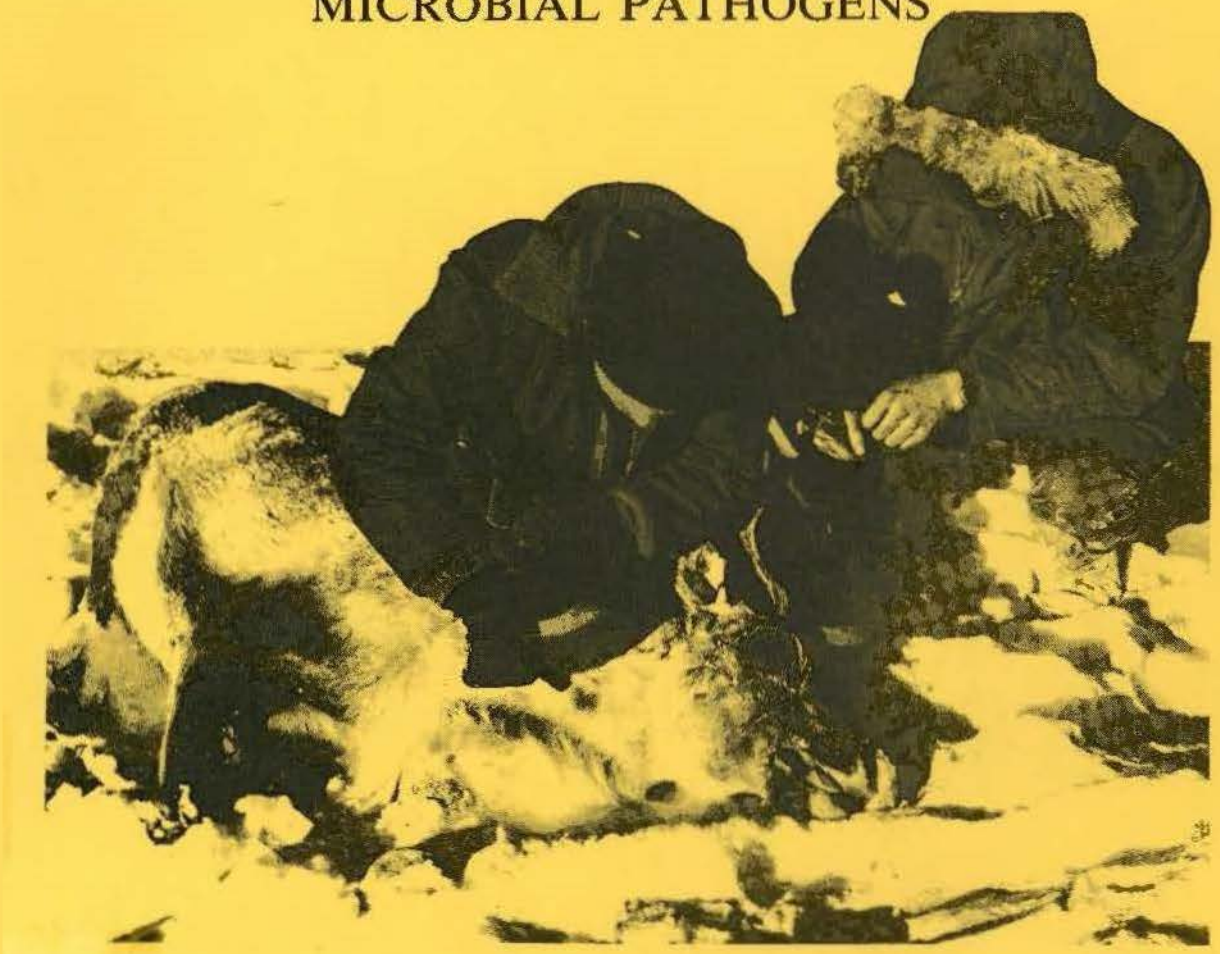
Alaska Department of Fish and Game
Division of Game
Federal Aid in Wildlife Restoration
Research Progress Report

SEROLOGIC SURVEY FOR MICROBIAL PATHOGENS



by
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Project W-22-6
Job 18.6R
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PROGRESS REPORT (RESEARCH)

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Job No: 18.6R Job Title: Serologic Survey for Microbial Pathogens

Period Covered: 1 July 1986-30 June 1987

SUMMARY

A serologic survey of selected wildlife species from Alaska was conducted. Evidence of exposure to infectious bovine rhinotracheitis was found in caribou (Rangifer tarandus) from the Fortymile and Porcupine Herds. Evidence of exposure to bovine viral diarrhea was found in both caribou herds for the first time, as well as in moose (Alces alces) from the Koyukuk River. Serum antibody prevalence of parainfluenza III remains very high in the Delta bison (Bison bison) herd, and also showed up for the 1st time in the Chitina bison, Koyukuk River moose, Dall sheep (Ovis dalli) from both the White Mountains and Dry Creek, and caribou from the Fortymile and Porcupine Herds. Serum antibody prevalence of leptospirosis in moose remained low. Evidence of epizootic hemorrhagic disease continued to be sporadic. Prevalence of tularemia and brucellosis in wolves (Canis lupus) followed established patterns. Evidence of Q fever appeared in bison for the 1st time. There was no evidence of respiratory syncytial virus exposure in Dall sheep. Muskoxen were free of evidence of any diseases.

CONTENTS

OBJECTIVE

The objective of this survey has been to monitor Alaskan wildlife populations for the occurrence of microbial disease agents which may have a detrimental effect upon the health of both individual animals and entire populations.

METHODS

Most blood samples were collected by ADF&G biologists who captured animals to meet objectives of other studies. General collection areas are indicated in Figure 1.

Most blood samples were allowed to settle at ambient or refrigerated temperatures for 6-36 hours and then centrifuged. Serum was then removed by aspiration. Sera were kept frozen until the time of testing. All serologic tests were performed by personnel of the National Veterinary Services Laboratories (USDA, Ames, Iowa). Serology is a suitable diagnostic tool for most viral and bacterial infections. Disease agents were selected for inclusion in this survey based upon past or potential problems with wildlife species in Alaska or other parts of the world.

Sera were tested for evidence of antibodies to:

(1) Brucella spp., by the buffered acidified plate antigen test (Angus and Barton 1984),

(2) tularemia, by the tube agglutination test (Owen 1970),

(3) Q fever and contagious ecthyma, by the complement fixation test (Erickson et al. 1975),

(4) Leptospira spp., by the microscopic agglutination test (Cole et al. 1973),

(5) infectious bovine rhinotracheitis, bovine viral diarrhea, epizootic hemorrhagic disease, and bluetongue, by the serum neutralization test (Thorsen and Henderson 1971),

(6) epizootic hemorrhagic disease and bluetongue, by the immunodiffusion test (Pearson and Jochim 1979),

(7) parainfluenza III, by the hemagglutination-inhibition test (Thorsen and Henderson 1971),

(8) respiratory syncytial virus, by the indirect fluorescent antibody test (Trigo et al. 1984).

Twelve Leptospira interrogans serovarieties were included in the tests: pomona, ballum, canicola, icterrohemorrhagiae, wolffi, grippotyphosa, hardjo, autumnalis, bataviae, tarassovi, australis, and pyrogenes. Minimum titers for all tests were established based upon natural or experimental infection of the species in question or of a domesticated species. Sera which met or exceeded these titers (plus those designated "positive" in the immunodiffusion test and brucellosis plate test) were considered to contain evidence of past infection by the agent in question. Hereafter, these samples may be referred to as "positive." All other samples may be referred to as "negative."

RESULTS AND DISCUSSION

In most cases, test results provided no evidence of exposure to a particular disease in a particular host species (Tables 1-6). This discussion will focus on those situations where evidence of previous exposure was found.

Three viral diseases, infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), and parainfluenza III (PI3), are commonly referred to, collectively, as the "bovine respiratory group." As this generic term implies, the viruses often cause upper respiratory infections (Dieterich 1981). Morbidity (rate of illness) may be high in an infected population, but mortality (rate of death) is usually low. Transmission usually occurs via aerosol droplet, but the venereal route may also play a role (Dieterich 1981). Serologic evidence of exposure has been previously reported for various wildlife species (Thorsen and Henderson 1971; Parks and England 1974; Stauber et al. 1980).

Serum antibody prevalence of IBR in the Porcupine Caribou Herd (PCH) during 1985 (3/17--18%; Table 1) corresponded quite well with the previously reported rate of 23% (Zarnke 1986). Titers of all 3 positive samples were low and thus somewhat subject to interpretation. This new data provides further evidence that in regard to exposure to IBR, the PCH aligns more closely with the Western Arctic and Central Arctic Caribou Herds (WAH and CAH, respectively) in comparison with more southerly caribou herds in Alaska where prevalence is very low.

Prevalence of IBR in the Fortymile Caribou Herd (FCH) during 1985 was quite high in a small sample (4/6--67%; Table 1). As was true for the PCH, the titers of all 4 positive FCH specimens were low. The high prevalence was a dramatic change from the previously reported rate of 0% for the FCH (Zarnke 1986) and aligns the FCH with the Arctic herds regarding IBR exposure.

We have not found any direct evidence of IBR-induced mortality in any caribou, although occasional cases of respiratory disease do occur. IBR does represent a threat as a debilitating agent which can predispose animals to other respiratory diseases. In this light, the higher prevalence of IBR in northerly caribou herds warrants additional investigation.

Serum antibody prevalence of BVD in the PCH during 1985 (2/17--12%; Table 1) was somewhat higher, but in general agreement with prevalences previously reported for the WAH (5%) and CAH (6%) (Zarnke 1986). The occurrence of positive animals was a change from past status when no evidence of BVD was found in the PCH (Zarnke 1986).

Prevalence of BVD in the FCH during 1985 was moderate in a small sample (1/6--17%; Table 1). This specimen represents the 1st evidence of BVD in the FCH. The titer of this specimen was low and therefore subject to question.

Prevalence of BVD in small samples of moose collected during 1985 from Game Management Unit 13 (GMU 13) and the Three-Day Slough portion of the Koyukuk River were surprisingly high (2/4--50% and 1/3--33%, respectively; Table 2). Titers were low in all cases. Only 1 previous moose specimen had shown evidence of BVD out of approximately 700 sera collected statewide during the past 15 years.

In the absence of evidence to the contrary, I perceive BVD as posing a minimal threat to the health of wildlife populations statewide.

Other than specimens from the Delta Bison Herd, all positive samples referred to below had very low titers and therefore the PI3 results are somewhat subject to interpretation.

Serum antibody prevalence for PI3 remains high in the Delta Bison Herd (41/41--100% for 1984 and 28/29--97% for 1985; Table 3). These rates agree with those for the past couple of years (Zarnke 1986). I continue to be concerned about the implications of these data for the health of the herd. This may be the next potential wildlife disease problem in Alaska.

The high prevalence of PI3 in the Chitina Bison Herd (4/4--100%; Table 3) was the 1st evidence of this disease in this herd, although only a small number of samples from previous years are available. Titers of all 4 specimens were low. Additional samples from this area will help to clarify the status of Chitina bison relative to PI3 exposure.

There was a high serologic prevalence of PI3 in a small sample of moose collected from the Three-Day Slough area of the Koyukuk River during 1985 (2/4--50%; Table 2). Both samples had low titers. The only previous evidence of PI3 in moose was a single animal from GMU 13 and 8/586 (1%) from the Kenai Peninsula (Zarnke 1986).

Serum antibody prevalence of PI3 was low in collections of Dall sheep from both the White Mountains and Dry Creek during 1985 (1/7--16% and 1/30--3%, respectively; Table 4). Titers were low in both cases. These samples represent the 1st evidence of PI3 in free-ranging sheep in Alaska. Considering the problems posed by this virus for sheep elsewhere in North America, this new development should not be taken lightly. Every opportunity should be taken to collect specimens from sheep for continued monitoring.

Prevalence of PI3 was moderate to high in the PCH and FCH during 1985 (2/19--10% and 5/6--83%, respectively; Table 1). Titers were low in all cases. These results represent the 1st evidence of PI3 exposure in these herds (Zarnke 1986).

Leptospirosis

Leptospirosis is caused by 1 or more so-called "serovarieties" of a spirochete known as Leptospira interrogans (Busch 1970). Symptoms may include chronic kidney infections (Diesch et al. 1970), hepatitis (Bishop et al. 1979), and/or abortion. Transmission usually occurs via contamination of water by leptospires which are shed in urine (Busch 1970). Also, the disease may be passed along the food chain from prey to predators (Reilly et al. 1970). Exposure to more than one serovar is not uncommon.

The low prevalence of leptospirosis in moose populations from various areas of the state (Table 2) is in general agreement with previously reported conditions (Zarnke 1986). Serovariety ballum was the most common, occurring in 2

specimens from the Three-Day Slough area. Other serovars included grippotyphosa from the Susitna study area, autumnalis from GMU 13 and pyrogenes from the White Mountains.

The occurrence of positive animals on the Koyukuk River and the White Mountains represents a northerly extension from previous reports, but should not be viewed as a big surprise. Apparently, leptospirosis is geographically widespread but occurs in only a small proportion of any moose population at any particular time.

Bluetongue - (BLU) and Epizootic hemorrhagic disease - (EHD)

BLU and EHD are viral diseases of wild and domestic ruminants. Symptoms may include anorexia, ataxia, dyspnea, and depression, but the 2 diseases are most often recognized post-mortem by acute subcutaneous and/or internal hemorrhaging (Hoff and Trainer 1978). The oral route may be important for transmission during enzootic periods, but arthropod vectors play a big role during epizootics (Hoff and Trainer 1978).

The situation surrounding EHD and BLU in Alaskan wildlife is more confusing than for most other diseases. On occasions when positive samples were detected by means of immunodiffusion tests, USDA personnel attempted to determine which of the 2 viruses (EHD or BLU) was responsible. This was done by means of implementing the more specific serum neutralization test. In virtually all cases where this was done, test results were inconclusive and were accompanied by the following comment: "significance of these results is difficult to evaluate in an area where no [overt disease] has ever been reported. The reaction may be due to exposure to an antigenically similar virus." Mention of antigenic variation and overlap are inherent in any discussion of these 2 viruses. Although discernible from each other, EHD and BLU are closely related antigenically. On the other hand, there are at least 19 distinct strains of BLU. It is not inconceivable that there is a distinct relative of EHD and BLU present in Alaskan wildlife. The proper means of addressing such a problem is to isolate and identify the disease agent in question. In the absence of clinical disease, the likelihood of isolating the agent is small.

There is some question regarding the transmission of either EHD or BLU in Alaska. In North America, a midge (Culicoides variipennis) is the most common vector of these viruses. There is some debate as to whether this particular gnat species exists in Alaska. I recently attempted to collect gnats for identification but failed due to difficult trapping conditions during long summer evenings. Certainly, members of the genus Culicoides do occur in Alaska and experience in

other parts of the world indicates that in the absence of the preferred vector species, other members of the genus will occupy this ecological niche and serve as vectors. In an attempt to answer several questions surrounding the significance of EHD/BLU in Alaska, I plan to continue attempts to collect Culicoides spp. both for vector identification and for virus isolation purposes.

Serum antibody prevalence of EHD was moderate in moose specimens collected from the Susitna Dam study area during 1982 (2/17--12%; Table 2). Collections from the same geographic area during 1980 and 1981 revealed no evidence of exposure to this virus (0/10 and 0/25, respectively; Table 2). These data are largely in agreement with past records where prevalence ranged from 1-5% in all areas from which adequate sample sizes were available (Zarnke 1986).

The regular, low-level occurrence of sera from various species and areas which are positive for evidence of EHD continues to be an enigma (Zarnke 1986).

The precise identity of the agent which is responsible for these serologic reactions and the identity of potential vectors remains unknown. To date we are unaware of any clinical cases of any hemorrhagic diseases in any of Alaska's wildlife. Therefore, it is tempting to conclude that EHD and/or BLU pose no threat. However, environmental conditions play a major role in the epizootiology of these diseases. Subtle changes in conditions can result in major changes in transmission rates. In this sense, the potential for serious outbreaks may exist. I do not perceive this potential to be great. I view the identification of potential vectors as the primary aspect of the epizootiology which needs to be addressed.

Brucellosis

Brucella suis IV is the causative agent of the type of brucellosis found in Alaska. The most well-studied host species include caribou and their associated predators (Neiland et al. 1968; Neiland 1975). Infection usually localizes in joints or reproductive organs, causing arthritis and/or abortion (Neiland et al. 1968). Transmission occurs venereally (Neiland et al. 1968), or via the food chain (Neiland 1970, 1975).

Serum antibody prevalence of brucellosis was low in wolves from the Arctic National Wildlife Refuge (GMU 26C) during 1984 (1/10--10%; Table 5). The disease is more or less common in caribou, which is considered to be the primary host species. Wolves are infected via the food chain.

Two test procedures were utilized for the diagnosis of brucellosis. Established standards require that specimens give a positive reaction in both systems in order to be considered indicative of previous exposure. Single specimens from collections on the Kenai Peninsula, Fairbanks vicinity (GMU 20A), GMU 26C during 1984, and GMU 26C during 1985, were positive in the less rigorous of the 2 tests, (the buffered antigen plate agglutination test) but failed the more stringent Standard Tube Test.

Q fever

Q fever is caused by the rickettsium Coxiella burnetti (Randhawa et al. 1977). The organism usually localizes in the respiratory tract. Although the disease is usually mild in domestic species, abortions can occur in sheep and goats. Death is rare (Bell 1981). Coxiella burnetti is shed in milk, feces, birth fluids, and placental tissues (Enright et al. 1969).

Antibody prevalence of Q fever was uncommon in bison from the Delta and Farewell herds during 1984 (1/6--17% and 1/3--33%, respectively; Table 3). These samples represent the 1st evidence of exposure to Q fever in Alaska's bison, although the data do fit the pattern of low prevalence in a wide variety of species (Zarnke 1986).

Tularemia

Tularemia is an acute, febrile, plague-like disease of wild lagomorphs and rodents caused by the bacterium Francisella tularensis. Snowshoe hares are the primary reservoir of tularemia in Alaska (Dieterich, 1981). Ticks usually transmit the disease to hares, particularly when the population density of hares is high. Transmission to predators usually occurs as a result of their preying on infected hares. Historically, serum antibody prevalence has been low in red fox and domestic dog populations in Alaska (Zarnke 1983).

Evidence of previous exposure to tularemia was found in wolves from GMU 20A (3/6--50%: Table 5), GMU 26C during 1984 (1/10--10%; Table 5) and GMU 26C during 1985 (1/5--20%; Table 5). All titers were low. The single positive specimen from GMU 26C in 1984 was from an animal that was also positive for brucellosis. Therefore, the possibility exists that the positive result in the tularemia test actually represents cross-reactive brucellosis antibody. In fact, the titers found in the current batch of samples were low enough that they are all susceptible to this criticism although I do not feel that the criticism is valid. The high prevalence reported here is in general agreement with that reported

previously for GMU 13 (Zarnke and Ballard 1987). Implications of this disease for individual wolves is hard to assess, but I suspect that otherwise healthy adults would recover from an uncomplicated bout with tularemia.

Respiratory Syncytial Virus

Respiratory syncytial virus (RSV) is a pathogen of the lower respiratory tract (Dunbar et al. 1986). Clinical signs of disease in domestic sheep (Ovis aries) include elevated body temperature and elevated respiratory rate (Lehmkuhl and Cutlip 1979). At necropsy, interstitial pneumonia is often present (Lehmkuhl and Cutlip 1979). Perhaps most importantly, RSV can serve as a predisposing agent for other serious diseases (Dunbar et al. 1986). Recent serologic surveys of free-ranging wildlife in the western U.S. have revealed 25-50% prevalences in bighorn sheep (Ovis canadensis) (Dunbar et al. 1985), mountain goat (Oreamnos americanus) (Dunbar et al. 1986), pronghorn (Antilocapra americana) (Johnson et al. 1986a), white-tailed deer (Odocoileus virginianus) (Johnson et al. 1986b) and mule deer (Odocoileus hemionus) (Johnson et al. 1986b). The virus has been isolated from a bighorn sheep lamb which was suffering from suppurative respiratory disease (Spraker et al. 1986).

This is the 1st time that RSV has been included in a wildlife survey in Alaska. It is encouraging to note that no evidence of exposure to this potentially serious pathogen was found in any of 14 Dall sheep specimens from Dry Creek or 22 samples from Sheep Creek.

ACKNOWLEDGMENTS

The author wishes to thank Jesse Venable for his assistance with the computerization of data involved in this project. I also acknowledge the contributions of sera by many Game Division personnel throughout the state. Without the cooperation of the State-Federal Animal Health Laboratory and the National Veterinary Services Laboratory this study would not have been feasible.

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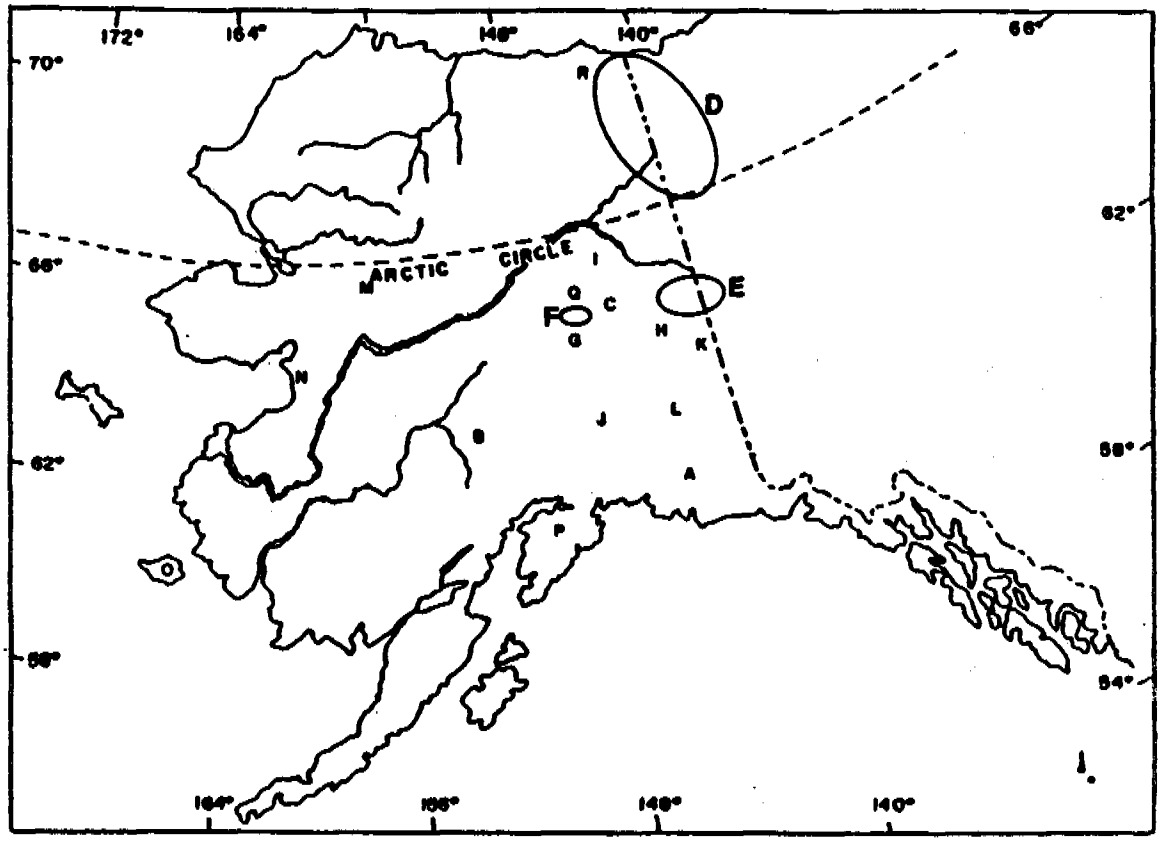

Fig. 1. Locations at which blood specimens were collected for disease survey.

A Chitina - bison

B Farewell - bison

C Delta Junction - bison, moose

D Porcupine Herd - caribou

E Fortymile Herd - caribou

F Delta Herd - caribou

G Dry Creek - Dall sheep

H Sheep Creek - Dall sheep

I White Mts. - Dall sheep, moose

J Susitna Study area - moose

K Tetlin - moose

L GMU 13 - moose

M Three-day Slough - moose

N Unalakleet - muskox

O Nunivak - muskox

P Kenai Peninsula - wolf

Q GMU 20 - wolf

R GMU 26C - wolf

Table 1. Serum antibody prevalence for 3 infectious diseases in caribou from the Porcupine, Fortymile, and Delta Herds, 1985.

Disease	Porcupine Herd	Fortymile Herd	Delta Herd
Infectious bovine rhinotracheitis SN 8[a]	3/17[b]	4/6	0/14
Bovine viral diarrhea SN 8	2/17	1/6	0/15
Parainfluenza 3 HI 8	2/19	5/6	0/15

[a] Name of test: SN = serum neutralization test; HI = hemagglutination - inhibition test. Numbers indicate minimum titers necessary to be considered as evidence of previous infection.

[b] Number postive/number tested.

Table 2. Serum antibody prevalence for 9 infectious diseases in moose collected from 6 areas of Alaska, 1980-85.

Disease	Susitna Study Area 1980	Susitna Study Area 1981	Susitna Study Area 1982	Tetlin 1984	White Mtns 1985	GMU 13 1984	GMU 13 1985	3-Day Slough 1984	3-Day Slough 1985	Delta Jct 1984	Delta Jct 1985
Brucellosis											
BAPA (±)[a]	0/10[b]	0/25	0/17	0/10	0/10	0/6	ND[c]	0/19	ND	0/3	ND
Leptospirosis											
MAT 100	0/10	1/25	0/17	0/10	1/10	1/6	ND	2/19	ND	0/3	ND
Q Fever											
CF 200	0/10	0/25	0/17	0/10	0/10	0/6	ND	0/18	ND	0/3	ND
Contagious ecthyma											
CF 5	0/9	0/17	0/15	0/10	0/5	0/4	ND	0/8	ND	0/3	ND
Epizootic hemorrhagic disease											
ID (±)	0/10	0/25	2/17	0/10	0/10	0/6	ND	0/19	ND	0/3	ND
Bluetongue											
ID (±)	0/10	0/25	0/17	0/10	0/10	0/6	ND	0/19	ND	0/3	ND
Infectious bovine rhinotracheitis											
SN 8	ND	ND	ND	ND	ND	ND	0/4	ND	0/3	ND	0/4
Bovine viral diarrhea											
SN 8	ND	ND	ND	ND	ND	ND	2/4	ND	1/4	ND	0/4
Parainfluenza 3											
HI 8	ND	ND	ND	ND	ND	ND	0/4	ND	2/4	ND	0/4

Table 2. Continued.

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[a] Name of test: SN = serum neutralization test; HI = hemagglutination - inhibition test; ID = immunodiffusion test; CF = complement fixation test; BAPA = buffered antigen plate agglutination test; MAT = microscopic agglutination test. Numbers indicate minimum titers necessary to be considered as evidence of previous infection. The (±) indicates that test is simply read as either positive or negative.

[b] Number positive/number tested.

[c] ND = not done.

Table 3. Serum antibody prevalence for 7 infectious diseases in bison collected from 3 areas of Alaska, 1984-85.

Disease	Chitina 1985	Farewell 1984	Delta 1984	Delta 1985
Epizootic hemorrhagic disease ID (±)[a]	0/4[b]	0/7	0/42	ND[c]
Bluetongue ID (±)	0/4	0/7	0/40	ND
Infectious bovine rhinotracheitis SN 8	0/4	0/7	0/40	0/5
Bovine viral diarrhea SN 8	0/4	0/7	0/35	0/8
Parainfluenza 3 HI 8	4/4	0/7	41/41	28/29
Q fever CF 20	0/1	1/3	1/6	ND
Brucellosis BAPA (±)	0/4	0/7	0/39	ND

[a] Name of test: SN = serum neutralization test; HI = hemagglutination - inhibition test; ID = immunodiffusion test; CF = complement fixation test; BAPA = buffered antigen plate agglutination test. Numbers indicate minimum titers necessary to be considered as evidence of previous infection. The (±) indicates that test is simply read as either positive or negative.

[b] Number positive/number tested.

[c] ND = not done.

Table 4. Serum antibody prevalence for 6 infectious diseases in Dall sheep collected from 3 areas of Alaska, 1984-85.

Disease	Dry Creek 1984	Sheep Creek 1984	Dry Creek 1985	Sheep Creek 1985	White Mountains 1985
Epizootic hemorrhagic disease					
ID (±)[a]	0/14[b]	0/22	ND[c]	ND	ND
Bluetongue					
ID (±)	0/14	0/22	ND	ND	ND
Respiratory syncytial virus					
IFA 20	0/14	0/22	ND	ND	ND
Infectious bovine rhinotracheitis					
SN 8	ND	ND	0/30	0/23	0/7
Bovine viral diarrhea					
SN 8	ND	ND	0/30	0/22	0/7
Parainfluenza 3					
HI 8	ND	ND	1/30	0/23	1/7

[a] Name of test: SN = serum neutralization test; HI = hemagglutination - inhibition test; ID = immunodiffusion test; IFA = indirect fluorescent antibody test. Numbers indicate minimum titers necessary to be considered as evidence of previous infection. The (±) indicates that test is simply read as either positive or negative.

[b] Number positive/number tested.

[c] ND = not done.

Table 5. Serum antibody prevalence for 3 infectious diseases in wolves collected from 3 areas of Alaska, 1984-85.

Disease	Kenai Peninsula 1984	Game Management Unit 20 1984	Game Management Unit 26C 1984	Game Management Unit 26C 1985
Brucellosis				
BAPA (±)[a]				
STT (25)	0/2[b]	0/6	1/10	0/6
Tularemia				
TAT 20	0/2	3/6	1/10	1/5
Leptospirosis				
MAT 100	0/2	0/7	0/10	0/6

[a] Name of test: BAPA = buffered antigen plate agglutination test; MAT = microscopic agglutination test; STT = standard tube test; TAT = tube agglutination test. Numbers indicate minimum titers necessary to be considered as evidence of previous infection. The (±) indicates that test is simply read as either positive or negative.

[b] Number positive/number tested.

Table 6. Serum antibody prevalence for 7 infectious diseases in muskoxen from 2 areas of Alaska, 1978 and 1984.

Disease	Unalakleet 1978	Nunivak Island 1978	Nunivak Island 1984
Contagious ecthyma			
CF 5[a]	0/0[b]	0/9	0/13
Epizootic hemorrhagic disease			
ID (±)	0/8	0/21	0/21
Bluetongue			
ID (±)	0/8	0/21	0/21
Infectious bovine rhinotracheitis			
SN 8	0/8	0/21	0/20
Bovine viral diarrhea			
SN 8	0/8	0/21	0/20
Parainfluenza 3			
HI 8	0/8	0/20	0/19
Q fever			
CF 20	0/0	0/12	0/19

[a] Name of test: SN = serum neutralization test; HI = hemagglutination - inhibition test; ID = immunodiffusion test; CF = complement fixation test. Numbers indicate minimum titers necessary to be considered as evidence of previous infection. The (±) indicates that test is simply read as either positive or negative.

[b] Number postive/number tested.