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SMALL MAMMAL SURVIVAL AND TRAPABILITY IN MARK-RECAPTURE MONITORING PROGRAMS FOR HANTAVIRUS

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ABSTRACT: Following the 1993 hantavirus pulmonary syndrome (HPS) epidemic in the southwestern United States, mammalogists and epidemiologists instituted long-term studies to monitor population density and prevalence of infection in rodents which constitute the reservoir for Sin Nombre virus (SNV). In this study, field techniques used in sampling small mammals for SNV infection were evaluated to determine if trapping and handling protocols were having significant effects on future trapability or mortality of animals. We compared rodent mark-recapture control plots, on which all rodents were simply measured, marked, and released on site, with experimental plots on which all animals were anesthetized with methoxyflurane, sampled for blood and saliva, measured, marked, and released. Blood samples were obtained from anesthetized animals on the experimental plots via a retro-orbital sinus puncture using a heparinized capillary tube. Dacron tipped oral swabs were used to collect buccal cells and saliva from the rodent's oral cavity. Field data were collected monthly from August 1994 to August 1996 at two sites in New Mexico (USA). Analyses were based on 3,661 captures of 1,513 individuals representing 21 species from three rodent families (Rodentia: Muridae, Heteromyidae, Sciuridae) and two species of rabbits (Lagomorpha: Leporidae). Overall, for most murid rodents (including five *Peromyscus* spp., *Neotoma albigula*, and *Onychomys leucogaster*) and one rabbit species (*Sylvilagus floridanus*), the handling/bleeding procedures had no significant effects on recapture rates or mortality. In contrast, several species of heteromyids (*Dipodomys ordii* and *Perognathus flavus*), one murid (*Reithrodontomys megalotis*) and one leporid (*S. auduboni*) suffered higher mortality rates, and heteromyid kangaroo rats (*D. ordii* and *D. merriami*) exhibited lower trapability as a result of the anesthesia and sampling procedures. In view of the overall non-significant influence of the sampling procedures on murid rodents, the anesthesia and blood/saliva sampling protocols described herein appear to be appropriate for hantavirus research, and may serve as a model for environmental monitoring of other zoonotic agents and their reservoirs.

Key words: Hantavirus, mark-recapture, Methoxyflurane, *Peromyscus* spp., rodent populations, Sin Nombre Virus, zoonosis.

INTRODUCTION

Rodents are known to carry and transmit a number of zoonotic agents, many of which are actually or potentially fatal to humans (Childs et al., 1995; Gage et al., 1995); for example rodent-borne hemorrhagic fever viruses annually are responsible for over 100,000 cases of human disease worldwide. Considerable scientific effort has been devoted to understanding the epizootiology of rodent-borne viruses in hopes of developing more effective strategies for disease prevention. While significant progress has been made over the last several decades in the identification and epidemiology of rodent-borne diseases, there exists today a need to examine the long-term interactions among rodent population dynamics and environmental factors in relation to zoonoses. To date, longitudinal mark-recapture studies have made important contributions to our understanding of the epidemiology of other rodent-borne hemorrhagic fever viruses (Childs et al., 1987; Mills et al., 1992, 1994), and recent outbreaks of newly discovered hantaviruses have led to an increase in such studies.

In the spring of 1993, an outbreak of hantavirus pulmonary syndrome (HPS) occurred in the southwestern United States. Thirty-four human cases were recorded with a mortality rate in excess of 50% (Centers for Disease Control and Preven-

tion, 1994a). The virus responsible for the HPS outbreak was a previously unknown species of the genus *Hantavirus* (family Bunyaviridae), now named Sin Nombre virus (SNV).

A variety of murid rodents (Sigmodontinae) are the primary hosts of hantaviruses, and hantavirus/host associations appear to be very specific, with closely related reservoir species harboring different species of *Hantavirus*. For example, the white-footed mouse, *Peromyscus leucopus,* is known to harbor the New York-1 virus, while another related species, the harvest mouse (*Reithrodontomys megalotis*), is host for *Hantavirus*-ELMCV (Hjelle et al., 1994a; b). Comparisons of phylogenetic trees among these viruses and their rodent hosts have shown the branching patterns to be virtually identical (Schmaljohn and Hjelle, 1997). The primary reservoir of SNV, the deer mouse (*Peromyscus maniculatus*), is found throughout much of North America (Hall, 1981), and appears to be infected with SNV over a considerable part of its range (Kaufman et al., 1994; Douglass et al., 1996; Mills et al., 1998). Additional murid rodent species may be infected (Childs et al., 1994; Center for Disease Control and Prevention, 1994b), although seropositive results reported in other species may be the result of incidental, non-contagious infections rather than a natural part of the SNV life cycle.

Given the large number of rodent species involved with HPS and other rodent-borne disease, any field sampling program should employ techniques appropriate for a wide range of small mammals. The reliability of rodent-zoonosis monitoring programs depend on accurate field data, and would be compromised if field techniques and study design (1) produced biased results, or (2) were statistically insensitive to spatiotemporal dynamics of rodents and their diseases. In the case of the rodent/hantavirus monitoring program in the Southwest, field sampling efforts of rodent-borne zoonoses were based upon repeated live-trapping of wild rodents. Captured animals generally were anesthetized and underwent considerable handling during blood and saliva sampling procedures prior to being marked and released. These procedures may have produced unintended effects on survivorship and subsequent trapability of the rodents, which, in turn, would bias the results of the density estimates and reduce accuracy of the monitoring program's results. In view of these potential problems, the purpose of this study was to evaluate trap data from the first 2 yr of the SNV/rodent monitoring program, and test the null hypothesis that the rodent handling procedures for sampling blood and saliva caused no significant differences in future trapability or mortality of the rodents when compared to rodent populations not subjected to anesthesia and blood/saliva collection.

MATERIALS AND METHODS

For our analyses, we selected two sites in New Mexico that were part of the CDC rodent/Hantavirus monitoring program. The first was a desert grassland site located 90 km south of Albuquerque, on the Sevilleta National Wildlife Refuge, Socorro County, New Mexico, USA (34°21.3′N, 106°53.1′W, Elevation 1,465 m). This study area was dominated by one-seed juniper (*Juniperus monosperma*), honey mesquite (*Prosopis glandulosa*), and a variety of desert grasses (*Sporobolus* spp. and *Bouteloua*, spp.).

The second site (Placitas) was a piñon-juniper woodland site located in Sandoval County in the foothills of the Sandia Mountains, Cibola National Forest, approximately 30 km north of Albuquerque, New Mexico, USA, (35°16.7′N, 106°18.6′W, Elevation 1,830 m). This area was dominated by piñon pine (*Pinus edulis*), one-seed juniper, and blue grama grass (*Bouteloua gracilis*).

At each study site, three permanently-marked trapping webs were established (Anderson et al., 1983; Buckland et al., 1993). The trapping web design was selected over the more traditional trapping grid because accurate absolute densities (number of animals per ha) could be estimated from the distance data generated on the webs, and because the estimation procedures required few assumptions about capture probabilities and animal movement patterns (see Buckland et al., 1993 for detailed discussions). Accuracy assessments of density

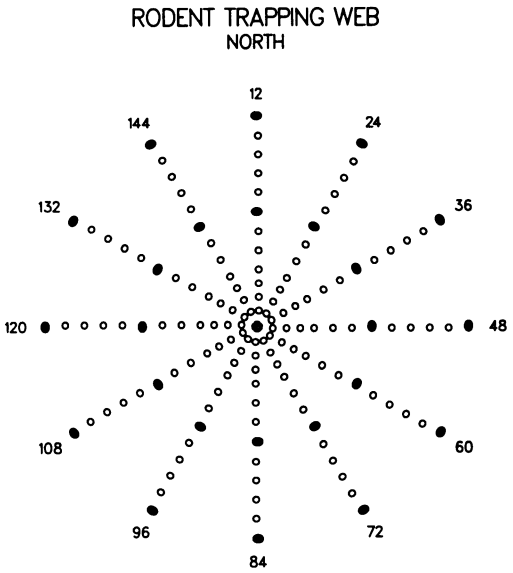

FIGURE 1. Diagram of trap distributions on a trapping web used in the rodent/Hantavirus monitoring program. Darkened circles show sites where both a Sherman® and a Tomahawk® trap were located; all other sites contained only one Sherman® trap. The first four trap stations were at 5 m intervals from the center, and the last eight stations were at 10 m intervals. Tomahawk® traps were placed at 50 and 100 m from the center of the web. Total web diameter was 200 m (3.14 ha).

estimates using trapping webs in both computer simulations and field studies have shown excellent correspondence between actual and estimated densities of organisms (Wilson and Anderson, 1985; Parmenter et al., 1989). Each web consisted of 12 radial 100 m long lines of trap stations, with each line having 12 stations (Fig. 1). The first four trap stations were at 5 m intervals from the center, and the last eight stations were at 10 m intervals. Generally, a single Sherman® (H. B. Sherman Traps, Tallahassee, Florida, USA) live trap was placed at each station, with four Sherman® traps at the web center. At trap stations located 50 m and 100 m from the center, a Tomahawk® (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) trap (61 × 15 × 15 cms) was placed adjacent to the Sherman® trap. Each trapping web covered an area of 3.14 ha. Webs were separated from one another by distances of at least 200 m. The same field research technicians were involved in sampling all sites and all webs.

The rodent monitoring program was begun in August 1994, with two trapping webs at each site. A third trapping web was added to each site in October 1994. Trapping webs were sampled monthly beginning in August 1994; data used in this study were collected from August 1994 to August 1996. All three webs at a given site were trapped simultaneously. Webs were trapped for three consecutive nights each month during the dark phase of the moon. Traps were baited with a mixture of rolled oats and grains. Cotton nestlets and peanut butter were added during winter months to help prevent rodent hypothermia. Traps were checked early each morning and periodically during the day in order to retrieve diurnal species. During field and laboratory sampling procedures, researchers followed the safety procedures described in Mills et al. (1995) and Centers for Disease Control and Prevention (1993).

One of the three webs at each site was the control. On this web, all animals underwent abbreviated processing immediately on the web site. These animals were identified to species using external morphological characteristics following Findley et al. (1975). Body mass, sex, reproductive status, and presence of scars or wounds were recorded. Rodents were then eartagged with metal fingerling tags (Gey Band and Tag Co., Norristown, Pennsylvania, USA) and released at their original point of capture.

The remaining two webs also were used for rodent density estimations, but in addition, the animals were sampled for blood and saliva. Initially, all small mammals captured on the plots were sampled for SNV infection, including rabbits and heteromyids which previously had not been implicated in any hantavirus infections (Childs et al., 1994). Through the summer, fall, and winter of 1994, heteromyid samples consistently revealed no infection with SNV, but did experience high levels of mortality during handling procedures. Thus, in February 1995, heteromyid rodents were no longer subjected to blood and saliva sampling.

Captured live animals were taken to a laboratory area off the web for processing; on the Sevilleta NWR site, a laboratory room at the University of New Mexico's Sevilleta Field Research Station, (Socorro Co., New Mexico, USA) was used; on the Placitas site, a mobile field laboratory was used. Rodents were anesthetized with methoxyflurane (Metofane™, Pitman-Moore, Inc., Mundelein, Illinois, USA) saturated cotton contained in stainless-steel tea balls in plastic bags. New bags were used for each animal and the tea balls were cleaned with 70% ETOH between animals to prevent cross-contamination of SNV. Each animal experienced a one time minimal exposure to a standard dosage (1 ml) of fresh methoxyflurane. Handling time of each animal was kept to a minimum by processing one animal per technician at a time.

Demographic data and body measurements were collected for these rodents. In addition, a blood sample (five drops) was extracted from the retro-orbital sinus using a heparinized microhematocrit tube. The blood sample was allowed to drip into a 2-ml cryovial and then submerged in a dewer containing liquid nitrogen. Orbital bleeding was stopped by closing the rodent's eye with a sterile gauze and applying gentle pressure to the orbit. Buccal cells and saliva were collected with a Dacron tipped oral swab and placed in a tube of viral media which also was frozen in liquid nitrogen. Finally, the animals were ear tagged with numbered fingerling tags, allowed to recover from anesthesia, and released at the point of capture. Following release of animals, all traps were washed in a solution of 5% Lysol (Lysol®—Reckitt & Coleman Inc., Montvale, New Jersey, USA) for at least 10 min, followed by two water rinses to remove the disinfectant residue. All instruments and surfaces that may have been exposed to virus were cleaned with 5% Lysol or 70% ethanol between animals.

The analyses tested for differences between the effects of the two rodent handling regimes: field mark-recapture techniques only, or mark-recapture procedures plus anesthesia and blood and saliva sampling. The variables of interest were (1) mortality of individuals associated with each of the two handling regimes, and (2) the effect of the procedures on recapture rates. Data from each site, as well as pooled site data, were analyzed, and only those species having at least 15 individuals from each handling regime were included in the analyses. For the mortality effect, species-specific trapping-event mortality percentages were calculated for each site (pooled over all months). Trapping-event mortality percentage was defined as the total number of deaths (either in traps or during handling) of each species divided by the total number of captures for that species. Differences in the mortality percentages of each species between the two handling regimes were tested using the Z-test for proportions (Welkowitz et al., 1982). For testing the effects of the different handling regimes on recapture rates, the number of individuals of each species captured in more than 1 mo was tallied, and then was divided by that species' total number of individuals captured during the 2 yr study. The species-specific percentages of recaptured individuals between the different handling regimes at each site were then tested with the Z-test for proportions. In addition, mortality patterns were analyzed for *P. boylii, P. maniculatus, P. truei, Neotoma albigula,* and *Dipodomys merriami.* Student's *t*-test was used to test for treatment effects on animal longevity (mean number of days alive following first capture, mean number of trap periods, and mean number of total captures). Chi-square analyses were used to test for treatment effects on temporal capture patterns (number of trap periods in which each animal was captured, and pattern of captures within each 3 day trapping period) (Welkowitz et al., 1982).

An assumption of the experimental design for testing the effects of handling procedures on the rodents was that the underlying probabilities of capture were equal for both treatment populations (control and bleed trapping webs). Differences in capture or recapture probabilities across trapping webs (due to, for example, differences in site topography, vegetation, or prevalence of predators) could have masked any effects of handling techniques. Species-specific capture and recapture probabilities were estimated using Program CAPTURE (Otis et al., 1978), which provided seven different estimators (M_o, M_h, M_b, M_t, M_{bh}, M_{th}, M_{tb}) based on various model assumptions concerning capture probabilities (see details in Otis et al., 1978; White et al., 1982; Chao, 1989; Chao et al., 1992). The assumptions of the models were: M_o (null model) assumed all animals had equal probabilities of capture on all trapping occasions; M_h (heterogeneity model) assumed each animal had its own capture probability; M_b (behavior model) assumed capture probabilities varied in response to previous captures; M_t (time model) assumed capture probabilities varied with time; M_{bh} assumed capture probabilities varied by individual animal and in response to capture; M_{th} assumed capture probabilities varied by individuals and through time; and M_{tb} assumed capture probabilities varied through time and in response to prior trap experiences. CAPTURE closure tests were performed to determine that the rodent data sets were equivalent to closed populations. Species data sets that contained at least 10 individuals per trapping web in any given month were included in these analyses. Mean species-specific capture probabilities were tested between treatments using Student's *t*-test (Welkowitz et al., 1982). All statistical analyses assumed a significance level of $P \leq 0.05$.

RESULTS

The analyses were based on 24 trapping periods between August 1994 and August 1996 and included 74,304 trap-nights (a trap night is one trap set for one night). The 3,661 trap captures yielded 1,513 individual small mammals belonging to two orders, four families, and 23 species (Table 1).

The two study sites differed in the abundance of different rodent families and

TABLE 1. Species list and capture summaries of rodents collected from Placitas and the Sevilleta National Wildlife Refuge (NWR), New Mexico.

Species	Placitas		Sevilleta NWR	
	Number of individuals	Number of captures	Number of individuals	Number of captures
Rodentia: Muridae				
Peromyscus boylii	216	543	6	12
Peromyscus eremicus	0	0	33	72
Peromyscus leucopus	17	29	19	37
Peromyscus maniculatus	57	101	49	96
Peromyscus nasutus	1	3	3	5
Peromyscus truei	177	350	54	121
Neotoma albigula	81	222	163	686
Neotoma micropus	5	8	0	0
Onychomys leucogaster	0	0	57	164
Onychomys arenicola	0	0	1	1
Reithrodontomys megalotis	17	18	54	71
Rodentia: Heteromyidae				
Chaetodipus intermedius	0	0	14	23
Dipodomys merriami	0	0	204	517
Dipodomys ordii	1	2	49	132
Perognathus flavescens	0	0	78	126
Perognathus flavus	43	86	18	28
Rodentia: Sciuridae				
Ammospermophilus interpres	4	5	0	0
Ammospermophilus leucurus	0	0	3	3
Spermophilus spilosoma	0	0	11	13
Spermophilus variegatus	1	3	0	0
Eutamias quadrivittatus	6	10	0	0
Lagomorpha: Leporidae				
Sylvilagus auduboni	0	0	44	111
Sylvilagus floridanus	27	63	0	0
Totals	653	1,443	860	2,218

rabbit species. The Heteromyidae and Muridae co-dominated the Sevilleta NWR site. At the Placitas site, murids were most abundant; five *Peromyscus* spp. made up 75% of the Placitas total number of rodents captured (Table 1). At Sevilleta NWR, the dominant species were *D. merriami, Perognathus flavescens,* and *N. albigula.* The two sites also had different species of rabbits; *Sylvilagus auduboni* at Sevilleta NWR, and *S. floridanus* at Placitas. Each site had its own complement of sciurid species, but at both sites these rodents were relatively rare. Sample sizes of sciurids were too small to perform any statistical analyses, and along with *Peromyscus nasutus, N. micropus, Onychomys arenicola* and *Chaetodipus intermedius* were not included in the analyses.

Species differed in their overall rates of recapture, ranging from 13% in *R. megalotis* to 74% in *D. ordii* (Table 2). *Neotoma albigula* and *Dipodomys* spp. are known to establish permanent territories around their nests and burrows, so the higher recapture rates were not unexpected. Approximately one half (48%) of the *Peromyscus* spp. individuals were captured more than once during the study.

Analysis of the handling effects on trapability indicated that, of 11 rodent species for which sufficient numbers were captured to allow statistical analysis, only two species (*D. merriami* and *D. ordii*) exhib-

TABLE 2. Results of differences between handling procedures for the rodent species captured on both study sites (Sevilleta NWR and Placitas). Animal treatments involved either field capture-mark-release procedures (Control) or similar procedures plus laboratory anesthesia and blood/saliva collections (Bleed). Both recapture and mortality effects were analyzed using a Z-test for proportions.

Species	Treatment	Total number of individuals	Number of individuals recaptured	Percentage recaptured	Z-Test *P*-Value	Number of capture events	Total number of deaths	Percentage mortality	Z-Test *P*-Value
Peromyscus boylii	Control	57	34	60	>0.05	154	15	10	>0.05
	Bleed	165	88	53		401	49	12	
Peromyscus eremicus	Control	9	4	44	>0.50	22	0	0	>0.20
	Bleed	24	10	42		50	1	2	
Peromyscus leucopus	Control	15	6	40	>0.20	23	1	4	>0.90
	Bleed	21	11	2		43	2	5	
Peromyscus maniculatus	Control	31	14	45	>0.90	55	3	5	>0.50
	Bleed	75	34	45		142	7	5	
Peromyscus truei	Control	60	25	42	>0.20	99	6	6	>0.05
	Bleed	171	77	45		372	28	8	
Neotoma albigula	Control	67	44	66	>0.20	249	11	4	>0.10
	Bleed	177	109	62		659	21	3	
Onychomys leucogaster	Control	24	16	67	>0.20	74	1	1	>0.90
	Bleed	33	19	58		90	3	3	
Reithrodontomys megalotis	Control	33	5	15	>0.20	45	1	2	<0.05
	Bleed	38	5	13		44	3	7	
Dipodomys merriami	Control	65	41	63	<0.02	186	13	7	>0.10
	Bleed	139	75	54		331	31	9	
Dipodomys ordii	Control	19	14	74	<0.05	55	1	2	<0.001
	Bleed	31	18	58		79	14	18	
Perognathus flavescens	Control	30	14	47	>0.05	58	1	2	>0.50
	Bleed	48	16	33		68	1	1	
Perognathus flavus	Control	24	10	42	>0.05	55	1	2	<0.001
	Bleed	36	10	28		59	8	14	
Sylvilagus auduboni	Control	17	9	53	>0.20	41	0	0	<0.05
	Bleed	27	12	44		70	4	6	
Sylvilagus floridanus	Control	5	1	20	>0.20	6	0	0	>0.90
	Bleed	22	11	50		57	0	0	

ited significantly lower recapture percentages due to the blood/saliva sampling regime (Table 2). All the other species, including the main reservoir of SNV, *P. maniculatus*, demonstrated no significant difference in recapture percentage based on handling procedures (Table 2).

Eight of the 10 rodent species for which sufficient sample sizes were obtained and one rabbit species (*S. floridanus*) exhibited no significant difference in mortality between handling treatments. However, two heteromyids (*D. ordii* and *P. flavus*), one murid (*R. megalotis*), and the desert cottontail (*S. auduboni*) showed significantly higher mortality rates from the handling procedures involved with anesthesia and blood and saliva sampling (Table 2). While all species suffered some level of mortality during trapping and handling, *D. ordii* and *P. flavus* proved especially susceptible to anesthesia and sampling procedures. *Peromyscus maniculatus*, the main SNV reservoir, exhibited no significant overall mortality effects from the handling procedures.

When the Placitas and Sevilleta NWR sites were analyzed separately, most of the overall comparisons were upheld (Ta-

TABLE 3. Results of differences between handling procedures for the rodent species captured on the Placitas study site. Animal treatments involved either field capture-mark-release procedures (Control) or similar procedures plus laboratory anesthesia and blood/saliva collections (Bleed). Both recapture and mortality effects were analyzed using a Z-test for proportions.

Species	Treatment	Total number of individuals	Number of individuals recaptured	Percentage recaptured	Z-Test *P*-Value	Number of capture events	Total number of deaths	Percentage mortality	Z-Test *P*-Value
Peromyscus boylii	Control	57	34	60		154	15	10	
	Bleed	159	85	53	>0.20	389	49	13	>0.05
Peromyscus leucopus	Control	12	4	33		17	0	5	
	Bleed	5	3	60	>0.20	12	0	0	>0.20
Peromyscus maniculatus	Control	17	7	41		32	1	3	
	Bleed	40	17	43	>0.50	69	7	10	<0.001
Peromyscus truei	Control	50	20	40		82	6	7	
	Bleed	127	58	46	>0.10	268	26	10	>0.10
Neotoma albigula	Control	21	12	57		42	0	0	
	Bleed	60	36	60	>0.50	180	4	2	>0.05
Reithrodontomys megalotis	Control	12	1	8		13	0	0	
	Bleed	5	0	0	>0.10	5	0	0	>0.90
Dipodomys ordii	Control	1	1	100		2	0	0	
	Bleed	0	0	—	—	0	0	—	—
Perognathus flavus	Control	17	10	59		48	1	2	
	Bleed	26	6	23	<0.001	38	6	16	<0.001
Sylvilagus floridanus	Control	5	1	20		6	0	0	
	Bleed	22	11	50	>0.20	57	0	0	>0.90

bles 3 and 4). However, some site-specific patterns emerged. At Placitas, *P. maniculatus* and *P. flavus* showed significant increases in mortality associated with the blood-sampling webs, and *P. flavus* exhibited reduced trapability (Table 3). However, six of the eight *P. maniculatus* deaths at this site were associated with cold weather (these animals were dead in their traps prior to handling because of hypothermia, following a storm in October, 1995). At Sevilleta NWR, the two *P. maniculatus* mortalities also were due to cold weather during trapping. Deaths from trapping and handling were not separated in the analyses because the circumstances of deaths were not consistently recorded during the initial phase of the study. The effects of handling on the Ord's kangaroo rat (*D. ordii*) and the desert cottontail (*S. auduboni*) at Sevilleta NWR resulted in significantly higher mortality rates (Table 4).

In analyzing the patterns of mortality for the species with larger sample sizes (Table 5), there was no significant effect of the anesthesia and sampling procedures on the subsequent pattern of trapping. In addition, for animals that subsequently died during the study, there were no significant differences between control and sampled populations in (1) the number of days between first capture and death, (2) the number of trap periods between first trap period and the period in which the animal died, or (3) the total number of capture events between first capture and death (Table 5).

The effect of handling on temporal mortality patterns also was evaluated by testing for differences in trapping history. Using the trap histories of all the animals that died during the study, there was no statistically significant effect of treatment on the number of trap periods that animals were captured for *P. maniculatus, P. truei, N. albigula,* and *D. merriami* (Table 6). Only *P.*

TABLE 4. Results of differences between handling procedures for the rodent species captured on the Sevilleta NWR study site. Animal treatments involved either field capture-mark-release procedures (Control) or similar procedures plus laboratory anesthesia and blood/saliva collections (Bleed). Both recapture and mortality effects were analyzed using a Z-test for proportions.

Species	Treatment	Total number of individuals	Number of individuals recaptured	Percentage recaptured	Z-Test *P*-Value	Number of capture events	Total number of deaths	Percentage mortality	Z-Test *P*-Value
Peromyscus boylii	Control	0	0	—		0	0	—	
	Bleed	6	3	50	—	12	0	0	—
Peromyscus eremicus	Control	9	4	44		22	0	0	
	Bleed	24	10	42	>0.50	50	1	2	>0.20
Peromyscus leucopus	Control	3	2	67		6	0	0	
	Bleed	16	8	50	>0.10	31	2	6	>0.10
Peromyscus maniculatus	Control	14	7	50		23	2	9	
	Bleed	35	17	49	>0.50	73	0	0	>0.10
Peromyscus truei	Control	10	5	50		17	0	0	
	Bleed	44	19	43	>0.20	104	2	2	>0.10
Neotoma albigula	Control	46	32	70		207	11	5	
	Bleed	117	73	62	>0.05	479	17	4	>0.05
Onychomys leucogaster	Control	24	16	67		74	1	1	
	Bleed	33	19	58	>0.20	90	3	3	>0.10
Reithrodontomys megalotis	Control	21	4	19		32	1	3	
	Bleed	33	5	15	>0.50	39	3	8	>0.10
Dipodomys merriami	Control	65	41	63		186	13	7	
	Bleed	139	75	54	<0.05	331	31	9	>0.05
Dipodomys ordii	Control	18	13	72		53	1	2	
	Bleed	31	18	58	>0.05	79	14	18	<0.001
Perognathus flavescens	Control	30	14	47		58	1	2	
	Bleed	48	16	33	>0.05	68	1	1	>0.50
Perognathus flavus	Control	7	0	0		7	0	0	
	Bleed	11	4	4	>0.20	21	2	10	>0.10
Sylvilagus audubonii	Control	17	9	53		41	0	0	
	Bleed	27	12	44	>0.20	70	4	6	<0.05

TABLE 5. Trapping histories of rodent mortalities during the 2 yr study. Only individuals that survived the first trapping period, but died subsequently, were used in this analysis. Values are means and standard errors.

Species	Treatment[a]	*n*	Mean number days alive	Mean number trap periods	Mean number total captures
Peromyscus boylii	Control	5	32.2 ± 0.4	2.0 ± 0.0	3.4 ± 1.1
	Bleed	7	50.1 ± 8.7	2.4 ± 0.3	4.0 ± 1.0
Peromyscus maniculatus	Control	2	95.5 ± 59.5	2.0 ± 0.0	2.5 ± 0.5
	Bleed	4	51.2 ± 9.3	2.2 ± 0.2	2.5 ± 0.5
Neotoma albigula	Control	8	97.8 ± 23.5	3.6 ± 0.9	6.1 ± 1.8
	Bleed	17	256.1 ± 53.4	6.6 ± 1.3	8.9 ± 1.7
Dipodomys merriami	Control	10	55.5 ± 8.1	2.1 ± 0.1	3.2 ± 0.8
	Bleed	11	68.0 ± 10.4	2.8 ± 0.3	3.6 ± 0.7

[a] Student's *t*-test analyses indicated no significant treatment differences in any category ($P > 0.05$).

TABLE 6. Distributions of rodent mortalities based on the number of trapping periods in which each individual was captured. Values are total numbers of mortalities (percentage of total mortalities) for each species during the 2 yr study; data pooled from both study sites.

Species	Treatment[a]	*n*	Captured only in one period	Captured only in two periods	Captured only in three periods	Captured in four or more periods
Peromyscus boylii	Control	15	10 (67)	5 (33)	0 (0)	0 (0)
	Bleed	49	43 (88)	4 (8)	1 (2)	1 (2)
Peromyscus maniculatus	Control	3	1 (33)	2 (67)	0 (0)	0 (0)
	Bleed	7	2 (29)	3 (43)	1 (14)	1 (14)
Peromyscus truei	Control	6	5 (83)	1 (17)	0 (0)	0 (0)
	Bleed	28	19 (68)	6 (21)	3 (11)	0 (0)
Neotoma albigula	Control	11	3 (27)	4 (37)	1 (9)	3 (27)
	Bleed	21	9 (43)	2 (9)	5 (24)	5 (24)
Dipodomys merriami	Control	13	7 (54)	5 (38)	1 (8)	0 (0)
	Bleed	31	20 (65)	6 (19)	3 (10)	2 (6)

[a] Chi-square analysis of One Period versus all others indicated that only *P. boylii* had significantly greater mortality in initial capture period ($P \leq 0.01$).

boylii had higher mortality in the first period of capture between treatments.

In contrast, when mortality patterns were analyzed within the standard 3 day trapping period each month, highly significant differences were observed for four of the five species tested (Table 7); *P. maniculatus* was the only species for which this result did not apply, although sample sizes for this species were very small. Animals undergoing the anesthesia and sampling procedures had a higher probability of dying during the first capture and handling event when compared to the control populations. The first capture event each month, was, of course, the time when the animals were sampled for blood and saliva.

Capture probabilities and recapture probabilities were estimated using the seven models of Program CAPTURE for populations of *N. albigula* (n = 11 webs) and *D. merriami* (n = 7 webs). Monthly sam-

TABLE 7. Summary of trapping histories of rodents that died during one of the 3-day trapping periods. Capture events defined as being trapped, handled, marked, released (Control) and anesthetized and sampled for blood and saliva (Bleed). Values are total numbers of mortalities (percentage of total mortalities) for each species during the 2 yr study; data pooled from both study sites.

Species	Treatment	*n*	Died during first capture event	Died during second consecutive capture event	Died during third consecutive capture event	Captured on nights 1 + 3, died during night 3 event	X^2[a] *P*-Value
Peromyscus boylii	Control	15	4 (27)	5 (33)	3 (20)	3 (20)	$P \leq 0.001$
	Bleed	49	26 (53)	16 (33)	7 (14)	0 (0)	
Peromyscus maniculatus	Control	3	2 (67)	0 (0)	1 (33)	0 (0)	$P > 0.05$
	Bleed	7	6 (86)	1 (14)	0 (0)	0 (0)	
Peromyscus truei	Control	6	4 (67)	1 (17)	0 (0)	1 (17)	$P \leq 0.01$
	Bleed	28	21 (75)	5 (18)	0 (0)	2 (7)	
Neotoma albigula	Control	11	6 (55)	3 (27)	0 (0)	2 (18)	$P \leq 0.05$
	Bleed	21	18 (86)	2 (9)	1 (5)	0 (0)	
Dipodomys merriami	Control	13	9 (69)	3 (23)	0 (0)	1 (8)	$P \leq 0.05$
	Bleed	31	28 (90)	2 (7)	0 (0)	1 (3)	

[a] Chi-square analyses were performed on first capture event versus all other capture patterns combined.

ple sizes were too small to test other species. Population closure was satisfied in 17 of 18 cases. Based on the CAPTURE results, the tests of treatment differences of species-specific capture probabilities showed no significant differences between control and bleed populations for initial capture probabilities; similarly, model M_b indicated no significant difference for within-month recapture probabilities.

DISCUSSION

The monitoring program reported here was initiated to provide information on the relationship of rodent population density, SNV infection, and HPS outbreaks. The sensitivity of the current trapping program design has proven successful in detecting statistical differences in wild rodent densities among sites and through time. The blood-saliva sampling procedures of this program did not influence trapability or mortality for most murid species, although one leporid, some murids and most heteromyids suffered higher mortality rates when anesthetized and sampled.

In any population of wild animals, there are individuals which, for a variety of reasons, have relatively low levels of tolerance for the physical, physiological, and psychological stress of being captured and handled by humans. Trapping effects on rodent body mass, trapability, and survival rates have been described in many field studies (Slade and Iskjaer, 1990; Wood and Slade, 1990; Slade, 1991; Kaufman et al., 1994; Vanblankenstein and Botzler, 1996). In rodent populations, the proportion of the population that is susceptible to trap mortality seems to be small; susceptible individuals may be older animals, weakened by age, disease, parasites, or non-resident dispersing individuals that may be malnourished. Trap deaths also may be caused by cold or hot temperatures or wet conditions (e.g., the arrival of an unexpected snow storm or thunderstorm), insufficient quantities of bait (prior to rodents entering the traps, ants and birds often remove considerable portions of bait in a short period of time, leaving insufficient amounts of bait for animals to maintain their body temperature through the night), malfunctioning traps, and predators entering the traps (rattlesnakes, weasels). Vigilant and conscientious field procedures generally keep these mortality factors to a minimum. However, some of the trapped and sampled animals still die in traps or during handling.

In a previous rodent-hantavirus survey in Montana during the summer of 1994, Douglass et al. (1996) trapped and bled 634 rodents from eight species without using anesthesia prior to handling and blood sampling; while mortality results were not reported, their results indicated no sampling effect on monthly recapture percentages between control and blood-sampling treatments. However, humane animal care and use concerns, coupled with an increased risk of disease transmission to investigators from conscious, agitated animals, make the use of anesthetic more desirable. In addition, the results of our study showed that the sampling procedures for anesthetizing and bleeding/swabbing for most small mammals did not result in a detectable increase in mortality above the "background" level observed on the control webs. However, given that a number of individuals were susceptible to trap-death during any 3 day trapping period, the results indicate that the more intensive handling of the blood-sampling procedures caused these susceptible animals to die on the first capture event, rather than the second or third event (Table 7). For these species, this phenomenon did not result in an overall increase in trap deaths, but rather shifted the temporal pattern of mortalities within the trapping period. In contrast, heteromyid rodents appeared to suffer higher mortality rates due to the anesthesia and handling.

These data point out that although these rodent handling methods and blood sampling techniques used in this study provide powerful tools for long-term monitoring of wild rodent populations, certain modifica-

tions may be required depending on the target species and the manipulations to be performed. For those studies interested in changes in population density alone, or in other questions that do not require the specimens to be anesthetized, this method is ideal and has little negative impact regardless of the species involved. However, there is some measurable effect on survival depending on species simply due to trapping mortality. Perhaps different techniques other than the ones used in this study may result in higher survival rates.

Our data demonstrate that certain species are more susceptible to anesthetic death and handling than others. For these species, change in basic protocols may become necessary. For example, a change of anesthetic protocol may be required for sensitive species, or perhaps heated cages and fluids while recovering from anesthesia would increase survival.

Quantitative longitudinal studies of population levels of most murid species can be achieved and blood and oral swab samples can be taken simultaneously for subsequent determination of infection. Fortunately, *P. maniculatus* and other major reservoir species of the viruses known to cause HPS are among those species that can be studied effectively with these methods.

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