A survey of free-ranging and captive wild ungulates for tuberculosis and other diseases
by Brian Randolph Hood

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences
Montana State University

Abstract:
Today, tuberculosis and other diseases are significant problems in the game ranching industry around the world.

The threat of disease and parasites associated with game ranching and their potential impact on wildlife, domestic livestock, and human health has prompted concern among wildlife managers, cattlemen, and the public (Merritt, 1992). An outbreak of tuberculosis in a southcentral Montana game ranch prompted this study to determine whether the disease had been transmitted to surrounding wildlife. A disease surveillance project was conducted on hunter-killed and specially collected free-ranging deer and elk in proximity to the game ranch. Specimens from 41 mule deer (Odocoileus hemionus) , 3 white-tailed deer (Odocoileus virginianus) , and one elk (Cervus elaphus nelsonI) were tested for tuberculosis, brucellosis, leptospirosis, epizootic hemorrhagic disease, bluetongue, parainfluenza virus, bovine viral diarrhea, infectious bovine rhinotracheitis, and various parasites. Specimens from game-ranched elk and pronghorn (Antilocaprae americana) were also collected and tested for comparison. Methods used for testing included gross and histopathologic examination, bacterial culture and identification techniques, serologic tests, and parasitological exams. Mycobacterial lesions were noted in 2 free-ranging mule deer, and Mycobacterium bovis was isolated from one of these upon culture. This was the first record of tuberculosis in free-ranging mule deer in the United States. The discovery received widespread publicity and prompted state legislation that more clearly defined regulatory responsibility for Montana game farms. Wild deer also revealed antibodies against leptospirosis, epizootic hemorrhagic disease, bovine viral diarrhea, and parainfluenza-3. Parasites identified from free-ranging Cervidae included lungworms, gastrointestinal helminths, tapeworms cysts, and ectoparasites. Incidental findings in free-ranging cervids included actinobacillosis-like lesions; lymphosarcoma; parasitic lesions in liver and lung specimens; and Sarcocystis sp. in cardiac muscle.

Examination of game-ranched animals revealed giant liver flukes and antibodies to bluetongue virus, in addition to many of the antibodies, diseases, and parasites found in the free-ranging animals. It is suggested that wildlife disease surveillance continue in regions' where serious diseases are known to exist in captive animals. Surveillance results might be used in preventing disease outbreak in Montana's native wildlife.

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APPROVAL

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Brian Randolph Hood

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Approved for the College of Graduate Studies

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ABSTRACT

Today, tuberculosis and other diseases are significant problems in the game ranching industry around the world. The threat of disease and parasites associated with game ranching and their potential impact on wildlife, domestic livestock, and human health has prompted concern among wildlife managers, cattlemen, and the public (Merritt, 1992). An outbreak of tuberculosis in a southcentral Montana game ranch prompted this study to determine whether the disease had been transmitted to surrounding wildlife. A disease surveillance project was conducted on hunter-killed and specially collected free-ranging deer and elk in proximity to the game ranch. Specimens from 41 mule deer (*Odocoileus hemionus*), 3 white-tailed deer (*Odocoileus virginianus*), and one elk (*Cervus elaphus nelsoni*) were tested for tuberculosis, brucellosis, leptospirosis, epizootic hemorrhagic disease, bluetongue, parainfluenza virus, bovine viral diarrhea, infectious bovine rhinotracheitis, and various parasites. Specimens from game-ranched elk and pronghorn (*Antilocaprae americana*) were also collected and tested for comparison. Methods used for testing included gross and histopathologic examination, bacterial culture and identification techniques, serologic tests, and parasitological exams. Mycobacterial lesions were noted in 2 free-ranging mule deer, and *Mycobacterium bovis* was isolated from one of these upon culture. This was the first record of tuberculosis in free-ranging mule deer in the United States. The discovery received widespread publicity and prompted state legislation that more clearly defined regulatory responsibility for Montana game farms. Wild deer also revealed antibodies against leptospirosis, epizootic hemorrhagic disease, bovine viral diarrhea, and parainfluenza-3. Parasites identified from free-ranging Cervidae included lungworms, gastrointestinal helminths, tapeworms cysts, and ectoparasites. Incidental findings in free-ranging cervids included actinobacillosis-like lesions; lymphosarcoma; parasitic lesions in liver and lung specimens; and *Sarcocystis* sp. in cardiac muscle. Examination of game-ranched animals revealed giant liver flukes and antibodies to bluetongue virus, in addition to many of the antibodies, diseases, and parasites found in the free-ranging animals. It is suggested that wildlife disease surveillance continue in regions where serious diseases are known to exist in captive animals. Surveillance results might be used in preventing disease outbreak in Montana's native wildlife.

INTRODUCTION

Activities associated with game farms have increased over the last two decades throughout North America (Essey, 1991). Producers are supplying venison, antlers, and controlled hunts.

A number of factors described in Miller and Thorne (1993) are conducive to the establishment and dissemination of serious diseases among game-ranched animals. Disease surveillance programs are lacking. Extensive movements of breeding stock provide many opportunities for contamination of healthy animals. Various aspects of the biology and behavior of captive cervids, including social structures and interspecific variations in susceptibility to parasitic and other infectious agents, can create a disease-prone environment. Finally, requirements for maintaining healthy captive herds are not well understood. As a result, poor husbandry practices can contribute to the spread of disease (Krogh and Jenson, 1988).

Game-ranched cervids present a threat to native wildlife of Montana by transmitting disease or parasites (Lanka and Guenzel, 1991). Several recent publications have highlighted potential problems of disease and parasite transmission between game-ranched wildlife and native wildlife (Geist, 1991, Lanka and Guenzel, 1991, Ferlicka, 1991, Thorne et al., 1991). There have been captive cervid

facilities in Montana quarantined due to outbreaks of bovine tuberculosis (Ferlicka, 1991). The disease can be lethal to wild ungulates (Lanka and Guenzel., 1991) and humans are endangered as well (Fanning and Edwards, 1991). Tuberculosis is only one of several diseases which could be detrimental to native wildlife.

In April 1993, gross and microscopic lesions consistent with tuberculosis were found in elk (*Cervus elaphus nelsoni*) from Elk Valley Game Ranch (R. Clarke, pers. comm.). The ranch is situated northeast of Hardin, Bighorn County, Montana. Culture results were positive for *Mycobacterium bovis*, the bacterium that causes bovine tuberculosis (J. Payeur, unpubl. data).

A 1967-68 study of mule deer in eastern Montana indicated that cattle and mule deer sharing common ranges could readily exchange parasitic diseases (Worley and Eustace, 1972). Pocket Creek Ranch borders the northern boundary of Elk Valley Game Ranch and includes 700 to 800 head of cattle. The former ranch is also home to large numbers of wild mule deer (*Odocoileus hemionus*), and smaller numbers of wild white-tailed deer (*Odocoileus virginianus*) and elk. Because of the concern for spread of tuberculosis from captive cervids to surrounding cattle and free-ranging wildlife, a disease surveillance project examining cervids from land adjacent to the game ranch was conducted.

The primary objective of the study was to determine

whether bovine tuberculosis had been transmitted to surrounding wildlife populations. A secondary objective was to survey for other diseases and parasites in a free-ranging mule deer population, and to compare their pathogens with the kinds and numbers found in the captive ungulates of the Elk Valley Game Ranch.

The study was conducted through cooperation with the Departments of Biology and Veterinary/Molecular Biology at Montana State University, the Montana Department of Fish, Wildlife, and Parks (MDFWP), the Montana Department of Livestock Veterinary Diagnostic Laboratory (MVDL), and the USDA Animal and Plant Health Inspection Services (APHIS). Funding was provided by the MDFWP, the Rocky Mountain Elk Foundation (project no. MT93214), and the Montana Wildlife Federation.

STUDY AREA

Elk Valley Game Ranch is located in southcentral Montana, northeast of Hardin (45°55'N, 107°32'W). Animals are held in captivity by a single woven wire fence, 3.5 meters in height. The ranch consists of two pastures totaling 1,050 hectares and a 650- hectare shooter pen where fee-based hunting is conducted (Rhyan et al, 1995). In 1993, the game ranch was home to approximately 150 elk, 16 bison (*Bison bison*), 19 pronghorn (*Antilocapra americana*), and 10 mule deer (Rhyan et al., 1995).

Pocket Creek Ranch is a privately owned cattle operation and borders the entire north end of Elk Valley Game Ranch. Its western border lies along the Bighorn River. An aerial survey revealed an estimate of 5.4-7.7 mule deer per square mile (MDFWP, unpubl. data). Smaller numbers of white-tailed deer were present in riparian habitat along the Bighorn River. Surveillance was conducted on approximately 10 square miles immediately north of the game ranch.

Both Pocket Creek Ranch and Elk Valley Game Ranch consist of central grassland and eastern Montana ponderosa pine forest vegetative rangeland types (Payne, 1973) with breaks and badlands-type terrain. Climate is semiarid continental with warm summers and cold winters. The annual mean temperature is approximately 7 °C, with extremes

ranging from -5.5 °C for January and 23 °C for July (NOAA, 1993). Yearly precipitation averages 33 centimeters.

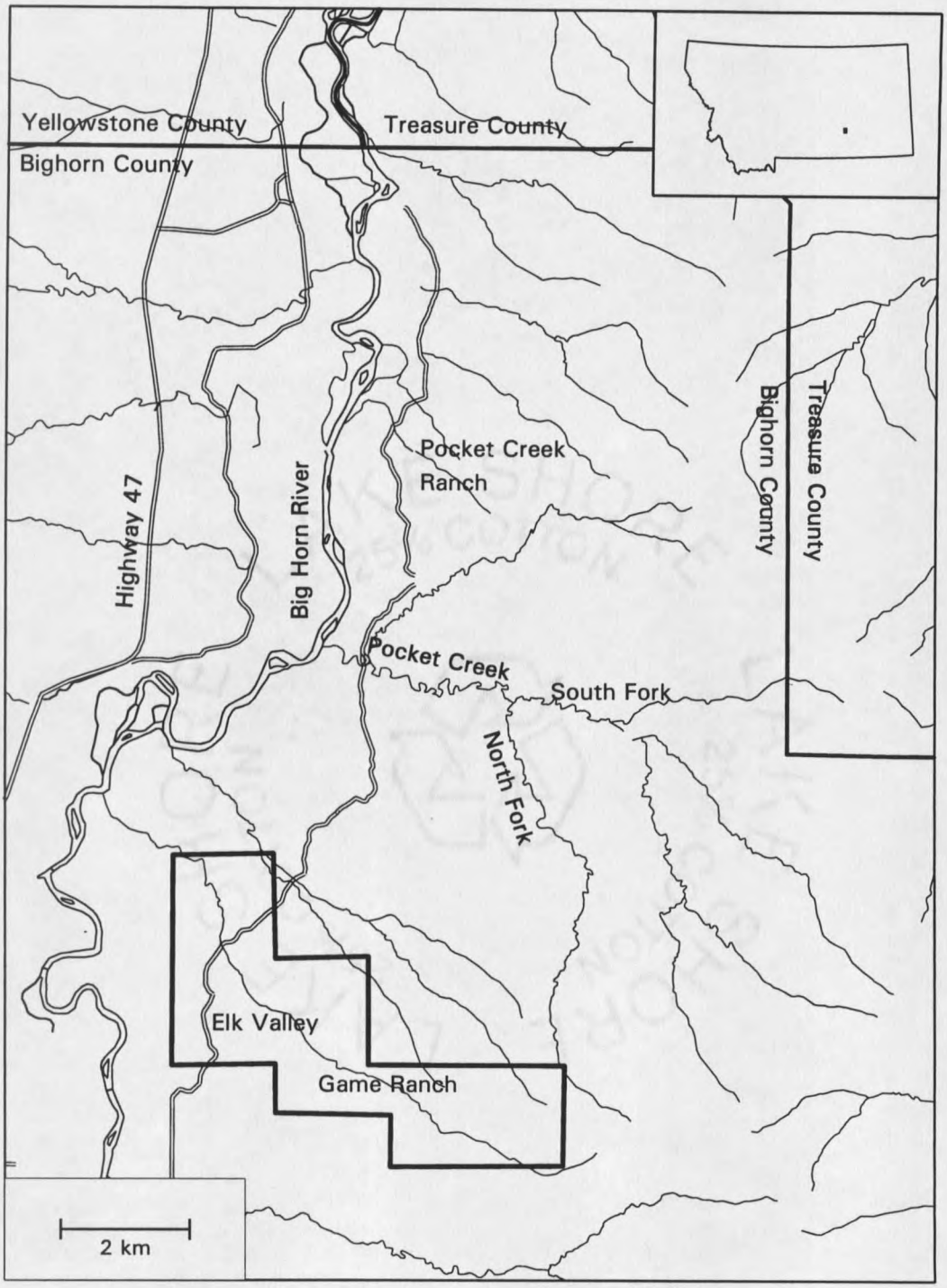

Figure 1. Map of study area.

METHODS

Specimen Collection

Field work began in the fall of 1993 and continued through the year 1994. Field specimens from Pocket Creek Ranch were collected from hunter-kills or by direct collection methods as permitted by MDFWP. In October and November of 1993, 2 white-tailed deer and 8 mule deer were collected by hunters. A special collection of 30 mule deer and 1 white-tailed deer took place on December 10, 1993. A second special collection of 3 mule deer took place on January 28, 1994. Thirty-five additional deer samples were taken during a third special collection in August of 1994. January 15, 1994, one elk was collected by MDFWP personnel 4 miles east of Pompey's Pillar, northwest of Pocket Creek Ranch.

Specimens from 39 captive elk and 10 captive pronghorn were collected from within Elk Valley Game Ranch for comparative evaluations from November 1993 to November 1994.

Sampling of hunter-killed cervids was accomplished by supplying hunters with collection kits. Each kit consisted of two garbage bags, one for the head and neck and one for the abdominal and thoracic viscera. The kit also included a numbered tag for each garbage bag and three numbered blood tubes. One tube contained the anti-coagulant heparin. Another contained the anti-coagulant EDTA. The last tube

was a serum-separator tube containing no anti-coagulant. Pick-up of hunter-killed specimens was accomplished on a regular basis. Direct collection methods were similar to hunter-killed collections, and was assisted by the USDA veterinary medical officer in Billings, Montana Department of Livestock veterinarians, and MDFWP personnel. Samples were placed in Rubbermaid Rough-neck containers and transported to the MDFWP Wildlife Laboratory where they were inspected.

Gross inspection

Heads and necks were dissected and tonsils and lymph nodes removed. Lymph nodes collected were the medial and lateral retropharyngeals, mandibulars, and parotids. Lungs were inspected for surface lesions, palpated, and dissected to expose lesions that could exist underneath the surface. From the thorax, samples of lung and heart were collected. The tracheobronchial and mediastinal lymph nodes were also collected. From the abdomen, the hepatic and mesenteric lymph nodes, spleen, liver, and kidneys were collected. Each tissue specimen was sliced with a scalpel and inspected thoroughly for gross lesions. Following inspection, half of each tissue specimen or lesion was placed in 10% neutral buffered formalin and half was frozen in a plastic bag.

Histopathology

Lymph nodes of the head fixed in formalin were sent to the USDA National Veterinary Services Laboratories (NVSL)

for processing and histologic examination by methods described in Rhyan et al. (1995).

Formalin-fixed tissues of the thorax and abdomen were routinely processed and embedded in paraffin at the MVDL. A microtome was used to cut five micrometer sections which were stained with hematoxylin and eosin. All sections were examined with a compound microscope and lesions recorded. Special stains were used as needed.

Bacteriology

Frozen specimens of tonsils, head and thoracic lymph nodes, and lungs were sent to the USDA NVSL for mycobacterial isolation attempts by methods described in Payeur et al.(1993). In some cases, attempts were also made to culture gross lesions observed at post-mortem examination.

Serology

All blood samples were submitted to the MDVL for testing. Whole blood was subjected to biochemistry and hematology evaluation. The tube without anti-coagulant was centrifuged to separate blood serum from cellular components. Serum was then tested for antibodies to brucellosis, leptospirosis, bluetongue, epizootic hemorrhagic disease, infectious bovine rhinotracheitis, bovine virus diarrhea, and parainfluenza virus 3.

Parasitology

The meninges of the brain and the brain cavities were

examined grossly for the presence of adult parasitic nematodes. The terminal 25 centimeters of the common carotid arteries and in some instances the proximal portion of the internal maxillary arteries were opened with surgical scissors and the lumina examined for adult nematodes. Often, auditory canals and nasal cavities were exposed to reveal arthropods. Specimens were placed in 70 percent ethanol for later study.

Ear ticks were identified according to the descriptions by Thorne et al. (1982). Identification of nose bot larvae genera was based on physical appearance and location of the parasite. Species identification of second and third-stage larvae was based on number of rows of spines on dorsal and ventral cuticular segments, on the number of spines in the upper row of the anal patch, and on the total number of spines on the anal patch (Bennett and Sabrosky, 1962).

The pleural surface of the lungs was examined externally for cysts or other abnormalities. The trachea was opened and examined. Air passages in all lobes of the lung were opened and examined. Cysts found on the surface of the lungs were opened by incision with a scalpel. Encysted tapeworm larvae were removed with forceps and fixed in a mixture of 95 parts 70 percent ethanol and five parts glycerin. At a later date, larval scolices were dissected and examined under a Leitz Ortholux compound microscope, where hooks could be counted and measured for identification

(Verster, 1969). Lungworms were removed from air passages, counted, and fixed in glycerine-alcohol. Preserved lungworms were cleared in glycerine and examined under an Ortholux microscope for specific identification (Ransom 1911).

Livers were serially incised and visually examined for scarring or pigmented lesions suggestive of fluke infection. Liver surfaces were also examined for tapeworm cysts. Flukes were removed from the liver with forceps and fixed in 70 percent ethanol. Adult trematodes were identified by location in host, length, and morphology (Thorne et al., 1982). The spleen was examined in a similar manner to the liver.

Regions of the gastrointestinal tract inspected for parasites were the abomasum, small intestine, and large intestine. The contents of each area were washed separately onto 24-, 60-, or 80-mesh screens. Gastrointestinal epithelium was also scraped to remove attached worms. Ingesta were then placed in labeled jars, and 10 percent formalin was added for preservation. Later, washed ingesta were examined in illuminated trays to recover worms. Recovered nematodes were separated by sex, counted, cleared in glycerin, and examined with an Ortholux compound microscope for identification.

When male nematodes were found, genus and species were identified by physical appearance, total worm length,

location in host, esophagus length, spicule length, and other distinguishing morphological characteristics such as cervical papillae or cuticular striations. In cases where only female nematodes were found, identification was often only possible to genera level. Identification of female worms was based on physical appearance, total worm length, location in host, egg size, esophagus length, length from vulva to tail tip, length from anus to tail tip and other unique characteristics such as vulvar shape, cervical papillae, and cuticular morphology. Three references were used in nematode identification; Thorne et al. (1982), Ransom (1911), and Gibbons and Khalil (1982).

Adult tapeworms were examined under a dissecting scope for identification. Cestodes were identified by worm size and proglottid morphology (Thorne et al., 1982).

The kidneys were also briefly scanned for parasites. Following inspection of the surfaces, kidneys were sliced in halves, exposing the renal pelvis. In some instances, other external parasites and tapeworm cysts found in the omentum were collected in addition to the usual postmortem material. Identification of ticks was based on the number of festoons and appearance of the spiracular plate (Thorne et al., 1982). Identification of deer keds was based on coxa and abdominal morphology (Furman and Catts, 1970).

Fecal samples were collected during post-mortem examination. Samples came from the rectum and varied in

weight from 5-30 grams. To extract larvae, the feces were subjected to the Baermann technique (Baermann, 1917). Fecal samples were retained in the Baermann apparatus for at least 12 hours, ensuring complete larval migration. Larva suspensions were withdrawn into a Petri dish and counted under a dissecting microscope. Identification at the generic level was accomplished using the dissecting microscope. In some instances, species was determined by measuring the larvae under an Ortholux compound microscope.

A modified Lane centrifugal flotation technique (Dewhirst and Hansen, 1961) was used to detect nematode and cestode ova, and coccidian oocysts in fecal samples. A saturated sodium chloride solution (sp. gr. 1.18-1.20) was used as the egg flotation medium. Eggs were identified and counted using an Ortholux compound microscope.

RESULTS

Bovine Tuberculosis

From October 1993-January 1994, tissue samples from 44 free-ranging deer collected from Pocket Creek Ranch, and one free-ranging elk collected near Pompey's Pillar, were tested for tuberculosis (Table 1).

Table 1. Tissues samples collected from Pocket Creek Ranch deer and Pompey's Pillar elk for tuberculosis survey.

Survey	Head	Thorax	Abdomen	Total
Hunter-killed				
White-tailed deer	1	2	2	2
Mule deer	4	8	4	8
Special Collection				
White-tailed deer	1	1	1	1
Mule deer	32	33	31	33
Elk	1	1	1	1
Total	39	45	39	45

At necropsy, lesions suggestive of tuberculosis were observed in 2 adult female mule deer. Gross lesions were visible in hepatic and thoracic lymph nodes of deer #168. The most evident changes occurred in the right tracheobronchial lymph node which was enlarged by approximately two times. Upon incision, the node was gritty in consistency and grayish yellow in color. Changes in this lymph node were diffuse. All other lesions visible in deer #168 were focal, ranged from 1-8 millimeters in size, and were also gritty upon incision. Gross lung lesions were not observed in this deer.

In deer 210, grossly visible lesions suggestive of tuberculosis were found in the right parotid and mandibular lymph nodes. These lesions were small (about 2 mm) in size, yellow, and also gritty upon incision. Gross lung lesions were not present in deer 210.

Histologic examination revealed microscopic lesions morphologically consistent with tuberculosis in sections of palatine tonsil, retropharyngeal lymph nodes, thoracic lymph nodes, and a hepatic lymph node from deer 168 (Rhyan et al., 1995). Rare, acid-fast bacilli were scattered in necrotic material, macrophages, and multinucleate giant cells of all lesions (Rhyan et al., 1995). Microscopically, the head lymph node lesions from deer 210 were examined and pyogranulomatous lymphadenitis was diagnosed. Lesions contained club colonies morphologically similar to those of *Actinomyces bovis* and *Actinobacillus lignieresi* in cattle (J. Rhyan, pers. comm.).

Also noted on histologic examination was a single microgranuloma suggestive of tuberculosis in one head lymph node of deer 173. However, acid-fast bacilli were not demonstrated in the lesion (Rhyan et al., 1995).

Mycobacterium bovis was isolated from pooled head lymph node specimens and from pooled thoracic lymph node specimens from deer 168 by methods described in Payeur et al. (1993) (Table 2). Attempts to culture *M. bovis* from all other deer were unsuccessful.

Table 2. Tuberculosis culture results for Pocket Creek Ranch deer and Pompey's Pillar elk.

Species	# positive/# examined	% positive
Mule deer	1/41	2.4
White-tailed deer	0/3	0.0
Elk	0/1	0.0
Total	1/45	2.2

Serology

From November 1993 to November 1994, blood serum was collected from animals taken from Pocket Creek Ranch and Elk Valley Game Ranch and tested by the MVDL for the presence of antibodies against brucellosis, leptospirosis, bluetongue, epizootic hemorrhagic disease, infectious bovine rhinotracheitis, bovine virus diarrhea, and parainfluenza virus 3 (Tables 3-6). Approximately one-third of Pocket Creek Ranch mule deer had antibodies to epizootic hemorrhagic disease virus, bovine virus diarrhea, and parainfluenza virus 3. The most prevalent antibodies detected in Pocket Creek Ranch white-tailed deer were against *L. interrogans* serovar *tarassovi*. Elk Valley Game Ranch elk had antibodies against epizootic hemorrhagic disease, bovine virus diarrhea, and parainfluenza virus 3. Most pronghorn collected from Elk Valley Game Ranch possessed antibody titers against epizootic hemorrhagic disease virus or *L. interrogans* serovar *tarassovi*. Animals from Elk Valley Game Ranch and Pocket Creek Ranch did not

present antibodies against infectious bovine rhinotracheitis.

When considering information from serologic studies, the reader is cautioned to be mindful that seropositive animals are not necessarily infected and that interpretation of serologic findings are subject to certain restrictions (Shotts, 1981). The demonstration of antibody from serum collected from an animal may mean that the animal is in the early stages of the disease or that the antibody noted represents a residual titer from a previous exposure. Antibodies against certain organisms may remain for several years.

Table 3. Serological test results from Pocket Creek Ranch mule deer.

Disease	# positive/# tested	% positive
Brucellosis	0/130	0
Leptospirosis-- *autumnalis* serovar	3/130	2
Leptospirosis-- *tarassovi* serovar	3/130	2
Bluetongue	0/130	0
Epizootic hemorrhagic Disease	29/95	31
Bovine viral diarrhea	11/35	31
Parainfluenza virus 3	13/35	37

Table 4. Serological test results from Pocket Creek Ranch white-tailed deer.

Disease	# positive/# tested	% positive
Brucellosis	0/12	0
Leptospirosis-- *autumnalis* serovar	1/12	8
Leptospirosis-- *tarassovi* serovar	3/12	25
Bluetongue	0/12	0
Epizootic hemorrhagic disease	0/11	0
Bovine virus diarrhea	0/1	0
Parainfluenza virus 3	0/1	0

Table 5. Serological test results from Elk Valley Game Ranch elk.

Disease	# positive/# tested	% positive
Brucellosis	0/39	0
Leptospirosis-- *autumnalis* serovar	1/39	3
Leptospirosis-- *tarassovi* serovar	3/39	8
Bluetongue	3/39	8
Epizootic hemorrhagic disease	6/26	23
Bovine viral diarrhea	12/39	31
Parainfluenza virus 3	5/39	13

Table 6. Serological test results from Elk Valley Game Ranch pronghorn.

Disease	# positive/# tested	% positive
Brucellosis	0/7	0
Leptospirosis-- *autumnalis* serovar	0/7	0
Leptospirosis-- *tarassovi* serovar	4/7	57
Bluetongue	0/7	0
Bovine viral diarrhea	4/7	57
Parainfluenza virus 3	1/7	14

Two elk collected from Elk Valley Game Ranch were suspect reactors for brucellosis using the standard plate agglutination test. However, both elk were negative on the rivanol and rapid card tests.

Blood sera collected from both Elk Valley Game Ranch and Pocket Creek Ranch were tested for 8 serovars, or subgroups, of *Leptospira interrogans*. Animals from Pocket Creek Ranch and Elk Valley Game Ranch did not test positive for the serovars *pomona, hardjo, grippotyphosa, icterohaemorrhagiae, canicola,* or *bratislava*. Therefore, only test results for the serovars *autumnalis* and *tarassovi* are presented in the tables.

Parasitology

Heads, carotid arteries, lungs, livers, mesenteries, and gastrointestinal contents from mule deer, white-tailed deer, elk, and pronghorn were examined to detect the presence of parasitic helminths (Table 7-9). All deer were

collected from Pocket Creek Ranch. Elk and pronghorn antelope were collected from Elk Valley Game Ranch.

Pocket Creek Ranch mule deer were infected with lungworms, gastrointestinal nematodes, and tapeworm cysticerci; the most prevalent parasitic helminth was *Taenia hydatigena* cysticerci. Worm burdens were generally low. Elk Valley Game Ranch elk were infected with small numbers of giant liver flukes and gastrointestinal nematodes. *Ostertagia* sp. and *Oesophagostomum venulosum* were most prevalent in sampled elk. Pronghorn collected from Elk Valley Game Ranch were infected with lungworms and large numbers of gastrointestinal nematodes. The most common parasitic helminth collected from pronghorn was *Haemonchus contortus*.