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EXPERIMENTAL INFECTIONS BY *Brucella suis* type 4 IN ALASKAN RODENTS

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Abstract: The susceptibility of nine species of rodents and one species of lagomorph to *Brucella suis* type 4 was studied experimentally. The rodent species included: guinea pig *(Cavia porcellus),* Scandinavian lemming *(Lemmus lemmus),* brown lemming *(L. sibiricus),* northern red-backed vole *(Clethrionomys rutilis),* varying lemmings *(Dicrostonyx stevensoni* and *D. rubricatus),* yellow-cheeked vole *(Microtus xanthognathus),* flying squirrel *(Glaucomys sabrinus)* and ground squirrel *(Citellus parryii).* The lagomorph, *Lepus americanus* (varying hare), was also studied. All of these species were readily infected by intraperitoneal inoculations of brucellae. Pathologic responses were not marked in most of these species. However, both species of varying lemmings responded dramatically to infections initiated by about as few as two cfu. All individuals of both species that were not killed eventually died from the infection.

INTRODUCTION

Brucella suis type 4 is enzootic in Alaskan reindeer and caribou[11] and occurs in humans[2,7,8] and various domestic and wild carnivores.[9,10] Brody *et al.*[2] found strong evidence that the source of human infection was caribou, but were unable to explain a high rate of positive serologic tests in a village where consumption of caribou was thought to be low. They hypothesized that rodents might also be a reservoir of infection.

While evidence of natural infections in Alaskan rodents is lacking, evidence of such infections in Eurasia is available.[3] Accordingly, we undertook a series of experiments on the susceptibility of indigenous, laboratory-reared rodents to brucellaceae, especially *B. suis* type 4. The results of our experiments are reported below. The untimely closure of the experimental facility prevented our carrying on our work as far as originally planned.

MATERIALS AND METHODS

Experimental Animals

With the exception of the guinea pig and the Scandinavian lemming, the animals used were indigenous to Alaska (Arctic and sub-Arctic). These included the northern red-backed vole, varying lemmings, brown lemming, and yellow-cheeked vole. All were laboratory reared. The varying hares, flying squirrels, and ground squirrels were adults, trapped alive, and held for a minimum of 4 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. *B. suis* type 4 was isolated from an Alaskan sled dog[9] and subcultured from stocks maintained by Dr. D.T. Berman (Dept. Vet. Sci., Univ. Wisconsin, Madison). The remaining species, *B. abortus* type 1 (W.H.O. Reference strain 544), *B. melitensis* type

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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, SEA, 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.[1] *Brucella* agar (BBL) was used as the base medium for isolation and counting.

Brucella agar, Baltimore Biological Laboratory, was used as the base medium for isolating and determining the number of microorganisms inoculated.

Serological Techniques

Agglutination tests were performed using the standard tube agglutination tests.[6]

Animal Infection Studies

Organisms were grown for 72 h 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) and is noted individually with the results below. 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 killed, dipped into absolute alcohol, flamed, macerated, and smeared onto selective media.[1] Tissue isolates were confirmed as *Brucella* on the basis of their agglutination by *Brucella* antiserum.

RESULTS

Guinea Pig (B. suis type 4)

Guinea pigs were injected intraperitoneally with 3.8×10^4 to 3.8×10^6 cfu of *B. suis* type 4. Animals were killed at 7, 14, and 77 days. Lesions typical of those described[3] 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 brucellae were found in the four animals injected in the liver (1/4), spleen (3/3), testis (3/4), and urine (4/4).

Dicrostonyx stevensoni (B. suis type 4)

Four series of experiments were performed with *D. stevensoni*. In three, they 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_{50}. The final experiment was to determine the susceptibility of *D. stevensoni* to three additional species of *Brucella*.

The first set of 18 *Dicrostonyx* were inoculated with 4×10^6 cfu of *B. suis* type 4. Two animals were killed on each of days 8, 14, 31, and 37 post infection (PI). Necropsies were performed on those that otherwise died during the course of this experiment. Abcesses were found on 10 of 14 livers, and enlarged spleens were found in animals that had died or were killed 22 days after inoculation. Occasional abcesses were found within the capsules of the kidneys. Large abcesses were found within the abdominal cavity along the mesentaries. In all instances pure cultures of *B. suis* type 4 were isolated from the abcessed tissues. Four females developed pus within their uteri and one male developed unilateral epididymitis.

Brucella suis type 4 was isolated consistently from the livers, spleens, kidneys, and heart blood samples of these animals. In three cases urine samples contained brucellae in concentrations ranging from 200 to 20,000 microorganisms per ml.

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The second and third experiments were performed to determine the LD_{50} and the effects of graded doses of *B. suis* type 4. The second experimental group received doses from 2 to 250,000 cfu intraperitoneally. The animals were maintained for 27 days when 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.

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 abcesses in the lower dose range, 35 to 350 cfu. Five of the males in this group also developed abcesses in the prostate glands. Two lemmings receiving 35 cfu survived 114 and 126 days. Both developed crippling abcesses in their feet. *B. suis* type 4 was the only organism isolated from the tissues and abcesses cultured in this group. The abcesses cultured included those observed on the feet of one of the two lemmings.

D. stevensoni (Other Brucella sp.)

The final experiment was to determine the susceptibility of *D. stevensoni* to three additional species of Brucella. *Brucella abortus, B. melitensis,* and *B. suis* type 1 were used to challenge. Three dilutions containing 4×10^7, 4×10^5, and 4×10^3 cfu of *B. abortus* and *B. melitensis,* and 6×10^6, 6×10^4, and 6×10^2 cfu of *B. suis* type 1 were used to inoculate separate groups of lemmings intraperitoneally. With the exception of those inoculated with *B. melitensis* (4×10^3 cfu) and *B. suis* type 1 (6×10^2 cfu) that had only two animals each, there were three animals inoculated at each dosage. Only two animals survived to the arbitrary limit of the experiment (28 days). Otherwise, the deaths occurred between 4 and 18 days in those infected with *B. abortus,* 11 to 27 days with *B. melitensis* and 4 to 26 days with *B. suis* type 1. All animals inoculated with 4×10^3 cfu or less survived until after the 21st day.

There were subtle differences between the lesions caused by infection with each of the species of *Brucella.* Abcesses developed on livers regardless of the

TABLE 1. Survival of *Dicrostonyx stevensoni* inoculated with *Brucella suis* type 4.

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

[1]Terminates 27 days
[2]Geometric mean
[3]Not calculated
[4]Experiment terminated

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infecting microorganism, and spleens became enlarged and developed abcesses. *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*.

Brucellae were consistently isolated from livers, spleens, kidneys, uteri, testes, and samples of heart blood. Samples of urine from 12 animals contained 50 to 10^5 brucellae per ml.

Dicrostonyx rubicatus

Nineteen *D. rubicatus* were challenged intraperitoneally with doses of *B. suis* type 4 ranging from 35 to 3.5×10^6 cfu. Eighteen of the 19 animals died between 14 and 37 days with one animal that received 3.5×10^5 cfu surviving until day 59 when it was killed. Abcesses developed progressively in livers of 11 of 19 animals, in 12 of 19 spleens, in three cases on the posterior aspect of the sternum, and subcutaneously in three animals. Pinpoint abcesses developed on livers between the 18th and 21st day of infection; by the 59th day (if the animal survived) the abcesses had enlarged to 1 to 2 mm in diameter. The spleen became enlarged as early as the 15th day in one animal receiving 3.5×10^5 cfu. However, the majority of the animals receiving 350 to 3,500 cfu and dying between the 15th and 37th day developed splenomegaly by the 25th day, and 11 developed either one or two large discrete abcesses on their spleen. Congested lungs were seen in 11 of the animals and abcesses were found in two. Brucellae were isolated consistently from the livers, spleens, kidneys and specimens of heart blood and in two of the three urine samples, in concentrations of 10^4/ml and 10^5/ml.

Varying Hare

Three varying hares were inoculated with 7.5×10^6 cfu *(B. suis* type 4) intraperitoneally. One was killed on each of days 14, 22, and 57. Brucellae were isolated from the uteri, axillary lymph nodes and spleens, and from a cysticercus (probably *Taenia pisiformis* Bloch, 1780) found in the abdominal cavity of the animal killed on day 14. Lungs, urine samples, ovaries, heart blood samples, livers, and kidneys were negative. When livers, lungs, kidneys, and spleens were cultured on the remaining animals, those killed on days 27 and 57, the organism was recovered only from the liver of the hare killed on day 57.

Ground Squirrel

Three ground squirrels were inoculated with 7.5×10^6 cfu *B. suis* type 4 intraperitoneally. One was killed at 14 days and the remaining two at 80 days. Small abcesses were seen on the livers (one at 14 days and another at 80 days) and on the spleens of both animals killed on the 80th day; otherwise the organs appeared normal. Brucellae were recovered from the livers and spleens of all animals and from a kidney, testis, and lung of the animal killed on the 14th day. The salivary gland and samples of blood and urine from the animals killed at 14 days were negative.

Flying Squirrel

Three flying squirrels were infected intraperitoneally with 7.5×10^6 cfu *B. suis* type 4. One died on the 41st day, and the others were killed at 14 and 80 days. At 14 days brucellae were isolated from the spleens, livers, kidneys, heart bloods, mesenteric lymph nodes, salivary glands, and one testis; urine samples were all 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 abcess seen

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on the spleen of one of the two animals killed at 80 days, the organs appeared normal; *B. suis* type 4 was recovered from the spleen and liver, but not the blood or urine of this animal.

Red-backed Voles

Ten northern red-backed voles were inoculated with 3.8×10^6 cfu *B. suis* type 4 intraperitoneally. They were killed at 8 (2 animals), 14 (2 animals), 37 (1 animal), and 80 days (5 animals). Except for abcesses seen on the liver of 1 animal killed on day 14, no other lesions were found until day 80. Those included pinpoint abcesses on the kidneys and enlargement of the spleens (two cases; in one of which the spleen was abcessed). There were no lesions common to all animals and, aside from the aforementioned exceptions, the organs looked normal.

Up to 37 days after inoculation the organism could be isolated from the majority of livers, spleens, and kidneys. At day 80 isolates were made from 1/5 livers and 1/1 urine samples, but not from five spleens, one kidney or one sample of heart blood.

Yellow-cheeked Vole

Doses of 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 killed. There were no lesions seen. Livers and spleens were cultured and were positive in 2 of 4 animals at the 8000 cfu dose level and in 1 of 4 at the 80 cfu level. Four animals developed *Brucella* agglutinins: 2 at 8000 cfu (1:160 and 1:80) and 2 at 80 cfu (1:40 and 1:20).

Scandinavian Lemming

Fourteen Scandinavian lemmings were inoculated intraperitoneally with doses of *B. suis* type 4 ranging from 10^1 to 10^7 cfu. Two animals, one each at dosages of 10^7 and 10^5 cfu, were killed at 28 days. No lesions were seen, but brucellae were isolated from the spleens of both animals and from the liver of one. The remaining 12 lemmings were sacrificed 50 days after inoculation. Microabcesses 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. Unexpectedly, of two of three animals receiving doses of 10 cfu, one exhibited liver necrosis and developed an abcess in the inguinal region and another had an abcess on the lung with adhesions causing it to adhere to an abcess on the rib cage. The remaining animal appeared normal. *B. suis* type 4 was isolated from all spleens of animals inoculated with 10^3 to 10^7 cfu, one of two livers (10^7 cfu dose), and one of three livers (10^5 cfu dose).

Brown Lemming

Twenty brown lemmings were inoculated intraperitoneally with doses of 3.6×10^6 cfu (5), 3.6×10^5 cfu (5), 3,500 cfu (5), and 35 cfu (5) *B. suis* type 4. During the period of the experiment (140 days) the lemmings suffered mortality which could not be attributed to infection by *B. 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, but there were no lesions attributable to infection in any individual except those from which *Proteus* or *Pseudomonas* were isolated. Brucellae were isolated from the liver, spleen, kidney, blood, and embryo of one lemming that died at 10 days (3.5×10^6 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 3.5×10^6 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 3.5×10^5 cfu dose level developed agglutinins although brucellae were isolated from the liver of one. There were no lesions seen, no agglutinins detected, nor isolations made from the remaining

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survivors (one at 3500 cfu dose level, and two at the 35 cfu dose level).

DISCUSSION

Rementsova[1,3] reviewed the literature on rodent brucellosis citing numerous reports on the susceptibility of hares, susliks *(Citellus* spp.) and voles to *B. abortus, B. melitensis,* or *B. suis.* Thorpe et al.[14] 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* spp. Neither of these studies utilized *B. suis* type 4.

Quite recently, experimental infections in voles, lemmings, and guinea pigs of *Brucella suis* type 4 have been reported by Gorban[3] and Gorban and Grekova.[4,5] They reported that field voles, *Microtus arvalis,* could be infected by as few as 50 cfu, [3,4] and that this biotype of *Brucella* was highly pathogenic in guinea pigs.

The animals included in this study, with the exception of the guinea pig and Scandinavian lemming, are indigenous to Alaska, and their ranges, though variable, are continuous with those of far ranging Alaskan mammals on which brucellosis studies have been reported elsewhere (caribou,[11] canids, [9] wild carnivores[10] and man).[2,7]

Varying lemmings *(Dicrostonyx* sp.) were found to be the most susceptible. Fatalities occurred when the inoculum was as low as 2 cfu with *D. stevensoni* and 20 cfu with *D. rubricatus.* Whether the difference was significant cannot be determined from the small number of animals used in each series.

D. stevensoni also proved susceptible to infection by *B. abortus, B. melitensis,* and *B. suis* type 1, but we did not determine the least number of cfu necessary for initiating infection.

With the exception of the yellow-cheeked vole, 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 2).

Overall, these Alaskan rodents showed a wide range of pathologic responses 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

TABLE 2. Survival times of Alaskan rodents infected with *Brucella suis* type 4.

Rodent	Number[1]	Dosage[2]	Time[3]	Positive Isolation[4]
Varying hare	1/1	7.5×10^6	57	liver 1/1
Ground squirrel	2/2	7.5×10^6	80	liver, spleen 2/2
Flying squirrel	1/1	7.5×10^6	80	liver, spleen 1/1
Red-backed vole	5/5	3.8×10^6	80	liver 1/5
Scandinavian lemming	3/3	9.6×10^6	28	spleen 2/2, liver 1/2
Brown lemming	2/2	3.5×10^6	140	liver, spleen 1/2
Yellow-cheeked vole	4/4	8.0×10^3	35	liver 1/5, urine 1/1
D. stevensoni	0/6	3.5×10^1	(48-126)	liver, spleen 6/6
D. rubicatus	0/18	3.5×10^1 to 10^6	(14-37)	liver, spleen 18/18

[1]Number of animals surviving per number not killed earlier
[2]Dosage expressed as cfu
[3]Days post exposure
[4]Number positive over number examined

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and Miller[12] have demonstrated that carnivores can become infected by ingesting 10^8 - 10^9 cfu of *B. suis* type 4. Thorpe *et al.*[10] found that orally administered doses of two to four logs higher than intraperitoneal inocula were necessary to initiate infection. Verger[15] infected 1 of 10, 7 of 10, and 7 of 10 mice fed 2.15×10^1, 2.15×10^2, and 2.15×10^3 cfu of *B. melitensis*, respectively. Rementsova[13] 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 species.

We feel that the most noteworthy result reported above is the high susceptibility and extreme pathologic response of species of *Dicrostonyx* (varying lemmings) to infection by *Brucella* spp. For these reasons we believe that the varying lemming may be ideally suited as an experimental host in studies on *Brucella* spp.

It is apparent that there are lapses in the data which affect the strength 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 free-living rodent populations should be sought. In any case, 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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