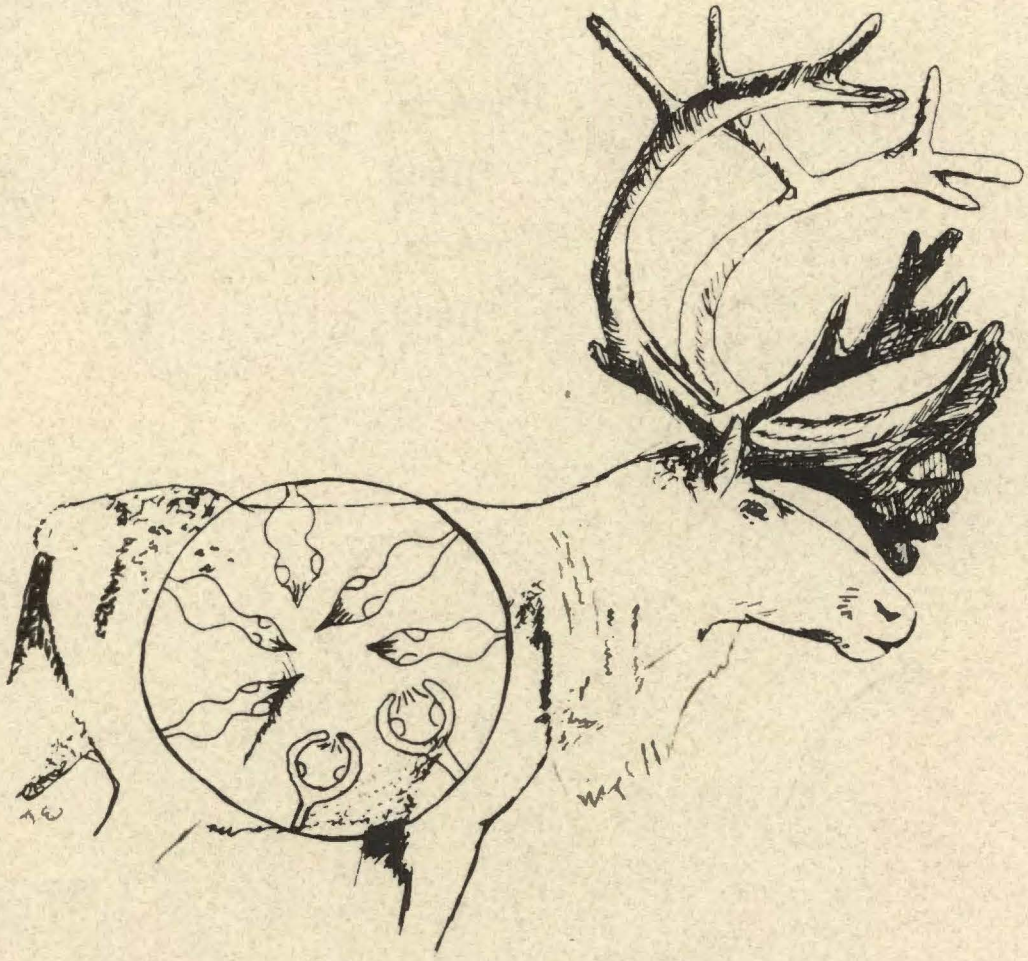
Alaska Department of Fish and Game
Division of Wildlife Conservation

Federal Aid in Wildlife Restoration
Research Progress Report
1 July 1991 - 30 June 1992
1 July 1992 - 30 June 1993

Serologic Survey for Microbial Pathogens

by

Rnadall L. Zarnke



Projects W-23-5 & W-24-1
Study 18.7
July 1993

Alaska Department of Fish and Game
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Randall L. Zarnke

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PROGRESS REPORT (RESEARCH)

State: Alaska

Cooperator: None

Project No.: W-23-5 Project Title: Wildlife Research and Management

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During the 1980s, the U.S. Department of Agriculture (USDA) provided serologic testing services for the Alaska Department of Fish and Game (ADF&G). This arrangement was terminated in 1990, reportedly due to budget reductions with USDA. Since that time, we have been trying to (1) reestablish a working relationship with USDA and (2) locate alternate testing labs. After a lengthy search, agreements were reached in late 1991 with (1) the University of California-Davis for testing bear and wolf sera and (2) the Wyoming State Veterinary Lab for testing all ruminant sera. These two arrangements will approximate the same scope of disease agents included in the USDA surveys. However, costs will be higher. Samples were sent to the two labs in late 1991. To date, no results are available.

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PROGRESS REPORT (RESEARCH)

State: Alaska

Cooperator: None

Project No: W-24-1 Project Title: Wildlife Research and Management

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Period Covered: 1 July 1992-30 June 1993

SUMMARY

A serologic survey of selected wildlife species from Alaska was conducted. There was little or no evidence of most disease agents in most host species. Based on serologic test results, some notable exceptions were apparent:

1. Antibody prevalence of parainfluenza 3 virus (PI3) in the Delta bison (*Bison bison*) herd remained extremely high.

2. Evidence of respiratory syncytial virus (RSV) was found in the Delta bison herd for the first time in 1990. No evidence was detected in 1991.

3. Sporadic evidence of epizootic hemorrhagic disease virus (EHD) was found in both caribou (*Rangifer tarandus*) and Dall sheep (*Ovis dalli*). These results agree with previously established patterns.

4. No evidence of exposure to *Brucella suis* IV was found in any caribou sera. These results are unexpected and may be due to the utilization of a new testing laboratory rather than an actual change in prevalence of this agent.

5. Antibody prevalence of respiratory group viruses was higher in caribou from the northern portion of the state as contrasted with herds from other areas.

Key Words: Alaska, serologic, survey, wildlife

CONTENTS

SUMMARY i
BACKGROUND 1
OBJECTIVE 2
METHODS 2
RESULTS AND DISCUSSION 3
Respiratory Group Viruses 3
Epizootic Hemorrhagic Disease 4
Brucella suis IV 4
ACKNOWLEDGMENTS 4
LITERATURE CITED 4
FIGURES 5
TABLES 7

BACKGROUND

There have been few documented instances of infectious diseases having a detectable impact on wildlife populations in Alaska. Brucellosis in caribou (*Rangifer tarandus*) and rabies in canids have been notable exceptions. In an effort to evaluate the disease status of various Alaskan wildlife populations, a serologic survey has been conducted throughout the state.

Disease surveys conducted by means of serologic tests have many advantages:

1. Blood samples are easy to collect.

2. It is not necessary to sacrifice animals to test for evidence of previous exposure to disease(s).

3. Periodic samples can be collected from the same animal(s) over an extended time frame, thus providing information on the timing of exposure.

4. Tests are relatively inexpensive to perform.

5. A single sample can be tested for evidence of many different diseases, rather than requiring a specific tissue or organ for each disease of concern.

6. Sera are stable for a long period of time (under adequate storage conditions), thus providing the basis for a functional archive system that can be analyzed in the future.

7. If the sample size is adequate, it is possible to evaluate the status of an entire population in relation to a disease.

8. If populations are monitored over a period of time, it is possible to determine changes in the disease status of the population.

9. Early warnings of such changes in disease status of a population allow for the consideration of human intervention into the disease process at the most opportune time and place.

Within a living animal, antibody molecules are produced in response to invading disease agents. For certain agents, antibody may decay to undetectably low levels over a relatively short period (ca. several months). For other agents, antibody may be more long-lived and may remain at detectable levels for many years. Furthermore, re-exposure to the same disease agent usually causes an increase in the level of antibody in circulation. These factors all confound attempts to correlate the level of antibody in the serum to the date of exposure of the host to the agent.

Perhaps the most reasonable means of determining the time frame during which an animal has been exposed to an infectious disease agent is to periodically collect serum specimens from a specific animal. However, in most cases, such periodic sampling schemes are not practical for free-ranging animals. Thus, determining the timing of exposure of either specific individuals or populations is difficult.

Test results for samples that have been collected during any particular year do not necessarily reflect the transmission pattern during that year. For example, animals with evidence of exposure may have been infected during previous years. However, analyzing such test results based upon the year in which the samples were collected may reveal long-term trends in the frequency of disease transmission. Although this approach of grouping samples according to the year in which they were collected may not be infallible, it serves a practical purpose and therefore has become an accepted technique for evaluating data. This sample grouping approach will be used throughout the discussion of the current study.

Alaska Department of Fish and Game (ADF&G) has conducted serologic surveys since the early 1960s. During the early years such surveys were limited in the scope of disease agents and host species that were investigated. Over the past decade, the survey has been expanded to include both more potential host species and more disease agents.

OBJECTIVE

The objective of this survey has been to monitor Alaskan wildlife populations for the occurrence of microbial disease agents that 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. Hunters collected and contributed samples from bison (*Bison bison*), caribou, Dall sheep (*Ovis dalli*), and Sitka blacktail deer (*Odocoileus hemionus sitkensis*). General collection areas are indicated in Figs. 1-2.

Most blood samples were allowed to settle at ambient or refrigerated temperatures for 6 to 36 hours and then centrifuged. Sera were then removed by aspiration and dispensed in vials. Sera were kept frozen until the time of testing. Most serologic tests were performed by personnel of the Wyoming State Veterinary Laboratory (Laramie, WY). 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 exposure to:

1. *Brucella* spp., by the standard plate test (U.S. Department of Agriculture undated),

2. *Leptospira* spp., by the microscopic agglutination test (National Veterinary Services Laboratory 1987). Five *Leptospira interrogans* serovarieties were included: *canicola, grippotyphosa, hardjo, icterohemorrhagiae*, and *pomona.*

3. epizootic hemorrhagic disease and bluetongue viruses by the immunodiffusion test (Pearson and Jochim 1979), and

4. infectious bovine rhinotracheitis, bovine viral diarrhea, parainfluenza 3, and bovine respiratory synctial viruses by the serum neutralization test (Carbrey et al. 1975).

Minimum titers for all tests were established based upon natural or experimental infection of the species in question or of a domesticated species. Sera that 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."

Two types of potential qualitative errors should be considered in evaluating the significance of serologic survey results: (1) samples from animals that have in fact been infected by the disease agent in question may be incorrectly categorized as "negative," and (2) samples from animals that have never been exposed to an agent may be incorrectly deemed "positive." Explanations for the former include: (1) natural antibody decay over time, (2) antibody degradation due to improper handling of the specimen, (3) establishment of the threshold titer value at a level that is too high, (4) improper inspection or evaluation of the test, and (5) inaccuracies in recording data. Explanations for the latter include: (1) presence of "nonspecific" reacting substances in the sample, (2) improper inspection or evaluation of the test, and (3) inaccuracies in recording data. With these disclaimers in mind, discussion of the test results may proceed.

RESULTS AND DISCUSSION

Serologic test results are presented in Tables 1-8. Little or no evidence of exposure to disease agents was found in most potential host species. This discussion will focus on those cases where evidence of exposure WAS found.

Respiratory Group Viruses

Serum antibody prevalences of infectious bovine rhinotracheitis virus, bovine viral diarrhea virus, parainfluenza 3 virus, and respiratory syncytial virus were higher in northern caribou herds (Teshekpuk, Central Arctic, and Fortymile) as contrasted with herds from other areas of Alaska (Tables 1-2). These results agree with previously established patterns (Zarnke 1991).

Prevalence of parainfluenza 3 virus remains high in the Delta bison herd (Table 3). There were no reports of respiratory distress in the Delta bison herd that may have been linked to PI3 exposure. In 1990, the first evidence of respiratory syncytial virus exposure in the Delta bison herd was detected. However, no evidence was detected in 1991.

Epizootic Hemorrhagic Disease

Evidence of sporadic exposure to epizootic hemorrhagic disease virus was found in caribou and Dall sheep (Tables 1, 2, and 4). These results agree with previously established patterns (Zarnke 1991).

Brucella suis IV

No evidence of exposure to *B. suis* IV was found in any caribou sera. These results were unexpected. Previous surveys have found rates exceeding 20% (Zarnke 1991). The current discrepancy may be related to a recent change to a new laboratory. Procedures and/or reagents at the new lab may provide a lower estimate of prevalence.

ACKNOWLEDGMENTS

I acknowledge the contributions of sera made by many hunters and wildlife professionals throughout the state. Without the cooperation of the Wyoming State Veterinary Laboratory this study would not have been feasible.

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Figure 1. Approximate home ranges of caribou (Rangifer tarandus)herds from which blood samples were collected for serologic survey.

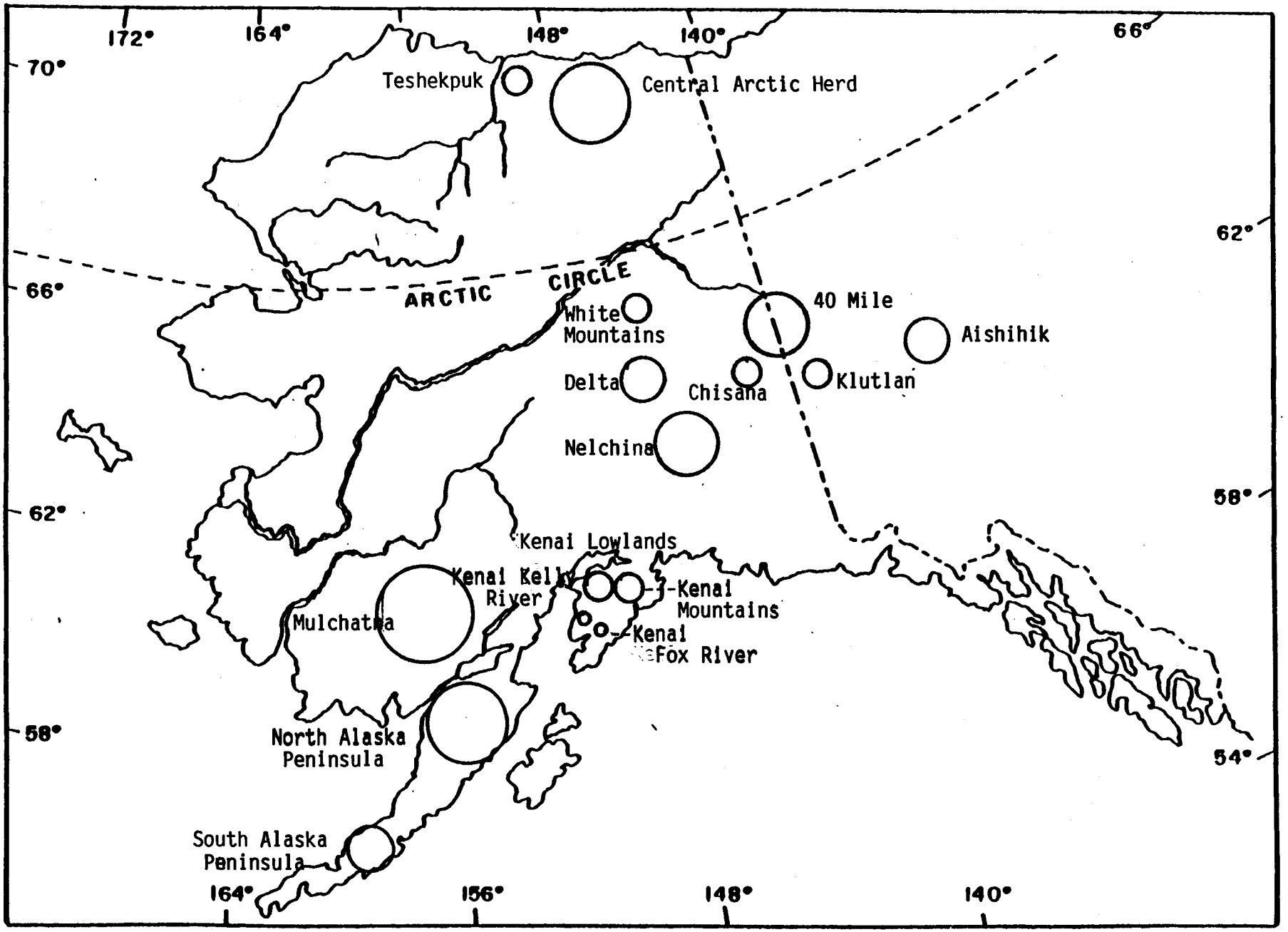


Figure 2. Locations at which blood samples were collected from listed species for serologic survey.

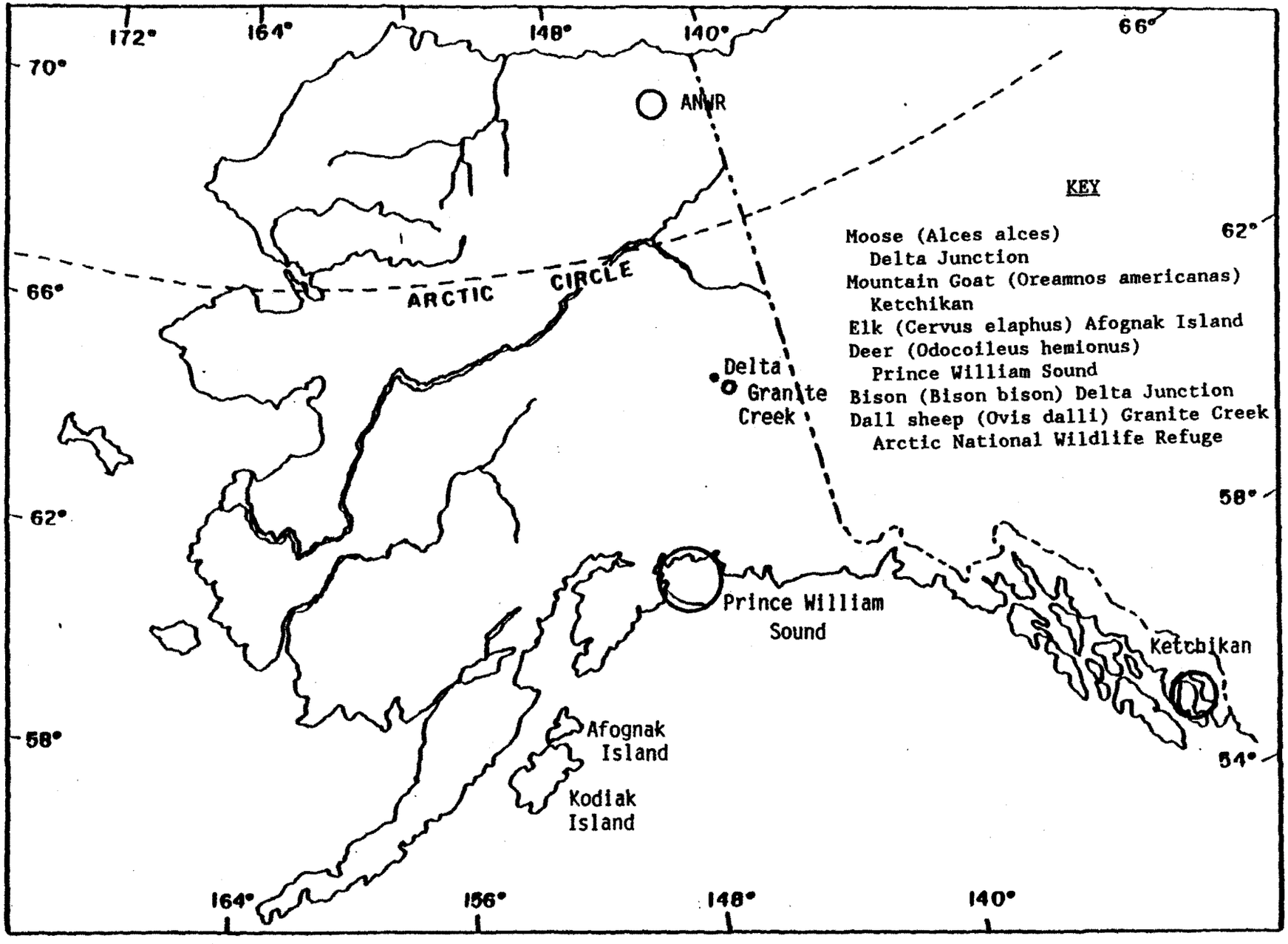


Table 1. Serum antibody prevalence of eight infectious disease agents in caribou from selected Alaskan herds in 1990.

	40 Mile	Chisana	Teshekpuk	Central Arctic	N. Alaska Peninsula	S. Alaska Peninsula	Mulchatna	Nelchina	Ethel Lake	Klutlan
Infectious bovine rhinotracheitis virus SN[a] 32[b]	0/9[c]	0/11	1/5	2/40	0/14	0/18	1/15	1/12	0/7	0/4
Bovine viral diarrhea virus SN(16)	2/14	0/12	4/5	11/41	0/14	0/18	0/15	0/12	0/7	0/4
Parainfluenza 3 virus SN(16)	0/14	0/12	1/5	11/41	0/14	0/18	0/15	0/12	0/7	0/4
Respiratory synctial virus SN(32)	0/14	0/12	0/5	1/41	0/14	0/18	0/15	0/12	0/7	0/4
Epizootic hemorrhagic disease virus ID(±)	0/14	0/12	0/5	0/41	1/14	0/18	0/15	0/12	0/7	0/4
Bluetongue virus ID(±)	0/9	0/8	0/5	0/36	0/14	0/18	0/15	0/12	0/7	0/4
Brucella suis IV SPT(50)	0/9	0/8	0/5	0/36	0/14	0/18	0/15	0/12	0/7	0/4
Leptospira interrogans MAT(100)	0/12	0/12	0/5	4/41	0/14	1/18	0/15	3/12	0/7	0/4

[a] Test method: SN = serum neutralization test, ID = immunodiffusion test, SPT = standard plate test, MAT = microscopic agglutination test.

[b] Number in parenthesis indicates minimum titer necessary to be considered evidence of exposure to agent in question. (±) indicates that test is interpreted as simply either "positive" or "negative."

[c] Number positive/number tested.

Table 2. Serum antibody prevalence of eight infectious disease agents in caribou from selected Alaskan herds in 1991.

	40 Mile	Chisana	Teshekpuk	Delta	White Mountains	Kenai Lowlands	Kenai Mountains	Kenai Kelly R.	Kenai Fox R.	Aishihik
Infectious bovine rhinotracheitis virus SN[a] 32[b]	0/39[c]	0/10	0/11	0/40	0/9	0/5	0/13	1/9	0/3	0/9
Bovine viral diarrhea virus SN(16)	12/39	0/10	2/11	0/40	0/9	0/5	0/13	0/9	0/3	0/19
Parainfluenza 3 virus SN(16)	0/39	0/10	1/11	0/40	0/9	0/5	0/13	0/9	0/3	0/19
Respiratory synctial virus SN(32)	0/39	0/10	0/4	0/40	0/9	0/4	0/13	0/9	0/3	0/10
Epizootic hemorrhagic disease virus ID(±)	0/39	0/10	0/11	1/40	0/9	0/5	0/13	0/9	0/3	0/19
Bluetongue virus ID(±)	0/39	0/10	0/11	0/40	0/9	0/4	0/13	0/9	0/3	0/19
Brucella suis IV SPT(50)	0/39	0/10	0/11	0/40	0/9	0/4	0/13	0/9	0/3	0/18
Leptospira interrogans MAT(100)	0/39	0/10	0/11	1/40	0/9	0/5	0/13	0/9	0/3	0/19

[a] Test method: SN = serum neutralization test, ID = immunodiffusion test, SPT = standard plate test, MAT = microscopic agglutination test.

[b] Number in parenthesis indicates minimum titer necessary to be considered evidence of exposure to agent in question. (±) indicates that test is interpreted as simply either "positive" or "negative."

[c] Number positive/number tested.

Table 3. Serum antibody prevalence of eight infectious disease agents in bison in Delta Junction, Alaska in 1990 and 1991.

	1990	1991
Infectious bovine rhinotracheitis virus SN[a] 32[b]	0/43[c]	0/20
Bovine viral diarrhea virus SN(16)	0/43	0/20
Parainfluenza 3 virus SN(16)	42/42	19/20
Respiratory synctial virus SN(32)	4/43	0/20
Epizootic hemorrhagic disease virus ID(±)	0/43	0/20
Bluetongue virus ID(±)	0/42	0/20
Brucella suis IV SPT(50)	0/41	0/20
Leptospira interrogans MAT(100)	1/43	0/13

[a] Test method: SN = serum neutralization test, ID = immunodiffusion test, SPT = standard plate test, MAT = microscopic agglutination test.

[b] Number in parenthesis indicates minimum titer necessary to be considered evidence of exposure to agent in question. (±) indicates that test is interpreted as simply either "positive" or "negative."

[c] Number positive/number tested.

Table 4. Serum antibody prevalence of eight infectious disease agents in Dall sheep in selected areas of Alaska in 1990 and 1991.

	Delta controlled Use area 1990	ANWR 1991	Granite Creek 1991
Infectious bovine rhinotracheitis virus SN[a] 32[b]	0/1[c]	0/19	0/19
Bovine viral diarrhea virus SN(16)	0/2	0/19	0/19
Parainfluenza 3 virus SN(16)	0/2	0/19	0/19
Respiratory synctial virus SN(32)	0/2	0/19	0/19
Epizootic hemorrhagic disease virus ID(±)	0/2	0/19	0/19
Bluetongue virus ID(±)	0/2	0/19	0/19
Brucella suis IV SPT(50)	0/2	0/19	0/19
Leptospira interrogans MAT(100)	0/1	0/19	0/19

[a] Test method: SN = serum neutralization test, ID = immunodiffusion test, SPT = standard plate test, MAT = microscopic agglutination test.

[b] Number in parenthesis indicates minimum titer necessary to be considered evidence of exposure to agent in question. (±) indicates that test is interpreted as simply either "positive" or "negative."

[c] Number positive/number tested.

Table 5. Serum antibody prevalence of eight infectious disease agents in deer in Prince William Sound and Kodiak Island, Alaska in 1989 and 1990.

	Prince William Sound 1989	Kodiak 1989	Kodiak 1990
Infectious bovine rhinotracheitis virus SN[a] 32[b]	0/2[c]	0/1	0/15
Bovine viral diarrhea virus SN(16)	0/2	0/1	0/14
Parainfluenza 3 virus SN(16)	0/2	0/1	0/15
Respiratory synctial virus SN(32)	0/2	0/1	0/14
Epizootic hemorrhagic disease virus ID(±)	0/2	0/1	0/15
Bluetongue virus ID(±)	0/2	0/1	0/15
Brucella suis IV SPT(50)	0/2	0/1	0/15
Leptospira interrogans MAT(100)	0/1	0/1	0/15

[a] Test method: SN = serum neutralization test, ID = immunodiffusion test, SPT = standard plate test, MAT = microscopic agglutination test.

[b] Number in parenthesis indicates minimum titer necessary to be considered evidence of exposure to agent in question. (±) indicates that test is interpreted as simply either "positive" or "negative."

[c] Number positive/number tested.

Table 6. Serum antibody prevalence of eight infectious disease agents in moose in Delta Junction, Alaska in 1990 and 1991.

	1990	1991
Infectious bovine rhinotracheitis virus SN[a] 32[b]	0/11[c]	0/4
Bovine viral diarrhea virus SN(16)	0/11	0/4
Parainfluenza 3 virus SN(16)	0/11	0/4
Respiratory synctial virus SN(32)	0/11	0/4
Epizootic hemorrhagic disease virus ID(±)	0/11	0/4
Bluetongue virus ID(±)	0/11	0/4
Brucella suis IV SPT(50)	0/11	0/4
Leptospira interrogans MAT(100)	0/11	0/4

[a] Test method: SN = serum neutralization test, ID = immunodiffusion test, SPT = standard plate test, MAT = microscopic agglutination test.

[b] Number in parenthesis indicates minimum titer necessary to be considered evidence of exposure to agent in question. (±) indicates that test is interpreted as simply either "positive" or "negative."

[c] Number positive/number tested.

Table 7. Serum antibody prevalence of eight infectious disease agents in mountain goat in Ketchikan, Alaska in 1991.

	1991
Infectious bovine rhinotracheitis virus SN[a] 32[b]	0/15[c]
Bovine viral diarrhea virus SN(16)	0/15
Parainfluenza 3 virus SN(16)	0/15
Respiratory synctial virus SN(32)	0/15
Epizootic hemorrhagic disease virus ID(±)	0/15
Bluetongue virus ID(±)	0/15
Brucella suis IV SPT(50)	0/15
Leptospira interrogans MAT(100)	0/15

[a] Test method: SN = serum neutralization test, ID = immunodiffusion test, SPT = standard plate test, MAT = microscopic agglutination test.

[b] Number in parenthesis indicates minimum titer necessary to be considered evidence of exposure to agent in question. (±) indicates that test is interpreted as simply either "positive" or "negative."

[c] Number positive/number tested.

Table 8. Serum antibody prevalence of eight infectious disease agents in elk in Ketchikan, Alaska in 1989.

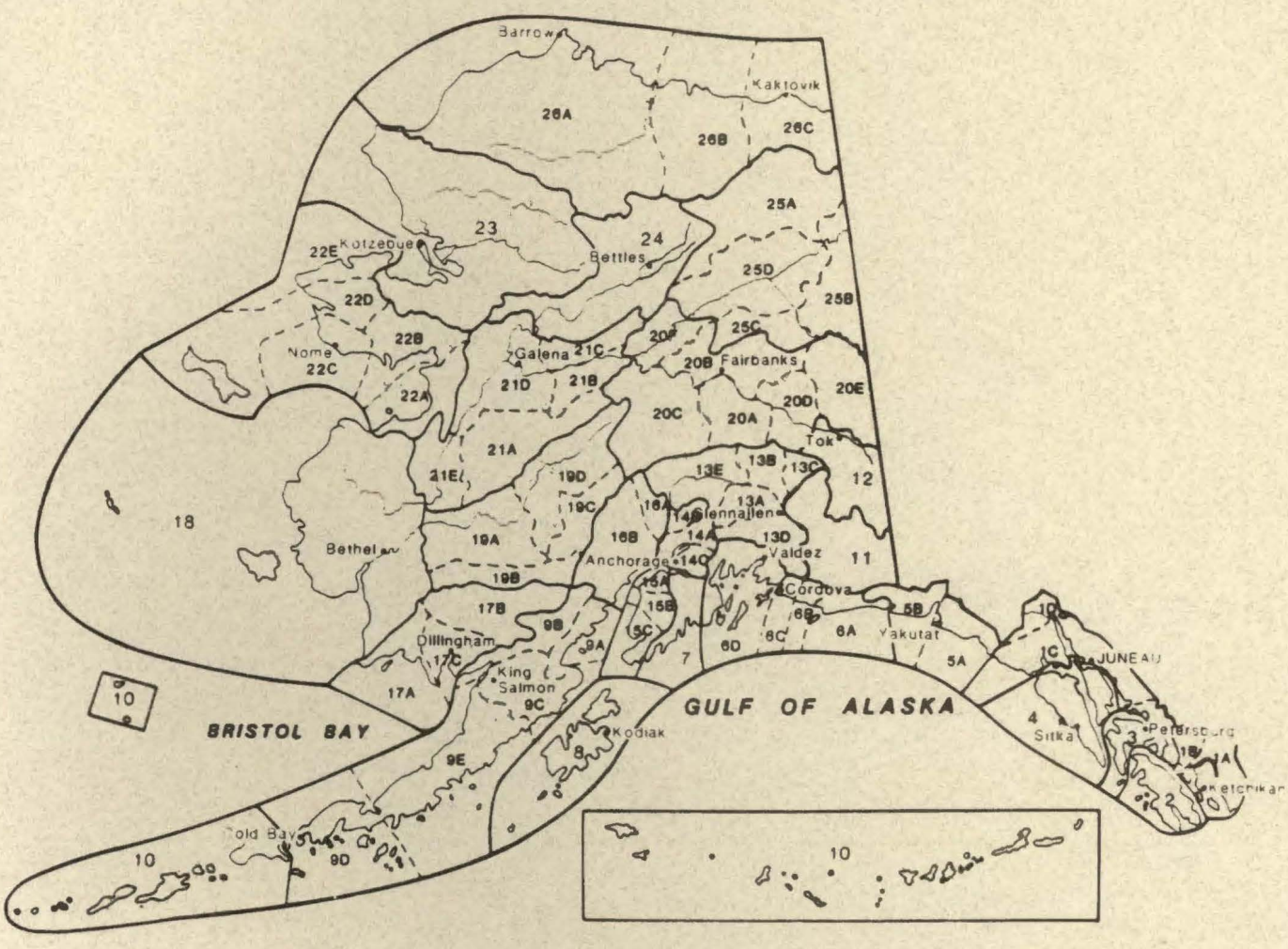
	1991
Infectious bovine rhinotracheitis virus SN[a] 32[b]	0/6[c]
Bovine viral diarrhea virus SN(16)	0/5
Parainfluenza 3 virus SN(16)	0/6
Respiratory synctial virus SN(32)	0/5
Epizootic hemorrhagic disease virus ID(±)	0/6
Bluetongue virus ID(±)	0/6
Brucella suis IV SPT(50)	0/6
Leptospira interrogans MAT(100)	1/5

[a] Test method: SN = serum neutralization test, ID = immunodiffusion test, SPT = standard plate test, MAT = microscopic agglutination test.

[b] Number in parenthesis indicates minimum titer necessary to be considered evidence of exposure to agent in question. (±) indicates that test is interpreted as simply either "positive" or "negative."

[c] Number positive/number tested.

Alaska's Game Management Units



Federal Aid in Wildlife Restoration

The Federal Aid in Wildlife Restoration Program consists of funds from a 10% to 11% manufacturer's excise tax collected from the sales of handguns, sporting rifles, shotguns, ammunition, and archery equipment. The Federal Aid program then allots the funds back to states through a formula based on each state's geographic area and the number of paid hunting license holders in the state. Alaska receives 5% of the revenues collected each year, the maximum allowed. The Alaska Department of Fish and Game uses the funds to help restore, conserve, manage, and enhance wild birds and mammals for the public benefit. These funds are also used to educate hunters to develop the skills, knowledge, and attitudes necessary to be reponsible hunters. Seventy-five percent of the funds for this project are from Federal Aid.