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# Hantavirus ecology in rodent populations in three protected areas of Argentina

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**Summary**

In this study, we identified hantavirus genotypes and their reservoirs and evaluated the spatial and temporal distribution of the virus in rodent population in three protected areas of Argentina over 3 years (2007–2010). A total of 837 rodents were captured with an effort of 22 117 trap-nights. We detected the genotype Lechiguanas in *Oligoryzomys nigripes* and *O. flavescens* and Pergamino in *Akodon azarae*. There was no correlation between seroprevalence and trap success of the host. The proportion of seropositive males was significantly higher than the proportion of seropositive females. The total length of seropositives was higher than that of seronegatives in each host species. Seropositive individuals were observed in warm months and not in cold months, which suggests an infection cycle. This investigation confirms that protected areas of central east Argentina are places with a variety of sylvan rodents species associated with different hantavirus genotypes where reservoirs are numerically dominant. Although there was more than one known reservoir of hantavirus, only one species had antibodies in each area. This can be explained because the transmission of the virus does need not only the presence of a rodent species but also a threshold density. Longevity of even a small proportion of the host population in cold months may provide a trans-seasonal mechanism for virus persistence. The seroprevalence detected was higher than the one found before in rodent populations of Argentina, and this explains the appearance of human cases in two of these three areas.

**keywords** hantavirus seroprevalence, protected natural areas, rodents, genotypes, population dynamics, ecology

## Introduction

Hantavirus pulmonary syndrome (HPS) is an increasingly recognized infectious disease caused by New World hantaviruses (family Bunyaviridae) (Peters 1998; Young *et al.* 1998). Each hantavirus is usually hosted by a single species of rodent belonging to subfamilies *Sigmodontinae* in America (Clement 2003). Virus transmission among rodents occurs mainly via saliva and blood (Glass *et al.* 1988; Hinson *et al.* 2004). Humans can be infected by contact with contaminated aerosols, saliva, urine or faeces from infected rodents (Padula *et al.* 2004).

In central east Argentina, four sigmodontine host species associated with hantavirus have been identified: *Oligoryzomys flavescens* (AND Lechiguanas and AND Plata), an omnivorous species that inhabits grasslands, forests and irrigated cropfields; *Oligoryzomys nigripes* (Juquitiva), a principally granivorous species endemic from Delta del Paraná ecoregion; *Necromys obscurus* (Maciel), an insectivore species that inhabits grasslands and edges of flooded fields and *Akodon azarae* (Pergamino), an omnivorous species that inhabits principally grasslands and habitats with high herbaceous cover (Levis *et al.* 2004; Padula *et al.* 2007). The last two hantaviruses genotypes have not been associated with human cases.

Protected natural areas are one of the places where contact between sylvan rodents and humans is possible; however, few studies on hantavirus have been carried out in these areas so far. In Argentina, three people who had visited protected areas (Calilegua, El Palmar and Islas de Santa Fé National Parks) were diagnosed with HPS during 2007 and 2008, being the parks the probable sites of infection. In view of these records, we focused our research on this type of areas.

The aims of this study were (i) to identify the hantavirus reservoirs; (ii) to identify the genotypes associated with each reservoir; (iii) to estimate seroprevalence of infection; (iv) to evaluate seasonal variation in seroprevalence; (v) to determine the distribution of the virus in the host population in three protected areas of central east Argentina.

## Materials and methods

### Study area

The study was conducted in three protected areas of central east Argentina: El Palmar (8500 hectares) and Pre-Delta (2458 hectares) National Parks (NP) and Otamendi Natural Reserve (NR; 3000 hectares) (Figure 1). El Palmar National Park (31° 51′ S; 58° 19′ W) is located on the west bank of the Uruguay River. In this NP, we determined seven study zones: a camping area surrounded by a forest; a shrubland; two riparian forests; a *Butia yatay* palm forest with shrubs; a *B. yatay* palm forest without shrubs and a forest dominated by exotic trees.

The Pre-Delta National Park (32° 03′S; 60° 38′ W) is located in the flood plain of the Paraná River and comprises a continental area and several islands. In this NP, we determined five study zones: two sectors of an island (incorporated to Islas de Santá Fé NP in 2010); a riparian forest of a small river; a continental forest and a lowland grassland.

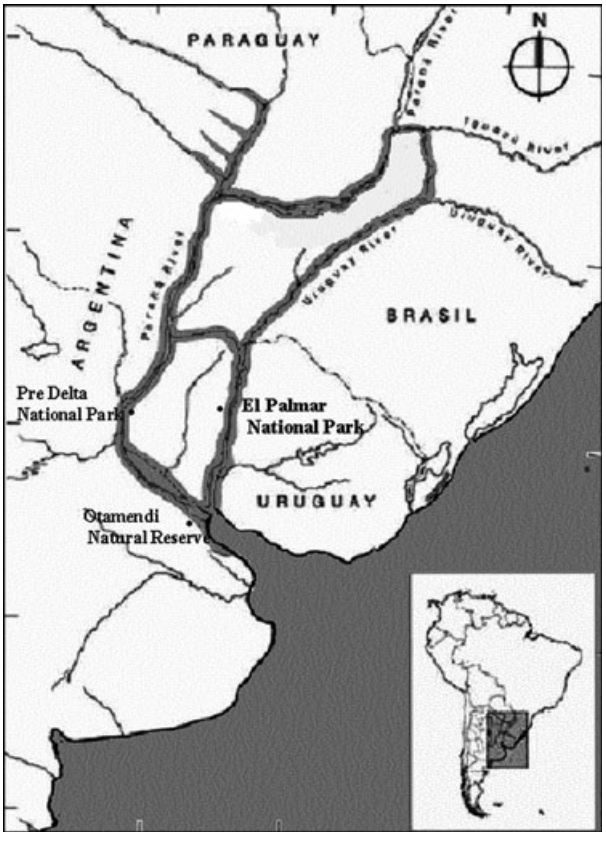


**Figure 1** Location of the study areas.

The Otamendi NR (34° 10′ S; 58° 48′ W) is located on the coast of the Paraná river. In this NR, we determined six zones of study: a riparian forest; a *Celtis tala* forest; a lowland grassland; a salty grassland and two highland grasslands, one characterized by a great invasion of *Ligustrum* sp. and the other one by intermittent presence of livestock.

### Rodent survey

Rodents were live-trapped seasonally for 3 years in El Palmar NP (April 2007–April 2010) and in Otamendi NR (September 2007–June 2010) and for 2 years in Pre-Delta NP (July 2008–August 2010). Between 250 and 300 live capture traps were placed in lines of 20–50 traps at 10-m intervals in each protected area and season. Trap lines were located in the same places during each season. Traps were baited with a mixture of peanut butter, fat and rolled oats. Trap success (TS) was estimated as: Number of rodents captured × 100/(number of traps × number of nights).

Captured rodents were anaesthetized and the sex, weight, presence of scars and body–tail length were recorded. The species were identified based on external morphology and skull characteristics. Then, all individuals were tagged with a uniquely numbered ear tag and were released at the point of capture.

An index of body condition was calculated for the reservoir species as,

$$\text{Body condition} = \frac{\sqrt[3]{\text{weight}}}{\text{total length}}$$

To detect AND virus-specific immunoglobulin G antibodies, enzyme-linked immunosorbent assays were performed on blood samples (Padula *et al.* 2000). Some individuals were sacrificed, and the tissues were stored in a nitrogen tank to determine the hantavirus genotype. The total RNA was extracted from lung tissues of seropositive rodents using Trizol (Invitrogen) and purified by the RNAid kit (Bio 101). Partial S- and M-segments were amplified by RT-PCR followed by a second round of nested PCR. Specific oligonucleotide primers, based on conserved regions of AND virus genome, were used. Amplification products were analysed on agarose gels and sequenced (Padula *et al.* 2000).

We amplified and sequenced a 480-nt fragment for S-segment (position 71–550). For El Palmar rodents, a 790-nt fragment (position 71–860) was also amplified for S-segment and for M-segment Gn 349-nt (59–407) and Gc 253-nt (2695–2947) fragments. All positions were referred to AH1 AND strain, Gene Bank No AF324902.

**Table 1** Total trap success (TS), *Oligoryzomys nigripes* (*On*) TS and percentage of seropositive (+) *O. nigripes* in each habitat type of El Palmar National Parks

| Habitat type | Total TS | *On* TS | % of (+) *On* |
|---|---|---|---|
| Riparian forest 1 | 0.95 | 0.95 | 31 |
| Palm forest without shrubs | 0.54 | 0.31 | 25 |
| Camping area | 1.17 | 2.88 | 17 |
| Forest dominated by exotic tree | 0.84 | 0.61 | 13 |
| Shrubland | 1.48 | 0.09 | 0 |
| Riparian forest 2 | 0.74 | 0.37 | 0 |
| Palm forest with shrubs | 0.15 | 0.15 | 0 |

In the camping area of El Palmar NP and in the island sectors of the Pre-Delta NP where people are likely to be in contact with rodents, the reservoirs captured were removed. Therefore, the virus dynamics was only observed in Otamendi NR, which acted as an undisturbed area.

### Data analysis

We compared the body condition between positive and negative rodents in each protected area by means of a Mann–Whitney test (Zar 1996). The association between the number of host captured and seroprevalence in different seasons was analysed by means of a Spearman's correlation per protected area and per zone whenever the amount of data was sufficient (Zar 1996). The association between the number of host captured and number of positive individuals per season and protected area was analysed by means of a Spearmen's correlation (Zar 1996). The proportion of individuals with hantavirus antibodies (seropositives) was compared between sexes by means of a test of difference of proportions (Zar 1996). The total lengths of seropositive and seronegative individuals were compared by an ANOVA test or a Mann–Whitney *U* test when data did not fulfil the ANOVA assumptions.

## Results

A total of 840 rodents were captured: 145 in El Palmar NP, 300 in Pre-Delta NP and 395 in Otamendi NR with an effort of 8832, 6024 and 7261 trap-nights, respectively. In El Palmar NP, we captured *O. nigripes* (66), *Calomys callidus* (48), *A. azarae* (21) and *Oxymycterus rufus* (10) with TS of 1.5. Hantavirus antibodies were detected in *O. nigripes*. This species was captured in all the study zones, but seropositive individuals were detected in four of these (Table 1); 45% of the total captures tallied with the host species, and 17% of these had antibodies for hantavirus (Tables 1 and 2). We detected viral genome in two (EP56, EP57) of three rodents with tissue samples available of the 11 seropositives individuals. Through sequencing and phylogenetic comparison, it was shown that the viral genotype present in EP56 and EP57 was AND Lechiguanas (Figure 2). Because this genotype is not usually reported for *O. nigripes*, a large fragment of the S-segment was amplified resulting in 97% in respect to Lechiguanas virus strain 22819 (AF482714). Two other fragments from M-segment were also sequenced, and 92% for Gn and 91% for Gc maximum identities were obtained with Lechiguanas virus strain Of22819 (AF028022).

**Table 2** Trap success (TS), percentage of females, and seroprevalence of hantavirus (seropr.) in *Oligoryzomys nigripes* (*On*) in El Palmar National Park

| Period | Trap-nights | Total captures | # *On* captured | *On* TS | *On* females (%) | # positive *On** | Total seropr. | Female seropr. | Male seropr. |
|---|---|---|---|---|---|---|---|---|---|
| Apr-07 | 540 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oct-07 | 900 | 23 | 11 | 1.22 | 45.5 | 2 | 0.18 | 0.2 | 0.17 |
| Nov-07 | 648 | 25 | 18 | 2.78 | 33.3 | 2 | 0.11 | 0 | 0.17 |
| Feb-08 | 876 | 12 | 6 | 0.68 | 60 | 1 | 0.17 | 0.33 | 0 |
| May-08 | 870 | 4 | 1 | 0.11 | 0 | 0 | 0 | 0 | 0 |
| Sep-08 | 744 | 7 | 2 | 0.27 | 0 | 0 | 0 | 0 | 0 |
| Dec-08 | 780 | 4 | 1 | 0.13 | 100 | 0 | 0 | 0 | 0 |
| Mar-09 | 837 | 10 | 4 | 0.48 | 75 | 1 | 0.25 | 0 | 1 |
| Jul-09 | 936 | 6 | 5 | 0.53 | 40 | 1 | 0.20 | 0 | 0.33 |
| Oct-09 | 900 | 10 | 5 | 0.56 | 20 | 2 | 0.40 | 0 | 0.50 |
| Jan-10 | 801 | 10 | 5 | 0.62 | 80 | 1 | 0.20 | 0 | 1 |
| April-10 | 870 | 14 | 8 | 0.92 | 0.25 | 1 | 0.13 | 0 | 0.17 |
| Total | 8832 | 145 | 66 | 0.68 | | 11 | 0.17 | | |

*Hantavirus genotype: Lechiguanas.

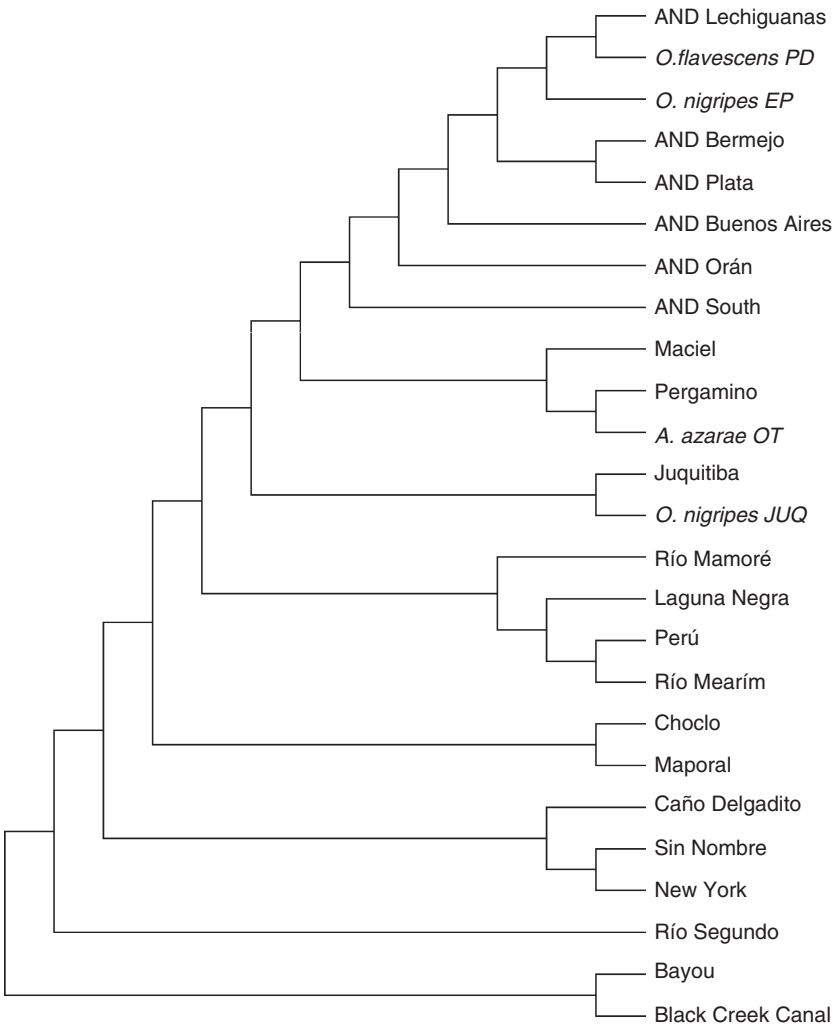


**Figure 2** Phylogenetic relationships among the nucleotide sequences of the N protein of different hantaviruses from America. A maximum parsimonious phylogenetic tree was generated on the basis of nucleotide sequence differences in the 480-nt region of the N gene using MEGA 4 software package (http://www.megasoftware.net), obtained from 2000 bootstrap reiterations. The strain sequences under study in this paper are in italics (EP, El Palmar; PD, Pre-Delta; OT, Otamendi).

**Table 3** Total trap success (TS), *Oligoryzomys flavescens* (*Of*) TS and percentage of seropositive (+) *O. flavescens* in each habitat type of Pre-dDelta National Parks

| Habitat type | Total TS | *Of* TS | % of (+) *Of* |
|---|---|---|---|
| Sector of an island 1 | 4.19 | 4.09 | 13 |
| Sector of an island 2 | 6.09 | 5.52 | 5 |
| Riparian forest | 3.71 | 0.52 | 2 |
| Lowland grassland | 7.35 | 1.21 | 0 |
| Continental forest | 5.07 | 0.77 | 0 |

In addition, we amplified and sequenced a fragment of mitochondrial DNA (386 pb), which showed a 95% identity with *O. nigripes* (GU185869) and 87% with *O. flavescens* (GU185880), the previously reservoir reported for AND Lechiguanas.

In Pre-Delta NP, we captured *O. flavescens* (133), *O. rufus* (80), *C. callidus* (53), *A. azarae* (27) and *Holochilus* sp. (7) with total TS of 4.8. Hantavirus antibodies were detected in *O. flavescens*. This species was captured in all the study zones, but positive individuals were detected in three of these (Table 3); 46% of the total captures tallied with the host species, and 8% of these had hantavirus antibodies (Tables 3 and 4). In this park, we detected the presence of viral genome in 7 of 10 seropositive rodents. Phylogenetic analysis of a 480-nt fragment of viral S-segment revealed that viral lineage was 99% identical to Lechiguanas virus strain 22819 (AF482714) (Figure 2).

In Otamendi NR, we captured *O. rufus* (132), *A. azarae* (122), *Scapteromys aquaticus* (74), *O. flavescens* (23), *Deltamys kempi* (20), *O. nigripes* (18) and *Calomys laucha* (6), with a total TS of 5.4. Hantavirus antibodies were

**Table 4** Trap success (TS) rate, percentage of females, and seroprevalence of hantavirus (seropr.) in *Oligoryzomys flavescens* (*Of*) in Pre-Delta National Park

| Period | Trap nights | Total captures | # *Of* captures | *Of* TS | *Of* females (%) | # positive *Of** | Total seropr. | Female seropr. | Male seropr. |
|---|---|---|---|---|---|---|---|---|---|
| Jul-08 | 780 | 19 | 8 | 1.03 | 25 | 1 | 0.13 | 0 | 0.17 |
| Oct-08 | 831 | 95 | 70 | 8.42 | 41.4 | 7 | 0.10 | 0 | 0.17 |
| Febr-09 | 750 | 19 | 4 | 0.53 | 75.0 | 0 | 0 | 0 | 0.00 |
| June-09 | 750 | 58 | 35 | 4.67 | 42.9 | 2 | 0.06 | 0 | 0.10 |
| Sep-09 | 750 | 63 | 12 | 1.60 | 25.0 | 0 | 0 | 0 | 0.00 |
| Dec-09 | 669 | 23 | 1 | 0.15 | 100.0 | 0 | 0 | 0 | 0.00 |
| May-10 | 750 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 |
| Aug-10 | 744 | 24 | 3 | 0.40 | 0 | 0 | 0 | 0 | 0.00 |
| Total | 6024 | 300 | 133 | 2.21 | | 10 | 0.08 | | |

*Hantavirus genotype: Lechiguanas.

**Table 5** Total trap success (TS), *Akodon azarae* (*Aa*) TS and percentage of seropositive (+) *A. azarae* in each habitat type of Otamendi Natural Reserve

| Habitat type | Total TS | *Aa* TS | % of (+) *Aa* |
|---|---|---|---|
| Lowland grassland | 5.60 | 1.06 | 39 |
| Highland grassland with livestock | 3.62 | 2.39 | 31 |
| Salty marshes | 4.74 | 3.55 | 29 |
| Riparian forest | 12.31 | 0.57 | 20 |
| *Celtis tala* forest | 2.15 | 0.75 | 13 |
| Grassland with invasion of *Ligustrum* sp. | 6.45 | 2.91 | 0 |

detected in *A. azarae* and *D. kempi* (only one individual). *Akodon azarae* was captured in all the study zones, and seropositive individuals were detected in all but one zone (Table 5); 31% of the captures tallied with *A. azarae,* and 22% of these had antibodies for hantavirus (Tables 5 and 6). Samples for molecular analysis were available from nine seropositive rodents. All these nine samples had viral genome, and the hantavirus genotype detected was Pergamino, identified from a 480-nt fragment of viral S-segment (97% identity with previous characterized strain 14403, AF482717; Figure 2).

We observed a significant positive association between the number of host captured and the number of positive individuals (El Palmar: $r = 0.88$, $P = 0.0002$, $N = 11$; Pre-Delta: $r = 0.79$, $P = 0.019$, $N = 8$; Otamendi: $r = 0.76$, $P = 0.017$, $N = 9$; Figure 3a.I,b.I,c.I). But there was no significant correlation between seroprevalence and the number of host captured in any of the three protected areas considering each protected area as a whole (El Palmar: $r = 0.50$, $P = 0.11$, $N = 11$; Pre-Delta: $r = 0.65$, $P = 0.08$, $N = 8$; Otamendi: $r = 0.42$, $P = 0.26$, $N = 9$; Figure 3a.II,b.II,c.II) or taking into account each habitat within each of the protected areas ($P > 0.05$; Figures 4–6).

The proportion of seropositive males was significantly higher than the proportion of seropositive females in the

**Table 6** Trap success (TS) rate, percentage of females, and seroprevalence of hantavirus (seropr.) in *Akodon azarae* (*Aa*) in the Otamendi Natural Reserve

| Period | Trap-nights | Total captures | # *Aa* captured | *Aa* TS | *Aa* females (%) | # positive *Aa** | Total seropr. | Female seropr. | Male seropr. |
|---|---|---|---|---|---|---|---|---|---|
| Sep-07 | 990 | 45 | 10 | 1.01 | 20 | 1 | 0.1 | 0 | 0.13 |
| Dec-07 | 942 | 34 | 7 | 0.74 | 0 | 3 | 0.43 | 0 | 0.43 |
| March-08 | 837 | 45 | 3 | 0.36 | 66.7 | 0 | 0 | 0 | 0.00 |
| July-08 | 867 | 29 | 5 | 0.58 | 60 | 0 | 0 | 0 | 0.00 |
| April-09 | 720 | 48 | 19 | 2.64 | 57.9 | 2 | 0.11 | 0 | 0.29 |
| Aug-09 | 717 | 39 | 17 | 2.37 | 23.5 | 5 | 0.29 | 0.25 | 0.31 |
| Dec-09 | 667 | 34 | 13 | 1.95 | 53.8 | 2 | 0.15 | 0 | 0.33 |
| March-10 | 771 | 41 | 13 | 1.69 | 41.7 | 5 | 0.42 | 0.40 | 0.43 |
| June-10 | 750 | 78 | 35 | 4.67 | 45.71 | 9 | 0.26 | 0.06 | 0.42 |
| Total | 7261 | 395 | 122 | 1.68 | | 27 | 0.22 | | |

*Hantavirus genotype: Pergamino.

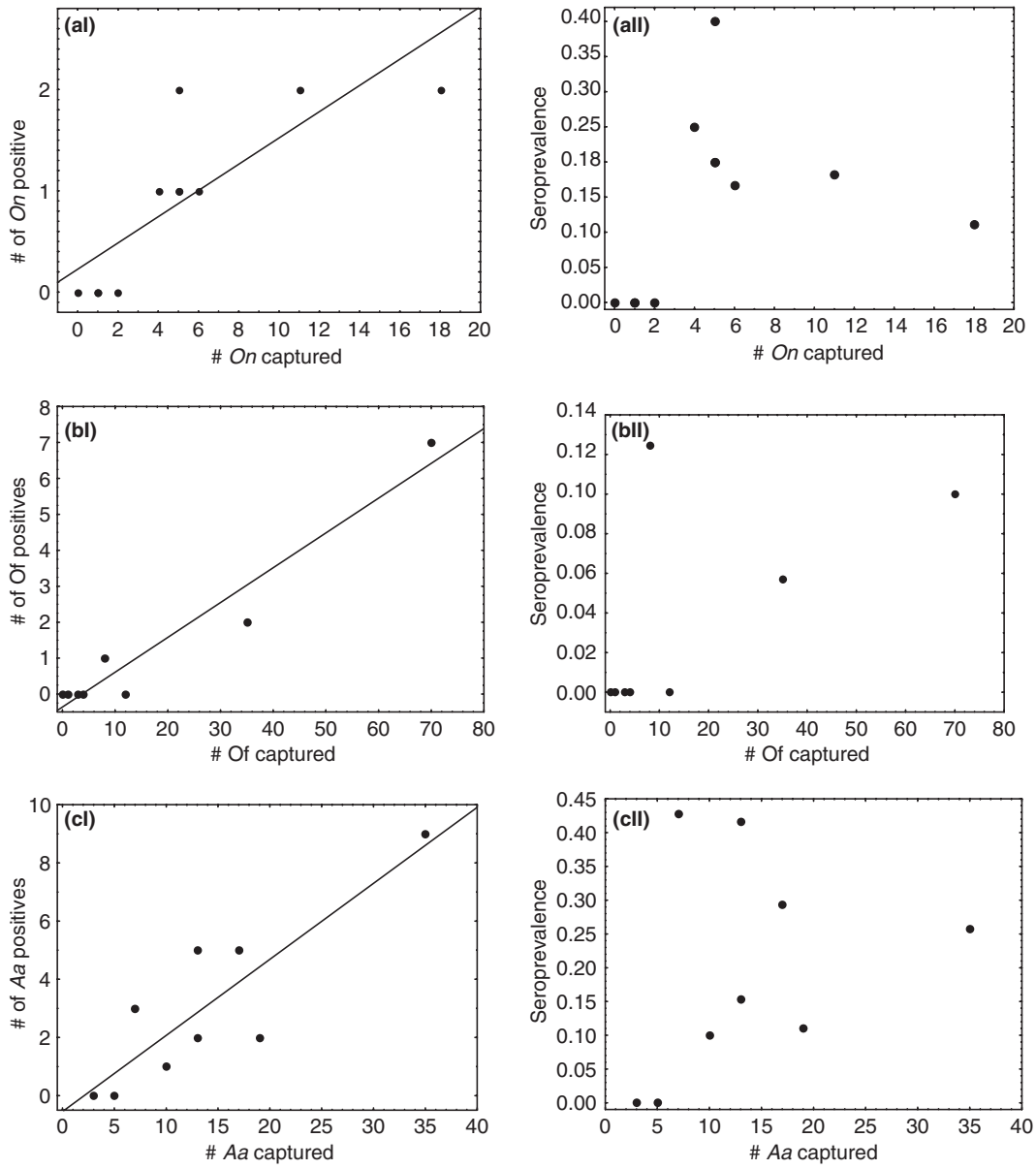


**Figure 3** Relationship between number of host captured and number of positive individuals (I) or prevalence (II) in El Palmar National Parks (NP) (a), Pre-Delta NP (b) and Otamendi Natural Reserve (c).

three protected areas (Figure 7), but we did not observe individuals of the host species with noticeable scars.

A total length of *O. flavescens* was significantly higher for seropositive (mean = 213 mm) than for seronegative individuals (mean = 197 mm) in Pre-Delta NP ($F_{1,126}$ = 11.32; $P$ = 0.001; Figure 8b). The same was observed for *A. azarae* in Otamendi NR (mean seropositive = 180 mm *vs.* mean seronegative = 169 mm; $F_{1,119}$ = 15.41; $P$ = 0.0001; Figure 8c). In El Palmar, we found a marginally significant difference between the total length of seropositive (mean = 249 mm) and seronegative (mean = 239 mm) *O. nigripes* ($U_{1,64}$ = 201.5; $P$ = 0.08; Figure 8a). There were no significant differences in body condition between positive and negative individuals in any protected area (NR Otamendi: $U_{1,110}$ = 1070, $P$ = 0.74; NP Palmar: $U_{1,61}$ = 236, $P$ = 0.36; NP Pre-Delta:

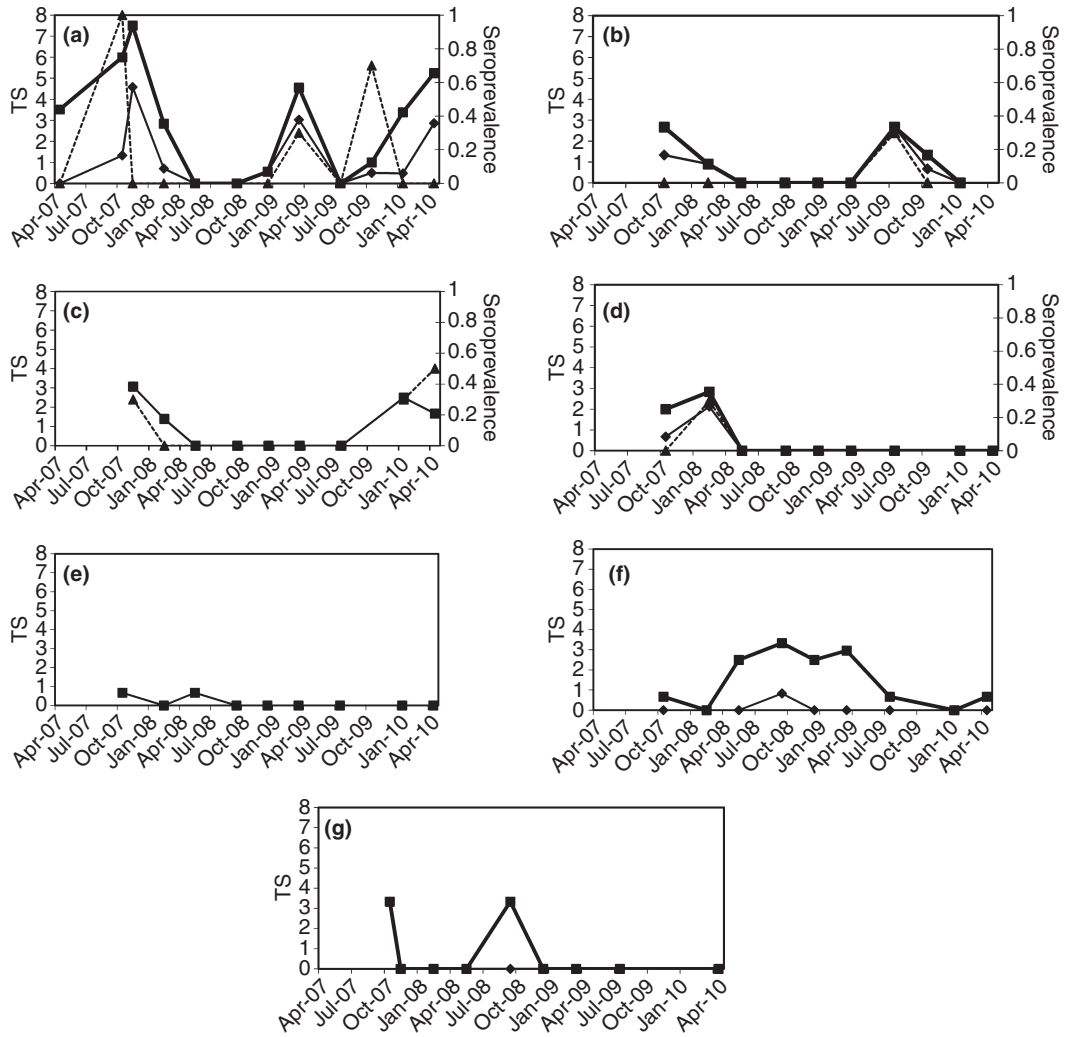


**Figure 4** Total trap success (TS) (thick solid line), *Oligoryzomys nigripes* TS (thin solid line) and seroprevalence (dotted line) in El Palmar National Parks for (a) Camping area; (b) forest dominated by exotic trees; (c) riparian forest 1; (d) palm forest without shrubs; (e) palm forest with shrubs; (f) shrubland; (g) riparian forest 2.

$U_{1,123} = 410.5$, $P = 0.134$). As regard the dynamics of seroprevalence over time, seropositive individuals were observed in warm months and not in cold months, coincidently with the low rodent abundance (Figure 6).

## Discussion

This investigation confirms that protected areas of central east Argentina are a zone of circulation for hantavirus of different genotypes (Levis *et al.* 1997; Martínez *et al.* 2001; Suárez *et al.* 2003), and reservoirs were found in a great variety of floristic habitats showing a high niche breadth.

All the three studied protected areas share two rodent species (*A. azarae* and *O. rufus*) and one genus (*Oligoryzomys*) and differ from one another in at least three species. Differences in rodent population composition are probably due to the fact that these natural areas either belong to different ecoregions (Delta del Paraná or Espinal) or are located in different latitudes; therefore, they present different environmental conditions and different vegetation structure and composition.

At least two hantavirus reservoirs were present in each protected areas. However, only one species had antibodies for hantavirus in each area, and the rodent species was not the same. This result suggests that transmission and

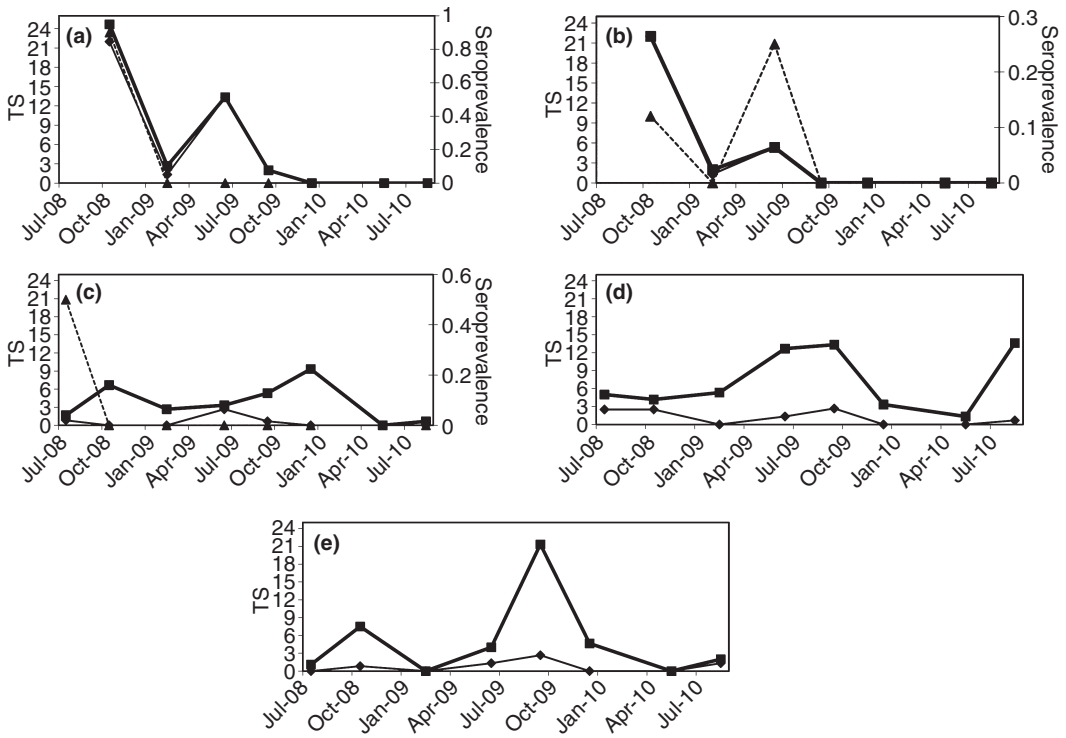


**Figure 5** Total trap success (TS ) (thick solid line), *Oligoryzomys flavescens* TS (thin solid line) and seroprevalence (dotted line) in Pre-Delta National Parks for (a) sector of an island 1; (b) sector of a island 2; (c) riparian forest 1; (d) lowland grassland; (e) continental forest.

persistence of the virus do not only depend on the presence of a reservoir species (and the presence of the virus) but also on certain population or environmental conditions, such as certain habitat characteristics or threshold density. This has been suggested in studies in Europe and North America (Boone *et al.* 1998; Escutenaire *et al.* 2002; Tersago *et al.* 2010) and in our study, where the species that presented hantavirus antibodies was the most abundant. We also observed spatial focality in the distribution of the virus within host population (i.e. hantavirus was not detected in all those habitats where the reservoir species was caught) as it has already been observed in other sigmodontine host systems in North and South America (Glass *et al.* 1988; Mills *et al.* 1999, 2007).

In the hosts, *O. flavescens* and *O. nigripes*, we determined the genotype Lechiguanas although this genotype is typical of *O. flavescens*. This result could be because of a spillover effect or the beginning of a host switch (Nemirov *et al.* 2002; Schlegel *et al.* 2009). Although we did not capture *O. flavescens* in El Palmar NP, it was present in owl's regurgitated pellet of the area (Gómez Villafañe, unpublished observation). More rodent tissue samples will be necessary to confirm these assumptions. Another case is the positive *D. kempi*, an uncommon host species of hantavirus, which also constitutes a spillover host. Spillovers are common in sympatric rodents and are believed to have little or no impact on hantavirus distribution (Schmaljohn & Hjelle 1997).

The host populations showing a higher prevalence of infection in male and adult rodents (Mills *et al.* 1999; Chu *et al.* 2003; Suárez *et al.* 2003; Madhav *et al.* 2007; Tersago *et al.* 2010). Hantavirus infections have traditionally been considered asymptomatic in their rodent host (Childs *et al.* 1989; O' Connor *et al.* 1997; Mills *et al.* 2007; Olsson *et al.* 2010). However, studies carried out in North America and Europe have suggested that hantavirus infection decreases survival and weight gain of their hosts (Douglass *et al.* 2007; Kallio *et al.* 2007). In agreement with the first authors, we did not find any detrimental effects on any aspect of growth or body condition of their host.

It has been proposed that aggressive encounters play an important role in hantavirus transmission during the breeding season, while behaviours such as communal nesting or mutual grooming could be acting as determinant factors in the dynamics of infection during the winter

 

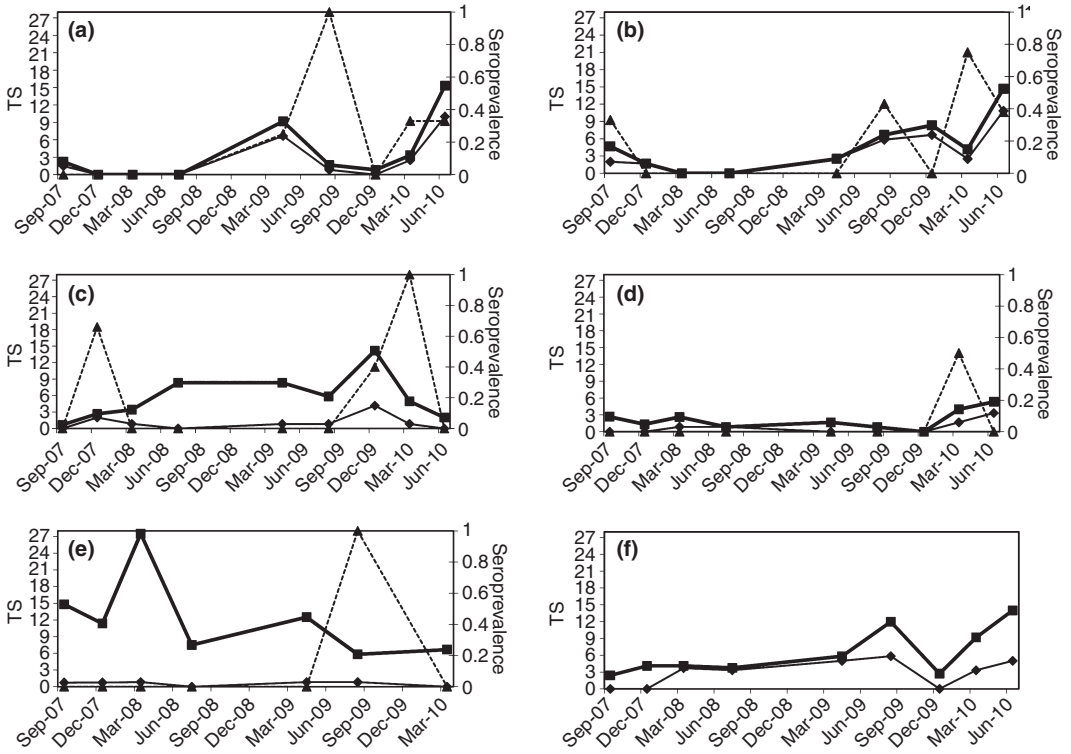


**Figure 6** Total trap success (TS) (thick solid line), *Akodon azarae* TS (thin solid line) and seroprevalence (dotted line) in Natural Reserve Otamendi for (a) highland grassland with livestock; (b) salty marshes; (c) lowland grassland; (d) *Celtis tala* forest; (e) riparian forest; (f) grassland with invasion of *Ligustrum* sp.

(Escutenaire *et al.* 2002; Tersago *et al.* 2010). The detection of positive individuals mainly in the warm season suggests that reproductive activity during the breeding season dominates hantavirus transmission among reservoir in our study sites (Abbott *et al.* 1999; Mills *et al.* 1999). The lack of detection of seropositive individuals in cold months does not represent their total absence. The positive individuals in the following months indicate the presence of seropositive individuals in winter, but in abundance too low for capturing. Longevity of even a small proportion of the host population may provide a trans-seasonal mechanism for virus persistence.

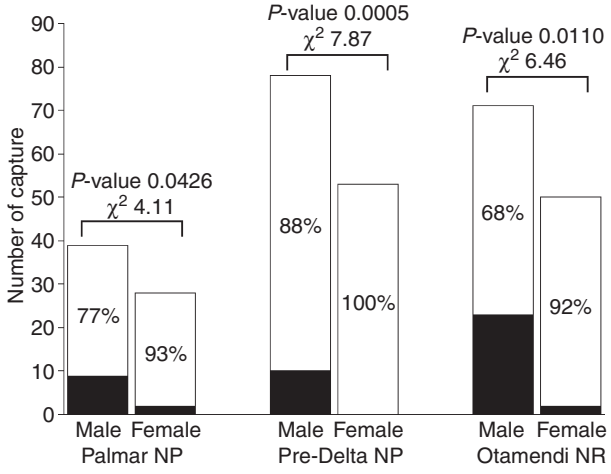


**Figure 7** Number of seropositive and seronegative individuals by sex and protected area. White bars: seronegative individuals; black bars: seropositive individuals.

The seroprevalence detected in the three protected areas was higher than that found for rodent populations in southern, central and northern Argentina (Levis *et al.* 1997; Pini *et al.* 2003; Piudo *et al.* 2005; Mills *et al.* 2007). As a result of this investigation, El Palmar and Pre-Delta NP have carried out contingency plans to decrease the probability of rodent–human contact.

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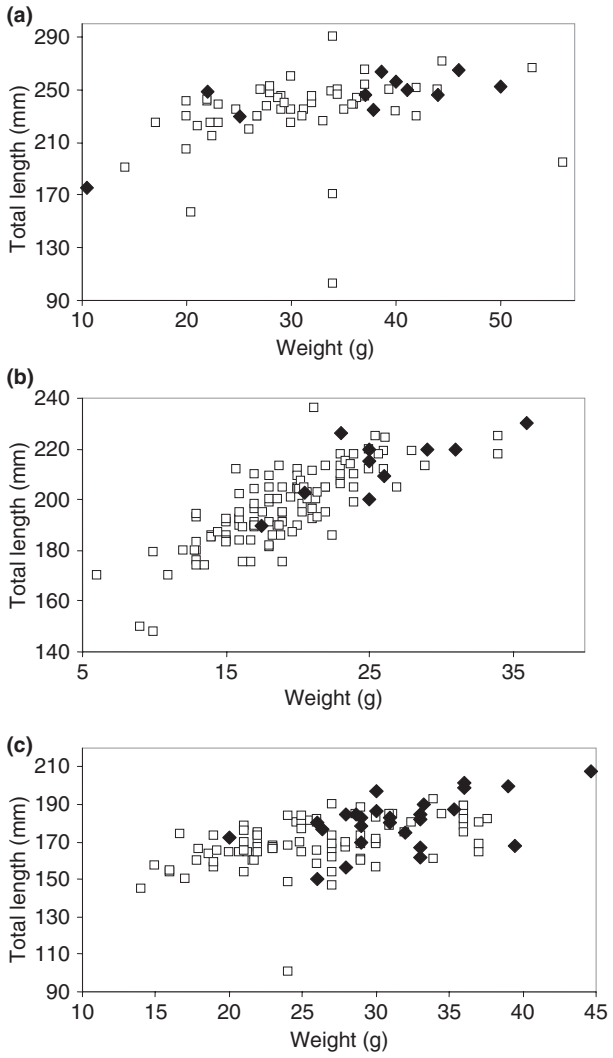


**Figure 8** Total length and weight of: (a) *Oligoryzomys nigripes* in El Palmar National Parks (NP), (b) *Oligoryzomys flavescens* in Pre-Delta NP and (c) *Akodon* azarae in Otamendi Natural Reserve. White squares: seronegative individuals; black rhombus: seropositive individuals.

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