

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

SEROLOGIC SURVEY
OF ALASKA WILDLIFE
FOR MICROBIAL PATHOGENS



by
Randall L. Zarnke
Project W-23-3
Study 18.6
September 1990

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Serologic Survey of Alaska Wildlife for Microbial Pathogens

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SUMMARY

Under the study title Serologic Survey for Microbial Pathogens, 3 projects were conducted. The manuscripts constitute the progress report for this study and have been submitted to the Journal of Wildlife Diseases.

Mycoplasma ovipneumoniae killed Dall sheep (Ovis dalli) in a Canadian zoo. Serologic tests of 251 free-ranging Dall sheep from Alaska revealed no evidence of exposure to this agent.

Parainfluenza 3 virus is a respiratory pathogen of ungulates, primarily domestic livestock. Serum antibody prevalence of this agent in the Delta Bison (Bison bison) Herd increased from 0% in 1977 to 100% in 1984. Domestic cattle are believed to be the source of this infection.

Dermacentor albipictus ticks cause hair loss in Canadian moose (Alces alces). The tick is not found in Alaska. Experiments involving captive ticks indicate that they could survive in Interior Alaska if introduced. Apparently, weather patterns near the Alaska-Canada border prevent natural spread of the tick into the state.

Key Words: bison, Dall sheep, Dermacentor albipictus, moose, Mycoplasma ovipneumoniae, parainfluenza 3, serologic survey, winter tick

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SEROLOGIC SURVEY FOR MYCOPLASMA OVIPNEUMONIAE IN FREE-RANGING
DALL SHEEP (OVIS DALLI) IN ALASKA

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ABSTRACT: Indirect hemmagglutination tests on sera from 251 Dall
sheep (Ovis dalli) from Interior Alaska collected during the
period 1979 to 1987 revealed no evidence of exposure to
Mycoplasma ovipneumoniae. Apparently, this potentially fatal
disease agent has not been introduced into free-ranging Dall
sheep populations. In the interest of continued health of Alaska
Dall sheep, strict enforcement of domestic animal health
regulations and prudent land use practices are clearly indicated.

Key Words: Mycoplasma ovipneumoniae, Dall sheep, Ovis dalli,
Alaska, serology.

Mycoplasma ovipneumoniae caused pneumonia in 10 and death of 3
Dall sheep (Ovis dalli) at the Metropolitan Toronto Zoo during
1986 (Black et al. 1988). Domestic sheep were believed to be the

source of M. ovipneumoniae. Black et al. (1988) concluded that M. ovipneumoniae is highly pathogenic for Dall sheep and posed questions regarding the occurrence and impact of this disease agent in free-ranging Dall sheep. The objective of the present study was to determine the serum antibody prevalence of M. ovipneumoniae in free-ranging Dall sheep from Interior Alaska, USA.

Blood samples were collected from Dall sheep at Sheep Creek (63°25'N, 143°50'W) ($n = 125$), Dry Creek (63°55'N, 147°25'W) ($n = 112$), and White Mountains (65°40'N, 141°20'W) ($n = 11$) by the Alaska Department of Fish and Game during population ecology studies from 1979 through 1987. Three samples were also collected from captive Dall sheep near Fairbanks (64°50'N, 147°50'W). Specimens were allowed to clot and settle for 12 to 36 hrs at ambient temperatures. Occasionally, samples were also centrifuged. Sera were collected by aspiration and frozen. Indirect hemagglutination tests (Cho et al. 1976) were conducted at the Ontario Veterinary College (Guelph, Ontario N1G 2W1, Canada). A titer of $\geq 1:16$ was established as indicative of previous exposure, based upon results of tests involving captive Dall sheep (Black et al. 1988). Known positive and negative control sera from Dall sheep involved in the Toronto Zoo epizootic were included with each series of tests.

Results of all tests were negative. Without sampling every animal in a population, we cannot be 100% certain that none of the sheep have been exposed to M. ovipneumoniae; however, confidence intervals for the Dry creek and Sheep Creek populations suggest that if M. ovipneumoniae is present in either population, we are 95% certain that prevalence is less than 3.2% (Johnson and Kotz 1969). Thus free-ranging populations represented in the current study are probably immunologically naive to M. ovipneumoniae. If M. ovipneumoniae were to be introduced into these populations, significant morbidity and mortality could result. A domestic animal pathogen (parainfluenza 3 virus) has recently been introduced into free-ranging bison in Alaska (Zarnke 1987). Hopefully, prudent land use practices and strict enforcement of domestic animal health regulations will prevent introduction of M. ovipneumonia and other disease agents into Dall sheep populations.

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SERUM ANTIBODY PREVALENCE OF PARAINFLUENZA 3 VIRUS IN A
FREE-RANGING BISON (BISON BISON) HERD FROM ALASKA

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ABSTRACT: Serum antibody prevalence of parainfluenza 3 virus in
the free-ranging Delta bison, herd which is found near Delta
Junction, Alaska (USA), increased from 0% to 100% during the
period 1977 to 1988.

Key Words: Bison, Bison bison, parainfluenza 3 virus, PI3,
serologic survey

The Delta bison (Bison bison) herd numbers from 300 to 400 and
ranges freely in a 800-km² area principally south and east of the
community of Delta Junction, Alaska (145°45'W, 64°00'N). This
herd was established by a transplant of 23 bison from Montana in
1928.

During the 1970's and early 1980's, major expansion of
agricultural industries occurred near Delta Junction (Engelbrecht
and Thomas 1987). Cattle and swine populations in the Delta
Junction area increased 2- to 3-fold between the mid-1970's and
mid-1980's (Alaska Agricultural Statistics Service 1988). IN
1988 cattle, horses, and swine all numbered in the hundreds;
sheep and goats were less numerous.

Bison do not routinely come into direct contact with domestic
livestock; however, part of the historic bison range has been
cleared and developed for agricultural purposes. During August
and September bison have been attracted to agricultural areas by
maturing grain crops. Direct and indirect interaction with
livestock may occur at such times.

Moose (Alces alces) are common within the home range of the Delta
bison herd. Bison may occasionally come into contact with Dall
sheep (Ovis dalli) and caribou (Rangifer tarandus) at the edges
of respective home ranges. There is no contact with other free-
ranging bison. A serologic survey was conducted to determine the
exposure of wildlife populations, especially bison, to livestock
pathogens in the area.

Blood samples from 409 Delta herd bison were collected by hunters
from animals which they had shot or by personnel of the Alaska
Department of Fish and Game (P.O. Box 605, Delta Junction, Alaska
99737) from tranquilized bison. Samples were collected from 43

Delta herd caribou, which range from 146°30'W to 149°00'W and from 63°45'N to 64°15'N. Thirty moose sera were collected from an area extending from 146°00'W to 149°00'W and 64°15'N to 64°45'N. Specimens were collected from 77 Dall sheep at Sheep Creek (143°50'W, 63°20'N). Blood was allowed to settle for 18 to 48 hrs at ambient or refrigerated temperatures and then centrifuged. Sera were collected and frozen.

Hemagglutination inhibition tests (HI) for evidence of exposure to parainfluenza 3 virus (PI3) were performed according to the method of Thorsen and Henderson (1971). Confirmatory PI3 tests on 78 bison sera were conducted at the same facility, using the serum neutralization procedure (Thorsen and Henderson, 1971). Bison sera were also periodically tested for evidence of exposure to: (1) infectious bovine rhinotracheitis, bovine viral diarrhea, and respiratory syncytial virus by the serum neutralization test (Thorsen and Henderson 1971); (2) epizootic hemorrhagic disease and bluetongue viruses by the immunodiffusion test (Pearson and Jochim 1979); (3) Coxiella burnetti by the complement fixation test (Erickson et al. 1975); (4) Brucella spp. by the buffered acidified plate antigen test (Angus and Barton 1984); (5) 12 serovarieties of Leptospira interrogans by the microscopic agglutination test (Cole et al. 1973); and (6) Mycobacterium bovis by the enzyme-linked immunosorbent assay test (Thoen et al. 1988).

Known positive and negative control sera were included with each batch of sera tested. A titer of 8 or greater was considered evidence of past exposure to PI3 for both methods, based upon standards established for other wildlife species (Thorsen and Henderson 1971, Parks and England 1974, Kingscote and Bohac 1986). Samples that met or exceeded this titer will be referred to as "positive." All others are referred to as "negative." Differences in antibody prevalence based upon gender of bison were tested for significance by means of the Chi-square test (Johnson 1980). Virus isolation was attempted from nasal swabs collected by hunters during 1984 ($n = 27$) and 1988 ($n = 16$), bison lung tissue during 1985 ($n = 17$), and lung lavage fluid during 1988 ($n = 17$).

Results of PI3 HI tests for bison are presented in Table 1. Titers ranged from 8 to 256. Results of neutralization tests confirmed positive results of HI tests in every case. All virus isolation attempts were unsuccessful.

No evidence of exposure to PI3 was found in bison samples collected prior to 1977. By 1983 antibody prevalence had reached nearly 100% and remained high for the remainder of the sampling period. A 1972 serologic survey of 41 bison from the National Bison Range in Montana revealed that "antibodies against parainfluenza-3 virus were present in all serum samples tested" (Heddleston and Wessman 1973). Apparently PI3 has been enzootic in that herd for many years. By contrast, the dramatic increase in antibody prevalence in the Delta Bison Herd is typical of the

pattern following introduction of a new disease agent into a susceptible population.

Ages were not available for most bison. Of 12 yearling bison sampled between 1983 and 1986, 11 were serologically positive for PI3. The only calf (6 mo old) sampled during this time was also positive. The calf's test result may have been due to passive transfer of maternal antibody; however, these data suggest that bison are exposed to PI3 early in life. There was no significant difference in sex-specific prevalence. This is expected for an agent transmitted primarily by means of respiratory aerosols.

Serum antibody prevalence of PI3 in other wildlife species was 0%. Serum antibody prevalence in bison for infectious bovine rhinotracheitis virus was 1/366, bovine viral diarrhea virus 6/316, respiratory syncytial virus 0/105, epizootic hemorrhagic disease virus 2/392, bluetongue virus 0/391, Coxiella burnetti 1/274, Brucella spp. 0/309, Leptospira interrogans 14/306, and Mycobacterium bovis 0/46.

Domestic livestock are believed to have been the source of PI3 which entered the Delta Bison Herd. Respiratory disease is not uncommon in cattle in this area. Serologic evidence indicated that cattle have been exposed to infectious bovine rhinotracheitis, bovine viral diarrhea, and PI3 (R. A. Dieterich, pers. commun.). Long-term, large-scale serologic survey results for cattle are not available.

To date, there have been no signs of disease nor any decrease in the high productivity in this closely monitored bison herd. Fortunately, by itself PI3 is considered to be a minor pathogen in most wildlife species (Karstad 1981). Other pathogens that could be transmitted from livestock to bison may not be so innocuous. In a parallel situation, pathogens from domestic sheep have been implicated as being partially responsible for declines of bighorn sheep (Ovis canadensis) populations in the contiguous United States (Turner and Payson 1982).

Wildlife populations in Alaska have been largely free of livestock diseases in the past (Zarnke 1986). Increased movement of livestock and geographic expansion of agriculture may pose a threat to the health of wildlife populations. The potential of pathogen exchange between domestic and wildlife species should play a role in the decision-making process related to the agriculture industry and management of wildlife.

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TABLE 1. Serum hemagglutination inhibition antibody prevalence of parainfluenza 3 virus in the Delta bison herd from Delta Junction, Alaska (USA).

Year	Sex		Total (%)	
	Male	Female		
1975	0/5a	0/3	0/8	(0%)
1976	0/5	0/8	0/13	(0%)
1977	0/14	0/12	1/26	(4%)
1978	9/18	4/12	13/30	(43%)
1979	4/6	4/5	8/11	(73%)
1980	1/3	1/2	2/5	(40%)
1981	19/33	4/12	23/45	(51%)
1982	9/26	8/27	17/53	(32%)
1983	14/14	30/31	44/45	(98%)
1984	18/18	23/23	41/41	(100%)
1985	10/11	18/18	28/29	(97%)
1986	13/13	38/38	51/51	(100%)
1987	22/22	20/20	42/42	(100%)
1988	7/7	3/3	10/10	(100%)
Total	126/195 (65%)	154/214 (72%)	280/49	(68%)

a Number positive/number tested.

FACTORS INFLUENCING THE POTENTIAL ESTABLISHMENT OF THE WINTER TICK (DERMACENTOR ALBIPICTUS) IN ALASKA

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ABSTRACT: The winter tick, (Dermacentor albipictus) is not known to occur in Alaska. Survival and development of free-living (i.e., nonhost-associated) stages of the tick were studied at 3 sites in central and southern Alaska. Female ticks survived and oviposited, and eggs hatched at all sites. Hatch success was low at 1 site where summer temperatures were low. Results suggest that establishment of winter ticks in Alaska following accidental translocation is possible, but several factors would affect such establishment.

Key Words: Winter tick, Dermacentor albipictus, tick survival and development, Acari, epizootiology, Alaska, experimental field study

The northern limit of Dermacentor albipictus in North America is not well-defined. Wilkinson (1967) has proposed that an isopleth of 1,500 accumulated day degrees over 42°F (5.6°C) could be the northern boundary. In western North America this isopleth, as presented by Wilkinson (1967), extends through the southern Northwest Territories and northern British Columbia (Canada) and the southern tip of Alaska (USA). Klein (1965) found "Dermacentor sp." during examination of 46 Sitka black-tailed deer (Odocoileus hemionus sitkensis) in southeastern Alaska. Samuel (1989) suggests that winter ticks may occur as far north as 62°N. Although both reports indicate that winter ticks occur in areas geographically close to Alaska, the winter tick apparently does not occur naturally in Alaska.

Dermacentor albipictus infests many species of mammals in North America. Moose (Alces alces) are a major host (Anderson and Lankester 1974), but elk (Cervus elaphus), deer (Odocoileus spp.), horses, and cattle (Bishopp and Trembley 1945, Arthur 1960) also may be infested. Moose populations are contiguous from Alberta and British Columbia to Alaska, but populations of winter ticks apparently are not (Wilkinson 1967, Samuel 1989). The question arises as to whether or not D. albipictus could

become established in Alaska if it were introduced into the state by means of a translocated host.

Objectives of this study were to determine if (1) engorged female ticks could survive in Alaska, (2) these same ticks could oviposit, and (3) any resulting eggs would hatch. Results of experiments from Edmonton, Alberta, Canada (53°33'N, 113°25'W) are presented for comparison.

Detached, engorged adult female *D. albipictus* ($n = 215$) that had fed on captive moose ($n = 146$ ticks) were collected in mid-April near Edmonton, Alberta (Canada) in 1984 and 1986 and from a freshly dead moose ($n = 69$ ticks) near Jackson, Wyoming (USA) (43°55'N, 110°40'W) in mid-April 1985. Ticks were stored at 3°C and sent to Fairbanks, Alaska (USA) (64°50'N, 147°50'W) within 8 days after collection.

To prevent escape of the ticks, individual ticks were placed in separate 20- x 75-mm glass vials. A 10-mm-diameter hole was drilled in the vial cap, and the hole was covered with a fabric mesh (largest opening in fabric approximately 0.10 x 0.25 mm). Several such vials were then placed inside a 22- x 27-cm zippered cylindrical bag constructed of the same fabric material. The bag was hung at ground level inside a 34- x 105-cm cylindrical hardware cloth cage (mesh size 3 mm). The cage was secured upright inside a 28- x 56-cm galvanized washtub that had been half-filled with soil and native vegetation. The number of ticks at each study site ranged from 15 to 24, depending upon availability of ticks in any particular year.

Tick enclosures were placed (1) on the roof of a shed near Fairbanks between 23 and 28 April 1984 to 1986, (2) on the roof of an office building in Palmer, Alaska (61°35'N, 149°10'W) between 23 and 27 April 1984 to 1986, and (3) at ground level near Soldotna, Alaska (60°30'N, 151°04'W) between 24 and 28 April 1984 to 1986 (Fig. 1). Two identical enclosures were placed on opposite sides of a building (north- and south-facing) at the Soldotna site. The climate, topography, geology, flora, fauna, etc., of these areas have been described previously (Selkregg 1974). Ticks were examined for survival and oviposition, and eggs were examined for eclosion at approximately 7- to 10-day intervals from late April until mid-October 1984 to 1986. Weather information for Alaska was obtained from the National Climatic Data Center (Climatological Data 1984, 1985, 1986). Day-degrees is defined as (Daily maximum temperature $F +$ Daily minimum temperature $F \div 2$) - 42.

In Edmonton, Alberta, 26 ticks were placed in screen-wire cages, as described by Drew and Samuel (1986), and placed in an open, early growth aspen (*Populus tremuloides*) copse at the University of Alberta Ellerslie Research Station on 24 April 1987. Cages used in Alberta were less elaborate, because (unlike Alaska) escape would not represent a threat of introduction and establishment of a new parasite. These cage differences may have

resulted in microhabitat differences and therefore affected tick development. Ticks were examined weekly until 2 January 1988.

Survival and oviposition of engorged female D. albipictus were high at all sites (Table 1). Eclosion success by mid-September was high at Fairbanks, Palmer, and Edmonton, but low at the two Soldotna sites. Eggs only hatched at Soldotna on one of five occasions.

The median date of oviposition was approximately 31 May at Edmonton, 1 June at Fairbanks, 6 June at Palmer, 16 June at Soldotna-south, and 29 June at Soldotna-north. Oviposition occurred over periods ranging from as short as 4 days at Fairbanks in 1984 and 7 days at Edmonton to as long as 72 days at Soldotna-south in 1986. The median date of hatch was approximately 26 August at Edmonton, 19 August at Fairbanks, and 28 July at Palmer (based on 1 year of data). No eggs hatched at Soldotna-north. Timing of hatch was not recorded for the single year in which eggs hatched at Soldotna-south.

At Fairbanks and Edmonton, egg masses ranged in size from 1 mm to 1 cm diameter. The larger masses contained an estimated 1,000-3,000 eggs. Subjective estimates of percent hatch for individual vials at Fairbanks ranged from zero to 75%. Approximately 90% of the eggs hatched at Edmonton. Such data were not collected at other sites.

Results from Alaska indicated that the environmental conditions necessary for survival and development of D. albipictus were met at Palmer and Fairbanks. Experiments there resulted in viable larvae during all 3 years. In contrast, few live larvae were produced at the two Soldotna sites. Because survival of female ticks and success of oviposition were high (77%) at Soldotna, it was the incubation period (length of time between egg-laying and hatching) that was most negatively affected. Drew and Samuel (1986) suggest that the incubation period for D. albipictus is temperature dependent. If so, the 30-year average of 1,226 day-degrees at Soldotna (Fig. 1) was probably below the minimum threshold for complete egg development and eclosion. Thirty-year average day-degrees for the Palmer and Fairbanks areas were 1,673 and 1,948, respectively.

If, as we contend, summer conditions at Palmer and Fairbanks are suitable for development of D. albipictus, the question arises as to why this parasite has not become established in Alaska. Moose populations are contiguous from Alaska to the Canadian provinces where D. albipictus is enzootic. Theoretically, the parasite could occur throughout the host's range as well. One plausible explanation is that a natural barrier, related to climate, exists in northwestern British Columbia and/or the Yukon Territory of Canada (Samuel 1989). Accumulated day-degrees above 42F (5.6C) constitute the basis for Wilkinson's (1967) proposed northern range limit for D. albipictus in Canada. Considering Wilkinson's (1967) isopleth (see his Fig. 5), Alaska weather data (Fig. 1),

and present results, it appears that D. albipictus could survive and become established in much of interior Alaska's moose range if it were able to broach this natural barrier.

Another closely related factor in tick survival is the length of winter. In Alaska, cold temperatures and snow cover tend to come earlier in autumn and last later in spring than in areas (e.g., central Alberta) where D. albipictus are typically present. Early cold and snow in autumn would bury and/or kill host-seeking larvae on vegetation. Late-winter cold and snow kills engorged adult female ticks recently disengaged from moose (Drew and Samuel 1986).

An alternative explanation for the absence of D. albipictus in Alaska relates to the prehistoric invasion of ancestral moose into North America. Invasion occurred in 2 waves separated by tens of thousands of years (Bubenik 1986). Descendents of the first wave populated much of North America, but not Alaska. Moose currently found in Alaska descended from the second wave. Perhaps D. albipictus evolved with those moose descended from the first invasion but not with those from the second invasion.

The acknowledged subspecies of moose are readily distinguishable. This suggests little mixing of populations. Perhaps the absence of D. albipictus in Alaska is a result of lack of movement of infested moose from the south. Other possible contributing factors include genetic differences of moose from Alaska and moose from more southerly areas, densities of moose, and habitat-tick relationships (Drew and Samuel 1986).

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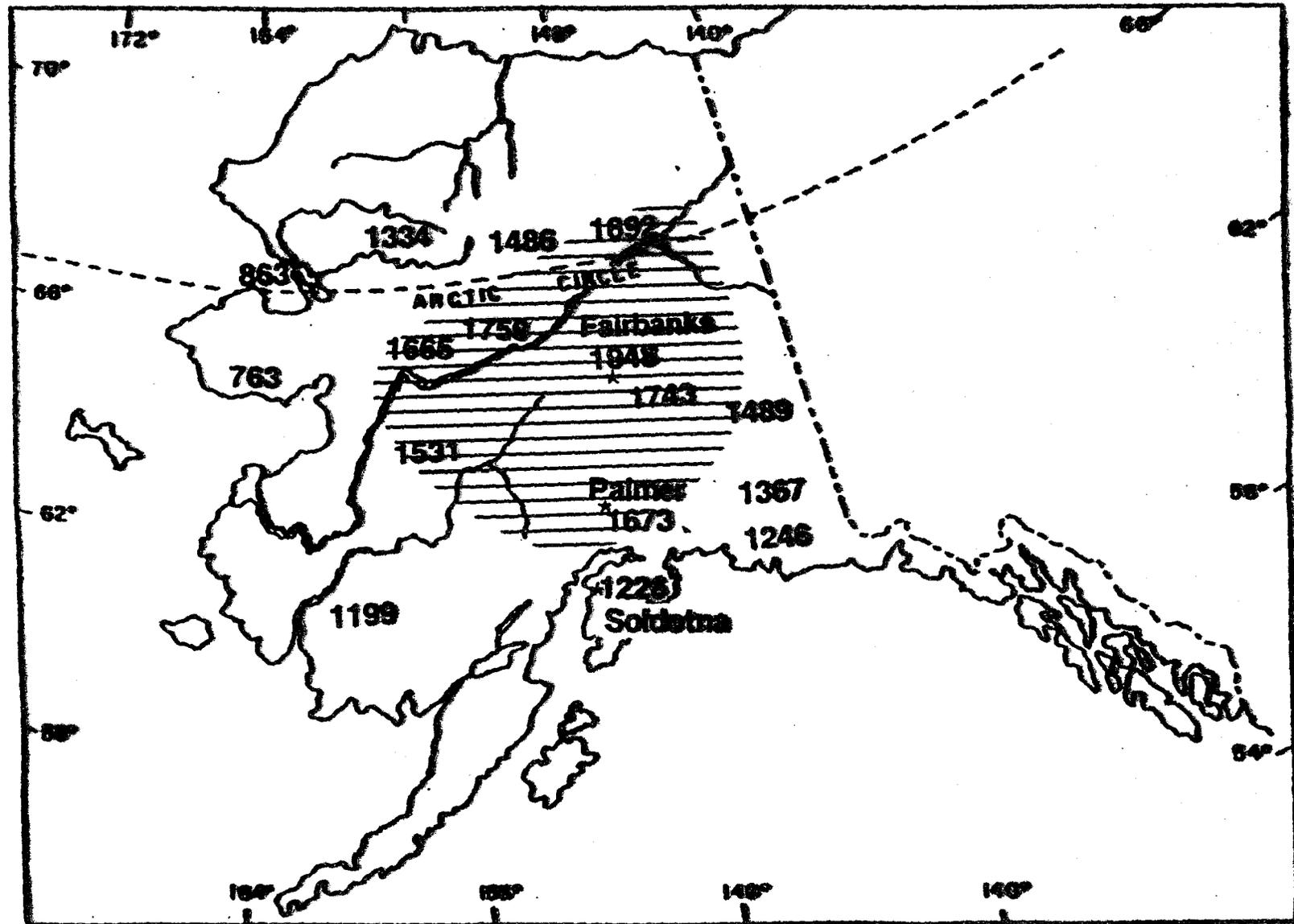


Fig. 1 Cumulative day-degrees above 42F (5.6C) for selected sites in Alaska. Shaded portion of map delineates 1,500 day-degree area (see text for details).

Table 1. Survival of adult female Dermacentor albipictus, oviposition and eclosion at four sites in Alaska.

Location	Date experiment begun	Number engorged female (EF) ticks	Number (%) of live EF on 15 June	Number (%) EF that oviposited by 15 July	Number (%) successful EF ^a
Fairbanks					
1984	23 April	16	10(63)	10(100)	10(100)
1985	25 April	18	14(78)	14(100)	12(86)
<u>1986</u>	<u>28 April</u>	<u>24</u>	<u>12(50)</u>	<u>12(100)</u>	<u>11(92)</u>
Total		58	36(62)	36(100)	33(92)
Palmer					
1984	27 April	19	19(100)	19(100)	19(100) ^b
1985	23 April	16	15(94)	12(80)	12(100) ^b
<u>1986</u>	<u>28 April</u>	<u>24</u>	<u>24(100)</u>	<u>21(87)</u>	<u>7(33)^c</u>
Total		59	58(98)	52(90)	38(84)
Soldotna-North					
1984	26 April	15	15(100)	15(100)	0
1985	24 April	18	15(83)	3(20)	0
<u>1986</u>	<u>28 April</u>	<u>24</u>	<u>11(46)</u>	<u>9(82)</u>	<u>0</u>
Total		57	41(72)	27(62)	0
Soldotna-South					
1984	24 April	17	17(100)	14(82)	10(71)
<u>1985</u>	<u>28 April</u>	<u>24</u>	<u>19(79)</u>	<u>19(100)</u>	<u>0</u>
Total		41	36(87)	33(92)	10(30)
Grand Totals 215		171(80)	148(86)	81(55)	
Edmonton, Alberta					
1987	24 April	26	22(85)	21(95)	20(95)

^a Successful engorged female = a blood-engorged female that laid eggs that hatched by 15 September.

^b Vials not checked between 27 June (0 hatched) and 14 October (all 12 hatched).

^c Experiment terminated 21 August.



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