Community ecology of small mammal populations in Panamá following an outbreak of Hantavirus pulmonary syndrome

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ABSTRACT: In late 1999 and early 2000, an outbreak of hantavirus pulmonary syndrome (HPS) occurred in and around Los Santos, on the Azuero Peninsula of southwestern Panamá. This HPS episode, resulting in 22% case fatality, was linked to the Costa Rican pygmy rice rat, *Oligoryzomys fulvescens costaricensis*, which harbored a then undescribed hantavirus, Choclo virus. In addition, Cherrie's cane rat, *Zygodontomys brevicauda cherriei*, was identified as carrying a distinct hantavirus, Calabazo virus with no known pathogenicity to humans. Herein we present the ecological results of the outbreak investigations in the Azuero region. A total of 164 animals were captured, of which 126 were potential small, non–volant mammal hosts of a hantavirus: rodents in the family Muridae. There were significant differences in small mammal community structure between case sites and a negative control site. Differences were manifest in ecological measures of species diversity and in species evenness and heterogeneity measures, as indicated by Pairwise Euclidian distances and Morisita indices of community similarity. Our analyses suggest that human activities (i.e., deforestation for cattle ranching) coupled with environmental factors (i.e., increased precipitation) may have synergistically coalesced for an increased risk of HPS to area residents. ***Journal of Vector Ecology* 29 (1): 177-191. 2004.**

Keyword Index: Calabazo virus, Choclo virus, Hantavirus ecology, Muridae, Panama, Sigmodontinae.

INTRODUCTION

Hantavirus pulmonary syndrome (HPS) is an increasingly recognized infectious disease associated with infection of humans by New World hantaviruses (family *Bunyaviridae*). The symptomatology of the disease, with a rapid onset of respiratory failure and a fatality rate of 38% to 69%, depending on the specific hantavirus, has been described extensively. Each hantavirus (small, tripartite, negative strand RNA virus) is usually hosted by a single species of rodent belonging to the murid subfamilies Sigmodontinae or Arvicolinae. Only hantaviruses associated with sigmodontine rodents are known to cause human disease in the Americas (15 of 20; Clement 2003). Transmission to humans occurs through inhalation of aerosolized excreta or saliva from infected rodents.

In December 1999, cases clinically consistent with HPS began appearing in the Azuero region of Panamá, a

peninsula extending south on the western Pacific slope of the country and including the provinces of Los Santos, Herrera, and portions of Veraguas (Figure 1). By February, twelve cases had been reported. Currently, close to 40 cases of HPS have been confirmed in Panamá, with a case fatality of 22%.

Results of the initial virological phase of the investigations have been published elsewhere (Vincent et al. 2000). Herein, we report substantive new data relating to the ecological and field aspects of the outbreak investigation that are pertinent to human disease and well-being; these data extend our knowledge considerably beyond the exclusively clinical aspects discussed by Vincent et al. (2000). Specifically, we examined the structure of the rodent communities in areas where human disease was apparent (or where seropositive rodents were found) and compared it to that in a relatively undisturbed site to test the hypothesis that in the Panamá HPS outbreak, the faunal communities associated with peridomestic habitats contributed to a greater risk of humans contracting HPS.

MATERIALS AND METHODS

Trapping and study sites

Thirteen study localities were located on the Azuero Peninsula of southwestern Panamá (Figure 1). The overarching climate regime of the Península del Azuero is characterized by extreme seasonality, with regional rainfall maxima between May and December and a dry season from January to April. However, subsumed within this regime is a strong gradient of precipitation, with the northeast portion of the peninsula receiving the least amount of precipitation and the southwest receiving the most. Highland areas of the peninsula receive more precipitation regardless of their geographic location on the peninsula. Dry deciduous forest occurs primarily in the drier eastern portion, with evergreen forest types predominating in the higher regions and the SW. Mangrove vegetation locally lines discrete portions of the coast. Patches of evergreen forest remain in the Cerro Hoya highlands of the southwestern portion of the

Figure 1. Localization of study area in Panamá with a box framing the Azuero Peninsula, where the outbreak of Hantavirus pulmonary syndrome occurred; case sites of HPS on the Peninsula are marked by the circles. The solid triangle marks the location of Cerro Hoya National Park, a control site with neither HPS nor hantaviral infection among animals tested. Inset: Panamá relative to North and South America.

Table 1. Taxonomic identities and numbers of species captured at each site sampled during the outbreak investigation in the Peninsula de Azuero region of Panamá (see Figure 1). Not included in the tally are 24 bats of seven species, captured at only two sampled localities; all tested negative for hantaviruses. In addition, seven rodents of four species were brought to us from three different areas; these are not included in the analyses either; all of these tested negative for hantaviruses as well. Included among these rodents were *Nyctomys sumichrasti*, a species which we otherwise did not sample. The *Marmosa* are not rodents, but rather marsupials in the order Didelphimorphia. The two murid rodents, *Mus* and *Rattus*, are Old World invasive species; remaining murids are in the New World subfamily Sigmodontinae, known for its extensive coevolved radiation of hantaviruses (Ksiazek et al. 1997, Levis et al. 1997, 1998, Monroe et al. 1999, Morzunov et al. 1998). The row titled "Rarefaction (5)" shows the results of the rarefaction analyses, i.e., species present when community size is standardized to $N = 5$ captures (see materials and methods for details).

Family	Species (below) Site (right)	1	2	3	4	5	6	7	8	9A	9B	10	11	12	13	Totals
Didelphidae	*Marmosa mexicana*	1			2									2		5
Didelphidae	*Marmosa robinsoni*														3	3
Dasyproctidae	*Dasyprocta punctata*														2	2
Echimyidae	*Proechimys semispinosus*														1	1
Heteromyidae	*Liomys adspersus*							1							7	8
Muridae	*Mus musculus*	1	3	1	2								1	1		9
Muridae	*Rattus rattus*	1				6	1	1			1		1			11
Muridae	*Oligoryzomys fulvescens costaricensis*		1	4	1			9	1							16
Muridae	*Oryzomys couesi*														2	2
Muridae	*Oryzomys talamancae*														14	14
Muridae	*Sigmodon hispidus*	5					1	7	1			3			3	20
Muridae	*Zygodontomys brevicauda cherriei*	6	4	3	7	1	3	17	5	3	1	2			2	54
Totals	(14 species) Individuals	14	8	8	12	7	5	35	7	3	2	5	2	3	34	145
	Species	5	3	3	4	2	3	5	3	1	2	2	2	2	8	
	Rarefaction (5)	3.0	2.6	2.6	2.8	1.7		2.7	2.4			2.0			3.4	
	Trap success (by individuals)	5.7	4.5	6.4	10.5	1.3	6.3	14.5	7.9	7.5	5.0	7.9	2.5	3.8	5.3	
	Trap success (by species)	1.7	1.7	3.2	3.8	1.3	3.8	2.1	3.9	2.5	5.0	3.2	2.5	2.5	1.9	

Table 2. Matrix of Euclidean distances among sites' small mammal community assemblages, based on calculations using biomass at each of the sites. The column headed "mean" represents the average Euclidean distance between the capture site in the left–most column and all other columns. CHNP refers to Cerro Hoya National Park.

Site	mean	1	11	10	2	4	3	9A	9B	8	12	5	6	Pocri	CHNP
1	393.73	0.00													
11	248.36	392.22	0.00												
10	297.92	182.94	224.73	0.00											
2	218.98	354.30	116.60	226.89	0.00										
4	265.66	347.20	218.00	271.37	114.30	0.00									
3	217.46	352.22	124.49	227.65	29.71	102.81	0.00								
9A	218.88	351.16	129.04	227.53	40.80	101.23	24.71	0.00							
9B	269.59	389.05	137.86	249.50	141.35	205.29	142.57	142.39	0.00						
8	212.62	348.44	81.80	203.52	52.68	143.59	49.03	52.14	127.30	0.00					
12	264.01	393.27	76.85	236.80	140.40	212.72	147.06	149.08	156.78	111.92	0.00				
5	231.38	379.00	34.59	218.24	91.88	189.58	96.63	97.50	127.01	52.61	82.28	0.00			
6	212.73	311.43	107.47	173.41	56.27	137.46	60.03	60.27	139.14	42.51	131.42	82.47	0.00		
Pocri	641.21	470.08	703.87	591.57	604.40	524.04	593.16	592.84	660.91	626.44	709.29	677.90	600.36	0.00	
CHNP	880.63	847.14	881.09	838.82	877.17	885.94	876.97	876.78	885.47	872.08	884.24	878.26	863.28	980.91	0.00

Table 3. Matrix of Morisita similarities among sites' small mammal community assemblages, based on calculations using biomass at each of the sites. The column headed "mean" represents the average Morisita similarity between the capture site in the left-most column and all other columns. CHNP refers to Cerro Hoya National Park.

Site	mean	1	11	10	2	4	3	9A	9B	8	12	5	6	Pocrí	CHNP
1	0.416	1.000													
11	0.067	0.027	1.000												
10	0.287	0.441	0.000	1.000											
2	0.605	0.391	0.290	0.245	1.000										
4	0.596	0.472	0.087	0.249	0.940	1.000									
3	0.615	0.457	0.178	0.255	0.974	0.964	1.000								
9A	0.580	0.436	0.000	0.254	0.911	0.926	0.960	1.000							
9B	0.276	0.198	0.000	0.110	0.403	0.410	0.416	0.408	1.000						
8	0.640	0.609	0.098	0.423	0.939	0.953	0.984	0.942	0.414	1.000					
12	0.017	0.082	0.078	0.000	0.032	0.005	0.009	0.000	0.000	0.000	1.000				
5	0.580	0.436	0.000	0.254	0.911	0.926	0.960	1.000	0.408	0.940	0.000	1.000			
6	0.641	0.812	0.117	0.648	0.885	0.859	0.872	0.821	0.374	0.935	0.015	0.821	1.000		
Pocrí	0.630	0.777	0.000	0.607	0.856	0.868	0.885	0.816	0.406	0.954	0.000	0.816	0.977	1.000	
CHNP	0.116	0.271	0.000	0.248	0.084	0.085	0.083	0.073	0.037	0.128	0.000	0.073	0.198	0.227	1.000

peninsula.

We visited 13 sites on the Azuero peninsula during the waning period of the HPS outbreak; small mammal trapping was conducted around all confirmed and suspected case sites, and one control site (Cerro Hoya National Park). Traps, baited with a mixture of crunchy peanut butter and cracked corn, with some extract of vanilla added, were set at approximately 10 m intervals in linear transects in a complex matrix of riparian vegetation, residential, and agricultural areas. The negative control (undisturbed) site consisted of late secondary and primary vegetation; traps there also were set in linear transects and spaced apart by 10 m. In all, 1,819 trap nights were conducted among all sites, of which 432 were in the negative control site. For all sites, mean trapping effort was 121 trap nights/visit (range 80–242).

Mammals were sampled and handled according to recommendations of Mills et al. (1995). Briefly, blood was obtained from the retroorbital sinus using heparinized capillary tubes. The animals were then euthanized using an overdose of an inhalant anesthetic (methoxyflurane, Pitman–Moore, Mundelein, IL; currently only available in the U.S. from Medical Developments Australia, Springvale, Victoria, Australia). The following data were recorded: species; sex; age; mass; reproductive condition; length of body, tail, hind foot, and ear; and presence and nature of external wounds. Blood and samples of spleen, liver, kidneys, heart, and lungs were collected in separate, labeled cryovials using clean sterilized instruments for each animal. All biological samples were immediately stored in liquid nitrogen. After processing, each animal voucher specimen was placed directly into 80% ethanol. All voucher specimens were deposited in the Museum of Southwestern Biology (University of New Mexico) or the Gorgas Memorial Institute (Panama City).

Ecological metrics and statistical analyses

We calculated Euclidean distances (additive and average) and Morisita's Index of Similarity (Morisita 1959, Krebs 1989). The two indices were calculated twice: (1) based on numbers of individuals for each species caught at each of the study sites, and (2) based on the biomass for each species. These metrics were used because they incorporate information regarding abundance of species at each site, whereas the more common similarity measures (Jaccard, Sørensen) use only presence–absence data (Clifford and Stephenson 1975, Krebs 1989, Romesburg 1984, Ruedas et al. 1994). Five of the 13 sites were discarded due to low rodent sample sizes ($n < 3$). Sites were then clustered based on

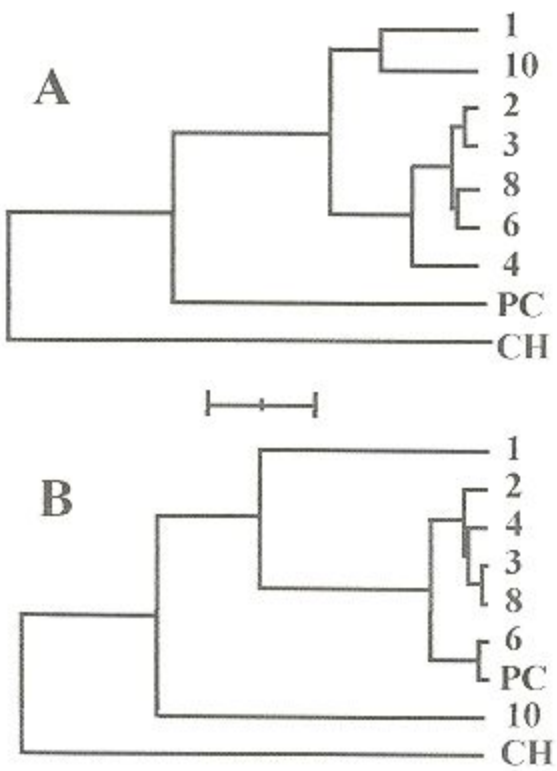

Figure 2. Phenograms depicting the community similarity among sites generated using the "unweighted pair–group method using arithmetic averages" or UPGMA algorithm (Sneath and Sokal 1973, Romesburg 1984; see Materials and Methods). Clustering was carried out using the Neighbor subprogram (version 3.573c) of Phylip (Felsenstein, 1993). A. Relationships among capture localities using as a metric Euclidean distances based on species and biomass (from Table 2). Scale bar equals 100 units. B. Relationships among capture localities using as a metric the Morisita Index of Similarity (Morisita, 1959), also based on species and biomass (from Table 3). Scale bar equals 100 units.

Table 4. Shannon species diversity, H' (calculated using natural logs), H'_{MAX} and H'_{MIN} (the theoretical maximum and minimum possible diversities), and evenness, J' (based on H'). N_1, the number of equally common species which would produce the same diversity as expressed by H', is derived from the alternative expression of the Shannon index: $N_1 = e^{H'}$. S is the actual number of species of small mammals captured at the site. The different total species noted for site 13 reflects that the *Dasyprocta punctata* were not included in the calculations for these metrics. The two specimens of this species were brought to the investigators by hunters, rather than being trapped, thereby violating the statistical assumption of equal probability of capture at all sites.

Site	H'	H'_{MAX}	H'_{MIN}	J'	N_1	S
1	1.119	1.386	0.794	0.807	3.062	4
10	0.673	0.693	0.500	0.971	1.960	2
2	0.974	1.099	0.736	0.887	2.649	3
4	1.089	1.386	0.940	0.785	2.971	4
3	1.011	1.099	0.868	0.921	2.749	3
8	1.040	1.099	0.868	0.946	2.828	3
6	0.950	1.099	0.950	0.865	2.586	3
7	1.225	1.609	0.514	0.761	3.404	5
13	1.855	2.079	0.951	0.892	6.389	8

Table 5. Pairwise comparisons of Shannon diversity indices between study sites. Comparisons were undertaken using the *t* test approach of Hutcheson (1970) for the Shannon formula, as described by Zar (1999). Symbols are: *, $0.05 < P < 0.1$; **, $0.02 < P < 0.05$; ***, $0.01 < P < 0.02$; ****, $0.005 < P < 0.01$; *****, $0.002 < P < 0.005$; ******, $0.001 < P < 0.002$; *******, $P < 0.001$; n.s., comparison not significantly different.

Sites	1	10	2	4	3	8	6	7	13
1	—								
10	*******	—							
2	*	*******	—						
4	n.s.	*******	n.s.	—					
3	n.s.	*******	n.s.	n.s.	—				
8	n.s.	*******	n.s.	n.s.	n.s.	—			
6	**	*******	n.s.	n.s.	n.s.	n.s.	—		
7	n.s.	*******	*****	n.s.	****	***	******	—	
13	*******	*******	*******	*******	*******	*******	*******	*******	—

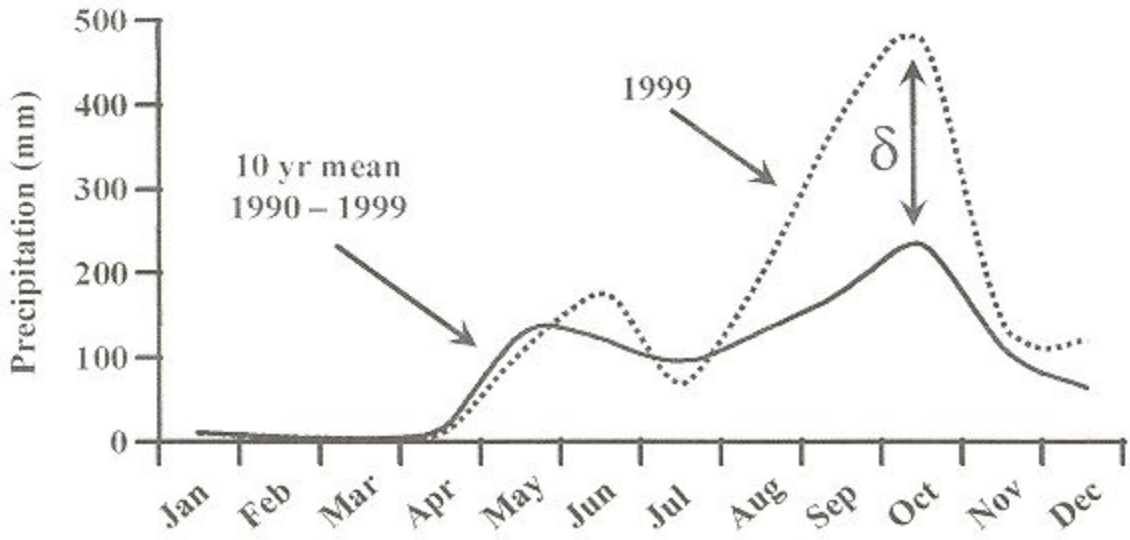

Figure 3. Deviation in precipitation for 1999 relative to a 10-year average. Note the large increase (a) in precipitation in the months immediately preceding the Panamá HPS outbreak. Data from the Meteorological Service of Panamá.

the pairwise metrics between small mammal communities (Euclidean distance and Morisita similarity) using the "unweighted pair-group method using arithmetic averages" or UPGMA algorithm (Sneath and Sokal 1973, Romesburg 1984). Clustering was carried out using the Neighbor subprogram (version 3.573c) of Phylip (Felsenstein 1989, 1993).

Species diversities (community heterogeneity) were calculated using the Shannon index (Shannon 1948), H' (using base e logarithms), as were H'_{max} and H'_{min} (the theoretical maximum and minimum possible diversities) and evenness, J', based on H'. The evenness measure J' has the advantage that it ranges from 0 to 1 and is based on the Shannon index. Krebs (1989) pointed out that an alternative form of the Shannon index is sometimes preferred (e.g., Hill 1973, Peet 1974): $N_1 = e^{H'}$, where N_1 is the number of equally common species that would produce the same diversity as expressed by H'; we therefore also present the N_1 values for the different sites. Differences between diversity indices among sites were evaluated using the t test approach of Hutcheson (1970) for the Shannon formula, as described by Zar (1999). Community similarity was examined using the Horn index of community overlap (Horn 1966, Brower et al. 1998); the correction of Rejmánek (1981) was applied when H'_A or H'_B were greater than H'_{AB}. Evenness of abundance of species was calculated as the ratio of the Shannon index, H', to H'_{max} (Pielou 1969). These measures of community similarity are preferred herein over species abundance models because they are distribution independent (Magurran 1988, Peet 1974, Ruedas et al. 1994, see Graham 1983, for a contrasting opinion).

A common problem in analysis of communities is lack of uniformity in captures across distinct sites, i.e., heterogeneity of sample sizes (Hayek and Buzas 1997). In order to uniformly compare communities that differed in sample sizes across sites, we standardized all samples to a common size of 5 individuals using the rarefaction technique of Sanders (1968) as modified independently by Hurlbert (1971) and Simberloff (1972). We used the implementation of Krebs (1989) in order to calculate the rarefactions for each sample.

RESULTS

Capture data

A total of 164 animals were captured. Of these, 145 were non-volant mammals trapped during the approximately 1,819 trap nights, a trap success rate of approximately 8%; 126 of the non-volant small mammals belonged to 7 species of the family Muridae (Table 1). This rodent family contains all the reservoir species of known New World hantaviruses.

Community ecology

Inspection of the inter-site Euclidean distances (Table 2) indicates there are statistical and qualitative differences among sites. The negative control site (Cerro Hoya National Park, site 13) is more distant from all

Table 6. Values of the Horn index of community overlap, R_o (Horn, 1966). The index varies from 0 (when the two communities have no species in common) to 1 (when species compositions and relative abundances are identical). Values with a superscripted star have been subjected to the correction of Rejmánek (1981) for instances where either H'_1 or H'_2 were greater than H'_{12}. The column headed "mean" shows the mean overlap of the community in the first column with all other communities sampled.

Localities	mean	1	10	2	4	3	8	6	7	13
1	0.663	1.000								
10	0.457	0.732*	1.000							
2	0.666	0.643	0.441	1.000						
4	0.672	0.738	0.471	0.838	1.000					
3	0.686	0.600	0.444	0.935	0.852	1.000				
8	0.798	0.872	0.701*	0.813	0.850	0.918	1.000			
6	0.729	0.920*	0.800*	0.812	0.745	0.732	0.942	1.000		
7	0.655	0.762	0.807*	0.652	0.690	0.801	0.809*	0.761	1.000	
13	0.308	0.362	0.473	0.191	0.195	0.203	0.390	0.383	0.264	1.000

other sites than those are from each other ($p = 0.0004$). Site 5 (Poeri) is also different ($p < 0.0001$) from the positive case sites. However, we consider Poeri among the positive sites because the habitat is similar to that surrounding case sites (preponderance of sugarcane and corn), and because hantavirus antibodies were detected there in both *Zygodontomys brevicauda cherriei* and *Oligoryzomys fulvescens costaricensis* (Vincent et al. 2000). The matrix of Morisita similarities (Table 3) presents a similar pattern. Cerro Hoya N P. is on average most dissimilar to the remaining sites. Site 11 and site 12 both have lower average similarities overall, but these are likely artifacts of low sample size as both sites each had only two species present.

As in the distances and similarities (see below), the Shannon species diversity index for Cerro Hoya NP, $H' = 1.855$, is significantly higher ($P < 0.001$) than that of all other sites (Tables 4 and 5). Site 10 was also significantly distinct in the Shannon index but did not greatly differ qualitatively; five specimens were captured at that site of only two species (Table 1). That site therefore differs from remaining sites (control and case) in having an unusually low Shannon diversity, $H' = 0.673$. These parameters can be further explored by considering the amount of overlap among the different sites sampled in the course of the study. Cerro Hoya NP has a mean Horn index of community overlap with other sites of $R_o = 0.308$, while for site 10 $R_o = 0.457$ (Table 6). Mean values of overlap for other sites range from 0.663–0.798. Thus, unusually diverse as well as unusually depauperate sites stand out from a general norm when using these ecological indices. The diagonal matrices can be visualized as trees depicting the ecological relationships among areas (Figure 3).

Rarefaction analyses

A potential problem in the foregoing analyses is that the increased diversity found in Cerro Hoya NP (negative control) was due not to any real, fundamental differences in community ecology between control and treatment sites, but rather to uneven trapping effort among sites. This is particularly so given that there was unevenness in trapping effort among sites due to the limitations imposed by trapping in and around residences in order to retroactively assess the proportion of serologically positive rodents at case sites. There are, in fact, significant effects in our data due to sampling effort: a linear regression between species richness and trap-nights was highly significant ($P = 0.001$, $R^2 = 0.617$). In order to account for this potential source of error, we undertook rarefaction analyses on the site collection data (Krebs 1989, Hayek and Buzas 1997). The results of the rarefaction analyses are presented in Table 1; the row

labeled "rarefaction" represents the number of species that would be expected based on a sample size of 5 specimens captured. Statistically assessing a difference between a single (control) sample and a population of case sites is difficult at best, but a nonparametric analysis of sites standardized to a sample size of 5 suggested that there was in fact a quantitative as well as qualitative difference between Cerro Hoya NP and sites where human cases of HPS occurred ($P = 0.02$).

DISCUSSION

The HPS outbreak in light of faunal considerations

The present work was designed to determine what species hosted the hantavirus causing the HPS outbreak in the Las Tablas region of Panamá. We nevertheless wished to address the hypothesis that the faunal communities associated with peridomestic settings contributed to a greater risk of contracting HPS. Although sample sizes were relatively small, our data indicate that there was a clear and significant difference between faunal communities in peridomestic settings and those in more pristine, undisturbed habitats.

The results of the analyses pertaining to continuously variable indices (distance and similarity) point to a clear and distinct trend: faunal communities where (1) the virus was present or (2) human cases of HPS were found, were significantly distinct from control localities (wherein neither virus nor HPS were present). These ecological differences are due to species diversities: faunal communities associated with cases of HPS were extremely depauperate in their biodiversity and associated properties. For example, Cerro Hoya NP was ecologically most distant (Table 2) and dissimilar (Table 3) and had the highest species diversity (Tables 4 and 5) from all other faunal communities sampled, even when standardized across sites (rarefaction analyses).

Our data provide empirical support for theoretical advances in ecological epidemiology, particularly as enumerated by Matuschka et al. (1992, 1999), Ostfeld and Keesing (2000a,b), and Schmidt and Ostfeld (2001). Although concentrating on multi-reservoir, vector-borne diseases (specifically Lyme disease), these authors suggested that there exists a so-called "dilution effect" in these biological systems. Previously, Macdonald (1952), Garrett-Jones (1964), and Molineaux et al. (1978) had applied similar concepts to malaria and malarial transmission dynamics, including the modeling tool known as the Macdonald equation (Spielman and Rossignol 1984). Specifically, "dilution effect" refers to the consequence of an excess of reservoir species of relatively low competency for a pathogenic organism. In a system such as Lyme disease or other vector-borne diseases, this is a straightforward, non-controversial theorem: large number of reservoir species may be somewhat competent (for Lyme disease, the list includes at least 11 mammal species in 9 genera of 6 families in 6 orders, as well as several bird species). Many of these potential hosts are, however, poor reservoirs and may be incapable of transmitting Lyme spirochetes (Matuschka et al. 1991, 1992). Attenuation would become a function, to a certain degree, of local biodiversity: the greater the potential number of hosts and lesser their competence, the greater the attenuation (Wilson et al. 1990, Schmidt and Ostfeld 2001).

A similar mechanism might operate in the case of directly transmitted zoonoses such as HPS where to date there is little evidence of vectored transmission (notwithstanding Houck et al. 2001). Theoretically, there is only one competent host for each hantavirus: the virus would be transmitted most efficiently in a single-species community. With each additional species, the proportion of potential virus transmitting interactions involving species of low competency would increase and the efficiency of virus transmission would decrease.

Also affecting the probability of hantaviral transmission to humans is the abundance of the host in the faunal community in proximity to humans. In our study, faunal communities with cases of HPS all had few species (low Shannon indices, Table 4) and were very similar both by inspection (Table 6, Horn index of community overlap, Table 2, Euclidean distances; and Table 3, Morisita similarities) and statistically (Table 5, pairwise comparisons of Shannon indices). The principal factor in general (albeit not in each instance) uniting these communities was the presence of the hantavirus hosts *Oligoryzomys fulvescens costaricensis* and *Zygodontomys brevicauda cherriei*. These rodent species thrive in disturbed habitats such as those generated by anthropogenic change on the Azuero Peninsula.

In addition, discrete differences in faunal composition exist among sites, the principal of these again relating to biodiversity. Sites wherein humans were likely to develop HPS were significantly more depauperate in species than sites without disease. Furthermore, case sites tended to be dominated by *Z. b. cherriei*, with the additional presence of one or more of any of three species: *M. musculus*, *O. f. costaricensis*, and *S. hirsutus*. *Zygodontomys b. cherriei* was present in 77% of the case sites, *M. musculus* in 54%, and *O. f. costaricensis* and *S. hirsutus* in 38%.

In contrast, the small mammal fauna of Cerro Hoya N.P. (negative control site) was not as dominated by any one species; of nine species present, the most common, *O. talamancae*, constituted 41% of the total captures; *Z. b. cherriei* made up 6%. The remaining eight species

were subequally prevalent with one to four captures each. In the positive control site, *Z. b. cherriei* constituted 49% of the captures; at case sites, *Z. b. cherriei* averaged 51%. One may argue that *Z. b. cherriei* were captured at Cerro Hova N.P., and that they would be an indication of disturbance: indeed *Z. b. cherriei* are indicative of disturbance. However, the *Z. b. cherriei* captured at Cerro Hova N.P. were in a field of sugar cane adjacent to the only residence present in many square kilometers. While there may be *Z. b. cherriei* in undisturbed habitats of Cerro Hova N.P., their prevalence there, if any, is likely to be low.

Four other species appear to be indicators of relatively undisturbed habitat: the marsupial *Marmosa robinsoni* and the Panamá endemic pocket mouse *Liomys adspersus*. The pocket mouse also was found in the positive control site, Poeri (site 7); however, the single specimen captured there was found in late secondary vegetation along a small stream. The remaining animals at that site were captured in fields of harvested corn and sugarcane. None of the common peridomestic species (*O. f. costaricensis*, *Z. b. cherriei*) were captured in this area of site 7. Finally, neither *Oryzomys* species, all forest specialists, were found in disturbed case sites.

Our small mammal trapping was concentrated around case houses. However, only *M. musculus* (house mice) were trapped *within* houses, and a very small number of rodents (and rodent species) were captured outside very close to houses. These were mainly *M. musculus*, but also, in lesser proportion, *Z. b. cherriei*. The preponderance of sylvatic sigmodontine rodents captured near residences (*O. f. costaricensis*, *S. hispidus*, and *Z. b. cherriei*) were captured in highly disturbed habitats near the residences (particularly sugarcane, corn fields, and meadows used for pasturing horses or cattle).

Accordingly, high risk factors for HPS are deduced to be the presence of corn and sugarcane fields near residences rather than factors associated with domestic exposure. It follows that high risk tasks are associated with these habitats (cane or corn harvesting; cane or corn processing). Because most families in the Azuero Peninsula, even if they live in a more urban setting, tend year-round to small landholdings for different crops, they would be at risk of contracting HPS. A successful program for reducing the incidence of HPS in Panamá therefore needs to include a strong outreach component.

The ecological context resulting from our trapping efforts may be better understood in comparison with other studies of the same area. These show that even early in the century, the community of rodents on the Azuero Peninsula was not very rich. Aldrich and Bole (1937) captured five sigmodontine species (*Oryzomys azuerensis* [= *couesi*], *O. talamancae*, *Sigmodon hispidus* [= *hirsutus*], *Nyctomys sumichrasti*, and *Zygodontomys b. cherriei*), one heteromyid (*Liomys adspersus*), one echimyid (*Proechimys semispinosus*), and one murine (*Rattus rattus*) rodent species during two mo of field work on the western side of the peninsula. The two most abundant rodent species were *S. hirsutus* and *Z. b. cherriei*. This early collecting, presumably under conditions of lesser human environmental disturbance, detailed a community very much resembling the one reported here, with the exception that *O. f. costaricensis* was present in our sample. Although Mendez (1993) reported that *O. f. costaricensis* occurs throughout Panamá, the specimen-based report of Carleton and Musser (1995) did not report this species from the peninsula. Ours is therefore the first report of the Costa Rican pygmy rice rat from the region.

Fleming (1970), collecting at the Rodman Naval Ammunition Supply Depot in Rodman Marine Base (near Balboa, 8°58'N, 79°36'25"W), found ten species of sigmodontine rodents, two of heteromyids, and one of murine rodents and suggested that *O. f. costaricensis* occurred in grasslands adjacent to forests but not in forests proper. Adler et al. (1997) reported on the community structure of a disturbed dry forest in nearby northern Colombia. These authors found a species-poor rodent community where the most abundant species were also the most widely distributed; these species also appeared to have benefited from forest clearing and agricultural activities. The species in question also exhibited large population fluctuations from year to year, a phenomenon which could have important future public health implications.

The HPS outbreak in light of climate considerations

The epidemiology of many zoonotic or vector-borne diseases occurring in humans, such as dengue fever, Hantavirus pulmonary syndrome, hemorrhagic fever, schistosomiasis, and malaria, is determined in large part by the ecology of the reservoir or vector (LeDuc 1989). Alteration of the environment and climate change are thought to have led to the recent emergence or increased incidence of many of these diseases throughout the world (Epstein 1995). The recent recognition of several new hantaviruses in North and South America is just one example of global climate effects that may have led to ecological conditions favorable to a carrier species (CDC 1993, Ksiazek et al. 1995). Epidemiology of human HPS is a direct reflection of the ecology of its small mammal reservoirs (Korpela and Lahdevirta 1978, LeDuc 1987, Xu et al. 1985, Kovats 2000), yet contributing factors leading to major population changes in these rodent hosts have not been fully elucidated. Unusual climatic conditions due to the El Niño–Southern Oscillation

(ENSO) phenomenon are hypothesized to have led to population explosions of wild rodents such as the one preceding the 1993 outbreak of HPS in the Southwestern U.S. (Parmenter et al. 1999). Similar models based on unusual climate conditions may have been at play in the Panamá HPS outbreak of early 2000. For example, Figure 3 shows the mean precipitation for the 10 years prior to the outbreak with that for 1999. The increased precipitation may have led to a population explosion of wild rodents on the Peninsula. Although this a tentative conclusion, it provides support for an association between precipitation and HPS outbreaks, possibly through the "cascade hypothesis" of Yates et al. (2002).

There are additional factors involved in the "why here, why now" question. The most obvious is an increased awareness of hantaviruses and HPS in the Americas. The symptoms of HPS are becoming better known, leading to detection of cases and characterization of the etiologic agents. The second factor is environmental: aerial perspectives of the Los Santos province area show a very dry, open, and deforested region. This is a longterm consequence of practices in commercial logging, crop management (primarily corn and sugarcane), and a focus on cattle, horse, and sheep herding. Recent studies have demonstrated that in Central America, deforestation in one area can have ecological effects considerable distances away, reducing rainfall and causing deforestation (Lawton et al. 2001). The rodent species hosting Choclo virus (*O. f. costaricensis*) was most closely associated with fields resulting after the clearing of corn and sugarcane and was found in grass near habitations and pastures used for cattle and horses. Finally, although no *Sigmodon hirsutus* were found with detectable antibody to hantaviruses in the present investigation, *Sigmodon* are known to harbor a hantavirus associated with human disease (Black Creek Canal), and several arenaviruses (Pirital, Tamiami). Thus, longterm agricultural practices in the Los Santos region have had a positive effect on populations of rodents potentially associated with hantaviruses and arenaviruses and increasingly augment the risk of HPS with growing human populations (1.26% growth rate, 2002 CIA est., for a doubling rate of 57 years). As demonstrated here and elsewhere, a growing body of data supports the conclusion that anthropogenically–driven environmental, and consequently faunal, change has important epidemiological consequences (Colwell et al. 1998, Cueto de Casas and Carcavallo 1995, Daszak et al. 2001, Epstein 1995, 1998, Epstein and Chikwenhere 1994, Githeko et al. 2000, Gratz 1999, Haines 1998, Hales et al. 1997, Matuschka and Spielman 1986, Molyneux 1997, 1998, Nicholls 1993, Rogers and Packer 1993, Sutherst 1998, Walsh et al. 1993).

The presence of the reservoir host of Choclo virus at any site will dictate the risk of contracting HPS. During our study, however, we only found *O. f. costaricensis* in 5 of 13 (38%) HPS case sites; absence of *O. f. costaricensis* in any specific sampling should therefore not be construed as absence of risk. Rather, this risk should be evaluated within the framework of the totality of the faunal characteristics discussed above. Long–term studies will have to be undertaken to more closely scrutinize population levels of *O. f. costaricensis*, since faunal communities change over time due to a variety of factors (Argemi et al. 1999). It is thus important to continue monitoring population dynamics of confirmed and potential hosts of hantaviruses in the region.

Our data suggest that a fortuitous conjugation of biotic and abiotic factors, especially anthropogenically–driven impoverishment of vegetative and faunistic communities, led, on the Azuero Peninsula of Panamá in late 1999, to circumstances enhancing the propagation of hantaviruses and their transmission to humans. These circumstances further led to the establishment of faunal communities dominated by one or two small rodent species that are competent reservoirs of hantaviruses, including Choclo, now known to cause HPS in Panamá. Although in this particular instance, the primary causative agent in the system appears to have been human change, other factors, such as unusual precipitation, may have exacerbated this epidemiological framework. Most critical, therefore, is the association of anthropogenically–distressed habitats and their consequent depauperate faunal communities with an increase in the risk of contracting HPS.

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REFERENCES CITED

Adler, G.H., J.J. Arboledo, and B.L. Travi. 1997. Diversity and abundance of small mammals in degraded tropical dry forest of northern Colombia. Mammalia 61: 361-370.

Aldrich, J.W. and B.P.J. Bole. 1937. The birds and mammals of the western slope of the Azuero peninsula. Sci. Publ. Cleveland Mus. Nat. Hist. 7: 1-196.

Argemi, M., M. Monclus, F. Mestres, and L. Serra. 1999. Comparative analysis of a community of drosophilids (Drosophilidae: Diptera) sampled in two periods widely separated in time. Zool. Syst. Evol. Res. 37: 203-210.

Brower, J.E., J.H. Zar, and C. von Ende. 1998. *Field and laboratory methods for general ecology*. 4th ed. McGraw-Hill, New York. 273 pp.

Carleton, M.D. and G.G. Musser. 1995. Systematic studies of oryzomyine rodents (Muridae, Sigmodontinae)—definition and distribution of *Oligoryzomys vegetus* (Bangs, 1902). Proc. Biol. Soc. Wash. 108: 338–369.

Clement, J.P. 2003. Hantavirus. Antiviral Res. 57: 121-127.

Clifford, H.T. and W. Stephenson. 1975. *An introduction to numerical classification*. Academic Press, New York. 229 pp.

Colwell, R., P. Epstein, D. Gubler, M. Hall, P. Reiter, J. Shukla, W. Sprigg, E. Takafuji, and J. Trtanj. 1998. Global climate change and infectious diseases. Emerg. Infect. Dis. 4: 451-452.

Curto de Casas, S.I. and R.U. Carcavallo. 1995. Climate change and vector–borne disease distribution. Soc. Sci. Med. 40: 1437-1440.

Daszak, P., A.A. Cunningham, and A.D. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Trop. 78: 103-116.

CDC. 1993. Update: Hantavirus infection–United States. MMWR 42: 517-519.

Epstein, P.R. 1995. Emerging diseases and ecosystem instability: new threats to public health. Am. J. Public. Health 85: 168-172.

Epstein, P.R. 1998. Global warming and vector–borne disease. Lancet 351: 1737.

Epstein, P.R., and G.P. Chikwenhere. 1994. Environmental factors in disease surveillance. Lancet 343:1440-1441.

Felsenstein, J. 1989. PHYLIP—Phylogeny Inference Package (Version 3.2). Cladistics 5: 164-166.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle. http://evolution.genetics.washington.edu/phylip.html.

Fleming, T.H. 1970. Notes on the rodents faunas of two Panamanian forests. J. Mammal. 51: 473-490.

Garrett-Jones, C. 1964. The human blood index of malaria vectors in relation to epidemiological assessment. Bull. Wld. Hlth. Org. 30: 241-261.

Githeko, A.K., S.W. Lindsay, U.E. Confalonieri, and J.A. Patz. 2000. Climate change and vector–borne diseases: a regional analysis. Bull. Wld. Hlth. Org. 78: 1136-1147.

Graham, G.L. 1983. Changes in bat species diversity along an elevational gradient up the Peruvian Andes. J. Mammal. 64: 559-571.

Gratz, N.G. 1999. Emerging and resurging vector–borne diseases. Annu. Rev. Entomol. 44: 51-75.

Haines, A. 1998. Climate warming and vector–borne disease. Lancet 351:1737–1738.

Hales, S., P. Weinstein, and A. Woodward. 1997. Public health impacts of global climate change. Rev. Environ. Health 12: 191-199.

Hayek, L.-A.C. and M.A. Buzas. 1997. *Surveying natural populations*. Columbia University Press, New York. 563 pp.

Hill, M.O. 1973. Diversity and evenness: a unifying notation and its consequences. Ecology 54: 427-432.

Horn, H.S. 1966. Measurement of "overlap" in comparative ecological studies. Am. Nat. 100: 419-424.

Houck, M.A., H. Qin, and H.R. Roberts. 2001. Hantavirus transmission: potential role of ectoparasites. Vector Borne Zoonotic Dis. 1: 75-79.

Hurlbert, S.H. 1971. The non–concept of species diversity: a critique and alternative parameters. Ecology 52: 577–586.

Hutcheson, K. 1970. A test for comparing diversities based on the Shannon formula. J. Theor. Biol. 29: 151-154.

Korpela, H. and J. Lahdevirta. 1978. The role of small rodents and patterns of living in the epidemiology of nephropathia epidemica. Scan. J. Infec. Dis. 10: 303-305.

Kovats, R.S. 2000. El Niño and Public Health. Bull. WHO 78: 1127–1135.

Krebs, C.J. 1989. *Ecological methodology*. Harper & Row, New York. 654 pp.

Ksiazek, T.G., S.T. Nichol, J.N. Mills, M.G. Groves, A. Wozniak, S. McAdams, M.C. Monroe, A.M. Johnson, M.L. Martin, C.J. Peters, and P.E. Rollin. 1997. Isolation, genetic diversity, and geographic distribution of Bayou virus (Bunyaviridae:

Hantavirus). Am. J. Trop. Med. Hyg. 57: 445–448.
Ksiazek, T.G., C.J. Peters, P.E. Rollin, S. Zaki, S. Nichol, C. Spiropulou, S. Morzunov, H. Feldmann, A. Sanchez, A. S. Khan, B.W.J. Mahy, K. Wachsmuth, and L.C. Butler. 1995. Identification of a new North American hantavirus that causes acute pulmonary insufficiency. Am J. Trop. Med. Hyg. 52: 117-123.
Lawton, R.O., U.S. Nair, R.A. Pielke, and R.M. Welch. 2001. Climatic impact of tropical lowland deforestation on nearby montane cloud forests. Science 294: 584-587.
LeDuc, J.W. 1987. Epidemiology of Hantaan and related viruses. Lab. Animal Sci. 37: 413-418.
LeDuc, J.W. 1989. Epidemiology of hemorrhagic fever viruses. Rev. Inf. Dis. 11: 730-735.
Levis, S., J.E. Rowe, S. Morzunov, D.A. Enria, and S.C. St. Jeor. 1997. New hantaviruses causing hantavirus pulmonary syndrome in central Argentina. Lancet 349: 998–999.
Levis, S., S.P. Morzunov, J.E. Rowe, D. Enria, N. Pini, G. Calderon, M. Sabattini, and S.C. St. Jeor. 1998. Genetic diversity and epidemiology of hantaviruses in Argentina. J. Infect. Dis. 177: 529-538.
Macdonald, G. 1952. The analysis of equilibrium in malaria. Trop. Dis. Bull. 49: 813-828.
Magurran, A.E. 1988. *Ecological diversity and its measurement*. Princeton University Press, Princeton, New Jersey. 179 pp.
Matuschka, F.R., R. Allgower, A. Spielman, and D. Richter. 1999. Characteristics of garden dormice that contribute to their capacity as reservoirs for Lyme disease spirochetes. Appl. Environ. Microbiol. 65: 707-711.
Matuschka, F.R., P. Fisher, M. Heiler, D. Richter, and A. Spielman. 1992. Capacity of European animals as reservoir hosts for the Lyme disease spirochete. J. Infect. Dis. 165: 479-483.
Matuschka, F.R., P. Fischer, K. Musgrave, D. Richter, and A. Spielman. 1991. Hosts on which nymphal *Ixodes ricinus* most abundantly feed. Am. J. Trop. Med. Hyg. 44: 100-107.
Matuschka, F.R. and A. Spielman. 1986. The emergence of Lyme disease in a changing environment in North America and central Europe. Exp. Appl. Acarol. 2: 337-353.
Mendez, E. 1993. *Los roedores de Panamá*. Panama City, Imprenta Pacifico. 372 pp.
Mills, J.N., T.L. Yates, J.E. Childs, R.R. Parmenter, T.G. Ksiazek, P.E. Rollin, and C.J. Peters. 1995. Guidelines for working with rodents potentially infected with Hantavirus. J. Mammal. 76: 716-722.
Molineaux, L., K. Dietz, and A. Thomas. 1978. Further epidemiological evaluation of a malaria model. Bull. Wld. Hlth. Org. 56: 565-571.
Molyneux, D.H. 1997. Patterns of change in vector–borne diseases. Ann. Trop. Med. Parasitol. 91: 827-839.
Molyneux, D.H. 1998. Vector–borne parasitic diseases—an overview of different changes. Int. J. Parasitol. 28: 927-934.
Monroe, M.C., S.P. Morzunov, A.M. Johnson, M.D. Bowen, H. Artsob, T. Yates, C.J. Peters, P.E. Rollin, T.G. Ksiazek, and S.T. Nichol. 1999. Genetic diversity and distribution of *Peromyscus*–borne hantaviruses in North America. Emerg. Infect. Dis. 5: 75-86.
Morisita, M. 1959. Measuring interspecific association and similarity between communities. Mem. Fac. Sci. Kyushu Univ., Ser E (Biol) 3: 65-80.
Morzunov, S.P., J.E. Rowe, T.G. Ksiazek, C.J. Peters, S.C. St. Jeor, and S.T. Nichol. 1998. Genetic analysis of the diversity and origin of hantaviruses in *Peromyscus leucopus* mice in North America. J. Virol. 72: 57–64.
Nicholls, N. 1993. El Niño–Southern Oscillation and vector–borne disease. Lancet 342: 1284-1285.
Ostfeld, R.S. and F. Keesing. 2000a. Biodiversity and disease risk: the case of Lyme disease. Conserv. Biol. 14: 722-728.
Ostfeld, R.S. and F. Keesing. 2000b. The function of biodiversity in the ecology of vector–borne zoonotic diseases. Can. J. Zool. 78: 2061-2078.
Parmenter, C.A., T.L. Yates, R.R. Parmenter, and J.L. Dunnum. 1999. Statistical sensitivity for detection of spatial and temporal patterns in rodent population densities. Emerg. Infect. Dis. 5: 118-125.
Peet, R.K. 1974. The measurement of species diversity. Annu. Rev. Ecol. Syst. 5: 285-307.
Pielou, E. C. 1969. *An introduction to mathematical ecology*. Wiley Interscience, New York. 286 pp.
Rejmánek, M. 1981. Corrections to indices of community dissimilarity based on species diversity measures. Oecologia 48: 290-291.
Rogers, D.J. and M.J. Packer. 1993. Vector–borne diseases, models, and global change. Lancet 342: 1282-1284.
Romesburg, H.C. 1984. *Cluster analysis for researchers*. Lifetime Learning Publications, Belmont, California, 334 pp.
Ruedas, L.A., J.R. Demboski, and R.V. Sison. 1994. Morphological and ecological variation in *Otopteropus cartilagonodus* Kock, 1969 (Mammalia: Chiroptera: Pteropodidae) from Luzon, Philippines. Proc. Biol. Soc. Wash. 107: 1-16.
Sanders, H.L. 1968. Marine benthic diversity: a comparative study. Am. Nat. 102: 243-282.

Schmidt, K.A. and R.S. Ostfeld. 2001. Biodiversity and the dilution effect. Ecology 82: 609-619.

Shannon, C.E. 1948. A mathematical theory of communication. Bell System Tech. J. 27: 379-423.

Simberloff, D.S. 1972. Properties of the rarefaction diversity measurement. Am. Nat. 106: 414-418.

Sneath, P.H.A. and R.R. Sokal. 1973. *Numerical taxonomy: the principles and practice of numerical classification*. W. H. Freeman, San Francisco, 573 pp.

Spielman, A. and P.A. Rossignol. 1984. Insect vectors. In, K. S. Warren and A. A. F. Mahmoud (eds.) *Tropical and Geographical Medicine*. pp. 167-183. McGraw-Hill, New York, NY. 1,175 pp.

Sutherst, R.W. 1998. Implications of global change and climate variability for vector-borne diseases: generic approaches to impact assessments. Int. J. Parasitol. 28: 935-945.

Vincent, M.J., E. Quiroz, F. Gracia, A.J. Sanchez, T.G. Ksiazek, P.T. Kitsutani, L.A. Ruedas, D.S. Tamin, L. Caceres, A. Garcia, P.E. Rollin, J.N. Mills, C.J. Peters, and S.T. Nichol. 2000. Hantavirus Pulmonary Syndrome in Panamá: identification of novel hantaviruses and their likely reservoirs. Virology 277: 14–19.

Walsh, J.F., D.H. Molineux, and M.H. Birley. 1993. Deforestation: effects on vector–borne disease. Parasitology 106(Suppl.): S55-S75.

Wilson, M.L., T.S. Litwin, T.A. Gavin, M.C. Capkanis, D.C. Maclean, and A. Spielman. 1990. Host-dependent differences in feeding and reproduction of *Ixodes dammini* (Acari, Ixodidae). J. Med. Entomol. 27: 945–954.

Xu, Z.–Y., C.–S. Gu, Y–L. Wu, X.–W. Zhang, and L. Kun. 1985. Epidemiological studies of hemorrhagic fever with renal syndrome, analysis of risk factors and mode of transmission. J. Inf. Dis. 152: 137-144.

Yates, T.L., J.N. Mills, C.A. Parmenter, T.G. Ksiazek, R.R. Parmenter, J.R. Vande Castle, C.H. Calisher, S.T. Nichol, K.D. Abbott, J.C. Young, M.L. Morrison, B.J. Beaty, J.L. Dunnum, R.J. Baker, J. Salazar–Bravo, and C.J. Peters. 2002. The ecology and evolutionary history of an emergent disease: Hantavirus pulmonary syndrome. Bioscience 52: 989-998.

Zar, J.H. 1999. *Biostatistical analysis*. 4th ed. Prentice–Hall, NJ. 929 pp.