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Spatial and temporal analysis of the distribution of hantavirus pulmonary syndrome in Buenos Aires Province, and its relation to rodent distribution, agricultural and demographic variables

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Summary We studied the spatial and temporal distribution of Hantavirus Pulmonary Syndrome (HPS) cases from 1998 to 2001 in the Buenos Aires Province, Argentina. HPS is a severe viral disease whose natural reservoir are rodents of the subfamily Sigmodontinae (Muridae) and which occurs in many countries of South and North America. We considered two spatial arrangements: cells of 18.5×18.5 km^2; and departments, the political subdivisions of the province, as spatial units. We tested the departure from a Poisson distribution of the number of cases per cell and per month with the Variance/Mean index, while the interaction between spatial and temporal clustering was tested by means of the Knox and Mantel tests. We constructed probability maps in which the HPS rates per department were considered Poisson variates according to population, area and the product of population and area. We analysed the relation between rodent distribution, environmental and demographic variables and HPS cases conducting preliminary univariate analysis from which we selected variables to enter in general linearized models. We found that both the spatial and temporal distribution of cases is strongly aggregated. The spatiotemporal interaction appears to be related to a strong seasonality and the existence of particular ecological conditions rather than epidemic transmission of the disease. The main explanatory variables for the distribution of HPS cases among the departments of the Buenos Aires Province were human population, the distribution of the rodent *Oxymycterus rufus* and evapotranspiration. The last two variables are probably indicators of favourable ecological conditions for the reservoirs, which encompass other variables not taken into account in this study.

keywords hantavirus pulmonary syndrome, spatial distribution, temporal distribution, rodents, demography, agriculture

Introduction

More than 100 years ago, epidemiologists and physicians started to explore the potentiality of the use of maps in order to understand the spatial dynamics of human diseases (Scholten & Lepper 1991; Verhasselt 1993). Information about the localization of epidemiological events is considered relevant both to generate hypotheses about this event and to design control measures which in general are directed to specific areas (Barreto 1993). The study of the spatiotemporal pattern of occurrence of cases of a disease may help understand the mechanism of transmission: while a purely spatial or temporal aggregation of cases may be related to the existence of local conditions or particular moments that favour the spread of the disease, interaction between the spatial and temporal aggregation has been associated with infectious diseases (Glass & Mantel 1969; Bailey & Gatrell 1995). In the case of vector-borne diseases, transmission involves the vector ecology, the environmental conditions that determine their distribution and, in the case of zoonosis, the ecology of natural reservoirs. The result is a complex interaction among multiple populations of pathogens, vectors and reservoirs, and between them and the environment (Mills & Childs 1998).

Hantavirus Pulmonary Syndrome (HPS) is a severe viral disease (40–60% case-fatality rate; Toro *et al.* 1998) caused by the genus *Hantavirus*, family Bunyaviridae. This virus has a long-time relation with their natural reservoirs, the New World rodents of the family Muridae, subfamily Sigmodontinae. According to Orellana (2003), virus transmission among rodents occurs principally via saliva and saliva-aerosol, and it is not necessary to be bitten to get infected, although other authors found association between wounds and infection (Calisher *et al.* 1999). Humans can be infected by contact with contaminated aerosols, urine or faeces from rodents, although there is some evidence of person-to-person transmission (Enría *et al.* 1996; Padula *et al.* 1998), also supported by the results of Orellana (2003).

In Argentina, the first cases of HPS were recorded and characterized in 1995 in the Southwest (López *et al.* 1996), but now the occurrence of cases is concentrated in three geographically isolated areas: north (Salta and Jujuy provinces), centre (Buenos Aires, Santa Fe and Entre Ríos provinces) and southwest (Neuquén, Chubut and Río Negro provinces). The virus associated to HPS cases in Argentina, Andes virus, was described as a new type of Hantavirus which circulates in Argentina, Chile and Uruguay with lineages characteristic of the areas (Padula *et al.* 2000). HPS cases are unevenly distributed in space, time, and within the human population in the Buenos Aires Province (BAP). They are more frequent in the northeast, near the shores of the Paraná and de la Plata rivers, during the spring and summer months, and inhabitants of rural areas are the most affected, especially those who work in tool or grain stores, stay in empty silos or houses, or transport grain (Martínez *et al.* 2001).

In Buenos Aires occurred 31.1% of the total cases of HPS registered in Argentina between 1998 and 2001 (Ministerio de Salud de la Nación 2002) and is one of the most affected provinces of Argentina, along with Salta and Jujuy.

Rodent reservoirs of HPS in Argentina are three species of the genus *Oligoryzomys*: *O. longicaudatus* (in the north and south), *O. chacoensis* (in the north) and *O. flavescens* in the centre (Levis *et al.* 1998; González della Valle *et al.* 2002; Padula *et al.* 2002). The reservoirs of two lineages of virus which caused HPS in BAP, AND Cent Buenos Aires and AND Cent Plata (Martínez *et al.* 2001) have not yet been identified.

Among other common sigmodontine species in BAP, *Calomys laucha* was identified as the reservoir of the virus Laguna Negra, the aetiological agent of HPS in Paraguay (Yahnke *et al.* 2001); but serological surveys of this species in the province of Buenos Aires provided negative results for Hantavirus antibodies (Calderón *et al.* 1999). *O. longicaudatus*, the reservoir of the Andes SOUT virus, has also been reported from the south of the province (Massoia 1973). Two other types of virus, which have not yet been associated to human disease, were described from species of the province: Maciel (in *Necromys obscurus*) and Pergamino virus (in *Akodon azarae*) (Levis et al. 1998). The small number of individuals of the other sigmodontine species (*Oligoryzomys delticola*, *Oxymycterus rufus*, etc.) surveyed for Hantavirus antibodies does not allow any conclusions as to their role as Hantavirus reservoirs. In *Holochilus brasiliensis* the prevalence was 3.3 ($n = 30$), while in *Holochilus sciureus*, a species of the same genus in Brazil, the seroprevalence was 28.8% ($n = 52$ animals, Vasconcelos *et al.* 2001).

The purpose of this work is to describe and explain the aggregational distribution of the HPS in BAP, to identify the spatiotemporal pattern of aggregation and to find environmental and demographic variables that may be useful to explain the pattern of occurrence of cases.

Materials and methods

Study area

Buenos Aires Province is located between 33°40′35″ and 41°8′49″ Lat. S and between 56°24′42″ and 63°10′35″ Lon. W, covering a total surface of 307 571 km^2. The total population is of 12 594 974 (census of 1991), with a mean density of 41 inhabitants/km^2, but the population is unevenly distributed between very dense urban areas (94%) and low population rural zones (6%). The climate is temperate with annual mean temperatures from 12 to 16 °C and rains ranging between 600 and 900 mm. The east portion has a strong oceanic influence. Most of the area is plain (less than 100 m above the sea level). It is included in the phytogeographic provinces of the Pampa, Monte and Espinal. Approximately 40% is farmland (corn, soya bean, wheat, linen, sorghum) and cattle breeding grounds. Most of the area is covered by grass, dominated by many species of *Stipa*, *Lolium* and *Paspalum* (Soriano 1991). Near the rivers and in the lowlands of the northeast the vegetation is characterized by *Scirpus giganteus* and *S. californicus*, meadows of *Cortaderia selloana*, *Baccharis* shrublands and on streambanks, the 'Monte Blanco' woodland (Cueto *et al.* 1995).

Rodent distribution

The rodent species that inhabit the province of Buenos Aires show different ranges of tolerance to environmental conditions, especially to mean summer temperature, precipitation and duration of the dry season. These ranges determine both their distribution within the province as well as their habitat requirements.
One group is formed by *O. flavescens*, *A. azarae*, *C. musculinus* and *C. laucha*, which have a wide range of tolerance and are widely distributed in the province and over a large array of habitats. Another group of species, corresponding to a Brazilian stock, is composed

by *H. brasiliensis*, *O. rufus*, *O. delticola*, *Scapteromys tumidus*, *Deltamys kempi* (cited by Redford & Eisenberg 1992 as *Akodon kempi*) and *Reithrodon auritus*. These species are mainly distributed in the north of the province, along the shores of the Paraná and de la Plata rivers, where the climate is temperate by the oceanic influence and the vegetation is typical of wet habitats with sandy soils. *Akodon molinae* and *Eligmodontia typus* belong to a third group, which are present to the south of the province, in regions of low temperatures and dry conditions (Galliari *et al.* 1991; Bilenca 1993). The knowledge of the ecology of these species is very different, while there are many studies for *C. laucha*, *C. musculinus*, *A. azarae*, *O. rufus, O. flavescens* and *O. delticola* in agroecosystems and in the Paraná Delta region, there is little information about the ecology of *D. kempi*, *Necromys obscurus*, *H. brasiliensis* or *S. tumidus*.

Data source

Data on occurrence of HPS cases and their spatial and temporal localization comes from Instituto de Enfermedades Infecciosas 'Dr Carlos G. Malbran', Ministerio de Salud de la Nación. Geographic, demographic and agricultural data were extracted from Bulletins of the INDEC (1991), from the IGM Atlas of Argentina (IGM 1998) and Ministerio de Agricultura y Ganadería de la Nación Argentina (1988). Data on the rural population come from ENHOSA (2002); climatic and hydrographic data from INA (2002).

Spatio-temporal patterns

For the analysis of the spatial pattern we located 85 cases of HPS between January 1998 and December 2001 on a map of the BAP (Marín & Rotay 1991). The scale of the map was about 1:371 000, and it was divided into cells of 0.5 × 0.5 cm, equivalent to 18.5 × 18.5 km (Figure 1). We used the Variance/Mean Index to test if the distribution of cases per cell was random (Index = 1), aggregated (Index > 1) or regular (Index < 1). The significance of the departure of the index from 1 was assessed calculating the Confidence Interval according to Rabinovich (1980). For the analysis of the temporal pattern we considered each month as a cell, and calculated the Variance/Mean relation in a similar way as for the spatial distribution. We analysed the spatial and temporal pattern considering all the time periods and independently for each year.

After plotting cases on the map we determined the rectangular coordinates of each point relative to a fixed origin (cell 0,0, Figure 1). Because of the precision of the data source, we did not have the exact localization of cases, and considered all cases located in the capital of the department. In the end, three values, characterizing each of the time–space locations, were obtained for subsequent analysis: the horizontal and vertical map positions and the coded number of the month of occurrence ranging from 0 to 47 (0 = first case considered).

For the analysis of the time–space clustering we used two approaches: the Knox 2 × 2 contingency table approach and the Test of Mantel (Mantel 1967). Both methods evaluate whether there is some positive relationship between the (unsigned) temporal distance and (unsigned) spatial distance between the members of each pair of cases (taking all the possible pairs = $n(n - 1)/2$), where n = total number of cases.

For the Knox test we considered critical distances ranging from 1 to the median value of the observed distances, both for space and time. For the Mantel test we estimated the r statistics using separation measures x_{ij} and y_{ij} given by: $x_{ij} = 1/(k_s + d_{ij})$ and $y_{ij} = 1/(k_t + t_{ij})$ where d_{ij} and t_{ij} are the Euclidean distance and time interval, respectively, between events i and j, and k_s and k_t are arbitrary constants equal to 0.5. To determine significance we compared the test statistics r with a randomization distribution obtained by conducting 999 iterations of the spatial and time distance matrixes. We used POPTOOLS version 3.2 (Hood 2002) for this purpose.

Seasonality

Seasonality in the occurrence of HPS cases was assessed by means of a Kruskal–Wallis non-parametric ANOVA, considering two possible arrangements of months according to season: cases occurring between March and May (autumn), between June and August (winter), September and November (spring) and December to February (summer), or cases occurring between January and March (summer), April and June (autumn), July and September (winter) as well as October and December (spring). In order to have four replicates (years) for each period, we included cases occurring in January and February 2002 in the first arrangement.

Probability maps

This analysis was conducted by considering the 127 departments (political subdivisions) of the BAP as the spatial units. This was the more detailed scale at which demographic and agricultural data were available. A Geographic Information System was used to manipulate all data (ArcView GIS 3.2a 1999; ArcView Spatial Analyst

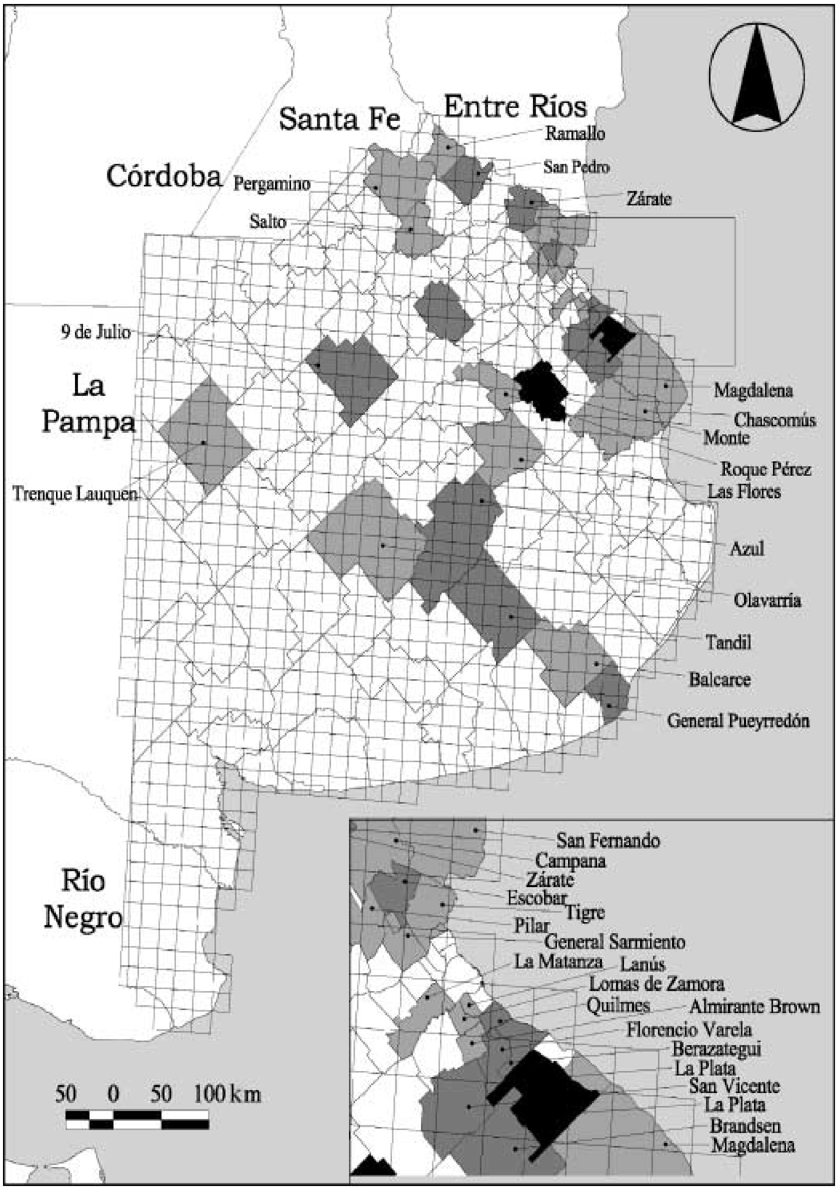

Figure 1 Political subdivision of Buenos Aires Province showing the grid of cells considered in the study (18.5 × 18.5 km each), and the distribution of cases among departments. Black: more than or equal to seven cases; dark grey: two to six cases; light grey: one case.

2.0a 2000). The projection used was Transverse Mercator, 66 degrees W central meridian and international 1909 spheroid. The layer of political subdivision was taken from Aeroterra SA (1995) soil database.

Probability maps based on the Poisson distribution (Choynowski 1959; Cressie 1993; Bailey & Gatrell 1995) were drawn in order to study the basic spatial pattern of HPS cases in BAP. In these maps the probability of occurrence of an event x depends only on the space considered (area, volume, time or inhabitants):

$$P(x) = u^x e^{-x}/x!$$

where u is assumed as a constant density and equals: # events x/space.

To build the maps the number of expected events E in department i are defined as:

$$E_i = n_i \hat{p}$$

where $\hat{p} = \sum H_i / \sum n_i$; H_i is the number of observed HPS cases in department i and n_i is the population of department *i*. Under the assumption that H_i are independent Poisson random variables with expected values h_i, and that $h_1/n_1 = \cdots = h_{125}/n_{125} = p$, an index of deviation from equal h_i/n_i can be defined:

$$ro_i = \sum_{x \geq Hi} E_i^x e^{-Ei}/x! \quad \text{for } H_i \geq E_i$$

or

$$ro_i = \sum_{x < Hi} E_i^x e^{-Ei}/x! \quad \text{for } H_i < E_i$$

A choropleth map based on ro_i is called a 'probability map'. Values of $ro_i < 0.01$ indicate that department *i*'s HPS rate departs from expected Poisson values, being unusually high (for $H_i \geq E_i$) or low (for $H_i < E_i$). In addition to using the rate as 'cases per inhabitant' (*n*) as defined earlier, other denominators were employed: *n* defined as population (*np*), *n* defined as area (*na*) and as the product of area and population (*nap*). In the three resulting maps we identified the departments with ro_i lower than 0.01.

Relation between the distribution of HPS cases and the environmental and demographic variables at the department (political subdivision) scale

We used the same spatial database that was used for probability maps. The hydrological and climatic layers (annual precipitations, mean annual temperature and evapotranpiration) were taken from INA (2002). The climatic layers were transformed from contour lines to raster grids of 10 km pixel size and the value of the nearest contour line was assigned to each pixel. The mean value over each department polygon was used as a variable.

Preliminary analyses

We used the Fisher Exact Test (Sokal & Rohlf 1995) to test for independence between HPS cases and presence/absence of 13 sigmodontine rodent species distributed among the departments of BAP: *A. azarae*, *A. molinae*, *Bibimys torresi*, *C. laucha*, *C. musculinus*, *D. kempi*, *H. brasiliensis*, *N. obscurus*, *O. delticola*, *O. flavescens*, *O. longicaudatus*, *O. rufus*, *R. auritus* and *Scapteromys aquaticus*. The analysis was carried out in three areas: the whole BAP, the northern section (NBAP) and the southern portion (SBAP). The N–S boundary was placed near Buenos Aires City latitude (34°35′ Lat. S) corresponding to the distribution of AND Cent Buenos Aires and AND Cent Plata lineages of Hantavirus (Martínez *et al.* 2001). Rodents that were present in all departments were excluded from the analysis, which is a drawback of our method, but we had not much detailed information about rodent distribution. Rodents with a distribution of at least six departments were considered for BAP, and in five or more for NBAP and SBAP. Those species which showed a significant association to HPS cases were included in Generalized Linear Models (GLMs) models as factors with two levels (present or absent).

We compared the medians of agricultural, demographic and climatic variables (Table 1) between the groups of departments with and without cases of HPS (Wilcoxon–Mann–Whitney test, and Bonferroni multiple comparisons correction). Variables measured as percentages and which showed modes below 20% or above 80% were arc-sin transformed: $y' = \arcsin(\text{sqrt}(y))$ (Crawley 1993; Zar 1996).

The association between HPS cases and agricultural, demographic, climatic and rodent variables was studied using GLMs (Nelder & Wedderburn 1972; McCullagh & Nelder 1989) with a stepwise multiple regression procedure (Donazar *et al.* 1993). The response variable was presence/absence of HPS cases (PA). The explanatory variables are shown in Table 1. To fit the model for HPS cases S-PLUS 6.0 software with ARCVIEW 3.2a add-on and SPATIAL STATISTICS modules were used.

We assumed a binomial distribution of errors and applied the logistic function as a link for the response variable. This link constrains the predicted values to lie between 0 and 1. The probability of a department having HPS cases (*p*) follows an S-shaped curve when the LP is a first-order polynomial (Crawley 1993): $p = e^{LP}/(1 + e^{LP})$, which can be linearized as: $\text{Ln}(p/(1 - p)) = \text{LP}$. To account for overdispersion the dispersion parameter was calculated by quasi-likelihood methods (McCullagh & Nelder 1989).

We computed the Pearson correlation coefficient between pairs of explanatory variables; when it surpassed 0.5 the variable responsible of the greater change in deviance was kept, while the other was excluded from further analyses.

When a model could not be improved any further, geographical coordinates were added as a variable to check for spatial dependence (Legendre 1993). Interaction terms between the significant variables were added to check if they contributed to a better fit of the model. The best models were selected by residual analysis. The standardized residuals were plotted against fitted values (to check lack of fit of the residuals) and against normal quantiles (to check for its normality). Misclassification error was checked with the Kappa index for unbalanced number of positive and negative cases (Titus & Mosher 1984). The parameters were resampled with the Jackknife method.

Table 1 Median, lower and upper quartiles of agricultural, demographic, climatic and location variables for the groups of departments with and without cases of HPS

		With cases of HPS			Without cases of HPS		
Variables	*P*/*Bonf.	Median	LQ	UQ	Median	LQ	UQ
Agriculture							
Area dedicated to agriculture (2)	0.0912	0.260	0.170	0.480	0.420	0.210	0.620
Perennial crops (1), +	0.0213	0.000	0.000	0.141	0.000	0.000	0.000
Annual crops (1), ×	0.1930	0.290	0.100	0.515	0.380	0.238	0.570
Annual pastures (1), +	0.0887	0.326	0.120	0.389	0.353	0.246	0.433
Perennial pastures (1)	0.0500	0.250	0.065	0.420	0.360	0.210	0.480
Natural grassland (2), +	0.1915	0.644	0.442	0.732	0.547	0.409	0.685
Areas adequate for agriculture but not planted (2), +	0.4420	0.243	0.141	0.400	0.199	0.100	0.318
Areas inadequate for agriculture	0.2096	0.172	0.100	0.243	0.199	0.141	0.243
Demography							
Log Population	0.0000*	11.22	10.42	12.41	9.83	9.33	10.45
Population density, ×	0.0000*	71.0	11.4	458.8	8.50	3.70	22.5
Rural population in small towns (<2000 inhabitants)	0.0006	0.065	0.000	0.197	0.224	0.106	0.327
Rural population not grouped in towns, ×	0.0002*	0.225	0.000	0.343	0.381	0.307	0.446
% Younger than 14 years ×	0.1407	27.10	24.90	29.00	26.15	24.78	27.43
14–64 years old (%)	0.0290	63.0	62.2	64.2	62.2	61.5	63.2
Older than 64 years, +	0.0017	−0.068	−0.211	0.400	−0.268	−0.426	−0.099
Unoccupied domiciles, +	0.0034	−0.687	−0.821	−0.014	−0.860	−0.960	−0.638
Domiciles with tap water (%), ×	0.8991	60.1	41.1	77.2	60.5	42.0	73.0
Analphabetism (3), +	0.0009	0.995	0.969	0.999	0.970	0.929	0.995
% Population with health assistance	0.4730	63.6	59.5	69.1	62.5	58.8	67.3
Foreign population, +	0.4431	0.841	0.646	0.957	0.889	0.800	0.949
No. of hospital beds per 1000 persons	0.0000*	3.7	2.7	5.0	6.15	4.18	8.73
Climate							
Evapotranspiration	0.0001*	700	700	700	686	600	700
Precipitation, ×	0.0003*	1000	900	1000	900	808	1000
Mean temperature, ×	0.0032	16	14	17	15.65	14.20	16.00
Location							
Log area (km^2), ×	0.0745	1152	313	3233	2017	928	3933

P: Significance according to the Wilcoxon–Mann–Whitney rank test for two samples. Asterisk indicates significance after Bonferroni multiple test correction.

(1) % of planted surface; (2) % of total surface; (3) % of total population that is unable to read and write.

+: Percentages transformed to: y- = sin(sqrt(y)).

×: Variables that were excluded from the GLM models because they were more than 50% correlated to another variable that had higher explanatory value.

Results

The 85 HPS cases analysed were distributed among 30 out of a possible 995 cells. Although a time series of 4 years is short, there is a trend to a decrease in the incorporation of new positive cells through the studied period, that is to say, new cases were more likely to occur in cells with previous cases ($\chi^2 = 31.39$; $P < 0.001$). By December 1999, the cumulative number of positive cells was the 86.67% of the total cells that were positive in December 2001 ($n = 30$).

Spatial and temporal patterns

According to the values of the Variance/Mean Index both the distribution of cases per cell and cases per month were aggregated (V/M = 10.85, CI = 0.911–1.089, and V/M = 2.55, CI = 0.585–1.585, respectively). The spatial pattern of cases within each year was also aggregated for 1998, 1999 and 2000 (V/M = 2.28, 2.5 and 2.20, respectively), while in 2001 it showed a regular distribution (V/M = 0.85). The temporal pattern was aggregated in 1999, 2000 and 2001 (V/M = 6.04, 2.35 and 3.17, respectively), while it did not differ from randomness in 1998 (V/M = 1.012).

Interaction between spatial and temporal aggregation of HPS cases

The spatiotemporal pattern of aggregation was marginally significant when analysed with the Mantel test ($r = 0.0413$; $P = 0.058$). The results of the Knox test showed significant spatiotemporal interactions at different spatial and temporal scales. We found that cases were close in space for almost every distance at temporal scales of 0 or 3 months, and close in time at spatial scales of more than four cells (4 × 18.5 km) (Table 2).

Seasonal distribution of cases

The median values of the number of cases significantly differed among the seasons when grouping D–F, M–M, J–A, S–N ($H = 8.455$, $P = 0.038$). The sum of ranks was highest for the summer months (D–F, 52) followed by the spring period (38), autumn (32) and the lowest, winter (14). *A posteriori* comparisons showed significant differences between D–F and J–A periods (alpha = 0.025). When grouping J–M, A–J, J–S, O–D, we found marginal differences among seasons ($H = 7.416$, $P = 0.060$). The summer was again the season with the highest sum of rank (47), followed by spring with 46.5, autumn (24) and winter (18.5), but there were no significant differences between any pair of seasons according to *a posteriori* comparisons.

Probability maps

We observed a patch (i.e. a cluster with more cases than expected) formed by the departments of 'Monte', 'La Plata' and 'Brandsen' in all models. The np model showed fewer cases than expected in 'La Matanza' and 'Moron'. 'San Pedro', 'Escobar' and 'San Vicente' showed more cases than expected according to two models (Figure 2).

Table 2 Spatial and temporal scales at which we detected significant spatiotemporal interactions according to the Knox test. The criteria used for closeness ranged from 0 (cases in the same cell) to the median value of the distribution of distances between pairs of cases, and from 0 (cases occurred at the same date) to the median value of the temporal distribution of distances in time between pairs of cases

	Temporal distance (months)												
Spatial distance (cells)	0	1	2	3	4	5	6	7	8	9	10	11	12
0	*			*									
0.5	*			*									
1	*						*	*					
1.5													
2	**			*									
2.5													
3				*									
3.5	*			*									
4	***	*	*	***	*	*	*	*	*				
4.5	**	*	*	*	*		*						
5	***	*		*	*								

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

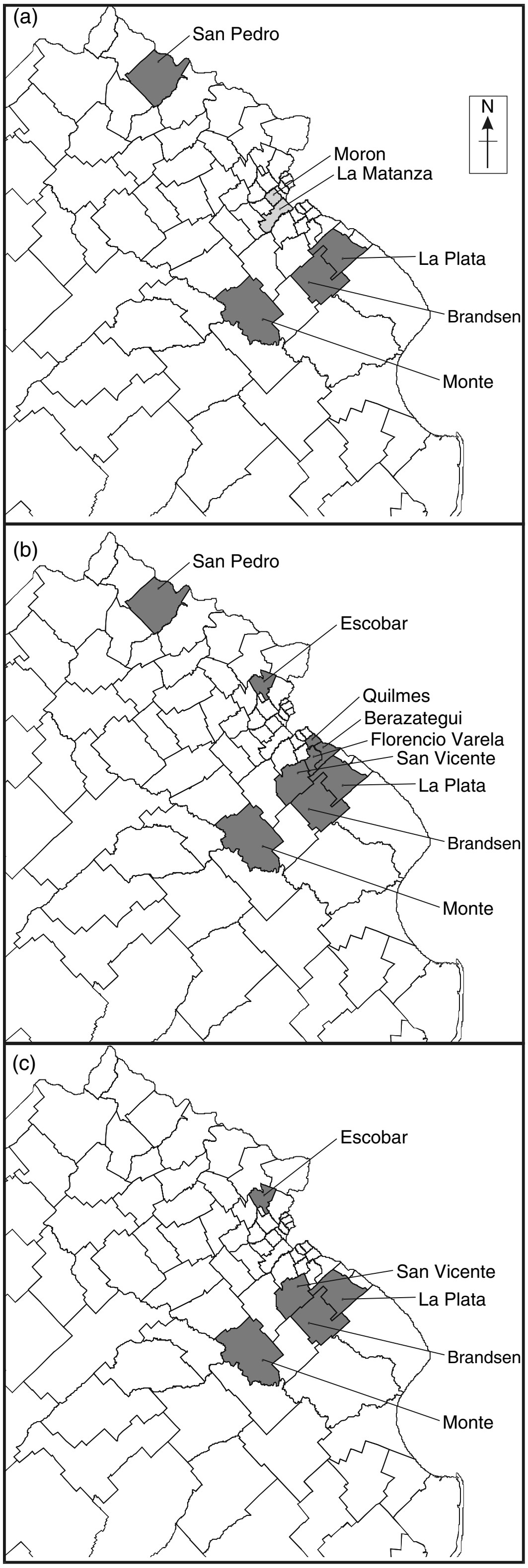

Figure 2 Probability maps. (a) *np* model, which considers that HPS rates per department varies according to population as a Poisson variable. In dark grey are shown those departments with a higher than expected HPS rate, and in light grey those whose rate is lower than expected. (b) *na* model, which considers that HPS rates per department varies according to the area as a Poisson variable. (c) *nap* model, which considers that HPS rates per department vary according to population and area as a Poisson variable.

Relation between rodent and HPS cases distributions

Only seven rodent species were considered for BAP: *D. kempi*, *H. brasiliensis*, *N. obscurus*, *O. rufus*, *R. auritus* and *S. aquaticus* (the other six sigmodontine species were very scarce or widely distributed). Four showed a significant positive association to HPS cases (*D. kempi*, *O. rufus*, *H. brasiliensis* and *S. aquaticus* (marginal)). *D. kempi*, *H. brasiliensis* and *O. rufus* were also associated to HPS cases in the north, as well as *R. auritus* (marginal). For the southern part of the province we did not find any significant relation between HPS cases and rodent presence (Table 3).

Association of environmental, rodent, agricultural and demographic variables with HPS cases

According to the median test, precipitation, evapotranspiration, temperature, logarithm of population and population density were higher in departments with HPS cases. On the other hand, hospital beds per inhabitant and rural population in small towns (<2000 inhabitants) were fewer (Table 1).

The groups of variables that showed more than 50% correlation (a minus sign indicates inverse relation) were: (1) Evapotranspiration, longitude, latitude, –area, precipitation and temperature; (2) Log population, population density, –rural population not grouped in towns and –perennial pastures; (3) percentage of implanted surface and annual crops; (4) older than 64 years, younger than 14 and houses with tap water. We kept for further analyses only the first of each group because of their better explanatory power in the GLM.

The logistic regression model with lower residual deviance (Model 1) included as explanatory variables: Log population, evapotranspiration, presence/absence of *O. rufus* and perennial crops (Table 4). No improvement was obtained after fitting interaction terms, latitude or longitude coordinates to the final model. Since after the jackknife resampling perennial crops was not significant, we refitted a second model keeping out this variable, (Model 2, Table 4). As in Model 1, log population, *O. rufus* presence and evapotranspiration (marginally)

Table 3 Association between rodent presence and HPS cases at a department scale (Fisher exact test of independence). We did not consider in the analysis those species that were present everywhere and those that were distributed in less than five departments

	BAP (127 departments)			NBAP (35 departments)			SBAP (92 departments)		
	#	*P*	Relation	#	*P*	Relation	#	*P*	Relation
D. kempi	9	0.0017	+	5	0.0258	+	0	–	
H. brasiliensis	27	0.0499	+	10	0.0413	+	17	0.3670	
N. obscurus	7	0.3932		0	–		5	0.6026	
O. rufus	17	0.0063	+	5	0.0014	+	12	0.2874	
R. auritus	22	0.1874		8	0.0767	+	14	0.7537	
S. aquaticus	7	0.0905	+	0	–		0	–	

#: number of departments in which each rodent species was present.

Table 4 GLM models for presence or absence of HPS cases

	Model 1		Jackknifed		Model 2		Jackknifed	
	Parameter	SD	Parameter	SD	Parameter	SD	Parameter	SD
Intercept	−20.818	4.570**	−20.835	4.801**	−15.880	3.355**	−15.890	3.478**
Log population	0.991	0.240**	0.992	0.269**	0.723	0.177**	0.723	0.195**
Oxymycterus rufus	0.925	0.355**	0.926	0.322**	0.826	0.306**	0.827	0.310**
Evapotranspiration	0.015	0.006**	0.015	0.006**	0.0116	0.0045**	0.0116	0.0050
Perennial crops	−3.924	1.653**	−3.929	2.196				
Residual deviance	99.27				106.70			
Residual degrees of freedom	122				123			
Deviance accounted for	33.6%				28.7%			
Dispersion parameter	0.870				0.857			
Kappa	0.534				0.464			

** Variable significant at $P < 0.01$ when t (parameter/SD) > 2.357. Null deviance, 149.54. Total degrees of freedom, 126.

contributed to explain the occurrence of HPS cases (Table 4).

The values fitted by the models can be interpreted as a probability (*Pr*) of a department having HPS cases. A department might be classified as having cases when $Pr > 0.5$ and not having HPS cases when $Pr < 0.5$. Model 1 (Table 4) classified correctly 92.4% of the departments without HPS cases and 57.1% of the departments with cases. This is 53.4% better than random classification (Kappa = 0.534). Model 2 classified correctly 91.3% and 51.4%, which was 46.4% better than random (Kappa = 0.464).

Discussion

We confirmed the observations of previous studies (Martínez *et al.* 2001) about the aggregated distribution of HPS cases at all three scales analysed (cells, departments and clusters of departments). The localities where cases occurred showed a higher probability of new occurrence, especially in summer months. The significant interaction between spatial and temporal patterns at many scales have been interpreted as evidence of an infectious spread of the disease (Glass & Mantel 1969) but in the case of HPS we consider that it may be related to a strong aggregation in each variable, caused by particular ecological conditions that occurs in some places and times (aggregation scales in time correspond to the 3-month period of the summer) and not in others, due to the spatial heterogeneity and seasonality, as was proposed by Manly (1997).

Only five of 127 departments showed a significant deviation in HPS rates from what was expected according to their population and area. Considering population and area separately, there were six and nine significant deviations, respectively. According to the GLM models, constructed after testing many demographic and environmental variables, human population was also the variable that most contributed to explain the presence of HPS cases, along with the presence of *O. rufus*, evapotranspiration and perennial crops (this last variable was not significant in the jackknifed model).

The regression models were built with an exploratory objective in mind, not to construct predictive models. The exclusion of correlated variables should be regarded with care, as important variables could have been excluded. For example, population showed association to HPS cases but was correlated to population density and inversely to rural population not grouped in towns. Evapotranspiration was correlated to longitude, latitude, temperature and precipitation, but geographic position also defines temperature and precipitation and these in turn evapotranspiration. An inverse relation between HPS cases and rural population not grouped in towns was contrary to that expected according to the distribution of rodent reservoirs, which are more abundant in rural habitats, and suggest that the probability of becoming ill is associated with the movements to rural habitats because of labour or recreation activities, and persons that move might represent a fixed percentage of the population in the area.

In vector-borne diseases, or when pathogens are maintained in nature by reservoirs, it is likely to find an uneven distribution of the pathogen both in space and time. The probability of encounter will not be a simple result of density but will also depend on the habitat use of humans and reservoirs, and their temporal (mainly seasonal) changes. High HPS rates may then be explained on the basis of the particular conditions that determine the distribution of the pathogen as well as according to human activities, since HPS mainly affects adult males that work in rural habitats (Martínez *et al.* 2001). The location of departments with cases coincides with the shores of the Río de la Plata and Paraná rivers, and with the basin of other minor rivers that flow into the Atlantic. Along these shores a wetland forest is developed where the rodent assemblage includes many species of the Brazilian stock associated to wet and mesic habitats, as *O. rufus*, *H. brasiliensis* and *S. aquaticus*, along with *A. azarae* and *O. flavescens*. The association of HPS cases to *O. rufus* and evapotranspiration may be indicators of these particular ecological conditions. We cannot exclude that other rodent species, which were excluded from the analysis because of their wide distribution, may be related to HPS cases. Our results suggest that more detailed information of rodent abundance at smaller spatial scales, which lacks in BAP, is needed. For example, the reservoir of the AND Cent Lechiguanas, *O. flavescens*, although present in most of BAP shows higher abundance in the wetlands along the shores of the la Plata and Paraná rivers (habitats characteristics of *O. rufus*) than in grasslands in the interior of the province. Another species which is abundant in wetlands is *H. brasiliensis*, which showed a prevalence of Hantavirus antibodies of 3.3% ($n = 30$) in central Argentina (Calderón *et al.* 1999). In *Holochilus sciureus*, a species of the same genus which is present in Brazil, a seroprevalence of 28.8% was found ($n = 52$ animals, Vasconcelos *et al.* 2001). So far no Hantavirus antibodies have been detected in *O. rufus*, but few individuals of this species were analysed; nine in Calderón *et al.* (1999) and 20 in the department of Berisso (Busch *et al.*, pers. comm.). Our results suggest that their role as potential reservoir of HPS must be further investigated.

Forty-three per cent of the departments in which HPS cases occurred were misclassified by the final model. In all of them *O. rufus* appears as absent. The quality of the data on rodent distribution, with many localities that lack studies about the rodent species present, may be contributing to this lack of fit, because this species is probably present in many of these departments. We cannot exclude, however, that other variables not taken into account may be contributing to the occurrence of cases.

We can postulate different hypotheses for the observed seasonal pattern of occurrence of cases, with higher rates in summer and spring months: an increase in the probability of interaction between human and rodents due to seasonal differences in human population density, in rodent density, in habitat use by humans, in habitat use by rodents or seasonal variations in the proportion of rodents infected with Hantavirus. We can drop the first hypothesis, because there are no such differences in human population density. With respect to sigmodontine rodents, the seasonal pattern of variation shows for most species a peak in autumn–winter months, with low density in spring–summer months (Busch & Kravetz 1992). *O. rufus*, however, suffers small changes in its population size among seasons, although it is more abundant in summer than in winter in the Delta region, and lives longer than the other species (Cueto *et al.* 1995; Sánchez López 1998). The third hypothesis is supported by the characteristics of persons who were infected, because cases were associated to recreational and labour activities in rural habitats (Martínez *et al.* 2001). Recreational activities in habitats that have risk of contact with rodents are typical in spring–summer time, coincident with the higher rates of HPS cases. We have no data to support the hypothesis of changes in habitat use by rodents, but an increase in the use of urban or suburban habitats may be expected in high density months, not when population is scarce. Finally, many works showed that Hantavirus prevalence is higher in adult animals than in juveniles (Glass *et al.* 1998; Yahnke *et al.* 2001), and in spring–summer months rodent populations show a higher proportion of adults than during autumn and winter.

Our conclusions about the association between HPS rates and environmental variables are restricted to the spatial scales considered, and may have been different at other scales. After this analysis, conducted at a broad scale, and with limitations on the quality of rodent and agricultural data, we consider that further investigations may be concentrated on local conditions that favour the occurrence of cases within departments, and in having a detailed knowledge of rodent species distribution and their role as Hantavirus reservoirs. We consider that departments where cases have occurred previously and those which harbour those habitats described as favourable for rodent communities associated to *O. rufus* are at special risk for the occurrence of new cases. On the other hand, our results highlight the necessity of paying attention to the consequences of land use changes, which enhance contact of human beings with wetland habitats. In the last years in the northeast of Buenos Aires Province, near La Plata (an area with high incidence of HPS cases), there was an increase of sites where the soil was extracted for brick fabrication, these sites were then abandoned and converted into artificial wetlands with a characteristic fauna and flora (Schnack *et al.* 2000).

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