were males. Captures of these species occurred in all habitat types but 75% of seropositive individuals were detected in the salty grassland, while the rest of the species were captured in the lowland marsh. No seropositive *A. azarae* were captured in the highland grassland. All seropositive individuals were captured in the breeding season (October and December). The other three rodent species captured did not present hantavirus antibodies.

RODENT MOVEMENT ANALYSIS

Data from 85 rodents were used to describe rodent movements. Combined rodents species did not show any significant correlation between the HBL and the movement estimators (TD, MLD, or TA). Nevertheless, when the analysis was made for each species separately, it showed that larger individuals of *A. azarae* traveled longer TD ($r_s = 0.39$; $P = 0.05$), while the other species did not show any significant associations.

The combined rodent species traveled an average TD of 79.8 m (min 11 m; max 274 m). *O. flavescens* traveled shorter distances ($n = 4$; mean = 17.5 m) than other species (*O. rufus:* $n = 39$; mean = 87.2 m; *S. aquaticus:* $n = 7$;

mean = 59.7 m; *A. azarae:* $n = 33$; mean = 86.9 m; $F_{3;81} = 3.27$; $P = 0.025$).

The combined species average MLD was 26.2 m (min 4 m; max 83 m). There were no differences among species ($H = 1.84$; $P = 0.604$).

The combined rodent species covered an average TA of 118.3 m² (min 0 m²; max 927 m²). The area covered by *O. flavescens* ($n = 4$; mean = 9.5 m²) was smaller than the area covered by *O. rufus* ($n = 38$; mean = 134.8 m²) and *A. azarae* ($n = 33$; mean = 125.0 m²; $F_{3;77} = 2.68$; $P = 0.052$). *S. aquaticus* used an area intermediate ($n = 7$; mean = 104.1 m²) between *O. flavescens* and the other two species.

Variability of TD of *O. rufus* ($n = 44$) was marginally explained by sex, with males traveling longer distances (114.2 m) than females (79.5 m); season, with individuals moving farther during the non-breeding season (132.7 m) than during the breeding season (61.2 m); and habitat type, with longer movements occurring in the highland grassland (118.8 m) than in the salty grassland (75.2 m). Individuals of *O. rufus* were not captured in the lowland marsh. The MLD variability for this species was explained by sex and reproductive season, with longer values for males (males = 38.2 m; females = 20.9 m) and during the non-breeding season (non-breeding = 37.7 m; breeding = 21.4 m) (Table 1). The TA variability of *O. rufus* was explained by reproductive season, with a longer value during the non-breeding (271.8 m) than during the breeding season (73.2 m) (Table 1).

Variability in TD, MLD, and TA for *A. azarae* ($n = 34$) was explained by habitat type (Table 1). Movements were longer in the highland grassland (TD = 149.6 m; TA = 304.6 m and MLD = 42.4 m) than in the lowland marsh (TD = 68.8 m; TA = 48.0 m and MLD = 25.4 m) as well as the salty grassland (TD = 62.9 m; TA = 59.3 m and MLD = 18.9 m). *A. azarae* was captured only during the breeding season.

Variability in MLD for *S. aquaticus* ($n = 10$) was explained by habitat type (Table 1), with longer distances traveled in the lowland marsh (MLD = 24.25 m) than in the salty grassland (MLD = 8.50 m). No significant model explained the variability in TD and TA for *S. aquaticus*. We captured no *S. aquaticus* in the highland grassland.

The paths of rodents marginally differed in their linearity ($F_{3;80} = 2.24$; $P = 0.089$), *O. rufus* paths being most tortuous (mean = 0.29) and *O. flavescens* being most linear (mean = 0.49), while *A. azarae* and *S. aquaticus* showed an intermediate degree of tortuosity (mean = 0.34 and 0.45, respectively). *O. rufus* was the only species with a significant model explaining the intraspecific variability in LI. The

Table 1. General Linear Model Analysis of Movement Estimators Among Species of Sigmodontinae Rodents in Otamendi Natural Reserve, including the Explanatory Variables: Sex, Reproductive Season, and Habitat Types.

Species	Response variables	Explanatory variables						
		Sex		Reproductive season		Habitat type		
		F	P	F	P	F	P	R^2
O. rufus	TD	3.10	0.088	7.41	0.009	3.69	0.062	0.24
	TA			8.04	0.007			0.16
	MLD	9.15	0.004	5.81	0.021			0.25
	LI			2.98	0.092	4.18	0.047	0.11
S. aquaticus	MLD					5.52	0.047	0.41
A.azarae	TD					7.08	0.003	0.31
	TA					7.30	0.003	0.32
	MLD					8.50	0.001	0.35

Only significant results are shown.

TD total distance, *MLD* maximum linear distance, *TA* total area, *LI* linearity index.

significant model showed that individuals created more linear paths during the breeding (0.34) than in the non-breeding season (0.18), as well as in the salty grassland (0.34) than in the highland grassland (0.18) (Table 1).

Low numbers of captures precluded the use of statistical analysis for *O. flavescens* ($n = 5$).

Microhabitat Selection

Seventy-five rodent paths were used for the analysis of microhabitat selection.

O. rufus was captured in the salty grassland and the highland grassland. *O. rufus* selected sites with low fresh grass cover values (Table 2). Fresh grass cover was negatively correlated with vegetation height and with fresh and dry *C. selloana* cover. *S. aquaticus* was present in the lowland marsh and in the salty grassland and showed a tendency to select sites with dense dry shrub cover (Table 2). Dry shrub cover was positively correlated with fresh broad leaf plant cover and negatively correlated with fresh grass cover.

A. azarae captured in every habitat type of the ONR, but less frequently in the lowland marsh than in the salty grassland ($z = -2.44$; $P = 0.037$) and the highland grassland ($z = -2.21$; $P = 0.068$). In all habitat types, *A. azarae* used sites randomly rather than selectively (Table 2).

O. flavescens was found in the lowland marsh and the highland grassland, and in these habitats, they selected sites with low dry shrub cover (Table 2).

Characteristics of the Paths Used by Rodents

Quantitative characteristics of 61 paths used by rodents were recorded. Fifty-nine percent (36 of 61) of all the paths showed overlapping with other paths (i.e., sharing of a section of a path among two or more individuals).

Sixty-nine percent (42 of 61) of all paths showed crossing (i.e., one path crossing another one); 39% (24 of 61) were crossings between individuals of the same species and 30% (18 of 61) were crossings between individuals of different species.

Forty-three percent of crossings occurred between paths of males (between males of *O. rufus*, between males of *A. azarae*, and between males of *O. rufus* with males of *A. azarae*), 26% between females (between females of *O. rufus*, between females of *O. rufus* with females of *S. aquaticus*, and between females of *O. rufus* with *A. azarae* females) and 31% between different sexes of the same species (*A. azarae* and *O. rufus*).

O. flavescens did not overlap or cross its paths with other individuals of their own or other species.

Seventy-two percent of individuals of all species (44 of 61) moved repeatedly and in both directions across sections of their own path. The crossing sections showed an intense and frequent use by rodents, and looked like "tunnels" covered by vegetation.

O. rufus and *A. azarae* used a diversity of nests or refuges constructed above the ground with dry vegetation. Two individuals of *O. rufus* used some of the thread to

Table 2. Generalized Linear Model Analysis for Microhabitat Selection of Sigmodontinae Rodents in Otamendi Natural Reserve.

Species	Explanatory Variables	Estimator	SE	Z	P
O. rufus	Intercept	−1.03	0.32	−3.23	**0.001**
	% fresh grasses	−0.01	0.01	−2.06	**0.040**
S. aquaticus	Intercept	−5.45	1.24	−4.39	<0.001
	% dry shrubs	0.94	0.58	1.62	0.105
A. azarae	Intercept	−0.86	0.94	−0.92	0.360
	Lowland marsh	−1.58	0.71	−2.23	**0.026**
	Salty grassland	0.10	0.47	0.21	0.833
O. flavescens	Intercept	8.63	4.76	1.81	0.070
	% dry shrubs	−5.74	1.78	−3.23	**0.001**

The values in bold correspond to P values less than 0.05
SE standard error.

construct their nests. No thread of any species was found to end in a burrow.

O. rufus used space horizontally, and non-vertically. Individuals of *O. rufus* moved through several types of vegetation, and some of their paths were below 0.5 m of dense layers of dry grasses, inside very moist tunnels. Every *O. rufus* path crossed through the base of plants of *C. selloana*.

S. aquaticus paths were not surrounded by enough vegetation as to be considered "tunnels." But similarly to *O. rufus*, *S. aquaticus* also used *C. selloana* plants as refuges, always at ground level. In the salty grassland, *C. selloana* paths crossed flooded zones with high coverage of sedges (family Cyperaceae).

A. azarae showed a broad horizontal and vertical use of space, climbing plants of *C. selloana* up to one meter height repeatedly along the same path (up to 10 times for a single path), moving above the vegetation, in contrast to paths of *O. rufus* and *S. aquaticus*, and used tunnel-like paths, as *O. rufus* and *S. aquaticus*. Both male and female seropositive *A. azarae* individuals crossed paths with others individuals of *A. azarae* (which were seronegatives) and both male and female *O. rufus*. Additionally, a snake was found in the same tunnel used by *A. azarae*. Also, we frequently found feces of *Cavia aperea* in tunnel-like paths in the salty grassland.

All the individuals of *O. flavescens* used space not only horizontally (29% of the points) but also vertically by climbing sedges or shrubs (71% of the recorded points), and did not use tunnel-like paths.

DISCUSSION

We captured four Sigmodontinae species in Otamendi Natural Reserve, two of which, *O. flavescens* and *A. azarae*,

are host of hantavirus in the region (Vadell et al. 2011, Palma et al. 2012).

In spite of the fact that *O. flavescens* has been identified as host of the Lechiguanas genotype (etiological agent of HPS in humans) in a location close to ONR (Suárez et al. 2003), antibodies were not detected in this species during this study. However, we do not rule out the presence of virus because the number of individuals analyzed was very small. Previous studies conducted in ONR (Bonaventura et al. 1991; Gómez Villafañe et al. 2012) reported slightly higher densities of *O. flavescens* in each habitat type, so the risk for human health could have previously been greater. However, we have to be cautious because the population dynamics of this species is irregular with periods of high densities followed by periods of low densities and their migration pattern remains poorly known (Crespo, 1966, Chiappero et al. 1997).

Hantavirus antibodies were found in individuals of *A. azarae*, demonstrating this species as a reservoir in ONR as has previously been reported (Vadell et al. 2011). *A. azarae* is host of the Pergamino hantavirus genotype, which up to date has not been associated with human disease (Vadell et al. 2011; Palma et al. 2012). The abundance of *A. azarae* found in this study was similar to that previously reported (Bonaventura et al. 1991; Suárez and Bonaventura 2001; Gómez Villafañe et al. 2012).

As we expected, rodent species, except *A. azarae*, were not found in all habitat types, showing a differential use of habitat that may be due to specific requirements of each species (Gómez Villafañe et al. 2012). *A. azarae* was the only species found in all habitat types and patches inside them. This pattern is consistent with previous descriptions of this species as omnivorous and opportunistic (Suárez

and Bonaventura 2001), commonly found in undisturbed grasslands or crop field edges (Bonaventura and Kravetz 1984; Mills et al. 1991; Busch and Kravetz 1992; Bilenca and Kravetz 1995; Gómez Villafañe et al. 2012). *A. azarae* inhabits all the habitats studied but seropositive individuals were only found in the salty grassland and the lowland marsh, where the vegetation is denser, showing focality of infection, as was proposed by other authors in several hantavirus–rodent systems (Abbott et al. 1999, Glass et al. 1998, Mills et al. 1999). The focus of concentration suggests that the presence of a reservoir is not enough to explain the circulation and persistence of the virus within the reservoir populations. Characteristics of the habitat or the host population, such as a threshold density, could be keys for the maintenance of infected individuals within the populations.

O. flavescens was captured in two of the three habitats studied and within the three habitats selected sites with low dry shrub cover and high fresh grass cover. Previous work described this species inhabiting stable and linear habitats such as crop field borders and railroad tracks (Mills et al. 1991, Busch and Kravetz 1992).

S. aquaticus is a characteristic species of temporarily flooded habitats (Bonaventura et al. 2003; Nava et al. 2003) such as the lowland marsh and the salty grassland. This species selected places with high dry shrub cover, though this selection is rare for a semiaquatic species. However, this microhabitat is positively correlated with fresh broad leaf cover and negatively correlated with green grass cover, with which *S. aquaticus* could be dietary related (Bonaventura et al. 2003).

O. rufus has been described as inhabiting wet grasslands (Bonaventura et al. 2003, Nava et al. 2003). We captured *O. rufus* in the highland and salty grasslands, in agreement with what has been previously described for this species. The lack of captures in the lowland marsh may have been related to the fact that lowland marsh is almost permanently covered with water, a characteristic not expected to be selected by a semi-fossorial species. Our findings in microhabitat selection were consistent with previous studies for *O. rufus* in the same region (Cueto et al. 1995, Bonaventura et al. 2003), and the use of space made by this species was consistent with its semi-fossorial habits and with its diet (Cueto et al. 1995, Coutinho et al. 2013).

In agreement with our third hypothesis (distance moved is related to body size), *O. flavescens*, the smallest species, moved over shorter distances than others. This movement pattern would restrict virus dispersal among habitat types. On the other hand, *A. azarae* being a larger species than *O.*

flavescens traveled longer distances showing that the dispersion of Pergamino virus could be larger than the dispersion of Lechiguanas. Additionally, *O. flavescens* had the most linear movements of the assemblage covering a lesser area than other species, probably because its scansorial habits allow it to overcome obstacles by climbing sedges or branches, instead of avoiding obstacles at ground level. In addition, the vertical use of habitat could expose shed virus to more sunlight which destroys the virus (Kraus et al. 2005).

In agreement with our fourth hypothesis, movements of *A. azarae* increased in distance with the size of the individual. Because size of individuals has also been associated with prevalence of hantavirus infection (Glass et al. 1998, Mills et al. 1999, Suárez et al. 2003, Vadell et al. 2011), individuals traveling large distances are more prone to be carrying the virus than individuals traveling shorter distances, favoring the dispersion of the virus in the environment. This dispersion would be especially favored in the highland grassland where individuals of *A. azarae* made longer and more tortuous movements than in the other two habitats. This habitat-related differential behavior could be due to low cover of herbs forcing individuals to move greater distances to obtain resources (Bilenca et al. 1992).

Another factor that influences the acquisition of the virus by rodent is the sex of the individuals, probably because aggressive contacts among males increase the probability of transmission (Padula et al. 2004). Because there is a general sex-related difference in prevalence, we studied the existence of differences in movement between sexes. We expected that males moved longer distances than females due to the search for mates which would result in increasing the risk of transmission. However, this hypothesis was fulfilled only for *O. rufus,* a species which is not a hantavirus host. Additionally, we expected movements to be different during the breeding season than the non-breeding seasons for all species; however, we found differences between sexes only for *O. rufus*. Daily movements were longer and more tortuous during the non-breeding season than during the breeding season, possibly as a result of an increased foraging time required during the non-breeding season because of the low availability of resources (Kravetz and De Villafañe 1981; Bonaventura and Kravetz 1984). Neither *A. azarae* nor *S. aquaticus* showed longer movements during one season than in another season. This lack of seasonal differences in movements does not influence the increase in virus transmission due to differences in the movements of individuals.

All species with the exception of *O. flavescens* had overlapping home ranges at an interspecific, intraspecific, intrasexual, and intersexual level. The absence of shared paths of *O. flavescens* could be related to the scansorial habits of this species (Coutinho et al. 2013) that result in the lack of the creation of a path in the vegetation which can result in use by other individuals of the same or different species. The tunnel-like paths below the vegetation cover had signs of frequent use by one or more species of rodents. The paths facilitate movement because the vegetation in the frequently used paths is crushed. The sharing of space throughout a greater habitat overlapping among rodent species probably results in an increase in the frequency of encounters among individuals facilitating the direct and indirect transmission of diseases (Wisnivesky 2003; Palma et al. 2012).

The overlapping of paths at an interspecific level could promote the horizontal transmission of pathogens among rodent species, allowing the occurrence of spillover and host-switching events, and therefore, the emergence of an infectious disease, since it depends on the type and intensity of contacts between the donor host or its viruses and the new host (Daszak et al. 2000, Parrish et al. 2008). In this sense, based on the fact that *A. azarae* has generalist habits, and that it shares paths with the rest of the species, we propose that this rodent species could play an important role as a link among species within the assemblage and could promote the spillover of hantavirus.

Acknowledgments

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