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Hantavirus Testing in Rodents of North-Central New Mexico 1993–1995

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by
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ABSTRACT

In 1993, an outbreak of a new strain of hantavirus in the southwestern US indicated that deer mice (*Peromyscus maniculatus*) was the primary carrier of the virus. In 1993, 1994, and 1995 the Ecological Studies Team (EST) at Los Alamos National Laboratory surveyed small mammal populations using live capture-recapture methods in Los Alamos County, New Mexico, to determine seroprevalence of hantavirus in this region. EST used trapping grids in 1993 and 1994 and used trapping webs in 1995. Grids were 120 m x 120 m (400 ft x 400 ft) with 144 trap stations at each grid. Three webs consisting of 148 traps each were used in 1995. Trapping took place over 4 to 8 consecutive nights. Programs CAPTURE and Distance were used to determine density estimates for grids and webs, respectively. Blood samples were analyzed in 1993 by the Centers for Disease Control and the University of New Mexico, School of Medicine. The 1994 and 1995 samples were analyzed by the University of New Mexico, School of Medicine. The deer mouse (*Peromyscus maniculatus*) was the most commonly captured species at all locations except one site where voles (*Microtus* spp.) were the most commonly captured species. Other species sampled included: harvest mice (*Reithrodontomys megalotis*), woodrats (*Neotoma* spp.), shrews (*Sorex* spp.), white-footed mice (*Peromyscus leucopus*), pinyon mice (*Peromyscus trueii*), and brush mouse (*Peromyscus boylii).* Results of the 1993, 1994, and 1995 testing identified a total overall seroprevalence rate among deer mice of approximately 5.5%, 4.2%, and 0%, respectively. Several other species tested positive for the hantavirus but it is uncertain if it is Sin Nombre virus. Further studies will be necessary to quantify seroprevalence rates in those species. Higher seroprevalence rates were found in males than females. Seroprevalence rates for Los Alamos County were much lower than elsewhere in the region.

INTRODUCTION

In the early 1980s, testing of small mammals in the western United States showed evidence of a strain of hantavirus infecting deer mice (*Peromyscus maniculatus*), rock mice (*Peromyscus difficilis*), California mice (*Peromyscus californicus*), Mexican woodrats (*Neotoma mexicana*), and bushy-tailed woodrats (*Neotoma cinerea*) (Tsai, et al. 1985). Other strains of hantavirus recognized in North America have been

identified in voles (*Microtus* and *Clethrionomys*), white-footed mice (*Peromyscus leucopus*), Norway rats (*Rattus norvegicus*), and house mice (*Mus musculus*) (Yanagihara 1990; Pyung-Woo, et al. 1985). In late spring-early summer 1993, a newly recognized strain of hantavirus was identified in the southwestern United States, including New Mexico, Arizona, Utah, and Colorado, and is currently referred to as the Sin Nombre Hantavirus. It was found to be primarily associated with the deer mouse but was also found in several other species (Childs, et al. 1994).

During the outbreak of the hantavirus disease in the southwestern US (1993), the Centers for Disease Control (CDC) requested that the Ecological Studies Team (EST) at Los Alamos National Laboratory (LANL) collect blood samples from rodents to obtain information on hantavirus seroprevalence (prevalence of hantavirus antibodies in mammal sera) in the LANL area. Subsequently, in 1994 and 1995, blood samples were analyzed by the School of Medicine at the University of New Mexico. Small mammal data collection included capture-and-release studies on rodent populations in three canyon systems, Guaje, Sandia, and Los Alamos Canyons, in Los Alamos County, New Mexico. Blood samples were collected in Los Alamos and Guaje Canyons in 1993 and 1994 and in Sandia Canyon in 1995. Due to the remoteness of the upper portion of Guaje Canyon, a field camp was established for a one-week period to collect data.

This study was not specifically designed to identify relationships between seroprevalence and small mammal population characteristics; further studies will be necessary to identify these relationships. This paper describes the small mammal studies, presents results of the small mammal population and density estimates, and provides results of the hantavirus testing. In addition, this paper does not describe in detail the collection of blood samples and safety procedures taken during the study to minimize risk of acquiring the virus. Detailed procedures for these activities can be found in Mills, et al. (1995) and Biggs and Bennett (1995).

In addition to the canyon systems, blood samples were also taken in 1993 from several locations throughout LANL. This sampling was used to determine seroprevalence of the hantavirus to aid in

development of Standard Operating Procedures (SOPs) for nonEST Laboratory personnel that may potentially be exposed to the virus under normal working conditions.

STUDY AREA

LANL is located in north-central New Mexico on the Pajarito Plateau, approximately 120 km (80 mi.) north of Albuquerque and 40 km (25 mi.) west of Santa Fe (Figure 1). The plateau is an apron of volcanic sedimentary rock stretching 33–40 km (20–25 mi.) in a north-south direction and 8–16 km (5–10 mi.) from east to west. The average elevation of the plateau is 2286 m (7500 ft). It slopes gently eastward from the edge of the Jemez Mountains, which are composed of a complex assemblage of volcanic rocks situated along the northwest margin of the Rio Grande rift. Intermittent streams flowing southeastward have dissected the plateau into a number of narrow mesas separated by deep, narrow canyons. The bedrock consists of Bandelier tuff erupted from the Jemez Mountains about 1.1 to 1.4 million years ago. The tuff overlaps other volcanics that in turn overlay the Puye Formation conglomerate (Env. Surv. Group 1988). This conglomerate intermixes with Chino Mesa basalts along the Rio Grande.

The LANL area is characterized by a semiarid, temperate mountain climate. In the summer months, temperatures typically range from a daily low of around 10° C (50° F) to a high of 27° C (80° F) (Bowen 1990). Winter temperatures generally range from near -10° C (15° F) to about 10° C (50° F) during a 24-hour period. Annual precipitation varies from 33 to 46 cm (13 to 18 in.), with most of it falling as rain in July and August.

The distribution of plant communities in the LANL region is determined largely by elevation. Plains and Great Basin Riparian-Deciduous Forest occurs at the lowest elevations in Los Alamos County along the

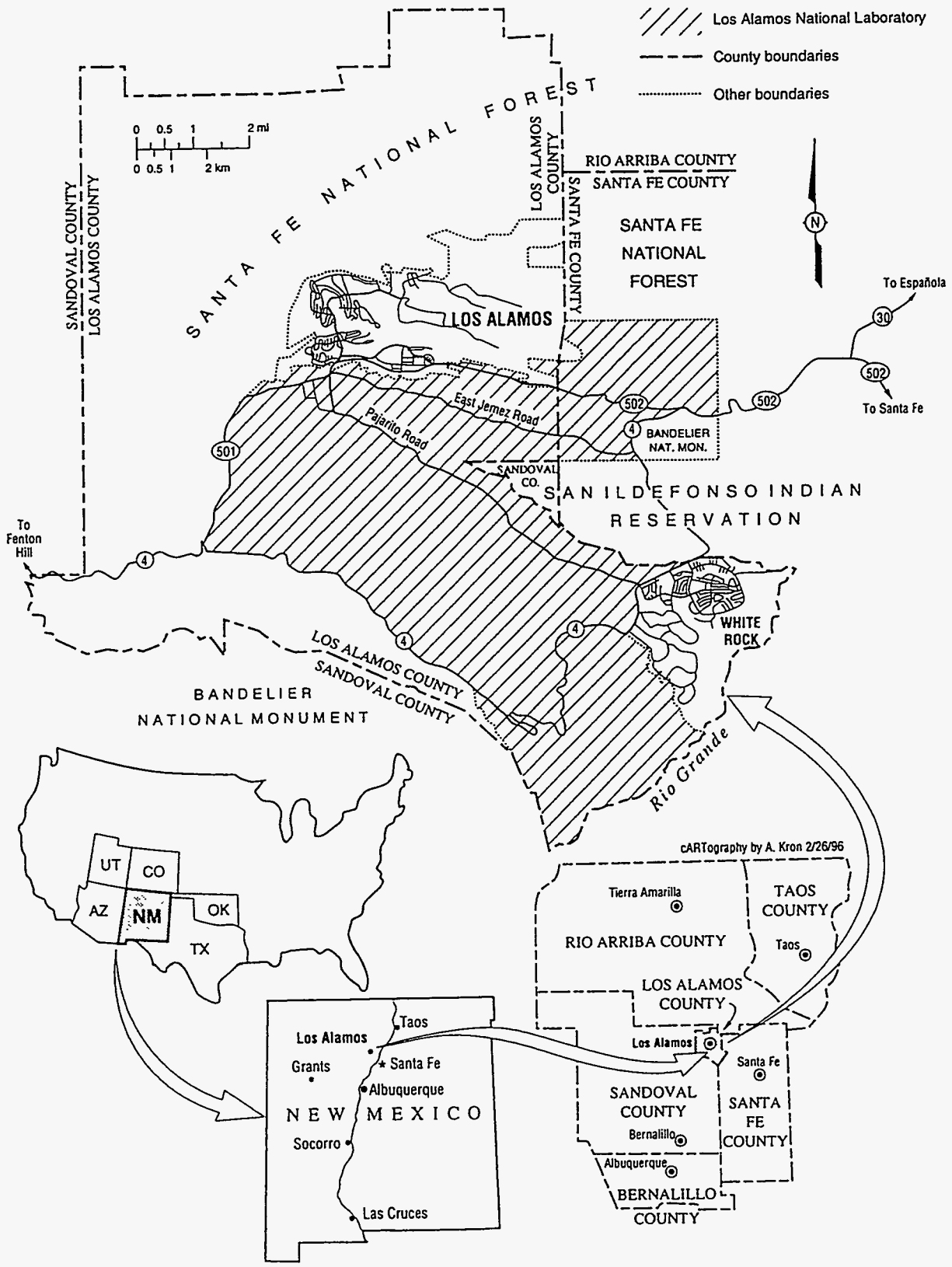


Figure 1. Location of Los Alamos National Laboratory.

Rio Grande floodplain (about 1524 m [5000 ft] above sea level). This vegetation type is characterized by stands of cottonwood (*Populus* spp.) and willow (*Salix* spp.), and nonnative species such as salt cedar (*Tamarix pentandra*) and Russian olive (*Eleagnus angustifolia*). Above the Rio Grande floodplain at elevations ranging from about 1700 to 1890 m (5600 to 6200 ft), one-seed juniper (*Juniperus monosperma*) becomes the most common overstory species, often intermixed with lesser amounts of piñon pine (*Pinus edulis*). Both of these tree species are typical of the Great Basin Conifer Woodland and together they form an open piñon-juniper woodland at elevations of 1890 to 2100 m (6200 to 6900 ft).

As the elevation increases towards the Jemez Mountains, the piñon-juniper woodland community gradually intergrades into Rocky Mountain Montane Conifer Forest. Ponderosa pine (*Pinus ponderosa*) becomes a dominant species at about 2100–2290 m (6900–7500 ft) on the higher mesa tops and along many of the north-facing canyon slopes. White fir (*Abies concolor*) and Douglas fir (*Pseudotsuga menziesii*) also grow along the north-facing slopes at intermediate elevations where they intermix with ponderosa pine to form a mixed-conifer community. Species of the Rocky Mountain Subalpine Conifer Forest and Woodland are more prevalent at the higher elevations of the Jemez Mountains.

Three small mammal trapping webs were placed in the upper portion of Sandia Canyon. The first two webs were centered within a cattail-dominated marsh with a ponderosa pine overstory (persistent, artificially flooded, palustrine wetland). The third web was placed in a transition area between the persistent, artificially flooded, palustrine wetland and the temporarily flooded palustrine wetland with a ponderosa pine overstory. This area was much drier than the habitat surrounding the first two webs and is more riparian than marsh in nature. The small mammal trapping sites located in Guaje and Los Alamos Canyons consisted of three habitat types; two distinct types—ponderosa pine and mixed-conifer, and a third in a transitional habitat type comprised of ponderosa pine and mixed conifer. A more detailed description of habitats sampled in Guaje and Los Alamos Canyons can be found in Biggs, et al. (1995).

METHODS

Due to the association between hantavirus and rodents, EST had to incorporate new techniques to allow for the collection of blood samples while collecting data on small mammals, and address health and safety issues associated with the hantavirus. The procedures for processing and bleeding animals and the personal protective equipment used in association with the collection of blood samples are not discussed in great detail in this report. A complete description of these procedures is given in Mills, et al. (1995) and Biggs and Bennett (1995).

In Guaje and Los Alamos Canyons, EST set up trapping grids in two habitat types (ponderosa pine and mixed-conifer) in 1993 and added a third in 1994 (ponderosa pine-mixed conifer). Grids were 120 m x 120 m (400 ft x 400 ft) with 144 trap stations each. In 1993, one grid was situated in the upper elevation of each canyon within mixed conifer habitat and one grid was in the lower portions of the canyons in ponderosa pine habitat. The third grid added in 1994 was setup similarly. The team used the computer program CAPTURE (White, et al. 1982) to estimate population size and density.

A web method of 148 traps was utilized in Sandia Canyon and data was analyzed (population density) using Buckland, et al. (1993) and Laake, et al. (1994). Trapping took place in 1994 and 1995, however, blood sampling for hantavirus took place only in 1995. Each web consisted of 12 lines of traps spaced at 5- to 10-m intervals with 4 traps placed in the center (Parmenter 1994). Each web was placed at least 200 m apart to prevent overlap of species between webs. Data on rodents for each web were pooled for analysis. Trapping took place over 4 to 8 consecutive nights. All traps were baited with a molasses-coated horse feed and peanut butter mixture.

In 1993, we placed two Sherman live-traps within 2 m (6.6 ft) of each trap station in Los Alamos and Guaje Canyons, making sure to set traps at least 1 m (3.3 ft) from obvious deer, elk, and other large mammal trails or bedding sites. In 1994 and 1995 sampling, only one trap per station was used. The field

crew baited traps in late afternoon and checked them in early morning to record nocturnal species. While awaiting checking, the traps were kept out of the sun and animals were removed as soon as possible. Trapping took place over 4 to 8 consecutive nights at each grid and web during each year of trapping and for a one-week period in 1993 around Laboratory facilities for a total of approximately 12,000 trap nights. In the morning, personnel collected traps that had been tripped.

At a centrally located (within the grid) processing station, animals were taken from the traps by shaking the animal into a ziplock bag which contained two cottonballs saturated with metaphane for anesthetization. Once the animal was anesthetized, personnel used a heparinized capillary tube to take a blood sample from the animal's interorbital area. The animal was then weighed, sexed, and measured. Additionally, animals were marked with size #FF rodent ear tags from the Salt Lake Stamp Co., Salt Lake City, Utah. Once measurements were taken from the animal, the animal was placed back into the trap, which was set in the shade, to revive. Once the animal revived, it was returned to the trap site and released at the original capture location. If the animal did not recover from the procedure, the specimen was double bagged and frozen. The blood samples were stored in coolers on dry ice. Capillary tubes used for collecting blood were placed in a Sharps container and heavily sprayed with Lysol.

Some of the blood samples collected in 1993 were tested by the CDC while the remaining portion of the 1993 samples and all of the 1994 and 1995 samples were tested by University of New Mexico, School of Medicine.

For purposes of data analysis, traps were assigned two numbers corresponding to an x-y coordinate (i.e., 1-1, 1-2, 1-3, etc.) with the first station (1-1) located at the northwest corner of the grid. The numbers were printed on pin flags placed at each trap station (the x-y coordinate). Additional flagging was placed above the trap station for ease in relocating. Species name, weight, body length, tail length, ear and foot length, and location of capture (x-y coordinate) were recorded.

EST used the program CAPTURE to estimate population size and density for grids. A nested-grid methodology was used to estimate density. Use of the nested-grid methodology compensates for possible "edge effect" (animals being drawn into the trap grid that normally would not occur there). The x-y coordinates for each capture were input to CAPTURE for use in density estimates. All species data were pooled to calculate density and populations of small mammals. Sample sizes for most individual species were insufficient to calculate population size or density. However, capture-recapture data on the deer mouse was large enough to be used.

Program Distance was used to estimate density of webs in Sandia Canyon. Estimating accurate densities by use of webs is based on the assumption that trapping continues until no new captures are recorded in the center of the web. Although this did not occur for voles, it did occur for deer mice.

RESULTS

For purposes of this study, seroprevalence rates among rodents in Los Alamos County are assumed to be dependent on small mammal population dynamics irrespective of habitat type and site location. This thereby assumes that seroprevalence rates are similar in small mammal populations by year throughout Los Alamos County regardless of site sampling locations.

Due to the implementation of new techniques (i.e., bleeding, anesthetizing of animals) added to the small mammal population study, concerns arose about the possibility of additional stress factors affecting recapture rates. Daily recapture rates (total number of tagged animals/total number of animals captured) for some of the sampling efforts presented in this paper and for some rodent studies in 1992 have been previously analyzed (Biggs, et al. 1995). Analysis of recapture data showed no significant differences between days when comparing between years when bleeding procedures where employed and years when these procedures were not used.

Table 1 lists all small mammal species tested for hantavirus in Los Alamos County from 1993 through 1995. A more detailed description and breakdown of species captured at each of these locations is given in Bennett and Biggs (In preparation) and Biggs, et al. (1995).

Table 1. Small Mammal Species Tested for Hantavirus, 1993–1995.

Long-tailed vole	*Microtus longicaudus*
Montane vole	*Microtus montanus*
Mexican woodrat	*Neotoma mexicana*
White-throated woodrat	*Neotoma albigula*
Silky pocket mouse	*Perognathus flavus*
White-footed mouse	*Peromyscus luecopus*
Brush mouse	*Peromyscus boylii*
Deer mouse	*Peromyscus maniculatus*
Pinyon mouse	*Peromyscus trueii*
Harvest mouse	*Reithrodontomys megalotis*
Water shrew	*Sorex palustrus*
Vagrant shrew	*Sorex vagrans*

The relative species composition for small mammals was calculated by habitat for each area sampled in the county (Table 2). Deer mice were the most common species captured in each habitat sampled. A stream channel with associated riparian vegetation occurred in all sampling areas and, as previously described, a cattail-dominated marsh occurs in one location. In all of these areas, deer mice were primarily captured in the drier habitats. Harvest mice, voles, and shrews were only captured in the more moist areas or in areas where surface water was present.

Table 2. Relative Percent Small Mammal Species Composition by Habitat, 1993–95.

SPECIES	Mixed Conifer	Mixed conifer/ Ponderosa pine	Ponderosa Pine	Ponderosa Pine/Marsh
Harvest mouse	0	0	5.0	18.2
Pinyon mouse	0	0	0	<1
Deer mouse	62.4	57.1	68.3	34.5
Brush mouse	5.1	42.9	21.8	8.8
White-footed mouse	0	0	2.0	0
Silky pocket mouse	0	0	0	0
Montane vole	1	0	2.0	15.8
Long-tailed vole	21.4	0	1.0	16.8
White-throated woodrat	2.6	0	0	0
Mexican woodrat	1	0	0	0
Shrew spp.	6.8	0	0	5.6

In each year of sampling, several species tested positive for hantavirus including montane vole, long-tailed vole, harvest mouse, and white-footed mouse (Table 3). However, because there were uncertainties in these species testing positive for the actual Sin Nombre virus, these positives are only viewed as unconfirmed. Therefore, results on analysis of deer mice only will be the main focus of the remainder of this section.

Table 3. Small Mammal Seroprevalence Rates by Year.

	NUMBER TESTED			% SEROPREVALENCE		
SPECIES	1993	1994	1995	1993	1994	1995
Deer mouse	57	53	33	8%	5%	0
Long-tailed vole	27	2	30	4%	0	0
Harvest mouse	4	2	38	25%	0	0
White-footed mouse	0	2	0	0	50%	0
Brush mouse	10	24	18	10%	0	0
Montane vole	0	1	9	0	0	11%

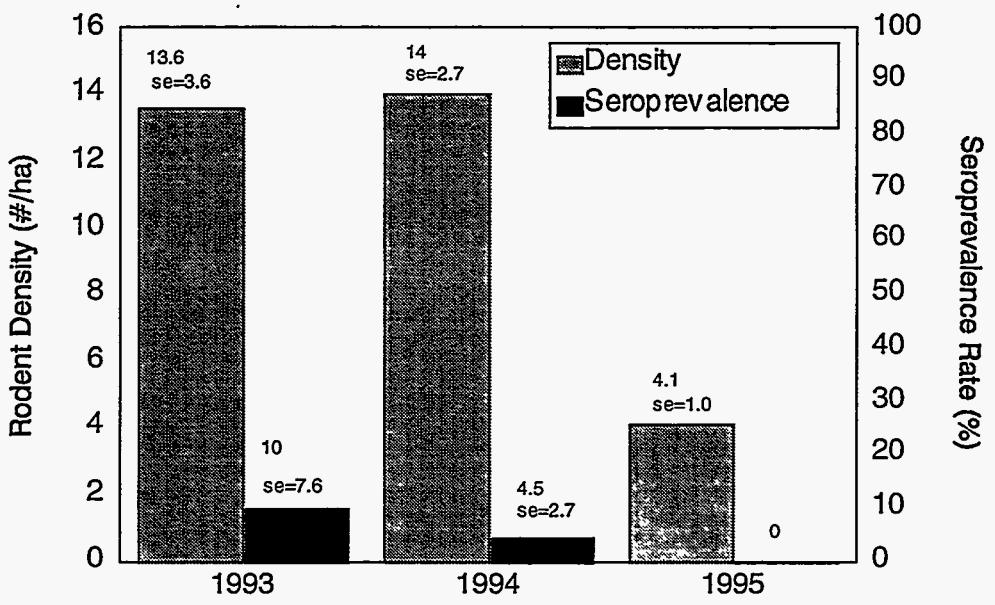
Table 4 provides information on density estimates for deer mice at each sampling location and the corresponding seroprevalence rates. Insufficient sample sizes prevented analysis from being conducted in lower Los Alamos Canyon. Density and seroprevalence data given in Table 4 were pooled and plotted by year (Figure 2).

Table 4. Density Estimates of Deer Mice for each Location Sampled for Hantavirus, Los Alamos County, 1993–95.

HABITAT	LOCATION[1]	POPULATION ESTIMATE	SE	SEROPREVALENCE RATE (%)
Mixed conifer	UGC (1994)	22	2.9	11 (n=19)
	UGC (1993)	19	1.1	5 (n=20)
	ULA (1994)	10	1.4	0 (n=18)
	ULA (1993)	15.2	5.8	0 (n=8)
Mixed C./Pond. pine	MGC (1994)	12	1.9	7 (n=14)
Pond. pine	LLA (1994)	0.1	0.1	0 (n=3)
	LLA (1993)	*		20 (n=5)
	LGC (1994)	12	1.3	0 (n=10)
	LGC (1993)	6.7	0.9	25 (n=12)
	SC3 (1995)	3.2	1.3	0 (n=3)
	SC1 (1995)	6.1	2.2	0 (n=13)
	SC2 (1995)	2.9	1.3	0 (n=18)

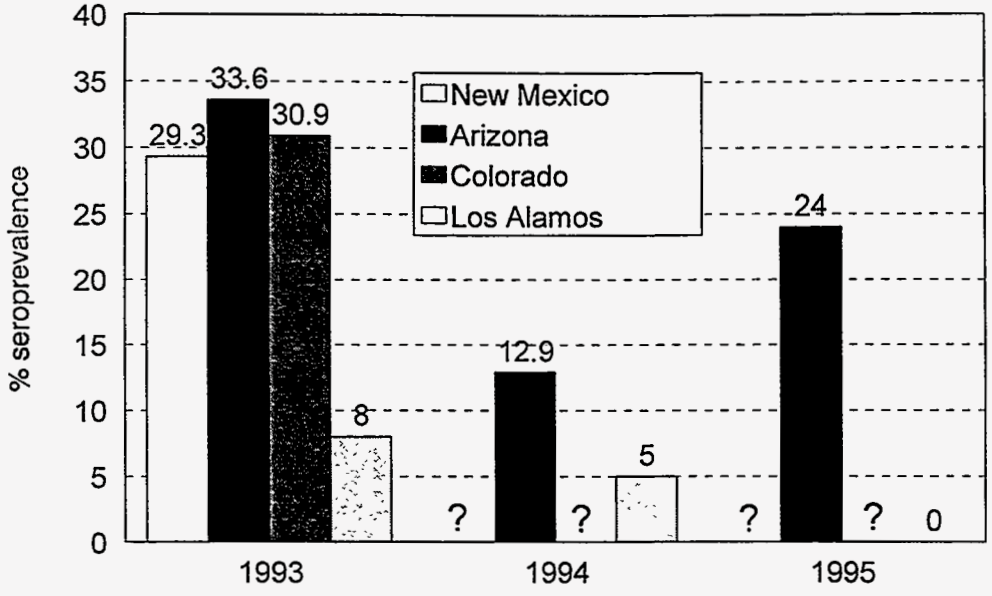
* Indicates sample size insufficient to run population and density estimate program or estimate was not provided by program.
[1] UGC=upper Guaje C.; MGC-middle Guaje C.; LGC=lower Guaje C.; ULA=upper Los Alamos C.; LLA=lower los Alamos C.; SC=Sandia C. (SC1,2,3 denotes web number).

Figure 2. Rodent Density Estimates and Seroprevalence Rates for Los Alamos County, 1993–95.



Los Alamos County and regional seroprevalence rates are provided in Figure 3. These are based on available data for three of the four-corners states (New Mexico, Arizona, and Colorado). Data is available for all states in 1993 and only for Arizona in 1994 and 1995. Seroprevalence rates in Los Alamos County were generally much lower than rates seen in all other locations during 1993. Compared to rates provided for Arizona in 1994 and 1995, Los Alamos County still showed much lower rates in deer mice tested for the virus.

Figure 3. Regional Seroprevalence Rates* For Deer Mice, 1993-95.



* seroprevalence rates are provided based on available data

references: unpublished data-Arizona Dept. of Health; unpublished data-Centers for Disease Control; Childs, et al., 1995

Finally, approximately 66% of all deer mice tested (n=143) were males and about 7.4% of all male deer mice tested positive for the virus while only 2.1% of the females tested positive.

DISCUSSION

The results presented in this paper are based on the assumption that the rate of infection of the Sin Nombre virus within a rodent population is independent of the immediate environment such as habitat type, condition of habitat (i.e., ecosystem health), inter- and intraspecific competition, food source availability, elevation, and topography (i.e., natural barriers preventing distinct rodent populations from interacting). It is unknown what role these factors play on the spread of infection among a rodent population but studies have shown that body mass, and therefore age, has been correlated with infection rates among male deer mice (Childs, et al. 1994). If this is the case, then factors such as food source availability, vegetative cover, predator-prey cycles, seasonal variation, as well as many other factors could play a significant role in the rate of infection among rodent populations both locally and regionally.

The bleeding procedure was of concern because it may affect the animals' behavioral responses to trappings. Although the procedure did not appear to have an affect on capture and recapture rates, additional studies will be necessary to more accurately determine any effects. Preliminary results of other studies have also shown the handling and bleeding procedure to have no affects on recapture rates (Yates, personal communication).

Deer mouse was the most common species captured in Los Alamos County during the sampling periods and has been identified as the primary host for the Sin Nombre virus in the four-corners area (Childs, et al. 1994). There were several small mammal species identified in our study as being infected by hantavirus but it is uncertain if this was the Sin Nombre virus or another strain of hantavirus. We calculated the density of deer mice at each sampling location and the seroprevalence rate among the population at each of those locations. The density of rodent populations in Los Alamos County has continually declined since 1991 to the present date although the deer mice densities were similar at our sampling locations from 1993 to 1994. There did not appear to be any correlation between the density of deer mice and the seroprevalence rate at the sampling locations. However, no statistical correlation analysis was run on this data. Analysis is currently underway on this data and data collected for two consecutive years at sampling locations in the four-corners area. Analysis will be conducted to determine if there are correlations between density of animals and the rate of infection in that particular population.

The seroprevalence estimates for our sampling locations are much lower than estimates from the four-corners area, where most of the human hantavirus infections have been reported. In and around the four-corners area, seroprevalence in rodents has been, on average, around 20% with higher rates for deer mice. Nationwide, seroprevalence rates for hantavirus in deer mice is approximately 14.4% (CDC; Hantavirus Conference, April 1995, Gallup, N.M.), which is about three times the rate found in Los Alamos County. Our data showed an approximately 65% drop in the seroprevalence rate of deer mice from 1993 to 1994. No deer mice sampled in 1995 tested positive for the virus. Large decreases were also observed for deer

mice in tested areas of New Mexico and the four-corners area (including New Mexico). However, much of this data is preliminary and has not yet been completely analyzed and reported.

EST is currently involved with long-term studies to attempt to identify the relationship of concentrations and outbreaks of the Sin Nombre virus to a variety of ecological parameters including food source use and availability, body condition, habitat condition, density, habitat type, and several other variables. As this data is collected and analyzed, it is possible certain relationships between the prevalence of the virus in rodent populations and their surrounding environment may be identified.

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