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SURVEY OF FREE-RANGING ELK FROM WYOMING AND MONTANA FOR SELECTED PATHOGENS

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ABSTRACT: From December 1991 through January 1995, a disease survey was conducted on herds of free-ranging, hunter-killed elk (*Cervus elaphus nelsoni*) from three areas in proximity to Yellowstone National Park (YNP), Wyoming (USA), after tuberculosis caused by *Mycobacterium bovis* was discovered in a captive herd of elk in the area. Complete or partial sets of specimens from 289 elk collected between December 1991 and January 1993 were examined histologically; no mycobacterial lesions were observed. Lesions of tuberculosis were not detected in tonsils or lymph nodes of the head from an additional 99 hunter-killed, adult elk from one area (area 2) collected in January 1995. Neither *M. bovis* nor *M. paratuberculosis* were isolated from any of the specimens cultured. Antibodies to *Brucella abortus* were detected in serum samples from 0%, 1%, and 1% of elk from three areas sampled (areas 1, 2, and 3), respectively. *Brucella abortus* biovar 1 was isolated from multiple tissues from one seropositive animal from area 3. Larvae with morphology consistent with *Dictyocaulus* sp. were found in 12%, 14%, and 0% of fecal specimens tested from areas 1, 2, and 3, respectively. *Pasteurella multocida* and *Actinomyces pyogenes* were isolated from a lung with purulent bronchopneumonia and abscesses.

Key words: Elk, *Cervus elaphus*, Yellowstone National Park, Survey, Brucella, Mycbacterium, brucellosis, tuberculosis, paratuberculosis, *Neolipoptena ferrisi*, Montana.

INTRODUCTION

Tuberculosis, caused by *Mycobacterium bovis,* has rarely been diagnosed in free-ranging cervids in North America. It has been reported in wild white-tailed deer (*Odocoileus virginianus*) in New York, USA (Levine, 1934; Friend et al., 1963), Ontario, Canada (Belli, 1962), and in Michigan, USA (J. Payeur, unpubl.). Gross lesions consistent with tuberculosis were observed in free-ranging elk (wapiti, *Cervus elaphus nelsoni*), mule deer (*Odocoileus hemionus*), and moose (*Alces alces*) in the Canadian National Buffalo Park near Wainwright, Alberta (Hadwen, 1942). Recently, tuberculosis was confirmed in a free-ranging elk in Manitoba, Canada, and a free-ranging mule deer in south central Montana, USA (Rhyan et al., 1995). In each of these instances, *M. bovis*-infected cattle, bison (*Bison bison*), or captive elk herds were in the vicinity and were considered likely sources of sporadic infection in wild ungulates. This occurrence of tuberculosis in captive herds of deer and elk in several states and provinces in North America (Essey and Koller, 1994) raised concerns about the health of wild cervid populations. This is especially true where captive cervids are kept in areas coterminous with high populations of their free-ranging counterparts.

In 1991, tuberculosis was diagnosed in a captive herd of 143 elk located near the northern border of Yellowstone National Park (YNP), Wyoming, USA (Thoen et al., 1992). Based on single cervical tuberculin skin testing (SCT), the herd had 28 responders, and at necropsy, one of nine SCT-positive animals had tuberculous lung lesions from which *M. bovis* was isolated. Based on laboratory tests at the National Veterinary Services Laboratories (NVSL), Ames, Iowa (USA), on specimens subsequently collected from that herd *M. bovis*

infection was confirmed in three additional elk (J. Payeur, unpubl.). Because of the proximity of the infected captive herd to the free-ranging northern YNP elk and the possibility of across-the-fence exposure of wild elk to *M. bovis* or escape of *M. bovis*-infected elk into the wild, a study was undertaken to survey the free-ranging elk population in the area for tuberculosis.

Our objective was to determine the prevalence of tuberculosis in hunter-killed elk of YNP. Because of the opportunity to collect a variety of specimens from a large number of carcasses, laboratory examinations for brucellosis, paratuberculosis, dictyocauliasis, fascioliasis, and other diseases were also conducted.

MATERIALS AND METHODS

Specimens were collected from hunter-killed elk during late-season hunts in three geographic areas. Area 1 was a private 54,000-ha ranch (45°30′N, 111°22′W) located in southwestern Montana. The resident elk herd was estimated at 2,500 animals, and there was little evidence for interaction of the resident herd with YNP elk (K. Alt, pers. comm.). Collections were done during 3-day hunts in early December of 1991 and 1992. Area 2 was a large tract of mostly public land, consisting of approximately 30,000 ha surrounding the upper Yellowstone River adjacent to the northern YNP boundary in the vicinity of Gardiner, Montana (45°03′N, 110°42′W). This area surrounded the private game farm from which tuberculous elk were identified in 1991. Specimens were collected from elk in accessible southern portions of area 2 during hunts in January and February of 1992. In addition, heads were collected from 3-yr-old and older elk being taken through the area 2 hunter check station in January 1995. The large majority of elk that winter in area 2 were from the northern Yellowstone herd (Craighead et al., 1972). Area 3 consisted of approximately 20,000 ha of mostly private land located on the upper Madison River (45°00′N, 111°35′W), 35 km from the northwest corner of YNP. Approximately half of the elk taken in late-season hunts in area 3 summer in the northwestern part of YNP along the headwaters of the Gallatin and Madison Rivers (K. Alt, pers. comm.). Specimens were collected in area 3 in January of 1992 and 1993.

Successful hunters were located during field-dressing procedures or as carcasses were dragged to vehicles. With hunter permission, each animal's age, sex, and any abnormalities were recorded. Ages were determined by teeth wear and replacement (Quimby and Gabb, 1957). Carcasses were then tagged for head removal at the check station, and specimens were collected from carcasses and viscera. A complete set of field specimens consisted of whole and heparinized blood samples obtained from the heart or body cavity, approximately 200 g of feces, any tissues with visible lesions, mammary gland, supramammary lymph nodes, uterus or placentome (testicles from males), amnionic fluid, liver (including hepatic lymph nodes), mesenteric lymph nodes, kidney, distal ileum, trachea, lungs, heart, and head. Samples of whole blood were also collected by hunters from additional elk in each area, and from 16 elk live-trapped in area 3 in March 1992.

Each day, field specimens were transported to the Montana Wildlife Research Laboratory (MWRL), Bozeman, Montana, where livers, hearts, and lungs were examined, and representative specimens were collected. Heads were dissected and palatine tonsils and lymph nodes (medial and lateral retrophopharyngeal, mandibular, and parotid) were collected and bisected. Half of each tissue specimen was frozen, and the other half was fixed in 10% neutral buffered formalin. Lymph nodes of the head, thorax, and abdomen were pooled in separate containers. Formalin-fixed and frozen tissues were sent to the NVSL for histopathologic and bacteriologic examination. Fecal specimens were divided and sent to the NVSL for mycobacterial culture attempts and to the Montana Veterinary Molecular Biology Laboratory (MVMBL), Bozeman, Montana, for examination for *Dictyocaulus* sp. Serum samples were sent to the Montana Veterinary Diagnostic Laboratory (MVDL) for serologic testing. One ectoparasite removed from the head of an area 1 cow elk was sent to the NVSL for identification.

Formalin-fixed tissue specimens were serially incised and examined for lesions. Representative portions of each specimen were embedded in paraffin; 5 μm sections were stained with hematoxylin and eosin. Selected sections were stained with Gram, Giemsa, Warthin-Starry, fuchsin acid fast (Henry and Cassidy, 1966), or Gomori's methenamine silver techniques (Grocott, 1955). All sections were examined by light microscopy and any lesions were recorded.

All specimens of uterus, amnionic fluid, mammary gland, and supramammary lymph nodes were cultured for *Brucella* spp. using the methods of Alton et al. (1988). In addition, retropharyngeal lymph nodes from the 1992 to 1993 season elk and other available tissues from elk seropositive for *Brucella* spp. were

TABLE 1. Numbers of elk from which tissue specimens and blood were collected and examined.

			Tissue specimens						
	Tissues collected	Blood collected	Head: tonsils & lymph nodes	Thorax: viscera & lymph nodes	Abdomen: viscera & lymph nodes	Ileum	Mammary gland	Reproductive organs[a]	Feces
Area 1	55	224	44	44	46	30	38	40U 2T	46
Area 2	248[b]	721	226[b]	142	141	134	74	104U 24T	122
Area 3	85	389[c]	75	62	63	58	42	55U 5T	59

[a] U: elk from which uterus was collected; T: elk from which testicles were collected.
[b] Number includes 99 elk from which only heads were collected in 1995.
[c] Number includes 16 elk live-trapped and sampled in 1992.

cultured. Isolates were biotyped using the methods of Alton et al. (1988). Specimens with gross or microscopic lesions considered suggestive of mycobacteriosis and all fecal specimens were cultured for mycobacteria using the procedures of Payeur et al. (1993). Pulmonary lesions from one animal were cultured for aerobic bacteria using the methods of Murray et al. (1995).

Livers were serially incised and visually examined for scarring or pigmented lesions suggestive of liver fluke infection. Thirty- to 50-g fecal specimens from each animal were examined for lungworm larvae using the Baermann (1917) technique. Specimens remained in the Baermann apparatus for a minimum of 12 hr to ensure complete larval migration. Larvae were then examined and identified by morphologic characteristics (Chapin, 1925). The ectoparasite was identified using the identification aids and procedures of Bequaert (1956) and Maa and Peterson (1987).

The standard plate agglutination test, standard tube agglutination test (Anonymous, 1965a), rivanol precipitation agglutination test, and the card test (Anonymous, 1965b) were used to detect antibodies to *Brucella* spp. In addition, the rapid screen test (Luchsinger and Pietz, 1972) and the complement fixation test (Martin and Ritchie, 1973) were also done. Serologic test results were interpreted using the recommendations of Morton et al. (1981).

RESULTS

Complete or partial sets of tissue specimens were collected from 55 elk from area 1, 149 elk from area 2, and 85 elk from area 3 from December 1991 through January 1993 (Table 1). Of the 289 elk from which sets of tissue were collected, 15 were female calves, 228 were adult females, 21 were bull calves, and 23 were adult bulls. Age and sex were not recorded for two animals. Ages ranged from 6 mo to 15 yr. In January 1995, only heads were collected from 99 additional female elk aged 3 yr and older from area 2 (Table 1).

Pulmonary abscesses and pleural adhesions were observed in one adult female elk from area 1; focal pneumonia was noted in one adult female elk from area 2. Gross lesions in other elk were minor and consisted of numerous tonsillar cysts or abscesses; occasional 1- to 5-mm, white to yellow, caseous foci in lymph nodes of the head; one firm, 2-cm-diameter red nodule in lung parenchyma; and occasional firm, 2- to 5-mm, white or pale yellow nodules in hepatic and pulmonary parenchyma. A single 2-cm pedunculated skin tumor was present on the ventral thorax of an aged female from area 2.

Microscopic lesions considered typical of tuberculosis in elk were not observed. Specially stained sections of pulmonary and tonsillar abscesses, lymph node lesions, and liver lesions were negative for acid-fast bacilli. Tissues from eight elk with inflammatory lung or lymph node lesions were cultured for mycobacteria. Cultures from seven of the elk were negative. *Mycobacterium avium* was isolated from a lung lesion from an elk from area 2. Histologically, the lesion was focal chronic fi-

TABLE 2. Results of serologic and bacteriologic tests for *B. abortus* in elk from the three study areas.

	Seropositive/ tested	Culture positive/ tested	Elk number	Serologic test results[a]						Culture results
				Plate	Card	RST	RIV	CF[b]	Tube	
Area 1	0/224	0/50								
Area 2	8/721	0/135	40	+100	+	4	+200	2 + 320	+200	Neg
			117	Neg	+	4	I200[c]	4 + 320	+50	Neg
			308	+50	+	4	+100	AC[d]	+200	ND[e]
			391	I100[c]	+	4	+200	4 + 80	+200	ND
			462	I100	+	4	+200	4 + 320	+200	ND
			538	Neg	+	4	+50	3 + 80	+100	ND
			775	+25	+	4	Neg	4 + 20	+200	ND
			838	125	+	4	+200	4 + 40	+100	ND
Area 3	3/389	1/63	5	+200	+	4	+200	AC	+200	+
			64	+25	+	4	+100	4 + 160	+100	ND
			151	Neg	+	4	+100	4 + 80	+100	ND

[a] Plate: standard plate agglutination test; Card: card test; RST: rapid screen test; RIV: rivanol precipitation agglutination test; CF: complement fixation test; Tube: standard tube agglutination test.

[b] Serum titer is the reaction reading (1+ to 4+) at the next higher dilution following the last 4+ reading.

[c] I: incomplete agglutination.

[d] AC: serum was anti-complementary.

[e] ND: culture was not done.

brinopurulent pneumonia with no demonstrable acid-fast bacilli.

Fecal specimens from 46, 122, and 59 elk from areas 1, 2, and 3, respectively, were cultured for *M. paratuberculosis*; results were negative. Additionally, sections of ileum from 222 elk and mesenteric lymph node sections from 250 elk were examined histologically, and no lesions suggestive of paratuberculosis were observed.

Serologic testing for brucellosis done on 224 specimens from area 1, 721 specimens from area 2, and 389 specimens from area 3 resulted in identification of zero, eight (1%), and three (1%) seropositive elk, respectively (Table 2). Two of the seropositive animals (40Y, an aged female; 117Y, a 3-yr-old pregnant female) from area 2 and one (5M, a 10-yr-old pregnant female) from area 3 were part of the sample from which sets of tissue specimens were collected. Cultures for *Brucella* spp. prepared from tonsils and lymph nodes of the head and from thoracic and abdominal lymph nodes of 40Y were negative. Cultures prepared from supramammary lymph nodes, amnionic fluid, uterus, ovary, and mammary gland of 117Y were also negative. *Brucella abortus* was isolated from ovary, uterus, and abdominal lymph nodes of 5M. The ovarian isolate was biotyped as *B. abortus* biovar 1. Cultures prepared from fetal tissues from 5M were negative. Cultures prepared from tissue specimens from 245 seronegative elk from the three areas were negative for *Brucella* spp.

Baermann tests for *Dictyocaulus* larvae were done on fecal specimens from 194 elk. Five (12%) of the 43 area 1 elk tested, 18 (14%) of the 133 area 2 elk tested, and none of the 18 area 3 elk tested were positive. Histologically, focal eosinophilic interstitial pneumonia considered a parasitic reaction was noted in lungs from several animals, and cross sections of nematodes morphologically consistent with *Dictyocaulus* spp. were present in airways of one animal.

Microscopically, the grossly observed 2- to 5-mm, white or pale yellow hepatic and pulmonary nodules were aggregates of lymphoid follicles occasionally containing a central aggregate of giant cells or partially surrounding an eosinophilic granuloma. These lesions were interpreted as inflammatory responses to degenerating para-

sites. Minor microscopic lesions consistent with a parasitic reaction were observed in various tissues; these included eosinophilic granulomas and focal eosinophilic lymphadenitis. Also observed in an abdominal lymph node of one area 1 elk were focal accumulations of giant cells with several associated unidentified microfilariae. The microfilariae were located extracellularly and in giant cells, where some had degenerated. The mean width of the organisms was 5 μm. Numerous sarcocysts were present in cardiac and skeletal myofibers of most elk examined from the three areas.

The ectoparasite collected from the head of an area 1 elk was identified as *Neolipoptena ferrisi* (National Veterinary Services Laboratories' Parasitology Reference Collection, Accession No. G91-126), a louse fly typically found on mule deer. No gross or microscopic evidence of fascioliasis was observed in the livers examined.

Chronic fibrinopurulent bronchopneumonia with focal abscessation occurred in lung tissue from one elk. *Pasteurella multocida* and *Actinomyces pyogenes* were isolated.

The grossly observed, white to yellow, caseous foci in lymph nodes of the head of several elk were actinobacillosis-like lesions. Microscopically, they consisted of encapsulated pyogranulomas surrounding deposits of Splendore-Hoeppli material containing colonies of tiny Gram-negative coccobacilli. They were noted on microscopic examination in 11 (25%) of 44 area 1 elk, 36 (16%) of 226 area 2 elk, and six (8%) of 75 area 3 elk from which lymph nodes of the head were examined. Additionally, similar lesions were noted in tonsils of several elk.

The pedunculated skin tumor was a fibropapilloma.

DISCUSSION

The late elk hunts at selected areas were well suited to a comprehensive disease survey because there was limited access to hunting areas and hunters routinely went through check stations. Also, the late hunts often provide excellent hunter success allowing sampling of numerous animals in a short time period. The collection of specimens from carcasses and viscera was labor intensive, requiring the presence of several skilled prosectors in the field. Although the 1995 study involved only the heads, it provided an abbreviated but still useful survey, especially pertaining to tuberculosis. In two past studies of naturally infected tuberculous captive elk, 15 (63%) of 24 (Rhyan et al., 1992) and 70 (52%) of 134 (Whiting and Tessaro, 1994) elk with *M. bovis* infection had lesions in head lymph nodes or tonsils.

The absence of histologic lesions of tuberculosis and paratuberculosis and negative culture results for *M. bovis* and *M. paratuberculosis* are consistent with the lack of previous reports of these diseases in YNP elk. In a recent survey of 795 hunter-killed elk from northwestern Wyoming, bovine tuberculosis was not detected (Williams et al., 1995). Paratuberculosis has been diagnosed in free-ranging ungulates, including bighorn sheep (*Ovis canadensis*) and a Rocky Mountain goat (*Oreamnos americanus*) (Williams et al., 1979), tule elk (*Cervus elaphus nannodes*) (Jessup and Behymer, 1981), and axis (*Axis axis*) and fallow (*Dama dama*) deer (Riemann et al., 1979). The disease also has been experimentally reproduced in elk, mule deer, and white-tailed deer (*Odocoileus virginianus*) (Williams et al., 1983) and has been reported in farmed red deer (*C. elaphus elaphus*) (Power et al., 1993). The isolation of *M. avium* from a lung lesion in this survey was considered incidental. The fibrinopurulent pneumonia was inconsistent with a mycobacterial lesion. *Mycobacterium avium* can cause tuberculous lesions in deer (de Lisle et al., 1995); however, it is more often isolated from deer without observed lesions (de Lisle and Havill, 1985).

The low prevalence of antibodies to *B. abortus* in elk from areas 2 (1.1%) and 3 (0.8%) in this study was consistent with results from a large serologic survey of northern YNP elk in the 1960s. In that

work, recently cited by Smith and Robbins (1994), 1.7% of 6,027 elk were *Brucella* spp.-test reactors. In a more recent serologic survey, (Aguirre et al., 1995), three (2%) of 143 northern YNP elk had *Brucella* spp. antibodies. This low seroprevalence was in marked contrast to results from northwestern Wyoming (USA) elk herds that were fed during winter months at feedgrounds. In a study of six feedground herds, the mean seroprevalence of brucellosis among adult female elk was 37% (range, 16 to 50%) (Herriges et al., 1992). Consistently low or zero seroprevalences have occurred on serologic surveys for brucellosis among free-ranging elk from other areas in the United States (McCorquodale and DiGiacomo, 1985).

Our finding of three elk seronegative on the standard plate agglutination test and positive on three or more other tests was similar to the results of Thorne et al. (1978), who found 31% of elk that were seropositive on other tests were negative on the standard plate test. The standard plate test is effective in detecting early *B. abortus* infection but may become negative in chronically infected animals (Morton et al., 1981).

Isolation of *B. abortus* biovar 1 from one of three seropositive elk cultured was consistent with the prevalence of isolation in previous field collections in various ungulate species. Thorne et al. (1978) isolated *B. abortus* biovar 1 from 17 (38%) of 45 elk, most of which were seropositive. Williams et al. (1993) cultured *B. abortus* from four of 11 seropositive Wyoming bison. Harrington and Brown (1976) isolated *B. abortus* biovar 1 from 137 (39%) of 355 cattle that were brucellosis reactors by one or more tests; other biotypes, strain 19, and *B. suis* were isolated from an additional 27 animals. Isolation of *B. abortus* from the minority of seropositive animals was expected. In chronic infections, distribution of the organisms may be limited to a few sites; within an infected lymph node, the organisms may be only focally distributed (McCullough et al., 1951). Hence, cultures from chronically infected animals will be negative unless portions of the lymph node or lymph nodes harboring the organism are cultured.

Prevalence of *Dictyocaulus* spp. in elk in this study was similar to previous surveys conducted during the winter. Barrett and Worley (1966) dissected lungs from 59 elk from approximately the same areas as sampled in this study and found three (16%) of 19 animals from the Gallatin drainage, two (8.7%) of 23 from the northern Yellowstone herd, and none of 17 elk from the Madison drainage to be infected. Bergstrom (1975) found 8%, 19%, and 15% infected elk in Teton County, Wyoming, during the winters of 1968, 1969, and 1972, respectively. Additionally, he demonstrated a marked increase in prevalence of infection during the spring months of each year. The *Dictyocaulus* spp. in elk has been referred to as *D. viviparus, D. viviparus* elk strain, and *D. hadweni*. Bergstrom (1982) states there are morphologic differences between *D. viviparus* and *D. hadweni*. Additionally, based on cross transmission experiments, there are differences in host resistance (Presidente et al., 1972).

Identification of the louse fly *N. ferrisi* on an elk is the first reported, but it probably represents an accidental occurrence. Mule deer are the usual hosts of *N. ferrisi*. Bequaert (1956) documented several similar instances of stray *N. ferrisi* found on hosts that share habitats with mule deer, including western white-tailed deer (*O. virginianus leucurus*) and pronghorn (*Antilocapra americana*). The only previous record of louse flies on free-ranging western elk is for another mule deer parasite (*Lipoptena depressa*) on Vancouver Island Roosevelt elk (*C. elaphus roosevelti*) (Blood and Smith, 1986).

The *P. multocida* pneumonia, actinobacillosis-like lymph node lesions, and fibropapilloma were not unique findings. *Pasteurella multocida* has been associated with pneumonia and septicemia in elk from the National Elk Refuge in Wyoming

(Franson and Smith, 1988). Actinobacillosis-like lesions have been previously observed in elk lymph nodes (Rhyan et al., 1992); however, the high prevalence (15%) in this study was surprising. The lesions were usually confined to a single lymph node and were noteworthy in that they grossly resembled lesions of tuberculosis. Fibropapillomas are common benign tumors in deer but are relatively uncommon in elk (Williams, 1982).

In summary, we did not find evidence of tuberculosis or paratuberculosis in northern Yellowstone or southwestern Montana free-ranging elk. The seroprevalence of brucellosis and percentage of *B. abortus*-culture-positive elk were low in both areas containing YNP elk and zero in the area remote from YNP. Periodic animal health surveys employing serology, gross examination and histopatholgy, fecal flotations, and culture techniques on specimens collected from hunter-killed animals are useful as a means of disease surveillance in wildlife populations.

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LITERATURE CITED

AGUIRRE, A. A., D. E. HANSEN, E. E. STARKEY, AND R. G. MCLEAN. 1995. Serologic survey of wild cervids for potential disease agents in selected national parks in the United States. Preventative Veterinary Medicine 21: 313–322.

ALTON, G. G., L. M. JONES, R. D. ANGUS, AND J. M. VERGER. 1988. Bacteriological methods. *In* Techniques for the brucellosis laboratory. Institut National de la Recherche Agronomique, Paris, France, pp. 13–62.

ANONYMOUS. 1965a. Standard agglutination test procedures for the diagnosis of brucellosis. Diagnostic Reagents Manual 65D. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Veterinary Services Laboratories, Ames, Iowa, 8 pp.

———. 1965b. Supplemental test procedures for the diagnosis of brucellosis. Diagnostic Reagents Manual 65E. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Veterinary Services Laboratories, Ames, Iowa, 23 pp.

BAERMANN, G. 1917. Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben. Geneeskundig Tijdschrift voor Nederlandsch-Indie 57: 131–137.

BARRETT, R. E., AND D. E. WORLEY. 1966. The incidence of *Dictyocaulus* sp. in three populations of elk in south-central Montana. Bulletin of the Wildlife Disease Association 2: 5–6.

BELLI, L. B. 1962. Bovine tuberculosis in a white-tailed deer (*Odocoileus virginianus*). Canadian Veterinary Journal 3: 356–358.

BEQUAERT, J. C. 1956. The Hippoboscidae or louse-flies (Diptera) of mammals and birds. Part II. Taxonomy, evolution and revision of American genera and species. Entomologica Americana (New Series) 36: 417–611.

BERGSTROM, R. C. 1975. Prevalence of *Dictyocaulus viviparous* infection in Rocky Mountain elk in Teton County, Wyoming. Journal of Wildlife Diseases 11: 40–44.

———. 1982. Lungworms of ruminants. *In* Diseases of wildlife in Wyoming, 2nd ed, E. T. Thorne, N. Kingston, W. R. Jolley, and R. C. Bergstrom (eds.). Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 206–208.

BLOOD, D. A., AND G. W. SMITH. 1986. Parasites from Roosevelt Elk on Vancouver Island. Contributions to Natural Science 7: 1–3.

CHAPIN, E. A. 1925. New nematodes from North American mammals. Journal of Agricultural Research 30: 677–681.

CRAIGHEAD, J. J., G. ATWELL, AND B. W. O'GARA. 1972. Elk migrations in and near Yellowstone National Park. Wildlife Monograph No. 29. The Wildlife Society, Washington, D.C., 48 pp.

DE LISLE, G. W., AND P. F. HAVILL. 1985. Mycobacteria isolated from deer in New Zealand from 1970–1983. New Zealand Veterinary Journal 33: 138–140.

———, M. A. JOYCE, G. F. YATES, B. J. WARDS, AND F. P. HOYLE. 1995. *Mycobacterium avium* infection in a farmed deer herd. New Zealand Veterinary Journal 43: 1–3.

ESSEY, M. A., AND M. A. KOLLER. 1994. Status of bovine tuberculosis in North America. Veterinary Microbiology 40: 15–22.

FRANSON, J. C., AND B. L. SMITH. 1988. Septicemic pasteurellosis in elk (*Cervus elaphus*) on the United States National Elk Refuge, Wyoming. Journal of Wildlife Diseases 24: 715–717.

FRIEND, M., E. T. KROLL, AND H. GRAFT. 1963. Tuberculosis in a wild, white-tailed deer. New York Fish and Game Journal 10: 118–123.

GROCOTT, R. G. 1955. A stain for fungi in tissue sections and smears using Gomori methenamine silver nitrate technique. American Journal of Clinical Pathology 25: 975.

HADWEN, S. 1942. Tuberculosis in the buffalo. Journal of the American Veterinary Medical Association 100: 19–22.

HARRINGTON, R., JR., AND G. M. BROWN. 1976. Laboratory summary of brucella isolations and typing: 1975. American Journal of Veterinary Research 37: 1241–42.

HENRY, E. J., AND D. R. CASSIDY. 1966. Rapid decalcification followed by a new fuchsin-methylene blue stain for acid-fast bacilli in frozen sections. Stain Technology 41: 345–346.

HERRIGES, J. D., E. T. THORNE, AND S. L. ANDERSON. 1992. Vaccination to control brucellosis in free-ranging elk on western Wyoming feed grounds. *In* The biology of deer, R. D. Brown (ed.). Springer-Verlag, New York, New York, pp. 107–112.

JESSUP, D. A., AND D. BEHYMER. 1981. Paratuberculosis in tule elk in California. Journal of the American Veterinary Medical Association 179: 1252–1254.

LEVINE, P. P. 1934. A report on tuberculosis in wild deer (*Odocoileus virginianus*). The Cornell Veterinarian 24: 264–266.

LUCHSINGER, D. W., AND D. E. PIETZ. 1972. A rapid screening test for brucellosis. Proceedings of the U.S. Animal Health Association Meeting 76: 115–126.

MAA, T. C., AND B. V. PETERSON. 1987. Hippoboscidae. *In* Manual of Nearctic Diptera, Vol. 2, J. F. Mc Alpine, B. V. Peterson, G. E. Hewell, T. J. Teskey, J. R. Vockeroth, and D. M. Wood (eds.). Agriculture Canada Monograph 28, Canadian Government Publishing Centre, Hull, Quebec, Canada, pp. 1271–1281.

MARTIN, W. H., AND W. H. RITCHIE. 1973. A microtiter technique for the complement fixation test for anaplasmosis. Proceedings of the U.S. Animal Health Association Meeting 77: 563–581.

MCCORQUODALE, S. M., AND R. F. DIGIACOMO. 1985. The role of wild North American ungulates in the epidemiology of bovine brucellosis: A review. Journal of Wildlife Diseases 21: 351–357.

MCCULLOUGH, N. B., C. W. EISELE, AND A. F. BYRNE. 1951. Incidence and distribution of *Brucella abortus* in slaughtered Bang's reactor cattle. Public Health Reports 66: 341–345.

MORTON, J. K., E. T. THORNE, AND G. M. THOMAS. 1981. Brucellosis in elk III. Serologic evaluation. Journal of Wildlife Diseases 17: 23–31.

MURRAY, P. R., E. J. BARON, M. A. PFALLER, F. C. TENOVER, AND R. H. YOLKEN. 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology Press, Washington, D.C., 1,482 pp.

PAYEUR, J. B., J. L. JARNAGIN, J. G. MARQUARDT, L. A. SCHAPER, AND B. M. MARTIN. 1993. Laboratory methods in veterinary mycobacteriology for the isolation and identification of mycobacteria. National Veterinary Services Laboratories, U.S. Department of Agriculture, Ames, Iowa, 117 pp.

POWER, S. B., J. HAAGSMA, AND D. P. SMYTH. 1993. Paratuberculosis in farmed red deer (*Cervus elaphus*) in Ireland. The Veterinary Record 132: 213–216.

PRESIDENTE, P. J. A., D. E. WORLEY, AND J. E. CATLIN. 1972. Cross-transmission experiments with *Dictyocaulus viviparus* isolates from Rocky Mountain elk and cattle. Journal of Wildlife Diseases 8: 57–62.

QUIMBY, D. C., AND J. E. GAAB. 1957. Mandibular dentition as an age indicator in Rocky Mountain elk. The Journal of Wildlife Management 21: 435–451.

RHYAN, J., D. A. SAARI, E. S. WILLIAMS, M. W. MILLER, A. J. DAVIS, AND A. J. WILSON. 1992. Gross and microscopic lesions of naturally occurring tuberculosis in a captive herd of wapiti (*Cervus elaphus nelsoni*) in Colorado. Journal of Veterinary Diagnostic Investigation 4: 428–433.

———, K. AUNE, B. HOOD, R. CLARKE, J. PAYEUR, J. JARNAGIN, AND L. STACKHOUSE. 1995. Bovine tuberculosis in a free-ranging mule deer (*Odocoileus hemionus*) from Montana. Journal of Wildlife diseases 31: 432–435.

RIEMANN, H., M. R. ZAMAN, R. RUPPANNER, O. AALUND, J. B. JORGENSEN, H. WORSAAE, AND D. BEHYMER. 1979. Paratuberculosis in cattle and free-living exotic deer. Journal of the American Veterinary Medical Association 174: 841–843.

SMITH, B. L., AND R. L. ROBBINS. 1994. Migrations and management of the Jackson elk herd. National Biological Service Resource Publication 199. U.S. Department of the Interior, Washington, D.C., pp. 38–39.

THOEN, C. O., W. J. QUINN, L. D. MILLER, L. L. STACKHOUSE, B. F. NEWCOMB, AND J. M. FERRELL. 1992. *Mycobacterium bovis* infection in North American elk (*Cervus elaphus*). Journal of Veterinary Diagnostic Investigation 4: 423–427.

THORNE, E. T., J. K. MORTON, AND G. M. THOMAS. 1978. Brucellosis in elk I. Serologic and bacteriologic survey in Wyoming. Journal of Wildlife Diseases 14: 74–81.

WHITING, T. L., AND S. V. TESSARO. 1994. An abattoir study of tuberculosis in a herd of farmed elk. Canadian Veterinary Journal 35: 497–501.

WILLIAMS, E. S. 1982. Neoplasias. *In* Diseases of wildlife in Wyoming, 2nd ed, E. T. Thorne, N. Kingston, W. R. Jolley, and R. C. Bergstrom (eds.). Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 261–274.

———, T. R. SPRAKER, AND G. G. SCHOONVELD. 1979. Paratuberculosis (Johne's disease) in bighorn sheep and a Rocky Mountain goat in Colorado. Journal of Wildlife Diseases 15: 221–227.

———, S. P. SNIDER, AND K. L. MARTIN. 1983. Experimental infection of some North American

wild ruminants and domestic sheep with *Mycobacterium paratuberculosis*: Clinical and bacteriological findings. Journal of Wildlife Diseases 19: 185–191.

———, E. T. THORNE, S. L. ANDERSON, AND J. D. HERRIGES, JR. 1993. Brucellosis in free-ranging bison (*Bison bison*) from Teton County, Wyoming. Journal of Wildlife Diseases 29: 563–567.

———, S. G. SMITH, R. M. MEYER, AND E. T. THORNE. 1995. Three-year survey for bovine tuberculosis in hunter-killed free-ranging elk (*Cervus elaphus nelsoni*) in northwestern Wyoming. Proceedings of the U.S. Animal Health Association Meeting 99: 631–637.

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