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Source: Journal of Wildlife Diseases, 17(2) : 183-189

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-17.2.183

EXPERIMENTAL *Brucella suis* TYPE 4 INFECTIONS IN DOMESTIC AND WILD ALASKAN CARNIVORES

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Abstract: Beagle dogs were readily infected by 1.3×10^8 colony forming units (cfu) of *Brucella suis* type 4 administered either on canned dog food, or intraperitoneally. Such infections were afebrile and otherwise asymptomatic and without any obvious gross lesions.

Inoculation of 10^8 cfu *B. suis* type 4 intraperitoneally into two gravid wolves *(Canis lupus)* resulted in infections in both animals. About 24 days later they gave birth, apparently at full-term, to two (both alive) and six (two alive and four dead) pups, respectively. Pups born alive died within 24 hours.

A black bear *(Ursus americanus)* infected with between 10^8 and 10^9 cfu yielded serologic and bacteriologic data similar to that derived from the observations on beagles and wolves. Two grizzly bears *(Ursus arctos horribilis)* were both infected by exposure to 1.3×10^9 cfu *B. suis* type 4 placed on canned dog food. Antibody titres reached very high levels within the first two months of infection.

INTRODUCTION

Brucellosis caused by *Brucella suis* type 4 is common in some Alaskan caribou *(Rangifer tarandus)* herds[9] and occurs in sled dogs, wolves, red foxes *(Vulpes fulva)*, and grizzly bears which feed on caribou.[7,8] The disease has been reported in arctic foxes *(Alopex lagopus)* and wolverines *(Gulo gulo)* on Siberian reindeer ranges.[11] Many predators and scavengers feeding on prey species in which *Brucella* is enzootic may eventually become infected and develop detectable serum antibodies.[8] The method of transmission of *Brucella* between free-ranging predators and effects of such infections under natural circumstances are unknown. However, reproductive failure of foxes on fur farms[12] and of beagle dogs in commercial kennels[2] as a consequence of *Brucella* spp. infection is known to occur.

Accordingly, it seemed desirable to experimentally infect gravid wolves and grizzly and black bears with *Brucella suis* type 4. We were particularly interested in examining the serologic and reproductive responses to infection in the bears and wolves, respectively. We also wondered whether dogs infected by *B. suis* 4 might be a source of infection for their owners via secretions or excretions. Weber[13] has reported *B. canis* in nasal and ocular secretions, saliva, feces, urine and vaginal mucus of experimentally infected beagles.

MATERIALS AND METHODS

Bacteriology. A strain of *Brucella* isolated from a sled dog from Kobuk, Alaska, in July, 1968[8] and identified by Drs. D.T. Berman and L.M. Jones, Department of Veterinary Science, University of Wisconsin as *B. suis* type 4 was used in our experiments. The organism was grown from lyophilized subcultures on *Brucella* agar [1] at 37 C for 72 h.

[1] Baltimore Biological Laboratories, Inc., cat. #11086, Baltimore, Maryland, USA.

Experimental inocula were prepared by suspending cells in peptone saline.[1] Stock suspensions were adjusted to approximately 2.0×10^9 cfu/ml using MacFarlane turbimetric comparison standards. 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 h.

Tissues for bacteriologic assay were dipped in 95% ethanol and flamed before being aseptically lacerated and streaked on *Brucella* agar plates. Venous or heart blood (2-5 ml) and urine and feces were cultured using the methods described by Alton and Jones.[1]

Typical colonies isolated from each tissue were identified with *Brucella abortus* antiserum [2] using the rapid slide agglutination procedure.[1] An estimate of cfu on agar plates from various tissues 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 serum from experimental animals were determined using commercial *Brucella abortus* smooth antigen.[2] Complement fixation titres were determined by Dr. D.T. Berman, Department of Veterinary Science, University of Wisconsin, using methods described elsewhere.[1]

Experimental Infections. Two experiments were done with beagle dogs obtained from the experimental colony maintained at the Arctic Health Research Center.

The first experiment involved 2 female and 1 male beagle, each about 1 year old. They were individually exposed to about 1.3×10^8 cfu *B. suis* type 4 placed on their daily ration of canned dog food on 5 December 1972. Blood samples were taken from the heart prior to exposure and on postinoculation (PI) days 14 and 28. They were euthanized 30 days PI and a variety of tissues cultured for *Brucella*.

In the second experiment, 2 females and 1 male beagle pup were used. They were all bled on 14 March 1973 and the male was infected on 20 March with about 1.5×10^8 cfu inoculated intraperitoneally. The 3 animals, 1 infected and 2 uninoculated controls, were then kept as cage-mates for 78 days when the experiment was terminated. They were bled for serologic testing three times during the course of the experiment.

Two pregnant wolves (#3214 and #3215) which had been bred in the second week of March were intraperitoneally inoculated with 2.1×10^8 cfu *B. suis* type 4 and euthanized 24 days later. Both had been held in captivity at the Naval Arctic Research Laboratory, Barrow, Alaska since they were captured as pups in the Brooks Mountain Range[4] and 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 indoor cages throughout the experiment.

In another experiment a yearling female black bear was infected intraperitoneally with between 10^8 and 10^9 cfu *B. suis* 4. She was euthanized and examined at necropsy 78 days later.

Two grizzly bear litter-mates (#2936 and #2937) approximately 10 months old were utilized in the final experiment. The cubs were individually caged and both were infected by placing approximately 1.3×10^9 cfu on their daily rations of canned dog food on 6 December. It was noted that neither bear ate all of its food on this occasion. On 8 and 9 December cub #2936 vomited. We did not make preinfection blood collections for serology or hemoculture. The study was terminated on PI day 83 and no necropsy was performed.

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[2] Difco Laboratories, Inc., Detroit, Michigan, USA.

RESULTS

Beagle Dogs. Daily temperature measurements gave no indication of any febrile responses. Attempts to isolate brucellae from urine and feces were unsuccessful, and the animals appeared normal throughout the experiment.

The serologic data and hemoculture results are shown in Table 1. *Brucella* was isolated from the following tissues from one or more of the three individuals (number isolates/number attempts) as follows: blood clot (3/3), liver (3/3), spleen (3/3), kidney (1/3), lung (1/2), salivary glands (1/3), tonsil (2/2), and mandibular (2/3), parotid (1/1), submandibular (1/1), medial retropharyngeal (3/3), superficial cervical (2/2), axillary (3/3), mesenteric (3/3), external iliac (2/2), popliteal (3/3) lymph nodes. *Brucella* was not isolated from the following (number of attempts in parentheses): uterus (2), urine sediments (3), blood (3).

The male from the second experiment developed the following post-exposure titres (days PI shown in parentheses): agglutination, 4+ 1:320(16), 4+ 1:160(37) and 4+ 1:80(78); and complement fixation, 4+ 1:80(16), 4+ 1:640(37), and 4+ 1:640(78). Both female cage-mates remained serologically negative throughout the experiment. *Brucella* was isolated from the spleen and mandibular, retropharyngeal and mesenteric lymph nodes, but not from the liver, kidney, urine, testis, parotid lymph node, or blood of the experimental male. *Brucella* was not recovered from similar tissues from the two females.

Wolves. Wolf #3214 gave birth 23 days PI to a pup that died a few hours afterward. At necropsy the only gross lesions present in this pup were several broken ribs and hemorrhages along the left side of the rib cage. The next day a second pup was born and discovered partially eaten a few hours later.

The agglutination titre of wolf #3214 was 1:20 at the time of exposure and 4+ 1:160 and 4+ 1:5280 on PI days 17 and 24, respectively. At necropsy there was splenomegaly and extensive fibrous tissue of a presumed inflammatory origin over the ventral half of the capsule. In addition, both uterine horns contained much caseous material from which a variety of contaminants, but no *Brucella*, were isolated. All other organs appeared normal. *Brucella* was isolated from the following tissues: liver, spleen, mammary gland, and mandibular, medial retropharyngeal, superficial cervical, axillary, mediastinal, mesenteric, external iliac and popliteal lymph nodes, but not from whole blood, lung, urine, or parotid and mandibular salivary glands. *Brucella* was isolated from the liver and spleen, but not whole blood, of the uneaten pup.

Wolf #3215 gave birth, 24 days PI to six pups (#3230-3235). Four were apparently

TABLE 1. Serologic titres and hemoculture results from 3 beagle dogs experimentally infected with *Brucella suis* type 4.

Days Post-Inoculation	Procedure	Dog Number and Sex		
		2938(F)	2939(M)	2940(F)
14	Tube agglutination titer[1]	4+, 1:160	4+, 1:640	4+, 1:160
	Complement fixation[1] titer	2+, 1:20	4+, 1:40	2+, 1:20
	Hemoculture	+	+	+
28	Tube agglutination titer	4+, 1:640	4+, 1:1280	4+, 1:640
	Complement fixation titer	4+, 1:160	3+, 1:320	4+, 1:80
	Hemoculture	+	+	+

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

dead at birth and the two others died within 24 h. Each of the four dead at birth showed signs of trauma, i.e. broken ribs and related hemorrhage. At necropsy the dam showed no signs of splenomegaly and both uterine horns contained caseous material. Otherwise, all other organs appeared normal.

Distribution of brucellae in the tissues of #3215 and her pups at necropsy are shown in Table 2. Tube agglutination titres of this animal on the day of infection and PI days 17 and 24 are as follows: 1+ 1:20, 4+ 1:160, and 4+ 1:1280, respectively.

Black Bear. Agglutination titers on PI days 36 and 78 were 1:160 and 1:800, respectively; hemocultures were negative. At necropsy the only gross lesion noted was apparent enlargement of both right and left axillary lymph nodes. *Brucella* was isolated from the spleen, urine and mandibular, medial retropharyngeal, parotid, superficial cervical, axillary nodes, mediastinal, mesenteric, external iliac and popliteal lymph nodes.

Grizzly Bears. Tube agglutination titres and hemoculture results on various days PI were as follows: *#2936,* 4+ 1:2560(24PI), positive hemoculture (34PI), 4+ 1:10240(57PI), 4+ 1:5120 and negative hemoculture (83PI); *#2937,* 4+ 1:2560 (24PI), negative hemoculture (34PI), 4+ 1:5120 (57PI), 4+ 1:1280, and negative hemoculture (83PI).

DISCUSSION

Beagle Dogs. The experiments showed that: 1) beagle dogs became infected with *B. suis* type 4 when exposed via contaminated food or intraperitoneal inoculation; 2) in such infections, *Brucella* are distributed throughout the lymphatic system; 3) *Brucella* may be present in both the kidney and salivary glands (and possibly in urine and saliva)

TABLE 2. Distribution of *Brucella suis* type 4 at 24 days post-inoculation in an experimentaliy infected wolf (#3215)[1] and her six pups (#3230-3235).

	Culture Results						
Tissue	#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+						

[1]Inoculated into the conjunctival sac.

although perhaps not with sufficient regularity or intensity to commonly act as a source of infection; 4) no clinical signs of brucellosis were observed; and 5) serologic responses of beagle dogs to *B. suis* type 4 are similar to those seen in other strains of brucellosis in canids.[2,5]

It appears that *Brucella suis* 4 could be transmitted from infected dogs to humans under favorable circumstances via contamination by urine or saliva. Human cases of the disease caused by *B. abortus, B. suis,* or *B. melitensis* contracted from canines are considered in reviews by Morse,[5] Rementsova,[12] and Nicoletti *et. al.*[10] And human cases of *B. canis* have been associated with infections in dogs. No doubt most cases of infection by *B. suis* 4 in arctic native peoples are the result of their taste for raw caribou or reindeer marrow, etc. or barehanded butchering and handling of freshly-killed infected animals. Nevertheless, programs to prevent this disease in rural people who subsist on caribou or reindeer must take into account the remote possibility of infection via *Brucella* infected dogs.

Wolves. Experimental infections of wolves with *B. suis* type 4 present much the same general picture as seen in beagle dogs: 1) the wolf is susceptible to *B. suis* type 4 infection: 2) *Brucella* are widely distributed at 24 days PI; 3) infection of the uterus and developing fetuses readily takes place and may lead to reproductive failure; 4) organisms may be shed in urine, saliva, and milk; and 5) serologic responses by wolves to *B. suis* type 4 are similar to those of other host species.

The suggested reproductive failure in wolves due to *B. suis* type 4 infection should be qualified. While it is clear enough that none of the pups survived what otherwise might be considered normal births, we cannot unequivocally rule out the possibility that they were killed by their mothers for behavioral reasons unrelated to *Brucella* infections. If the pups had been allowed to live, they might have grown into normal adult animals.

Eating of aborted fetuses and placental materials is common in Beagle dogs infected with *Brucella canis*[2] and has been studied experimentally in hosts infected with other strains of Brucella.[6] Whether *B. suis* type 4 which naturally infects wolves on Alaskan[7,8] and Siberian[11] reindeer ranges, is a significant cause of reproductive failure in wolves is unresolved and it appears unwise to dismiss this possibility in advance of obtaining further experimental and/or natural evidence.

Grizzly Bears. Information on naturally-occurring, infectious diseases of bears is scarce,[3,14] but it appears that infection of grizzly bears by *B. suis* type 4 is a commonplace event on some caribou ranges in northern Alaska.[8] The relatively high prevalence rates (up to 90%) of *Brucella*-antibodies in free-ranging grizzlies may be explained by the susceptibility of bears to infection via contaminated food reported here. 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. 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 presented above, rangiferine brucellosis in bears is com-

parable, 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 susceptible to infection by *B. suis* type 4 via natural means of transmission and invasion of *Brucella* through oral mucous membranes.

During acute stages of infection *B. suis* type 4 tends to congregate in these species in high numbers in lymph nodes distributed throughout the body.

Brucella suis type 4 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.

Acknowledgements

We wish to thank Dr. R.L. Rausch, who was the Leader, Zoonotic Disease Section of the now defunct Arctic Health Research Center, for his help in maintaining and manipulating experimental animals. His discussion of our work with us was always helpful.

We must also thank personnel of the Naval Arctic Research Laboratory and of the Alaska Department of Fish and Game who helped obtain the wolves and bears, respectively, with which we worked.

Dr. B.L. Deyoe, National Animal Disease Laboratory, Ames, Iowa, very kindly took us into his laboratory where over a period of several days we were exposed to some of the common techniques used in *Brucella* research. He also gave this manuscript a critical reading.

This work was supported in part by Federal Aid in Wildlife Restoration Funds, Project W-17-6.

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Received for publication 17 August 1979