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# **Serologic Survey of Alaska Wildlife for Microbial Pathogens**

**Randall L. Zarnke**

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This is a progress report on continuing research. Information may be refined at a later date.

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### SUMMARY

A serologic survey of selected wildlife species from Alaska was conducted. There was little or no evidence of exposure to most disease agents in most host species. Some notable exceptions were apparent:

- 1 Antibody prevalence of 3 respiratory viruses (infectious bovine rhinotracheitis [IBR], bovine viral diarrhea [BVD], and parainfluenza 3 [PI3]) remained high in the Western Arctic caribou (*Rangifer tarandus*) herd (WAH).
- 2 Prevalence of BVD and PI3 remain high in the Porcupine caribou herd.
- 3 There was no evidence of exposure to the respiratory viruses in the Galena Mountains caribou herd.
- 4 Evidence of PI3 was found in the Nushagak and Northern Alaska Peninsula caribou herds for the first time. Pneumonia has been found in calves from the Northern Alaska Peninsula Herd for the past several years. There may be a link between these 2 observations.
- 5 Prevalence of BVD and PI3 remain moderate and stable in the moose (*Alces alces*) population near Kotzebue.
- 6 Evidence of PI3 exposure was found in moose from the Togiak area. This represents a major change from previous surveys.
- 7 Antibody prevalence of *Brucella* sp. remains low in both the WAH and wolves (*Canis lupus*) from Unit 20A near Fairbanks.

- 8 Evidence of exposure to *Leptospira interrogans* serovar *icterohemorrhagiae* was high in the WAH and low in wolves from Unit 20A.
- 9 Prevalence for canine distemper virus was 0% in wolves from all areas included in the current survey. This is the first time there has been no evidence of exposure to this agent.
- 10 Prevalence for infectious canine hepatitis virus was high in wolves from all areas included in the survey.
- 11 Prevalence for canine parvovirus was moderate in wolves from all areas.
- 12 Prevalence for canine coronavirus in wolves was substantially higher than previous surveys. In addition, prevalence exhibited a distinct seasonal pattern. Prevalence was high in the spring and low in the autumn.

**Key words:** Alaska, disease, serologic survey, wildlife.

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## BACKGROUND

There have been few documented instances of infectious diseases having a detectable effect on wildlife populations in Alaska. Brucellosis in caribou 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, providing information on the timing of exposure.
- 4 Tests are relatively inexpensive.

- 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 time (under adequate storage conditions), 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 time, it is possible to determine changes in the disease status of the population.
- 9 Early warning 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, reexposure 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 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 scope to disease agents and host species that were investigated. Over the past decade the survey has been extended to include both more potential host species and more disease agents.

## OBJECTIVE

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. General collection areas are indicated in Figures 1–4.

Most blood samples were allowed to settle at ambient or refrigerated temperatures for 6–36 hours and then centrifuged. Sera were then removed by aspiration and dispensed in vials. Sera were kept frozen until the time of testing. Serologic tests were performed by personnel of the Wyoming State Veterinary Laboratory (Laramie, Wyoming, USA) and the Washington State University (Pullman, Washington, USA). 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 Infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), parainfluenza III (PI3), respiratory syncytial virus (RSV), canine distemper virus (CDV), infectious canine hepatitis virus (ICH), canine parvovirus (CPV), and canine coronavirus (CCV), by the serum neutralization test (Thorsen and Henderson 1971).
- 2 Epizootic hemorrhagic disease and bluetongue, by the immunodiffusion test (Pearson and Jochim 1979).
- 3 *Leptospira* spp., by the microscopic agglutination test (Cole et al. 1973).
- 4 *Brucella* spp. by the buffered *Brucella* antigen and standard plate tests (US Department of Agriculture, undated).

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**

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

### **RESPIRATORY VIRUSES**

Four viral diseases, IBR, BVD, PI3, and RSV, 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. Major effects on individual animals occur via lowered body condition, decreased weight gain, and increased susceptibility to other infectious diseases. 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, BVD and PI3 continues to be high in the Western Arctic caribou herd (Table 1). Prevalence of BVD and PI3 remains high in the Porcupine caribou herd (Table 2). We have only seen a few cases of clinical respiratory disease in these herds during the past 20 years. There was no evidence of exposure to any of the respiratory viruses in the Galena Mountains Herd (Table 3). This is in contrast to results of the most recently published survey (Zarnke 1998). This discrepancy may be due to the small sample size in the current survey.

For the first time, we found evidence of exposure to PI3 in both the Northern Alaska Peninsula Herd and the Nushagak Herd (Table 4). We have been investigating pneumonia in the Northern Alaska Peninsula Herd for the past several years. We previously found histologic evidence of both verminous (parasitic) and bacterial pneumonia. However, there was no previous evidence of viral infection. None of the 60 animals collected from this herd between 1990 and 1995 had serologic evidence of exposure to any of the respiratory viruses. Perhaps these current serologic results reflect a long-term change in the epizootiology of respiratory pathogens in the southwestern portion of the state.

Antibody prevalence of both BVD and PI3 remain moderate and stable in the moose population from the northwest portion of the state (Table 5). We assume that presence of these agents in the moose population is related to the occurrence of these same agents in caribou from this region.

We also found serologic evidence of PI3 exposure in moose from the Togiak area (Table 5). This is the first significant evidence of exposure in moose south of the Arctic. This observation may be related to the same epizootiologic phenomena responsible for the sudden appearance of PI3 in caribou from this region.

#### ***BRUCELLA SPP.***

*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 through the food chain (Neiland 1970, 1975).

Antibody prevalence of *Brucella* spp. returned to a low level in the Western Arctic caribou herd (Table 1). The combined prevalence for the 1996–1998 period is on the low end of the range for this herd.

Prevalence of *Brucella* spp. exposure has been low (<10%) in wolves from Unit 20A in past years. In the current survey, we found 1 “positive” in 1997 (#306) and 3 in 1998 (including #306) (Table 6). Overall prevalence for the 4-year period 1995–1998 was in concurrence with long-term trends. All 3 of these wolves were young females (age 17–29 months). Wolf #306 had a successful litter in 1998. One of the others was trapped by a private individual during the season immediately following her capture by ADF&G. The other wolf is a member of a pack that is not routinely monitored. Therefore, the reproductive productivity of this wolf is unknown.

#### ***LEPTOSPIRA INTERROGANS***

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 through contamination of water by leptospire that 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 1 serovar is not uncommon.

Antibody prevalence for *L. interrogans* has traditionally been low in wolves from Unit 20A (Zarnke 1998). Results for the current survey continue that trend (Table 6).

Antibody prevalence for *L. interrogans* serovar *icterohemorrhagiae* was 16% (18/111) in the Western Arctic Herd during 1998. This value is substantially higher than found in previous surveys. All "positive" samples had a titer of 100, the minimum threshold for discriminating between negative and positive status. No explanation for the high prevalence is readily apparent.

#### **CANINE DISTEMPER VIRUS**

Signs of CDV infection may include discoloration and ulceration in the mouth, swollen foot pads, loss of appetite, decreased mobility, difficult breathing and neurologic



abnormalities. Previous studies reported antibody prevalences between 2% and 12% (Zarnke and Ballard 1987).

Antibody prevalence of canine distemper virus ranged from 6–52% for wolves from 11 geographic areas during our most recent serologic survey (Zarnke 1998). Low prevalences had been the norm in previous surveys (Zarnke and Ballard 1987; Stephenson et al. 1982). Prevalence was 0% in all areas during the current survey (Table 6). The sudden disappearance of this virus is difficult to explain. Directors of the lab where the serologic tests were conducted assured us they have been using not only the same procedure but also the same technician for the past 20 years. Therefore, they claim the results are accurate.

### **INFECTIOUS CANINE HEPATITIS VIRUS**

Signs of ICH virus infection (also known as canine adenovirus) may include nasal discharge, decreased mobility, loss of appetite, blood in feces, clouding of the eyes, and occasionally convulsions leading to paralysis and death. Previous surveys in Alaska reported high antibody prevalences (Zarnke and Ballard 1987).

Antibody prevalence for this agent has always been high in Alaska wolves (Stephenson et al. 1982; Zarnke and Ballard 1987; Zarnke 1998). Prevalences in the current survey continued this trend (Table 6).

### **CANINE PARVOVIRUS**

CPV was first reported in domestic dogs in 1978. A wide variety of free-ranging canids have been exposed. CPV infection can range from inapparent to fatal in domestic dogs. Infection affects the heart and/or the gastrointestinal tract. A previous survey of wolves in Southcentral Alaska reported that prevalence increased from 0% in the 1970s to approximately 50% during the early 1980s (Zarnke and Ballard 1987).

In the most recent ADF&G survey, antibody prevalence of canine parvovirus ranged from 17–73% in wolves from 11 areas of Alaska (Zarnke 1998). In the current survey, prevalence ranged from 23–67% (Table 6). Thus, the current results are in general agreement with previous data.

### **CANINE CORONAVIRUS**

Some experts believe that CCV and CPV operate synergistically to cause gastrointestinal dysfunction. Interest in CCV is relatively recent. There are no previous published CCV serologic surveys on free-ranging wolves.

Antibody prevalence of canine coronavirus ranged from 0–19% in wolves from 11 areas of the state during the most recent survey (Zarnke 1998). In the current survey, prevalences ranged from 26–70% (Table 6). In addition, a distinct seasonal pattern has emerged. Antibody prevalence averaged 75% during the spring collection period (March–April) in Unit 20A during 1995–1998. In the autumn period (October–November), prevalence averaged 23%. Prevalence was 0% (0/27) for the pup cohort (age

5 months) captured during the autumn. Prevalence was 72% (26/36) for the yearling cohort (age 10 months) captured during the spring. Apparently, most transmission occurred during the winter months. Prevalence for the adult cohort fell from 81% in the spring to 36% during the autumn. Apparently, antibody decay occurs quite rapidly.

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**PREPARED BY:**

Randall L Zarnke  
Wildlife Biologist II

**APPROVED BY:**

\_\_\_\_\_  
Wayne L Regelin, Director  
Division of Wildlife Conservation

**SUBMITTED BY:**

Kenneth R Whitten  
Research Coordinator

\_\_\_\_\_  
Steven R Peterson, Senior Staff Biologist  
Division of Wildlife Conservation

Figure 1 Locations where blood samples were collected from wolves (*Canis lupus*) in Alaska for serologic survey

Figure 2 Locations where blood samples were collected from moose (*Alces alces*) in Alaska for serologic survey

Figure 3 Locations where blood samples were collected from caribou (*Rangifer tarandus*) in Alaska for serologic survey

Figure 4 Locations where blood samples were collected from caribou (*Rangifer tarandus*) in the Northwest Territories, Canada for serologic survey

<b>HERD</b>	
<b>1</b>	<b>HART RIVER</b>
<b>2</b>	<b>BONNET PLUME</b>
<b>3</b>	<b>MAYO</b>
<b>4</b>	<b>ETHEL LAKE</b>
<b>5</b>	<b>MOOSE LAKE</b>
<b>6</b>	<b>TAY RIVER</b>
<b>7</b>	<b>REDSTONE</b>
<b>8</b>	<b>FINLAYSON</b>
<b>9</b>	<b>NAHANNI</b>
<b>10</b>	<b>LA BICHE</b>
<b>11</b>	<b>SMITH RIVER</b>
<b>12</b>	<b>LITTLE RANCHERIA</b>
<b>13</b>	<b>WOLF LAKE</b>
<b>14</b>	<b>ATLIN</b>
<b>15</b>	<b>CARCROSS/SQUANGA</b>
<b>16</b>	<b>IBEX</b>
<b>17</b>	<b>PELLY HERDS</b>
<b>18</b>	<b>TATCHUN</b>
<b>19</b>	<b>KLAZA</b>
<b>20</b>	<b>AISHIHIK</b>
<b>21</b>	<b>KLUANE</b>
<b>22</b>	<b>CHISANA</b>
<b>23</b>	<b>MENTASTA</b>
<b>24</b>	<b>NELCHINA</b>
<b>25</b>	<b>FORTYMILE</b>
<b>26</b>	<b>PORCUPINE</b>

## Tables



Table 1 Serum antibody prevalence of 7 infectious disease agents in caribou (*Rangifer tarandus*) from the Western Arctic Herd, Alaska, 1996–1998

Disease agent	1996	1997	1998
Infectious bovine rhinotracheitis virus SN <sup>a</sup> (32) <sup>b</sup>	14/71 <sup>c</sup>	1/75	35/113
Bovine viral diarrhea virus SN (16)	27/70	24/73	59/111
Parainfluenza 3 virus SN (32)	18/61	27/68	32/112
Respiratory syncytial virus SN (32)	0/56	0/59	0/113
Epizootic hemorrhagic disease virus ID (±)	0/70	0/72	0/105
<i>Leptospira interrogans</i> bacterium MAT (100)	1/70	0/75	18/111
<i>Brucella suis</i> biovar 4 buffered <i>Brucella</i> sp. antigen; ± standard plate test (50)	2/71	0/75	8/113

<sup>a</sup> Test method: SN = serum neutralization test, ID = immunodiffusion test, MAT = microscopic agglutination test.

<sup>b</sup> Number in parentheses 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.”

<sup>c</sup> Number positive/number tested.

Table 2 Serum antibody prevalence of 7 infectious disease agents from selected caribou (*Rangifer tarandus*) herds in the Yukon Territories, Canada

Disease agent	Herd/Year								
	Porcupine			Aishihik	Klaza	Kluane	Tatchun	Wolf Lake	Carcross
	1994	1996	1997	1996	1996	1996	1996	1996	1997
Infectious bovine rhinotracheitis virus SN <sup>a</sup> (32) <sup>b</sup>	0/9 <sup>c</sup>	0/35	0/28	0/22	0/6	0/9	0/5	0/23	0/4
Bovine viral diarrhea virus SN (16)	7/9	12/32	12/19	0/23	0/6	0/9	0/5	0/22	0/4
Parainfluenza 3 virus SN (32)	1/8	2/27	1/10	0/23	0/6	0/9	0/4	0/19	0/3
Respiratory syncytial virus SN (32)	0/9	0/25	0/16	0/22	0/6	0/9	0/5	0/23	0/4
Epizootic hemorrhagic disease virus ID (±)	0/9	0/36	0/29	0/23	0/6	0/9	0/4	0/23	0/3
<i>Leptospira interrogans</i> bacterium MAT (100)	0/9	0/36	0/20	0/23	0/6	0/9	0/5	0/23	0/4
<i>Brucella suis</i> biovar 4 buffered <i>Brucella</i> sp. antigen; ± standard plate test (50)	0/10	0/38	0/14	0/23	0/6	0/9	0/5	0/23	0/4

<sup>a</sup> Test method: SN = serum neutralization test, ID = immunodiffusion test, MAT = microscopic agglutination test.

<sup>b</sup> Number in parentheses 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.”

<sup>c</sup> Number positive/number tested.

Table 3 Serum antibody prevalence of 7 infectious disease agents from selected caribou (*Rangifer tarandus*) herds in Alaska

Disease agent	Herd/Year			
	Galena Mountain	Chisana		Fox River and Killey River <sup>a</sup>
	1993	1996	1997	1997
Infectious bovine rhinotracheitis virus SN <sup>b</sup> (32) <sup>c</sup>	0/4 <sup>d</sup>	0/5	0/4	0/8
Bovine viral diarrhea virus SN (16)	0/4	0/5	0/4	0/8
Parainfluenza 3 virus SN (32)	0/4	0/5	0/4	0/8
Respiratory syncytial virus SN (32)	0/4	0/5	0/4	0/8
Epizootic hemorrhagic disease virus ID (±)	0/4	0/5	0/4	0/8
Bluetongue virus ID (±)	0/4	0/5	0/4	0/6
<i>Leptospira interrogans</i> bacterium MAT (100)	0/4	0/5	0/4	0/8

<sup>a</sup> Combined sample of 2 caribou from the Fox River Herd and 6 caribou from the Killey River Herd.

<sup>b</sup> Test method: SN = serum neutralization test, ID = immunodiffusion test, MAT = microscopic agglutination test.

<sup>c</sup> Number in parentheses 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.”

<sup>d</sup> Number positive/number tested.

Table 4 Serum antibody prevalence of 7 infectious disease agents from 4 caribou (*Rangifer tarandus*) herds in Southwest Alaska

Disease agent	Herd/Year			
	Mulchatna	Nushagak Peninsula	N AK Peninsula	S AK Peninsula
	1996	1997	1997	1997
Infectious bovine rhinotracheitis virus SN <sup>a</sup> (32) <sup>b</sup>	0/20 <sup>c</sup>	0/19	0/28	0/19
Bovine viral diarrhea virus SN (16)	0/20	0/19	0/29	0/19
Parainfluenza 3 virus SN (32)	0/20	5/19	3/29	0/19
Respiratory syncytial virus SN (32)	0/20	0/19	0/29	0/19
Epizootic hemorrhagic disease virus ID (±)	0/20	1/19	0/29	0/19
Bluetongue virus ID (±)	0/20	0/18	0/28	0/18
<i>Leptospira interrogans</i> bacterium MAT (100)	0/20	0/18	0/23	0/16

<sup>a</sup> Test method: SN = serum neutralization test, ID = immunodiffusion test, MAT = microscopic agglutination test.

<sup>b</sup> Number in parentheses 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.”

<sup>c</sup> Number positive/number tested.

Table 5 Serum antibody prevalence of 8 infectious disease agents in moose (*Alces alces*) from several areas of Alaska

Disease agent	Lake Clark	Noatak	Selawik River			Moose Research Center		Yukon Flats	Togiak
	1996	1997	1996	1997	1998	1997	1998	1998	1998
Infectious bovine rhinotracheitis virus SN <sup>a</sup> (32) <sup>b</sup>	0/29 <sup>c</sup>	0/39	0/15	0/18	0/37	0/11	0/9	0/30	0/32
Bovine viral diarrhea virus SN (16)	0/29	3/39	0/15	1/18	2/37	0/11	0/10	0/30	0/32
Parainfluenza 3 virus SN (32)	0/30	4/32	4/14	6/17	16/37	0/11	0/10	0/30	10/34
Respiratory syncytial virus SN (32)	1/30	0/37	0/15	0/18	0/37	0/11	0/10	0/30	0/34
Epizootic hemorrhagic disease virus ID (±)	0/30	0/35	0/13	0/17	0/37	0/11	0/10	0/30	0/34
Bluetongue virus ID (±)	0/30	ND <sup>d</sup>	ND	ND	0/37	0/11	0/10	0/30	0/33
<i>Leptospira interrogans</i> bacterium MAT (100)	0/30	0/38	0/15	0/18	1/37	1/11	0/10	1/30	0/34
<i>Brucella suis</i> biovar 4 buffered <i>Brucella</i> sp. antigen; ± standard plate test (50)	ND	0/39	0/15	0/18	0/37	ND	ND	0/30	ND

<sup>a</sup> Test method: SN = serum neutralization test, ID = immunodiffusion test, MAT = microscopic agglutination test.

<sup>b</sup> Number in parentheses 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.”

<sup>c</sup> Number positive/number tested.

<sup>d</sup> Not done.

Table 6 Serum antibody prevalence of 6 infectious disease agents in wolves (*Canis lupus*) from selected areas of Alaska

Disease agent	Area/Year									
	Palmer 1999	Unit 20A			Fortymile			Yukon-Charley		
	1995	1996	1997	1998	1996	1997	1998	1997	1998	
Canine distemper virus SN <sup>a</sup> (10) <sup>b</sup>	0/27 <sup>c</sup>	0/36	0/42	0/64	0/52	0/11	0/34	0/43	0/16	0/13
Infectious canine hepatitis SN (10)	19/27	31/36	38/42	63/64	50/52	10/11	29/34	41/44	14/16	12/13
Canine parvovirus SN (25)	18/27	12/36	25/42	22/64	30/52	3/11	6/35	12/44	5/16	2/13
Canine coronavirus SN (25)	19/27	22/36	25/42	34/64	28/52	2/11	4/35	17/44	10/16	10/13
<i>Leptospira interrogans</i> bacterium MAT (100)	0/27	0/36	0/42	1/64	1/52	0/11	0/35	0/44	0/16	1/13
<i>Brucella suis</i> biovar 4 buffered <i>Brucella</i> sp. antigen; ± standard plate test (50)	0/27	0/36	0/42	1/64	3/52	0/5	1/34	0/44	0/16	0/13

<sup>a</sup> Test method: SN = serum neutralization test, ID = immunodiffusion test, MAT = microscopic agglutination test.

<sup>b</sup> Number in parentheses 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.”

<sup>c</sup> Number positive/number tested.