*Hantavirus Testing in Small Mammal
Populations of Northcentral New Mexico*

Los Alamos
NATIONAL LABORATORY

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HANTAVIRUS TESTING IN SMALL MAMMAL POPULATIONS OF NORTHCENTRAL NEW MEXICO

by

James Biggs, Kathy Bennett, Teralene Foxx, Mary Salisbury, Eric Pacheco, Laura Payne, Alethea Banar, Delia Raymer, Tim Haarmann, David Keller, and Dan Dunham

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 and 1994, the Ecological Studies Team (EST) at Los Alamos National Laboratory surveyed small mammal populations in Los Alamos County, New Mexico, primarily for ecological risk assessment (ecorisk) studies. At the request of the Centers for Disease Control (CDC) and the School of Medicine at the University of New Mexico, EST also collected blood samples from captured animals for use in determining seroprevalence of hantavirus in this region due to the recent outbreak of this virus in the four-corners region of the Southwest. The deer mouse was the most commonly captured species during the trapping sessions. Other species sampled included harvest mice (*Reithrodontomys megalotis*), least chipmunk (*Eutamias minimus*), long-tailed vole (*Microtus longicaudus*), Mexican woodrat (*Neotoma mexicana*), and brush mouse (*Peromyscus boylii*). The team collected blood samples from trapped animals following CDC's suggested guidelines. Results of the the 1993 and 1994 hantavirus testing identified a total overall seroprevalence of approximately 5.5% and 4.2%, respectively. The highest seroprevalence rates were found in deer mice sera (3–6%), but results on several species were inconclusive; further studies will be necessary to quantify seroprevalence rates in those species. 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 Lee, 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 Se 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, 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. During 1993 and 1994, we collected various biological data for an ecological risk assessment (ecorisk) in the LANL region, at which time blood samples were collected for hantavirus testing. We modified the project design, incorporating and expanding on CDC suggestions for collecting blood samples from small mammals. Ecorisk data collection included capture-and-release studies on rodent populations in two canyon systems, Guaje and Los Alamos Canyons, in Los Alamos County, New Mexico. Due to the remoteness of the upper portion of Guaje Canyon, a field camp was established for a one week period to collect data. We established trapping grids on US Forest Service and Laboratory property. For comparative purposes, the grids were located in similar habitats in the two canyon systems. The primary purpose of the small mammal studies was to estimate population characteristics, including size and density for the study areas. The 1993 data was the first collected for the regional ecorisk study and was, in part, used to modify the design of the 1994 small mammal studies for the ecological risk project.

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. (*In prep.*)¹ and Biggs and Bennett (*In prep.*)².

In addition to the ecological risk assessment studies, 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

Los Alamos National Laboratory (LANL) is located in northcentral 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.

¹ Mills, J.N., T.L. Yates, J.E. Childs, R.R. Parmenter, T.G. Ksiazek, P.E. Rollin, and C.J. Peters. *In Preparation*. Guidelines for Working with Rodents Potentially Infected with Hantavirus.

² Biggs, J.R. and K. Bennett. *In preparation*. Application of “Guidelines for Reduction of Hantavirus Infection” to Field Studies of Rodent Populations in Northern New Mexico. Los Alamos National Laboratory, Los Alamos, N.M.

Figure 1. Location of Los Alamos County, New Mexico.

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 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.

The ecorisk trapping sites were located in two canyon systems (Figure 2). Three habitats were selected for sampling within each canyon; two distinct types—ponderosa pine and mixed-conifer, and a third in a transitional habitat type comprised of ponderosa pine and mixed conifer. The upper area of Guaje Canyon

Figure 2. Location of Trapping Sites
in Los Alamos and Guaje Canyons.

is characterized by mixed-conifer vegetation with an overstory of Douglas fir, spruce (*Picea engelmannii*), thinleaf alder (*Alnus tenuifolia*), and, in lesser quantity, ponderosa pine. Shrubs include cliffbush (*Jamesia americana*), serviceberry (*Amelanchier* spp.), and oak (*Quercus* spp.). The most common understory species are cutleaf coneflower (*Rudbeckia laciniata*) and several species of grasses and forbs. The upper area of Los Alamos Canyon is similar in overstory and shrub composition with the addition of white fir. Geranium (*Geranium jamesii*) and strawberry (*Fragaria americana*) are common in the understory. The upper canyons are relatively narrow with steep slopes. The middle sites of both canyons are characterized by Douglas fir, white fir, Engelmann spruce, limber pine (*Pinus flexilis*), aspen (*Populus tremuloides*), Gambel oak (*Quercus gambelii*), chokecherry (*Prunus virginiana*), New Mexico locust (*Robinia neomexicana*), cliffbush, rose (*Rosa woodsii*), raspberry (*Rubus strigosus*) and various grass and forb species. The terrain in middle Guaje Canyon is generally level to slightly sloping on the south-facing slope and relatively steep on the north-facing slope, whereas the terrain in middle Los Alamos Canyon is steeper on the south facing slope than on the north-facing slope.

The lower portions of the canyons support open ponderosa pine habitat, dominated by an overstory of ponderosa pine with lesser amounts of juniper (*Juniperus monosperma*). Common shrubs here include barberry (*Berberis fendleri*), oak, and rose. The understory in this habitat is sparse and consists mostly of grass species. Understory cover is greater in lower Guaje canyon than lower Los Alamos.

A stream channel runs through all study sites in both upper and lower canyon areas but water flow is perennial in the upper portions of the canyons only; in the middle and lower areas, water flow is intermittent.

The additional sites selected for hantavirus testing in 1993 were in Canada del Buey, a dry canyon consisting of essentially three habitat types. Indications of a piñon-juniper woodland are found on the south-facing slope with one-seed juniper and piñon pine scattered along the slope. The canyon bottom is characterized by ponderosa pine and a mixture of shrubs with the most common being chokecherry,

currant (*Ribes* spp.), and sumac (*Rhus trilobata*). Oak is also relatively common. The north-facing slope has influences of mixed-conifer with a mixture of fir and ponderosa pine. Oak dominates the north-facing slope and is more common there compared to the canyon bottom or south-facing slope. The locations selected around the buildings were generally highly disturbed but consisted of various forbs and grasses in some areas.

METHODS

The following methodology describes procedures used for the ecorisk small mammal studies. The additional trapping that took place around LANL in 1993 consisted of placing traps in such a way to ensure “high-intensity, short-duration” capture of small mammals for collection of blood samples.

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. (*In prep*) and Biggs and Bennett (*In prep*).

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 to estimate population size and density.

No extraordinary procedures were required for setting up grids and traps, but specialized clothing was necessary for the remaining portion of the study because of the risk of contact with rodents infected with hantavirus (these are discussed in detail in Mills, et al. (*In prep*); and Biggs and Bennett (*In prep*). In 1993, we placed two Sherman live-traps within 2 m (6.6 ft) of each trap station, 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, only one trap per station was used. The bait consisted of sweet feed (a molasses-coated horse feed) or a peanut butter and oats mixture. The field crew baited traps in late afternoon and checked them in early morning to record nocturnal species. Trapping took place over four consecutive nights at each grid during each year of trapping and for a one week period around Laboratory facilities for a total of approximately 9,100 trap nights. In the morning, personnel collected traps that had been tripped. While awaiting checking, the traps were kept out of the sun and animals were removed as soon as possible.

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 Sharp's 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 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.

The Statistical Analysis System (SAS) was used analyze data on capture/recapture rates and for all analysis on seroprevalence data. A one-way parametric Analysis of Variance (AOV) and Student-Newman Keuls (SNK) multiple range test were used to determine if mean daily small mammal capture rates were statistically different between years. A one-way AOV was also used to analyze recapture data to determine if handling procedures significantly affected recapture rates.

EST used the program CAPTURE (White et al. 1982) to estimate population size and density. 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.

RESULTS

Figure 3 shows mean daily capture rates recorded for 1991–1994. As shown in the graph, capture rates (includes recaptures) in 1991 and 1992 were moderate, ranging from about 15->25%. In 1993 and 1994, however, we experienced very poor capture rates which were not only evident in the ecorisk trapping locations but elsewhere at the Laboratory during similar live-trapping sampling. Analysis (AOV and SNK multiple range test) showed that the mean daily capture rates observed during the four consecutive

years are statistically different ($\alpha=0.05$). Capture rates for 1991 were significantly higher than the subsequent years, and 1992 rates were significantly higher than 1993 and 1994. Capture rates were not significantly different between 1993 and 1994.

Figure 3. Mean Daily Small Mammal Capture Rates, Los Alamos County 1991-94.

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 these studies and for some of the studies in 1992 have been plotted and are shown in Figure 4. Only two nights of data were collected in 1992. Analysis of recapture data showed no significant differences between days when comparing between years.

Figure 4. Daily Recapture Rates for Small Mammal Studies, Los Alamos County, 1992-94.

Table 1 lists all small mammal species captured during trapping sessions in Los Alamos and Guaje Canyons. This list also includes incidental captures of diurnal species. Table 2 lists each species by the habitat they were captured in, either ponderosa pine, mixed-conifer, or the transition area between these two. Deer mice were captured in all trapping locations except middle Los Alamos Canyon. Shrews and voles were only captured in the upper locations of each canyon, and deer mice and a small number of harvest mice were the only species captured in the ponderosa pine habitat of the lower portions of each canyon.

Table 1. Small Mammal Species Captured during the 1993–94 Ecological Risk Studies, Los Alamos and Guaje Canyons.

Least chipmunk	<i>Eutamias minimus</i>
Colorado chipmunk	<i>Eutamias quadrivittatus</i>
Long-tailed vole	<i>Microtus longicaudus</i>
Weasel	<i>Mustela frenata</i>
Mexican woodrat	<i>Neotoma mexicana</i>
Brush mouse	<i>Peromyscus boylii</i>
Deer mouse	<i>Peromyscus maniculatus</i>
Harvest mouse	<i>Reithrodontomys megalotis</i>
Water shrew	<i>Sorex palustris</i>
Vagrant shrew	<i>Sorex vagrans</i>

Table 2. Small Mammal Species Captured during the 1993–94 Ecological Risk Studies, Los Alamos and Guaje Canyons, By Habitat.

Species	Habitat					
	Ponderosa Pine		Transition Area		Mixed Conifer	
	LG	LLA	MG	MLA	UG	ULA
Least chipmunk						x
Colorado chipmunk				x		x
Long-tailed vole					x	x
Weasel						x
Mexican woodrat					x	
Brush mouse				x	x	x
Deer mouse	x	x	x		x	x
Harvest mouse		x				
Water shrew					x	
Vagrant shrew					x	
Shrew (unidentified species)						x

Table 3 provides information on population estimates and density estimates for small mammals captured during the study. Insufficient sample sizes prevented analysis from being conducted on all individual species except deer mice. Data was pooled for all nocturnal species to obtain overall small mammal population and density estimates.

Table 3. Small Mammal Population and Density Estimates for Guaje and Los Alamos Canyons, Los Alamos County, 1993–1994.

SITE	POPULATION ESTIMATE	SE	DENSITY ESTIMATE animals/ha	SE
Lower Guaje Canyon				
1993	*		*	
1994	19	1.65	*	
Middle Guaje Canyon				
1994	21	4.50	*	
Upper Guaje Canyon				
1993	48	6.58	39	11.54
1994	24	2.88	7.5	5.41
Lower Los Alamos C.				
1993	*		*	
1994	10	1.85	7.6	3.71
Middle Los Alamos C.				
1994	11	1.49	*	
Upper Los Alamos C.				
1993	38	4.24	25	
1994	14	1.90		

* Indicates sample size insufficient to run population and density estimate program.

Table 4 gives overall hantavirus testing results for Los Alamos County. The table also shows the number of animals of each species for which hantavirus tests were inconclusive. Further testing will be necessary to determine seroprevalence rates in those species. As shown in Figure 5, seroprevalence rates in Los Alamos County were generally much lower than rates for 1993–94 in other locations in New Mexico or the four-corners area of New Mexico, Arizona, Utah, and Colorado. Seroprevalence rates were highest in deer mice. Approximately 8.9% of all male deer mice tested positive for the virus while only 2.4% of the females tested positive.

Table 4. Summary of Hantavirus Seroprevalence in Los Alamos County, 1993–94

SPECIES	NUMBER		POSITIVE		QUESTIONABLE	
	1993	1994	1993	1994	1993	1994
Deer mouse	62	61	5 (8%)*	3 (5%)		
Long-tailed vole	27	2	1 (3.5%)	0		
Harvest mouse	4	2	0	0	1	
White-footed mouse **	0	2	0	1(50%)*		
Brush mouse	10	24	0	0	1	
* Numbers in parenthesis indicate percent seroprevalence rates						
** Species identification unconfirmed						

Figure 5. Regional Seroprevalence Rates For All Rodents, 1993-94.

*1993 data for four-corners includes Az., Co., and N.M.; 1994 data includes Az., Co., N.M., Ut.; 1993 data for Los Alamos C. includes three questionables (for seroprevalence) for one deer mouse, brush mouse, and harvest mouse; 1994 data for Los Alamos C. includes one unconfirmed species identification.

DISCUSSION

When EST initiated the trapping sessions, the only capture rates available were from 1991 and 1992 data.

The capture rates were fairly high in these surveys. This was not the case for the 1993 surveys, when sample sizes were low. As indicated in Table 3, most of the estimates are within generally acceptable standard errors, with the exception of density estimates and standard errors for all species in the upper canyons. However, the modeling procedure of the CAPTURE program usually selected the null estimator, which suggests small sample sizes were used in the analysis. The CAPTURE program is less accurate as the population size decreases, particularly when the population size is under 50 (White et al. 1982).

Because population estimates for this study were under 50, the results calculated by CAPTURE may not be reliable. EST may modify future studies, if conducted, to compensate for the small population sizes.

These would increase sample sizes to more accurately estimate populations and densities of small mammals in the study areas and thus seroprevalence rates.

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. The seroprevalence estimates for Guaje and Los Alamos Canyons and for various areas of the Laboratory 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. Most of the samples that tested positive for hantavirus were from deer mice, although a relatively high percentage were questionable for several other species. Our data showed an approximately 40% drop in the seroprevalence rate of deer mice from 1993 and 1994. 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.

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