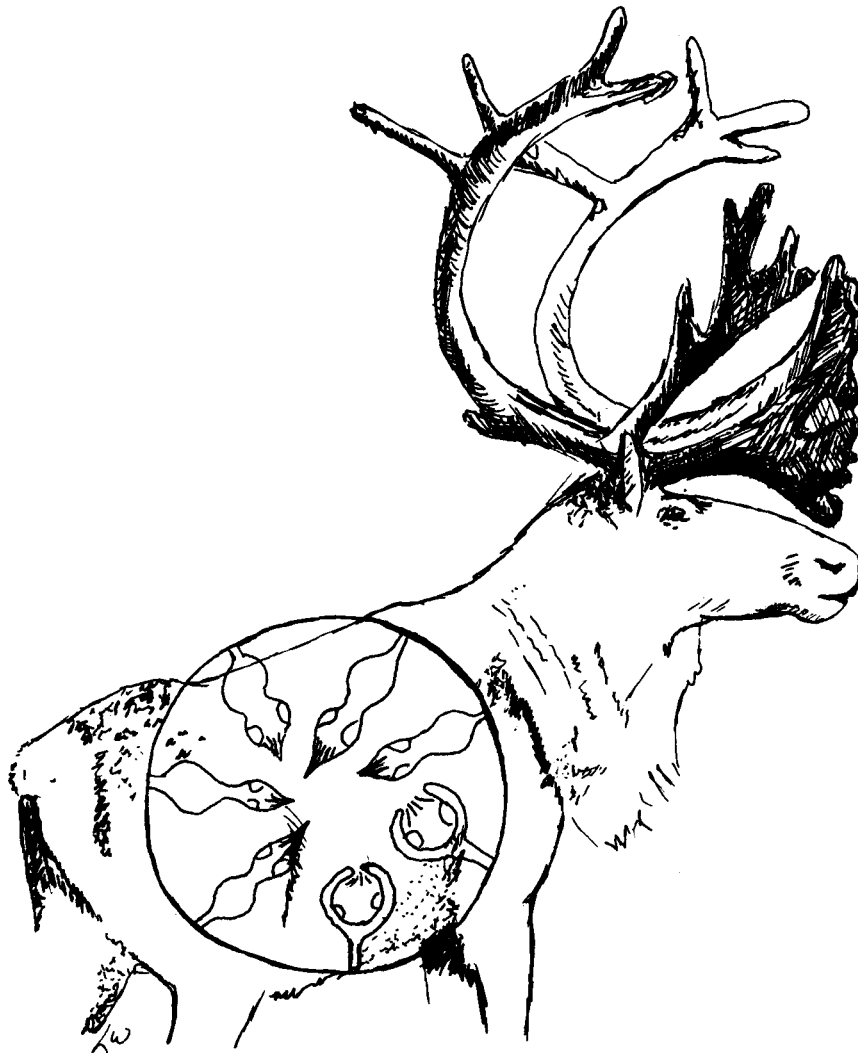


ALASKA DEPARTMENT OF FISH AND GAME

JUNEAU, ALASKA

CARIBOU DISEASE STUDIES

By: Kenneth A. Neiland



STATE OF ALASKA  
Jay S. Hammond, Governor

DIVISION OF GAME  
Robert A. Hinman, Acting Director  
Donald McKnight, Research Chief

DEPARTMENT OF FISH AND GAME  
Ronald O. Skoog, Commissioner

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## FINAL REPORT (RESEARCH)

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Cooperator: Kenneth A. Neiland

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### SUMMARY

The prevalence of the larvae (hydatid cyst) and adults of the tapeworm *Echinococcus granulosus* in more than 3000 Alaskan herbivores and carnivores, respectively, is reported. In caribou, the hydatid cyst appears to occur somewhat more commonly in females and is most frequently seen in older animals.

It appears that the protozoan, coccidial-like parasite, *Sarcocystis*, may occur in the cardiac muscle of most (all?) adult caribou. It has been demonstrated that canids (dogs experimentally) are the final host in which sporocysts infective for caribou are produced. A moderate exposure of a short-yearling reindeer to sporocysts produced in a beagle dog fed on caribou heart did not produce significant pathology.

For the first time serologic indications of infection by *Brucella* were seen in two bison from the Delta-Clearwater herd. Relatively high titres were also seen in a moose on the Colville River. Experimental infections of indigenous species of Alaskan wild carnivores and rodents with *Brucella suis* 4 are reported. Lemmings of the genus *Dicrostonyx* may be the most susceptible host for *Brucella* thus far investigated. Grizzly bears develop very high serologic titres when fed on food experimentally contaminated with *B. suis* 4, the rangiferine biotype. Pups born to experimentally infected wolves were dead at birth or died soon after.

Retention of placental materials is a regular birthing disorder of comparatively low frequency in caribou of the Western Arctic herd (WAH). The disorder was seen less frequently in the WAH following the recent decline in population. Only about 20 percent of the animals retaining placental materials also had serum antibodies against *B. suis* 4.

A bibliography on the species of *Sarcocystis* occurring in wild animals and certain domestic hosts is presented.

First-draft manuscripts on experimental studies on rangiferine brucellosis (*B. suis* 4) infections in Alaskan carnivores and rodents are presented.

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## BACKGROUND

Caribou (*Rangifer tarandus*) are important for sport and subsistence purposes in Alaska. Individual segments of major populations may at times occupy the same range as that used by commercial reindeer herds. Thus, disease conditions (including pathogenic infestations of parasites) in caribou which can be transmitted to man or domestic animals or which are significantly harmful to the caribou are of obvious concern. The caribou in some areas are plagued by more potentially serious parasites or disease conditions than most of the other Alaskan wildlife species. Brucellosis, foot rot, warble and bot flies, and gastro-intestinal roundworms, are all prevalent in all North American caribou herds and either directly cause or contribute to serious disease conditions in caribou, reindeer, man and/or his animals. Brucellosis is a particularly significant zoonotic disease of Alaskan caribou whose prevalence is documented (Neiland et al. 1968) though not well understood.

The present study, a continuation of one in progress since 1962, was primarily concerned with fully documenting the natural history (i.e. epidemiology) and pathology of rangiferine brucellosis. It seems likely that it may be cyclic with an as yet unknown periodicity. We cannot yet be sure that all of the pathological conditions (e.g. placental retention), which we suspect to be caused by the disease only, involve this pathogen. We do not know whether the disease will essentially "die-out" in caribou herds, only to be reintroduced from some non-rangiferine reservoir host species in which it may occur, perhaps in "quiet" form, or whether reindeer and/or caribou serve as both reservoir and secondary hosts. While

these and other questions are of great scientific interest, they also point the way toward "practical" management goals. If we find that "rangiferine" brucellosis is indeed a disease of *Rangifer* spp. and does not necessarily involve a regular reservoir host system, then we will also likely find that whereas the disease continues at a low endemic level in "close herded" reindeer, it will likely disappear in wide-ranging caribou. A recurrence of epidemic levels of the disease in caribou could be expected to recur whenever substantial contact between caribou and infected reindeer occurred, particularly after prolonged absence of the disease from caribou. If reindeer do play the role of reservoir for the disease, the management solution is to remove them from known caribou range. If a non-rangiferine reservoir may also be involved, as is the case with porcine brucellosis (wild rabbits, Europe) or bovine brucellosis (wild foxes, Argentina), then effective control is more difficult. Because of the known involvement of foxes in Argentina, the proven involvement of dogs on occasion in human brucellosis, and our scant data on Eskimo sled dogs, one cannot help but wonder whether wild or semi-domestic canines are possible reservoirs of Alaskan rangiferine brucellosis.

#### OBJECTIVES

To determine the incidence and distribution of potential pathogens in Alaskan caribou and alternate or reservoir hosts.

To determine whenever possible or practical the extent that such organisms may contribute to mortality or lowered productivity or economic value of affected caribou populations.

To determine the extent to which wildlife pathogens depreciate the value of caribou for use as food by humans or may be a threat to domestic animal industry.

#### PROCEDURES

Our primary effort in rangiferine disease studies is focused on the long-term study of brucellosis in caribou. In this respect we are continuing our close cooperation with the Animal Disease Eradication Division, U. S. Department of Agriculture, which is monitoring the disease in reindeer. In these studies the following specific procedures are emphasized:

1. Serological surveillance of brucellosis prevalence in major caribou herds, particularly those in the Nelchina and Arctic areas.
2. Confirmation by isolation of suspected brucellar infections.
3. Serological studies on potential reservoir host species.
4. Aerial surveillance of the occurrence of animals displaying gross symptoms (i.e. limping, retention of afterbirth) of brucellosis during calving.

5. Surveillance from the ground of concentrations of animals during the spring and fall migrations through Anaktuvuk Pass in the Arctic to detect and collect specific animals for bacteriological and/or other studies.
6. Routine autopsies of animals taken for subsistence purposes by native or sport hunters or specifically for the purposes of various scientific studies (e.g. radiation studies, disease and parasite studies, etc.).
7. Examination of specimens submitted to our laboratory by the public.
8. Preparation of a definitive bibliography of the "Diseases, Parasites, and Disorders of Caribou and Reindeer."
9. Publication of data at suitable intervals.

#### FINDINGS

During the past several years we have been concerned with continuing studies on four major categories of caribou parasite and disease problems. These problems were analyzed during this time either through data analysis, laboratory experimentation and/or field studies. Each of these parasite or disease entities is considered separately in the following sections.

##### A. Hydatid Infections

The barren-ground caribou plays an important role as an intermediate host in the epizootiology of *Echinococcus granulosus* in Alaska. Over the years my associates and I have accumulated data on the adult and larval stages of this cestode parasite in over 3000 animals (11 possible host species). These unpublished data are summarized for comparative purposes in Table 1. It can be seen that the principal, wild host to the adult worm in Alaska is the wolf (*Canis lupus*). However, it should be noted that wherever domestic dogs run loose (e.g. Matanuska Valley, see Table 1) or are fed on infected game (e.g. Anaktuvuk Pass), they also can serve as the final host to the worm. Caribou are the principal host to the larval stage (i.e. the so-called hydatid cyst) in those areas where moose (*Alces alces*) and/or blacktail deer (*Odocoileus hemionus sitkensis*) are not present or relatively abundant. In this respect it should be noted that the negative data reported for blacktail deer in Table 1 are explained by the fact that the great majority of deer we have examined have come from areas in Southeastern Alaska where no wolves are present. At the same time the infected wolves reported in Table 1 have come from areas where we have not examined deer. Natural infections in deer and mountain goats (*Oreamnos americanus*) in Southeastern Alaska have been reported by Rausch and Williamson (1959).

The prevalence of the hydatid infection in several caribou herds is summarized in Table 2. The apparent higher prevalence of the parasite in members of the Alaska Peninsula herd may be due to sampling error or it may be that the somewhat more mild and also seasonally prolonged,

Table 1. Summary of incidence of adults or larvae of Echinococcus granulosus in Alaskan animals.

Host Species	Number Examined	Number Infected	Percent Infected
<u>Alces a. gigas</u>	1116	117	10.5
<u>Bison bison</u>	144	2(?) <sup>1</sup>	?
<u>Canis familiaris</u>	19	1	5.3
<u>C. latrans</u>	5	0	0
<u>C. lupus</u>	90	11	12.2
<u>Lynx lynx</u>	543	0	0
<u>Odocoileus hemionus sitkensis</u>	115	0	0
<u>Oreamnos americanus</u>	6	0	0
<u>Ovibos moschatus wardi</u>	3	0	0
<u>Ovis dalli dalli</u>	84	0	0
<u>Rangifer tarandus granti</u>	1105	69	6.2

<sup>1</sup> Diagnoses uncertain. Specimens not taken by assistant.

Table 2. The incidence of larval Echinococcus granulosus (hydatid cysts) in various Alaskan caribou herds.

Herd	Incidence <sup>1</sup>		Percent Infection
	Number Examined <sup>2</sup>	Positive	
Arctic	426(18)	20	4.7
Alaska Peninsula	79(7)	8	10.1
Nelchina	593(51)	38	6.4
Delta	1(0)	1	--
Porcupine	3(0)	0	--
Steese-Fortymile	3(0)	2	--
Total	1105	69	6.2

<sup>1</sup> All age classes including 76 negative calves.

<sup>2</sup> Number of calves in parentheses.

favorable climatic conditions (i.e. for *Echinococcus* eggs) may provide greater opportunity for infection. We do not think that wolf densities and therefore *Echinococcus* egg production are substantially higher on the Peninsula than elsewhere.

The data presented in Table 3 suggest that the prevalence and intensity of infection by hydatid cysts in caribou are directly related to the age of the animal. That is, the older the animal the more likely it is that: 1) it will be infected (i.e. have been exposed) and 2) relatively speaking, it will be infected more heavily. Whether or not there is a significant decline in prevalence or increase in intensity of infection among the oldest age classes (i.e. 10+ years) cannot be determined from the relatively small number of old caribou examined. However, if the data do accurately portray the prevalence of cysts in older animals, then it does not appear that this parasite is very pathogenic in caribou. If it were, infected animals would drop out of the population at some average age and prevalence rates and infection intensities in animals beyond this age would be proportionately lower. This does not appear to be the case, but it may be a matter of inadequate sampling.

The data on the relationship between sex of host and prevalence of infection are considered in Table 4.

It appears that hydatid cysts may occur somewhat more often in females. But the data are equivocal. The method used to age most of the caribou (i.e. comparative wear of teeth) is not without error. Accordingly, if in fact our sample were made up of more older females and younger males than we suppose to be the case, then one could expect more infections in females. That is, the rate of infection goes up with age (see Table 3), and if the average, true age of the female segment of the sample is older, the prevalence of hydatid cysts should be greater.

In any event the data were subjected to two statistical tests by Dr. Samuel Harbo, Division of Life Sciences, University of Alaska. The Wilcoxon Paired-Sample Test, which is considered to be the most "powerful" in this specific instance, suggested that the apparent result could come about through random chance 10-20 percent of the time. The less "powerful" Sign Test indicated less probability (i.e. 7%) of this being a random-chance event.

If indeed it is true that females are more often infected, I do not believe that this is due to some inherent difference in susceptibility to infection between males and females. It seems more likely that this is a matter of differential exposure. That is, during the summer when females and fawns are predominantly separated from males into maternity bands, they may also be more closely attended by wolves than males. As a consequence there would be greater exposure to fresh, unfrozen, viable *Echinococcus* eggs.

Different prevalences of parasites in male and female cervids has been previously reported. Neiland (1963) observed more frequent infestation



Table 3. The incidence and intensity of infections of larval Echinococcus granulosus in different age classes of barren-ground caribou.

Age Class <sup>1</sup>	Number <sup>2</sup> Examined	Incidence		Intensity	
		Number Infected	Percent Infected	Number of cysts	Average
1 yr.	76	none	--	--	--
1+ yrs.	87	none	--	--	--
2+ yrs.	133	3	2.3	1-2	1.3
3-5 yrs.	394	22	5.6	1-10	2.0
6-9 yrs.	171	19	11.1	1-6	2.4
10+ yrs.	30	3	10.0	2-6	3.7

<sup>1</sup> Ages determined by tooth replacement and relative wear.

<sup>2</sup> Includes animals of unrecorded sex.

Table 4. The incidence of larval Echinococcus granulosus in male and female barren-ground caribou of different age classes.

Herd <sup>1</sup> Age Class	Incidence					
	Male			Female		
	Number Examined	Number Infected	Percent Infected	Number Examined	Number Infected	Percent Infected
<u>Arctic</u>						
1 yr.	10	none	--	8	none	--
1+ yrs.	12	none	--	12	none	--
2+ yrs.	23	1	4.3	34	2	6.0
3-5 yrs.	66	2	3.0	127	7	5.5
6-9 yrs.	13	1	7.7	62	5	8.1
10+ yrs.	1	none	--	13	1	7.7
unknown	none	none	--	1	1	--
Total	125	4	3.2	257	16	6.2
<u>Alaska Peninsula</u>						
1 yr.	2	none	--	5	none	--
1+ yrs.	5	none	--	4	none	--
2+ yrs.	6	none	--	8	none	--
3-5 yrs.	14	none	--	13	none	--
6-9 yrs.	3	1	33.0	12	4	3.0
10+ yrs.	none	--	--	1	none	--
unknown	none	none	--	3	3	--
Total	30	1	3.3	46	7	15.2
<u>Nelchina</u>						
1 yr.	26	none	--	25	none	--
1+ yrs.	34	none	--	20	none	--
2+ yrs.	31	none	--	31	none	--
3-5 yrs.	62	4	5.4	112	9	8.0
6-9 yrs.	25	2	8.0	56	6	10.7
10+ yrs.	3	none	--	12	2	16.6
unknown	7	7	--	6	6	--
Total	188	13	6.9	262	23	8.8

Table 4. Continued.

Herd <sup>1</sup> Age Class	Incidence					
	Male			Female		
	Number Examined	Number Infected	Percent Infected	Number Examined	Number Infected	Percent Infected
<u>All Herds</u>						
1 yr.	38	none	--	38	none	--
1+ yrs.	51	none	--	36	none	--
2+ yrs.	60	1	1.7	73	2	2.7
3-5 yrs.	142	6	4.2	252	16	6.3
6-9 yrs.	41	4	9.7	130	15	11.0
10+ yrs.	4	none	--	26	3	11.5
unknown	7	7	--	10	10	--
Total	343	18	5.2	565	46	8.1

<sup>1</sup>

See Fig. 3.

of male caribou by larvae of the warble fly *Oedemagena tarandi* and these observations were confirmed by Kelsall (1968). Prestwood et al. (1971) have noted that in southeastern United States white-tailed deer. (*Odocoileus virginianus*), the young males are most frequently infected with the lungworm *Dictyocaulus viviparus*.

## B. Sarcocystis

Our work with this parasitic protozoan was initiated less than two years ago when the work of others revealed that it was not uniformly, pathologically benign as had been supposed over the past century since its discovery. We first set out to determine the prevalence of *Sarcocystis* spp.(?) in Alaskan big game herbivores (the intermediate host) by histologic examination of sections of heart and various skeletal muscles collected from hunter kills. During June 1977 we collected 10 caribou does with newborn fawns from the Western Arctic herd to use in our prevalence studies and for investigations on the life cycle and on the occurrence of the "sarcocyst" in various major muscle systems.

The prevalence data accumulated to date are summarized in Table 5. It seems noteworthy that all heart tissue samples from caribou thus far examined histologically have been found infected with this parasite. And in further comparison with other wild hosts in Alaska, the infections seen in caribou are substantially heavier. It appears that the opportunity for infection of caribou by *Sarcocystis* is high. However, this does not necessarily suggest that this parasite is of low pathogenicity. For example, species of this parasite long known to commonly parasitize domestic animals have been shown to be highly pathogenic when the initial exposure is high enough (see Fayer et al., Appendix I). One might characterize this relationship by the old truism, "the poison's in the dose." Whether or not this is true of any of the species occurring in Alaskan wildlife awaits thorough investigation.

In order to determine the distribution of the parasite in the various major muscles of an animal and also to determine whether prenatal transmission takes place, we collected 10 does with newborn fawns from the Western Arctic caribou herd during June 1977. We specifically selected does with retained placental materials because we also wondered if this condition might be caused by *Sarcocystis* infections. The data on the distribution of sarcocysts in eight different muscle tissues are summarized in Table 6. It is noteworthy that although all sections of heart tissue as well as the majority of the other muscle tissues (except uterine muscle) were infected, neither the heart nor tongue of the accompanying fawns contained cysts. Furthermore, it appears that esophageal muscle may also be as commonly infected as heart muscle. It may be that the sarcocysts seen in esophageal muscle are a distinct species from the one occurring in heart muscle. This possibility will be investigated by comparing the morphology of sporocysts produced by feeding esophageal tissue to beagles with sporocysts derived in this way from heart muscle.

Table 5. Summary of prevalence of Sarcocystis in Alaskan wildlife, March 15, 1978.

Species	Area	Number Examined	Number Infected
Bison	Big Delta herd	29	1
Blacktail Deer	SE Alaska	20	4
Caribou	Western Arctic herd	55*	51*
	Porcupine herd	6	6
	Delta herd	1	1
Carnivores	Interior Alaska	2	-
Moose	SE Alaska	11	1
	Interior Alaska	14	2
Mountain Goats	SE Alaska	6	2
Seals	N Pacific & Bering Sea	30	-
Sheep	Alaska Range, Region III	25	14

\* All samples of heart tissue infected. Negative results based on samples of skeletal muscle only. The recently born fawns from infected dams, all negative, not included.

Table 6. Occurrence of Sarcocystis in tissues of 10 adult female caribou with newborn fawns.\*

Tissue	Number Infected
Heart	10/10
Esophagus	10/10
Intercostal muscle	8/9
Masseter	7/10
Tongue	6/10
Diaphragm	6/10
Lower hindleg muscle	6/8
Uterus	0/10

\* All females with retained placental materials. All fawns free of sarcocysts, two found dead. Animals collected in early June 1977 on calving grounds of Western Arctic herd.

## Life Cycle

Even though the major intermediate life-cycle stage of *Sarcocystis*, i.e. the sarcocyst, was first discovered over a century ago, it was not until recent years that the two-host nature of the life cycle was determined. In all the species thus far studied in detail, it has been shown that the cycle follows a typical predator-prey plan. That is, the sexual stages occur in a carnivore (which therefore is termed the definitive host) and the asexual stages occur in a prey species (which is termed the intermediate host).

We have shown (and our work has been corroborated by Dr. R. L. Fayer, U.S.D.A., Beltsville) that canids (beagles experimentally, no doubt wolves under natural conditions) can be infected by feeding them fresh caribou heart. For this purpose we used heart tissue from the animals we collected on the fawning grounds of the Western Arctic herd in June 1977. About 12-30 days after feeding on the fresh caribou heart, typical sporulated sporocysts were demonstrated in the feces of the experimental beagles.

In order to complete the life cycle it was only necessary to feed these sporocysts to a susceptible caribou. For this purpose we used a suspension of about 150,000 sporocysts prepared by our collaborator Dr. Fayer. The number used was judged to be sufficient to cause pathologic symptoms, but not to kill the experimental animal during the 90-day observation period. During this time various regular observations on the health of the animal were made (e.g. weight change, temperature, and several blood chemistry parameters). No significant signs of ill health were seen, but when the animal was sacrificed at the end of the experimental period, examination of frozen tissue sections revealed numerous sarcocysts. (We have not yet prepared permanent stained sections of the tissues collected at necropsy.)

Our findings in this preliminary investigation of the pathologic potential of this rangiferine species of *Sarcocystis* must be qualified as follows. The only animal we had available for the experiment was a five-month-old reindeer fawn which had been held in an outside pen. We cannot rule out the possibility that the animal might have been previously exposed to *Sarcocystis* (i.e. a species from domestic animals in dogs) which had an immunizing effect. We collected a serum sample at the start of the experiment to serologically evaluate this possibility but the Beltsville Laboratory, U.S.D.A., has not completed the analysis. It also may be that the experimental dose (i.e. about 150,000 sporocysts) was too low. And it may be that this rangiferine species of *Sarcocystis* is well adapted to its "normal host" and causes significant pathologic responses only when first exposures are severely high.

## Discussion

The first report of *Sarcocystis* in *Rangifer*, which I have at hand is that of Hadwen (1922) who worked with Alaskan reindeer. He stated that,

"Reindeer, especially older animals, are very commonly infested with Sarcosporidia. It is quite usual to find numerous cysts in the esophagus and other muscles of reindeer killed for meat, which otherwise seemed to be in the best of health and condition." He further noted that esophageal cysts were distinctly larger than those seen in heart or skeletal muscle. He also described another sarcosporidian as the causative agent of so-called "corn-meal" disease of connective tissues in reindeer. He named it *Fibrocystis tarandi* although it is now known as *Besnoitia tarandi*. Regarding the "probable effects" of this latter parasite on caribou and reindeer he stated, "The massive infections found in the two cases examined leave little doubt that the animals were adversely affected.... It is probable that in addition to mechanical effects the parasite may also cause injury by their secretions and excretions." We have commonly seen *Besnoitia tarandi* in caribou, but have not as yet made any attempt to demonstrate its life cycle or measure its pathologic potential. (Apparently Bergman, 1913, working in Sweden, was the first to note *Sarcocystis* in *Rangifer* but I do not have his paper and cannot determine from other sources what he reported.)

Griuner (1927) reported on the occurrence of *Sarcocystis* in heart muscle of reindeer in the Tobolsk Region of the Soviet Union. He studied three animals that had been infected and in each he noted "macroscopic abnormality" of the heart. He evidently saw no other pathologic changes, but suggested that such infections were "possibly lethal." Babudieri (1932), in his monographic treatment of the genus *Sarcocystis*, stated that the species reported in reindeer (and various African antelopes as well) is *S. fusiformis* Railliet, 1897. Yakimoff and Sokoloff (1934) described the species of *Sarcocystis* originally reported from Soviet reindeer by Griuner (1927) as *S. griuneri* new species. They examined sections of heart muscle from 100 animals and saw sarcocysts in all sections, but made no comments on pathogenicity. Murie (1935), in his report on caribou in Alaska and the Yukon, noted Hadwen's earlier report (1922) on rangiferine sarcosporidia but did not offer any original observations. Yakimoff (1936) reported further studies on *Sarcocystis griuneri* Yakimoff and Sokoloff, 1934, using material from 15 reindeer from Lapland. He described the anatomy of the cysts and spores in detail and concluded: "I in no manner doubt that severe infections of *Sarcocystis* in reindeer has sure significance for the health of this animal."

Justoff (1937) presented views regarding the pathogenicity of *Sarcocystis* in reindeer which are opposed to those of Yakimoff and others. Based upon his histologic studies of infected esophageal and diaphragmatic tissue from reindeer and in consideration of this work on this parasite in swine he concluded: "*Sarcocystis* do not change the normal structure of the muscle tissue, the change of the muscle tissue infected with *Sarcocystis*, as it was described by some other investigators,

namely by Koselkin, Kononoff, Petroff and Etremoff--is due not to *Sarcocystis*, but to the general disease of organism." In view of our modern understanding of the life cycle and pathogenic mechanism of *Sarcocystis* infections, Justoff's opinions have little validity except in regard to the final, non-pathogenic cyst-stage itself.

Gibbs (1960) reported on the apparent occurrence of *Sarcocystis* in Canadian barren-ground caribou. He stated: "Although no gross lesions were seen on post-mortem examination, spores of a species of *Sarcocystis* were found in the blood smears. The pathogenicity of this organism is doubtful and unless the infestation was a very heavy one it would cause little trouble." Aside from the uncertainty regarding the true identity of the spores reported by Gibbs, it should be kept in mind that it wasn't until around 1974 that the life cycle and pathogenicity of some common species of *Sarcocystis* in domestic animals became known. All older claims discounting the pathologic potential of *Sarcocystis* must be viewed with reserve. I am not aware of any other publications on *Sarcocystis* in reindeer or caribou.

However, recent studies on *Sarcocystis hemionilatrantis* n. sp. by Hudkins and Kistner (1977) appear to have direct and significant applicability to our current interests in this kind of parasite. This species of *Sarcocystis* was commonly seen in mule deer fawns in eastern Oregon in 1974 where the herd had declined in recent years. And it is probably identical to the species described from 68 percent of 877 yearling and adult mule deer in California by Sayama (1952). It was first established by Hudkins and Kistner (loc. cit.) that coyotes serve as the definitive host for further development of the sarcocyst-stage occurring in mule deer fawns. They completed the full cycle by feeding sporocysts produced in fawn-fed coyotes to mule deer fawns taken into isolation 2-7 days after birth. They summarized the extremely interesting results of their experiments as follows:

Fifteen coyotes (*Canis latrans*) shed sporulated sporocysts in their feces after eating freshly ground skeletal muscles from a mule deer (*Odocoileus hemionus hemionus*) infected with microscopic-sized cysts of *Sarcocystis*. Sporocysts were shed intermittently from 12 to 36 days after ingestion of the infected meat. Sporocyst size averaged 14.4 x 9.3 microns.

Eleven mule deer fawns orally inoculated with these sporocysts became infected and 9 of 11 died between post-inoculation days (PID) 27 and 63. Clinical signs of anorexia, weight loss, pyrexia and weakness were evident prior to death...uninoculated control animals consisting of three mule deer fawns, two lambs and one calf remained healthy during the experiment.... Mortality rates for the dosage levels... (i.e. of sporocysts)...  $1.0 \times 10^6$ ,  $2.5 \times 10^5$  and  $5.0 \times 10^4$  were 100 percent, 75 percent and 75 percent, respectively.... Developing or mature muscle cysts... (i.e. sarcocysts)...were not found in fawn tissue until PID 60.



Thus it is clear that a commonly occurring, cervine species of *Sarcocystis* will cause severe pathology, including death, in its regular intermediate host. That *S. hemionilatrantis* is specifically adapted to mule deer is proven by the failure of attempts by these workers to experimentally infect cattle and sheep with the same coyote-reared sporocysts with which they fatally infected mule deer fawns. Thus it would seem that the mere fact that a parasite occurs quite commonly in a given host is, at least in some cases, insufficient reason to conclude that the parasite is of low or no pathogenicity. The poison's in the dose!

The only interesting question remaining unanswered about *Sarcocystis hemionilatrantis* involves the degree to which it reduces annual recruitment of mule deer fawns under natural conditions. Indeed, it is interesting to speculate that fawns weakened by infections of this parasite are more susceptible to predation by coyotes. And this would be of importance to the welfare of the parasite. If the parasite were so pathogenic that it killed the intermediate host soon after exposure and before adequate development of obligatory life cycle stages, then the life cycle would be broken. However, if the pathogenicity of the parasite is such that average, natural levels of infections are not rapidly fatal, but only debilitating to the point of favoring predator-induced mortality, then completion of the life cycle is also favored. And it should be noted that in an evolutionary frame of reference, it is the completion of the life cycle and survival of the species that is most important, not the long-term survival of the individual parasite.

We hope to investigate in similar detail the species of *Sarcocystis* occurring in Alaskan caribou and reindeer. The form we see in caribou heart tissue may be identical to the one called *S. grieneri* Yakimoff and Sokoloff, 1934.

### C. Brucellosis

Members of the genus *Rangifer* (reindeer and caribou) are generally known to be infected with *Brucella suis* biotype 4 throughout their circumpolar distribution wherever adequate serologic testing has been done. The disease is generally chronic, but can assume an acute form during first pregnancies resulting in abortion. In its chronic form it causes joint infections and sterility (orchitis) in males. It also sometimes is the cause of placental retention which will be considered in detail in section D of this report.

In Alaska the disease is well documented in the Western Arctic and Nelchina caribou herds (Neiland et al. 1968, and various administrative reports) and is also known to occur in the Delta herd and various herds of reindeer.

The disease is generally notorious as a potentially serious disease of humans, particularly the non-rangiferine strains of *Brucella suis*, and also *B. abortus* and *B. melitensis*. Humans contract the disease by eating or handling uncooked infected material (e.g. milk, meat, etc.). The disease can also be contracted by various non-human carnivores and it has been well documented in Alaska in naturally infected sled dogs, grizzly bears (*Ursus arctos*), wolves, and red foxes (*Vulpes vulpes*) by

Neiland (1970, 1975). The interested reader can refer to the literature cited in Neiland (1975) for documentation on rangiferine brucellosis in Eurasian hosts.

In order to evaluate the effects of the disease on wild carnivores in Alaska and to better understand its epizootiology, we carried out extensive laboratory experimentation during the early part of this report period. At that time I had access to the microbiologic facilities of the now defunct Arctic Health Research Center and I worked in close collaboration with Mr. Lawrence G. Miller, chief bacteriologist of the facility.

The results of our work in preliminary draft form for publication are included as Appendices II and III to this report. Our findings may be briefly summarized as follows. For full details and discussion the reader should refer to the draft manuscripts.

#### Carnivores (Appendix II)

We infected two grizzly bears in a natural way by contaminating a single daily food ration with a laboratory culture of *B. suis* 4 first isolated from a sled dog (Neiland 1970). Both bears rapidly developed extremely high antibody titres (1:10,000). Because these bears were to be used in a rabies experiment we had no further contact with them.

We infected two gravid wolves by intraperitoneal and conjunctival routes, respectively. About 24 days later they gave birth, apparently at full-term, to two (both alive) and six (two alive and four dead) pups, respectively. Those pups born alive died within 24 hours in both cases. Trauma may have played a part in the death of all pups. However, seven of the eight pups were infected by *B. suis* 4. One pup eaten shortly after birth was not available for examination.

Experimental infections of a black bear (*Ursus americanus*) and several beagle dogs yielded serologic and bacteriologic data similar to these seen in the wolves and in infections of dogs by other species of *Brucella*.

#### Rodents (Appendix III).

We studied experimental infections of *B. suis* 4 in nine species of rodents and also a lagomorph (varying hare). All of these were readily infected by intraperitoneal inoculation of various "challenge-levels" of organisms. Brucellae were routinely isolated at necropsy from liver and spleen, or other tissues, but pathologic responses in most of these hosts were not marked. However, infections in two species of varying lemmings (i.e. *Dicrostonyx stevensoni* and *D. rubricatus*) always produced severe pathology even when initial challenge-levels involved less than 10 colony-forming units (C.F.U., might be as few as 1 cell per C.F.U.). All those animals not sacrificed early in the experiment inevitably developed massive, lethal infections. It seems safe to conclude that any individual specimen of *D. stevensoni* or *D. rubricatus* exposed to

even very few (less than 10) C.F.U. will become infected and die. In nature, infected *Dicrostonyx* could well be a source of infection for rodent eaters. It might also be noted that wherever *Dicrostonyx* shares habitat with infected *Rangifer* spp., brucellosis (other diseases?) might well play a significant role in the population dynamics of varying lemmings.

### Serologic Surveys

Most of the serologic data accumulated during this report period on the prevalence of *Brucella* antibodies in Alaskan wildlife involve potential host species other than *Rangifer*. Nevertheless, this seems to be an appropriate place to present them. The data summarized in Table 7 do not include test results on sera collected from 10 caribou taken on the Western Arctic caribou fawning grounds in June 1977, nor on the samples collected from bison (*Bison bison*) during the annual fall hunt (1977) at Delta Junction. These have not been reported yet by the consulting laboratory that does the tests. Samples collected during NPR-A studies on grizzlies in 1977 are not yet available.

Both the positive and some of the negative results presented in Table 7 are worthy of special consideration.

### Bison

For about the past 15 years we have been concerned about the possibility of introduction of the rangiferine strain of *Brucella* from the infected Delta caribou herd into the Delta bison herd. We also have considered that a strain of *Brucella abortus* which has been found at least once in a local milk cow might also infect the bison herd. Elsewhere (e.g. Yellowstone Park and Utah) bison have been known for many years to be infected with *B. abortus*. The two suspect reactors reported in Table 7 are the first that we have seen in several hundred which have been tested. Whether these two animals had any gross signs of infection is not known. None were reported by the hunters or the field biologists (ADF&G) who guided them.

Now that an attempt is being made to start up a large-scale dairy operation in the Delta-Clearwater agricultural area, there is even more reason to view the infection of bison as a distinct possibility.

### Moose

The high-titred serum sample taken from a moose near Umiat on the Colville River is the first unequivocal evidence of a naturally infected moose in Alaska. Neiland et al. (1968) reported the somewhat suspicious occurrence of a serologically positive moose hit by a car on the Seward Highway not far from pens where experiments on *Brucella suis* 4 in reindeer were being conducted. Otherwise, a substantial amount of negative data has been collected on animals from areas where moose don't have close contact with infected caribou. However, moose along the Colville River occupy habitat also commonly frequented by caribou from the infected

Table 7. Prevalence of brucella antibodies in various Alaskan wildlife.<sup>1</sup>

Species	Locality	Sample Size	Number Positive	Titres
Grizzly bear	Brooks Range	3	-	0
Brown bear	Alaskaland Zoo	1	0	-
Bison	Delta Junction	34	2	1:50(AG)
Moose	Tanana Flats	92	0	-
	Colville River	8	1	1:200(AG) <sup>3</sup>
Muskox	Nunivak Island	7	0	-
Dall sheep	Dry Creek	8	0	-
Wolf	Glennallen	4	0	-
	Tanana Flats	49	0	-

<sup>1</sup> All samples tested by tube agglutination (AG), mercapto ethanol (ME), U.S.D.A. card (CT), and complement fixation (CF) tests.

<sup>2</sup> Titre values equaling or exceeding the following values were considered positive: AG(1:50); ME(1:25); CT(+); CF(1:20).

<sup>3</sup> 1:200(AG); 1:200(ME); +(CT); 1:100(CF).

Western Arctic herd. It seems quite likely that this is the reservoir of infection from which this first natural infection of an Alaskan moose was derived.

Brucellosis has been only rarely reported in moose in other areas. Jellison et al. (1953) reported a case in a moose in Montana and Corner and Connell (1958) reported a case or two in animals from Elk Island National Park, Alberta, Canada. I am not aware of any reports of this disease in European moose.

### Wolf

It is interesting to note that all sera from wolves taken on the Tanana Flats were negative. Elsewhere in Alaska, where wolves feed extensively on caribou, serologic evidence suggests that infection by *Brucella suis* 4 is common. Neiland (1975) reported that 11 of 28 samples of serum taken from wolves killed by Inupiat subsistence hunters of Anaktuvuk Pass were positive in the sensitive, complement fixation test. The negative data on wolves from the Tanana Flats strongly suggest that these animals do not regularly prey on the infected Delta caribou herd. However, infected animals may now be rare in the Delta population.

### D. Placental Retention

Retention of placental components for an abnormal time following birth is widely seen in various mammals. Our early knowledge of this condition in Alaskan caribou was summarized by Neiland et al. (1968). I noted in 1963 that a substantial percentage of does (25%) with retained placentas may lose their fawns within a day or so of birth. And Neiland (1974) reported an eyewitness account of the birth of a fawn followed by placental retention. Accordingly, we have made a special effort over the years to monitor the prevalence of placental retention, particularly in the western Arctic, and under favorable conditions to determine early postpartum loss of fawns. These data are summarized in Table 8.

It is quite clear that placental retention is a regularly occurring birthing disorder in the Western Arctic herd. In some years (i.e. when survey conditions were favorable and in one instance the weather was comparatively harsh) relatively high proportions of fawns born to affected does were lost within a few days postpartum. Of greatest possible interest is the apparent decrease in the prevalence of the disorder in the years 1975-77 following the crash of the herd. Assuming for sake of discussion that the decrease is real, and not just a product of sampling error, it is not surprising or unexpected that this might occur.

Placental retention is caused in various animals by a variety of factors which may act singly or in concert. A number of infectious agents can cause retention. Of these agents, species of *Brucella*, particularly *B. suis* 4 which is known to occur in the Western Arctic herd, are noteworthy. During the years 1969-71 we had a helicopter at our disposal on the fawning grounds for the purpose of collecting does

Table 8. Prevalence of placental retention in the Western Arctic caribou herd.

Year	Sample Size	Retained Placentas	Lost* Fawns
1963	2,130	107(5.0%)	27(25%)
1965	787	25(3.4%)	-
1966	2,075	33(1.6%)	1
1967	2,846	6(0.2%)	2
1968**	2,037	52(2.6%)	30(57.7%)
1969	4,357	44(0.9%)	9
1970	2,217	28(1.3%)	-
1971	3,331	67(2.0%)	29(43%)
Total	19,780	362(1.8%)	86(38%)***
1975	180	5(2.8%)	-
1976	1,847	9(0.5%)	-
1977	1,483	14(0.9%)	2
Total	3,510	28(0.8%)	-

\* Fawns lost by does with retained placentas.

\*\* Relatively severe weather conditions.

\*\*\*Three-year average (1963, 1968 and 1971).

with retained placentas. Of 42 affected does only 8 (19%) showed *Brucella* titres of 1:40 or higher (Neiland 1972). While some active *Brucella* infections may not stimulate the production of agglutinating antibodies, one would not expect this to happen in 80 percent of the cases of concern. Serologic indications of past exposure to one or more serotypes of the bacterial genus *Leptospira* were also seen, but the results of attempts to isolate leptospire were all negative.

It seems likely that some cases of placental retention are directly related to the specific nutritional state of the affected doe (which also could affect susceptibility to infectious agents). Reduction in herd size might be expected to result in some improvement in average nutrition, and, also at the same time, reduced transmission of infectious agents. While the etiology of placental retention remains largely an enigma, its effect on the Western Arctic herd (and other Alaskan herds) apparently has been small in the past and is evidently smaller now.

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PREPARED BY:

Kenneth A. Neiland  
Game Biologist

SUBMITTED BY:

John Coady  
Regional Research Coordinator

APPROVED BY:

Robert P. Hinman  
Acting Director

Donald E. McKnight  
Research Chief



## Appendix I

The following collection of bibliographic citations include the majority of the world literature on the species of Sarcocystis which occur in wildlife. There are also appropriate references to related forms whose life cycles occur in domestic animals (in part or in whole) and a few citations of articles concerned with the closely related genus Besnoitia.

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## APPENDIX II

### Observations on Experimental Rangiferine Brucellosis Infections in Domestic and Wild Alaskan Carnivores

Kenneth A. Neiland<sup>1</sup> and Lawrence G. Miller<sup>2</sup>

<sup>1</sup> Alaska Department of Fish and Game, Fairbanks

<sup>2</sup> Arctic Health Research Center, College, Alaska  
(Now at: PHS - DHEW - DQS, Denver, Colorado)

## Abstract

Beagle dogs were readily infected by about  $10^8$  C.F.U. of *Brucella suis* type 4 administered either on canned dog food, intraperitoneally or into the conjunctival sac. Such infections are afebrile and otherwise asymptomatic and without obvious, gross pathological changes. Brucellae concentrate in all major lymph nodes regardless of site of infection. Infection of salivary glands and the kidney may take place. Serologic responses are similar to those observed in infections of canids by other strains of *Brucella*.

Two gravid wolves (*Canis lupus*) were infected by about  $10^8$  C.F.U. administered intraperitoneally and into the conjunctival sac, respectively. About 24 days later they gave birth, apparently at full-term, to two (both alive) and six (two alive and four dead) pups, respectively. Those pups born alive died within 24 hours in both cases. Trauma may have played a part in the death of all the pups. Seven of the eight pups were infected by brucellae. One pup was eaten shortly after birth and was not available for examination.

The serologic and bacteriologic character of the infection in wolves is comparable to that seen in dogs.

Two grizzly bears (*Ursus arctos horribilis*) were both infected by exposure to about  $10^9$  C.F.U.-aliquots of *B. suis* type 4 placed on each of their respective portions of canned dog food. Within the first two months of infection antibody titres reached levels as high as 1:10240. At the end of the third month of infection, they were fatally infected for experimental purposes with rabies and the original brucellosis infections were not further studied. A black bear (*Ursus americanus*) infected with between  $10^8$  and  $10^9$  C.F.U. yielded serologic and bacteriologic data similar to those derived from the observations on beagles and wolves.

## Introduction

Rangiferine brucellosis caused by *Brucella suis* type 4 is common in some Alaskan caribou (*Rangifer tarandus*) herds.<sup>10</sup> It also occurs in sled dogs, wolves, red foxes and grizzly bears which feed on caribou.<sup>8,9</sup> The disease has been reported in Arctic foxes (*Alopex lagopus*) and wolverines (*Gulo gulo*) on Siberian reindeer ranges,<sup>12</sup> but we have not had the opportunity to examine these species in Alaska. Probably all predators and/or scavengers which feed on prey species in which *Brucella* is enzootic will eventually become infected and develop detectable serum antibodies.<sup>9</sup> Whether or not these infections are transmissible under natural circumstances between individual, free-ranging predators is unknown, as are the effects such infections might have. However, reproductive failure of foxes on fur farms<sup>14</sup> and of beagle dogs in commercial kennels<sup>2</sup> as a consequence of infection by *Brucella* spp. is well known.

Because of the abortifacient character of *Brucella* spp. in a variety of host species, and also the widespread concern over the welfare of Alaskan wildlife, particularly wolves and grizzlies, in the face of accelerated resource development, it appeared worthwhile to experimentally evaluate the effects of rangeliferine brucellosis on canids and ursids. We were also concerned over the possibility that infected dogs might transmit the disease to their owners. Many instances of canine to human transmission have been recently reviewed.<sup>11</sup>

Unfortunately, although the preliminary results reported below were of considerable interest and cojency, untimely termination of our experiments was required when the experimental faciltiy was deactivated. Accordingly, under the circumstances, we see little prospect of being able to carry this line of experimentation to a logical termination. Therefore, we deem it worthwhile to publish our incomplete and somewhat fragmentary results at this time.

#### Materials and Methods

The strain of *Brucella suis* type 4 used in our experiments was isolated from a sled dog from Kobuk, Alaska, in July 1969. This organism was identified by Drs. D. T. Berman and L. M. Jones, Department of Veterinary Science, University of Wisconsin. A lyophilized subculture of this isolate was used in our experiments. The organism was grown on brucella agar (BBL #11086) at 37°C for 72 hours. All experimental inocula were prepared by suspending cells in peptone saline.<sup>1</sup> Stock suspensions were adjusted to approximately  $2.0 \times 10^9$  colony forming units (C.F.U.) per ml using MacFarlane turbimetric comparison standards. Then, decimal dilutions were prepared using a Vortex mechanical mixer to insure uniform suspensions. Three aliquots of suitable dilutions were spread on brucella agar plates and counted at 72 hours. We generally inoculated the animals either intraperitoneally or via conjunctival sac unless otherwise noted.

Tissues for bacteriological assay were dipped in 95 percent ethanol and flamed before being sterily lacerated and streaked-out on brucella agar plates. Blood cultures were prepared using 2-5 ml aliquots of freshly withdrawn, citrated veinous or heart blood in brucella "broth."

Urine and feces were cultured on brucella agar to which were added cycloheximide, bacitracin and polymixin B. as prescribed by Alton and Jones.<sup>1</sup>

Typical colonies from each suspected tissue-isolate were typed using *Brucella abortus* antiserum (Difco) in a rapid slide agglutination procedure.<sup>1</sup> The relative number of C.F.U. in various tissues streaked-out on agar plates was recorded as follows: 1-5 colonies, 1+; 6-20 colonies, 2+; 21-50 colonies, 3+; more than 51 colonies, 4+. Tube agglutination titres of sera from experimental animals were determined according to published procedures<sup>1</sup> using commercial *Brucella abortus* smooth antigen (Difco). Complement fixation titres were determined in the laboratory of Dr. David T. Berman, Department of Veterinary Science, University of Wisconsin, using methods described elsewhere.<sup>1</sup>

Beagle dogs were obtained from the experimental colony maintained at the Arctic Health Research Center since 1962 without introduction of new breeding stock at any later time. The two wolves, both pregnant bitches, were obtained from the experimental colony at the Naval Arctic Research Laboratory, Barrow, Alaska. Both had been caught as pups in the Brooks Mountain Range and had been successfully bred in captivity several times. The black bear cub was captured as a nuisance animal in the environs of Fairbanks, Alaska. Both grizzly bear cubs were captured in the vicinity of Tok, Alaska.

The dogs, bears and wolves were fed individually appropriate amounts of various commercially prepared wet and dry dog foods and canned milk daily and allowed free choice of water. The wolves and bears were tranquilized with phencyclidine hydrochloride (Sernalyn, Bio-Ceutic Laboratories) administered via a Palmer Cap-Chur gun prior to handling. Unless otherwise noted, the animals were all individually caged indoors.

## Results

### Beagle Dogs

Two experiments were done with beagles. These are reported separately below. Both were concerned in part with possible natural modes of transmission.

#### Experiment #1

The first experiment involved three beagles (2 females and 1 male) each about one-year-old. They were individually exposed to about  $1.3 \times 10^8$  C.F.U. placed on their daily ration of canned dog food on December 5, 1972. Blood samples were taken from the heart prior to exposure and on December 19 and again on January 2, 1973. They were sacrificed 30 days post-exposure and a variety of tissues were screened for brucellae. These results are presented in Table 1. Observations on blood cultures and serum agglutination titres are shown in Table 2.

No gross pathological signs were noted at necropsy. Attempts to isolate brucellae from urine and feces failed. The animals appeared normal in all respects throughout the experimental period. Daily temperature measurements gave no indication of any febrile responses.

#### Experiment #2

In this experiment, three beagle pups (1 male, #2993, and 2 females, #2994 and #2995) were used. They were all bled on March 14, 1973, and the male (#2993) was infected on March 20 with about  $1.5 \times 10^8$  C.F.U. inoculated intraperitoneally. The three animals, one infected and two controls, were then kept as cage mates until June 6, 1973, when the experiment had to be terminated. They were bled three times during the course of the experiment. The results of the bacteriological examination

Table 1. Distribution of Brucella suis type 4 in the tissues of experimentally infected beagle dogs.

Tissue	<u>Occurrence of brucella</u> <u>Dog Number and Sex</u>		
	2938(F)	2939(M)	2940(F)
Liver	+	+	+
Spleen	4+	2+	2+
Uterus	-	n/a	-
Kidney	-	-	+
Bladder	-	-	-
Lung	+	-	----
Testis	n/a	-	n/a
Salivary Gland, mandibular	----	+(R) <sup>1</sup>	+(L) <sup>2</sup>
Salivary Gland, maxillary	+(L)	----	----
Lymph Node, mandibular	4+(R,L)	----	4+(R,L)
Lymph Node, parotid	----	3+	----
Lymph Node, sub-mandibular	----	----	4+(R)
Lymph Node, medial retropharyngeal	4+(L)	4+(R)	2+(L)
Lymph Node, superficial cervical	----	3+(R)	4+(L)
Lymph Node, axillary	4+(R)	3+(L)	4+(L)
Lymph Node, mesenteric	4+	4+	4+
Lymph Node, external iliac	4+	----	4+(R,L)
Lymph Node, submammary	3+(R,L)	----	4+(R,L)
Lymph Node, popliteal	4+	4+(R)	4+(R)
Tonsil	----	+(L)	+(R)
Blood, sediments	-	-	-
Blood, clot	+	+	+

<sup>1</sup> Right side (R)

<sup>2</sup> Left side (L)

Table 2. Serologic titres and results of cultures of blood obtained from beagle dogs experimentally infected with Brucella suis type 4.

Date	Procedure	Results <sup>1</sup> Dog Number and Sex		
		2938(F)	2939(M)	2940(F)
12/19/72	Serology, agglutination	4+, 1:160	4+, 1:640	4+, 1:160
	Serology, complement fixation	2+, 1:20	4+, 1:40	2+, 1:20
	Blood culture	+	+	+
1/2/73	Serology, agglutination	4+, 1:640	4+, 1:1280	4+, 1:640
	Serology, complement fixation	4+, 1:160	3+, 1:320	4+, 1:80
	Blood culture	+	+	+

<sup>1</sup> A complete reaction at a given dilution is given as 4+. Incomplete reactions are recorded as 2+ or 3+.

of tissues collected at necropsy are given in Table 3. The serologic results are reported in Table 4.

The animals appeared normal throughout the experimental period and no gross lesions were observed at necropsy.

### Wolves

Two pregnant wolves (#3214 and #3215) which had been bred in the second week of March 1973, were utilized in the following experiment. They had been held in captivity at the Naval Arctic Research Laboratory, Barrow, Alaska, since they were captured as pups in the Brooks Mountain Range in June 1967<sup>5</sup>. Both had successfully produced litters in the past under conditions of close captivity. They were sent to the Arctic Health Research Center in early May where they were held in individual cages indoors throughout the experimental period. The experimental manipulation of these two animals is separately described below.

Wolf (#3214): This animal was infected with  $2.1 \times 10^8$  C.F.U. via the intraperitoneal route on May 4. On May 27 it gave birth to a pup (#3225) which died later in the day. At necropsy, the pup (#3225) showed no gross lesions, but several ribs were broken and there was apparent hemorrhaging along the left side of the rib cage. On May 28 a second pup was born alive but was discovered partially eaten a few hours later.

On June 7 wolf #3214 was euthanized and necropsied. Splenomegaly was evident and there was extensive fibro-inflammatory tissue over the ventral half of the capsule. In addition, both uterine horns appeared to contain caseous material. Otherwise all other organs appeared normal. A number of tissues were taken for bacteriological examination. Data on the distribution of brucellae in the tissues of the bitch (#3214) and the pup (#3225) are presented in Table 5. Serologic data are presented in Table 7.

Wolf (#3215): This animal was infected on May 4 by introducing  $2.1 \times 10^8$  C.F.U. into the conjunctival sac. On May 28 it gave birth to six pups (#3230, 3231, 3232, 3233, 3234 and 3235). Four of these were presumed dead at birth and the two others died within 24 hours. Several of the pups showed some signs of trauma, i.e. broken ribs and consequent hemorrhaging. Otherwise, there were no gross lesions attributable to the experimental infection of the bitch.

On June 7, #3215 was euthanized and necropsied. Splenomegaly was not evident in #3215. The spleen was about one-half the size of that of #3214 and no inflammatory tissue was seen. Both uterine horns contained apparently caseous material as seen in #3214. Otherwise, all other organs appeared normal. Data on the distribution of brucellae in the tissues of #3215 and her pups are shown in Table 6. Serologic data are reported in Table 7.

Table 3. Distribution of Brucella suis type 4 in an experimentally infected beagle pup and its two normal, control cage-mates.

Tissue	<u>Occurrence of brucellae</u> <u>Dog Number and Sex</u>		
	2993(M)	2994(F)	2995(F)
Liver	-	-	-
Spleen	+	-	-
Kidney	-		
Urine	-		
Testes	-		
Salivary gland, maxillary			-
Salivary gland, parotid	-		-
Lymph Node, mandibular	2+		
Lymph Node, retropharyngeal	2+		
Lymph Node, mesenteric	+		
Blood	-	-	-

Table 4. Serologic observations on a beagle pup experimentally infected with Brucella suis type 4 and its two normal, control cage-mates.

Date	<u>Serologic Titre<sup>1</sup></u> <u>Dog Number and Sex</u>					
	2993(M)		2994(F)		2995(F)	
	AGGL	CF	AGGL	CF	AGGL	CF
3/14	-	-	-	-	-	-
4/5	4+, 1:320	>4+, 1:80	-	-	-	-
4/26	4+, 1:160	>4+, 1:640	-	-	-	-
6/6	4+, 1:80	>4+, 1:640	-	-	-	-



Table 5. Distribution of Brucella suis type 4 in an experimentally infected wolf (#3214)<sup>1</sup> and her pup (#3225).

Tissue	<u>Culture Results</u>	
	#3214	#3225
Liver	+	4+
Spleen	2+	2+
Blood	-	-
Lung	-	
Urine	-	
Uterine horn, right	contaminated	
Uterine horn, left	contaminated	
Mammary gland	4+	
Salivary gland, parotid	-	
Salivary gland, mandibular	-	
Lymph Node, mandibular	4+	
Lymph Node, medial retropharyngeal	4+	
Lymph Node, superficial cervical	4+	
Lymph Node, axillary	4+	
Lymph Node, mediastinal	4+	
Lymph Node, mesenteric	4+	
Lymph Node, external iliac	4+	
Lymph Node, submammary	-	
Lymph Node, popliteal	4+	

<sup>1</sup> Inoculated intraperitoneally.

Table 6. Distribution of Brucella suis type 4 in an experimentally infected wolf (#3215)<sup>1</sup> and her six pups (#3230-3235).

Tissue	Culture Results						
	#3215	#3230	#3231	#3232	#3233	#3234	#3235
Liver	4+	3+	+	4+	2+	+	4+
Spleen	+	3+	-	+	-	+	4+
Blood	-	-	-	-	-	-	4+
Lung	contaminated						
Urine	+						
Uterine Horn, right	4+						
Uterine Horn, left	4+						
Salivary Gland, parotid	-						
Salivary Gland, mandibular	4+						
Lymph Node, medial retropharyngeal	4+						
Lymph Node, superficial cervical	4+						
Lymph Node, axillary	4+						
Lymph Node, mediastinal	2+						
Lymph Node, mesenteric	4+						
Lymph Node, external iliac	4+						
Lymph Node, submammary	4+						
Lymph Node, popliteal	2+						

<sup>1</sup> Inoculated into the conjunctival sac.

Table 7. Serologic observations on experimental infections of Brucella suis type 4 in two pregnant wolves (#3214 and #3215).

Date	Specimen Number	Agglutination Titre
May 4	3214	1:20
May 4	3215	1:20
May 21	3214	4+1:160
May 18	3215	4+1:160
June 7	3214	4+1:5280
June 7	3215	4+1:1280

We also examined for brucellae the mandibular lymph nodes and parotid salivary gland of a wolf killed near Anaktuvuk Pass during April 1973, with negative results. Only obvious contaminants were recovered.

#### Black Bear

A yearling female black bear was infected on March 21, 1973, with between  $10^8$  and  $10^9$  C.F.U. of *Brucella suis* type 4 injected into the peritoneal cavity. On April 26 a blood culture gave negative results, but a slide agglutination titre between 1:80 and 1:160 was observed. On June 6 it was euthanized and necropsied. At that time we observed a slide agglutination titre of 1:800. The only gross pathology observed at necropsy was the apparent enlargement of both the right and left axillary lymph nodes, both of which subsequently were found to harbor *B. suis* type 4. The distribution of brucellae in some tissues of this animal is given in Table 8.

#### Grizzly Bears

Two litter mates, probably born about February 1972, were utilized in this experiment. The cubs were individually caged and both were infected by placing approximately  $1.3 \times 10^9$  C.F.U. on their respective daily rations of canned dog food on December 6, 1972. It was noted that neither bear ate all of its food on this occasion. On December 8 and 9 one of the cubs (#2936) vomited. Because of the potentially adverse effects of tranquilizing the animals, we decided to minimize this possible risk. Therefore, we did not make pre-infection observations on serologic titres or whether brucellae could be isolated from the blood. Data on serology and blood culture are given in Table 9.

The ultimate termination of the experiment was initiated on March 5, 1973, by experimentally infecting both bears with the strain of rabies enzootic in Alaskan foxes to which they both succumbed.<sup>13</sup> After exposure to rabies we did not again handle the animals.

### Discussion

#### Beagle Dogs

Morse<sup>6</sup> in 1951 and Rementsova<sup>14</sup> in 1962 reviewed the literature on canine brucellosis caused by the earlier known strains of *Brucella abortus*, *B. suis* and *B. melitensis*. More recently Carmichael and Kennedy<sup>2</sup> have summarized information on the form of canine brucellosis specifically caused by *Brucella suis* type 5, a distinct strain which was discovered to be the cause of epidemic abortion in beagle dog colonies. Relatively little is known about the bio-medical character of the form of canine brucellosis specifically caused by the rangiferine brucellosis agent, i.e. *Brucella suis* type 4, which thus far has only been reported in domestic canines in Alaska.<sup>8,9</sup>

Table 8. The distribution of Brucella suis type 4 in an experimentally infected black bear.

Tissue	Culture Results
Liver	-
Spleen	2+
Lung	-
Ovary	-
Urine	+
Salivary Gland, parotid	-
Lymph Node, mandibular	4+
Lymph Node, medial retropharyngeal	3+
Lymph Node, parotid, left	2+
Lymph Node, superficial cervical	3+
Lymph Node, axillary, right	3+
Lymph Node, axillary, left	2+
Lymph Node, mediastinal	3+
Lymph Node, mesenteric	3+
Lymph Node, external iliac	3+
Lymph Node, right popliteal	3+

Table 9. Data on the serologic and bacteriologic examination of blood of grizzly bears experimentally infected with Brucella suis type 4.

Date	Specimen Number	Results	
		Serology	Blood Culture
January 5	2936	4+, 1:2560	
	2937	4+, 1:2560	
January 15	2936		positive
	2937		negative
February 7	2936	4+, 1:10240	
	2937	4+, 1:5120	
March 5	2936	4+, 1:5120	negative
	2937	4+, 1:1280	negative

The results of our experiments described above and summarized in Tables 1-4 show that: 1) beagle dogs are readily infected with rangiferine brucellosis via contaminated food or intraperitoneal inoculation; 2) in such infections, brucellae are distributed in large numbers throughout the lymphatic system in all major regional nodes; 3) brucellae may be present in both the kidney (and urine?) and salivary gland(s) (and saliva?) although perhaps not with sufficient regularity or intensity to commonly act as a source of infection; 4) neither febrile nor other gross, inflammatory signs were observed; and 5) serologic responses of beagle dogs to rangiferine brucellosis are similar to those seen in other forms of canine brucellosis.

Our original reasons for experimenting with rangiferine brucellosis in canids are not negated by the results we report above. Primarily we were concerned over the possibility that the disease might be an abortifacient as is the case in canid infections caused by *Brucella abortus*<sup>6,7</sup>, *B. melitensis*<sup>6,14</sup>, and a non-rangiferine strain of *B. suis*<sup>2</sup> (i.e. *B. suis* type 5). Our preliminary experiments were designed to familiarize ourselves with brucellar infections in canids including the general susceptibility and spread of rangiferine brucellosis in the organs of canids. Our plans to infect pregnant animals later were confounded by the untimely closure of our experimental facilities. Nevertheless, under the present circumstances, we can see no good reason to doubt that rangiferine brucellosis may act, under both natural and experimental conditions, as an abortifacient. The results of experimental infections of wolves reported above and considered in the next section, support this conclusion, and it is with the reproduction of wild canids that we primarily are interested.

We were also concerned whether canid infections might serve as a source of human infection with rangiferine brucellosis. The literature contains numerous references to canid-derived human infections by one or another of the strains of the three species of *Brucella*.<sup>6,11,14</sup> While most often these infections have apparently resulted from association with aborted material, they may also occur via unexpected pathways. For example, Rementsova<sup>14</sup> cites a case in which the disease was transmitted to a person that was bitten by an infected dog. Our observations of brucellae in the salivary glands of three experimental beagle dogs reported in Table 1 suggest that salivary transmission of rangiferine brucellosis from canids to humans (or other canids) might also occur. Organisms present in the kidneys (see Table 1) might also be present in urine and be transmitted to other hosts via contamination. More work needs to be done to fully evaluate the degree to which canid infections by rangiferine brucellosis may pose a threat to human health.

### Wolves

Experimental infections of wolves with *Brucella suis* type 4 present much the same general picture as seen in beagle dogs. The data presented above and summarized in Tables 5, 6, and 7 indicate that: 1) the wolf evidently is readily susceptible to rangiferine brucellosis; 2) brucellae

concentrate in nodes throughout the lymphatic system; 3) infection of the uterus and developing fetuses readily takes place; 4) organisms are probably shed in urine, saliva and milk; 5) rangiferine brucellosis may lead to reproductive failure in wolves; and 6) serologic responses by wolves to *Brucella suis* type 4 are similar to those of other host species.

Conclusion #5 presented above should be qualified. While it is clear enough that none of the pups survived what otherwise might be considered normal births for any significant time (i.e. full term, normal appearing fetuses), we cannot unequivocally rule out the possibility that they were killed by their mothers for behavioral reasons unrelated to our experimental manipulations. If the pups had been allowed to live, they might have grown into essentially normal adult animals, and indeed, on past occasions, both bitches, captives since they were pups, had proven capable of successful breeding under conditions of close captivity. Therefore, one cannot help but wonder whether brucellar infections in lower animals are also complicated by the neuro-psychiatric aberrations (behavioral disorders) so frequently seen in human cases of brucellosis and sometimes caused by porcine as well as other species of *Brucella*.<sup>15</sup> If this is the case, the killing and/or eating of newborn pups might be an example of *Brucella*-induced psychoneurotic behavior in a lower animal. The eating of aborted fetuses and placental materials is commonplace in cases of *Brucella suis* type 5 in beagle dogs.<sup>2</sup> Whether rangiferine brucellosis, which naturally infects wolves on Alaskan<sup>8,9</sup> and Siberian<sup>12</sup> reindeer ranges, is a significant cause of reproductive failure is unresolved and it appears unwise to dismiss this possibility in advance of further experimental evidence.

### Grizzly Bears

Information on naturally-occurring, infectious diseases of bears is scarce.<sup>3,4</sup> This is probably more a matter of lack of opportunity in the past to study wild bear populations than any unusually protective resistance of bears to microbial infections. Be this as it may, it appears that infection of grizzly bears by rangiferine brucellosis is a commonplace event on some caribou ranges in northern Alaska.<sup>9</sup> If the susceptibility of bears to infection via contaminated food we reported above is typical, then it is somewhat less surprising that we encountered such relatively high prevalence rates (up to 90%) of *Brucella*-antibodies in free-ranging grizzlies. High prevalence of antibodies might also be a result, in part, of the relatively high titre-levels that evidently occur during early stages of infection in grizzly bears (see Table 9). This assumes that host species or individuals that produce relatively high titres initially will maintain recognizable titres longer. In this case a population composed of such individuals would build up a high prevalence of antibodies even though the relative exposure rate was comparatively low and stable.

While we have no independent knowledge of grizzly bear biology in Arctic Alaska which suggests that these populations of bears may have reproductive problems, we cannot help but point out the abortifacient

character of the disease in other carnivores. Judging from the serologic and bacteriologic information on an experimentally infected black bear cub present above and summarized in Table 8, rangiferine brucellosis in bears is comparable, at least in these respects, to similar infections in canids. We see no reason to conclude that abortion will not occur under the proper circumstances.

#### General Conclusions

Canids and ursids are readily susceptible to rangiferine brucellosis via natural means of transmission involving passage of brucellae across mucus membranes of the buccal cavity and conjunctival sac.

*Brucella suis* type 4 tends to congregate in these species in high numbers in lymph nodes distributed throughout the body regardless of the initial site of infection.

*Brucella suis* type 4 commonly invades the salivary glands and probably also the mammary glands and kidneys, thus providing for the shedding of brucellae in saliva, milk, and urine.

Reproductive failure is a probable, but essentially unproven, consequence of ill-timed infections.

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APPENDIX III

Observations on Experimental Rangiferine  
Brucellosis Infections in Domestic and  
Wild Species of Alaskan and Scandinavian Rodents

Lawrence G. Miller<sup>1</sup>  
Arctic Health Research Center, Fairbanks, Alaska 99701

and

Kenneth A. Neiland  
Alaska Department of Fish and Game, Fairbanks, Alaska 99701

<sup>1</sup> Now at: PHS-DHEW-DQS, Denver, Colorado

ABSTRACT: The susceptibility of nine species of rodents and one species of lagomorph to several strains of *Brucella*, but principally the rangiferine strain *Brucella suis* type 4, was studied experimentally. The rodent species included the following forms: guinea pig (*Cavia caviae*), Scandinavian lemming (*Lemmus lemmus*), Northern red-backed vole (*Clethrionomys rutilus*), varying lemmings (*Dicrostonyx stevensoni* and *D. rubricatus*), brown lemming (*Lemmus sibiricus*), yellow-cheeked vole (*Microtus xanthognathus*), flying squirrel (*Glaucomys sabrinus*) and ground squirrel (*Citellus parryi*). The lagomorph, *Lepus americanus* (varying hare), was also studied.

All of these potential host species were readily infected by intraperitoneal inoculations of  $10^6$  cfu, or less in some cases, of *B. suis* type 4. Subsequently, the organism was isolated from one or more tissues, principally liver and spleen, of each species. Pathologic responses were not marked in most of these hosts.

Both species of varying lemmings, i.e. *D. stevensoni* and *D. rubricatus* responded dramatically to infections initiated by as few as two cfu. All individuals of both species that were not sacrificed eventually died from the infection. The most common gross pathologic sign encountered was extreme hypertrophy of the spleen which occurred in all individuals. Microabscesses were commonly seen in the liver. Longer term infections also resulted in abscessation of the joints of the feet, subcutaneous tissue, kidneys, lungs, and uterus or testes. In some cases, substantial numbers of organisms were excreted in the urine.

Trial infections with *Brucella abortus*, *B. melitensis* and *B. suis* type 1 were also easily accomplished in *Dicrostonyx stevensoni*. Symptomatology and pathology more or less similar to that seen in infections of *B. suis* type 4 in this host were observed.

It was concluded that rodents might play a role in the circulation of *B. suis* type 4 among various sympatric, potential hosts.

It was suggested that because of the high susceptibility of *Dicrostonyx* spp. to infection by *Brucella* spp., varying lemmings should prove to be of considerable value in on-going research on brucellosis control procedures. They may also be a useful host in investigations of basic mechanisms of disease resistance and/or host susceptibility.

## INTRODUCTION

Rangiferine brucellosis occurs as a disease of humans in Alaska, Canada and Eurasia. Huntley, et al.<sup>4</sup> conducted a serological survey among Eskimo, Indian, and Aleut populations and found serological evidence that the disease was prevalent among the native populations of Alaska dependent upon caribou for food. They also isolated a species of *Brucella* later identified as *Brucella suis* type 4 (Meyer<sup>5</sup>). The organism has been isolated from human infections as recently as 1974 (F. Pauls, pers. comm.). Brody, et al.<sup>2</sup> endeavored to define the epidemiology of brucellosis

further, and although they did obtain strong evidence that the source of human infection was caribou, they were unable to explain a higher rate of positive serologies in a village where the consumption of caribou was thought to be considerably less. They hypothesized that rodents might also be a reservoir of infection in this instance.

Neiland, et al.<sup>7</sup> extended studies on Alaskan brucellosis with their observations in caribou. They pointed out at that time that dogs had been overlooked as a reservoir of human disease in Alaska. The first known case of rangiferine brucellosis in the domestic dog, confirmed with the isolation of *B. suis* type 4 in an Alaskan sled dog, was reported by Neiland<sup>6</sup>. He suggested at that time that wild rodents should not be overlooked as possible sources of infection and that "sylvatic brucellosis in Alaska may prove to be as widely distributed among the wild host species as it is elsewhere."

Lacking evidence of natural infections in the Alaska's indigenous rodent population, and with knowledge of natural infections reported in rodents elsewhere (see Rementsova<sup>9</sup>), a series of experiments were undertaken to ascertain the susceptibility of these rodents to brucellaceae, especially *B. suis* type 4 (*B. rangiferi*). The results of these preliminary experiments are the subject of this report. The untimely closure of the experimental facility prevented our carrying on the work to a logical conclusion.

## MATERIALS AND METHODS

### Animal Species

With the exception of the guinea pig (*Cavia cavae*) and the Scandinavian lemming (*Lemmus lemmus*, Linnaeus), the animals used were indigenous to Alaska (Arctic and sub-Arctic). The wild rodents, Northern red-backed vole (*Clethrionomys rutilus*, Pallas), varying lemmings (*Dicrostonyx stevensoni*, Nelson, and *D. rubicatus*, Richardson), brown lemming (*Lemmus sibiricus*, Kerr), and yellow-cheeked vole (*Microtus xanthognathus*, Leach), were laboratory reared. The varying hares (*Lepus americanus*, Erxleben), flying squirrels (*Glaucomys sabrinus* Shaw), and ground squirrels (*Citellus parryi*, Richardson) were adults, trapped alive, and held for a minimum of four weeks before being inoculated. Both sexes were used, but a 1:1 ratio could not be maintained.

### Bacterial Species

The organisms were obtained in a lyophilized state. *Brucella suis* type 4 was isolated from an Alaskan sled dog<sup>6</sup> and subcultured from stocks maintained by Dr. D. T. Berman. The remaining species, *B. abortus* type 1 (W.H.O. Reference strain 544), *B. melitensis* type 1 (W.H.O. Reference strain 16M), and *B. suis* type 1 (W.H.O. Reference strain 1330) were from stocks maintained by the National Animal Disease Laboratory, ARS, USDA, Ames, Iowa.

## Bacteriological Techniques

The bacteriological techniques for isolation, propagation, maintenance of strains, viable counting, and serological identification were those described by Alton and Jones<sup>1</sup>. *Brucella* agar (BBL) was used as the base medium for isolation and counting.

## Animal Infection Studies

Organisms were grown for 72 hours on *Brucella* agar, and decimal dilutions of the organism were prepared in peptone saline. Viable counts were made from the same series of decimal dilutions as were used for inoculation. Dosage is expressed as colony forming units (cfu). The animals were inoculated intraperitoneally and observed closely during the duration of the experiments. Animals inoculated with different species were kept in separate isolation rooms.

Tissues for bacteriological analysis were taken aseptically from animals found dead or sacrificed, dipped into absolute alcohol, flamed, macerated, and smeared onto selective media (Alton and Jones<sup>1</sup>). Tissue isolates were confirmed as *Brucella* on the basis of their agglutination by *Brucella* antiserum.

## RESULTS

### Guinea Pig

The guinea pig was injected intraperitoneally with  $3.8 \times 10^4$  to  $3.8 \times 10^6$  cfu of *B. suis* type 4. Animals were sacrificed at 7, 14, and 77 days. Lesions typical of those described (Hausler and Koontz<sup>3</sup>) for other species of *Brucella* were observed and were found to become progressively more pronounced with time and increased dosage. Isolations were made from at least one tissue from each animal. At 77 days, the microorganism was found in the liver (1/4), spleen (3/3), testes (3/4), and urine (4/4).

### *Dicrostonyx t. stevensoni*

Four series of experiments were performed with *D. t. stevensoni*. In three, the lemmings were challenged with *B. suis* type 4. The first experiment was to determine whether the animals were susceptible to infection with this microorganism. The next two experiments were attempts to determine the effects of lesser doses and to approximate the LD<sub>50</sub>. The final experiment was to determine the susceptibility of *D. t. stevensoni* to three additional species of *Brucella*.

The first set of 18 *Dicrostonyx* were inoculated with  $4 \times 10^6$  cfu of *B. suis* type 4. Two animals were sacrificed at 8, 14, 31, and 37 days post infection. Those that died during the course of this experiment

were also necropsied. Abscesses were found on 10 of 14 livers, and enlarged spleens were found in animals that had died or were sacrificed 22 days after inoculation. An occasional abscess was found within the capsule of the kidney. Large abscesses were found within the abdominal cavity along the mesentery. Four females developed pus within their uterus and one male developed unilateral epididimitis.

*B. suis* type 4 was isolated consistently from the liver, spleen, kidney, and heart blood of these animals. In three cases the urine contained *Brucella* in concentrations ranging from 200 to 20,000 microorganisms per ml.

The second and third experiments were performed to determine the LD<sub>50</sub> and the effects of graded doses of *B. suis* type 4 on this species of lemming. The second experimental group of *Dicrostonyx* consisted of a nearly uniform group of young lemmings, who received doses from 2 to 250,000 cfu intraperitoneally. The animals were maintained for 27 days when, for premature reasons, the experiment was terminated.

The third experiment was a repeat of the second using doses of 35 to 35,000 cfu in decimal increments. The discrepancy between the mean death time (Table 1) in each experiment cannot be explained. The variables that probably contributed to differences include: 1) The animals in the second experiment were young, mature animals of a nearly equal sex ratio whereas the third set was composed of animals 6 to 12 months older, of which 20 of the 24 were males; 2) Changes in the virulence of the *Brucella* strain may account for the discrepancy, however the mean death time (34 days) of animals infected with  $4 \times 10^6$  cfu in the first experiment is very similar to that seen in the third experiment (range 22-56).

Generally there was no appreciable difference in the lesions seen in the animals inoculated with graded doses, except that a greater number of animals developed large abscesses in the lower dose range, 35 to 350 cfu. Five of the males in this group also developed abscesses in the prostate glands. Two lemmings receiving 35 cfu survived 114 and 126 days. Both developed crippling abscesses in their feet.

*D. t. stevensoni* - Other *Brucella* sp.

*B. abortus*, *B. melitensis*, and *B. suis* type 1 were used to challenge *D. t. stevensoni*. Three, one-hundred fold dilutions beginning with  $4 \times 10^7$  cfu (*B. abortus* and *B. melitensis*) and  $6 \times 10^6$  cfu (*B. suis* type 1) were used to inoculate separate groups of lemmings intraperitoneally. Only two animals survived to the arbitrary limit of the experiment (28 days), otherwise the deaths occurred between 4 to 18 days (*B. abortus*), 11 to 27 days (*B. melitensis*), and 4 to 26 days (*B. suis* type 1), with all animals inoculated with  $4 \times 10^3$  cfu or less surviving until after the 21st day.

Table 1. Survival of Dicrostonyx stевensoni inoculated with Brucella suis type 4.

Dose	Experiment 2 <sup>1</sup> Days survived			Experiment 3 Days survived		
	#	Mean <sup>2</sup>	Range	#	Mean	Range
2.0 x 10 <sup>5</sup>	4	12	(11-13)			
3.5 x 10 <sup>4</sup>				5	51	(39-96)
2.0 x 10 <sup>4</sup>	3	15	(14-16)			
3.5 x 10 <sup>3</sup>				6	41	(32-47)
2.0 x 10 <sup>3</sup>	4	13	(12-15)			
3.5 x 10 <sup>2</sup>				6	53	(48-59)
2.0 x 10 <sup>2</sup>	4	--- <sup>3</sup>	(21-27)			
35				6	72	(48-126)
20	4	27				
2	4	27				

<sup>1</sup> Terminates 27 days

<sup>2</sup> Geometric mean

<sup>3</sup> Not calculated

There were subtle differences between the lesions caused by infection with each of the species of *Brucella*. Abscesses developed on livers regardless of the infecting microorganism, spleens became enlarged and developed abscesses. *B. melitensis* appears to have caused a greater effect on the respiratory system with six of the seven lemmings showing some form of lesion (fluid in the pleural cavity or lung consolidation), as opposed to only two of the six infected with *B. abortus* and three of the nine infected with *B. suis* type 1 developing lesions in the respiratory tract. In addition, five of the animals infected with *B. melitensis* developed fibrous exudates on the surfaces of their spleens or livers, a condition seen in only one other animal, that infected with *B. abortus*.

The infecting microorganism was consistently isolated from the liver, spleen, kidney, uterus or testes, heart blood, and the urine (50 to  $10^5$  per ml) of 12 out of 13 animals from which urine was available.

#### *Dicrostonyx rubicatus*

Nineteen *D. rubicatus* were challenged intraperitoneally with doses of *B. suis* type 4 ranging from 35 to  $3.5 \times 10^6$  cfu. Eighteen of the 19 animals died between 14 and 37 days with 1 animal that received  $3.5 \times 10^5$  cfu surviving until day 59 when it was sacrificed. Abscesses developed progressively in the liver, spleen, in three cases on the posterior aspect of the sternum, and subcutaneously. The liver developed pinpoint abscesses between the 18th and 21st day of infection; by the 59th day, if the animal survived, the abscesses had enlarged to 1 to 2 mm in diameter. The spleen became enlarged as early as the 15th day in one animal receiving  $3.5 \times 10^5$  cfu. However, the majority of the animals receiving 350 to 3500 cfu and dying between the 15th and 37th day developed splenomegaly by the 25th day, and 11 of the *Dicrostonyx* developed either 1 or 2 large discrete abscesses on their spleen. Congested lungs were seen in 11 of the animals and abscesses were found in 2. Brucellae were isolated consistently from the liver, spleen, kidney, and heart blood. In two of the three urines cultured, brucellae were found in concentrations of  $10^4$  and  $10^5$  per ml.

#### Varying Hare

Three varying hares, *Lepus americanus*, were inoculated with  $7.5 \times 10^6$  cfu intraperitoneally. One was sacrificed on each of the days 14, 22, and 57. *Brucella* was isolated from the uterus, axillary lymph nodes, and spleen and from a cysticercus (probably *T. pisiformis*, Bloch 1780) found in the abdominal cavity of the animal killed on day 14. The lungs, urine, ovary, heart blood, liver and kidney were negative. When liver, lungs, kidney and spleen were cultured on the remaining animals, the organism was recovered solely from the liver of the hare killed on day 57.

#### Ground Squirrel

Three ground squirrels, *Citellus parryi*, were inoculated with  $7.5 \times 10^6$  cfu intraperitoneally. One was killed at 14 days and the remaining

2 at 80 days. Small abscesses were seen on the liver (one 14 day and one 80 day animals) and on the spleen of both animals killed on the 80th day; otherwise the organs appeared normal. The organism was recovered from the liver and spleen of all animals and from the kidney, testes, and lungs of the animal killed on the 14th day. The urine, salivary gland, and blood from the 14 day animals were negative.

#### *Glaucomys sabrinus*

Three flying squirrels, *Glaucomys sabrinus*, were infected intraperitoneally with  $7.5 \times 10^6$  cfu *B. suis* type 4. One died on the 41st day, and the others were sacrificed at 14 and 80 days. At 14 days brucellae were isolated from the spleen, liver, kidney, heart blood, mesenteric lymph nodes, salivary gland, and one of the testes; urine was negative. The organs of the squirrel that died at 41 days appeared normal, and no brucellae were isolated from the spleen, liver, blood or kidney. With the exception of a small abscess seen on the spleen of the animals sacrificed at 80 days, the organs appeared normal. *B. suis* type 4 was recovered from the spleen and liver, but not the blood or urine.

#### *Clethrionomys rutilus*

Ten Northern red-backed voles, *Clethrionomys rutilus*, were inoculated with  $3.8 \times 10^6$  cfu intraperitoneally. They were sacrificed at 8 (2 animals), 14 (2 animals), 37 (1 animal), and 80 days (5 animals). Except for abscesses seen on the liver of 1 animal killed on day 14, no other lesions were found until day 80. Those lesions included pinpoint abscesses on the kidney, an abscessed accessory sex gland, enlargement of the spleen (two cases); in one case the spleen was abscessed. There were no lesions common to all animals, and aside from the aforementioned exceptions, the organs looked normal.

The results of the bacteriological examination of the organs is seen in Table 2. Up to 37 days after inoculation, the organism could be isolated from numerous tissues. After that time the number of isolates decreased. In one instance the presence of an abscess in an accessory sex gland was coupled with the isolation of *Brucella suis* from the urine.

#### *Microtus xanthognathus*

Doses of 0.8, 80, and 8000 cfu of *B. suis* type 4 were given to 12 yellow-cheeked voles (4 animals per dose). All survived for 35 days, when they were sacrificed. There were no lesions seen. Livers and spleens were cultured. Livers and spleens were positive in 2 of 4 animals at the 8000 cfu dose level and in 1 of 4 at the 80 cfu level. An additional animal at this level had a positive liver. Serum was obtained from all but one animal at the 8000 cfu level. Four animals developed *Brucella* agglutinins, 2 inoculated with 8000 cfu (1:160 and 1:80) and 2 inoculated with 80 cfu (1:40 and 1:20), with a single animal at both levels positive for both organism and agglutinins.



Table 2. Results of cultures on C. rutilus inoculated with B. suis type 4.

Tissue	Days post-inoculation			
	8	14	37	80
Liver	2/2 <sup>a</sup>	2/2	1/1	1/5
Spleen	2/2	2/2	1/1	0/5
Kidney	2/2	1/2	1/1	0/1
Heart Blood	1/2	1/2		0/1
Urine	0/2			1/1 <sup>b</sup>
Lungs	1/2			
Uterus		1/1		

<sup>a</sup> is the number positive/number cultured

<sup>b</sup> this animal had the abcessed accessory sex gland

### *Lemmus Lemmus*

Fourteen Scandinavian lemmings were inoculated intraperitoneally with doses of *B. suis* type 4 ranging from  $10^1$  to  $10^7$  cfu. Two animals,  $10^7$  and  $10^5$  cfu, were killed at 28 days. No lesions were seen, but brucellae were isolated from the spleen of both animals and from the liver of one. The remaining 12 lemmings were sacrificed 50 days after inoculation. Microabscesses seen at the juncture of the stomach and mesentery of an animal receiving  $10^5$  cfu, and a cyst in the lower abdomen of one receiving  $10^3$  cfu were the only lesions observed in the animals inoculated with  $10^3$  to  $10^7$  cfu. However, of the 3 animals receiving doses of 10 cfu, 1 exhibited liver necrosis and developed an abscess in the inguinal region; another had an abscess on the lung with adhesions causing it to adhere to an abscess on the rib cage; the third animal appeared normal. *B. suis* type 4 was isolated from the spleens of animals inoculated with  $10^3$  to  $10^7$  cfu, 1 of 2 livers ( $10^7$  cfu dose), 1 of 3 livers ( $10^5$  cfu dose), and from the abscess seen in the animals were negative on culture.

### *Lemus sibiricus*

Twenty *Lemus sibiricus* were inoculated intraperitoneally with doses of  $3.6 \times 10^6$  cfu (5),  $3.6 \times 10^5$  cfu (5), 3500 cfu (5), and 35 cfu (5). During the period of the experiment (140 days) the lemmings suffered mortality which could not be attributed to infection by *Brucella suis* type 4. A *Proteus* sp. was isolated from one and a *Pseudomonas* sp. from four others. Three animals were killed by cage mates. A necropsy was performed on each animal, there were no lesions attributable to infection in any except those from whom the *Proteus* and *Pseudomonas* were isolated. *Brucella* was isolated from the liver, spleen, kidney, blood, and embryo of 1 lemming that died at 10 days ( $3.5 \times 10^6$  cfu) and from the heart blood of another that was killed by a cage mate at 52 days (3500 cfu). Seven animals survived to the 140th days. Of these, two had received  $3.5 \times 10^6$  cfu and developed agglutinin titres of 1:320 and 1:160; *B. suis* type 4 was recovered from the liver and spleen of one. Neither of the two survivors at the  $3.5 \times 10^5$  cfu dose developed agglutinins although *Brucella* was isolated from the liver of one. There were no lesions seen, nor agglutinins detected nor isolations made from the remaining survivors (1 at 3500 cfu dose level, and 2 at the 35 cfu dose level).

### DISCUSSION

Rementsova<sup>9</sup> reviewed the literature on rodent brucellosis citing numerous reports on the susceptibility of hares, susliks (*Citellus* sp.) and voles to *B. abortus*, *B. melitensis*, or *B. suis*. Thorpe et al.<sup>10</sup> performed experimental studies with four species of *Brucella* on selected wildlife, laboratory, and domestic animals, and found that species of rats, lagomorphs, and squirrels were more resistant than wild mice exposed to the same *Brucella* sp. None of the studies cited by Rementsova<sup>9</sup> or Thorpe et al.<sup>10</sup> knowingly utilized *B. suis* type 4.

The animals included in this study, with the exception of the guinea pig and Scandinavian lemming, are indigenous to Alaska. Although their ranges vary, they are continuous with those of other rodents studied and those of far ranging mammals on which brucellosis studies have been reported elsewhere (caribou, Neiland et al.<sup>7</sup>; canids, Neiland<sup>6</sup>; wild carnivores, Neiland and Miller<sup>8</sup>; and man, Huntly et al.<sup>4</sup> and Brody et al.<sup>2</sup>).

Subspecies of the varying lemming were found to be the most susceptible. Fatalities occurred when the inoculum was as low as 2 cfu with *D. t. stevensoni* with 20 cfu with *D. t. rubicatus*. Whether the difference was significant cannot be determined from the small number of animals used in each series.

*D. t. stevensoni* also proved susceptible to infection by *B. abortus*, *B. melitensis*, and *B. suis* type 1. We did not determine the least number of cfu necessary for initiating infection, however the apparent high susceptibility and the ease with which these animals may be handled recommend them as a model for further studies in brucellosis.

With the exception of *M. xanthognathus*, with which the highest challenge dose was 8000 cfu, individual animals of the remaining species survived doses of greater than  $10^6$  cfu for the length of that particular experiment (Table 3).

Overall, these Alaskan rodents exhibited a wide range of disease state and outcome which have a bearing upon their possible role in the transmission of *Brucella* in the wild and in providing a reservoir for human infection. There is ample evidence that brucellosis can be caused by ingestion of the organism. Neiland and Miller<sup>8</sup> have demonstrated that carnivores can become infected by ingesting  $10^8 - 10^9$  cfu of *B. suis* type 4. Thorpe et al.<sup>10</sup> found that orally administered doses of two to four logs higher than an intraperitoneal inoculum were necessary to initiate infection. Verger<sup>11</sup> infected 1 of 10, 7 of 10, and 7 of 10 mice fed  $2.15 \times 10$ ,  $2.15 \times 10^2$ , and  $2.15 \times 10^3$  cfu of *B. melitensis*, respectively. Rementsova<sup>9</sup> cites numerous examples of infection in rodents inoculated by the oral route.

Rodents living on ranges traversed by infected caribou, or contaminated with *B. suis* type 4 in any manner, would have opportunity to feed upon aborted fetuses or forage or drink water contaminated by excreta. Once infected the rodents might become part of the transmission cycle; 1) by serving as reservoirs for survival of the microorganism; 2) by contaminating grasses and water through excretion of the microorganism in their urine and feces; 3) by infecting wild carnivores that prey upon them; and 4) by spreading the organism through contact with others of their species. Furthermore, rodents might transmit brucellosis to man directly through contaminating foods or indirectly through sled dogs that have become infected by eating the rodents. However, despite the high probability that rodents might be naturally infected, evidence regarding this possibility is not yet available for free-living Alaskan rodents.

Table 3. Maximum survival times of Alaskan rodents and a lagomorph infected with Brucella suis type 4.

Rodent	Number <sup>1</sup>	Dosage <sup>2</sup>	Time <sup>3</sup>	Positive Isolation <sup>4</sup>
Varying hare	1/1	$7.5 \times 10^6$	57 days	liver 1/1
Ground squirrel	2/2	$7.5 \times 10^6$	80 days	liver, spleen 2/2
Flying squirrel	1/1	$7.5 \times 10^6$	80 days	liver, spleen 1/1
Red-backed vole	5/5	$3.8 \times 10^6$	80 days	liver 1/5
Scandinavian lemming	3/3	$9.6 \times 10^6$	28 days	spleen 2/2, liver 1/2
Brown lemming	2/2	$3.5 \times 10^6$	140 days	liver, spleen 1/2
Yellow-cheeked vole	4/4	$8.0 \times 10^3$	35 days	liver 1/5, urine 1/1
<u>D. t. stevensoni</u>	3/4	$2.0 \times 10^2$	27 days	liver, spleen 3/3
<u>D. t. rubicatus</u>	4/4	$2.0 \times 10$	27 days	liver, spleen 3/3

<sup>1</sup> Number of animals surviving per number allowed to survive.

<sup>2</sup> Dosage expressed as cfu.

<sup>3</sup> Time from injection.

<sup>4</sup> Number positive over number examined.

It is apparent that there are lapses in the data which affect the validity of our tentative conclusions. Further work is necessary to define the infective dose of *B. suis* type 4 needed to initiate infection in all species, particularly via the oral route. There is a need to more closely monitor the period of excretion of the microorganism, the length of time the organism can be recovered from the tissues, and of the eventual outcome of infection in each species of rodent. Evidence confirming the presence of *B. suis* type 4 in the rodent population will need to be obtained. However, the data support the need for continuing studies into the role of the rodent populations in the transmission of brucellosis in wildlife and human population in Alaska and elsewhere.

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