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CARIBOU DISEASE REPORT

by
Kenneth A. Neiland

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JOB PROGRESS REPORT (RESEARCH)

State: Alaska

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SUMMARY

The retained placenta condition occurs with unknown frequency in the Nelchina caribou herd.

The birth of a fawn and the post-parturient activities for three hours of cow and fawn are described. The cow retained placental materials throughout the observation period.

Two, first-draft manuscripts on natural and experimental rangiferine brucellosis in domestic and wild Alaskan carnivores are presented.

BACKGROUND

Placental retention and associated early loss of caribou (*Rangifer tarandus*) calves is well known in the Arctic caribou herd. This condition has also been seen in a few animals on the Porcupine caribou calving ground near Barter Island. While we knew of one occasion several years ago when a field biologist thought he had seen a retained placenta on the Nelchina caribou calving grounds, it has always appeared that the problem probably did not occur in that herd. However, no one had made any close surveys of this herd during calving in recent years. Therefore, considering the great, recent decline in numbers in the herd, it was decided that it was necessary to closely observe the progression and success of calving as part of the attempt to understand the decline. In the following section we report our observations in this regard made from May 23 to May 30, 1973, on the calving segment of the Nelchina caribou herd on Upper Kosina Creek in the Nelchina Basin.

For several years we have been gathering serologic data on the natural occurrence of rangiferine brucellosis, caused by *Brucella suis* type 4, in sled dogs and various wild carnivores associated with the Arctic caribou herd in which the disease is enzootic. We have found serologic evidence of exposure in dogs from nearly every village we have visited on the range of the Arctic herd, but not in dogs from two villages on the range of the Porcupine herd. However, brucellar antibodies were more or less commonly found in grizzly bears (*Ursus arctos*) from both the Arctic caribou range (about 93% prevalence) and Porcupine caribou range (about 35% prevalence). Wolves (*Canis lupus*) and red foxes (*Vulpes fulva*) taken by subsistence hunters of Anaktuvuk Pass also yield occasional serologic titres. Because most other strains of brucellosis are well known as abortifacient agents in a variety of hosts including canids and other carnivores, it was decided that it was necessary to experimentally investigate rangiferine brucellosis infections in canids and ursids. We also decided to experimentally investigate the potential of various indigenous species of rodents to act as alternate hosts of rangiferine brucellosis. In Europe, hares serve as the reservoir for a strain of brucellosis which also infects pigs causing considerable loss of reproduction. "Self-maintaining" brucellar infections in certain indigenous rodents have been reported in Russia.

The results of our serologic surveys of sled dogs and wild carnivores have been prepared for publication and the manuscript is presented in the following section. I have also prepared a jointly-authored manuscript on the experiments with carnivores. It too is presented below. A co-authored manuscript dealing with rodent infections is now being prepared by my colleague in this research.

FINDINGS

We will consider first the results of our Nelchina calving ground survey. The two, currently-available manuscripts dealing with rangiferine brucellosis are presented *in toto* in the second part of this section.

A. Calving Ground Surveys

Because of inadequate funding it was not possible to duplicate our usual survey procedure. Normally, we aerially survey fawning bands throughout a fawning area with a helicopter for three to four hours each of five days during the peak of fawning. We count the number of fawns present in a band and record the number of retained placentas per hundred fawns. Since we could not afford to operate continuously with the helicopter we used it only to fly to and from the fawning area along Kosina Creek. Individual biologists were set out every few miles up and down the creek on vantage points where animals could be closely examined with binoculars or spotting scopes. The counts of fawns, yearlings and other age and sex classifications will be reported by the Caribou Research Leader, Region II, Mr. Gregory Bos, in his progress report for this period.

We only saw one instance of an unquestioned example of placental retention. This occurred in an animal which I observed throughout the actual time of birth and for several hours afterwards. My field notes are presented below with only slight editing. It is also worth noting that we saw two other instances of animals showing fresh-appearing, retained placental materials. In these two cases we do not know how long the placental materials had been retained and we did not attempt to land the helicopter and continue to observe either animal.

A Calf is Born

On May 29, 1973, we were put out on Kosina above the main forks of the creek above the point that Clarence Creek enters Kosina Creek. The weather was mostly clear with occasional snow squalls moving by. About 1:10 p.m. I came up on three cows and a yearling and noticed that one of the cows (unantlered) was beginning to give birth. At that time about 10-12 inches of both front (?) legs of the fetus were protruding. The cow was standing in a declivity surrounded by low, dwarf birch (about 2 ft. high) about 200 yards from my place of concealment. Whenever the cow laid down I could not clearly see all of her body, particularly her posterior end. Every few minutes she would stand up and lie down again changing her position. Frequently, while fully recumbent on her sternum she would throw her head about.

About 1:30 p.m. she began moving her head more vigorously and rolled over on her right side from a normal, sternally recumbant position. I assume the calf was fully expelled at this time. Next, she stood up and appeared to be eating (placenta?). At this time she faced toward me and I could not determine that the fetus had indeed been expelled. When she lay back down it appeared that she was licking her calf.

By about 1:40 p.m. the cow appeared to be fully alert to her surroundings. Every few minutes she would raise her head, look about and move her ears to hear from various directions.

The fawn first stood up about 2:02 p.m. and after a few seconds lay back down. The cow still apparently licking fawn. At about 2:33 p.m. the fawn stood up, walked off a few feet in a "humped-up" posture and then returned to the cow who continued grooming the fawn.

Cow stood up at 3:08 p.m. umbilicus still hanging out. Apparently continuing to chew on fetal membranes. Laid down again 3:12 p.m. Fawn stood up about 3:13 p.m., very wobbly, fell down, up again and then back down about 3:14 p.m. Up again, almost immediately, taking a few steps over to cow, standing very wobbly. Apparently fawn has not yet had a chance to feed. Fawn up again 3:17 p.m., very wobbly, fell down.

Cow stood up 3:18 p.m. and laid down 3:19 p.m. Umbilicus still retained. Cow up again and down, 3:28 p.m. and 3:29, respectively.

Fawn up and wobbling around several feet from mother at 3:37 p.m. and then back and nuzzling muzzle of cow. Fawn up again 3:40 p.m. and nuzzling muzzle of cow. Cow licking tail-end of fawn. Fawn down 3:42 p.m.

Fawn up 4:07 p.m. Cow rises 10 seconds later, apparently hears something. Fawn wobbly, appears to be looking for udder. Cow down 4:09 p.m. Fawn remaining up, nuzzling cow's muzzle and down 4:10 p.m. Fawn back up 4:12 p.m., wobbly. Down 4:13 p.m.

Cow up 4:21 p.m. Umbilicus retained. Cow down 4:22 p.m.

Helicopter arrived 4:35 p.m. Spooked cow and fawn. Umbilicus still retained as cow and fawn move off down the canyon.

In conclusion, I might note that the umbilicus and other *in utero* placental structures were retained for at least three hours after birth of the fawn. It appeared that the cow experienced some degree of difficulty in expelling the fetus. Whether such apparently difficult births more often result in retention of placental materials is hard to say. In any case, more opportunities to observe such births would probably be helpful in understanding the causation and consequences of this abnormal condition.

B. Publications

Two publications in first-draft form on natural and experimental rangiferine brucellosis in Alaskan, domestic and wild carnivores are presented verbatim in the following pages.

PREPARED BY:

Kenneth A. Neiland
Game Biologist III

APPROVED BY:

Director, Division of Game

SUBMITTED BY:

Research Chief, Division of Game

Richard H. Bishop
Regional Research Coordinator

Abstract

Antibodies against rangiferine brucellosis, *Brucella suis* type 4, are commonly found in the serum of various domestic and wild Alaskan carnivores which feed on caribou, *Rangifer tarandus granti*, in arctic Alaska.

Sled dogs from five native villages on the range of the Arctic caribou herd, but not from two villages on the range of the Porcupine caribou herd, are more or less commonly infected.

Wolves (*Canis lupus*) and red foxes (*Vulpes fulva*) are less commonly infected.

The first evidence of the natural infection of a species of bear, the grizzly (*Ursus arctos horribilis*), by *Brucella* is presented. About 90 percent of those bears associated with the Arctic caribou herd and 30 percent of those associated with the Porcupine caribou herd show serologic signs of exposure to *Brucella*, presumably the enzootic strain present in Alaskan caribou.

It is concluded that infection of predators by enzootic strains of *Brucella* present in prey species (e.g. ruminants) is common to many areas of the world. Evidence from the literature and unpublished experimental data suggest that such infections may interfere with reproduction, but additional study is needed to clearly resolve this question.

Introduction

Several Alaskan herds of caribou, *Rangifer tarandus granti*, are more or less commonly infected by *Brucella suis* type 4 for which caribou and reindeer evidently serve as reservoir hosts.[4] Serologic evidence from sled dogs and wolves (*Canis lupus*) and isolation of the organism from the former demonstrated that *B. suis* type 4 also infects naturally exposed carnivorous species.[3] It was suggested that rangiferine brucellosis might also occur in other carnivores (e.g. bears) which fed on infected caribou. The present communication announces the first evidence of the natural infection of an ursid species, the barren-ground grizzly (*Ursus arctos horribilis*), by a member of the genus *Brucella*. We also wish to report the evidence of the rangiferine strain of *Brucella* in red foxes (*Vulpes fulva*). Additional data on the maintenance and distribution of serologic titres in sled dogs are also presented.

Materials and Methods

The procedures used to determine tube agglutination and complement fixation titres are those described by Alton and Jones[1] as employed in the laboratory of Dr. David T. Berman, Department of Veterinary Science, University of Wisconsin, Madison. All serology, unless otherwise noted, was performed under the supervision of Dr. Berman by his technical staff under a contract between the Alaska Department of Fish and Game and the University of Wisconsin.

Serum samples from grizzly bears were obtained from animals tranquilized with Sernalyn administered with a Palmer Cap-Chur gun from a helicopter. Sera from wolves and red foxes were harvested from whole blood samples collected by Nunamiut subsistence hunters of Anaktuvuk Pass, Brooks Mountain Range, Alaska. Blood samples were taken from sled dogs under the restraint of their masters. In each case serum samples were withdrawn from whole blood samples which had been allowed to settle overnight. Sera were preserved by freezing.

Results

Sled Dogs

Data on the prevalence of serologic titres in sled dogs from a number of native villages in arctic Alaska are shown in Table 1. The location of these villages in respect to the Arctic and Porcupine caribou herds is shown in Fig. 1. With the exception of Gambell on St. Lawrence Island in the Bering Sea, Ft. Yukon on the Yukon River, and Arctic Village and Old Crow within the range of the Porcupine herd, the remainder of the villages are all within the normal range of the Arctic caribou herd. It is noteworthy that only villages associated with the Arctic caribou herd had dogs with *Brucella* titres.

The titres of the team mates of the sled dog from Kobuk from which *Brucella suis* type 4 was originally isolated[3] were followed for a year. These data are shown separately in Table 2.

Wolves and Red Foxes

The results of serologic testing of some wild canids are presented in Table 3. Again we note that all reactors are associates of the Arctic caribou herd.

Grizzly Bears

Serologic data on grizzly bears are given in Table 4.

Discussion

Sled Dogs

The data presented above make it clear that wherever sled dogs are fed any appreciable amount of caribou from a herd infected by *Brucella suis* type 4 (e.g. Arctic caribou herd), they will commonly become infected and develop significant serologic titres. Perceptible titres will be maintained for at least 10 months in some cases. These limited observations conform, as far as they go, with those on maintenance of titres in infections of *B. suis* type 5 reported by others[14] in which perceptible titres may be present as much as 30 months post-infection.

The lack of reactors amongst the 49 sled dogs of Arctic Village and Old Crow is unexpected. These animals are fed in part on caribou from the Porcupine herd (see Fig. 1) which inhabits northeastern Alaska and the northern Yukon Territory. While we have no serologic data on the

Table 1. Occurrence of brucellosis reactors in Alaskan sled dogs.[1]

Number	Name	Locality	Date	Antibody Titres[2] AGGL	CF
17	German	Anaktuvuk Pass	6/67	2+, 1:640	---
18	Netah	" "	6/67	2+, 1:160	---
21 other dogs		" "	6/67	neg.	---
	Red	" "	5/71	2+, 1:320	4+, 1:160
	Trika	" "	5/71	3+, 1:40	neg.
19 other dogs		" "	5/71	neg.	neg.
10 dogs		Ambler	5/70	neg.	neg.
	Smokie	Kobuk	5/70	4+, 1:640	4+, 1:160
7 team members		"	5/70	neg.	neg.
19 dogs		Ft. Yukon	6/70	neg.	neg.
6879	Darkie	Ft. Yukon	8/70	neg.	neg.
2884		"	8/70	neg.	neg.
1970-1		Gambell	9/70	neg.	neg.
27 other dogs		"	9/70	neg.	neg.
1970-54		Pt. Hope	9/70	2+, 1:160	2+, 1:40
26 other dogs		"	9/70	neg.	neg.
1970-67		Wainwright	9/70	neg.	1+, 1:40
1970-70		"	9/70	neg.	4+, 1:40
1970-75		"	9/70	2+, 1:640	4+, 1:320
1970-78		"	9/70	2+, 1:40	4+, 1:40
23 other dogs		"	9/70	neg.	neg.
3004	Captain	Barrow	10/70	4+, 1:320	neg.
4 other dogs		"	10/70	neg.	neg.
30 dogs		Arctic Village	9/72	neg.	neg.
19 dogs		Old Crow	8/72	neg.	neg.

[1] Additional reactors from Kobuk are shown in Table 2.
[2] AGGL - standard tube agglutination test; CF - complement fixation text; *Brucella abortus* smooth antigen.

Table 2. Serologic observations[1] on a dog team infected with rangiferine brucellosis in Kobuk, Alaska.

Name of Dog	May 1969 Aggl. Test	May 1969 CF Test	July 1969 Aggl. Test	July 1969 CF Test	July 1969 USDA Card Test	Sept. 1969 Aggl. Test	Sept. 1969 CF Test	Dec. 1969 Aggl. Test	Dec. 1969 CF Test	May 1970 Aggl. Test	May 1970 CF Test
Beaver			Neg.	Neg.	Neg.	Neg.	1:20	Neg.	Neg.		
Fannie	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Harry			Neg.	1:20	Neg.	1:320	1:640	Neg.	?	Neg.	2+, 1:10
Jumbo			1:640	1:640	+	1:20	1:20	1:160	1:160		
King	Neg.	Neg.	1:160	1:10	+	Neg.	Neg.			Neg.	Neg.
Lucy[2]	1:2560	1:640	1:640	1:1280	+						
Mila	Neg.	Neg.	Neg.	1:10	Neg.	Neg.	Neg.				
Moose			1:640	1:1280	+	1:80	1:10	1:160	1:40	1:80	1:10
Nellie			Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Wendy	Neg.	Neg.	Neg.	Neg.	Neg.	1:160	1:80	Neg.	Neg.	Neg.	Neg.

[1] All procedures employed *Brucella abortus* smooth antigen.
[2] *Brucella suis* type 4 isolated at sacrifice, July 1969 (Neiland 1970).

occurrence of *Brucella* reactors in this herd, it comes in contact and may intermix with the Arctic herd ranging throughout northwestern and northcentral Alaska in which reactors do occur. Furthermore, grizzly bears of northeastern Alaska (i.e. the eastern Brooks Range), that also feed on animals from the Porcupine herd, do show a reactor rate of about 30 percent (see Table 4). Thus, one must conclude that the lack of reactors among sled dogs associated with the Porcupine herd may be simply a matter of sampling error. It is possible that the prevalence of infected animals in the Porcupine herd was (is?) lower than in the Arctic herd. It should also be noted that free-ranging grizzlies no doubt eat more caribou than do sled dogs in Arctic Village and Old Crow where dogs are fed substantial amounts of commercial products or fish. Thus, it seems quite possible that the relative exposure rates of dogs in Arctic Village and Old Crow are so low, that larger numbers of animals would have to be tested in order to be reasonably sure of detecting reactors.

The data on persistence of titres in a naturally exposed team of sled dogs (Table 2) suggest that many of the titres reported in Table 1 may have resulted from exposures as much as a year or so in the past. Thus, while sled dogs may serve as convenient "*Brucella*-sentinels" for caribou herds with which they are associated, they cannot be assumed to necessarily represent current levels of infection in such herds.

Published information on the relationship between antibody levels and the course of rangiferine brucellosis infections in dogs suggests that agglutination and complement fixation titres in excess of about 1:300 (perhaps less?) are certainly indicative of active infection.[3] The data in Table 2 show that titres may be intermittent; rising, falling and again rising over a period of time. Further information on the course of antibody levels in dogs will be reported in a paper dealing with experimentally infected dogs, wolves and bears presented elsewhere.[5]

Rangiferine brucellosis has also been observed in working dogs in the Taimyr and Chukot regions of Siberia.[7] Working dogs also function as "*Brucella*-sentinels" where they are in contact with other host-parasite combinations, e.g. sheep-*B. melitensis*.[2]

Wolves and Red Foxes

We have earlier reported the natural occurrence of rangiferine brucellosis agglutination titres of 1:20 to 1:160 in three of seven wolves from the Brooks Mountain Range of northern Alaska.[3] Additional data on the prevalence of titres in wolves of this area are shown in Table 3. Lumping these data with those reported earlier produces a prevalence rate of about 45 percent (10/22). While the comparatively small number of samples restricts us from making broad statements about the exposure of wolves throughout arctic Alaska, it seems clear enough that the wolf's favorite item of diet in northern Alaska may not be an entirely unmixed blessing. Although we do not have any field data which are suggestive of the possible effects of rangiferine brucellosis on wolves, information on experimental infections presented elsewhere[5] does suggest the possibility of reproductive failure.

Table 3. Brucellosis reactors in some wild Alaskan canids.

Species	Specimen Number	Locality	Titre (*B. abortus* smooth antigen) Complement Fixation		Agglutination	
Wolf	7354	Anaktuvuk Pass	neg.		neg.	
	7355	" "	neg.		neg.	
	7388	" "	1:40		1:20	
	7389	" "	neg.		1:20	
	7390	" "	neg.		neg.	
	7391	" "	neg.		neg.	
	7392	" "	1:160		1:160	
	3160	" "	4+, 1:20		neg.	
	3161	" "	4+, 1:320		4+, 1:160	
	A50,658	" "	2+, 1:80		neg.	
	A50,660	" "	3+, 1:160		inc., 1:320	
	A50,663	" "	neg.		neg.	
	A50,665	" "	4+, 1:640		3+, 1:640	
	A50,666	" "	3+, 1:10	Reactor	neg.	Reactor
	A50,667	" "	neg.	Prevalence	neg.	Prevalence
(pup)	3864	" "	neg.	(11/28)	neg.	(7/27)
(pup)	3865	" "	neg.		neg.	
(pup)	3866	" "	neg.		neg.	
	3867	" "	4+, 1:320		4+, 1:40	
	3930	" "	neg.		neg.	
	3931	" "	4+, 1:40		neg.	
	3932	" "	4+, 1:40		neg.	
	3933	" "	neg.		neg.	
	3572	Sheenjek River	neg.		-	
	3573	Tanana Flats	neg.		neg.	
	3574	Tanana Flats	neg.		neg.	
	3814	Anaktuvuk Pass	neg.		neg.	
	3815	" "	neg.		neg.	
Red Fox	3164	Seward Peninsula	neg.		neg.	
	3165	" "	neg.		neg.	
	3166	" "	neg.		neg.	
	3167	" "	neg.		neg.	
	3108	Anaktuvuk Pass	2+, 1:20	Reactor	neg.	Reactor
	3128	" "	neg.	Prevalence	neg.	Prevalence
	A50,659	" "	neg.	(2/11)	neg.	(1/11)
	A50,664	" "	neg.		neg.	
	A50,668	" "	neg.		neg.	
	A50,669	" "	4+, 1:640		inc., 1:320	
	3816	" "	neg.		neg.	

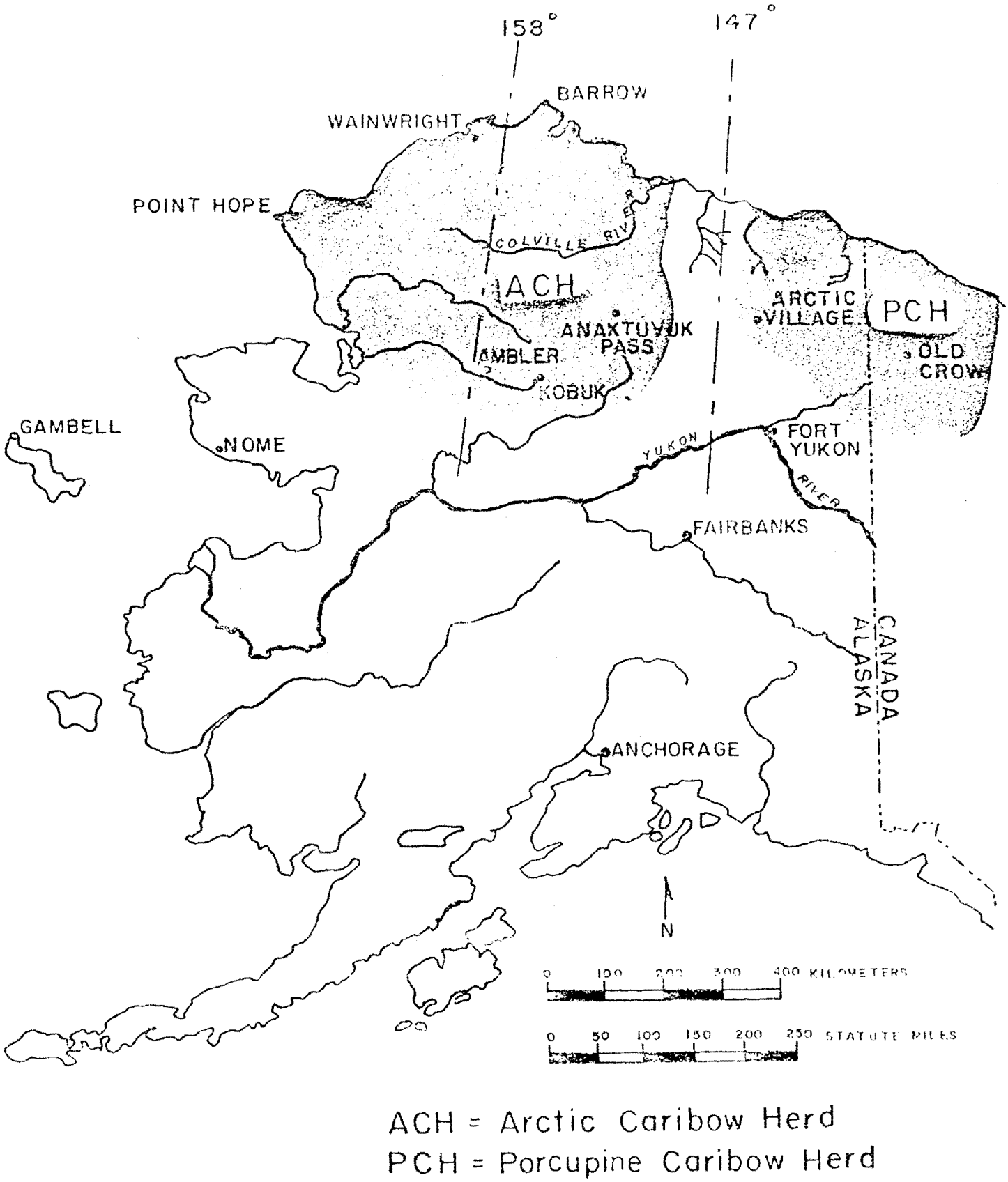

Fig. 1. The ranges of the Arctic and Porcupine caribou herds and associated native villages.

The red fox also is susceptible to infection by *B. suis* type 4 under natural conditions (Table 3). It seems probable that such infections occur less often in foxes which no doubt, except for aborted or stillborn fetuses, rarely get more than the picked-over leavings of caribou killed by wolves or bears.

Infection of wolves and red and arctic (*Alopex lagopus*) foxes with *B. suis* type 4 also has recently been demonstrated on Siberian reindeer ranges.[6,8,9] Twelve of 110 wolves[8] yielded *Brucella* cultures while even fewer red foxes, 3 of 136, were serologic reactors.[9]

Epizootiologically equivalent observations have been made in Africa on the transmission of *Brucellae* from various herbivorous reservoirs which serve as the prey of predators and/or scavengers.[11,12] In these instances hyenas, jackals and wild dogs have been found to carry antibodies against enzootic strains of *Brucella* which occur in antelopes.

Natural infections in indigenous species of wild foxes have been reported in Argentina where brucellosis is a common disease in range cattle.[13] Rementsova[10] has summarized the information available through 1962 on the occurrence of various strains of *Brucella* in wolves, foxes and other wildlife. A number of instances are noted in which commercially-reared or wild foxes infected by various strains of *Brucella* have aborted or produced stillborn kits.

Grizzly Bears

We believe the data presented in Table 4 are the first to be reported which demonstrate the presence of *Brucella* antibodies in the blood of a species of bear. Considering the high proportion of reactors we observed, it appears that grizzly bears associated with both the Arctic and Porcupine caribou herds are subject to frequent exposure to infected animals.[5] Experimental data presented elsewhere[5] suggest that grizzlies are readily susceptible to *B. suis* type 4 via contaminated food and produce high antibody levels.

The apparently lower prevalence of rangiferine brucellosis antibodies in bears associated with the Porcupine caribou herd suggests a lower prevalence of infected animals in that herd. As already noted, this hypothesis is further supported by limited serologic data on sled dogs from the Porcupine caribou range (Arctic Village and Old Crow) presented in Table 1.

Current research on the life history of grizzly bears in the Brooks Mountain Range suggests that their reproductive success is comparable to that of grizzlies elsewhere (pers. comm. Harry Reynolds, ADF&G). Whether or not rangiferine brucellosis may adversely affect bears, particularly their reproduction, is unknown. Unfortunately it was not possible in a series of experiments reported elsewhere[5] to investigate this possibility.

Table 4. Brucellosis reactors in some grizzly bears from the Alaskan Arctic.

Date	Sex	Specimen Number	Locality	Titre (*B. abortus* antigen) Complement Fixation	Tube Agglutination
1971	M	3000	Western Brooks Range[1]	4+, 1:80	4+, 1:40
	M	3001	" " "	4+, 1:40	2+, 1:40
	M	3002	" " "	4+, 1:20	4+, 1:20
	M	3004	" " "	4+, 1:20	2+, 1:20
	M	3005	" " "	4+, 1:20	neg.
	F	3006	" " "	--	4+, 1:160
	M	3007	" " "	4+, 1:160	3+, 1:80
	F	3008	" " "	4+, 1:20	2+, 1:80
	F	3009	" " "	4+, 1:320	3+, 1:80
	F	3010	" " "	3+, 1:40	3+, 1:20
	M	3011	" " "	3+, 1:40	3+, 1:40
	F	3012	" " "	2+, 1:160	3+, 1:80
	F	3013	" " "	4+, 1:80	3+, 1:20
	F	3014	" " "	4+, 1:40	neg.
	F	3015	" " "	4+, 1:80	4+, 1:80
	M	3016	" " "	neg.	neg.
	F	3017	" " "	4+, 1:40	2+, 1:40
Antibody Prevalence				15/16 (94%)	14/17 (82%)
	F	3913	Eastern Brooks Range[2]	neg.	2+, 1:40
	M	3916	" " "	>1:640	>4+, 1:40
	F	3917	" " "	1:20	neg.
	F	3918	" " "	2+, 1:20	4+, 1:40
	M	3920	" " "	neg.	3+, 1:40
	F	3924	" " "	>1:640	>4+, 1:40
	M	3927	" " "	4+, 1:40	3+, 1:20
	M	3956	" " "	3+, 1:20[3]	2+, 1:10[3]
	F	3957	" " "	neg.[3]	2+, 1:10[3]
	F	3960	" " "	neg.[3]	4+, 1:10[3]
	11 specimens		Eastern Brooks Range	neg.	neg.
Antibody Prevalence				6/21 (29%)	9/21 (43%)

[1] Arctic Alaska between longitudes 147° and 158°
[2] Arctic Alaska east of longitude 147°
[3] These titres were determined by Dr. B. L. Deyoe, National Animal Disease Laboratory, U.S.D.A., Ames, Iowa.

General Conclusions

On the basis of our own limited data on natural infections, experimental data presented elsewhere[5] and a considerable array of information presented by others, we conclude that strains of *Brucella* enzootic in various wild, reservoir-host species (principally ruminants) are regularly transmitted to the predators which prey on them. It also seems likely that whenever infection takes place during the proper stage of pregnancy, reproductive failures may occur. However, much additional field work and experimentation are required to fully evaluate the overall effects of brucellosis on predator populations.

Acknowledgments

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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 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.[10] It also occurs in sled dogs, wolves (*Canis lupus*), red foxes (*Vulpes fulva*)[8,9] and grizzly bears (*Ursus arctos horribilis*) which feed on caribou.[8,9] The disease has been reported in Arctic foxes (*Alopex lagopus*) and wolverines (*Gulo gulo*) on Siberian reindeer ranges,[12] 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[9] will eventually become infected and develop detectable serum antibodies. Whether or not these infections are transmissable 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[14] and of beagle dogs in commercial kennels[2] 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 affects of rangiferine 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.[11]

Unfortunately, although the preliminary results reported below were of considerable interest and cojency, untimely termination of our experiments was required when the experimental facility 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.[8] 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.[1] Stock suspensions were adjusted to approximately 2.0×10^9 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.[1]

Typical colonies from each suspected tissue-isolate were typed using *Brucella abortus* antiserum (Difco) in a rapid slide agglutination procedure.[1] 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[1] using commercial *Brucella abortus* smooth antigen (Difco). Complement fixation titres were determined in the laboratory of Dr. David T. Berman, Department of Veterinary Science,[1] University of Wisconsin, using methods described elsewhere.

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 Mtn. Range and had been successfully bred in captivity several times. The black bear (*Ursus americanus*) 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 female and 1 male) each about one year old. They were individually exposed to about 1.3×10^8 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 thirty 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.

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

Tissue	Occurrence of brucella Dog Number and Sex 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)[1]	+(L)[2]
" " , maxillary	+(L)	-----	-----
Lymph Node, mandibular	4+(R,L)	-----	4+(R,L)
" " , parotid	-----	3+	-----
" " , sub-mandibular	-----	-----	4+(R)
" " , medial retropharyngeal	4+(L)	4+(R)	2+(L)
" " , superficial cervical	-----	3+(R)	4+(L)
" " , axillary	4+(R)	3+(L)	4+(L)
" " , mesenteric	4+	4+	4+
" " , external iliac	4+	-----	4+(R,L)
" " , submammary	3+(R,L)	-----	4+(R,L)
" " , popliteal	4+	4+(R)	4+(R)
Tonsil	-----	+(L)	+(R)
Blood, sediments	-	-	-
Blood, clot	+	+	+

[1] Right side (R)
[2] 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[1] 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	+	+	+

[1] A complete reaction at a given dilution is given as 4+. Incomplete reactions are recorded as 2+ or 3+.

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 1.5×10^8 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 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 Mtn. Range in June, 1967.[5] 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 2.1×10^8 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 2.1×10^8 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.

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

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

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

	Serologic Titre[1] Dog Number and Sex					
	2993(M)		2994(F)		2995(F)	
Date	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)[1] and her pup (#3225).

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

[1] Inoculated intraperitoneally

Table 6. Distribution of *Brucella suis* type 4 in an experimentally infected wolf (#3215)[1] 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	contamin.						
Urine	+						
Uterine Horn, right	4+						
Uterine Horn, left	4+						
Salivary Gland, parotid	-						
" " , mandibular	4+						
Lymph Node, medial retropharyngeal	4+						
" " , superficial cervical	4+						
" " , axillary	4+						
" " , mediastinal	2+						
" " , mesenteric	4+						
" " , external iliac	4+						
" " , submammary	4+						
" " , popliteal	2+						

[1] 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

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.

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 1.3×10^9 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[13] of rabies enzootic in Alaskan foxes to which they both sucumbed. After exposure to rabies we did not again handle the animals.

Discussion

Beagle Dogs

Morse[6] in 1951 and Rementsova[14] 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[2] have summarized information on the form of canine brucellosis specifically caused by *Brucella suis* type 5, a distinct strain which was discovered

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+
" " , medial retropharyngeal	3+
" " , parotid, left	2+
" " , superficial cervical	3+
" " , axillary, right	3+
" " , axillary, left	2+
" " , mediastinal	3+
" " , mesenteric	3+
" " , external iliac	3+
" " , 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

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.[8,9]

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*[6,7], *B. melitensis*[6,14], and a non-rangiferine strain of *B. suis*[2] (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*.[6,11,14] While most often these infections have apparently resulted from association with aborted material[14], they may also occur via unexpected pathways. For example, Rementsova[14] 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 the other species of *Brucella*.[15] If this is the case, the killing and/or eating of new-born 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.[2] Whether rangiferine brucellosis, which naturally infects wolves on Alaskan[8,9] and Siberian[12] 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.[3,4] 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.[9] 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 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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