THE ROLE OF RODENTS AS A POTENTIAL RESERVOIR FOR *PASTEURELLA MULTOCIDA* ON THE NATIONAL ELK REFUGE, WYOMING

by

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

ABSTRACT

Hemorrhagic septicemia (HS) is a fatal disease affecting domestic and wild ruminants caused by the bacterium *Pasteurella multocida*. Although uncommon in the U.S, outbreaks of HS in elk (*Cervus elaphus*) occurred on the National Elk Refuge (NER) in the winters of 1986, 1987, 1993, 1995, 1999, and 2001. DNA fingerprinting of *P. multocida* from the 1987 and 1993 outbreaks (B:3,4 HhaI 036/HpaII 001) revealed the same organism was responsible for mortality in both years. However, testing has failed to find this genetic variant in healthy elk on the NER, suggesting reservoirs other than elk might play a role in HS epidemiology. I investigated the potential for rodents to serve as biological reservoirs for bacteria responsible for HS on the NER. Rodents are known to harbor *P. multocida*, may be carriers of variants capable of causing HS, and have been observed at sites where elk are fed during winter on the NER. I used mark-recapture techniques to determine densities of rodents on feedgrounds, feed-storage areas and other sites and removal trapping to collect tissues to determine prevalence of *P. multocida* in rodents on the NER. Weather conditions, age-gender class, and feedground characteristics also were assessed as risk factors for HS. I captured 849 small mammals, 283 of which (mostly *Peromyscus maniculatus*) were sampled for *P. multocida*. None were positive for *P. multocida*. These data did not support the hypothesis that rodents serve as a reservoir for HS; however, my detection sensitivity was low due to small sample sizes. Snow depth was the only weather variable significantly associated with the incidence of HS on the NER. The positive association between snow and number of elk dying from HS may be due to increased survival of bacteria in the environment under wet conditions. Calves and cows were found to be at a higher risk than males greater than one year of age, but, with winter feeding, there is no evidence that cows and calves were more stressed from nutrient shortages or crowding than males. All analyses of feedground characteristics failed to find a relationship between these characteristics and HS.

INTRODUCTION

Background

Hemorrhagic septicemia (HS), also known as septicemic pasteurellosis, is a peracute to acute, generally fatal disease, principally affecting domestic livestock and wild ruminants, including cattle, water buffalo (*Bubalus bubalis*), and cervids (Nordkvist and Karlsson 1962, Jones and Hussaini 1982, Franson and Smith 1988, Carter and De Alwis 1989, Campbell and Saini 1991). It is characterized by a rapid course, edematous swelling in the head-throat-brisket area (in bovids), swollen and hemorrhagic lymph nodes, and the presence of numerous subserous petechial hemorrhages (Carter and De Alwis 1989).

Although HS is not common in North America it can kill swiftly and may kill a large proportion of a population. Death can occur within 24-36 hours after the first recognized signs. Because transmission is by aerosol or direct contact, the disease can spread rapidly, especially in areas where large numbers of animals congregate. High mortality can be seen in all age groups; however, HS mostly affects young animals. It therefore has the potential to suppress recruitment and impair population growth. HS can cause great economic loss. An outbreak of HS that occurred in Zambia resulted in deaths of more than 10,000 cattle and financial losses in the millions of dollars (Francis et al. 1980). Although HS in not known to be a threat to human health, public concern regarding food safety could be raised due to natural fears of diseases, and therefore, harm local economies.

HS is caused by specific strains of the bacterium *Pasteurella multocida (P. multocida)*. At least 17 species of *Pasteurella* are currently distinguished on the basis of phenotypic traits (Bisgaard 1995). Strains within species are further characterized by somatic and capsule typing methods (Carter 1955, Heddleston et al. 1972, Rimler and Rhoades 1987). There are sixteen major somatic types (1 through 16) and five different capsular serogroups (A, B, D, E, and F) used to characterize and differentiate *P. multocida* isolates. Current serological nomenclature utilizes both these features. For example, *P. multocida* of capsule serogroup A and somatic serotype 3 is designated as A:3 (Wilson 1992).

Serotypes B:2 and E:2 cause HS in cattle and water buffalo in enzootic areas of Asia and Africa. The E:2 serotype has been isolated only from ungulates in Africa (Wilson 1995). The B:2 serotype is rarely reported in the United States, though was isolated from bison (*Bison bison*) in Yellowstone National Park in 1922 (Rhoades and Rimler 1992) and beef calves in 1993 in California (Blanchard et al. 1993). A B:3,4 strain was confirmed in HS outbreaks among bison calves at the National Bison Range, Montana, during 1965 and in dairy cattle from Pennsylvania in 1968 (Kradel et al. 1969). More recently, HS was the cause of death in two neonate pronghorn (*Antilocapra americana*) and a B:1 serotype was isolated from one of them on Hart Mountain National Antelope Refuge, Oregon in 1997 (Dunbar et al. 2000).

DNA fingerprinting is a molecular technique used to further characterize *P. multocida* isolates based on DNA fragments resulting from application of endonucleases. The banding pattern on an electrophoretic gel of these fragments is referred to as the

DNA profile of that organism. DNA fingerprinting reveals relationships in isolated that cannot be distinguished by serotyping, and provides a more precise identification of *P. multocida* (Wilson 1992). DNA profiles can help elucidate relationships among serotypes. For example, using the DNA fingerprinting method, identical fingerprint profiles were found between the HS outbreaks from bison calves at the National Bison Range and dairy cattle from Pennsylvania. These profiles were distinct from the HS outbreaks at the NER. The serotypes of *P. multocida* causing the 1987 and 1993 HS outbreaks in elk at the NER were identical and was identified as serotype B:3,4 and profile HhaI 036/HpaII 001(HhaI and HpaII are restriction endonucleases). The unique HhaI 036/HpaII 001 profile found at the NER suggests that a specific B:3,4 serotype may be an endemic cause of HS on the NER (Wilson 1995). The source of this organism is unknown. The carrier status of *P. multocida* serotype B:3,4 among elk on the NER is unresolved.

Epidemiology

The presence of healthy animals carrying the agent of HS has been documented in endemic areas of Asia, Africa and several middle eastern countries (Carter and DeAlwis 1989). HS has been documented in wild and restricted-range bison (Gochenour 1924, Heddleston et al. 1967), dairy cattle (Kradel et al. 1969), beef calves (Blanchard et al. 1993), and free-ranging elk (Franson and Smith 1988, Roffe et al. 1993, Wilson et al. 1995). To date, the limited studies of HS in the U.S. have not found healthy animals carrying the agent of HS.

Expression of HS or any other disease is dependent on the interactions among the three components of the epidemiological triad—host, agent and environment. Anything that affects a host's susceptibility to infection, modifies an agent's pathogenicity, or alters environmental conditions that affect microbial survival, replication or transmission, will affect the course of the disease.

The host's susceptibility to HS infection varies by host species. The B:2 serotype (Asian serotype) has been reported among pigs (*Sus* spp.), buffalo (*Bubalus* spp.), and elephants (*Elephas maximus*) in Sri Lanka and among beef calves in California (Carter 1982). The E:2 serotype (African serotype) has been reported among cattle in Africa. However, the B:2 and E:2 serotypes are non-pathogenic to dogs, chickens and ducks (Bain et al., 1982). One experiment in Sri Lanka showed that goats placed in close contact with buffalo clinically affected with B:2 serotype remained clinically normal and developed no carrier state or immune response (Wijewardana et al. 1986).

The host's susceptibility to HS infection also varies with age, with greater susceptibility among young animals. HS is more likely to be fatal in younger than in older animals because of an absence of acquired immunity or because of poor physical condition. One extensive survey in Sri Lanka showed that 65% of all HS deaths among cattle and 77% of all HS deaths among buffalo were in animals less than two years of age (De Alwis and Vipulasiri 1980). Another analysis of one outbreak showed the most susceptible age group to be six months to two years (De Alwis 1976).

The ability of hemorrhagic septicemia-causing *Pasteurella multocida* (HSPm) to induce disease depends on its virulence and pathogenicity. HSPm is highly virulent but

can vary in its pathogenicity. For example, in one study, a highly virulent strain of HSPm, obtained from a bison that died in YNP in 1922, was injected into a 12-month-old heifer and the animal succumbed within 18 hours. But in this same study, one normal cow and one yearling were inoculated with comparatively large amounts of the same culture. Neither of these two animals reacted sufficiently to even show a rise in body temperature. Researchers were unable to determine why these two animals were immune to the disease caused by the organism presented this way (Gochenour 1924).

Environmental conditions such as geographic location, climate and husbandry practices can favor HSPm survival. Favorable environments can include husbandry practices that can stress the host and compromise its immune system. Webster (1924, cited in Rosen 1981) showed that when physiologic stresses —such as poor forage conditions and crowding on a winter range—are applied to a herd, the host's resistance is lowered, the bacteria's virulence may increase, and clinical disease ensues. External environmental conditions that enable the organism to survive longer outside the host, and thereby facilitate transmission between animals also favor disease expression. HS has been usually associated with wet, humid weather, and an increased incidence has been recorded during wet seasons (De Alwis 1992). The increased incidence has been attributed to the longer survival of the organism in wet conditions and waterways serving as a source of dissemination from carcasses and other infective material (De Alwis 1989).

The manner in which all three components of the epidemiological triad—a susceptible host, a capable agent, and suitable environment—interact in expression of HS and the salient features of the cycle are best described by De Alwis 1992:

> "An outbreak is believed to begin when a 'latent carrier' animal becomes active and sheds virulent organism infecting in-contact susceptible animals. Once a clinical case is established, dissemination of infection will increase and the magnitude of the outbreak that will result will depend on the proportion of immune to non-immune animals in the herd. When an active carrier animal is introduced into a virgin area with a highly susceptible population, an explosive outbreak can result, whereas in endemic areas the animals potentially at risk are only the hitherto unexposed animals, mainly those born after the previous outbreak. Where annual seasonal outbreaks occur in endemic areas, only a small number of animals will die. It is possible that the organism sifts through the animal population producing 'arrested infections' and the animals acquiring immunity until an animal is reached where the balance (agent, host, environment) is tipped in favor of clinical disease."

HS on the National Elk Refuge

Outbreaks of hemorrhagic septicemia occurred in elk on the National Elk Refuge in Jackson, Wyoming during the winters of 1986, 1987, 1993, 1995, 1999, and 2001. The largest outbreak occurred in 1993. Normal elk mortality on the NER is 1 to 1.5% of the total population on all four feedgrounds for an entire year. From January through April 1993, mortalities due to HS alone were 1 to 1.5% of the population at two of the four feedgrounds; 4 to 5.5% of the population at the other two feedgrounds. These numbers of HS deaths are likely underestimated since the scavenger base on the NER is high. Approximately 95% of elk carcasses are unsuitable for gross examination (Cole personal communication) because within 24 hours most carcasses can be sufficiently scavenged by coyotes (*Canis latrans*) and ravens (*Corvus corax)* such that appropriate tissues for bacterial culture of *P. multocida* are not available. The NER staff however, was able to use positive results from bone marrow samples to estimate timing and

duration of the outbreak. The strain of *P. multocida* causing the 1993 (and 1987) outbreaks was identified as serotype B:3,4.

Because of the acute nature of the disease, identifying HS-infected animals prior to death can be difficult. With peracute or acute septicemic pasteurellosis, death is often the first or only sign reported (Miller 2001). Chronic manifestations of HS do not appear to occur frequently. During the 1993 outbreak, animals in apparently good condition died within 24 hours. The best clinical indicator of HS was an elk's reluctance to come to the feedline when food pellets are delivered (Roffe, personal communication). The most common manifestation of clinical illness was ears held in a horizontal position and reluctance to move. One spike bull was observed unable or unwilling to move to a feedline no further than three meters from his position. Other clinically ill animals appeared to be depressed and were observed salivating, and experience respiratory distress. Behavioral signs were not reported in many infected animals because most elk cannot be observed intensively. Most elk remained standing until just before death.

Because the source of *P. multocida* and the carrier status of *P. multocida* serotype B:3,4 among elk on the NER were unknown, an attempt was made to identify B:3,4 in healthy elk. *Pasteurella* is commonly found in the upper respiratory passages of animals and carrier states are thought to be an important disease mechanism for disease initiation, therefore tonsil and mandibular lymph nodes samples were taken from 210 hunter-killed elk from the Greater Yellowstone Ecosystem including the NER in 2000. Thirty-three *Pasteurella* isolates were obtained from the 210 elk and only seven of these were serogroup B. One of these seven was an isolate serotyped at B:3,4,7, and the other six

isolates serotyped as B:3,4. The six B:3,4 isolates were composed of four identical organisms and two unique organisms. Nineteen different DNA profiles were found in all 33 isolates. None of these 19 profiles matches the DNA profile type of the *Pasteurella* that had previously been responsible for HS in NER elk (Mark Wilson, unpublished data 2000). Therefore, it was concluded that the tissues from the 210 hunter-killed elk did not yield the B:3,4 *Pasteurella* that causes HS in elk. The rarity of HS among ruminants in the U.S., the finding of a specific DNA type in HS outbreaks in elk, and the necropsy findings of good to excellent body condition in HS-affected elk suggest that the disease may not caused by a commensal strain of *P. multocida* (Roffe et al 1993). Therefore, sources other than resistant elk need to be considered as a reservoir for the infective strain.

Although clinical HS occurs principally in cattle and water buffalo, the role of other species as reservoirs that may or may not be clinically affected is unknown. *P. multocida* is widespread among many terrestrial and aquatic species of mammals and birds, any of which could potentially serve as reservoirs (Biberstein 1981). Some of these potential reservoirs on the NER include waterfowl, coyotes, corvids, and rodents. Deer mice (*Peromyscus* spp.) and ravens have been observed on the feedline among the elk on the NER (Roffe pers. comm.; Swanekamp observation). Rodent have been sufficiently abundant in feed storage areas to warrant poisoning by NER staff. Rodent excretions and secretions could contaminate feed and possibly transmit HSPm to elk.

Several studies (Schipper 1947) suggest that rodents are a possible source of HSPm. Studies have shown that some rodents are not only resistant, but can also act as

carriers or transmit the organism through bites. Schipper (1947) examined 102 Norway rats (*Rattus norvegicus*) and found 14 with *P. multocida*. The Norway rat not only carried the organism but also was resistant to intraperitoneal inoculation of 0.5 ml of an undiluted culture of *P. multocida*. Upon examination of 156,000 rodents, *P. multocida* was isolated from 85 rats (*Rattus norvegicus*), 37 house mice (*Mus musculus*), 6 common voles (Arvicolinae), 8 field mice (several genera), and 1 bank vole (*Clethrionomys*) (Rosen 1981). Another study (Quan et al 1985) recovered *P. multocida* from specimens submitted for plague tests and found 79% (192 of 243) of the *P. multocida* isolates were from rodents, 10% were from lagomorphs, and 7% from carnivores. In one experiment, the abdomen of a dog was shaved and two rats carrying the organisms were allowed to bite through the skin. The dog died 23 days later from pasteurellosis (Schipper 1947).

HS has only been reported from feedground elk and not free-ranging, unfed elk; therefore, management that includes feeding may initiate or exacerbate the disease. The purpose of this study was to examine the epidemiology of HS in elk and to test the hypothesis that rodents are a reservoir of HSPm at the NER. Our goal was to determine whether current refuge management practices could be enhancing HS incidence, prevalence and potential transmission by rodents and thus have implications for managing other diseases of elk. Currently, the state of Wyoming conducts brucellosis vaccination on NER elk. This management requires elk to be habituated and remain on feed to maintain the high densities essential to effective vaccine delivery. This study may provide information for wildlife managers on how current and future management might affect the course of HS disease.

To test the hypothesis that rodents may serve as a reservoir, I determined the presence and prevalence of HSPm in rodent populations, and compared them to the temporal appearance of HSPm in elk on the feedgrounds. I also looked at relationships among population sizes of elk, relative densities of rodents, and the prevalence of HSPm in rodents and elk. Environmental factors — including climate, age, and habitat characteristics of feedgrounds associated with historical outbreaks of HS in elk— were also assessed.

METHODS

Trapping

I used two methods to determine if rodents could serve as a potential reservoir for HSPm on the NER (Figure 1): mark-recapture trapping and removal trapping. Mark-recapture trapping was used to study species distribution and density of rodents in relation to hemorrhagic septicemia prevalence in elk on the feedgrounds. Removal trapping was used for tissue collection to determine presence and prevalence of *P. multocida* in the rodent populations. For rodents to be a possible reservoir for HSPm,: 1) rodents had to be present; and 2) HSPm must reside in rodents for some period of time. I trapped during summer and winter. Although elk may not be present in summer on the feedgrounds, a relationship may exist between summer rodent distribution and abundance, and HS prevalence in elk. The finding of rodents harboring HSPm at any time would also indicate the potential of these mammals to serve as a reservoir for the organism. I trapped in winter to determine the presence of rodents on the feedgrounds when HS mortality is known to occur.

For summer trapping, I trapped 26 locations on the NER consisting of 13 primary grids, seven supplementary grids, three feed storage areas, and three ancillary sites. I sampled the 13 primary grids and feed storage areas from June through October in 2000 and 2001. The seven supplementary grids were trapped opportunistically between May and October of 2001 depending on available time and land cultivation activities. Ancillary sites were trapped in July of 2001. Two primary grids were trapped only in

2000 because underwater submergence from irrigation during 2001 precluded systematic trapping.

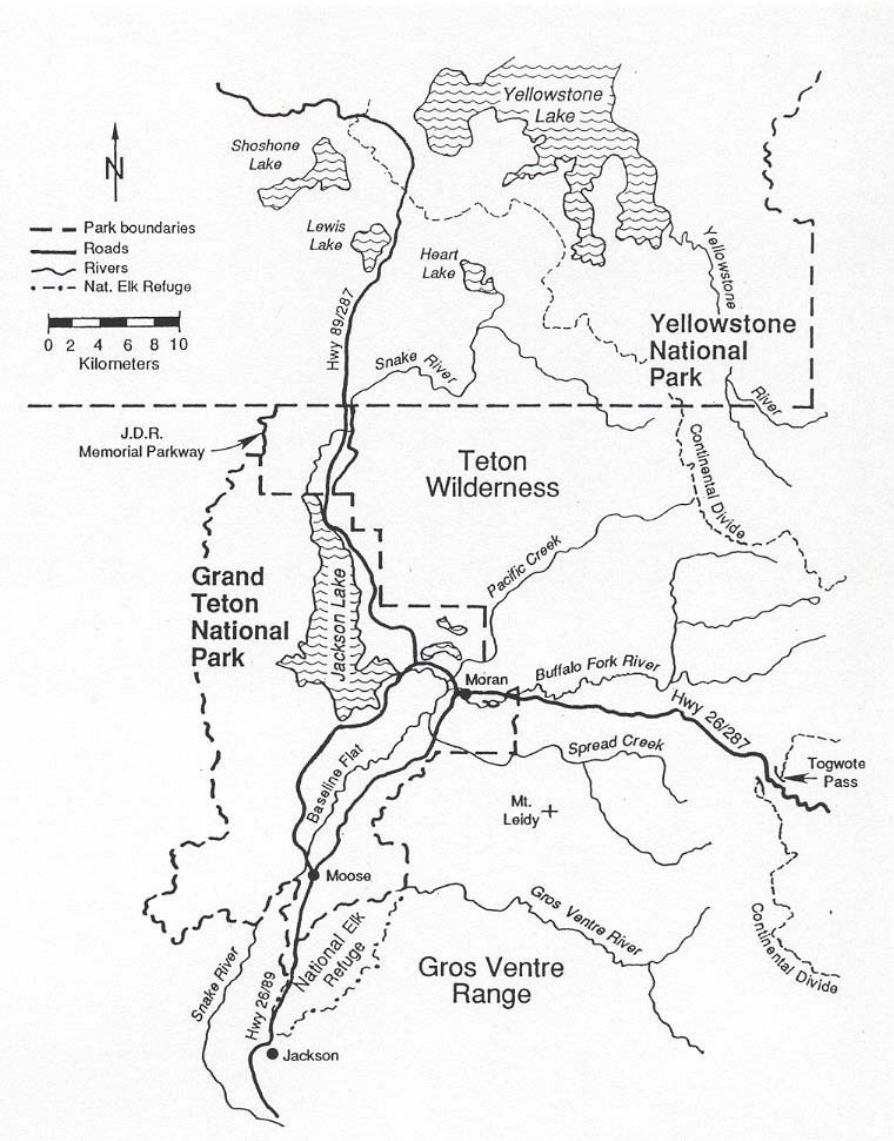

Figure 1. The National Elk Refuge shown in relation to the northern Jackson Hole region (from Migrations and Management of the Jackson Elk Herd 1994)

During winter, I trapped for rodents on the core feedground areas and in the feed storage sheds. Winter trapping was conducted during elk feeding operations in 2001 and 2002 during the months of January, February, and March. The 13 primary grids were located on the four feedgrounds--Shop, Nowlin, Poverty, and McBride (Figure 2). The general study area is described by Cole (1969), Boyce (1989), and Smith (1998). The Shop feedground is dominated by subirrigated bluegrass (*Poa sp*.) and cultivated smooth brome (*Bromus inermis*). The Nowlin feedground is dominated by cultivated fields of creeping meadow foxtail (*Alopecurus arundinaceus*) and timothy (*Phleum pratense*) grass. The Poverty and McBride feedgrounds are similar to each other and are dominated by wheatgrass (*Agropyron sp*.) and bluegrass. At the time of this study, Shop, Nowlin and McBride feedgrounds were subject to irrigation, cultivation, or a combination of both; Poverty was not irrigated or cultivated. These sites were specifically chosen for rodent trapping because they are the main winter feedgrounds on the NER and provide the nucleus for elk aggregation and HS transmission.

I selected the 13 primary grid sites on the basis of high (core feedground) and low (off core feedground) density elk use in each feedground, and the dominant plant community types within these areas. I defined "core feedground" as the main area where feed is deployed and the "off core feedground" as the adjacent area where elk might congregate but where food is not deployed. I placed grids in the major plant communities within each core feedground and in the same plant community types "off" the core feedground. Wetlands were excluded because it was not feasible to trap these areas (Table 1).

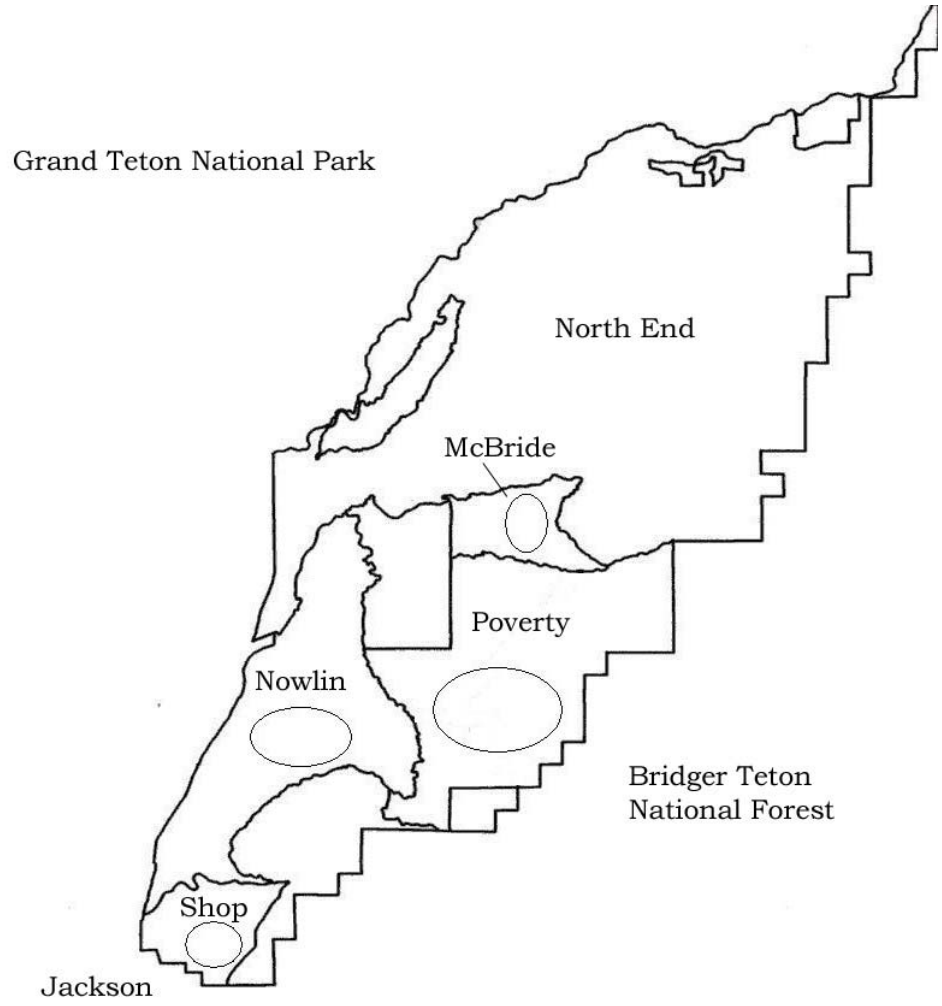

Figure 2. Schematic map of management areas within the National Elk Refuge near Jackson, Wyoming. Circles represent general core feedground areas (modified from NER Plant community types and management units map produced by the USGS, April 2000).

Table 1. Trapped areas in dominant plant community types and dominant plant species found within the Shop, Nowlin, Poverty, and McBride feedgrounds on the NER.

	Shop	Nowlin	Poverty	McBride
Cultivated Fields				
Agropyron cristatum			X	
Agropyron intermedium				X
Alopecurus arundinaceus		X		
Bromus inermis-- mixed grass	X			
Elymus junceus				
Phleum pratense--Poa species		X		
Poa pratensis				
Grasslands				
Agropyron species--Poa species (on flats)			X	X
Agropyron species--Poa species (Miller Butte)		X		
Agropyron species--Stipa species (Gros Ventre hills and slopes)				X
Subirrigated Poa species	X			
Wetlands				
Carex species--Juncus species				
Shrublands				
Artemesia tridentata--Artemesia tripartita--grass (on slopes)			X	
Artemesia tridentata--Poa species (on flats)				

Nowlin originally had three grids, but one grid subsequently was abandoned, again, due to flooding. Poverty had two grids and McBride had four grids. This resulted in a minimum of two grids per feedground and a total of 13 grids with 11 trapped in both 2000 and 2001. This design allowed me to compare relative rodent densities between core and off core feedground areas and among feedgrounds. This set of grids covered <1% of the surface area of each feedground but was the maximum I initially estimated that I could effectively sample multiple times in a single field season.

In 2001, I increased sampling effort by adding supplementary and ancillary trapping sites. Seven of the supplementary grids were located in the Shop and Nowlin feedgrounds, and two were located in the Poverty feedground. I trapped these locations more frequently because my trapping effort in 2000 resulted in small sample sizes and I wanted to increase tissue sample numbers to increase the probability of detection of *P. multocida.* The supplementary sites also allowed me to investigate specific areas in the Shop and Nowlin feedgrounds where epizootic HS occurred in 1993. The two supplementary grids at Poverty provided additional coverage of the largest feedground.

There are three feed storage areas at the NER. Two are located adjacent to the Nowlin feedground and one is located outside the McBride feedground. Storage areas concentrate rodents at high densities because they provide food and cover. Elk sometimes remain near the feedsheds during the winter and may come into direct contact with rodents. Feed trucks and other equipment are stored in the sheds, and they could carry rodents to the feedgrounds providing an additional route for direct contact between rodents and elk.

Because the main objective of this study was to determine if rodents are a reservoir for *P. multocida*, I also trapped three ancillary sites in 2001 that had the potential for maintaining rodents at high densities. These sites included the sleigh storage area, a gravel pit, and an elk trap adjacent to the Nowlin and Poverty feedgrounds. These sites had equipment, old lumber and large boulders which provided suitable habitat for rodents. The sleighs might also serve as a way to carry rodents out to the Nowlin feedgrounds where a large percentage of HS occurred.

Summer Sampling

I used Sherman live-traps (8 x 8 x 23cm, H. B. Sherman Traps, Tallahassee, FL) for rodent captures. The physical dimensions of the Sherman traps allowed me to catch small mammals ranging in size from shrews (Soricidae) to Uinta ground squirrels (*Spermophilus aramatus*) and bushy-tailed woodrats (*Neotoma cinerea*). All traps were baited with rolled oats and peanut butter. Cotton batting was provided as insulation to maximize comfort and survival of trapped animals. For the summer trapping, a grid system of 100 traps per grid covering one hectare was used. The grid was configured as 10 traps in 10 rows spaced at approximately 10-m intervals. Grid systems are a standard survey method for determining the presence or absence of target small mammal species (Tew et al 1994). Traps were set for three consecutive nights (considered one trapping session), and captured mammals were released early each morning. A three-night trapping period is commonly used for small-mammal trapping to ensure empirical and statistical validity. (ad hoc Committee on Acceptable Field Methods in Mammalogy

1987). This level of effort provides adequate assurances that one or more small mammals are likely to be trapped if small mammals are present in the grid, given the constraints of the size of the trap (Padgett-Flohr and Shellhammer 2002). Each grid was sampled once per month and four grids were trapped concurrently at two feedgrounds during a trapping session. Trap treadles were set for a sensitivity to capture animals as small as shrews.

Winter Sampling

'Winter' traps consisted of the same Sherman live-traps used in summer trapping, wrapped in 1.4-cm foam placed inside a piece of steel pipe approximately 53cm long and 20cm in diameter. This trapping assemblage provided extra insulation for the rodent and allowed me to sample directly on the feedline with traps that were resistant to trampling or accidental trigger by elk and bison. Winter traps were baited in the same manner as summer traps.

I used a snowcat (Thiokol®) to place sixteen to 20 'winter' traps in a single line approximately 100m apart on or adjacent to the elk feedline during feeding operations. The snowcat permitted trap transport across large snow-covered areas and minimized elk disturbance because of their habituation to the machine. The trap line was moved daily in conjunction with the daily feeding regime. The daily feeding regime required that food be deployed in a different location within the respective feedground from the previous day. Therefore, winter traps were left out for only one trap-night, and only one feedground was trapped at a time. I attempted to trap each feedground one day per week.

I trapped on a single feedground each day during winter feeding (generally January through March) provided snow was on the ground. Given the constraints of the winter trap design and trapping protocol—time consuming assemblage, heavy weight, restrictions to feeding regimes—a trap line was the best method for live-trapping any rodents present. Placing traps adjacent to the feedline increased the chances of catching any rodents attracted to the alfalfa pellets. The obvious limitations of winter trapping are a reduction in trap effort (300 trap nights in summer versus 20 traps nights in winter. My winter trap-lines were always within core feedground areas, but were not present for consecutive days in the same location and, therefore, may not have given trap-shy animals a chance to habituate to the trap. Feed storage sheds were also trapped in the winter using the summer protocol (~100 Sherman live-traps set for three consecutive nights at each shed).

Population Estimates

All rodents captured were marked with numbered fingerling fish tags (National Band and Tag Company, Newport, KY). Data collected on each individual included: weight (to the nearest 0.5g), species, gender, ear-tag number and location. I reported results of live-trapping as minimum number alive (MNA), a commonly used index of population size when trapping numbers are low (Krebs 1966). The MNA is an estimate based on the sum of animals known to be alive during a particular capture (trapping) session. The MNA is determined by the number of captured individuals during a trapping session, plus those known to be alive captured in both previous and subsequent

sessions that were not captured during the current trapping session. Population results were also reported as "catch-per-unit-effort" in order to make comparisons between sampling points.

Sampling For *P. multocida*

Removal trapping for tissue collection was conducted in October of 2000, March and October of 2001, and March of 2002, after the population density was established for a given area. Rodents were randomly selected within trapping grids and euthanized by cervical dislocation following American Veterinary Medical Association guidelines on humane euthanasia (JAVMA 2001 218:669-696). Two samples from each rodent were collected for *Pasteurella* isolation; retropharyngeal lymphoid tissue (including tonsil) and distal colon. Retropharyngeal lymphoid tissue was chosen because *P. multocida* is known to harbor in the tonsilar crypts. Distal colon tissue was chosen because *P. multocida* maintains viability through the intestinal tract and bacteria in the distal colon would have the highest probability of being excreted in the feces. Tissue samples were placed separately in an Amies culture tube, refrigerated, and sent the next day using overnight delivery to Caine Veterinary Research Center in Caldwell, Idaho. Amies culture tubes provide the best transport media to maintain *Pasteurella* organisms if they are present (Ward, personal communication). At the Caine research center, tissue samples were cultured for bacteria with *Pasteurella*-selective media to isolate the HSPm organism (Moore et al 1994). A positive control sample was included with each shipment as a quality control for the effects of shipping, handling and culture

methodology on *P. multocida* recovery. Ten microliters of the HSPm organism were suspended in 2 milliliters of physiological saline. The *Pasteurella* suspension was then inoculated into a mouse by intraperitoneal injection. If HSPm organisms are present, the mouse dies within 24 hours (De Alwis 1992). Tissues from positive control mice were collected and handled in the same manner as sample rodents. All samples and controls were kept bacteriologically isolated from one another, by disinfecting between tissue collection and by using two different processing stations—one for controls, one for tissue samples.

Elk Tissue Sampling

I opportunistically sampled elk carcasses collected by NER personnel during the winter of 1998-1999, 2000-2001 and 2001-2002 to determine an index of HSPm prevalence in elk over a winter season. I arranged to have bacteria isolated from elk tissues identified to determine if DNA-types and/or sero-types carried by rodents and elk were the same. *P. multocida* has been documented to persist for several months in the nasopharnyx and tonsil crypts (De Alwis 1992) so I extracted tonsilar or pharyngeal lymphoid tissues, or a combination of both, from intact elk carcasses. Most of the time, carcasses were too heavily scavenged for lymphoid samples; therefore, bone marrow (femur) samples were taken instead. Tissues from intact carcasses provide the best opportunity to get quality samples for HSPm and for commensal *Pasteurella* isolation (Franson and Smith 1988). If the elk was septicemic at death, marrow samples provide the next best reasonable chance of finding the HS organism (Carter and De Alwis 1989).

However, marrow samples can only be used as an index of *P. multocida*, and are not as reliable as other tissues as an indication of true presence. Positive samples confirm the presence of *P. multocida*. Elk that died of HS may still be negative in bone marrow if insufficient amount of bacteria is present to suitable culture.

Lymphoid and marrow samples were submitted to Caine Veterinary Center in Caldwell, Idaho for Pasteurella isolation (Moore 1994). I sent samples that were culture-positive for *P. multocida* to National Veterinary Services Laboratory (NVSL) in Ames, Iowa for DNA fingerprinting of *Pasteurella* isolates (Wilson et al 1992).

Relationship Between Weather And HS Occurrence

HS has been associated with wet, humid weather, and an increased incidence has been recorded during wet seasons. These conditions presumably favor survival and spread of HSPm (De Alwis 1992). Expression of HS has also been associated with changes in seasonal weather patterns or inclement weather that possibly reduce host resistance (De Alwis and Vipulasiri 1980, Franson and Smith 1988). Although outbreaks of HS in the NER are limited to winter, I examined the relationships between incidence of HS and several climate variables.

Elk mortality data came from records maintained by NER staff and climate data were obtained from USFS Jackson Climate station (Site No. 4910, Elev. 6230 ft) in Jackson, WY. The elk mortality records include age, gender, date and location by

feedground. Mortalities attributed to HS were based on gross pathologic findings, bacterial culturing, or both. I used six variables from the climate records: minimum, maximum, and average air temperature; total precipitation; snow water equivalent; and depth of snow on the ground. Snow depth at the Jackson weather station was used only as an index. The Jackson weather station is located approximately 1 km south of the NER on top of Snow King Mountain, and snow depths do not accurately reflect the depths on the NER. Because most of the confirmed HS cases occurred in January, February, and March, analyses were restricted to the incidence in these three months. I used the combined data from only 1986 and 1993 to examine the relationship between specific weather conditions and HS because these were the two years with the most HS occurrence.

I used a bivariate correlation to determine if there was a linear relationship between each daily weather variable and HS and among weather variables. The results of the bivariate correlation indicated high covariance among independent variables (weather), suggesting a principle component analysis (PCA) was appropriate. I entered five of the six weather variables into the PCA. Average temperature was omitted to avoid redundancy because it is calculated from the maximum and minimum temperature. I then applied a bivariate correlation between principle component one, principle component two, and the daily incidence of HS. I also evaluated this relationship by imposing lag periods of one through seven days on the daily incidence of HS to account for the incubation period of HS which can be two to five days. Using another bivariate correlation, I determined if there was a relationship between the mean of each weather

variable by week and the incidence of HS by week. In this weekly correlation, I included a diurnal temperature variable, which is the degrees difference between maximum and minimum temperature, to evaluate extreme fluctuations in temperature, and imposed a two-week lag period on this relationship.

Age-gender Class Risk Assessment

I assessed age-gender class as a risk factor for HS using a Fisher's exact test to compare the age-gender distribution of the population at risk to the age-gender distribution of the HS population. The age-gender distribution for the population at risk was based on data from classification counts conducted on the Refuge each year during feeding operations. Each February, observers riding on the feeding vehicles count the number of elk wintering on the NER and classify them as calves (< 1 year), cows (females ≥ 1 year), spikes (males 1- 2 years) or bulls (males ≥ 2 years) (NER files, Smith 1998). If each of the four age-gender classes were equally susceptible to HS, then the proportion of each class reported in mortality reports as dying from HS for each feedground (which identify elk found dead by gender, age, and feedground) should mirror the proportion of each class generated from counts on each feedground. I considered spikes and bulls equally at risk to cows and calves even though they tend to band together separate from larger cow-calf groups, because they do use the same feedlines as cows and calves, and the proximity required for HS transmission is unknown.

I restricted the analysis to elk at the two southern feedgrounds—Shop and Nowlin—because HS mortalities at the two northern feedgrounds—Poverty and McBride—were considerably lower, suggesting less exposure for Poverty and McBride elk. Data for elk at the Shop and Nowlin feedgrounds were analyzed separately because feedground characteristics may influence exposure risks. I excluded six elk deaths at the southern feedgrounds from the analysis because the ages of these elk were not reported.

Feedground Risk Assessment

Differences in feedground characteristics might influence the incidence of HS. In my analysis, I tested the hypothesis that incidence of HS on the NER varied among feedgrounds. I applied a one-way ANOVA to the number of HS cases, with feedground as the factor. I also examined the feedground-HS relationship using linear regression on three habitat characteristics that might influence the incidence of HS: 1) percentage of wetland within each feedground; 2) percentage of native grassland within each feedground; and 3) the density of elk on each feedground. I hypothesized that the native grassland percentage may affect expression of HS because HS is relatively rare or nonexistent in elk herds wintering on native range. Irrigation or cultivation techniques may modify local environmental conditions affecting *P. multocida* survival. I included number of elk by feedground (an index of elk density) as another independent risk factor because the mechanisms of HS transmission are favored under higher density conditions.

RESULTS

Small Mammal Trapping And Processing

I captured 849 small mammals distributed among six species. The population estimates are shown in Table 2. Deer mice (*Peromyscus maniculatus)* were the dominant species captured and accounted for 95% of all small mammals captured. Voles (*Microtus sp*.) accounted for 4%, and Uinta grounds squirrels (*Spermophilus aramatus*), a bushy-tailed woodrat (*Neotoma cinerea*), shrews (*Sorex sp.)* and short-tailed weasels (*Mustela erminea*) accounted for the remaining 1% of the small mammals captured.

Of the total 849 captures, 803 were captured during summer, and 46 were captured during winter. For summer trapping, 32% of the animals were caught in the 13 primary grids, 16% in the supplementary grids, 6% in the ancillary sites, and 46% were caught in the feed sheds. The greatest number of captures on the primary grids came from the Poverty (n=100) and McBride (n=136) feedgrounds, which have abundant bare ground interspersed with sparse stands of wheatgrass both on and off the core feedground areas. More small mammals were caught in grids off the core feedground areas than grids on the core feedground areas in all feedgrounds.

The Shop site had the highest species diversity of the four feedgrounds. In addition to deer mice, eight voles, one shrew, and three weasels were trapped at the Shop feedground in the primary and supplementary grids. The deer mouse was the only species captured at the Poverty feedground. Nowlin-1 feed shed and McBride feed shed had the most animals trapped of all three feed sheds.

Table 2. Minimum number alive (MNA) for deer mice and numbers of other species of small mammals captured on the National Elk Refuge from 2000-2002.

Location	General Habitat	Trap nights	CPU	Pm	Mi	Sa	Nc	So	Me	Total
Primary grids										
Shop exclosure	Cultivated Fields	100	0.000	0	1					1
Shop E	Grassland	900	0.000	0	1				2	3
Shop NW	Grassland	1800	0.000	0	1				1	2
Shop roadside	Grassland	1800	0.006	11	3			1		15
Nowlin S	Cultivated Fields	1800	0.007	12						12
Nowlin flood	Cultivated Fields	300	0.000	0						0
Nowlin N	Cultivated Fields	1800	0.002	4	1					5
Poverty A	Upland Grassland	1800	0.028	50						50
Poverty B	Upland Grassland	1800	0.028	50						50
McBride AgInt (core)	Cultivated Fields	1800	0.010	18	2					20
McBride AgInt (off)	Cultivated Fields	1800	0.014	26	1					27
McBride AgPoa (core)	Upland Grassland	1800	0.021	37					1	38
McBride AgPoa (off)	Upland Grassland	1800	0.028	51						51
Supplementary grids										
Shop 5	Cultivated Fields	600	0.017	10	1					11
Shop 6	Cultivated Fields	700	0.031	22	1					23
Shop 7	Grassland	300	0.003	1						1
Shop 8	Grassland	500	0.040	20						20
Nowlin MB	Upland Grassland	900	0.024	22						22
Poverty Ctwd	Cultivated Fields	400	0.085	34						34
Poverty lowCh	Shrubland	400	0.070	28						28
Feed sheds										
Nowlin 1 (west)	n/a	1708	0.088	138	11	3				152
Nowlin 2 (east)	n/a	2038	0.046	90	7	1	1	1		100
McBride	n/a	1463	0.103	140				1		141
Specific sites										
Sleigh area	n/a	75	0.187	14						14
Elk Trap	n/a	108	0.065	7						7
Gravel Pit	n/a	60	0.367	22						22
Total				807	30	4	1	3	4	849

CPU = catch/unit effort; Pm = *Peromyscus maniculatus*; Mi = *Microtus spp*; Sa = *Spermophilus aramatus*; Nc = *Neotoma cinerea*; So = *Sorex sp*; Me = *Mustela erminea*; Total = number of animals per site

Of the total 46 winter captures, 44 were deer mice captured in feed sheds. Only two animals were caught on the feedground areas. One was a deer mouse captured in snow-filled Flat Creek, just south (off core) of the McBride feedground. The second was a short-tailed weasel caught off the core feedground area of Shop. I collected tissues from 283 animals (98% were *P. maniculatus*) for *P. multocida* culture. Of these, 107 came from the primary grids on the feedgrounds, 121 from all three feed sheds, and 55 from the supplemental grids and ancillary sites. A shipment of 32 samples representing 15 different mice and a short-tailed weasel was delayed due to events of '9-11', and resulting overgrowth of non-target bacteria prevented proper analysis. These 32 samples were from the core and off-core areas of the McBride feedground.

No *Pasteurella* organisms were isolated from the usable samples from the251 animals. Sixteen of the colon samples and one of the lymph node samples from deer mice were overgrown with *Proteus sp*. that could have prevented detection of *Pasteurella*. A nonspeciated *Actinobacillus* isolate, a member of the Pasteurellaceae family, was cultured from the lymph node of one mouse. *P. multocida multocida* was isolated from both the colon and lymph node samples of 13 positive control mice except one in which *P. multocida multocida* was isolated only from colon, but not from the lymph node.

Elk Tissue Results

I had samples of elk tissues from three winters with which to determine HS prevalence. The minimum numbers of elk that died during these winters were 119 (1998-

1999), 36 (2000-2001), and 47 (2001-2002). No samples were obtained for *P. multocida* isolation in 1999-2000 because no carcasses were collected that year. Because carcasses are rapidly scavenged by coyotes and other animals, only 27 of the 202 total carcasses could be salvaged for tissue or femur sampling (Table 3).

Table 3. Number of elk sampled by year, sample quality (based on postmortem condition), tissue sampled, and whether *P. multocida* was isolated

Year	No. elk Sampled	Carcass Quality	Tissue Sampled	Result for presence of *P. multocida*
1998 - 1999	16	scavenged	femur	16 negative
2000 - 2001	4	scavenged	femur	1 positive, 3 negative
2001 - 2002	7	3 intact 4 scavenged	tonsil/retropharyngeal femur	1 positive, 2 negative 4 negative

Of the 27 elk carcasses sampled, *P. multocida* was recovered from two. *P. multocida* was recovered from the marrow of an adult female that died at the Shop feedground in February 2001. This animal had appeared to be in good condition with ample fat reserves in the femoral marrow (Smith, personal communication). However, the sample was not typed for DNA because the sample was also being used for another study. *P. multocida* was isolated from the tonsil and retropharyngeal lymph nodes of one adult female from the Nowlin feedground. The DNA profile of the isolate, using the Hha1 restriction endonuclease did not match profiles of organisms associated with HS outbreaks in 1986 and 1993.

Two other types of *Pasteurella* were isolated from two carcasses in 2002. A non-hemolytic *P. trehalosi* biovariant 2 was isolated from the retropharyngeal lymph node of

one adult bull from the McBride feedground. *P. gallicida* was isolated from the tonsil of a second adult bull, but the source of this carcass was unknown.

Weather And HS Occurrence Results

There were 169 confirmed cases of HS over the period 1986 to 2001 during the months of January through April (Appendix A). Only two cases were reported in April. No cases were reported in May through December. Therefore, April through December were eliminated from the analyses. The bivariate correlation between daily weather variables and HS resulted in significant but low correlations. Weather variables were also highly correlated with each other (Table 4); therefore, individual effect of each variable on the incidence of HS could not be analyzed.

Table 4. Bivariate correlation (Spearman) between daily climate variables and daily incidence of HS in 1986 and 1993 for the months of January, February, and March (n = 180)

	T MIN	T MAX	T AVG	PPT	SWE	SNWD
T MAX	0.782 (**)					
T AVG	0.949 (**)	0.927 (**)				
PPT	0.290 (**)	0.004	0.169 (*)			
SWE	-0.038	-0.087	-0.092	0.186 (*)		
SNWD	-0.410 (**)	-0.467 (**)	-0.475 (**)	0.185 (*)	0.458 (**)	
HS	0.101	0.041	0.066	0.094	0.156 (*)	0.375 (**)

T MIN = Minimum temperature, T MAX = Maximum temperature, TAVG = Average temperature, PPT = Precipitation, SWE = Snow water equivalent, SNWD = Snow depth, HS = Hemorrhagic septicemia. **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed)

PCA produced pseudovariables in which five factors were extracted from five input variables. The first factor accounted for 48% of the total variance inherent in the data set, the second 28% (Table 5 and Table 6). I retained these first two factors to enter into a bivariate correlation with HS based on the Kaiser criterion which drops all components with eigen-values under 1.0.

Table 5. The percentage of total variance accounted for, and the associated eigenvalues, for 5 extracted factors in a factor analysis of 5 variables

	Initial Eigenvalues		
Components	Total	% of Variance	Cumulative %
1	2.423	48.454	48.454
2	1.414	28.283	76.737
3	0.724	14.473	91.210
4	0.253	5.054	96.264
5	0.187	3.736	100.000

Table 6. Loadings of daily climate variables with the five principle components

Daily	Principle Components				
Climate variables	1	2	3	4	5
Minimum temperature	0.788	0.505	0.157	-0.015	-0.314
Maximum temperature	0.835	0.255	0.377	0.168	0.259
Total Precipitation	0.067	0.788	-0.603	0.012	0.102
Snow water equivalent	-0.628	0.594	0.404	-0.292	0.067
Snow depth	-0.840	0.347	0.173	0.372	-0.080

The bivariate correlation between factor one and the daily incidence of HS produced no significant results (Table 7). The correlation between factor two and the

daily incidence of HS produced positive but weak significant results (Table 7) in seven of the eight periods analyzed. The correlation between factor two and the six-day lag was not statistically significant.

Table 7. Bivariate correlation (Spearman) between factor 1, factor 2, and daily incidence of HS with a 7 day lag for combined years of 1986 and 1993 (n = 180).

Factor 1	Incidence of HS	p value	Factor 2	Incidence of HS	p value
0 day lag	-0.065	0.384	0 day lag	0.238	0.001
1 day lag	-0.083	0.267	1 day lag	0.222	0.003
2 day lag	-0.092	0.219	2 day lag	0.245	0.000
3 day lag	-0.071	0.345	3 day lag	0.186	0.013
4 day lag	-0.029	0.695	4 day lag	0.203	0.006
5 day lag	-0.035	0.645	5 day lag	0.163	0.029
6 day lag	-0.006	0.933	6 day lag	0.113	0.130
7 day lag	0.024	0.748	7 day lag	0.164	0.027

Analysis of weather variables by week showed that snow depth was significantly and positively correlated with the weekly incidence of HS. The correlation was strongest during the week of reported HS death and decreased over the two-week lag period (Table 8).

Table 8. Bivariate correlation (Spearman) between mean climate variables by week and incidence of HS by week (n = 30 weeks), with a two-week lag period

Climate variables	lag none	p value	lag 1 week	p value	lag 2 week	p value
Minimum temperature	-0.064	0.758	-0.163	0.425	-0.136	0.506
Maximum temperature	-0.055	0.788	-0.142	0.488	-0.128	0.534
Average temperature	-0.099	0.630	-0.175	0.391	-0.166	0.417
Precipitation	0.149	0.466	0.147	0.472	0.056	0.786
Snow water equivalent	0.300	0.136	0.260	0.200	0.236	0.247
Snow depth	0.632	0.001	0.564	0.003	0.441	0.024
Diurnal temperature	0.101	0.622	0.146	0.477	0.185	0.367

Age-Gender Class Risk Results

Calves and cows were at higher than expected risk for HS at the Shop and Nowlin feedgrounds (Table 9). Spikes and adult bulls were not disproportionately at risk.

Table 9: Cumulative incidence of HS by age-gender class (calves, cows, spikes, bulls) for the six years of HS occurrence: HS = the proportion of elk that died from HS; no HS = the population at risk that did not die from HS.

	Shop				Nowlin		
Age-gender Class	HS	no HS	p-value	Age-gender Class	HS	no HS	p-value
Calf	29	2636	p = 0.012	Calf	37	1588	p = 0.00
Other Classes	30	5373		Other Classes	48	10620	
Cows	24	4425	p = 0.026	Cows	33	7645	p = 0.00
Other Classes	35	3584		Other Classes	52	4563	
Spikes	4	701	p = 0.816	Spikes	3	720	p = 0.488
Other Classes	55	7308		Other Classes	82	11488	
Bulls	2	247	p = 0.704	Bulls	12	2257	p = 0.399
Other Classes	57	7762		Other Classes	73	9951	

Feedground Risk Assessment Results

All feedgrounds—Shop, Nowlin, Poverty, and McBride were dominated by cultivated fields and grassland community types (Fig. 3). Although the elk at Shop and Nowlin feedgrounds incurred higher losses than Poverty and McBride (Figure 4, Table

10), the mean number of HS cases did not vary significantly among feedgrounds (ANOVA; $p = 0.453$, $df = 3$). The regression analysis between percentage of wetland, percentage of native grassland, density of elk, and the annual incidence of HS produced non-significant relationships (wetland $p = 0.40$, grassland $p = 0.17$, elk density $p = 0.66$).

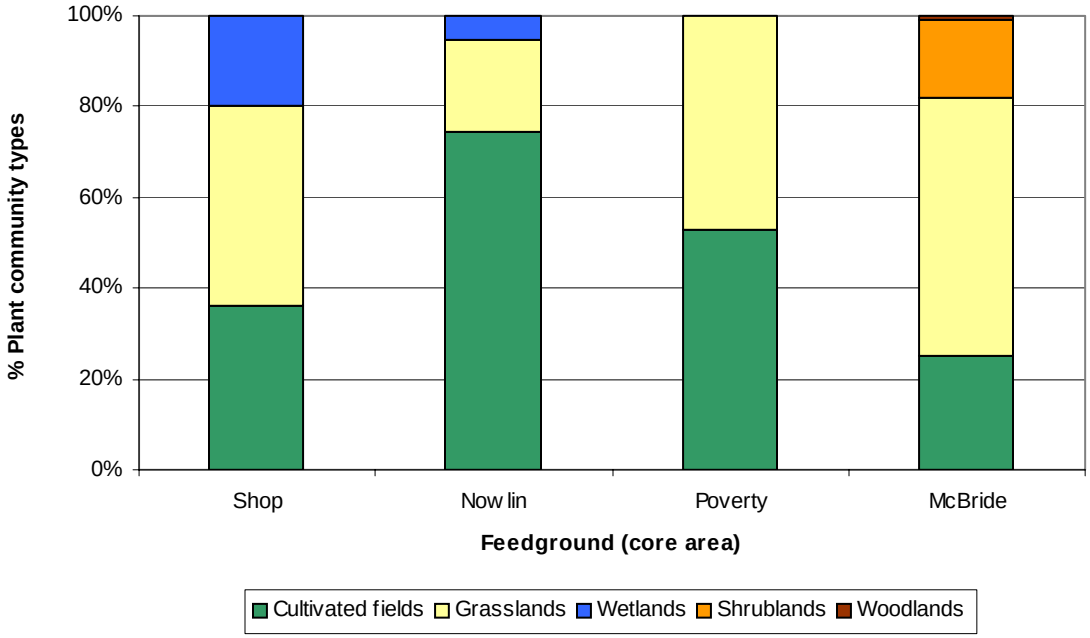

Figure 3. Dominant plant community types by feedground area, on the National Elk Refuge

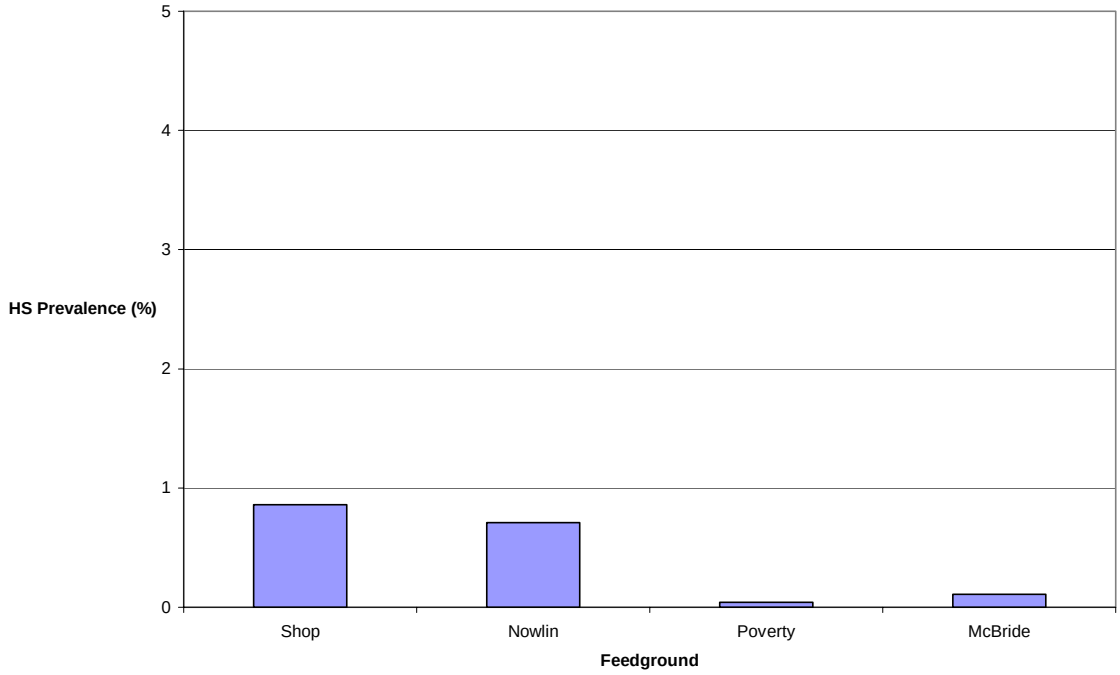

Figure 4. Prevalence of HS in elk by feedground on the NER based on the proportion of animals at risk at each feedground (n = 6 years)

Table 10. Incidence of HS in elk by year and feedground on the NER (n = 6 years).

	Feedground			
Year	Shop	Nowlin	Poverty	McBride
1986		32	4	2
1987		2	1	8
1993	59	51	1	2
1995		2		1
1999	3			
2001	1			
Total	63	87	6	13

DISCUSSION

I found no evidence to support the hypothesis that the rodents on the NER served as a reservoir for HS during the period of this study. *P. multocida* was not isolated from any rodent tissues collected, indicating either zero prevalence or an undetectable prevalence.

My ability to detect low levels of HSPm prevalence may have been limited by small sample sizes. Many of grids (13 of 20) yielded minimal population estimates of 25 animals or less. In order to have 95% confidence at detecting 2% or more HSPm prevalence, my sample size would have had to be 25 animals, the entire population at each grid site. My samples sizes of 10 to 16 animals provide a 95% confidence of detecting prevalence at 10% or greater. Thus my sampling was insufficient to detect *P. multocida* at low prevalences. Studies show that prevalence for effective reservoirs harboring the HSPm organism can be as low as one percent (Mohan et al., 1968; Singh, 1948; Wijewantha and Karunaratne, 1968). The rodent population may also be at densities too low to support *P. multocida,* though what densities are required to support the HSPm organism in a rodent population are unknown.

Even though I believe my samples make a convincing case for a low potential for rodents to serve as a reservoir for HS, I cannot eliminate the possibility that rodents in the NER could carry *P. multocida*. I may have sampled at a time when rodent population densities were low. Rodent populations are known to fluctuate inter-and intra-annually, and at high densities, I may have been able to more easily capture *P. multocida* carriers.

Infected rodents may exist as reservoirs in places I did not trap (for example, in wetland areas) or my choice of trap type and/or trap placement may have been ineffective for sampling rodents that interacted with elk. Systematic sampling, had I been provided with sufficient personnel and equipment to trap more extensively, might have allowed me to collect a more even sample across the NER thus avoiding conclusions based on a few hectares.

It should also be noted that my lack of finding *P. multocida* in rodents applies almost exclusively to deer mouse (*P. maniculatus*) as 95% of my total captures were deer mice. Captures of other rodent species were rare and it is possible that other rodents, which perhaps were abundant in previous years, may serve as a reservoir for HSPm. For example, NER personnel reported seeing large numbers of Uinta ground squirrels in past years, but low numbers during my study. It is unknown if Uinta ground squirrels carry HSPm and ground squirrels, which hibernate, are unlikely to directly contact elk in winter, but Quan et al. 1986 found *P. multocida* in the *Spermophilus* species, in 91 of the 192 rodents captured.

P. multocida was recovered from only two elk samples. The DNA profile of one of these samples had not been found in previous studies. It is possible that this B:3,4 strain may be one of only several B:3,4 strains existing in elk on or near NER (Wilson, 1995). I was unable to eliminate elk as potential reservoirs, because the sample size of 27 was small and the majority of the samples (20 of 27) were femur samples. Femur samples underestimate the prevalence of HS because the disease could have killed the elk before *P. multocida* was present in sufficient quantities to be detected. My sample size

would likely identify carriers only if the percentage of carriers in the population was high. The percentage of HSPm carriers has been detected as low as 1% (Mohan et al., 1968; Singh, 1948; Wijewantha and Karunaratne, 1968). In a different study determining the existence of carrier birds for avian cholera, just one of 298 healthy lesser snow geese was found to be a carrier for the pathogenic *P. multocida*, serotype 1 (Samuel et al., 1997).

Further testing would be required to determine if coyotes or ravens are reservoirs for HS. Two coyotes (*Canis latrans*) and one bobcat (*Lynx spp*.) were fed the liver and other tissues of a mule deer (*Odocoileus hemionus*) that had died of hemorrhagic septicemia, without provoking clinical disease (Quortrup, 1942). This incident suggests coyotes may be immune to HSPm and therefore could potentially serve as a reservoir. Ravens also could be worth investigating—more than 100 species of free-ranging wild birds are known to have been naturally infected with *P. multocida*, although this is of serotype 1 that causes avian cholera (Botzler 1991).

The results of the weather analysis by week indicate that snow depth was positively associated with incidence of HS. HS increases during wet seasons in tropical climates which suggests that wet conditions favor survival and spread of HSPm (De Alwis 1992), and Franson and Smith (1988) hypothesized that the muddy conditions on feedgrounds may have exacerbated the 1987 HS outbreak on the NER.However, HS on the NER occurs during winter when most precipitation occurs in the form of snow with, because of low humidity, low moisture content. In fact, snow depth alone may be a poor indicator of moisture on the NER. If moisture were an important factor in HS incidence,

high snow depth in conjunction with warm temperatures (i.e. precipitation with sufficient warmth to make it available as water) would be a better indicator of moisture. I found no significant relationship between temperature and HS incidence on the NER. The positive association between snow depth and HS incidence may be due to enhanced transmission of the bacteria when snow is abundant. It is possible that once an outbreak is initiated, snow may allow *P. multocida* to better survive outside a living host and infect elk that rely on snow as a water source. There are no studies that show snow as a direct factor associated with HS, but Nordkvist (1962) hypothesized that the HSPm organism may have accumulated on snow drifts, favoring the spread of the organism and contributing to an HS outbreak in reindeer in Sweden.

P. multocida is not known to survive long in the abiotic environment; however, snow may extend survival of the organism in infected carcasses. Snow may act as a natural incubator by insulating and creating a preferred temperature for *P. multocida* survival in carcasses. Under laboratory conditions, *P. multocida* survived up to 113 days in soil held at 3C, and five to six days in water at 5C-6C (Botzler 1991). In another study, *P. multocida* was proven to survive in chicken carcasses for at least two months when placed in an icebox (Rosen and Bischoff 1950). The temperature conditions of these two studies are similar to those to which NER elk carcasses on the snow would be exposed. Also, the duration of HS outbreaks on the NER was observed to be up to 105 days, similar to the durations of *P multocida* survival in these two studies.

PCA results did little to clarify the relationship between weather and HS incidence. Based on bivariate correlation, a weak relationship appeared to exist between

factor two, which was primarily an estimate of "moisture," and daily HS occurrence. The relationship may have been stronger if I had access to variables that more accurately reflected soil moisture or the temperature-moisture combination that had the highest potential to preserve *P. multocida* in snow or in carcasses. It would be warranted to further explore the relationship of moisture to HS occurrence in a study that collects detailed moisture data from the feedgrounds, as well as from the elk summer range.

Wind speed and direction may be equally or more important than moisture and temperature variables. I only had anecdotal information on wind and HS, but gusty wind conditions were documented during peak mortality in 1986. Gusty winds may have induced huddling behavior in the elk herd, which could have aided in aerosol transmission of pathogens through close contact.

I found that cows and calves were at higher risk of contracting HS than bulls and spikes. Sin Sri Lanka 65% of losses to HS in buffaloes and 77% in cattle occurred in animals under two years of age (De Alwis and Vipulasiri 1980). Another study in that country indicated that, in domestic water buffalo, the most vulnerable age was six months to two years (De Alwis et al., 1976). Franson and Smith (1988) showed that the mean age of HS deaths in elk on the NER was two years old in the winter of 1986 and four-years-old in 1987. Both values were under the mean age of elk in the NER. Elk calves are presumably more susceptible than older elk because they are unable to mount as strong an immune response to HS following exposure than can older animals

Several possibilities may explain the greater susceptibility to HS of cows than bulls. Elk cows may be infected by infected calves due to their prolonged exposure from

close contact. Cows may also be under physiological stress due to lactation or pregnancy thereby reducing the animal's resistance to infection HS. The incidence of HS in elk may be related to habitat type (Alwis 1981) or population size or density. All of the feedgrounds were dominated by native and cultivated grassland so I could not effectively test for the effects of different dominant habitat types on HS incidence. Feedgrounds did differ somewhat in the proportion of wetlands included. If moisture were important in maintaining *P.* multocida or transmitting HS on the NER (Franson and Smith 1988, Carter and De Alwis 1989, De Alwis 1992), the incidence of HS could be affected by the availability of wetland areas, though wetland areas may have other characteristics negatively correlated with HS. At Shop and Nowlin feedgrounds, where the highest HS incidence occurred, small portions of the core feedgrounds are wetlands. No wetlands occur on the other two feedgrounds. I found that population of elk on the feedgrounds had no relation to incidence of HS. Similar results were found in one study where HS mortalities were not correlated to herd size in cattle or buffalo (De Alwis 1981). HS in ungulates remains a poorly understood disease that could benefit from following the investigation approaches used to study avian cholera. Both diseases are caused by *P. multocida*, although avian cholera is caused by a different serotype and DNA type. Avian cholera serotype 1 has been found to exist at very low prevalences, so HSPm also may exist at very low prevalences. Both diseases have the capacity to swiftly kill many animals in what has been clinically observed as "sudden death". And, in North America, both diseases are associated with cold, wet weather.

Although HS is rarer than avian cholera, it has reoccurred periodically to the NER. This suggests that the disease could be endemic to the NER, with wetland areas as the likely geographic reservoirs, or elk may be the reservoirs. Both possibilities should be investigated, but both investigations would be best done during an active, or following recent, outbreaks of HS. To test the possibility that the disease is endemic to the NER, a baseline of sediment- and water-sample data should be established now. During an outbreak of HS sediment and water samples should be collected from the wetlands areas for HSPm analyses. It also would be beneficial to collect samples from areas of the feedgrounds other than wetlands, particularly areas of snow-melt inundation. This would provide a more direct approach to measuring moisture as a factor in HS. A design might include sampling, following the outbreaks, in spring when the elk start to leave, during fall when they start to return and the following winter when they are present again. If the bacteria are present during the outbreak and not afterwards, it likely means that HSPm is not able to survive in the environment long enough to be endemic or cause recurrent outbreaks.

Methods for isolation of *P. multocida* in water and sediment samples are described in Samuel et al. 2003. In this study the authors failed to find avian cholera serotype 1 after outbreaks, which suggested that carriers were a more important factor. However, the methods applied in this study for *P. multocida* isolation may have been insensitive. Bacteria need to be present in concentrations of two to 18 organisms per milliliter in wetland water samples using the culture methods described in Samuel 2003.

A polymerase chain reaction (PCR) analysis, which can detect the organism in lower concentrations, might be a better approach for HS detection.

Elk that survive an HS outbreak may become carriers of HSPm and may have the potential to cause future outbreaks. This hypothesis can be tested in two ways. First, use passive surveillance to test more hunter-killed elk, specifically ones on the NER, in an attempt to find the organism. The best chance to isolate HSPm would be in tissue from tonsils . One study showed that HSPm persisted in the tonsils and was isolated from these tissues 229 days after experimental infection of HS. Second, use an active surveillance system, taking blood samples during and after outbreaks at pre-designated time intervals. If antibodies were found in elk surviving the outbreak, this would indicate that elk were infected but survived and, therefore, could be carriers. Further testing would be necessary to determine if seropositive surviving elk mounted an immune response sufficient to kill the pathogen or if a carrier state can be sustained.

Confirmation of elk as carriers would require isolating live *P. multocida* organisms with the DNA profile and serotype responsible for the HS outbreak from elk that survived the outbreak and using these isolates in challenge studies of naïve animals. This method was used in a study of avian cholera in lesser snow geese, where a *P. multocida* serotype 1 was isolated from one of 298 lesser snow geese and was found to be pathogenic when injected into another bird (Samuel 1997).

CONCLUSION

My data did not support the hypothesis that rodents are a reservoir for HS in the NER, but my detection sensitivity for HSPm was low, and other factors may have affected my results. Because reservoirs may carry the organism at very low prevalences under non-epizootic conditions, rodent sampling during epizootic HS would provide a better indication if rodents are potential reservoirs. Current disease surveillance should continue, including testing elk for *P. multocida*, and routine DNA fingerprinting of *P. multocida* isolates during outbreaks. Further testing from wintering elk on the NER would be needed to confirm whether *P. multocida* B:3,4 HhaI 036/HpaII is consistently the cause of HS on NER, and whether this specific DNA profile can be found in normal healthy elk.

Weather variables analyzed in this study produced equivocal results, with a potential relationship to HS incidence observed in 1993, but not in 1986. Calves and cows were shown to be at a greater risk than other age-gender classes. Although higher HS incidence was clearly observed at the Shop and Nowlin feedgrounds, the feedground characteristics evaluated in this study showed no significant relationship to HS incidence.

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APPENDICES

APPENDIX A

OCCURRENCE OF HS OUTBREAKS ON THE NATIONAL ELK REFUGE

Appendix A. Monthly incidence of hemorrhagic septicemia on the National Elk Refuge, 1986 -2001

	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
January	4	0	0	0	0	0	0	32	0	0	0	0	0	0	0	1
February	24	4	0	0	0	0	0	39	0	3	0	0	0	3	0	0
March	10	7	0	0	0	0	0	40	0	0	0	0	0	0	0	0
April	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Total	38	11	0	0	0	0	0	113	0	3	0	0	0	3	0	1