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Source: Journal of Wildlife Diseases, 36(4) : 700-704

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-36.4.700

Journal of Wildlife Diseases, 36(4), 2000, pp. 700–704

EFFECTS OF BLEEDING NONANESTHETIZED WILD RODENTS ON HANDLING MORTALITY AND SUBSEQUENT RECAPTURE

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ABSTRACT: Handling mortality and recapture rates of wild rodents that were bled from the retro-orbital capillary plexus without anesthesia were assessed. In 9,670 captures of seven species of rodents from 1994 through 1998, we found no difference in handling mortality in bled mice compared to those from trapping grids where mice were not bled. Recapture rates of rodents on control (non-bleeding grids) and rodents on bleeding grids was not significantly different for any species. We conclude that bleeding in the absence of anesthesia does not affect immediate mortality or subsequent recapture.

Key words: Anesthesia, handling mortality, hantavirus, *Peromyscus maniculatus,* recapture rates.

INTRODUCTION

The infection of humans by rodent borne hantavirus in the United States (Childs et al., 1994) stimulated a series of longitudinal studies of rodent populations (primarily *Peromyscus* spp.) in both the southwestern states (Abbott et al., 1999; Calisher et al., 1999; Kuenzi et al., 1999; Mills et al., 1999) and Montana (Douglass et al., 1996). To track serologic evidence of infection, rodents have been bled from the retro-orbital capillary plexus using techniques described by Mills et al. (1995). In order for these studies to reflect reality, it is essential that the procedure used for blood sampling not modify population dynamics processes such as survival.

In the southwestern USA, where rodents were anesthetized prior to bleeding, Swann et al. (1997) and Parmenter et al. (1998) reported no difference in survival (recapture rates) between bled and non bled rodents for most species encountered, including *Peromyscus spp.* In Montana, however, animals were not anesthetized but were simply bled and released (Douglass et al., 1996). In this paper we examine the effects of bleeding non-anesthetized rodents on their subsequent survival.

The Animal Care and Use Committee (1998) of the American Society of Mammalogists recommends the mortality risk of anesthesia be weighed against the advantages of using anesthesia. The advantages of anesthetizing animals prior to bleeding are that anesthesia may reduce discomfort to animals and, depending on how it is administered, anesthesia may reduce the risk that researchers are bitten. The avoidance of bite wounds is extremely important when sampling animals potentially carrying zoonotic diseases. Disadvantages of anesthetizing prior to bleeding include possible long-term physiological damage to mice (Kosek et al., 1972), and a substantial increase in handling time.

During our study (Douglass et al., 1996), sub-freezing temperatures were encountered during most months except July and August. During cold weather, using anesthesia on animals in field situations puts animals at risk of hypothermia. Animals under anesthesia must be kept warm and monitored as they recover prior to release less they succumb to hypothermia. Due to the large number of individuals that we captured on a daily basis (up to 80 mice) it was not feasible to adequately warm and monitor all anesthetized individuals during their recovery period. We therefore chose to bleed animals with out anesthesia because we felt any transitory discomfort experienced outweighed the risk of increased mortality that might result from the use of anesthesia under cold ambient temperatures.

MATERIALS AND METHODS

This study was conducted from June 1994 through October 1998 at three locations in western Montana (45°40′N to 47°46′N, 113°00′N to 113°46′W) and three in central Montana (46°31′ to 48°56′N, 108°35′ to 112°31′W). Elevations ranged 738 to 1,957 m.

We constructed three 1 ha, live-trapping grids at each of six locations using 100 Sherman live traps spaced at approximately 10 m intervals. We placed peanut butter, oatmeal (not rolled oats) and synthetic cotton in each trap before placing it on the grid for three consecutive days each month. Traps were checked early each morning. All captured animals were marked with individually numbered ear tags. Body mass, sex and reproductive status were recorded. We bled animals on two grids at each location and used the third grid as a non-bled control. We bled mice within 100 m of grids. Animals were released near the point of capture. At five locations (15 grids) we trapped from June through September 1994 and from May through October from 1995 through 1998. At one location (three grids) we trapped every month from June 1994 through October 1998. We used handling and bleeding techniques suggested by Mills et al. (1995) except that we did not anesthetize animals. Additional details on methods can be found in Douglass et al. (1996).

To determine if the lack of anesthesia before bleeding caused mortality, we compared handling mortality and recapture rates between grids on which we bled animals and control grids on which we only captured, marked and released animals. We calculated handling mortality and recapture rates in the same manner as Parmenter et al. (1998). Handling mortality was defined as the total number of deaths in traps or during handling divided by the total number of captures. We calculated recapture rates as the total number of individuals of a given species recaptured in subsequent months divided by the number of individuals initially marked and released. We used the Z test for proportions (Zar, 1984) to compare handling mortality and recapture rates between bled and non-bled animals.

The composition of field crews changed from 1994 through 1998. During the course of the investigation, 12 different investigators bled mice. All but two had no previous experience obtaining blood samples from rodents before working on the project. During their tenure each investigator handled control animals as well as collected blood samples. Our results, therefore, contain data from the training phase of 10 people.

In our analysis we included only the most commonly captured rodents. These were deer mice (*Peromyscus maniculatus*), meadow voles (*Microtus pennsylvanicus*), montane voles (*M. montanus*) boreal red backed voles (*Clethrionomys gapperi*), Richardson's ground squirrels (*Spermophilus richardsonii*), red tailed chipmunks (*Tamias ruficaudus*) and yellow pine chipmunks (*T. amoenus*).

RESULTS

From June 1994 through October 1998 we captured 5321 individuals of the seven most common species 9,670 times (Table 1). We were unable to detect any statistically significant effect of bleeding on handling mortality (Table 1). Mortality varied among species with the lowest mortality for commonly captured species being 1.2% for Richardson ground squirrels. The highest mortality occurred in the *Microtus* spp. (5.7%), handling mortality for *P. maniculatus* was 2.7% for bled animals and 2.2% for non-bled animals.

Most mortality for all species was trap-induced mortality usually resulting from insufficient oatmeal in traps. Mortality for ground squirrels and chipmunks was mostly from hyperthermia caused by diurnal captures. During the summer we closed traps during the day but during the spring and fall unseasonably warm days caused occasional mortality. *Microtus* spp. were the most sensitive species to handling. During training, investigators occasionally killed individuals by simple handling and or bleeding.

Recapture rates varied by species ranging from 18.2% for *Microtus* spp. to 45.8% for deer mice (Table 2). The percentage of bled and unbled individuals recaptured was virtually identical for deer mice, 45.2 versus 45.8% respectively. In the microtine rodents (*M. pennsylvanicus, M. montanus* and *C. gapperi*), recapture rates were slightly higher in control versus bled animals but differences were not statistically significant. Recapture rates for *S. richard-*

TABLE 1. Handling mortality for rodents captured on six control (non-bled) and 12 bled grids in Montana from 1994 through 1998. Mortality effects were analyzed using a Z-test for proportions.

Species	Treatment	Capture events	Number of deaths	Percentage mortality	Z-test P-value
Peromyscus maniculatus					
	Control	3,225	88	2.7	0.15
Microtus spp.[a]	Bled	5,155	112	2.2	
	Control	105	6	5.7	0.88
Clethrionomys gapperi	Bled	321	17	5.3	
	Control	19	0.0	0.0	—[c]
Spermophilus richardsonii	Bled	326	17	5.2	
	Control	77	1	1.3	0.95
	Bled	81	1	1.2	
Tamias spp.[b]	Control	4	0.0	0.0	—[c]
	Bled	357	17	4.8	
Totals		9,670	259		

[a] Includes *Microtus pennsylvanicus* and <20 *M. montanus.*

[b] Includes *Tamias ruficaudus* and *T. amoenus.*

[c] Sample sizes too small to test statistically.

sonii and *Tamias* spp. also did not differ between control and bled individuals.

DISCUSSION

Our overall handling mortality, including bleeding without anesthesia was relatively low compared to other studies. In New Mexico, Parmenter et al. (1998) found handling mortality to vary with species ranging from 0% to 18%, with an average mortality of 5.3% for deer mice. In Arizona handling mortality ranged from 0% to 18% depending on species (Swann et al., 1997).

We found no statistically significant differences in handling mortality between bled and non-bled individuals for any of the species we compared. Similar results have been obtained by others. Swann et al. (1997) found no effect of bleeding on handling mortality of desert rodents in southern Arizona and Parmenter et al. (1998) found no effect of bleeding on handling mortality in most species with the exception of three heteromyid species.

Recapture rates in our study were similar to to other hantavirus studies that used antesthesia. In southern Arizona, Swann et al. (1997) found recapture rates ranging from 0 to 57% (mean of 48% bled and 53% non-bled). Recapture percentages in the New Mexico study (Parmenter et al., 1998) ranged from 0 to 100% (mean of 45.6%). The percentage of deer mice recaptured at least once after initial capture in our study was 45.2% for bled animals and 45.8% for non-bled animals compared to an average of 45% for deer mice at three locations in New Mexico (Parmenter et al., 1998). In Arizona, recapture rates for 2 *Peromyscus spp.* ranged from 37% to 54% (Swann et al., 1997).

Recapture rates in both Arizona and New Mexico were determined for grids that were trapped continuously. Our estimates were from girds most of which (15 of 18) were trapped for 6 mo intervals. Consequently, we probably under estimated recapture rates because of the 6 mo period between the last trapping period of one year and the first of the succeeding year.

We found no statistically significant differences in recapture rates between bled and non-bled individuals for any of the species we compared. This is consistent with our initial findings that minimum survival rates (Krebs, 1966) were equal between bled and non-bled populations (Douglass et al., 1996). Parmenter et al. (1998) also found bleeding to have no ef-

TABLE 2. Recapture rates for rodents on six control (non-bled) and 12 treated grids in Montana from 1994 through 1998. Recapture rates were compared using a Z-test for proportions.

Species	Treatment	Number of individuals captured	Number of individuals recaptured	Percentage recaptured	Z-test P-value
Peromyscus maniculatus	Control	1,705	781	45.8	0.69
	Bled	2,677	1,211	45.2	
Microtus spp.[a]	Control	83	17	20.5	0.64
	Bled	264	48	18.2	
Clethrionomys gapperi	Control	10	4	40	0.18
	Bled	258	51	19.8	
Spermophilus richardsonii	Control	54	16	29.6	0.30
	Bled	51	20	39.2	
Tamias spp.[b]	Control	3	1	33.3	0.87
	Bled	216	76	35.2	
Totals		5,321	2,225		

[a] Includes *M. pennsylvanicus* and <20 *M. montanus.*
[b] Includes *T. ruficaudus* and *T. amoenus.*

fect on subsequent recapture of deer mice but they did find bleeding to reduce recapture rates for three species in the family heteromyidae. Swann et al. (1997) also found reduced recapture rates associated with bleeding heteromyids, but no affect on any murid species. Although not statistically significant, we found slightly lower recapture rates in bled versus non-bled voles. Microtines have been reported to suffer reduced survival from non-anesthetized bleeding (Gaines and Krebs, 1971; Fraze et al., 1990).

We can only speculate on the relative advantages of anesthesia in protecting researchers. If mice are anesthetized before being handled either by placing the trap in a bag with the anesthetic or if the anesthetic is placed in a bag with the animal as suggested by Mills et al. (1995), then bites will be avoided. If mice are grasped by the investigator and then anesthetized via a facial cone (Swann et al., 1997), then the risk of bites is probably not significantly reduced.

Whether bleeding without anesthesia increases discomfort (Parmenter et al., 1998) is unknowable. We feel that the risks to rodents presented by hypothermia with the use of anesthesia, particularly under cold and or wet ambient conditions, are greater than risks presented by transient, though possibly intense, discomfort caused by not using anesthesia. Also, if the length of time an animal is removed from its normal environment is important to its well-being, bleeding without anesthesia, because it is more efficient, may be considered a benefit to the animal.

ACKNOWLEDGMENTS

The field assistance of C. Rognli, K. W. Coffin, T. Vossberg, and T. Swank is greatly appreciated. Funding for this project was provided by the United States Centers for Disease Control and Prevention, Deaconess Medical Research and the Montana Department of Fish, Wildlife and Parks.

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