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Brucella suis type 4 in foxes and their role as reservoirs/vectors among reindeer

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BRUCELLA SUIS TYPE 4 IN FOXES AND THEIR ROLE AS RESERVOIRS/VECTORS AMONG REINDEER

A

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BRUCELLA SUIS TYPE 4 IN FOXES AND THEIR

ROLE AS RESERVOIRS/VECTORS AMONG REINDEER

by

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ABSTRACT

BRUCELLA SUIS TYPE 4 IN FOXES AND THEIR
ROLE AS RESERVOIRS/VECTORS AMONG REINDEER

Field and laboratory studies were conducted to test the hypotheses that (1) the reindeer/caribou organism, Brucella suis type 4, is incidentally transmitted to reindeer predators such as foxes but does not cause reproductive disease in them, and (2) infected predators such as foxes are terminal hosts and do not serve as reservoirs of infection for reindeer.

In field collections, serologic prevalence of brucellosis was similar for male and female foxes (Vulpes vulpes and Alopex lagopus). B. suis type 4 was isolated from female Vulpes and Alopex. No association between reproductive status of foxes and brucellosis infections was observed.

Serologic titers in Vulpes experimentally infected by oral exposure to Brucella suis type 4 were detected first by the standard tube and plate agglutination tests which were followed by the buffered Brucella antigen, rivanol, and complement fixation tests.

Brucella suis type 4 was isolated from the feces 4 to 6 days post-exposure (PE) and from the oral cavity for as long as 3 weeks PE in Vulpes challenged with 10^9 or 10^{11} colony forming units. Brucella suis type 4 was isolated frequently from regional lymph nodes in the

head up to 18 weeks PE, and from only more distant nodes at 22 and 66 weeks PE. Organisms did not localize in the reproductive tract.

Clinical effects of brucellosis in Vulpes experimentally-infected were not observed. Pathologic lesions were not detected in the male or non-gravid female reproductive tract. Due to breeding failure, effects of Brucella suis type 4 on the pregnant fox reproductive tract were not determined in experimental infections. Gross and microscopic pathology was limited to lymph nodes.

Fox to fox transmission attributed to aerosols from products shed by infected foxes occurred readily. Transmission from Vulpes to lemmings (Dicrostonyx rubricatus) that were exposed to urine from infected fox occurred infrequently.

Transmission from infected Vulpes to two reindeer (Rangifer tarandus) occurred under conditions of close confinement. Ingestion of organisms passed mechanically in the fox feces was considered the probable source of infection. Fox saliva containing Brucella was also implicated in transmitting the organism through bites or aerosols.

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INTRODUCTION

Brucellosis is a zoonotic disease of wild and domestic animals caused by several members of the genus Brucella. In preferential hosts brucellosis characteristically causes abortion in females and pathologic changes in the genital tract of males. It may also affect the reticuloendothelial system, mammary glands, and joints. Clinical signs tend to be less severe in atypical hosts.

Transmission usually occurs through direct contact with aborted fetuses and the accompanying membranes and fluids. Animals become infected by inhaling or ingesting the contaminated material. In reindeer, swollen, fluid-filled joints and exudate or purulent abscesses have been shown to contain large numbers of organisms and are probably additional sources of transmission. Venereal transmission is probably of minor importance. Cold, moist conditions that prevail in the Arctic are ideal for long-term survival of the organism in the environment.

In studies conducted by the Alaska Department of Fish and Game, B. suis type 4 was isolated from Alaskan sled dogs feeding on reindeer (Rangifer tarandus) and caribou (Rangifer tarandus granti) (Neiland 1970), and positive serologic reactions were reported from wolves (Canis lupus) and grizzly bears (Ursos arctos horribilis) (Neiland 1975).

In studies conducted in Texas, coyotes (Canis latrans) fed B. abortus type 1 were shown to shed the organism in the feces up to 4 days after infection, and brucellosis-negative domestic cattle held

with these coyotes became infected (Davis et al. 1988).

Dogs have been recognized as being biological or mechanical vectors of brucellosis in livestock. Some workers feel dogs are terminal hosts but recognize the risks of transmission. The overall role of dogs in the total picture of bovine brucellosis is considered small.

The incidence of brucellosis in reindeer herds on the Seward Peninsula has increased in the last 20 years (Dieterich 1981). Loss of reproductive potential through abortions, death of young fawns, and sterility in males are the major impacts of the disease. Lameness associated with infected joints and enlarged, abscessed testicles are detrimental to the survival and comfort of individual reindeer.

The Reindeer Herder's Association has listed reindeer disease control as a top research priority. Eradication of brucellosis through test and slaughter methods used in the domestic livestock industry are not practical. It is almost impossible to locate all of the reindeer in a herd to corral them at one time, and current serologic techniques do not identify all infected animals.

Vaccination is one way to help control a disease. Research has been conducted for several years by R. A. Dieterich at the University of Alaska Fairbanks, to test the efficacy of various vaccines in preventing brucellosis infections in reindeer. In conjunction, epidemiologic studies have been conducted on reindeer in the field.

Studies for this thesis were initiated in 1977 to learn more about the pathogenesis of brucellosis in reindeer predators, scavengers and small mammals and their role in the epizootiology of the disease in

reindeer. An extensive literature review was conducted on the nature of brucellosis in typical and atypical hosts to facilitate comparison of the disease among species. Red foxes (Vulpes vulpes) and arctic foxes (Alopex lagopus)are probably primarily important as scavengers, but red foxes also prey on young fawns. Grizzly bears, although less numerous, are significant predators. Arctic ground squirrels (Spermophilus parryii) commonly occur in tundra areas. Identifying the nature of the disease in these animals is necessary in the overall program of brucellosis control.

Field and laboratory studies were conducted to test the hypotheses that (1) the reindeer/caribou organism, B suis type 4, is incidentally transmitted to reindeer predators such as foxes but does not cause reproductive disease in them, and (2) infected predators such as foxes are terminal hosts and do not serve as reservoirs of infection for reindeer.

LITERATURE REVIEW

HISTORY

Brucellosis is a zoonotic bacterial disease of wild and domestic animals caused by organisms of the genus Brucella. Each of several species of Brucella tends to infect a preferential host causing abortion in females and pathologic changes in the genital tract of males. It may also affect the reticuloendothelial system, mammary glands and joints (McCullough 1980).

Vague references to abortion or fever diseases exist in the literature as far back as the time of Hippocrates. More accurate records and descriptions began in the mid to late 1800's with the reports of an undulating fever in the residents and British troops serving on the island of Malta and other areas in the Mediterranean. In 1886 David Bruce described microorganisms, later named Micrococcus melitensis, in the spleens of human patients that had died with the disease (Brown 1976).

The Malta Fever Commission, appointed in 1904, conducted exhaustive studies on the organism, its survivability and transmission. The commission reported M. melitensis was viable in sterilized tap water for 37 days, in damp soil for 72 days, in the sun for a few hours. The organism could be isolated from the blood of patients but was not transmitted by mosquitoes. It was excreted in the urine but not in sweat. After workers isolated the organism from the blood of a goat,

they found some of the goats in every herd on the island were infected. Goats could excrete enormous numbers of the organism in the milk without showing any sign of being sick or any visible change in the milk. The Commission concluded humans were most commonly infected by ingesting infective food, mainly milk (Giltner 1933).

Bang and Stribolt in 1897 named the bacillus isolated from the placenta of an aborting cow and her fetus Bacillus abortus. The disease in cattle is still called Bang's disease. In 1916 Good and Smith reported on a bacillus being the etiologic factor in the production of infectious abortion of swine. Alice Evans in 1918 reported on the similar characteristics of the organisms isolated from different animal species. Huddleson reported in 1929 that there were three distinct species, Brucella melitensis of goats, B. abortus of cattle, and B. suis of swine, with other hosts aside from the primary host in each case (Giltner 1933).

Hagan (1937) reported the organism could pass from the alimentary tract to the lymph nodes; and that calves, chickens, dogs and cats so infected rarely suffered from the disease, but that they could pass the organism out with the feces. He stated the organism could localize causing necrosis and suppuration as in purulent bursitis and orchitis in cattle, poll evil and fistulous withers in horses, and rarely, orchitis and epididymitis in dogs. Finally, he stated the organism had a predilection for genital organs and would localize in the uterus.

Historically, brucellosis has caused severe economic losses due to reproductive failure and poor production in livestock worldwide.

Because infected animals can transmit the disease to man, the public health concern is significant. Bovine brucellosis has been successfully reduced or eradicated in some countries by using a test and slaughter program in conjunction with calfhood vaccination. Eradication efforts have not been as successful in sheep and goats, and the disease in these species continues to be a significant problem in developing countries (Alton et al. 1975). "The gravity of brucellosis in terms of human illness and economic loss remains a matter of major concern to national public and animal health authorities" (Matyas and Fujikura 1984).

ETIOLOGY

The Brucella organism, a small, gram-negative coccobacillus, is an obligate intracellular parasite and lacks a capsule, flagella or exotoxins. Six species make up the genus Brucella: B. melitensis (3 biotypes), B. abortus (9 biotypes), B. suis (4 biotypes), B. neotomae, B. ovis, and B. canis. Most strains can be identified as to species and biotype by standard biochemical and serologic tests. A few strains require examination by oxidative metabolic tests (Alton et al. 1975).

B. abortus, B. melitensis, B. suis, and B. neotomae occur naturally as smooth colonies and can dissociate to a rough form on laboratory media. B. ovis and B. canis occur naturally in the rough form and do not cross agglutinate with the smooth forms. Endotoxin activity is present in cultures of smooth strains and perhaps some of the rough strains as well (Jones et al. 1976; Moreno et al. 1984).

Two surface antigens, A (predominant in abortus) and M (predominant in melitensis), occur in varying ratios which determines specificity of antibody responses. In general, B. suis contains a mixture of A and M antigens; B. ovis and B. canis lack A and M antigens. Smooth Brucella species with A and M antigens are indistinguishable on agglutination tests (McCullough 1980).

The brucella organism has been reported as being viable for 5 months in artificially infected soil at low temperatures. It can live 15 days in the sun and 34 days on the surface of the ground in the shade. It survives in river water from a few days to 4 months; in frozen meat from 4 or 5 months to 2 years (Poljakow 1963).

Cattle are the typical host for B. abortus; sheep and goats for B. melitensis; swine for B. suis; sheep for B. ovis; dogs for B. canis; and the desert wood rat (Neotoma lepida) for B. neotomae. Reindeer and caribou are the natural hosts for B. suis type 4. Cross infections do occur, and nearly all domestic animal species are susceptible to some degree (Jubb et al. 1985).

In people, B. melitensis is the most invasive and causes the most serious infections. B. suis is highly invasive but tends to localize causing suppuration and necrosis. B. abortus is less invasive and causes a milder disease. B. canis is slightly invasive, causing a relatively mild disease with few complications (Hoff and Nichols 1974; Hoff and Schneider 1975; Kahrs et al. 1978; Morisset and Spink 1969; Polt and Schaefer 1982; Porter 1976; Swenson et al. 1972). B. neotomae and B. ovis have not been implicated in human disease (McCullough

1980; Meyer 1974).

Infected hosts produce IgM and IgG against bacterial surface antigens. IgM appears first followed by IgG. IgM may persist in chronic infections. There is also a delayed type hypersensitivity response to the organism and its components. The cell-mediated response largely determines the outcome of the disease (McCullough 1980).

BRUCELLA SP. IN TYPICAL HOSTS

CATTLE - Brucella abortus

Transmission

Aborted fetuses or the associated placental and uterine discharges are the usual sources of bovine infection with B. abortus. The usual route is alimentary, but the disease can also be transmitted conjunctivally, through the skin, or by artificial insemination. Natural venereal transmission is possible but rare (Jubb et al. 1985).

Pathogenesis

After entering the animal, organisms are carried free or in phagocytic cells to the regional lymph nodes which become enlarged due to lymphatic and reticuloendothelial cell hyperplasia and inflammation (Porter 1976; Thoen and Enright 1986). Infection may be overcome in the regional nodes. If the organisms are not killed, bacteremia and further phagocytosis follow. The disease spreads hematogenously to various tissues, especially the liver, spleen and other lymph nodes during the acute regional lymphadenitis phase. Disintegration of phagocytic cells

results in more circulating viable bacteria (Jubb et al. 1985).

Bacteremia may persist for months depending on the resistance or susceptibility of the host. Bacteremia may become intermittent and recur at parturition. Localization tends to occur during the early bacteremic phase and is usually limited to the spleen, mammary glands, mammary lymph nodes or pregnant uterus. Localization in the male occurs in the lymphoid tissues, testes or accessory glands. Occasionally localization occurs in the synovial structures resulting in a purulent tenosynovitis, arthritis or bursitis. Infected females may secrete Brucella in the colostrum, even in the presence of local antibodies, or less commonly in the milk. Mastitis tends to be focal without gross changes. There is little predilection for the kidneys, ovaries, bone marrow or mesenteric lymph nodes. The organism appears not to be excreted in the urine or feces (Jubb et al. 1985).

In females, B. abortus has a particular affinity for the pregnant endometrium and fetal placenta. Responsible factors may include erythritol found in the gravid uterus and a growth stimulant for Brucella in vitro, and the immune status of the pregnant uterus (Bosseray 1983; Thoen and Enright 1986). A uterus under the influence of progesterone, including a pregnant uterus, is more susceptible to any bacterial infection. The organism goes from the chorionic epithelium to the placental stroma, blood vessels, and fetus. Frequently only portions of the individual bovine placentomes develop lesions (Thoen and Enright 1986). Likewise in mouse infection models, placentas are independent units for bacterial colonization and proliferation (Bosseray 1980). The

non-pregnant uterus is relatively resistant (Jubb et al. 1985).

Outcome of the infection depends on the age of the host and the route of infection, the reproductive status of the animal, its resistance, and the dose and virulence of the organism. Young animals tend to be more resistant up to the age of puberty; the disease tends to persist in mature animals. The disease may take weeks to develop and may persist for months (Jubb et al. 1985).

Pathology

Mononuclear cells, the host's basic defense cell, and polymorphonuclear leukocytes (PMN's) attempt to localize the bacteria. The organism is capable of surviving and multiplying within the phagocyte and is thus protected from antibodies and chemotherapeutic agents (Porter 1976; Thoen and Enright 1986). Infected lymph nodes become large and hyperplastic without a clear distinction between the cortex and medulla. Hemorrhages are frequently seen in the medulla. Sinuses are infiltrated with PMN's and eosinophils. Germinal centers and proliferative activity are obvious. Plasma cells may accumulate in the medullary sinuses. Fibrosis and necrosis tend to be absent (Jubb et al. 1985).

Intrauterine lesions progress slowly, range from mild to severe, and are not pathognomonic (Thoen and Enright 1986). If the lesions are minor, the offspring may be non-viable or normal. Abortions usually occur during the 7th or 8th month. Although there is little sign of endometritis early, severe endometritis is present by the time abortion is inevitable. Great numbers of organisms are shed into the environment

during birthing. Placental lesions are not uniform, and there may be an abundant exudate between the endometrium and chorion. The placenta may be retained. The organism is usually cleared from the uterus in a few weeks or longer (Brown 1976; Jubb et al. 1985).

The fetus is usually edematous with blood-tinged subcutaneous fluid. The abomasal fluid may be turbid rather than clear. Pneumonia with scattered foci of bronchitis and bronchopneumonia is the important fetal lesion in bovine brucellosis. A granulomatous response may be seen in lymphatic tissue, liver and sometimes kidney. Mononuclear cells as well as PMN's are present (Jubb et al. 1985; Thoen and Enright 1986).

Bulls may suffer acute orchitis. If it is unilateral, sterility may still ensue due to thermal degeneration or inflammatory products mixing with the opposite testis. Swelling of the testis is not always obvious, but there may be pressure necrosis, fibrinopurulent exudate, total testicular necrosis, and liquifaction to pus. Perforation of the tunic or scrotum is rare. A necrotizing epididymitis is usually present (Jubb et al. 1985).

SHEEP AND GOATS - Brucella melitensis

Transmission

Sheep and goats are normally infected by B. melitensis, but can be infected by B. abortus. Transmission occurs by ingestion of contaminated abortion products (Jubb et al. 1985; Thoen and Enright 1986).

Pathogenesis

The disease in goats may be relatively asymptomatic to severe, but tends to be similar to that seen in cattle with B. abortus. An acute mastitis develops early, and the organism is shed in the milk. Goats may be infected for years. Sheep are less severely affected than goats. Abortions in sheep and goats occur late in pregnancy (Jubb et al. 1985; Thoen and Enright 1986).

Pathology

B. melitensis causes more necrosis and less exudation in the sheep placental tissue than B. abortus causes in cattle (Jubb et al. 1985; Thoen and Enright 1986).

SHEEP - Brucella ovis

Transmission

B. ovis infects sheep causing epididymitis in rams and placentitis in pregnant ewes. Venereal transmission is usually responsible for ram to ewe or ram to ewe to ram infection (Jubb et al. 1985; Thoen and Enright 1986).

Pathogenesis

Bacteremia may persist for two months. The organism usually localizes in the tail of the epididymis, but can also be found in the spleen, kidney and liver. Infected rams can pass large numbers of organisms in their semen for several months. B. ovis can infect the placenta and cause abortion. It is relatively non-pathogenic in the non-pregnant ewe (Jubb et al. 1985; Thoen and Enright 1986).

Pathology

Edema with an infiltration of lymphocytes and macrophages is seen in the infected epididymis. Epithelial hyperplasia, interstitial fibrosis, luminal obstruction and sperm stasis follow. Although there is no primary orchitis, immune-mediated lesions resulting from extravasation of sperm may result. In experimental animals, gross lesions are not seen in the liver, kidney or spleen (Jubb et al. 1985; Thoen and Enright 1986).

Placental lesions resemble those caused by other brucellae, but are less severe than those caused by B. melitensis or B. abortus (Meyer 1969b; Thoen and Enright 1986). The organism can be cultured from other organs, but lesions are not seen. Fetuses show little histologic evidence of systemic infection even though their gastric contents may be heavily infected. A mild pneumonia may be seen, and an acute interstitial nephritis is common (Jubb et al. 1985; Thoen and Enright 1986).

PIGS - Brucella suis

Transmission

Pigs are usually infected with B. suis but are also susceptible to B. melitensis and B. abortus. The disease is transmitted similarly to that in cattle, but venereal transmission occurs more frequently (Jubb et al. 1985).

Pathogenesis

As opposed to B. abortus in cattle, B. suis in swine tends to be

mainly a disease of the reticuloendothelial system with inflammatory disturbances and focal granulomatous lesions. After entering the host, the organism causes a local lymphadenitis followed by a bacteremia that may persist for months or years. It has an affinity for the joints and skeleton including the vertebral column. Joints of the legs, especially the knees and hocks, are frequently affected with pain, swelling, and fluid accumulations. It also localizes in the mammary glands, lymph nodes, spleen, liver, kidney, bladder, brain, and especially the male and female genitalia. Abortion is less frequent than in cattle, occurs early in gestation, and may be overlooked. There is a high incidence of stillborn piglets or weak piglets born at term. Also, there is probably a high incidence of undetected embryonic deaths. Boars are as susceptible as females. Suckling piglets are less susceptible than weaners, but a few can carry the disease to adulthood (Jubb et al. 1985; Thoen and Enright 1986; Wilson and Smith 1984).

Pathology

The organism grows and multiplies in the phagocytes producing granulomatous lesions with possible caseous necrosis. Small granulomas and caseous exudate independent of an association with pregnancy can be seen in the uterus and fallopian tubes. Females may have a uterine discharge for two years. Lesions in the male are seen in the testes and accessory organs. The bacteria are shed in the urine and may be shed in the semen for life. Fetuses may have a subcutaneous edema (Jubb et al. 1985).

DOGS - Brucella canis

Etiology

In 1966 a Brucella organism was recognized as being the cause of abortions in colonies of Beagle dogs in the U.S. The disease has subsequently been recognized in several countries (Azuma et al. 1977; Bosu and Prescott 1980; Kruedener 1975; Kumagai et al. 1975; Myers and Varela-Diaz 1980; Weber and Schultz 1976). Although Meyer (1969a) presented a strong case for classifying the organism as a B. suis biotype, the organism was eventually named B. canis.

B. canis cross reacts with B. ovis, is similar to rough abortus and melitensis, has biochemical characteristics and perhaps antigenic determinants similar to B. suis (especially rough forms), and lacks the LPS-endotoxin associated with smooth brucellae (Bowser et al. 1975 Carmichael 1976; Carmichael and Bruner 1968; Dees et al. 1981; Diaz et al. 1968; Jones et al. 1968; Meyer 1969a). Growth does not appear to be stimulated by erythritol in vitro, but testing is highly media dependent (Jones et al. 1968). Lowrie and Kennedy (1972) demonstrated the presence of erythritol in canine uteri, but noted the concentration was lower than that found in ungulates.

Transmission

The disease is transmitted mainly through the oropharynx by aborted fetal tissue and fluids which can contain up to 10^{10} organisms per ml. It can also be transmitted venereally (Carmichael 1976; Currier et al. 1982; Moore and Gupta 1970; Thoen and Enright 1986). Serikawa et al.(1981b) felt contaminated urine from infected male dogs might play a

role in disseminating the disease.

In controlled experiments and field observations, transmission did not occur between infected, non-pregnant females and uninfected females housed together or between infected and uninfected males housed together. However, an uninfected bitch in estrus placed with an infected male was readily infected (Carmichael 1976). Jones (1984) reported that separating susceptible animals in a breeding kennel, even with partial walls, was not enough to prevent transmission.

Pathogenesis

A bacteremia develops 1-3 weeks following exposure and persists for months to years (Carmichael et al. 1984; Jubb et al. 1985; Thoen and Enright 1986). Prolonged bacteremia is probably due to slow killing of the organism by the host (George 1975). Lymphoid tissues, including the Peyer's patches, are usually affected, and the organism can persist in lymphatic tissues months after bacteremia ceases (Carmichael and Kenney 1970).

Pathology

Besides abortion, B. canis also causes recurrent anterior uveitis, discospondylitis (Carmichael 1976; Henderson et al. 1974; Hubbert et al. 1980; Riecke and Rhoades 1975), meningitis, focal nonsuppurative encephalitis, osteomyelitis, pyogranulomatous dermatitis, draining scrotal ulcers, and internal abscesses (Meyer 1983). Arthritis is not observed (Carmichael and Kenney 1970).

Placentitis and chronic granulomatous endometritis are seen in infected pregnant females. Retained placentas in aborting females are

not observed; however, a vaginal discharge lasting 1-6 weeks after abortion is common. The discharge is serosanguinous, sometimes opaque, viscous, grayish-green, and strongly culture-positive. Commonly, an infected female fails to conceive or whelp, and it is likely that early, undetected embryonic deaths occur (Carmichael and Kenney 1968, 1970). Pups that are born weak usually die within 24 to 48 hours (Carmichael and Kenney 1970; Jubb et al. 1985). Fetuses show pneumonia, endocarditis and hepatitis (Jubb et al. 1985). Surviving pups, though appearing healthy, may have generalized lymphadenopathy and be bacteremic (Krakowa 1977).

Males harbor the organism in the epididymis and prostate as well as the spleen and lymphatics (Carmichael 1976). Orchitis is not consistent. However, in natural and artificial infections, testicular degeneration and atrophy, often unilateral, are seen (Carmichael and Kenney 1968; George et al. 1979; Thoen and Enright 1986). Isolation from semen is inconsistent (Carmichael and Kenney 1970), especially during the first 6 weeks after infection, but intermittent shedding has been observed up to 60 weeks (Thoen and Enright 1986). The organism can be isolated from the prostate and epididymis for 6 months. Anti-sperm antibodies are produced, and this autoimmune response results in infertility (George and Carmichael 1984; Rosenthal et al. 1984; Serikawa et al. 1981a; Serikawa et al. 1983; Serikawa et al. 1984; Thoen and Enright 1986).

B. canis does not cause clinically apparent disease in non-gravid females. In experimental infections, usually only enlarged lymph nodes

draining the site of inoculation are seen (Carmichael and Kenney 1970). Histologic lesions include hyperplasia of the lymphatic tissue, focal hepatitis, inconsistant mild placentitis, and micro-lesions in the testis, epididymis and prostate (Moore and Kakuk 1969; Spink 1970). Gleiser et al. (1971) reported hyaline thickening of the basement membranes of glomerular capillaries and inflammatory lesions in the interstitium of the prostate, epididymis and testis. Uterine lesions are not seen in non-gravid females (Carmichael and Kenney 1970).

DESERT WOOD RATS - Brucella neotomae

B. neotomae was recognized when Stoenner et al. (1959) detected Brucella agglutinins and subsequently isolated organisms from 7/107 desert wood rats. No significant lesions were seen associated with the infection. Brucella was not detected in the remainder of over 6,000 small mammals sampled during the study. The epizootiology remains unclear.

REINDEER/CARIBOU - Brucella suis type 4

History

Reindeer were introduced into Alaska in the late 1800's to help replace the decreasing supply of renewable resources traditionally used for food and clothing by Native populations in Northwest Alaska (Stern et al. 1980). Lesions compatible with brucellosis were described in reindeer in the 1920's (Hadwen and Palmer 1922), and the organism may have been introduced into Alaska with the original shipments of animals.

Forty-nine cases of brucellosis in humans were serologically diagnosed in Alaska between 1939 and 1953. The source of these cases was originally thought to be B. abortus in unpasteurized milk from cattle or swine. Toshach (1955) characterized two strains isolated from Canadian Eskimos as B. melitensis. Subsequent serotesting and bacterial isolations from humans and caribou implicated caribou in infections in both Alaskan and Canadian natives (Brody et al. 1966; Huntley et al. 1963; Toshach 1963). Meyer (1966) solved much of the confusion by determining the strains isolated from Eskimos and reindeer in Alaska, Canada and Russia were indistinguishable from each other. The organism was eventually classified as B. suis type 4.

Transmission

The disease in reindeer and caribou is transmitted by contaminated abortion or birthing fluids and membranes as in other species, but also by draining abscesses and infected joints.

Conditions existing in the reindeers' habitat are ideal for long-term survival of the organism in the environment. Vashkevich (1973) conducted studies on survival of the organism in the Soviet Union and reported that it could be isolated from areas of tundra with boggy type moisture and peaty boggy soil from 20 cm deep after 86 days, from frozen feces at 151 days, and from superficial layers of frozen soil at 260 days.

Pathogenesis

The pathogenesis of brucellosis abortion in reindeer and caribou appears to be similar to that in domestic cattle. Abortion in reindeer occurs 1 - 2 months before normal fawning time which is in early May

(Rausch 1978). Fawns born alive may die shortly after birth. Females aborting one year may produce live fawns the next (Dieterich 1981). Clinical signs such as swollen testicles, abscesses and fluid-filled joints (carpal, tarsal and fetlock) with accompanying lameness are seen in a small portion of chronically-infected reindeer (R. Dieterich, pers. comm.).

Pathology

Lesions in reindeer and caribou are more similar to those seen in swine infected with B. suis than in cattle infected with B. abortus. Lymphadenopathy is seen in experimental infections in reindeer. Abscesses containing a thick green purulent material are found in the mammary glands, reproductive organs, liver, kidney, abdominal cavity or as subcutaneous enlargements (Dieterich 1981). Perforation of the scrotum is commonly seen in bulls with orchitis (R. Dieterich, pers. comm.).

Neiland et al. (1968) described orchitis, epididymitis, bursitis, synovitis, metritis, abortion and retained placentas in Alaskan caribou. He speculated some of the retained placentas were related to malnutrition rather than brucellosis. Retained placentas associated with brucellosis are rare in Alaskan (R. Dieterich, pers. comm.) and Russian (Zabrodin et al. 1980) reindeer.

Significance

Reindeer numbers on the Seward Peninsula increased to an estimated 600,000 in the 1930's. The population has decreased to 20-25,000 at the present time. Factors contributing to the decline may include poor management, overgrazing, loss to caribou herds, predation and disease

(Stern et al. 1980).

Serologic results indicated the incidence of the disease in reindeer herds on the Seward Peninsula increased sharply from 1969-1973 (Dieterich 1980). By 1979 the incidence was 14% and 16% in two herds that had previously been negative. Subsequent testing indicated prevalence rates as high as 30 to 50% in some herds.

Increased demands for red meat, antler markets in the Orient, and the allocation of grazing lands to native corporations as part of the Alaska Native Claims Settlement Act have stimulated an interest in expanding the reindeer industry. The reindeer herders have indicated disease control is among their highest research priorities.

Human Infections

Rangiferine brucellosis is recognized as having public health significance in Alaska (Dieterich 1980) as well as in the Soviet Union (Grekova and Gorban 1978; Gudoshnik 1975). In 1964 approximately 20% of the residents of Fort Yukon and Arctic Village were seropositive for brucellosis. Eleven percent of 763 people tested during a serologic survey of seven villages from 1962-1964 were seropositive, and eight clinical cases were reported (Brody et al. 1966). Seventeen human cases were reported between 1966 and 1975 (Dieterich 1980). In one Russian community, 26.2% of 292 residents were seropositive (Gudoshnik 1975).

BRUCELLA SP. IN ATYPICAL HOSTS

Although each Brucella species tends to infect a preferential host, cross infections do occur. When transmitted to an abnormal host, the organism tends to localize in the mammary gland and reticulo-endothelial system rather than in the uterus and fetal membranes (Wilson and Miles 1975).

Cattle

Cattle are usually infected with B. abortus but may be infected with B. melitensis if in close contact with infected goats or sheep (Arshakuni et al. 1972; Hagan 1937; Wilson and Smith 1984). The organism tends to localize in the udder, and abortion is rare (Wilson and Miles 1975).

B. suis has been isolated from cattle in close association with feral (Cook and Noble 1984) or domestic swine (Elder 1946). Although B. suis is rarely found in mammary glands, infected cattle may excrete the organism in the milk (Wilson and Smith 1984). Experimental infection is difficult except by intramammary exposure (Deyoe 1970; Washko et al. 1948; Washko et al. 1951). Lesions and abortions are not induced (Norton and Thomas 1979). Isolation of the organism from infected cows is rare (Elder 1946; Nicoletti 1981; Washko et al. 1948). With either B. melitensis or B. suis, the disease in cattle tends to be self-limiting (Wilson and Miles 1975; Wilson and Smith 1984).

B. canis may be transmitted conjunctivally to cattle, but the pathogenicity is low (Deyoe 1970; FAO/WHO 1971; Pickerill 1970b).

Sheep and Goats

Early workers felt sheep were fairly resistant to Brucella species other than B. melitensis. B. abortus seldom and B. suis never causes abortion in sheep and goats (Manley 1968; Wilson and Miles 1975). Sheep can be infected conjunctivally with B. canis, the organism can be isolated at necropsy, but the disease appears to be self-limiting and has no effect on reproduction (Deyoe 1970; Pickerill 1970b).

Pigs

B. abortus has been encountered in mandibular lymph nodes of swine, but has never been known to spread from swine to swine. Neither B. abortus nor B. melitensis causes abortion in pigs (Deyoe 1970; Meyer 1964). B. canis is not considered very pathogenic for swine (Deyoe 1970; FAO/ WHO 1971).

Horses

Abortions in pregnant mares have been reported following infection with B. abortus (Denny 1973; Hagan 1937; McCaughey and Kerr 1967). However, suppurative lesions such as poll evil or fistulous withers are the usual signs. Arthritis and tenosynovitis are less frequently seen. Horses can transmit the disease back to cattle by shedding organisms in draining lesions. B. suis occasionally infects horses causing bursal lesions (Denny 1973; Jubb et al. 1985; McCauaghey and Kerr 1967; Wilson and Smith 1984).

Dogs

Brucellosis transmission to dogs was first reported in 1906 after B. melitensis was isolated from stray dogs on Malta. In 1931 B. suis

was isolated from testicular pus of a dog with a history of listlessness and stiffness in the rear legs. After the testicle was removed, the dog returned to normal even though he maintained a serologic titer (Planz and Huddleson 1931).

In an extensive literature review on livestock brucellosis in dogs, Morse (1951) reported that both spontaneous and experimental infections had involved isolated cases of a few dogs. Most authors consider dogs to be fairly resistant to livestock brucellosis (Margolis et al. 1945). Karlson and Clausen (1940) cited a report on one dog that was fed one quart of raw milk daily from infected cattle for several years and yet showed no clinical signs or any gross lesions at necropsy. In another experiment, milk from four cows shedding Brucella in one or two quarters was pooled and fed ad libitum to 14 puppies. None of the puppies seroconverted, and Brucella was not isolated from tissues or excreta (Morse et al. 1951a).

Brucellosis in dogs can frequently be detected serologically, but clinical signs are rare (Kimberling et al. 1966; Pannwitz and Meissner 1971). Lesions in dogs may be relatively mild even in the face of an almost overwhelming infection (Feldman et al. 1935a; Kerby et al. 1943). Small granulomas in the liver, kidney and lymph nodes are neither specific nor even necessarily related to the infection (Deyoe 1970; Jubb et al. 1985; Makkawejsky and Karkadinowskaya 1964; Wipf 1952). Seropositive females often have normal litters (Feldman et al. 1935b).

Infections in dogs tend to be self-limiting (Currier et al. 1982) and not persistent (Meyer 1983). However, B. abortus, B. melitensis,

and B. suis have been reported to occasionally cause abortion and metritis in dogs (Apartsev 1972; Bicknell et al. 1976; Ferney et al. 1984; Fincher and Puckett 1961; Hagan 1937; Kimberling et al. 1966; Meyer 1983; Morse et al. 1953; Taylor et al. 1975; van der Hoeden 1933). Other reported signs include orchitis (Davis 1937; Hall 1974; Klein 1959; Leitch 1969; Meyer 1983; Nolan 1940), epididymitis (Love et al. 1952; Meyer 1983; Whitby et al. 1936), muscle soreness (Bloxam 1972), polyarthritis (Clegg and Rowison 1968; McErlean 1966), stiffness, and posterior paresis (McErlean 1966; Meyer 1983). Lesions are often associated with the reticuloendothelial system (Makhashvii 1973; Margolis et al. 1945; Margolis et al. 1947; Meyer 1983). Infection can usually be traced to dogs being fed meat contaminated with abortion by-products (Bicknell and Bell 1979; Bicknell et al. 1976; Ferney et al. 1984; Meyer 1974) or from being in close contact with aborting livestock (Akhmedov 1960; Apartsev 1972; Barreto et al. 1978; Brown 1976; Wilson and Miles 1975).

Deyoe (1970) compared the pathogenesis of B. canis and B. suis type 3 by experimentally challenging dogs conjunctivally with 5 x 10^6 CFU (colony forming units) of each organism. Lesions seen in dogs infected with B. suis type 3 were similar to those seen in swine infected with field strain B. suis. Reproductive disorders were observed in all sexually mature bitches infected with B. suis but not with B. canis.

Deyoe (1970) concluded that dogs were not resistant to infections with B. suis type 3. He observed dissemination of the organism, persistent infection, marked pathologic changes, abortions, and isolation of

the organism from the vagina, milk and respiratory passages.

It has long been recognized that dogs can at least act as mechanical or biologic vectors of B. abortus, B. suis or B. melitensis (Fincher and Puckett 1961; Kiok and Grunbaum 1978; Lagneau 1969; Phillipon et al. 1969; Pogorzhel'skaya 1941; Rementsova 1962a; Salem et al. 1975; Vaughan 1969), and that they can represent a problem in a control or eradication program (Kiok and Grunbaum 1978: Lacombe 1962; Makkawejsky and Karkadinowskaya 1964; Mainil 1983; Martin et al. 1981; Pogorzhel'skaya 1941; Prior 1976; Zamora et al. 1967). In nearly all cases, transmission from dogs back to livestock can be traced to the dogs' consumption of contaminated materials (FAO/WHO 1971; Currier et al. 1982; Leitch 1969; Love et al. 1952; McErlean 1966; Pannwitz and Meissner 1971).

B. abortus has been isolated from dogs' urine (Bicknell and Bell 1979; Hall 1974; Makkawejsky and Karkadinowskaya 1964; Morse et al. 1951b), feces (Morse et al. 1951b), blood (Makkawejsky and Karkadinowskaya 1964), and joints (Clegg and Rowison 1968). In an experiment in which nine dogs were orally infected with B. abortus and kept for 6 months with 11 pregnant heifers, two bitches aborted, B. abortus was isolated from the abortion material, and six of the heifers eventually aborted with B. abortus again being recovered. The authors felt dogs should be included in a sanitation program (Kiok and Grunbaum 1978).

The possibility of dogs being a reservoir for brucellosis in pigs was suggested when B. suis type 1 was repeatedly isolated from the semen of a dog with discospondylitis and epididymitis (Barr et al. 1986). In another case, an entire large pig unit was re-stocked after liqui

dation of the original brucellosis-infected population. No signs of brucellosis were seen after the first breeding, but increasing numbers of abortions began occurring later. The boars were seropositive; the sows were seropositive; and the watchdog on the farm was seropositive. The dog was considered to be the reservoir maintaining the disease from the first infected herd and transmitting it to the clean herd (Kormendy and Nagy 1982). B. suis has been isolated from the spermatic cord (Harrington and Brown 1976) and testes of dogs (Hellman and Sprenger 1978). Nicoletti et al.(1967) reported on a case of swine to dog, then dog to human transmission following abortion in the dog.

An early study conducted on sheep dogs in the Soviet Union found 21 of 75 hemoculture positive and 21 of 75 seropositive (Eremin 1939). In a later study of 13 sheep farms, 29 of 501 dogs tested were seropositive for brucellosis (Chermisin 1963). Islamov (1972) cultured B. melitensis from seropositive sheep dogs killed 5 months after the probable transmission time during lambing and felt the dog was a more efficient maintenance host than previously thought. In another study, B. melitensis was cultured from both dead and normal puppies born to a female infected by being fed 10^8 organisms in milk, and the author concluded dogs and puppies were a potential source of transmission to sheep (Islamov 1973).

Bicknell et al. (1976) concluded the prevalence of dogs shedding brucellae organisms was very low but must be considered as risks for livestock and humans. Some have stated dogs are a terminal host (Carmicheal and Kenny 1968; Jones et al. 1968) and are not a significant

reservoir (Kolesnik 1966). Lacombe (1962) concluded that the role of the dog in transmission of the disease to man and other animals was undeniable but not well defined.

The consensus in bovine epidemiology is that non-bovine hosts are probably insignificant in the total reservoir. When the disease does occur in other animals, it is self-limiting. Transmission back to cattle probably occurs only under conditions of close association (Crawford and Hidalgo 1977).

Cats

Relatively little is known about natural brucellosis infections in domestic cats. Cats are generally considered resistant to infections with Brucella species (Jubb et al. 1985). Early reports indicated lesions in joints and organs could be caused by inoculations with B. abortus or B. melitensis (Wilson and Smith 1984). Experimental infection with B. canis did not induce abortions or even significant antibody titers (Pickerill 1970b). In a serologic survey of domestic cats, 1 of 114 from animal shelters and 5 of 56 from animal hospitals had significant titers, but no bacteriology was done (Randhawa et al. 1977a). Rementsova (1962a) felt cats in rural situations probably participate in the maintenance of infection in farm animals.

Laboratory animals

B. abortus, B. melitensis and B. suis are all pathogenic for lab animals. Guinea pigs are the most susceptible. The organisms affect mainly the reticuloendothelial system causing a non-hyperemic enlargement of the lymph glands, enlarged spleen, and circular necrotic foci in the

spleen and liver. Abscesses are sometimes seen in the testes or epididymides. Occasionally lesions occur in joints, bones or other organs (Wilson and Miles 1975).

B. abortus causes chronic, non-fatal infections in rats and mice infected by large, oral doses. If infected intraperitoneally, they may shed the organism in the urine and feces for a short time (Wilson and Miles 1975).

B. suis causes more purulent lesions (Wilson and Miles 1975) and markedly enlarged spleens (Deyoe 1970). In experimental infections B. canis can cause peritoneal and pleural adhesions, granulomatous changes in the reticuloendothelial system, but no deaths (Carmichael and Bruner 1968). B. ovis likewise has a relatively low pathogenicity for laboratory animals (Wilson and Miles 1975).

Poultry

Fowl can be infected with B. abortus, B. melitensis or B. suis either orally or intraperitoneally. They seem to be fairly resistant unless infected with large doses. Infections can cause diarrhea and an interruption in laying. Mortalities have been reported, especially with B. melitensis infections (Abdallah et al. 1983; Emmel and Huddleson 1929; Wilson and Miles 1975).

In a natural situation, 2 of 50 chickens housed with aborting cattle were seropositive and one was culture positive (Angus et al. 1971). Pinigin and Zabrodin (1970) isolated B. abortus from a crow.

Passage of B. abortus through fowl increases the virulence of the organism for fowl, but decreases the virulence for guinea pigs. Factors

responsible may include the effect of a higher body temperature or a different physiology of the fowl (Angus et al. 1971). Brucellosis in poultry is considered to be of minor economic or epidemiologic importance (Angus et al. 1971; FAO/WHO 1981; Stephen et al. 1978; Witter 1981).

BRUCELLA SP. IN WILDLIFE

General

Brucellosis has been detected serologically in many species throughout the world. Moore and Schnurrenberger (1981b) cited references to the disease in camels (unspecified sp.), fallow deer (Dama dama), elk (Cervus canadensis), sika deer (Cervus nippon), white-tailed deer (Odocoileus virginianus), mule deer (Odocoileus hemionus), moose (Alces alces), roe deer (Capreolus capreolus), giraffe (Giraffe cameloparadalis), antelope (Antilocapra americana), kudu (Tragelaphus strepsiceros), black-tailed jackrabbit (Lepus californicus), brown hare (Lepus europaeus), western porcupine (Erethizon hydrochaeres), capybara (Hydrochoerus hydrochoerus), coyote (Canis latrans), wolf (Canis lupus), red fox (Vulpes vulpes), and gray fox (Urocyon cinereoargenteus). In addition, Witter (1981) listed references to bison (Bison bison), Dall sheep (Ovis dalli), several species of chamois, several species of deer, spotted hyena (Crocuta crocuta), jackel (Canis mesomelas), grizzly bear (Ursus arctos horribilis), feral swine (Sus scrofa), bobcat (Lynx rufus), raccoon (Procyon lotor), badger (Taxidea taxus), rabbit (Sylvilagus floridanus), mice (Mus musculus), desert wood rat, ticks (Ornithodorus spp. and Dermacenter spp.), and fleas (Orehopeus sexdentatus). Sachs (1966) detected serologic titers in impala (Aepyceros melampus), wildebeest (Connochaetes taurenius albojubatus), Grant's gazelle (Gazella granati), and Thomson's gazelle (G. thomsonii).

Wild Swine

Pavlov et al. (1960) considered several wildlife species as being possible reservoirs for brucellosis in domestic swine in Bulgaria. B. suis was most prevalent in wild boars, but was also detected in hares, foxes and dogs. Many of the foxes examined had parts of aborted swine fetuses and placentas in their stomachs at necropsy. He concluded wild swine posed a serious problem for domestic swine, but that other species were of minor importance. Wood et al. (1976) felt the control programs for brucellosis in domestic swine in South Carolina should take into account the prevalence of brucellosis in wild swine.

Hares

Brucellosis in hares was first described in 1941 in Germany when B. abortus was isolated from subcutaneous abscesses. The organism was subsequently isolated from hares in Switzerland and Czechoslovakia, and B. melitensis was reported in hares in France. Subsequent attempts to experimentally infect hares with B. abortus were unsuccessful (Bendtsen et al. 1956). Russian workers reported 1.5% of the hares in animal husbandry regions were seropositive for brucellosis (Rementsova 1962b).

The porcine strain of B. suis type 2 was isolated from hares in Denmark in 1951 (Bendtsen et al. 1956). B. suis type 2 is now recognized in wild hares and domestic swine in continental Europe from Denmark to the Ural Mountains in the U.S.S.R. (Meyer 1974). Most cases in hares are associated with outbreaks in swine (McCaughey 1968). Lesions in hares include nodules of varying sizes with purulent, necrotic tissue

mainly in the testes, mammary glands, and less often in the lungs, liver and uterus (Fenske 1963; Vitovec et al. 1966; Wilson and Smith 1984).

Hare brucellosis has been detected in areas without swine brucellosis but with many cases of bovine brucellosis, and Jacotot and Vallee (1954) and Klahn (1962) speculated that B. abortus was transmitted to hares and transformed to B. suis type 2.

Fenske (1963) described hare brucellosis as being difficult to control. It is difficult to eradicate the hares, and new hares are often introduced for breeding stock. Stoll and Manz (1971) isolated B. suis from a rat (Rattus norvegicus) and felt rats could serve as mechanical vectors of the hare disease by spreading the organism through the urine. Most authors feel transmission from hares back to swine occurs readily and that hares present an etiologic hazard for breeding swine (Fenske 1963; Jacotot and Vallee 1954).

Ungulates

Brucellosis was first detected in North American wild ruminants in 1917 when Mohler reported the disease in bison in Yellowstone National Park. Numerous serologic surveys have been conducted, but the prevalence of brucellosis in wild ungulates does not appear to be significant except in elk on winter feedgrounds in Wyoming (Thorne 1982).

Elk are infected with B. abortus type 1 and probably originally acquired the infection from domestic cattle. Clinical signs seen in elk include abortion, premature birth, or the birth of nonviable calves. Carpal bursitis is seen in chronic infections. Transmission from elk to cattle was demonstrated under experimental conditions of close confine-

ment. Recommendations were made that cattle and elk not share feedgrounds during the early spring months when abortions are likely to occur (Thorne 1982).

Natural infections characterized by emaciation, weakness and death have been reported in a few moose (Corner and Connell 1958; Fenstermacher and Olsen 1942; Jellison et al. 1953). Clinical signs including an elevated temperature, increased white blood cell count, mild diarrhea and lethargy as well as lesions at necropsy were seen in a moose infected with B. suis type 4 at the University of Alaska Fairbanks (R. Dieterich pers. comm.). It has been speculated that brucellosis causes a generalized, fatal infection in moose which results in few seropositive animals being detected in the field.

Carnivores

Serologic titers were detected in racoons, badgers, skunks, coyotes and bobcats which had probably been in contact with infected livestock in California (Hog 1978).

Corbel et al. (1983) experimentally infected three young badgers with B. abortus type 1 by injecting 1.8×10^{11} CFU of strain 544 conjunctivally. Post-infection nasal, lacrimal, urine and fecal cultures were negative. The organism was isolated from lymph nodes, spleen and tonsil at necropsy. No gross lesions were seen.

Binninger et al. (1980) reported 18/332 (5%) black bears (Ursus americanus) in Idaho were seropositive for brucellosis on the tube agglutination test, and that the prevalence was significantly higher in males. He speculated that because the males had a larger home range,

they had more opportunity for exposure. Zarnke and Yuill (1981) found B. abortus in 1/283 black bears in Alberta but did not know the source of infection.

Davis et al. (1979) conducted studies on coyotes in east central Texas where there was a high prevalence of brucellosis in cattle. Nine of 51 coyotes (18%) were seropositive and 7/43 were culture-positive for B. abortus type 1. Culture-positive results were obtained from two vaginal swabs, the gastric contents of three newborn pups, and the spleen of a female that was live-trapped and later euthanized. Transmission of brucellosis, including abortion, was demonstrated from infected coyotes to seronegative heifers in one of four experimental trials (Davis et al. 1988). Workers felt the hot, dry conditions as opposed to the previous cool, rainy conditions may have been at least partially responsible.

After several experiments, Davis concluded that coyotes could be readily infected orally if the challenge inoculum was greater than 10^9 or 10^{10} CFU, that the organism could be recovered from the feces, that vertical transmission between coyotes was more likely to occur than horizontal transmission, and that coyote to cattle transmission was not a major factor in perpetuation of the disease in cattle (D. Davis pers. comm.).

Brucellosis has been implicated serologically and bacteriologically in abortions in mink (Mustela vison) on fur farms in the Soviet Union and the U.S. (Bispins and Lollinger 1963; Dukur 1973; Prichard et al. 1971). Lesions in experimentally infected mink included plasma cell

infiltration, especially in the liver and kidney, and glomerulonephritis (Bispins and Lollinger 1963).

Foxes

Brucellosis was first reported in red foxes on fox farms in the Soviet Union in 1938 (Duker 1973). Pinigin et al. (1970b) referred to reports of serotiters in blue foxes (Alopex lagopus) on farms. Meat from brucellosis-infected farm animals was held responsible for natural infections. Studies in the 1950's demonstrated infected Vulpes females could have abortions or still births. Aborting females or sterile males had a poor appetite and a suppurative conjunctivitis. The disease caused no increase in body temperature or changes in pulse or respiration in silver foxes. Decreased hemoglobin, transient leukocytosis, enlarged thymuses and spleens, and hemorrhages in the thymuses and kidneys were reported. Regional lymph nodes were enlarged and sometimes hypertrophied. Histologic lesions in silver foxes included an "extraordinary development of lymphoid and PMN proliferates, distributed in the form of nodules along the connective tissue of various organs." Giant cell accumulations were seen in some animals. Bacteremias were extremely transient (Dukur 1973).

McCaughey (1968 and 1969) reported positive serotiters in 2/5 foxes in 1968 and in 4/32 red foxes in 1969 in England. Davies et al. (1973) detected one high serologic reaction and isolated B. abortus from one of 87 wild red foxes in West Wales in an area of two known brucellosis-infected dairy herds. Szyfres and Tome (1966) and Parnas et al. (1969) reported the isolation of B. abortus from foxes (Dusicyon

gymnocercus antiquus) and (D. griseus) in Argentina.

B. abortus type 1 was isolated from two wolves and a red fox in Wood Buffalo National Park in Canada (Tessaro 1986). They were probably exposed by preying or scavenging on cattle, elk or bison. The author felt the role of wild carnivores in maintenance of the disease was poorly understood, and that the sample size was usually too small to accurately evaluate prevalence.

Scanlan et al. (1984) experimentally infected gray foxes (Urocyon cinereoargenteus) with 4.4×10^{10} B. abortus in dog food. Foxes were necropsied at 49 days post-exposure. The organism was isolated from several lymph nodes but not from lungs, livers, spleens or kidneys. Brucella was isolated from one fox that was seronegative at necropsy. Scanlan concluded that foxes were susceptible to brucellosis but remained unclear as to the foxes' role in porcine or bovine brucellosis.

Foxes and dogs are easily susceptible to porcine brucellosis, but are considered victims more than active carriers (Milanov et al. 1966).

Red foxes infected orally with 10^9 B. canis developed a bacteremia 4-5 weeks after inoculation which persisted until necropsy at 14 weeks. Agglutination titers developed similarly to those in dogs. B. canis was recovered from the urine of one fox at necropsy. The author felt foxes were susceptible and should be considered as wild reservoirs (Pickerill 1970a).

McCaughey (1968) felt foxes were unlikely to maintain persistent infections and that they probably served as mechanical rather than

biologic spreaders. McDiarmid (1975) felt it was unlikely brucellosis infections would persist in foxes or play any important role in the general epidemiology.

Opossums and Raccoons

Schnurrenberger et al. (1985) collected serologic and bacteriologic samples from over 200 feral mammals and birds on a farm with B. abortus infected cattle calving and aborting. B. abortus type 1 was isolated from 4/14 opossums (Didelphis virginiana) and 1/6 raccoons. Serotiters were detected in 1/4 opossums, 1/1 raccoons and 1/1 gray foxes. A serologic titer was detected in only one raccoon sampled 20 months later. The authors felt these results suggested brucellosis was unlikely to be maintained within a population through intraspecies transmission. They also felt there was little evidence to suggest that wild animals transmitted the disease to cattle.

Five female and eight male opossums were later infected experimentally with 10^8 to 10^9 CFU of B. abortus type 1. The organism was isolated from feces of six animals at day 1 and from one animal on days 2 and 3 post-exposure. B. abortus was isolated from tissues and lymph nodes of ten animals at necropsy. The presence of B. abortus in tissues did not always correspond with serologic reactions. The organism was not isolated from urine or saliva. The authors concluded it was unlikely opossums were important in the transmission of B. abortus to cattle via excreta or bites (Moore and Schnurrenberger 1981a).

Rodents

Many authors state that rodents act as scavengers and are capable of

transmitting brucellosis to domestic animals through their excreta by contaminating pastures, food and water (Cook et al. 1966; Fitch and Bishop 1938; Karkadinovsky 1936; Lord and Flores 1983; McCaughey 1968; McDiarmid 1975; Rementsova 1962b; Salem et al. 1975; Verger 1970). Seropositive susliks (formerly Citellus; currently Spermophilus) were repeatedly seen eating aborted fetal remains in areas of brucellosis-infected cattle (Rementsova 1962b).

Others believe that although rodents and small mammals become infected through association with infected domestic animals, evidence does not support transmission in reverse (Boerr et al. 1980; Bosworth 1940; Verger 1972).

Vest et al. (1965) detected serologic reactions in only 28 of over 16,000 rodents, lagomorphs and birds sampled in western Utah. Thorpe (1967) concluded from experimental infections that deer mice (Peromyscus maniculatus), pinyon mice (P. truei), and montane meadow mice (Microtus montanus) were relatively susceptible to all strains of Brucella; that desert wood rats, Ord and chisel-toothed kangaroo rats (Dipodomys ordii and D. microps), white-tailed antelope squirrels (Ammospermophilus leucurus), and black-tailed jack rabbits (Lepus californicus) were more resistant. He felt rodents could easily become infected in nature, but that there was no evidence to show that they passed the organisms in their excreta or other body wastes.

Menton (1937) tested 200 rats from infected farms and from a slaughterhouse where infected animals were killed and found no positive or suspicious agglutination reactions. Bosworth (1937) said the wild rat

was more resistant to brucellosis than the white rat, and that rats tended to eliminate the disease fairly rapidly from their systems. McCaughey (1968) and McDiarmid (1975) agreed that rats were relatively resistant to B. abortus in that infections required a high inoculum. Renoux (1985) stated the gray rat (Rattus norvegicus) was susceptible to B. melitensis in nature and that it could be a carrier, but only if it lived in a highly infected environment. McCaughey (1968) felt that if the disease were eliminated in livestock, it was unlikely to persist in rats.

Meyer (1974) stated that brucellosis in rodents was self-limiting and that such animals have not become a reservoir for infection. In a later publication she stated that all the available evidence indicated Brucella travelled to, not from, rodents. "Recent evidence shows the population density of rodents is insufficient to support continuous transmission of the organisms, especially with fluctuating cycles" (Meyer 1976).

Miscellaneous

Galouzo (1958) reported that brucellosis could be transmitted by blood sucking arthropods. Pinigin and Zabrodin (1970) reported that ticks (Dermacentor nuttalli) retained Brucella organisms during metamorphosis from the larval to adult stage, and that the organism could be transmitted by ticks from infected to non-infected guinea pigs. Rementsova (1962a) isolated Brucella from ticks taken from cattle and herd dogs. Renoux (1957) concluded that insects could be implicated in the transmissions of brucellosis to domestic animals and man.

BRUCELLA SUIS TYPE 4 IN ATYPICAL HOSTS

Ungulates, Carnivores and Wild Rodents

Davidov (1974) reported that rangiferine strains were less pathogenic for sheep than B. melitensis, less pathogenic for swine than B. suis, and less pathogenic for cattle than B. abortus.

Grekova and Gorban (1978) reported numerous references to spontaneous infections of wild and game animals with organisms characteristic of B. suis type 4. They added that cultures from arctic fox, ermine (Mustela erminea), wolves and reindeer were extremely pathogenic for guinea pigs.

Pinigin and Zabrodin (1970) cultured B. suis type 4 from 12/110 wolves, 12/370 arctic foxes, and 1/9 wolverines (Gulo gulo), but from none of 50 lemmings (Lemmus obensis), 19 muskrats (Ondatra zibethica) or 6 sables (Martes zibellina). Zabrodin (1970) felt both wild and domestic reindeer were the main reservoirs of infection. In other studies, Pinigin et al. (1970b) reported on the isolation of the reindeer organism from 9/530 arctic foxes, and from three dogs associated with reindeer (Pinigin et al. 1970a).

Petukhova et al. (1971) reported isolating the reindeer organism from 15 wild reindeer, 6 wolves, 10 arctic foxes and 1 muskrat. He also felt they had acquired the infections from domestic reindeer.

Neiland (1970 and 1975) found positive serologic reactions in sled dogs being fed reindeer or caribou. B. suis type 4 was isolated from lymph nodes, but no lesions were seen at necropsy.

Serotiters were detected in 10/22 (45%) wolves sampled in the Brooks Range, in 16/17 (94%) grizzly bears sampled from the western Brooks Range, and 10/21 grizzly bears from the eastern Brooks Range (Neiland 1975). Zarnke (1983) later reported a higher prevalence of brucellosis in grizzly bears from the North Slope than in grizzly and black bears from southcentral Alaska which correlated with the corresponding prevalence in caribou. One of five red foxes from Anaktuvuk Pass and none of four from the Seward Peninsula were seropositive (Neiland 1975). Zarnke and Ballard (1987) reported a higher prevalence of brucellosis in wolves that preyed on caribou than in wolves that preyed on moose.

Neiland and Miller (1981) experimentally infected dogs (beagle hounds), wolves, a black bear and two grizzly bears with B. suis type 4. Typical serologic responses were seen, and the organism was isolated at necropsy. Neiland linked reproductive problems in the wolves to the infection.

Rausch (1978) demonstrated serotiters in non-infected cattle in contact with reindeer experimentally infected with B. suis type 4.

Rodents - Experimental Infections

Miller and Neiland (1980) experimentally infected several species of rodents indigenous to Alaska. All animals were infected by the intraperitoneal route. Lemmings (Dicrostonyx stevensoni and D. rubricatus) were the most susceptible with fatalities occurring with doses as low as 2 - 20 CFU. Abscesses were seen on the livers and spleens; organisms were isolated from liver, spleen, kidney, heart and urine. Splenic abscesses

were seen in ground squirrels and flying squirrels (Glaucomys sabrinus). Liver abscesses were seen at day 14 post-inoculation and kidney abscesses at day 80 in red-backed voles (Clethrionomys rutilis). No lesions were seen in yellow-cheeked voles (Microtus xanthognatus), but they also received a lower infective dose. Gorban (1977) reported a generalized infectious process in the tundra vole (Microtus oeconomus) infected with 50 cells of B. suis type 4.

Miscellaneous

B. suis type 4 was cultured from reindeer warble fly larvae (Oedemagena tarandi) by Soviet workers. In field studies, the warble fly was implicated in transmitting the disease from infected to healthy reindeer (Vashkevich 1978).

Available literature on brucellosis in typical hosts and atypical hosts including wildlife supports the hypotheses that (1) the reindeer/caribou organism, B. suis type 4, is incidentally transmitted to reindeer predators such as foxes but does not cause reproductive disease in them, and (2) infected predators such as foxes are terminal hosts and do not serve as reservoirs of infection for reindeer. The following studies were conducted to test these hypotheses.

METHODS AND MATERIALS

FIELD STUDIES

Animal Collections

Thirty-four red foxes, four arctic foxes, eight grizzly bears, three arctic ground squirrels, one river otter (Lutra canadensis), one wolverine (Gulo gulo), and two arctic hares (Lepus othus) were collected on the fawning grounds of a brucellosis-infected reindeer herd 17 km north of Nome in April and May from 1977 to 1983. Animals were shot and necropsied as soon as possible.

Serology

Blood was collected by jugular venipuncture or heart puncture. Serum was harvested and frozen for later serologic testing. The standard plate (SP), buffered Brucella antigen (BBA), and rivanol (Riv) tests were conducted at the University of Alaska Fairbanks using standard procedures (U.S. Department of Agriculture a,b). The standard tube (ST), 2-mercaptoethanol (ME), and complement fixation (CF) tests were conducted at the National Animal Disease Center, Ames, Iowa, in 1977 and 1978. Procedures used for the ST and ME were done according to standard methods (U.S. Department of Agriculture a,b); the CF test was done according to Hill (1963). From 1979 to 1984 the CF test was conducted by Margaret Meyer, University of California, Davis, using automated methods. Serology for B. canis was conducted at the Alaska State/Federal

Laboratory, Palmer, Alaska, using the salt-ME tube-test method.

Bacteriology

Mandibular, popliteal, and internal iliac lymph nodes, and portions of heart, liver, lung, kidney, spleen, tonsils, and reproductive tracts were collected as available from foxes for bacteriologic examination. Tissues collected from grizzly bears included mandibular, retropharyngeal, popliteal, superficial cervical, femoral, internal iliac, and superficial inguinal or supramammary lymph nodes, testes, epididymides, uterus, cervix, heart, liver, lung, kidney, and spleen. Heart, liver, lung, kidney, spleen, and testes or uterus were collected from the wolverine, three arctic ground squirrels, and one arctic hare. Mandibular and popliteal lymph nodes, spleen, liver, testes, and epididymides were cultured from the other hare. No tissues were collected from the river otter.

Tissues were collected as soon as possible after death and frozen. Culturing was conducted later in an isolation suite in the Arctic Health Research Building, University of Alaska Fairbanks, using standard methods (U.S. Department of Agriculture c).

Tissues were dipped in alcohol, flamed or air-dried, halved, the surface minced, and inoculated onto a plate of trypticase soy agar (TSA)[1] and a more selective medium containing (per liter) tryptose broth[2] - 25 g; agar[3] - 20 g; Tergitol 7 - 0.15 ml; Tween 40 - 25 ml;

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[1]Trypticase Soy Agar, Baltimore Biological Laboratories, Cockeysville, MD.
[2]Tryptose Broth, Difco Laboratories, Detroit, MI.
[3]Bacto-Agar, Difco Laboratories, Detroit, MI.

broth[2] - 25 g; agar[3] - 20 g; Tergitol 7 - 0.15 ml; Tween 40 - 25 ml; ethyl violet- 1.4 mg; sodium lauryl sulfate-1.44 g; and CNV[4] - 500 mg (B. Deyoe, pers. comm.). Plates were incubated at 37° C under atmospheric conditions. Representative isolates were submitted to the National Animal Disease Center, Ames, Iowa, for confirmation as being typical of B. suis type 4.

Portions of tissues from four Vulpes sampled in 1979 were pooled and homogenized in tryptose broth in a Ten Broek tissue grinder or Virtis blender. A suspension (1 ml) was inoculated intraperitoneally into one guinea pig, and 0.25 ml was inoculated intraperitoneally into each of two lemmings (Dicrostonyx rubricatus) according to Alton et al. (1975). Guinea pigs were sacrificed at 3 weeks post-inoculation (PI) and lemmings at 5 weeks. Heart, liver, lung, kidney, spleen, and reproductive tracts were cultured for B. suis type 4 according to standard methods (U.S. Department of Agriculture c). Blood was collected by heart puncture, serum harvested, and serologic tests performed as previously described.

Histopathology

Portions of heart, liver, lung, kidney, spleen, and reproductive tract were preserved in 10% buffered formalin. Slides were prepared by Bay Histology, San Rafael, California. Tissues were stained with hemotoxylin and eosin (H & E) and by Brown and Brenn's technique for bacteria.

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[2]Tryptose Broth, Difco Laboratories, Detroit, MI.
[3]Bacto-Agar, Difco Laboratories, Detroit, MI.
[4]Cholestimethate, nystatin, vancomycin, Difco Laboratories, Detroit, MI.

LABORATORY INFECTIONS - Part 1

Animals

Three young (estimated age of 12 to 14 weeks) male red foxes were trapped near Delta Junction, Alaska, during the summer of 1981. A mature female was obtained from the Institute of Arctic Biology, University of Alaska Fairbanks. Blood samples were collected by jugular venipuncture for pre-exposure brucellosis serology, complete blood counts (CBC's), and serum chemistry profiles. Serologic tests were negative; hematologic values (packed cell volume PCV , white blood count WBC , and differential WBC) and serum chemistry values were within reference values.

All foxes were vaccinated for canine distemper, infectious canine hepatitis, leptospirosis, parvovirus[5], and rabies[6]. Foxes were treated with dichlorvos[7] and praziquantel[8] for intestinal parasites.

Housing

All four foxes were individually housed in dog cages (2 m x 1 m x 0.75 m) in an animal holding room in the Arctic Health Research Building, University of Alaska Fairbanks, for several months to become acclimated to captivity and handling. In 1982, during the breeding season for Vulpes in interior Alaska (January through March), one male

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[5]Adenomune 7-L, Tech America, Omaha, NE.
[6]Rabguard TC, Norden Laboratories, Lincoln, NE.
[7]Task, Solvay Veterinary Inc., P.O. Box 7348, Princeton, NJ.
[8]Droncit, Haver, Mobay Corporation, Animal Health Division, Shawnee, KS.

natural conditions. Foxes were fed a mixture of dry cat food[9] and canned dog food[10]. An effort was made to curtail disturbances in order to keep stress to a minimum.

During the third week of March, the foxes were transferred to individual cages (0.9 m x 1 m x 1.1 m) in the the canine isolation area. Reduced air pressure was maintained in the isolation area, and exhaust air was passed through absolute filters. Personnel entered through shower-in-shower-out air locks and wore protective clothing. A pass-through autoclave and sewage kill tanks were used to prevent environmental contamination.

Challenge Inoculum

B. suis type 4 used for experimental infections was originally isolated from a reindeer and had been used in several experimental infections in reindeer in other studies at the University of Alaska. Several cultures of this isolate had been stored as slants covered with milk in a freezer at -60° C. Before being used in foxes, a slant of the organism was thawed and transferred to fresh media.

For all animal inoculations, 48-hour slants of the B. suis type 4 were washed with sterile physiological saline and adjusted to 44% transmittance on a Klett-Summerson colorimeter with a blue filter No. KS-42. One ml of a 10^{-4} dilution of this suspension was inoculated intraperitoneally into each of three guinea pigs. Standard plate counts were done on the suspensions to determine the number of organisms in the

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[9]Ralston Purina Co., Checkerboard Square, St. Louis, MI.
[10]Alamo Products, Co., P.O. Box 4500, Lehigh Valley, PA.

were done on the suspensions to determine the number of organisms in the challenge dose.

Guinea pigs were necropsied at 8, 9, and 11 weeks post-inoculation (PI). Serologic tests (SP, BBA, and Riv) were conducted on available serum collected at necropsy. Gross lesions were noted. Heart, liver, lung, kidney, spleen, prefemoral lymph node, and testes or uterus were collected for bacteriologic culturing. Portions of these tissues were preserved in 10% buffered formalin for histologic examination.

Experimental Protocol

During the third week of March 1982, the male and female fox previously housed together were separated and each fed 50 g of hamburger containing approximately 10^7 CFU of B. suis type 4 on each of four consecutive days for a total infective dose of 8.34×10^7 CFU. The two males housed individually were each fed 50 g of hamburger containing approximately 10^9 CFU of B. suis type 4 on each of four consecutive days for a total infective dose of 4.9×10^9 CFU.

Serology/ Bacteriology/ Hematology

Blood samples were collected by jugular venipuncture weekly or bi-weekly for serology, bacteriology, and a CBC. Foxes were sedated with 10-15 mg zylazine[11] and 0.25- 0.37 mg atropine[12] for sampling.

Serologic tests were conducted as previously described. Blood (5 ml) for hemoculture was inoculated into a trypticase soy agar slant

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[11]Rompun, Haver-Lockhart, Bayvet Division, Cutter Laboratories, Inc., Shawnee, KS.
[12]Atropine Sulfate, Anpro Pharmaceutical, Arcadia, CA.

Hemocultures were incubated at 37°C under atmospheric conditions for 5 weeks before being discarded as negative. Bacterial growth was subcultured on TSA plates for further identification.

Shedding of B. suis type 4

To monitor shedding of the organism, oral and vaginal or seminal fluid/urine swabs were taken for bacteriologic culturing when the animals were handled for blood collection. Urine was collected from the cages whenever a relatively fresh sample could be obtained. Swabs were cultured on a TSA plate and also on a plate of selective media.

Feces were collected daily for the first few weeks. For culturing, 1 g of feces was placed in 9 ml of sterile peptone-saline (1% peptone and 0.5% sodium chloride) and mixed well. After being refrigerated at 4°C for 30 minutes, the samples were centrifuged at 2500 rpm for 90 minutes. One loopful of supernatant fluid was inoculated on a plate of TSA and another loopful inoculated on selective media previously described. The supernatant fluid was decanted and the pellet resuspended in 1 ml sterile peptone-saline. One loopful was inoculated on a plate of TSA and another loopful on the selective media. Plates were incubated at 37°C under atmospheric conditions (D. Perry, pers. comm.).

Lemmings

To biologically monitor shedding of brucellae from the foxes, lemmings (Dicrostonyx rubricatus) were housed in shallow pans with gridwork lids which were placed below the grates of the fox cages. Lemmings had been considered to be very susceptible to B. suis type 4 (B. Deyoe, pers. comm.), and it was thought organisms shed by the

foxes could be detected better by lemmings becoming infected through exposure to the fox urine than by standard culture methods on samples from the foxes. The lemmings were kept below the foxes for approximately 2 weeks, removed to be held in another room for an additional 3 to 4 weeks, and a replacement lemming introduced. Blood was collected by heart puncture at necropsy for serologic evaluation. Tissues collected for bacteriology included heart, liver, lung, kidney, spleen, and testes or uterus. Portions of these tissues were preserved in 10% buffered formalin for histopathologic examination.

Necropsy

Foxes were sacrificed by intravenous injection of T-61[13] at various intervals from 7 to 22 weeks post-exposure (PE). Tissues collected for bacteriologic examination included mandibular, retropharyngeal, superficial cervical, femoral, popliteal, internal iliac, mesenteric, mediastinal, tracheobronchial, supramammary or superficial inguinal lymph nodes, and portions of heart, liver, lung, kidney, spleen, tonsils, salivary glands, reproductive organs, and bladder. Tissues were cultured as previously described according to standard procedures.

Portions of collected tissues were preserved in 10% buffered formalin for microscopic examination as previously described.

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[13]Euthanasia Solution, Hoechst-Roussel, Agri-Vet Company, Somerville, NJ.

LABORATORY INFECTIONS - Part 2

Animals

Four young (estimated 12 - 14 weeks) red foxes (three males and one female) were trapped near Delta Junction, Alaska, during the summer of 1982. In addition, one young female red fox was obtained from the U.S. Fish and Wildlife Service after having been confiscated from a local trapper in October 1982. One male red fox was donated by a local trapper in January 1983. Other color phases of Vulpes vulpes including two adult male silver foxes, two adult female silver foxes, one adult female amber, and one adult female pearl fox were purchased from a local fox farmer in December 1982. The four silver foxes were considered good breeding stock.

Blood was collected from all foxes for pre-exposure brucellosis serology, CBC's, and serum chemistry profiles. Serologic tests were negative; hematologic and serum chemistry values were within reference values. All foxes were vaccinated and treated for parasites as previously described.

Housing

Foxes were housed in individual cages (2 m x 1 m x 0.75 m) in animal holding rooms in the Arctic Health Research Building, University of Alaska Fairbanks, until January 1983. Males and females were housed together as six pairs from January through early April to include the normal breeding season for Vulpes in interior Alaska (January through March). Light-dark cycles in the animal rooms were adjusted to simulate

natural conditions. Foxes were fed as previously described.

An effort was made to minimize excitement and stress at all times. Vulvar swelling and redness, indicators of the estrous cycle, were not monitored on a regular basis during the time the foxes were housed as pairs (January through early April) in preference to reducing the stress caused by additional handling. Activities of each pair were recorded on video tape for a few hours on alternate days. Video tapes were observed for behavior associated with breeding. Semen was collected from each male in late March by digital manipulation and examined for quality. Vulvas of all females were checked at that time. Following challenge exposure, the vulvas were examined for redness and swelling, and the testes were examined for firmness (an indication of sexual activity) or swelling (an indication or orchitis or epididymitis) whenever the foxes were handled for serologic or bacteriologic sampling.

Challenge Inoculum

The challenge organism had recently been passed through guinea pigs as previously described to confirm its virulence.

To further verify virulence of the challenge organism, a guinea pig was challenged intraperitoneally with 3.0×10^4 CFU of the first day's challenge inoculum. A second guinea pig was challenged intraperitoneally with 2.7×10^4 CFU of the second day's challenge inoculum. A third guinea pig and a lemming were similarly challenged with 2.4×10^4 and 2.6×10^3 CFU, respectively, on the third day. On the fourth day, one guinea pig was inoculated intraperitoneally with 2.5×10^4 CFU, one lemming intraperitoneally with 2.5×10^3 CFU, and one lemming subcutane-

ously with 2.5 x 10^3 CFU.

Lemmings were sacrificed at approximately 3 weeks and guinea pigs at 5 weeks PI. Blood was collected by heart puncture for serology. Tissues cultured for B. suis type 4 included heart, liver, lung, kidney, spleen, prefemoral lymph node, and bladder. Portions of tissues were fixed in 10% buffered formalin for microscopic examination as previously described.

Experimental Protocol

In early April foxes were transferred to individual cages in the canine isolation area as previously described. Four foxes (two males and two females) held in one room were each fed approximately 10^8 CFU B. suis type 4 in 50 g of hamburger on each of four consecutive days for a total infective dose of 1.21 x 10^9 CFU. In a second room, two males and two females were each fed approximately 10^{10} CFU B. suis type 4 in 50 g of hamburger on each of four consecutive days for a total infective dose of 1.06 x 10^{11} CFU.

To standardize housing conditions, and because only two rooms with carnivore cages were available in the isolation area, one male and one female fox were placed in additional individual cages in the isolation room with the four foxes challenged with 10^{11} CFU B. suis type 4 to serve as non-challenged reproductive, clinical, and histopathologic controls.

Serology/ Bacteriology/ Hematology

Samples for serologic, bacteriologic, and hematologic examination were collected as described in the previous experiment. However, foxes

used in this experiment were not chemically immobilized prior to sampling. Every effort was made to consistently handle each fox with as little excitement and stress as possible.

Shedding of B. suis type 4

To monitor shedding of the organism, oral, vaginal, and seminal fluid/urine swabs were collected and cultured as previously described.

Feces were collected daily for the first few weeks and cultured as previously described.

Lemmings

To biologically monitor shedding of brucella organisms, lemmings were placed in shallow pans beneath the cages of all experimentally challenged foxes as previously described. Tissues cultured included heart, liver, lung, kidney, spleen, testes or uterus, mandibular lymph node, salivary gland, and bladder.

Necropsy

Foxes were sacrificed by intravenous injection of sodium pentobarbital[14] at varying intervals from 3 to 18 weeks PE. Tissues were collected for bacteriologic and histologic examination as previously described.

[14]Euthanol-6, Veterinary Companies of America, Tempe, AZ.

ORAL INFECTIVE DOSE OF BRUCELLA SUIS TYPE 4 FOR LEMMINGS

A retrospective study was conducted to determine the number of orally administered B. suis type 4 organisms required to produce infection in lemmings.

Two lemmings received 3.7×10^6, two received 3.7×10^4, two received 3.7×10^2, and two received 3.7 CFU B. suis type 4 in normal saline as drops in the mouth. One lemming in each group was sacrificed 4 weeks PE, and the other in each group at 5 weeks.

Blood was collected by heart puncture for serology. Heart, liver, lung, kidney, spleen, urine, salivary gland, and reproductive organs were cultured for the presence of B. suis type 4 organisms. Portions of these tissues were preserved in 10% buffered formalin for histopathologic examination and stained as previously described.

FOX TO REINDEER TRANSMISSION

Experimental Protocol

In mid-April one male and one female fox held together as a breeding pair were each fed 50 g of hamburger containing approximately 10^{10} CFU B. suis type 4 on each of four consecutive days for a total challenge exposure of 1.06×10^{11} CFU. They were then transferred to an animal containment room (10.5 m^2) with two adult male reindeer previously determined to be serologically negative for brucellosis. All four animals had unrestricted movement within the room. Two wooden nest boxes were provided within the room for the foxes. Light-dark cycles were adjusted to simulate natural conditions. Feed and water were provided ad libitum. Fox feed was as previously described. Reindeer feed consisted of a commercial grain and pellet mixture[15].

Serology/ Bacteriology/ Hematology

Samples for bacteriologic, serologic, and hematologic examination were collected as described for foxes in other phases of the study. Blood samples were collected by jugular venipuncture weekly or bi-weekly from the reindeer for serologic examination and hemoculture. Animals were not sedated for sampling.

Necropsy

Both reindeer were sacrificed after 4 months by a captive bolt shot in the head. Tissues collected for bacteriologic examination included

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[15]Quality Texture, Fisher Mills Inc., Seattle, WA.

retropharyngeal, mandibular, parotid, superficial cervical, subiliac, popliteal, medial iliac, mesenteric, mediastinal, tracheobronchial, and superficial inguinal lymph nodes, and portions of heart, liver, lung, kidney, spleen, biceps femoris muscle, testes, epididymides, seminal vesicles, urine, and salivary gland. Tissues were cultured as previously described.

Portions of heart, liver, lung, kidney, spleen, and muscle tissue were collected from the reindeer for histologic examination as previously described.

The male and female fox were housed together as a pair until July the following year (1984) when they were both sacrificed. Samples were collected at necropsy as previously described.

Offspring of Foxes

Three surviving pups were born to the female fox in early June 1983. The mother and her young were then placed in a separate room from the male and the two reindeer. When the young were 6 weeks of age, the mother was returned to the room with the male.

Samples for serologic and bacteriologic examination were collected from the pups after 6 weeks as described for the other foxes. They were sacrificed at 3, 4, and 13 months of age, and tissues were collected for culture and histopathologic examination.

RESULTS

NATURAL INFECTIONS

Serology

Animals collected in the field were considered seropositive for brucellosis if the reaction was ≥ +25 on the ST or SP, ≥ I25 (incomplete at 1:25) on the Riv or ME tests, ≥ 1@20 on the CF test (according to Hill), > 19 on the automated CF test, or positive on the BBA. Due to limited quantities of serum, not every test was run on each sample.

Thirteen of 34 (38%) red foxes collected from 1977 to 1983 were serologically positive by these criteria (Tables 1 and 2). The ST test was conducted on six samples and was considered positive on one (17%) of those. The SP test was positive on 11 of 32 (34%) samples; the BBA on 8 of 31 (26%); the ME on 1 of 6 (17%); the Riv on 5 of 29 (17%); and the CF test on 6 of 26 (23%). No significant differences were detected among frequencies of tests being considered positive (chi-square; $p>0.01$). Three of 19 (16%) samples were considered suspicious for *B. canis* antibodies.

There was no significant difference between the number of male (7/14) and female (6/20) red foxes that were seropositive (chi-square; $p>0.01$).

Two of four (50%) arctic foxes collected in 1980 were seropositive (Table 3). The SP test was considered positive on two of the four (50%)

Table 1. Serologic test results for brucellosis in red foxes collected near Nome, Alaska, 1977-1980.

Pathology Number	Sex	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF	*B. canis*[1]
2558	M	+100	I25	+	I200	I50	3@200[2]	NT[3]
2561	F	N25	+200	NT	N25	N25	0@10	NT
2564	M	NT	+50	+	NT	NT	NT	NT
2565	M	N25	+200	-	N25	N25	0@10	NT
2569	M	N25	N25	-	N25	N25	0@10	NT
2749	F	NT	NT	NT	NT	NT	NT	NT
2877	F	NT	+25	-	NT	N25	124	NT
2879	M	NT	+50	+	NT	NT	19	NT
2880	M	N25	N25	+	N25	N25	0	NT
2906	F	NT	+25	-	NT	NT	0	NT
2911	M	N25	N25	-	N25	N25	0	NT
3082	F	NT	+400	+	NT	+400	87	N25
3084	M	NT	I100	-	NT	N25	NT	I50
3085	F	NT	N25	-	NT	N25	0	N25
3087	F	NT	N25	-	NT	N25	NT	NT
3098	F	NT	N25	-	NT	N25	0	NT
3115	F	NT	NT	NT	NT	NT	NT	NT

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
[2] Expressed as degree of reaction @ given dilution
[3] Not tested
[4] Numerical reaction on automated CF test

Table 2. Serologic test results for brucellosis in red foxes collected near Nome, Alaska, 1981-1983.

Pathology Number	Sex	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	*B. canis*[1]
3210	F	NT[3]	N25	-	NT	N25	3	N25
3211	M	NT	N25	-	NT	N25	0	N25
3212	M	NT	N25	-	NT	N25	NT	N25
3216	M	NT	+200	+	NT	+400	80	N25
3217	F	NT	N25	-	NT	N25	1	N25
3218	M	NT	N25	-	NT	N25	3	N25
3238	F	NT	N25	-	NT	N25	1	N25
3239	F	NT	N25	-	NT	N25	2	N25
3356	F	NT	N25	-	NT	N25	NT	N25
3357	M	NT	N25	-	NT	N25	NT	N25
3365	M	NT	N25	-	NT	N25	4	N25
3367	F	NT	N25	-	NT	N25	0	N25
3371	F	NT	+50	+	NT	+400	83	N25
3372	F	NT	N25	-	NT	N25	0	N25
3373	F	NT	N25	-	NT	N25	1	+100
3380	F	NT	+400	+	NT	+400	84	+100
3494	F	NT	N25	-	NT	N25	4	NT

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
[2] Numerical reaction on automated test
[3] Not tested

Table 3. Serologic test results for brucellosis in arctic foxes collected near Nome, Alaska, 1980.

Pathology Number	Sex	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	*B. canis*[1]
3089	F	NT[3]	I50	+	NT	N25	0	+100
3091	M	NT	N25	-	NT	N25	0	N25
3095	F	NT	+100	-	NT	N25	0	N25
3096	M	NT	N25	-	NT	N25	9	N25

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25 dilution
[2] Numerical reaction on automated test
[3] Not tested

foxes tested; the BBA test was positive on one of four (25%); and the Riv and CF tests were negative on all four animals. The serologic titer on one female was considered suspicious for B. canis antibodies. Both of the seropositive arctic foxes were females; neither was pregnant nor showed signs of recent whelping.

Comparing pairs of the primary serologic tests on all foxes as both being either positive or negative on the same sample, the Riv and CF tests agreed 100% of the time; the BBA and Riv tests, 94% of the time; the BBA and CF tests, 93%; the SP and Riv, 82%; the SP and BBA, 80%; and the SP and CF tests, 79% of the time.

Two of eight (25%) grizzly bears collected from 1979 - 1981 were seropositive (Table 4). No significant differences existed among the frequencies of serologic tests considered positive (chi-square; p 0.01). The SP test was considered positive on two of eight (25%); the ME test on one of four (25%); the Riv test on two of eight (25%); and the CF test on one of eight (12.5%). Serotiters were not detected on the BBA test. Titers were negative on all three samples tested for B. canis antibodies. Both seropositive bears were males.

Two of three male arctic ground squirrels were seropositive (Table 5). The SP test was positive on two of three and the BBA on one of three. The Riv test was negative on one sample on which it was run; the CF test was not run on any samples.

The otter, wolverine and two arctic hares were seronegative on all tests (Table 5).

For all groups of animals tested, the better agreement between the

Table 4. Serologic test results for brucellosis in grizzly bears collected near Nome, Alaska, 1978-1981.

Pathology Number	Sex	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF	*B. canis*[1]
2745	F	N25	N25	-	N25	N25	0	NT[2]
2746	F	N25	N25	-	N25	N25	0	NT
2750	M	N25	N25	-	N25	N25	0	NT
2752	M	+100	I50	-	+50	+50	2@100[3]	NT
3097	F	NT	N25	-	NT	N25	0	NT
3240	M	NT	N25	-	NT	N25	0	N25
3241	M	NT	N25	-	NT	N25	0	N25
3246	M	NT	+400	-	NT	+100	4[4]	N25

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25 dilution
[2] Not tested
[3] Expressed as degree of reaction @ given dilution
[4] Numerical reaction on automated test

Table 5. Serologic test results for brucellosis in miscellaneous mammals collected near Nome, Alaska, 1979-1981.

Pathology Number	Species	Sex	SP[1]	BBA	RIV[1]	CF[2]
2913	Arctic ground squirrel	M	+400	+	NT[3]	NT
2914	Arctic ground squirrel	M	I200	_	NT	NT
3242	Arctic ground squirrel	M	N25	-	N25	NT
3214	River otter	F	N25	-	N25	1
3215	Wolverine	M	N25	-	N25	4
3219	Arctic hare	F	N25	-	N25	0
3369	Arctic hare	M	N25	-	N25	0

1 += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
2 Numerical reaction on automated test
3 Not tested

BBA and Riv, BBA and CF, or Riv and CF than between the SP and BBA, Riv, or CF was consistent with the nature of the tests. The SP test is designed to detect both IgM and IgG, but the others are designed to be more specific for IgG.

In summary, of a total of 38 foxes collected, 15 were seropositive and 21 were seronegative by the defined criteria. Serologic data was not available for two. In addition, two of seven grizzly bears and two of three arctic ground squirrels were seropositive. An otter, wolverine, and two arctic hares were seronegative for brucellosis.

Bacteriology

Culture results from red foxes collected from 1977-1983 are presented in Tables 6-11. B. suis type 4 was not isolated from tissues of red foxes collected in 1977, 1978, 1979, 1981 or 1983 (Tables 6, 7 9 and 11). The organism was recovered from the iliac lymph node of one female (#3082) in 1980 (Table 8). In 1982 B. suis type 4 was isolated from lymph nodes, spleen and tonsils of two females (#'s 3372 and 3380) and also from an ovary of one of those (#3380) (Table 10). Numbers 3082 and 3372 were pregnant and #3380 was not at the time of collection.

B. suis type 4 was isolated from the liver, lung and spleen of one female arctic fox collected in 1980 (Table 12).

None of the tissues from guinea pigs or lemmings inoculated with pooled tissue homogenates of red foxes collected in 1979 were culture-positive for B. suis type 4 (Table 13).

Lymph nodes and organs collected from grizzly bears from 1978 - 1981 were all culture-negative for B. suis type 4 (Table 14). Likewise,

Table 6. Culture of B. suis type 4 from tissues of red foxes collected near Nome, Alaska, 1977-1978.

Tissue	Sex	Pathology Number 2558 M	2561 F	2564 M	2565 M	2569 M	2749 F
Heart		-[1]	-	-	-	-	-
Liver		-	-	-	-	-	-
Lung		-	-	-	-	-	-
Kidney		-	-	-	-	-	-
Spleen		-	-	-	-	-	-
Testis/ Uterus		-	-	-	-	-	-
Mandibular L.N.		-	-	-	-	-	-
Popliteal L.N.		-	-	-	-	-	-
Int. Iliac L.N.		-	-	-	-	-	-

[1] Negative results

Table 7. Culture of B. suis type 4 from tissues of red foxes collected near Nome, Alaska, 1979.

Tissue	Sex	Pathology Number 2877[1] F	2879[1] M	2880[1] M	2906[1] F	2911 M
Heart		-[2]	-	-	-	-
Liver		-	-	-	-	-
Lung		-	-	-	-	-
Kidney		-	-	-	-	NE[3]
Spleen		-	-	-	-	-
Testis/ Uterus		-	-	-	-	-
Mandibular L.N.		-	-	-	-	NE
Retropharyngeal L.N.		NE	NE	-	NE	NE
Popliteal L.N.		NE	NE	-	-	NE
Int. Iliac L.N.		NE	NE	-	-	NE

[1] Tissue homogenates later inoculated into lemmings
[2] Negative results
[3] Not examined

Table 8. Culture of B. suis type 4 from tissues of red foxes collected near Nome, Alaska, 1980.

Tissue	Sex	Pathology Number 3082 F	3084 M	3085 F	3087 F	3098 F	3115 F
Heart		NE[1]	NE	NE	-[2]	NE	NE
Liver		-	-	-	NE	-	NE
Lung		NE	NE	-	-	-	NE
Kidney		-	-	-	NE	NE	NE
Spleen		-	-	-	NE	-	NE
Testis/ Uterus		-	-	-	NE	-	NE
Amnionic fluid		-		NE	NE	NE	NE
Prostate			-				
Mandibular L.N.		NE	NE	NE	NE	NE	NE
Popliteal L.N.		NE	NE	NE	NE	NE	NE
Int. Iliac L.N.		+	NE	NE	NE	NE	NE

1 Not examined
2 Negative results

Table 9. Culture of B. suis type 4 from tissues of red foxes collected near Nome, Alaska, 1981.

Tissue	Sex	Pathology Number 3210 F	3211 M	3212 M	3216 M	3217 F	3218 M	3238 F	3239 F
Heart		NE[1]	NE	NE	-[2]	NE	NE	NE	NE
Liver		-	-	-	-	-	-	NE	NE
Lung		NE	NE	NE	-	-	-	-	NE
Kidney		-	-	-	-	-	-	-	NE
Spleen		-	-	NE	-	-	-	NE	-
Testis/ Uterus		-	-	-	-	-	-	-	-
Amnionic fluid		NE				NE		-	-
Prostate			-	-	NE		NE		
Mandibular L.N.		-	-	-	-	-	-	-	-
Popliteal L.N.		NE	NE	-	-	-	NE	-	-
Int. Iliac L.N.		-	NE	-	-	-	-	-	-
Mediastinal L.N.		NE	NE	NE	NE	-	-	NE	NE

[1] Not examined
[2] Negative results

Table 10. Culture of B. suis type 4 from tissues of red foxes collected near Nome, Alaska, 1982.

		Pathology Number							
Tissue	Sex	3356 F	3357 M	3365 M	3367 F	3371 F	3372 F	3373 F	3380 F
Heart		NE[1]	NE	NE	NE	NE	NE	NE	NE
Liver		-[2]	-	-	NE	NE	NE	NE	NE
Lung		NE	NE	-	NE	NE	NE	NE	NE
Kidney		-	-	NE	NE	NE	NE	NE	NE
Spleen		-	-	-	-	-	+	-	+
Testis/ Uterus		-	-	-	NE	-	-	-	-
Amnionic fluid		NE			NE	-	-	NE	NE
Epididymis			-	-					
Ovary		-			NE	NE	NE	-	+
Cervix		-			NE	NE	NE	-	NE
Mandibular L.N.		-	-	-	-	-	+	-	+
Popliteal L.N.		NE	NE	-	-	-	+	-	NE
Int. Iliac L.N.		NE	NE	-	NE	-	+	-	+
Tonsil		NE	NE	-	-	NE	+	NE	+

[1] Not examined
[2] Negative results

Table 11. Culture of B. suis type 4 from tissues of a red fox collected near Nome, Alaska, 1983.

	Pathology Number	
Tissue	Sex	3494 F
Liver		-1
Lung		-
Kidney		-
Spleen		-
Uterus		-
Amnionic fluid		-
Mandibular L.N.		-
Popliteal L.N.		-
Int. Iliac L.N.		-
Tonsil		-

[1] Negative results

Table 12. Culture of B. suis type 4 from tissues of arctic foxes collected near Nome, Alaska, 1980.

		Pathology Number			
Tissue	Sex	3089 F	3091 M	3095 F	3096 M
Heart		-[1]	-	-	-
Liver		+[2]	-	-	-
Lung		+	-	-	-
Kidney		-	-	-	-
Spleen		+	-	-	-
Testis/ Uterus		-	-	-	-

[1] Negative results
[2] Positive results

Table 13. Serologic and bacteriologic results of guinea pigs and lemmings inoculated with tissue homogenates from four red foxes collected near Nome, Alaska, 1979.

Pathology Number	Species	Fox Number	SPT	BBA	Culture
2942	Guinea Pig	2877	N25[1]	-[2]	-
2957	Lemming	2877	N25	-	-
2958	Lemming	2877	N25	-	-
2943	Guinea Pig	2879	NT[3]	NT	-
2959	Lemming	2879	NT	NT	NC[4]
2960	Lemming	2879	N25	-	-
2944	Guinea Pig	2880	N25	-	-
2961	Lemming	2880	N25	-	-
2962	Lemming	2880	N25	-	-
2949	Guinea Pig	2906	N25	-	-
2971	Lemming	2906	NT	NT	NC
2972	Lemming	2906	NT	NT	NC

[1] Negative at 1:25 dilution
[2] Negative results
[3] Not tested
[4] Not cultured

Table 14. Culture of B. suis type 4 from tissues of grizzly bears collected near Nome, Alaska, 1978-1981.

Tissue	Sex	Pathology Number 2745 F	2746 F	2750 M	2752 M	3097 F	3240 M	3241 M	3246 M
Heart		-[1]	-	-	-	NE[2]	NE	-	-
Liver		-	-	-	-	NE	NE	-	-
Lung		-	-	-	-	NE	NE	-	-
Kidney		-	-	-	-	NE	NE	-	-
Spleen		-	-	-	-	NE	NE	-	-
Testis/ Uterus		-	-	-	-	NE	NE	NE	NE
Epididymis/ Cervix		-	-	NE	NE	NE	NE	NE	NE
Mandibular L.N.		-	-	-	-	NE	NE	-	-
Retropharyngeal L.N.		-	-	-	-	NE	NE	-	-
Popliteal L.N.		-	-	-	-	NE	NE	-	-
Superficial Cervical L.N.		-	-	-	-	NE	NE	-	-
Femoral L.N.		-	-	-	-	NE	NE	-	-
Int. Iliac L.N.		-	-	-	-	NE	NE	-	-
Superficial Inguinal/ Supramammary L.N.		-	-	-	-	NE	NE	-	-

[1] Negative results
[2] Not examined

all samples collected from three arctic ground squirrels, one wolverine, and two arctic hares were negative (Tables 15).

In summary, B. suis type 4 was isolated from four of 38 foxes collected. Seven grizzly bears, three arctic ground squirrels, two arctic hares, one river otter, and one wolverine were all culture-negative.

Reproductive Status

Nine of the 20 female red foxes collected were pregnant. One of those (#3082) was both seropositive and culture-positive for B. suis type 4. Number 3371 was seropositive but culture-negative, and #3372 was seronegative but culture-positive. The remaining six were seronegative and culture-negative.

Three additional females showed signs of recent whelping (enlarged uterus, milk in mammary glands). One of those (#2906) was seropositive but culture-negative. The other two were seronegative and culture-negative.

Eight of the female red foxes showed no signs of pregnancy or recent whelping. One (#3380) was both seropositive and culture-positive. Two (#'s 2561 and 2877) were seropositive but culture-negative.

Neither female arctic fox was pregnant. One (#3089) was both seropositive and culture-positive; the other (#3095) was seropositive but culture-negative.

No trends between pregnancy or recent whelping and either seropositive or culture-positive results were seen. Likewise, no trends between non-pregnancy and either seropositive or culture-

Table 15. Culture of B. suis type 4 from tissues of three arctic ground squirrels, a wolverine, and two arctic hares collected near Nome, Alaska, 1979-1982.

		Pathology Number					
		Arctic Ground Squirrels			Wolverine	Arctic Hares	
		2913	2914	3242	3215	3219	3369
Tissue	Sex	M	M	M	M	F	M
Heart		-[1]	-	-	-	-	NE[2]
Liver		-	-	-	-	-	NE
Lung		-	-	-	-	-	NE
Kidney		-	-	-	-	-	NE
Spleen		-	-	-	-	-	-
Testis/ Uterus		-	-	-	-	-	-
Epididymis/ Cervix		NE	NE	NE	NE	NE	-
Mandibular L.N.		NE	NE	NE	NE	NE	-
Popliteal L.N.		NE	NE	NE	NE	NE	-

[1] Negative results
[2] Not examined

positive results was seen. There was no significant difference between the number of reproductive females being seropositive and/or culture-positive and the number of non-pregnant females being seropositive and/or culture-positive (chi-square; $p>0.01$).

Gross and Microscopic Pathology

Occurrence of gross and microscopic lesions observed in red foxes collected from 1977-1983 are presented in Tables 16-21. White foci were observed on the liver of a male red fox (#2558) collected in 1977 (Table 16). A thick, creamy uterine discharge was seen in the uterus of a non-pregnant female fox (#3098) collected in 1980 (Table 18). The uterus of a pregnant female red fox (#3239) collected in 1981 contained a gray, caseous exudate (Table 19). A calcified abscess was seen in a culture-positive iliac lymph node of a female red fox (#3380) collected in 1982 (Table 20). No other gross lesions suggestive of an infection with a Brucella organism were seen in red or arctic foxes (Tables 16, 17, 18, 19, 20, 21 and 22).

Most of the foxes examined were in very good body condition. Reindeer fawn hair was found in the stomachs of nine red foxes and adult hair in the stomachs of an additional three.

Microscopic foci of mononuclear cells were seen in the livers of five female red foxes (#'s 2561, 3356, 3372, 3373 and 3380), and three male red foxes (#'s 2258, 2880 and 3365)(Tables 16, 17, 18, 19, 20 and 21). Similar lesions were seen in the liver of one female arctic fox (#3089) (Table 22).

Scattered, white foci were seen on the livers of two female and two

Table 16. Presence of gross/microscopic lesions in tissues of red foxes collected near Nome, Alaska, 1977-1978.

		Pathology Number					
Tissue	Sex	2558 M	2561 F	2564 M	2565 M	2569 M	2749 F
Heart		-/-[1]	-/-	-/-	-/-	-/-	-/-
Liver		+[2]/+[3]	-/+[4]	-/-	-/-	-/-	-/-
Lung		-/-	-/-	-/-	-/-	-/-	-/-
Kidney		-/-	-/-	-/-	-/-	-/-	-/-
Spleen		-/-	-/-	-/-	-/-	-/-	-/-
Testis/ Uterus		-/NE[4]	-/NE	-/NE	-/NE	-/NE	-/NE

[1] No lesions observed
[2] Small, white foci on liver
[3] Several foci of mononuclear cells in liver
[4] Small accumulation of mononuclear cells in liver
[5] Not examined

Table 17. Presence of gross/microscopic lesions in tissues of red foxes collected near Nome, Alaska, 1979.

Tissue	Sex	Pathology Number 2877 F	2879 M	2880 M	2906 F	2911 M
Heart		-[1]/NE[2]	-/NE	-/-	-/-	-/-
Liver		-/NE	-/NE	-/-	-/-	-/-
Lung		-/NE	-/NE	-/-	-/-	-/-
Kidney		-/NE	-/NE	-/-	-/-	-/-
Spleen		-/NE	-/NE	-/-	-/-	-/-
Testis/ Uterus		-/NE	-/NE	-/NE	-/-	-/-

1 No lesions observed
2 Not examined

Table 18. Presence of gross/microscopic lesions in tissues of red foxes collected near Nome, Alaska, 1980.

		Pathology Number					
Tissue	Sex	3082 F	3084 M	3085 F	3087 F	3098 F	3115 F
Heart		-/-[1]	-/-	-/-	-/-	-/-	-/-
Liver		-/-	-/-	-/-	-/-	-/-	-/-
Lung		-/-	-/-	-/-	-/-	-/-	-/-
Kidney		-/-	-/-	-/-	-/-	-/-	-/-
Spleen		-/-	-/-	-/-	-/-	-/-	-/-
Testis/ Uterus		-/NE[2]	-/-	-/NE	-/NE	+[3]/NE	-/NE

[1] No lesions observed
[2] Not examined
[3] Thick, creamy uterine discharge

Table 19. Presence of gross/microscopic lesions in tissues of red foxes collected near Nome, Alaska, 1981.

		Pathology Number							
Tissue	Sex	3210 F	3211 M	3212 M	3216 M	3217 F	3218 M	3238 F	3239 F
Heart		-/-[1]	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Liver		-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Lung		-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Kidney		-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Spleen		-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Testis/ Uterus		-/NE	-/NE[2]	-/NE	-/NE	-/NE	-/NE	-/NE	+[3]/NE

[1] No lesions observed
[2] Not examined
[3] Gray, caseous uterine exudate

Table 20. Presence of gross/microscopic lesions in tissues of red foxes collected near Nome, Alaska, 1982.

	Pathology Number							
Tissue Sex	3356 F	3357 M	3365 M	3367 F	3371 F	3372 F	3373 F	3380 F
Heart	-/-[1]	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Liver	-/-	-/-	-/+[2]	-/-	-/-	-/+[2]	-/-	-/+[2]
Lung	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Kidney	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Spleen	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Testis/ Uterus	-/NE	-/NE[3]	-/NE	-/NE	-/NE	-/NE	-/NE	-/NE
Other	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+[4]/NE

[1] No lesions observed
[2] Few foci of mononuclear cells in liver
[3] Not examined
[4] Iliac lymph node enlarged, abscessed

Table 21. Presence of gross/microscopic lesions in tissues of a red fox collected near Nome, Alaska, 1983.

	Pathology Number	
Tissue	Sex	3494 F
Heart		-/-[1]
Liver		-/-
Lung		-/-
Kidney		-/-
Spleen		-/-
Uterus		-/NE[2]

[1] No lesions observed
[2] Not examined

Table 22. Presence of gross/microscopic lesions in tissues of arctic foxes collected near Nome, Alaska, 1980.

		Pathology Number			
Tissue	Sex	3089 F	3091 M	3095 F	3096 M
Heart		-/-[1]	-/-	-/-	-/-
Liver		-/+[2]	-/-	-/-	-/-
Lung		-/-	-/-	-/-	-/-
Kidney		-/-	-/-	-/-	-/-
Spleen		-/-	-/-	-/-	-/-
Testis/ Uterus		-/NE	-/NE[3]	-/NE	-/NE

[1] No lesions observed
[2] Small accumulation of mononuclear cells in liver
[3] Not examined

male grizzly bears (#'s 2745, 2746, 2750 and 2752), but microscopic lesions were not observed (Table 23).

No gross or microscopic lesions were seen in samples collected from the arctic ground squirrels, wolverine, or arctic hares (Table 24).

Table 23. Presence of gross/microscopic lesions in tissues of grizzly bears collected near Nome, Alaska, 1978-1981.

	Pathology Number							
Sex	2745 F	2746 F	2750 M	2752 M	3097 F	3240 M	3241 M	3246 M
Tissue								
Heart	-/-[1]	-/-	-/-	-/-	-/-	-/NE[2]	-/-	-/-
Liver	+[3]/-	+[3]/-	+[3]/-	+[3]/-	-/-	-/NE	-/-	-/-
Lung	-/-	-/-	-/-	-/-	-/-	-/NE	-/-	-/-
Kidney	-/-	-/-	-/-	-/-	-/-	-/NE	-/-	-/-
Spleen	-/-	-/-	-/-	-/-	-/-	-/NE	-/-	-/-
Testis/ Uterus	-/-	-/-	-/-	-/-	-/-	-/NE	-/-	-/-

1 No lesions observed
2 Not examined
3 Scattered white foci on liver

Table 24. Presence of gross/microscopic lesions in tissues of three arctic ground squirrels, a wolverine, and two arctic hares collected near Nome, Alaska, 1979-1982.

		Pathology Number					
		Arctic Ground Squirrels			Wolverine	Arctic Hares	
		2913	2914	3242	3215	3219	3369
Tissue	Sex	M	M	M	M	M	M
Heart		-/-[1]	-/-	NE/NE[2]	-/-	-/-	NE/NE
Liver		-/-	-/-	NE/NE	-/-	-/-	NE/NE
Lung		-/-	-/-	NE/NE	-/-	-/-	NE/NE
Kidney		-/-	-/-	NE/NE	NE/NE	-/-	NE/NE
Spleen		-/-	-/-	NE/NE	NE/NE	NE/NE	NE/NE
Testis/ Uterus		-/-	-/-	NE/NE	NE/NE	NE/NE	NE/NE

[1] No lesions observed
[2] Not examined

PATHOGENESIS OF BRUCELLA SUIS TYPE 4 IN FOXES

Virulence of Challenge Organism

Results of laboratory animal inoculation with B. suis type 4 are presented in Table 25. The culture used for experimental infections in 1982 was passed through guinea pigs #3331, 3335, and 3345. Numbers 3488, 3489, and 3490 were used in 1983. Guinea pigs #3514, 3515, 3510, and 3511, and lemmings #3497, 3512, and 3513 were inoculated on the actual days of challenge in 1983.

Results of serologic tests, bacteriologic cultures, and gross and microscopic examinations were consistent with those seen in previous laboratory animals infected with virulent B. suis type 4 at the University of Alaska Fairbanks.

Serology

The SP, BBA, Riv, and CF tests have comprised the routine battery of tests used in the reindeer-brucellosis research program and were considered the primary serologic tests for this phase of the study. Tables and figures of data used to evaluate these tests are presented in this section. Results of the ST and ME tests, which were conducted on selected samples for comparative purposes, are included in tables of results of individual animals in the Appendix.

Results of the ST were similar but not always identical to those on the SP. Likewise, results on the ME were not always identical to those on the Riv test (Appendix, Tables 43-54).

Geometric mean brucellosis titers for the SP, Riv and CF tests are

Table 25. Pathogenicity of challenge strain B. suis type 4 for laboratory rodents.[a]

Pathology Number	Challenge Inoculum (CFU)	Time PE Necropsy (Weeks)	Serologic Results	Culture Results	Gross Lesions	Microscopic Lesions
Guinea Pigs						
3331	$1.0x10^5$	8	+	+[b]	+[c]	-
3335	$1.0x10^5$	9	+	+[d]	+[e]	+[f]
3345	$1.0x10^5$	11	+	+[g]	+[c,h]	+[f,i]
3488	$1.0x10^6$	5	+	+[j]	+[k]	+[l]
3489	$1.0x10^6$	6	+	+[d]	-	NE[m]
3490	$1.0x10^6$	7	+	+[d]	+[c,k]	+[n,o]
3514	$3.0x10^4$	6	+	+[d]	+[c,k]	-
3515	$2.7x10^4$	6	+	+[d]	+[c]	+[f,i]
3510	$2.4x10^4$	5	+	+[d,p]	+[c,k]	-
3511	$2.5x10^4$	5	+	+[q]	+[c]	+[f]
Lemmings						
3497	$2.6x10^3$	1	NE	+[r]	-	+[f]
3512[s]	$2.5x10^3$	3	-	-	-	-
3513	$2.5x10^3$	3	-	+[t]	-	+[f]

(footnotes appear on following page)

Table 25. (continued)

[a] All animals challenged intraperitoneally unless specified otherwise
[b] Liver and lung
[c] Small, white foci on the liver
[d] Liver, spleen
[e] Small, white foci on liver and spleen
[f] Foci of mononuclear cells in the liver
[g] Liver, spleen, kidney, and testes
[h] Pus in testicle
[i] Foci of mononuclear cells in lung
[j] Heart, liver, lung, kidney, spleen
[k] Enlarged spleen
[l] Foci of mononuclear cells in kidney, liver, lung
[m] Not examined
[n] Foci of mononuclear cells in the uterus
[o] Foci of mononuclear cells, neutrophils and necrosis in the liver
[p] Prefemoral lymph node
[q] ver, lung, kidney, spleen, prefemoral lymph node
[r] Liver, lung, spleen
[s] Challenged subcutaneously
[t] Liver, lung, bladder

presented in Figures 1-4.

In 1982, serologic reactions were not detected in the male fox challenged with the lower dose (8.34 $x10^7$ CFU) (Appendix, Table 43). Diagnostic titers were initially detected at 3 weeks PE on the SP and Riv tests and at 5 weeks PE on the BBA and CF tests in the female fox challenged with that dose (Appendix, Table 44). In the two males receiving the higher dose (4.9 x 10^9 CFU), strong titers were detected on all tests at 2 and 3 weeks PE respectively (Appendix, Tables 45 and 46).

In 1983, positive reactions were detected on the SP test at 1-3 weeks PE in foxes challenged with 1.06 x 10^{11} and at 2-3 weeks PE in foxes challenged with 1.06 x 10^9 CFU. The BBA, Riv and CF tests were considered positive 2-3 weeks PE in the foxes challenged with the higher dose and 2-4 weeks PE in foxes challenged with the lower dose (Appendix, Tables 47-54).

In all groups of foxes, significant titers were detected on all tests for the duration of the experiments.

To evaluate accuracy of individual serologic tests, experimental foxes were considered infected following challenge if B. suis type 4 was subsequently isolated from blood cultures or tissues at necropsy. Prior to challenge, negative serologic tests were considered correct. Following challenge, positive serologic tests were considered correct, and negative tests were considered incorrect.

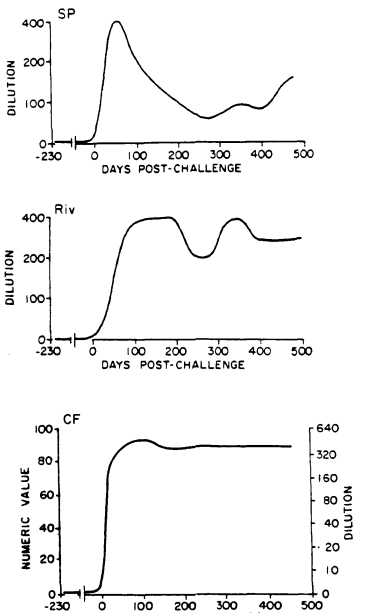

Figure 1. Geometric mean titers of two foxes challenge exposed in March 1982 with 8.34 x 10^7 CFU B. suis type 4. Test abbreviations: (end points are given in parentheses): SP (1:400), Riv (1:400), and CF (100) tests. Day 0 is first day of challenge exposure.

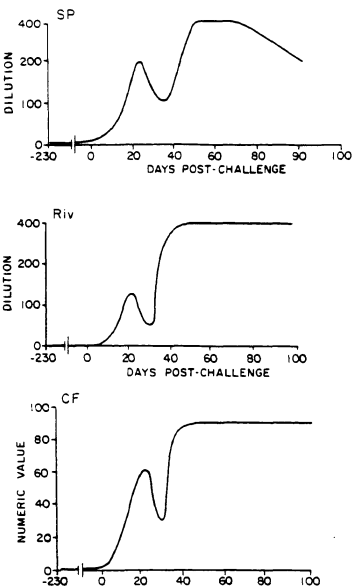

Figure 2. Geometric mean titers of two foxes challenge exposed in March 1982 with 4.9 x 10^9 CFU B. suis type 4. Test abbreviations: (end points are given in parentheses): SP (1:400), Riv (1:400), and CF (100) tests. Day 0 is first day of challenge exposure.

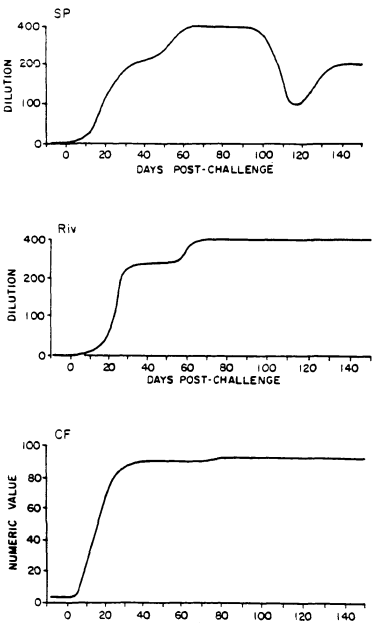

Figure 3. Geometric mean titers of four foxes challenge exposed in April 1983 with 1.21 x 10^9 CFU B. suis type 4. Test abbreviations: (end points are given in parentheses): SP (1:400), Riv (1:400), and CF (100) tests. Day 0 is first day of challenge exposure.

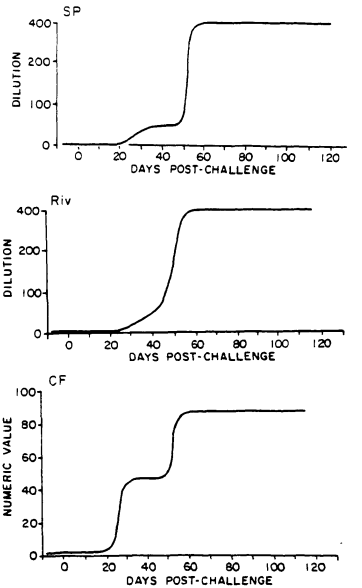

Figure 4. Geometric mean titers of six foxes challenge exposed in April 1983 with 1.06×10^{11} CFU B. suis type 4. Test abbreviations: (end points are given in parentheses): SP (1:400), Riv (1:400), and CF (100 or 1:640) tests. Day 0 is first day of challenge exposure.

Using these criteria, sensitivity and specificity of each serologic test were evaluated. Sensitivity of a serologic test is defined as:

$$\frac{\text{Number true positives}}{\text{Number true positives + Number false negatives}} \times 100$$

Specificity is defined as:

$$\frac{\text{Number true negatives}}{\text{Number true negatives + Number false positives}} \times 100$$

Serologic tests were also evaluated in pairs for agreement in both being either positive or negative on the same sample.

One of two foxes challenged with the lower dose (8.34×10^7 CFU) in 1982 was considered not infected. Serologic reactions were not detected, and B. suis type 4 was not isolated from hemocultures or tissues at necropsy. Negative serologic reactions were considered correct on this fox. Sensitivity for each of the SP, BBA, Riv and CF tests on the two foxes in this group was 75%. Specificity for each test was 100%. The SP and BBA, SP and Riv, SP and CF, BBA and Riv, BBA and CF, and the Riv and CF agreed on 100% of the samples from the two foxes.

In the two males receiving the higher challenge dose (4.9×10^9 CFU) in 1982, the SP and Riv, SP and CF, and Riv and CF tests agreed with each other 100% of the time. The SP and BBA, BBA and Riv, and the

BBA and CF tests agreed 96% of the time. Sensitivity of the BBA test was 92%; sensitivity of each of the SP, Riv and CF tests was 87.5%. Each of the four tests had 100% specificity.

On tests conducted on four foxes challenged with the lower dose (1.21×10^9 CFU) in 1983, the BBA and Riv, BBA and CF, and Riv and CF tests agreed on 100% of the samples; the SP and BBA, and SP and CF tests on 88%; and the SP and Riv tests on 84%. The sensitivity was 79% for the SP, 63% for the BBA, and 60% for the CF test. Specificity was 100% for all four tests.

Six foxes were challenged with 1.06×10^{11} CFU in 1983. The BBA or Riv and CF tests agreed on 98% of the samples; the BBA and Riv tests on 97%; the SP and BBA or CF tests on 95%; and the SP and Riv tests on 94%. Sensitivity was 98% for the SP, 94% for the BBA, 92% for the CF, and 91% for the Riv test. Specificity was 100% for each test.

Grouping results of all experimentally-infected foxes, the sensitivity of the SP test was 91%; BBA test, 87%; CF test, 86%; and Riv test, 85%. The specificity of each test was 100%, meaning no test was falsely positive.

Evaluating pairs of serologic tests on the four groups of foxes collectively, the Riv and CF tests agreed 100% of the time. The BBA and CF tests had 99% agreement; the BBA and Riv tests, 98%; the SP and BBA tests, 95%; and the SP and Riv, and SP and CF tests, 94%.

In summary, serologic reactions to brucellosis tended to be detected on the SP tests earlier than on the BBA, ME, Riv, or CF tests. Titers to brucellosis detected by the SP test declined relative

to those detected by the BBA, Riv, or CF tests in the later stages of infection in foxes challenged with 10^9 or 10^{11} CFU. Diagnostic titers were detected by all tests for the duration of the experiments. Sensitivity of the SP test was greater than that of the BBA, Riv, or CF tests.

Specificity was 100% for all four tests. Agreement between the SP and either the BBA, Riv, or CF tests was not as good as between the BBA and Riv, BBA and CF, or Riv and CF tests. These results were consistent with the concept that the SP test detects both IgM and IgG, while the other tests are more specific for IgG.

Bacteriology

B. suis type 4 was isolated in hemoculture from the female fox challenged with the lower dose (8.34×10^7 CFU) and neither of two foxes challenged with the higher dose (4.9×10^9 CFU) in 1982 (Appendix, Tables 43-46). In 1983, the organism was isolated from hemocultures of three of four foxes challenged with 1.21×10^9 CFU, and from two of six foxes challenged with 1.06×10^{11} CFU (Appendix, Tables 47-54).

B. suis type 4 was not isolated from oral, genital, fecal or urine samples from any of the four foxes experimentally infected in 1982 (Table 26). The organism was isolated from oral swabs (1 fox), fecal samples (3 foxes), and urine (1 fox) from three of four foxes challenged with 1.21×10^9 CFU in 1983. Oral swabs from three and fecal samples from all six foxes challenged with 1.06×10^{11} CFU were culture-positive (Table 26).

B. suis type 4 was isolated 14 times from the fecal pellet portion of the culture suspension, but only eight times from the supernatant

Table 26. Culture of B. suis type 4 from oral, genital, fecal or urine samples from foxes orally challenged with various infective doses.

Sample	Infective Dose (CFU) 8.34×10^7	4.9×10^9	1.21×10^9	1.06×10^{11}
Oral Swab	0/2[1]	0/2	1/4[2]	3/6[3]
Genital Swab	0/2	0/2	0/4	0/6
Fecal Sample	0/2	0/2	3/4[4]	6/6[5]
Urine Sample	0/2	0/2	1/4[6]	0/6

[1] Number of foxes culture positive/Number of foxes sampled
[2] Fox #3516 positive at 2 & 3 weeks PE
[3] 3 foxes positive 1 week PE; 1 fox positive 2 weeks PE; 2 foxes positive 3 weeks PE
[4] 2 foxes positive on each of days 2, 3, and 4 of challenge; 2 foxes positive 1 day PE
[5] 2 foxes positive day 2 of challenge; 3 foxes positive day 3 of challenge; 1 fox positive day 4 of challenge; 2 foxes positive 1 day PE; 1 fox positive 4 days PE; 1 fox positive 6 days PE
[6] Fox #3547 positive at necropsy, 13 weeks PE

fluid. Portions of positive fecal samples were later re-cultured to quantitate the brucellae organisms, but numbers were too low to evaluate.

Clinical Effects

Few statistically significant differences in blood parameters were seen between groups of foxes. The mean total WBC was significantly higher at 5 weeks PE in the two foxes challenged with 8.34×10^7 CFU than in the two challenged with 4.9×10^9 CFU in 1982 (t test; $p<0.05$). The mean total WBC at 20 weeks PE of two foxes challenged with 1.06×10^{11} CFU in 1983 was significantly lower than in two non-challenged, naturally infected foxes at the same time (t test; $p<0.05$) (Tables 27-30).

No clinical signs of brucellosis in foxes were seen.

Reproduction

No pairs of foxes in this part of the study produced offspring. Behavior such as riding, playing, and vocalization commonly seen in foxes during the breeding season was not observed either directly or on video tapes examined. To reduce handling stress, swelling of the vulvas and firmness of the testes, indicators of estrus and active sperm production respectively, were not examined routinely during the time the foxes were held together as pairs (January through early April). Semen examined from all male foxes late in March 1983 contained only dead sperm which indicated lack of recent sexual activity (N. Duenger, pers. comm.). No swelling or redness of the vulvas were observed at that time. Following experimental challenge, no swelling of the vulvas or firmness or swelling of the testes were observed at

Table 27. Blood values of two foxes orally challenged with 8.34 x 10^7 CFU B. suis type 4 in 1982.

Weeks Post-Exposure	PCV[1]	WBC[2]	Percent Neutrophils	Percent Lymphocytes	Percent Monocytes	Percent Eosinophils	Percent Basophils
2[3]	56.0[4]	5300	58.0	28.0	10.0	2.0	0.0
	57.0	5000	72.0	18.0	2.0	8.0	0.0
3[3]	57.0	5900	74.0	18.0	4.0	2.0	0.0
	58.0	2700	62.0	20.0	6.0	12.0	0.0
4[3]	55.0	3700	86.0	6.0	6.0	2.0	0.0
	58.0	5900	70.0	28.0	2.0	0.0	0.0
5[3]	52.0	9000*	78.0	12.0	8.0	2.0	0.0
	50.0	7800*	86.0	8.0	6.0	0.0	0.0
6[5]	52.0	6200	54.0	20.0	14.0	10.0	0.0
7[3]	42.0	4400	62.0	20.0	6.0	12.0	0.0
	47.0	5800	76.0	4.0	18.0	2.0	0.0
8[5]	48.0	6000	76.0	12.0	8.0	4.0	0.0
9[5]	51.0	4000	66.0	24.0	0.0	10.0	0.0
10[5]	49.0	5800	58.0	18.0	10.0	14.0	0.0
12[5]	44.0	5060	66.0	19.0	3.0	12.0	0.0
14[5]	51.0	8700	80.0	11.0	8.0	1.0	0.0
16[5]	47.0	4000	58.0	18.0	8.0	16.0	0.0

1 Packed cell volume (percent)
2 White blood cells/mm^3
3 n=2
4 Individual values
5 n=1
* Significant difference between this group and foxes challenged with 4.9 x 10^9 CFU ($p<0.05$).

Table 28. Blood values of two foxes orally challenged with 4.9 x 10^9 CFU *B. suis* type 4 in 1982.

Weeks Post-Exposure	PCV[1]	WBC[2]	Percent Neutrophils	Percent Lymphocytes	Percent Monocytes	Percent Eosinophils	Percent Basophils
2[3]	56.0[4]	7700	68.0	18.0	6.0	6.0	0.0
	54.0	5500	70.0	16.0	8.0	6.0	0.0
3[3]	53.0	6600	66.0	24.0	6.0	3.0	0.0
	46.0	3400	54.0	38.0	4.0	2.0	0.0
4[3]	52.0	3800	56.0	34.0	6.0	4.0	0.0
	53.0	5500	70.0	30.0	0.0	0.0	0.0
5[3]	37.0	5700*	74.0	10.0	14.0	2.0	0.0
	45.0	5800*	58.0	32.0	10.0	0.0	0.0
7[3]	48.0	8000	58.0	34.0	6.0	2.0	0.0
	52.0	5100	66.0	26.0	8.0	0.0	0.0
8[3]	52.0	5400	52.0	38.0	10.0	0.0	0.0
	53.0	6600	66.0	22.0	10.0	2.0	0.0
9[3]	55.0	6700	62.0	26.0	6.0	6.0	0.0
	55.0	7000	68.0	20.0	4.0	8.0	0.0
10[5]	52.0	5600	48.0	38.0	6.0	8.0	0.0
12[5]	48.0	5610	54.0	26.0	5.0	14.0	0.0
14[5]	53.0	4200	51.0	38.0	9.0	2.0	0.0
16[5]	42.0	5300	50.0	34.0	6.0	10.0	0.0
17[5]	47.0	5400	60.0	24.0	4.0	10.0	0.0
19[5]	50.0	2000	42.0	34.0	14.0	10.0	0.0
20[5]	48.0	2900	46.0	44.0	6.0	4.0	0.0
22[5]	53.0	3600	55.0	39.0	6.0	0.0	0.0

Table 28. (continued)

[1] Packed cell volume (percent)
[2] White blood cells/mm^3
[3] n=2
[4] Individual values
[5] n=1
* Significant difference between this group and foxes challenged with 8.34 x 10^9 CFU ($p<0.05$).

Table 29. Mean blood values of four foxes orally challenged with 1.21 x 10^9 CFU B. suis type 4 in 1983.

Weeks Post-Exposure	PCV[1]	WBC[2]	Percent Neutrophils	Percent Lymphocytes	Percent Monocytes	Percent Eosinophils	Percent Basophils
-12[3]	44.0[4] (8.8)	5200 (3535)	49.7 (9.1)	35.3 (9.9)	8.7 (6.1)	6.0 (6.9)	0.0
-1[5]	52.0 (2.0)	3700 (1212)	58.0 (15.9)	30.7 (13.2)	9.3 (6.4)	2.0 (2.0)	0.0
1[5]	46.0 (2.6)	4367 (493)	57.3 (12.2)	36.7 (11.4)	5.3 (5.0)	1.3 (2.3)	0.0
2[3]	47.0 (3.6)	3775 (957)	51.5 (6.4)	39.5 (6.2)	5.5 (2.5)	3.0 (3.5)	0.0
3[3]	46.0 (2.5)	2850 (1475)	53.7 (11.8)	37.7 (12.9)	7.7 (6.9)	1.2 (2.5)	0.0
4[6]	48.0[7] 43.0	3800 5600	64.0 54.0	30.0 36.0	4.0 10.0	2.0 0.0	0.0 0.0
5[6]	45.0 48.0	7300 5700	25.0 50.0	64.0 26.0	4.0 4.0	8.0 10.0	0.0 0.0
7[6]	46.0 49.0	8420 5170	27.0 38.0	60.0 43.0	2.0 2.0	13.0 17.0	0.0 0.0
10[8]	49.0	6270	66.0	30.0	0.0	4.0	0.0
13[8]	50.0	4730	34.0	44.0	10.0	12.0	0.0

1 Packed cell volume (percent)
2 White blood cells/mm^3
3 n=4
4 Mean (standard deviation)
5 n=3
6 n=2
7 Individual values
8 n=1

Table 30. Mean blood values of six foxes orally challenged with 1.06×10^{11} CFU *B. suis* type 4 in 1983.

Weeks Post-Exposure	PCV[1]	WBC[2]	Percent Neutrophils	Percent Lymphocytes	Percent Monocytes	Percent Eosinophils	Percent Basophils
-12[3]	46.8[4] (4.0)	5448 (1492)	55.6 (9.3)	36.0 (8.5)	5.0 (2.0)	3.4 (2.7)	0.2 (0.4)
-1[3]	53.0 (4.6)	4650 (836)	42.7 (11.6)	47.7 (11.6)	6.7 (2.1)	2.3 (1.9)	0.0
1[5]	49.0 (2.7)	6460 (2575)	59.2 (10.7)	34.2 (11.6)	4.6 (3.6)	2.0 (2.0)	0.0
2[5]	48.8 (3.7)	4540 (804)	48.4 (8.4)	32.4 (7.1)	13.2 (6.4)	6.0 (4.9)	0.0
3[3]	51.8 (4.9)	4916 (1292)	52.4 (9.8)	32.0 (9.0)	10.4 (5.2)	5.2 (3.6)	0.0
5[3]	50.0 (2.8)	5966 (2276)	49.7 (16.9)	38.0 (14.3)	5.0 (3.9)	7.0 (1.7)	0.0
7[3]	45.0 (4.3)	6120 (2810)	44.7 (13.4)	41.7 (10.9)	2.8 (1.7)	10.2 (2.5)	0.3 (0.8)
10[5]	48.0 (2.7)	6534 (2552)	49.0 (14.2)	43.0 (12.3)	0.8 (0.8)	6.8 (4.3)	0.3 (0.8)
12[5]	48.2 (3.7)	6270 (1507)	37.6 (10.7)	49.2 (13.9)	3.4 (1.9)	6.8 (4.1)	0.2 (0.4)
14[3]	47.5 (4.2)	5500 (2092)	43.5 (15.8)	42.5 (13.8)	7.3 (1.5)	7.7 (4.9)	0.0
16[6]	48.7 (3.6)	5865 (2456)	41.3 (10.2)	44.0 (11.1)	5.3 (5.9)	10.2 (4.8)	0.0
18[7]	50.7 (3.2)	5243 (2200)	43.7 (13.8)	46.0 (17.4)	4.0 (3.5)	6.3 (0.6)	0.0
20[8]	52.0[9] 56.0	4070* 3960	32.0 34.0	56.0 56.0	8.0 2.0	4.0 8.0	0.0 0.0

Table 30. (continued)

Weeks Post-Exposure	PCV[1]	WBC[2]	Percent Neutrophils	Percent Lymphocytes	Percent Monocytes	Percent Eosinophils	Percent Basophils
22[8]	48.0	5610	54.0	34.0	12.0	0.0	0.0
	47.0	6270	39.0	46.0	8.0	7.0	0.0
23[8]	48.0	9350	45.0	43.0	2.0	10.0	1.0
	51.0	5830	46.0	46.0	2.0	6.0	0.0
25[8]	57.0	5500	27.0	59.0	3.0	10.0	1.0
	51.0	4950	24.0	66.0	4.0	6.0	0.0
27[8]	45.0	6270	28.0	56.0	10.0	6.0	0.0
	52.0	6820	40.0	50.0	4.0	6.0	0.0
29[8]	52.0	1870	30.0	54.0	8.0	8.0	0.0
	43.0	2200	32.0	52.0	10.0	6.0	0.0
33[8]	52.0	7810	41.0	45.0	8.0	6.0	0.0
	48.0	7480	37.0	53.0	6.0	4.0	0.0
38[8]	57.0	5830	24.0	64.0	8.0	4.0	0.0
	53.0	8580	42.0	36.0	10.0	12.0	0.0
44[8]	55.0	3740	34.0	46.0	16.0	4.0	0.0
	48.0	3520	42.0	48.0	6.0	4.0	0.0
46[8]	53.0	4400	38.0	55.0	4.0	4.0	0.0
	46.0	6710	36.0	58.0	1.0	5.0	0.0
48[8]	51.0	4730	39.0	51.0	2.0	3.0	1.0
	48.0	7540	38.0	51.0	5.0	6.0	0.0
51[8]	56.0	4290	67.0	27.0	2.0	4.0	0.0
	51.0	4620	48.0	38.0	7.0	7.0	0.0
54[8]	58.0	5610	44.0	36.0	9.0	9.0	0.0
	52.0	5390	50.0	39.0	6.0	5.0	0.0
56[8]	50.0	4400	26.0	65.0	4.0	5.0	0.0
	46.0	5390	28.0	51.0	3.0	7.0	1.0

Table 30. (continued)

Weeks Post-Exposure	PCV[1]	WBC[2]	Percent Neutrophils	Percent Lymphocytes	Percent Monocytes	Percent Eosinophils	Percent Basophils
66[8]	53.0	4730	55.0	32.0	8.0	5.0	0.0
	46.0	8030	43.0	51.0	2.0	4.0	0.0

[1] Packed cell volume
[2] White blood cells/mm^3
[3] n=6
[4] Individual values
[5] n=5
[6] n=4
[7] n=3
[8] n=2
[9] Individual values
* Significantly lower than in two non-challenged foxes ($p<0.05$)

any time samples were collected for bacteriologic examination. No signs of pregnancy were observed at necropsy.

Necropsy - Bacteriology

Culture results on individual animals are shown in the Appendix, Tables 55-58. Results according to type of tissue are presented in the text.

B. suis type 4 was isolated at necropsy from the mandibular, retropharyngeal, femoral, popliteal, internal iliac, and mesenteric lymph nodes from foxes challenged with either dose in 1982. In addition to these sites, the organism was isolated from the superficial cervical, tracheobronchial and supramammary lymph nodes, and liver, kidney, spleen, bladder, tonsils, salivary glands and thymus from foxes challenged in 1983 (Table 31).

B. suis type 4 was isolated from all the above mentioned lymph nodes and organs from foxes necropsied between 3 and 18 weeks PE. Salivary glands were culture-positive in only two of the foxes necropsied 3-9 weeks PE. Mesenteric lymph nodes from four of five foxes necropsied in the 13-18 week interval were culture-positive. The internal iliac lymph node was the only culture-positive tissue of one fox necropsied at 22 weeks PE. The popliteal lymph node was the only culture-positive tissue in one of two foxes sacrificed at 66 weeks PE (Table 32).

Necropsy - Pathology

Gross and microscopic lesions observed in experimentally-infected foxes were mainly confined to the lymph nodes and were characterized by

Table 31. Culture of B. suis type 4 from tissues of foxes challenged orally with different infective doses and sacrificed 3 to 22 weeks post-expoure.

Tissue	Infective Dose (CFU) 8.34×10^7	4.9×10^9	1.21×10^9	1.06×10^{11}
Mandibular L.N.	1/2[1]	1/1	3/4	4/4
Retropharyngeal L.N.	1/1	1/2	3/4	4/4
Superficial Cervical L.N.	0/0	0/1	1/2	2/3
Femoral L.N.	1/1	0/0	1/1	0/0
Popliteal L.N.	1/2	1/2	3/3	1/3
Int. Iliac L.N.	1/2	2/2	3/4	3/4
Mesenteric L.N.	1/2	0/0	0/0	3/4
Tracheobronchial L.N.	0/0	0/0	3/3	0/2
Supramammary L.N.	0/1		0/0	1/1
Superficial Inguinal L.N.	0/0	0/0	0/1	0/1
Epididymis	0/1	0/1	0/1	0/0
Seminal Vesicle	0/1	0/1	0/0	0/0
Prostate		0/1	0/1	0/2
Testis	0/1	0/2	0/2	0/2
Ovary	0/1		0/1	0/2
Uterus	0/1		0/2	0/2
Heart	0/2	0/2	0/4	0/4
Liver	0/2	0/2	2/4	0/4

Table 31. (continued)

Tissue	Infective Dose (CFU) 8.34×10^7	4.9×10^9	1.21×10^9	1.06×10^{11}
Lung	0/2	0/2	0/4	0/4
Kidney	0/2	0/2	1/4	1/4
Spleen	0/2	0/2	3/4	0/4
Bladder	0/1	0/2	1/4	0/4
Tonsils	0/0	0/0	3/4	3/4
Salivary Gland	0/0	0/0	2/4	0/4
Thymus	0/0	0/0	1/2	1/4

[1] Number of foxes culture positive/number of foxes sampled

Table 32. Culture of B. suis type 4 from tissues of foxes challenged orally and sacrificed at various times post-exposure.

Tissue	Weeks Sacrificed Post-Exposure 3-9	13-18	22	66
Mandibular L.N.	3/5[1]	6/6	0/0	0/1
Retropharyngeal L.N.	4/4	5/6	0/1	0/2
Superficial Cervical L.N.	1/2	2/3	0/1	0/2
Femoral L.N.	0/0	1/2	0/0	0/0
Popliteal L.N.	4/5	1/3	0/1	1/2
Int. Iliac L.N.	3/5	5/6	1/1	0/2
Mesenteric L.N.	0/1	4/5	0/0	0/2
Tracheobronchial L.N.	2/3	1/3	0/0	0/1
Supramammary L.N.	0/0	1/2	0/0	0/0
Superficial Inguinal L.N.	0/1	0/1	0/0	0/0
Epididymis	0/3	0/0	0/0	0/0
Seminal Vesicle	0/2	0/0	0/0	0/0
Prostate	0/2	0/2	0/0	0/1
Testis	0/3	0/3	0/1	0/1
Ovary	0/1	0/3	0/1	0/1
Uterus	0/2	0/3	0/0	0/1
Heart	0/5	0/6	0/1	0/2

Table 32. (continued)

Tissue	Weeks Sacrificed Post-Exposure 3-9	13-18	22	66
Liver	1/5	1/6	0/1	0/2
Lung	0/5	0/6	0/1	0/2
Kidney	0/5	2/6	0/1	0/2
Spleen	2/5	1/6	0/1	0/2
Bladder	0/3	1/6	0/0	0/2
Tonsils	2/3	4/5	0/0	0/2
Salivary Gland	2/4	0/5	0/1	0/2
Thymus	1/2	1/4	0/0	0/0

[1] Number of foxes culture positive/Number of foxes sampled

hyperplasia and inflammatory foci.

A swollen retropharyngeal lymph node seen in the female fox challenged with 8.34 x 10^7 CFU and necropsied at 16 weeks PE was characterized microscopically by follicular hyperplasia. The internal iliac lymph node was hyperemic (Table 33). B. suis type 4 was isolated from both lymph nodes.

Follicular and medullary cord hyperplasia were observed histologically in the popliteal lymph node of one fox (#3403) challenged with 4.9 x 10^9 CFU and necropsied at 22 weeks PE (Table 33). This lymph node was culture-negative.

Lymph nodes including the retropharyngeal, superficial cervical and popliteal were swollen in three of the four foxes challenged with 1.21 x 10^9 CFU (Table 34). B. suis type 4 was isolated from five of the six swollen lymph nodes. Microscopic lesions characterized by follicular and medullary cord hyperplasia, cortical foci of neutrophils and macrophages, and germinal centers with macrophages, hyperemia and necrosis were seen in five lymph nodes of two foxes in this group sacrificed at 7 and 13 weeks PE (Table 34). All five of these lymph nodes were culture-positive for B. suis type 4. In addition, one small cluster of mononuclear cells was seen in the salivary gland (culture-positive) of one fox.

Swollen retropharyngeal, popliteal, and internal iliac lymph nodes were seen in one of four foxes challenged with 1.06 x 10^{11} CFU and necropsied 14-18 weeks PE (Table 35). Two of these lymph nodes were culture-positive. Microscopic lesions consisting of follicular, para-

Table 33. Presence of gross/microscopic lesions in two foxes orally challenged with 8.34×10^7 CFU and two foxes foxes orally challenged with 4.9×10^9 CFU *B. suis* type 4 in 1982.

		Pathology Number			
Tissue	Sex	3402[1] M	3405[2] F	3404[3] M	3403[4] M
Mandibular L.N.		-[5]/NE[6]	-/-	-/NE	-/-
Retropharyngeal L.N.		NE/NE	+[7]/+[8]	-/NE	-/-
Popliteal L.N.		-/NE	-/-	-/NE	-/+8,9
Internal Iliac L.N.		-/NE	+7/+[10]	-/NE	-/NE
Testis/ Uterus		-/-	-/-	-/-	-/-
Prostate/ Epididymis		-/- NE/NE		NE/NE NE/NE	NE/NE -/-
Heart		-/NE	-/-	-/-	-/-
Liver		-/-	-/-	-/-	-/-
Lung		-/-	-/-	-/-	-/-
Kidney		-/-	NE/NE	-/-	-/-
Spleen		-/-	-/-	-/-	-/-
Tonsil		NE/NE	NE/NE	NE/NE	-/-

1 Challenge dose = 8.34×10^7; sacrificed 7 weeks PE
2 Challenge dose = 8.34×10^7; sacrificed 16 weeks PE
3 Challenge dose = 4.9×10^9; sacrificed 9 weeks PE
4 Challenge dose = 4.9×10^9; sacrificed 22 weeks PE
5 No lesions observed
6 Not examined
7 Swollen
8 Follicular hyperplasia
9 Medullary cord hyperplasia
10 Hyperemic

Table 34. Presence of gross/microscopic lesions in four foxes orally challenged with 1.21 x 10^9 CFU B. suis type 4 4 in 1983.

Tissue	Pathology Number			
Sex	3516[1] M	3517[2] F	3537[3] F	3547[4] M
Mandibular L.N.	-/-[5]	-/NE[6]	-/NE	-/NE
Retropharyngeal L.N.	+[7]/-	+[7]/-	+[7]/+[8]	-/-
Superficial Cervical L.N.	NE/NE	+[7]/-	+[7]/+[9]	-/-
Popliteal L.N.	+[7]/-	-/NE	-/+[10]	NE/NE
Internal Iliac L.N.	-/-	-/-	-/+[10]	-/NE
Tracheobronchial L.N.	-/NE	-/NE	NE/NE	-/+[11]
Testis/ Uterus	-/-	-/-	-/-	-/-
Epididymis/ Ovary	-/-	-/-	-/-	-/-
Heart	-/-	-/-	NE/NE	-/-
Liver	-/-	-/NE	-/-	-/-
Lung	-/-	-/-	-/-	-/-
Kidney	-/-	-/-	-/-	-/-
Spleen	-/NE	-/-	-/NE	-/-
Tonsil	-/-	-/-	-/-	-/-
Salivary Gland	NE/NE	-/-	-/+[12]	-/-
Thymus	-/-	-/NE	-/-	-/NE

Table 34. (continued)

[1] Sacrificed 3 weeks PE
[2] Sacrificed 4 weeks PE
[3] Sacrificed 7 weeks PE
[4] Sacrificed 13 weeks PE
[5] No lesions observed
[6] Not examined
[7] Swollen
[8] Medullary cord hyperplasia
[9] Cortical foci of neutrophils and macrophages
[10] Follicular hyperplasia
[11] Germinal centers containing active macrophages, hyperemia, necrosis
[12] One cluster of mononuclear cells

Table 35. Presence of gross/microscopic lesions in four foxes orally challenged with 1.06 x 10^{11} CFU B. suis type 4 in 1983.

Tissue	Pathology Number			
Sex	3548[1] M	3549[2] F	3552[3] M	3559[4] F
Mandibular L.N.	-[5]/+[6]	-/+7,8	-/+7	-/+6
Retropharyngeal L.N.	-/+7,8	+9/+6,10	-/-	-/+10
Superficial Cervical L.N.	NE[11]/NE	-/NE	-/+6	-/-
Popliteal L.N.	NE/NE	+9/+6	-/NE	NE/NE
Internal Iliac L.N.	-/+8	+9/+7,8	-/+7,10	-/-
Mesenteric L.N.	NE/NE	-/-	-/-	-/-
Tracheobronchial L.N.	NE/NE	-/-	NE/NE	NE/NE
Testis/ Uterus	-/-	-/-	-/-	-/-
Epididymis	-/-		-/-	
Prostate/ Cervix	-/-	-/-	-/-	NE/NE
Heart	-/NE	-/-	-/-	-/-
Liver	-/-	-/-	-/-	-/-
Lung	-/-	-/-	-/NE	-/-
Kidney	-/-	-/-	-/NE	-/-
Spleen	-/-	-/-	-/NE	-/-
Tonsil	-/-	-/+12	-/-	-/-
Salivary Gland	-/-	-/NE	-/-	-/-
Thymus	-/-	-/-	-/-	-/-

Table 35. (continued)

1 Sacrificed 14 weeks PE
2 Sacrificed 15 weeks PE
3 Sacrificed 16 weeks PE
4 Sacrificed 18 weeks PE
5 No lesions observed
6 Follicular hyperplasia
7 Medullary cord hyperplasia
8 Germinal centers containing active macrophages, necrosis
9 Swollen
10 Paracortical hyperplasia
11 Not examined
12 Inflammatory foci in epithelium

cortical, and medullary cord hyperplasia, and germinal centers with active macrophages and necrosis were seen in one or more lymph nodes of all four foxes (Table 35). Nine of the eleven lymph nodes with microscopic lesions were also culture-positive for B. suis type 4.

Microscopic examination of testes for each fox indicated they were normal for the time of year. Few spermatocytes and no spermatozoa were seen in testes of two foxes necropsied in May. Testes of foxes necropsied in July, August or October were inactive. Uteri examined were typical of those from non-estrus, non-gravid females. Bacteria were not seen in any tissues or lymph nodes stained by Brown and Brenn's technique.

In summary, a challenge dose on the order of 10^7 CFU B. suis type 4 was not adequate to consistently produce infection. Serologic titers to brucellosis followed typical patterns. The SP test detected antibody response before the Riv, BBA, of CF tests, but titers on the latter three tests remained higher longer.

B. suis type 4 was isolated from oral, genital, and/or fecal samples in three of four foxes challenged with 10^9 CFU in 1983. The organism was isolated from similar samples of all six foxes challenged with 10^{11} CFU.

No clinical signs of infection or effects on reproduction were seen.

At necropsy, B. suis type 4 was isolated from six lymph nodes of one of two foxes challenged with 10^7 CFU. Of two foxes challenged with 10^9 CFU in 1982, four lymph nodes were culture-positive at 9 weeks PE in one fox, and one lymph node was culture-positive at 22 weeks PE in the

second fox.

Of four foxes challenged with 10^9 CFU in 1983 and sacrificed from 3-13 weeks PE, B. suis type 4 was isolated from six different lymph nodes plus salivary gland (2), tonsils (3), liver (2), spleen (3), kidney (1), and thymus (1). The organism was isolated from seven different lymph nodes plus tonsils (3), kidney (1), and thymus (1) in four foxes challenged with 10^{11} CFU and sacrificed between 14 and 18 weeks PE.

Swollen lymph nodes were the only gross lesions consistent with brucellosis infection observed at necropsy. Severity and frequency of microscopic lesions increased with the level of challenge dose.

TRANSMISSION OF BRUCELLA SUIS TYPE 4 - FOX TO FOX

Serology

A pair of non-challenged foxes were housed for 20 weeks in the same room with four other foxes challenged with 1.06×10^{11} CFU B. suis type 4. Positive serologic reactions were detected in the non-challenged female on the SP, Riv and CF tests at 5 weeks PE, and on the BBA test at 6 weeks. With the exception of the CF test, brucellosis titers peaked at 10 weeks, then declined until the time of necropsy at 20 weeks. Reactions on the CF test remained high from 5 to 20 weeks following exposure (Appendix, Table 59).

The SP test was positive at weeks 7, 10 and 18 in the non-challenged male fox. The BBA test was positive at week 18 only. A low titer was detected only at week 10 on the Riv test. The CF test was positive from week 7 to 20 inclusive (Appendix, Table 60).

Bacteriology

B. suis type 4 was not isolated from hemocultures of the two foxes considered naturally infected (Appendix, Tables 59-60), nor were any oral or genital cultures positive. Fecal samples were not cultured.

Clinical Signs

The mean WBC in the non-challenged foxes was significantly higher at 20 weeks PE than that of two other foxes that had been challenged with 1.06×10^{11} CFU and housed in the same room. (t-test; $p<0.05$). Hematologic values for the foxes themselves were considered normal (Table 36).

Table 36. Blood values of two unchallenged foxes housed in the same room with four foxes orally challenged with 1.06 x 10^{11} CFU *B. suis* type 4 in 1983.

Weeks Post-Exposure	PCV[1]	WBC[2]	Percent Neutrophils	Percent Lymphocytes	Percent Monocytes	Percent Eosinophils	Percent Basophils
-12[3]	36.0[4]	5830	69.0	27.0	3.0	0.0	0.0
	44.0	2750	26.0	64.0	6.0	4.0	0.0
-1	50.0	6200	64.0	34.0	0.0	2.0	0.0
	46.0	4600	26.0	64.0	10.0	0.0	0.0
2	48.0	5000	52.0	32.0	16.0	0.0	0.0
	46.0	4800	48.0	64.0	8.0	0.0	0.0
3	40.0	3200	56.0	32.0	14.0	0.0	0.0
	48.0	4200	38.0	52.0	10.0	0.0	0.0
5	42.0	3000	46.0	36.0	14.0	4.0	0.0
	48.0	2700	56.0	36.0	8.0	0.0	0.0
7	39.0	4070	66.0	30.0	2.0	2.0	0.0
	40.0	5390	37.0	51.0	2.0	10.0	0.0
10	44.0	7260	60.0	37.0	2.0	1.0	0.0
	46.0	5390	23.0	73.0	1.0	3.0	0.0
12	46.0	8140	72.0	22.0	1.0	5.0	0.0
	44.0	4620	37.0	50.0	1.0	12.0	0.0
14	44.0	6380	54.0	28.0	12.0	6.0	0.0
	44.0	8910	34.0	52.0	10.0	4.0	0.0
16	44.0	5600	42.0	49.0	2.0	7.0	0.0
	45.0	4700	37.0	60.0	2.0	5.0	0.0
18	44.0	5720	60.0	37.0	4.0	2.0	0.0
	45.0	3190	48.0	42.0	6.0	4.0	0.0
20	44.0	5170*	54.0	26.0	14.0	6.0	0.0
	45.0	5500*	33.0	55.0	9.0	3.0	0.0

Table 36. (continued)

[1] Packed cell volume (percent)
[2] White blood cells/mm^3
[3] n=2 for all samplings
[4] Individual values
* Significantly higher than in infected foxes in same room ($p<0.05$)

Reproduction

No offspring were born to this pair, and no sign of pregnancy was seen at necropsy.

Necropsy - Bacteriology/Pathology

B. suis type 4 was isolated from the mandibular, retropharyngeal, and supramammary lymph nodes of the female, and from the mesenteric and tracheobronchial lymph nodes of the male at necropsy (Table 37).

Retropharyngeal and superficial cervical lymph nodes were enlarged in both foxes. Popliteal and internal iliac lymph nodes were enlarged in the male (Table 38). One (retropharyngeal of the female) of six of these swollen lymph nodes was culture-positive (Appendix, Table 61).

Microscopic lesions seen in lymph nodes of both foxes included mild hyperplasia, follicular and paracortical hyperplasia, plus foci of neutrophils, macrophages, and necrosis (Table 38). Four of eight of these lymph nodes, two each from the male and female, were culture-positive (Appendix, Table 61).

Gross examination of reproductive organs was normal. Microscopically, the uterus appeared normal for a non-estrus, non-gravid female. Testes were not examined microscopically.

In summary, development of serologic titers to brucellosis in these foxes five weeks following the challenge of other foxes in the room suggests that transmission probably took place during the first two weeks. Isolation of B. suis type 4 from lymph nodes draining the head

Table 37. Culture of B. suis type 4 from tissues of two non-challenged foxes held for 20 weeks in the same room with four foxes orally challenged with 1.06×10^{11} CFU B. suis type 4 in 1983.

Tissue	Foxes Positive/Foxes Cultured
Mandibular L.N.	1/2
Retropharyngeal L.N.	1/2
Superficial Cervical L.N.	0/2
Femoral L.N.	0/0
Popliteal L.N.	0/2
Internal Iliac L.N.	0/2
Mesenteric L.N.	1/2
Tracheobronchial L.N.	1/2
Supramammary L.N.	1/1
Superficial Inguinal L.N.	0/0
Epididymis	0/1
Testis	0/1
Ovary	0/1
Uterus	0/1
Heart	0/2
Liver	0/2
Lung	0/2
Kidney	0/2
Spleen	0/2
Tonsil	0/2

Table 37. (continued)

Tissue	Foxes Positive/Foxes Cultured
Salivary Gland	0/2
Bladder	0/2
Thymus	0/1

Table 38. Presence of gross/microscopic lesions in two non-challenged foxes held in the same room for 20 weeks with four foxes orally challenged with 1.06×10^{11} CFU *B. suis* type 4 in 1983.

Tissue	Sex	Pathology Number 3562 F	3563 M
Mandibular L.N.		-[1]/+[2,7]	-/+[3,4]
Retropharyngeal L.N.		+[5]/+[4,6,7]	+[5]/+[3,7]
Superficial Cervical L.N.		+[5]/-	+[5]/+[4,7]
Popliteal L.N.		-/-	+[5]/+[7]
Internal Iliac L.N.		-/-	+[5]/-
Tracheobroncial L.N.		NE[8]/NE	-/+[9]
Mesenteric L.N.		NE/NE	-/+[9]
Supramammary L.N.		-/-	
Testis/ Uterus		-/-	-/-
Heart		-/-	-/-
Liver		-/-	-/-
Lung		-/-	-/-
Kidney		-/-	-/-
Spleen		-/-	-/-
Tonsil		-/-	-/-
Salivary Gland		-/-	-/-
Thymus		-/-	-/-

Table 38. (continued)

[1] No lesions observed
[2] Paracortical hyperplasia
[3] Hyperemia
[4] Foci of neutrophils and macrophages
[5] Swollen
[6] Areas of necrosis
[7] Follicular hyperplasia
[8] Not examined
[9] Mild hyperplasia

as well as from the tracheobronchial lymph nodes would be compatible with transmission by aerosols. Failure to isolate organisms from the blood or from body secretions or excretions and a relatively short duration of antibody titers to brucellosis indicates these foxes had a lower infective dose than those experimentally challenged.

TRANSMISSION OF BRUCELLA SUIS TYPE 4 - FOX TO LEMMING

Serology/Bacteriology/Pathology

Fifty-seven different collared lemmings were housed under fox cages during the two phases of this study to biologically monitor shedding of B. suis type 4.

In the first phase, no gross or microscopic lesions were observed in lemmings exposed to the two foxes (#'s 3402 and 3405) receiving the lower dose (8.34×10^7 CFU) in 1982. Microscopic lesions characteristic of brucellosis infections were observed in the livers of four different lemmings exposed to the two males (#'s 3404 and 3403) receiving the higher challenge dose (4.9×10^9 CFU) (Table 39).

Gross and microscopic lesions were observed in two different lemmings exposed to a fox (#3517) challenged with the lower dose (1.21×10^9 CFU) in 1983. Microscopic lesions alone were seen in two lemmings exposed to a second fox (#3547) receiving the same challenge dose (Table 39).

Microscopic lesions characteristic of brucellosis infections were observed in eight different lemmings exposed to three different foxes (#'s 3548, 3549, and 3559) challenged with 1.06×10^{11} CFU in 1983. One of these lemmings (#3536) was also seropositive. Another of these lemmings (#3498) was culture-positive, seropositive, and had gross lesions on the liver and spleen at necropsy (Table 39).

Table 39. Evidence of transmission of brucellosis from red foxes to collared lemmings.

Lemming Number	Fox Number	Weeks P.E. Exposed to Fox	Serologic Results	Culture Results	Gross Lesions	Micro-scopic Lesions
3396	3402	3-5	-[1]	-	-	-
3400	3402	3-5	-	-	-	-
3438	3402	7-9	-	-	-	-
3395	3405	3-5	-	-	-	-
3401	3405	3-5	-	-	-	-
3439	3405	5-7	-	-	-	-
3444	3405	7-9	-	-	-	-
3451	3405	9-12	-	-	-	-
3453	3405	12-13	-	-	-	-
3445	3405	13-17	-	-	-	NT[3]
3397	3404	3-5	-	-	-	+[4]
3399	3404	3-5	-	-	-	-
3437	3404	5-7	-	-	-	-
3443	3404	7-9	-	-	-	+[4]
3394	3403	3-5	-	-	-	+[4]
3398	3403	3-5	-	-	-	+[4]
3436	3403	5-7	-	-	-	-
3442	3403	7-9	-	-	-	-
3450	3403	9-12	-	-	-	-
3452	3403	12-13	-	-	-	-
3454	3403	13-17	-	-	-	-
3459	3403	17-22	-	-	-	-
3503	3516	0-1	-	-	-	-
3520	3516	1-2	-	-	-	-
3530	3516	2-4	NT	NT	NT	NT
3502	3517	0-1	-	-	+[6]	+[4]
3519	3517	1-2	-	-	+[7]	+[4]
3529	3517	2-4	-	-	-	-

Table 39. (continued)

Lemming Number	Fox Number	Weeks P.E. Exposed to Fox	Serologic Results	Culture Results	Gross Lesions	Micro-scopic Lesions
3504	3537	0-1	NT	-	-	NT
3521	3537	1-2	NT	-	-	-
3531	3537	2-4	-	-	-	-
3542	3537	4-7	-	-	-	-
3505	3547	0-1	-	-	-	-
3522	3547	1-2	-	-	-	+5
3532	3547	2-4	-	-	-	+4
3543	3547	4-7	-	-	-	-
3546	3547	7-13	-	-	-	-
3498	3548	0-1	+	+	+8	+4,9
3523	3548	1-2	-	-	-	+4
3523	3548	2-4	-	-	-	-
0191	3548	4-7	NT	NT	NT	NT
3555	3548	7-14	-	-	-	-
3499	3549	0-1	-	-	-	+4
3524	3549	1-2	-	-	-	+4
3534	3549	2-4	-	-	-	-
3544	3549	4-7	-	-	-	-
3556	3549	7-14	-	-	-	+5
3500	3552	0-1	-	-	-	-
3525	3552	1-2	-	-	-	-
3535	3552	2-4	-	-	-	-
3545	3552	4-7	-	-	-	-
3557	3552	7-16	-	-	-	NT
3501	3559	0-1	-	-	-	+4
3526	3559	1-2	-	-	-	-
3536	3559	2-4	+	-	-	+5
3541	3559	4-7	NT	-	-	NT
3564	3559	7-18	-	-	-	+4

Table 39. (continued)

[1] Negative results
[2] Weeks post-exposure of foxes to B. suis type 4
[3] Not tested
[4] Foci of mononuclear cells in liver
[5] One tiny mononuclear accumulation
[6] Enlarged mandibular lymph node and uterus
[7] Pinpoint white foci on liver
[8] Spleen enlarged; pinpoint white foci on liver
[9] Prominent macrophages in spleen

In summary, serologic tests and culture techniques were not sensitive indicators of transmission of B. suis type 4 from red foxes to collared lemmings as originally anticipated. Microscopic lesions in the livers of lemmings exposed to foxes receiving a challenge dose of 10^9 or 10^{11} CFU were a more sensitive indicator of exposure. Only one lemming exposed to a fox receiving the highest challenge dose (10^{11} CFU) was positive by serologic, cultural, and gross and microscopic pathologic methods.

ORAL INFECTIVE DOSE OF BRUCELLA SUIS TYPE 4 FOR LEMMINGS

Because so few isolations of B. suis type 4 were made from lemmings exposed to infected foxes, eight other lemmings were orally challenged with known numbers of organisms to determine an infective dose necessary to establish a detectable infection.

A kidney abscess was seen in one of two lemmings challenged with 3.7 CFU, and microscopic lesions were observed in the salivary glands of both (Table 40).

Microscopic lesions were seen in the liver and kidney of one of two lemmings challenged with 3.7×10^2 CFU. The second lemming was negative on serologic tests and culture, and no gross or microscopic lesions were observed (Table 40). Microscopic lesions were seen in the salivary gland of one lemming challenged with 3.7×10^4 CFU. Gross and microscopic lesions were seen in the liver. B. suis type 4 was isolated from the heart, liver, spleen, and salivary gland of the second lemming challenged with the same dose (Table 40).

Table 40. Oral challenge of lemmings to B. suis type 4.

Lemming Number	Infective Dose (CFU)	Time P.E. of Sacrifice (Weeks)	Serology Results	Culture Results	Gross Lesions	Microscopic Lesions
3572	$3.7x10^0$	4	-[1]	-	+[2]	+[3]
3576	$3.7x10^0$	5	-	-	-	+[3,4]
3541	$3.7x10^2$	4	-	-	-	-
3575	$3.7x10^2$	5	-	-	-	+[5]
3570	$3.7x10^4$	4	-	+[6]	+[7]	+[8]
3574	$3.7x10^4$	5	-	-	-	+[3]
3569	$3.7x10^6$	4	NT	NT	NT	NT
3573	$3.7x10^6$	5	+	+[9]	+[7,10]	+[3,4,8]

1 Negative results
2 Kidney abscess
3 Several foci of mononuclear cells in salivary gland
4 Neutrophils and necrosis in salivary gland
5 Foci of mononuclear cells in liver and kidney
6 Heart, liver, kidney, spleen, salivary gland
7 White, pinpoint liver abscesses
8 Foci of mononuclear cells and necrosis in liver
9 Liver, lung, spleen, salivary gland
10 Salivary gland abscess

One lemming challenged with 3.7×10^6 CFU died 4 weeks post-challenge. Tissues were too autolyzed for examinations. The other lemming receiving this dose was seropositive, and B. suis type 4 was isolated from the salivary gland, liver, lung and spleen. Abscesses, characterized microscopically by inflammatory cells and central necrosis, were seen on the liver and in the salivary gland (Table 40).

Therefore, gross and microscopic lesions were detected in collared lemmings receiving an oral challenge dose of 10^0 or 10^2 CFU B. suis type 4. However, a dose of 10^4 CFU was required to also be detected culturally, and a dose of 10^6 CFU was required to stimulate a serologic response.

TRANSMISSION OF BRUCELLA SUIS TYPE 4 - FOX TO REINDEER

FOXES

Serology

Serologic titers to brucellosis were detected 1 week PE on the SP test in one fox and the ST test in the second fox housed with the two reindeer. Both foxes were positive on the BBA and CF tests at 2 weeks PE, and on all tests by 3 weeks PE. Titers remained high on all tests on the male for 23 weeks PE. Following that, titers on the SP test fluctuated between 25 and 50 until the fox was sacrificed at 66 weeks PE. Titers on the other tests continued to be high. Titers remained high on all serologic tests on the female until she was sacrificed at 66 weeks PE (Appendix, Tables 62 and 63).

Analysis of individual serologic tests was included with results from four other foxes challenged at the same time with the same dose (1.21×10^{11} CFU) in another phase of the study.

Bacteriology

B. suis was not isolated from hemocultures from either of the two foxes (Appendix, Tables 62 and 63). However, four hemocultures were contaminated by other bacteria.

B. suis type 4 was not isolated from oral or genital swabs from the two foxes. Several cultures were contaminated with Proteus sp.

Few fecal samples were available to culture. However, a fecal sample from day 3 of challenge in the female and day 4 of challenge of the male were culture-positive for B. suis type 4.

Clinical Signs

No clinical signs were observed in the two foxes. Hematologic values are presented in Table 30.

Reproduction

This was the only pair of foxes in the study to successfully reproduce. Pups were born 52 days (which equals the gestation period of red foxes) after the adults were introduced into the reindeer room. Results of samples collected from these offspring follow.

Both adult foxes were sacrificed at 66 weeks PE after being housed as a pair through a second breeding season. No offspring were produced the second year, and no sign of pregnancy was seen in the female at necropsy.

Necropsy - Bacteriology/Pathology

B. suis type 4 was isolated from the internal iliac lymph node from the female, but from no tissues or lymph nodes from the male (Appendix, Table 64). No gross or microscopic lesions were seen in lymph nodes or tissues of either fox (Table 41). These findings were consistent with a brucellosis infection of long duration. Microscopic examination indicated the testes and uterus were inactive.

OFFSPRING OF FOXES

Serology

Serologic titers to brucellosis were detected in one (#3565) of three pups born 52 days PE to the pair of infected foxes housed with the two reindeer. The SP, Riv and CF tests were positive at 13 weeks of

Table 41. Presence of gross/microscopic lesions in two foxes orally challenged with 1.06×10^{11} CFU *B. suis* type 4 in 1983 and sacrificed 66 weeks PE.

Tissue	Sex	Pathology Number 3629 M	3630 F
Mandibular L.N.		-/-[1]	-/NE[2]
Retropharyngeal L.N.		-/-	-/-
Superficial Cervical L.N.		-/NE	-/-
Popliteal L.N.		-/-	-/-
Internal Iliac L.N.		-/-	-/-
Mesenteric L.N.		NE/NE	-/-
Testis/ Uterus		-/-	-/-
Prostate/ Ovary		-/-	-/-
Heart		-/-	-/-
Liver		-/-	-/-
Lung		-/-	-/-
Kidney		-/-	-/-
Spleen		-/-	-/-
Tonsil		-/-	-/-
Salivary Gland		-/-	-/-
Thymus		-/-	-/-

[1] No lesions observed
[2] Not examined

age, and the BBA was also positive at 14 weeks. No titers were detected in the other two pups (Appendix, Tables 65-67).

Bacteriology

B. suis type 4 was not isolated from hemocultures of these three pups (Appendix, Tables 65-67). Oral, genital, and fecal samples were not collected.

Necropsy - Bacteriology/Pathology

B. suis type 4 was isolated from the mandibular, retropharyngeal, and superficial cervical lymph nodes, and also from the spleen and thymus of the seropositive female (#3565) necropsied at 14 weeks of age. No organisms were isolated from tissues of the other two seronegative pups necropsied at 20 and 60 weeks of age (Appendix, Table 68).

Microscopic lesions were seen in the retropharyngeal lymph nodes of all three pups (Table 42). Only one of these (#3565) was culture-positive. The cause of the microscopic lesions in the other two pups could not be determined.

Detection of serologic titers to brucellosis in only one pup at 13 weeks of age indicated transmission probably occurred from the mother after birth rather than in utero. The culture-positive retropharyngeal lymph node suggests an oral route of exposure.

Table 42. Presence of gross/microscopic lesions in three foxes born to parents each orally challenged with 1.06×10^{11} CFU *B. suis* type 4 in 1983.

Tissue	Pathology Number 3565	3577	3633
Mandibular L.N.	-[1]/NE[2]	-/-	-/NE
Retropharyngeal L.N.	-/+[3,4]	-/+[3]	-/+[5]
Superficial Cervical L.N.	-/NE	-/NE	-/NE
Popliteal L.N.	NE/NE	-/NE	NE/NE
Internal Iliac L.N.	-/NE	NE/NE	-/NE
Tracheobronchial L.N.	-/-	NE/NE	NE/NE
Heart	-/-	-/-	-/-
Liver	-/-	-/-	-/-
Lung	-/-	-/-	-/-
Kidney	-/-	-/-	-/-
Spleen	-/-	-/-	-/-
Tonsil	-/-	-/-	-/-
Salivary Gland	-/-	-/-	-/-
Thymus	-/-	-/-	-/-

1 No lesions observed
2 Not examined
3 Follicular hyperplasia
4 Foci of neutrophils and necrosis
5 Paracortical hyperplasia

REINDEER

Serology

Positive serologic reactions were detected on the SP test in both reindeer 1 week after the foxes they were housed with were challenged. The BBA test was first positive in the two reindeer at 2 and 5 weeks, the Riv at 3 and 5 weeks, and the CF test at 2 and 5 weeks. Titers remained high on all tests on one of the reindeer (#3230) until he was sacrificed at 17 weeks (Appendix, Table 69). Titers on the other reindeer (#3554) rose from week 3 to 10, then began to decline. When he was sacrificed at 17 weeks, the Riv test was negative, the SP test reaction was I50, the BBA test was positive, and the CF test was still high at 86 (Appendix, Table 70).

Bacteriology

B. suis type 4 was isolated from a hemoculture of one of the reindeer (#3230) 3 weeks following fox challenge (Appendix, Table 69). No hemocultures from the other deer were culture-positive (Appendix, Table 70).

Clinical Effects

No clinical signs of brucellosis were seen in the reindeer.

Necropsy - Bacteriology/Pathology

Both reindeer were sacrificed 17 weeks after being housed with the infected foxes. B. suis type 4 was isolated from the mandibular, retropharyngeal, parotid, superficial cervical, and popliteal lymph nodes as well as the lung, jaw abscess and anorectal abscess of one

(#3230) of the two reindeer (Appendix, Table 71).

A jaw and anorectal abscess were the only gross lesions seen. No remarkable microscopic lesions were seen.

These results indicate that reindeer #3230 probably received a relatively higher exposure dose than #3554.

DISCUSSION

PROTOCOL

Wild-trapped red and fox farm silver, pearl and amber foxes used in experimental infections are all color phases of the red fox. Reds carry the dominant color genes (AABB), while Alaskan silver foxes are genotypically aaBB. Modifying genes have yielded such color variations as burgundy, pearl, and amber (Ables 1975; Nes et al. 1983). Use of different color phases should not have affected experimental results.

As a species, red foxes are extremely excitable. Psychotic-like behavior including aggression, withdrawal, catatonia, panic, and flight has been reported in foxes brought into captivity as adults. Fear of open spaces, movement, white objects, sounds, eyes or lenses, large objects, and people has also been reported. The stress of captivity can make them disturbed, confused, or depressed (Keeler 1975).

An effort was made in all phases of experimental infections to keep stress on the foxes to a minimum. Most of the red foxes used had been trapped in the wild as young and held in captivity for several months before experiments were initiated. Three brought in as older animals were definitely more excitable. Those purchased from a fox farm seemed well adapted to captivity. In the second phase of the study, all foxes could be handled for sampling without being sedated.

The experimental challenge was patterned after studies conducted in Texas on coyotes to facilitate comparison of results.

PATHOGENESIS OF BRUCELLA SUIS TYPE 4 IN FOXES

SEROLOGY

IgM is the major immunoglobulin initially produced in an immune response. It is followed in a few days by the production of IgG which eventually predominates in the later stages (weeks to months) (Tizard 1977). Thus, IgM is characteristic of a recent infection while IgG is more characteristic of a chronic infection.

Serologic diagnosis of brucellosis is generally made on the basis of a battery of tests. Most criteria have been derived from diagnostic standards in cattle. Serologic tests for brucellosis are usually not carried out to end points, but to a standard dilution for each test.

Serum agglutination tests (ST and SP) have historically been the principal methods used. The SP test was designed as a more simple, less time-consuming equivalent of the ST test. Both tests detect IgM and IgG agglutinating antibodies. IgM is a much more efficient agglutinator due to its pentameric structure (FAO/WHO 1986).

Supplementary tests such as the BBA, ME and Riv were designed to distinguish non-specific from specific reactions. In cattle, one of the IgG isotypes (IgG_1) is the predominant reactor in the low acid conditions of the BBA test. Treatment of serum with sulfhydryl reducing agents such as 2-mercaptoethanol dissociates the IgM molecule but leaves the IgG intact. Rivanol, an acridine dye (6,9-diamino-2-ethoxyacridine), selectively precipitates more IgM than IgG (FAO/WHO

1986).

The CF test is widely used as a supplemental test on samples that are suspicious on standard agglutination reactions, or as a confirmatory test on samples that are positive on the BBA test. Many workers feel the CF test is the most accurate serologic method for diagnosis of brucellosis in cattle, sheep and goats (Alton et al. 1975). IgG fixes complement, but the complement fixing ability of IgM depends on the conditions of the procedure. IgM may not be detected as well in a CF test system using warm fixation (FAO/WHO 1986). Warm fixation was used in both CF tests (Hill's and automated) used in these experiments.

It is expected that the SP and ST tests would be the first to detect a brucellosis infection, that positive reactions on the BBA, 2-ME, Riv and CF tests would soon follow, and that titers on the CF test would persist the longest.

Experimental Infections

Results from experimentally infected foxes indicated the serologic response was typical of that seen in brucellosis infections in most species. Serotiters were detected first on the ST or SP tests. Decline in titers on the SP test relative to those on the Riv or CF tests, reflecting a decreased production of IgM, was seen in the later stages of infection in foxes challenged with 10^9 or 10^{11} CFU.

Even though titers on the SP declined, diagnostic titers were maintained on all tests for the duration of the experiments. Of the two foxes held for 66 weeks, titers remained higher in the female.

Sensitivity of the SP test was greater than that of the BBA, Riv

or CF tests in 1983 because it detected the initial incubation stages of infection (IgM) the other tests missed.

Although the ST and SP tests detect the same antibody activity, the ST test detected serologic reactions before any of the other tests on foxes challenged in 1982. Workers in other fields recognized early that not all sera gave the same reaction on the SP and ST tests (Pietz 1970), and the same appears true for fox sera. Sensitivity of the SP test on foxes challenged in 1982 was the same as that of the BBA, Riv or CF tests. Because the ST test was conducted on a limited number of samples, sensitivity was not calculated.

In other species, results on the Riv and ME tests are not always identical even though they both theoretically detect only IgG (Crawford and Hidalgo 1977). Results of these tests on selected fox serum samples usually parallelled, but were not always the same as, each other.

Pairs of serologic tests for brucellosis are frequently compared. In these experiments, the Riv and CF tests tended to have the best agreement, while the SP test paired with other tests had the least agreement. This is consistent with the nature of the tests detecting different immunoglobulin fractions. It is difficult to compare individual tests with those in other species due to different diagnostic criteria used.

Serologic tests for B. canis antibodies were run on representative samples or as quantity allowed. Titers of +100 to B. canis were detected in two foxes in 1983. These titers, considered suspicious (Alton et al. 1975), were probably either non-specific or due to a

cross reaction with a related organism (Flores-Castro and Carmichael 1978; Hoff et al. 1974; Randhawa et al. 1977b).

Natural Infections

Isolation of the organism is the only sure way to confirm brucellosis infection. Because B. suis type 4 was isolated from only four foxes, it was difficult to make correlations between individual serologic tests and culture results. However, information gained from patterns on serologic test results on experimentally infected foxes could be applied to naturally infected foxes collected in the field.

Most foxes tended to be positive on several serologic tests if they were positive on one at all. No differences were seen in the relative frequencies of the various serologic tests being positive.

Of the animals that were not positive on all serologic tests, #3089 (female arctic fox) probably had acquired a recent infection since her serologic results (positive on only the SP and BBA) indicated primarily a IgM response. Isolation of the Brucella organism confirmed her infection.

Number 3084 (male red fox) and #3095 (female arctic fox) were positive on only the SP. Their exposures may have been even more recent since no organisms were isolated from their tissues.

As in other species of animals, a diagnosis of brucellosis in foxes should not be made on any one serologic test. Results from a battery of tests should be used to support a conclusion.

Serologic titers were indicators of prior exposure to the Brucella organism but not necessarily a measure of the immune status to the

disease. Cell-mediated immunity is recognized as playing an important role in the outcome of brucellosis infections. Procedures for the use of cell-mediated immunity as a diagnostic test for any species infected with brucellosis have not been standardized and are currently not recommended (FAO/WHO 1986). Therefore, aspects of cell-mediated immunity were not addressd in this study.

Suspicious titers to B. canis were detected in three red foxes and one arctic fox. These were probably non-specific or heterologous reactions.

CLINICAL EFFECTS

Effects of brucellosis infections in experimentally challenged foxes were not reflected in hematologic parameters. Leukopenias have sometimes been associated with, but not necessarily the result of, human brucellosis infections (Crosby et al. 1984). Rises or falls in total white blood counts in individual foxes did not follow a consistent pattern. Blancou et al. (1982) reported captive foxes had significantly more white blood cells in January than June, but this was not observed in these studies.

Clinical signs such as those occasionally reported in dogs with B. abortus, B. melitensis, or B. suis infections or those typically seen in dogs with B. canis infections were not seen in foxes infected with B. suis type 4.

REPRODUCTION

Natural Infections

Brucellosis was not observed to be associated with gross or microscopic lesions in the reproductive tract of males or females collected in this study. Nor was any relationship observed between reproductive status and brucellosis infection. Foxes were collected in the spring during the time they would normally be pregnant or lactating, yet some females showed neither indication of pregnancy nor of pathology in the reproductive tract. There was no statistical difference (chi square; $p>0.01$) in the number of pregnant and non-pregnant females that were seropositive and/or culturepositive for brucellosis. No difference was detected in prevalence of exposure between males and females.

Many complicating factors contribute to successful or unsuccessful fox reproduction. According to Allen (1984), increases in ovulation rates, embryonic litter sizes, and declines in prenatal mortality in red foxes are a function of increasing age of the female. In studies in North Dakota, the more adult males per female, the lower the overall ovulation rate and litter size, and the higher the prenatal mortality. Most pre-natal mortality occurred in the first third of gestation in females over 1 year of age (Allen 1984).

Barker (1943) examined the ovaries and placental sites of 865 female foxes. Corpora lutea were present in most of the yearling and all of the older animals. She concluded that most reproductive failures occurred in yearling females early in gestation, and that adult foxes

lost their young after placental sites were well developed.

Several workers have attributed variation in successful breeding to density-dependent factors and an unstable food supply (Englund 1970; Englund 1980; Lindstrom 1983; Macdonald 1980). Macdonald (1980) felt a status-linked reproductive suppression could occur among foxes that lived as groups in certain habitats and could explain variation in female productivity within and between habitats.

In a different situation, Macdonald (1979) reported on non-breeding females, "helpers", that helped care for pups in red fox groups. VonSchantz (1981), conducting studies in southern Sweden, reported "non-breeding" females regularly became pregnant, but either aborted or deserted their young. These non-breeding, beta females were probably related to each other and to the breeding, alpha females. The beta females used less desirable habitat than the alpha females and would visit the alpha dens more as back-up than as "helpers."

Arctic fox populations cycle up and down approximately every 4 years in many places including Alaska. Cycles seem to correlate with prey availability. Fox and lemming cycles are interrelated, but the mechanism is unclear (Chesemore 1967 and 1975). Reproductive success has been associated with an adequate food supply (Burgess 1984; MacPherson 1962).

It can be appreciated from the literature that reproduction in wild foxes is very complex. Even successful reproduction in fox-farm foxes is as much an art as a science. Considering the behavioral and ecological factors involved in fox reproduction, it would be difficult to assess

the effects of brucellosis infections on reproductive failures in field situations.

Experimental Infections

The breeding season for Vulpes in interior Alaska is usually considered to be from January through March with peak activity occurring in February and March (J. Rice and E. Follmann, pers. comm.). Foxes used for experimental infections in these studies were housed as pairs from January through late March in 1982 and January through early April in 1983.

In spite of efforts to keep stress at a minimum at all times and controlling light cycles to simulate the natural photoperiod, breeding was not successful in foxes used for experimental infections except for the pair housed with the reindeer. Vulvas of foxes on fox farms are customarily examined every 3 days for redness and swelling during the breeding season. After swelling is first observed, the vulva is examined every day. Ovulation occurs on the second day after swelling begins to decrease (N. Duenger, pers. comm.). To avoid the stress of handling, these examinations were not conducted on a routine basis during the time the foxes were housed as pairs in these studies.

Conditions of confinement of the foxes in these studies did not appear to be conducive to sexual arousal. During the time the foxes were housed as pairs, no indications of sexual interest or activity were observed either directly or on the video tapes examined. Semen containing only dead sperm from all males examined in late March indicated there had been no recent sexual activity (N. Duenger,

pers. comm.). Vulvas examined at that same time had no swelling or redness associated with estrus. During the time the foxes were housed individually following experimental challenge, testes on the males remained soft which is associated with sexual unreadiness rather than firm which is associated with sexual activity. Vulvas on the females remained unswollen and pale.

Another pair of foxes, not part of this study, were housed together under conditions similar to those in which the experimental foxes were housed as pairs prior to being challenged. Behavior and genital appearance in the non-experimental pair of foxes was similar to that of the experimental pairs of foxes, i.e. no indications of sexual activity or interest were observed. In late March, testes of this non-study male were also soft, and semen examined contained only dead sperm. However, this male was subsequently used in an artificial insemination study. Semen was collected from this fox two or three times a week until late May which is considered well past the breeding season for Vulpes. After the first collection, semen viability and motility progressively improved, and testes remained firm until collections ceased. Maintaining good semen quality well into May is common in Vulpes used for artificial insemination of Alopex females whose estrous cycles are later than those of Vulpes (N. Duenger, pers. comm.). This situation served as additional evidence the housing situation used for all the foxes was not conducive to sexual activity. The problem for all the foxes housed in the facilities used in this study appeared to be psychologic rather than pathologic.

Mondain-Monval et al. (1977) reported vixens in captivity may have a delayed estrus of as much as 2 months compared to wild vixens. This was apparently the case in the female fox that conceived after the calculated breeding season when housed in the open room with two reindeer for these experiments. Also, perhaps the foxes preferred the openness of the room as opposed to the confinement of cages even though captive reared foxes usually feel more comfortable in a smaller space. Even though this pair was housed either individually or as a pair in an open-room situation through another breeding season, they did not reproduce a second time.

Although some of the females used for experimental infections were young, foxes can breed their first year (Ables 1975; Mondain-Monval et al. 1977), and age was not considered to be the primary problem in breeding failure.

Duker (1973) reported reproductive failures in fox-farm foxes fed meat from brucellosis-infected farm animals in the Soviet Union (see p. 54). These foxes also had a suppurative conjunctivitis which is not consistent with brucellosis infections. These foxes may have been infected with more than one organism, and conclusions drawn by the author may be misleading.

Because the foxes apparently failed to breed and because both the non-challenged male and female became infected, the effects of brucellosis on the gravid female reproductive tract could not be evaluated in this study.

Although infections with B. suis type 4 did not appear to affect

the reproductive tract, it is interesting to note that all four culture-positive foxes from the field were females. Of the two foxes necropsied 66 weeks PE, the only culture-positive lymph node was from the female. These findings suggest B. suis type 4 may establish infections easier or persist longer in female foxes.

BACTERIOLOGY

Hemocultures

Isolation of B. suis type 4 from hemocultures of foxes was intermittent as has been reported in other species infected with smooth brucellae (Jubb et al. 1985). Hemocultures were not run on all samples due to lack of sufficient quantity of blood.

Oral, Genital, Urine and Fecal Cultures

B. suis type 4 was detected in oral swabs in 1983 for as long as 3 weeks PE. Salivary glands were probably infected at the time of challenge and continued to disseminate the organisms. Lacombe (1962) cited reports of other workers in France having isolated brucellae organisms from salivary glands of dogs associated with livestock.

No genital swabs were culture-positive. However, most cultures were overgrown with Proteus sp. which makes detection of Brucella very difficult even if it is present.

Morse et al. (1951b) isolated Brucella from urine 4 and 8 hours after feeding dogs abortion material from cows infected with strain 2308. Serikawa et al. (1981b) felt urine was an important source

of transmission of B. canis infections. B. suis type 4 was isolated from only one fox urine sample, and that was collected at necropsy. Attempts to obtain clean urine samples when the foxes were handled for blood collection proved futile as the foxes would urinate spontaneously as soon as they could sense handling was imminent. The importance of urine in transmission of B. suis type 4 infections in foxes remains unclear.

In similar experiments conducted with B. abortus in coyotes in Texas, an infective dose of about 10^{10} CFU was considered the minimum needed to induce detectable shedding in the feces (D. Davis, pers. comm). This is in close agreement with results found in foxes in these experiments. Shedding in the feces was readily detected in foxes receiving a challenge dose on the order of 10^9 or 10^{11} in 1983. Lack of detection of shedding of the brucella organism in 1982 may have been related to the smaller challenge dose (10^7) used for two of the foxes. Reasons for failing to detect shedding in the two foxes challenged with 10^9 CFU in 1982 were not determined.

Shedding of organisms in fox feces was detected up to 6 days PE, but only 4 days PE in the coyotes. Morse et al. (1981b) isolated Brucella from feces of dogs 2 hours after feeding them aborted fetuses and placentas from cows infected with B. abortus strain 2308.

Lymph Nodes and Other Tissues

Isolation of B. suis type 4 from regional lymph nodes (mandibular and retropharyngeal) of experimentally-infected foxes was typical of brucellosis infections caused by organisms gaining entry through

mucous membranes of the head (Porter 1976). Digestive enzymes degrade many bacteria, but the brucellae in this study had the benefit of the protection of the meat protein used in the challenge procedure. Leptospira have been shown to be protected from gastric juices by culture media or by enteric coated capsules (Reilly 1966). Isolation of B. suis type 4 from mesenteric lymph nodes was the result of intestinal passage.

Failure to isolate B. suis type 4 from salivary glands from foxes sacrificed after 18 weeks PE indicated infection was overcome in those glands by that time.

Isolation of B. suis type 4 from only the internal iliac or popliteal lymph node in the later stages of infection was consistent with brucellosis infections seen in other species. Brucella organisms first colonize local lymph nodes in the area of entry, then spread hematogenously to more distant nodes (Jubb et al. 1985; Payne 1959).

The only reproductive-related organs culture-positive for B. suis type 4 were the supramammary lymph nodes from a female challenged with 10^{11} CFU and from the non-challenged female.

Isolation of B. suis at necropsy was related to the challenge dose as well as the time of necropsy. Infection was not detected at all in one fox challenged with 10^{7} CFU. In similar experiments, only 1/6 coyotes orally challenged with 10^{6} CFU B. abortus seroconverted or was culture positive at necropsy (D. Davis, pers. comm) Carmichael (1976) however, was able to infect dogs with 10^{6} CFU B. canis.

Culture results from foxes collected in the field were more varied.

Probable exposure of these foxes to brucellosis was from February or March, when reindeer abortions begin, through early May when most fawns are born. Most foxes were collected in late April to early May. Exposure dose would have been more inconsistent in field-infected than in experimentally-infected foxes.

Isolation of B. suis type 4 from several lymph nodes and high antibody titers on serologic tests were consistent with a recent exposure and generalized infection in fox #3380. The ovary of this fox was the only culture-positive reproductive organ from field-collected foxes.

Serologic titers detected by only the SP and BBA tests in female arctic fox #3089 suggested a recent exposure. Brucella was recovered from the liver, lung, and spleen, but lymph nodes were not cultured. Zaiarniuk and Nikulina (1976) cultured over 3,000 captive arctic foxes fed infected reindeer meat and isolated B. suis type 4 from the spleens of only seven. Isolation of organisms from the liver and spleen of foxes appears rare.

Several culture-positive lymph nodes from fox #3372 indicated a generalized infection, even though titers were not detected on serologic tests. Perhaps the infection had been localized in the lymph nodes long enough that brucellae were not circulating to stimulate antibody production. Seropositive, culture-negative animals are usually seen during the incubation stage of brucellosis infections in other species (Crawford and Hidalgo 1977). However, if the organism had had time to spread to several lymph nodes in this fox, the fox should have had time

to produce antibodies.

Recovery from only the internal iliac lymph node of #3082 suggested an infection in the later stages, but high antibody titers on all serologic tests indicated a more recent, generalized infection. It is possible this fox had had a previous infection and had recently been re-exposed to Brucella organisms.

Failure to isolate the organism from a large number of foxes did not prove absence of the organism. Brucellae are slow-growing organisms easily outgrown by contaminating bacteria, but contaminants were not a problem in the cultures. Brucellae are also intracellular organisms, but tissue surfaces were minced prior to plating to release any bacteria present from within the cells. Fox lymph nodes are very small, often less than pea-sized, and it is possible there were too few organisms present to detect. Perhaps some brucellae in the smallest tissues were inadvertently killed in the disinfection process to eliminate contaminating bacteria. However, histologic examination of tissues also failed to reveal Brucella organisms in tissues.

PATHOLOGY

Natural Infections

White foci were observed on the liver of one fox, and microscopic foci of mononuclear cells were observed in the livers of nine foxes collected in the field. Although abscesses on the liver or spleen are often a feature of brucellosis infections in laboratory animals, none

were seen in experimentally-infected foxes. These foci seen in field-collected animals were considered unrelated to brucellosis.

One iliac lymph node from a fox collected in the field was enlarged, abscessed and also culture-positive for B. suis type 4. Abscesses are typical in B. suis type 4 infections in reindeer.

Uterine exudates appeared to be independent of brucellosis-infection status. A caseous uterine exudate independent of the association with pregnancy has been reported in swine infected with B. suis (Jubb et al. 1985). However, the female fox collected with a similar uterine exudate was seronegative and culture-negative for Brucella. She was also pregnant. A non-pregnant female with a creamy uterine exudate was also seronegative and culturenegative. No lesions were seen in the male reproductive tracts.

In general, pathology observed in foxes collected in the field was not remarkable. Pinigin et al. (1970b) examined 530 wild arctic foxes and reported lesions were limited to enlarged spleens in two and a few swollen lymph nodes in 12. Brucellosis infections in foxes collected in the field appeared to be short-lived and overcome by natural host defenses.

Experimental Infections

Gross and microscopic lesions observed in lymph nodes of experimentally-infected foxes characterized by hyperplasia and inflammatory foci were typical of B. abortus infections in cattle (Jubb et al. 1985). However, necrotic foci observed were more typical of B. suis infections in swine.

More numerous and more severe lesions were observed as the experimental challenge dose increased. These lesions were observed in foxes sacrificed up to 22 weeks PE, but none were observed in two foxes necropsied 66 weeks PE indicating the infection had been overcome by that time.

B. suis type 4 was isolated from most lymph nodes with histologic lesions indicating the lesions probably were associated with the brucellosis infection. Interpretation of lesions in culture-negative samples was more difficult because lack of culture does not necessarily mean lack of infection.

Lesions in foxes were confined to the reticuloendothelial system and were similar to those described in domestic dogs infected with B. abortus, B. melitensis, or B. suis (Margolis et al. 1945; Meyer 1983). Abortion, metritis, orchitis, epididymitis, or discospondylitis occasionally reported in domestic dogs infected with B. abortus, B. melitensis, or B. suis were not seen in foxes experimentally infected or in the non-challenged controls that became infected. Most Brucella species do not cause lesions in the reproductive tract in atypical hosts, and many do not cause lesions in the non-pregnant uterus of typical hosts (Jubb et al. 1985). The lack of gross or microscopic lesions in the non-gravid female fox reproductive tract was consistent with brucellosis infections seen in other host species (Jubb et al. 1985). However, because the foxes apparently failed to breed and because both the non-challenged male and female controls became infected, the pathologic effects of brucellosis on the gravid female reproductive

tract could not be evaluated in this study.

No pathology associated with the male reproductive tract was observed in these studies.

BRUCELLA SUIS TYPE 4 IN GRIZZLY BEARS AND MISCELLANEOUS SMALL MAMMALS

Grizzly Bears

Only male grizzly bears collected in this study were seropositive. Males and females were both collected in the same area relative to the infected reindeer herd, and it did not appear there was any difference in opportunity for exposure to brucellosis as speculated by Binninger et al. (1980).

Reasonably high titers on all serologic tests (with the exception of a negative BBA test) suggested grizzly bear #2752 may have had a generalized infection at the time of collection. Bear #3246, which was positive on only the SP and Riv tests, may have been more recently exposed.

Serologic results from the bears were not confirmed by bacterial isolations. Infections may have been in the early incubation stages and organisms not yet disseminated. It is also possible the bears were exposed to enough organisms to evoke a serologic response but not enough to establish an infection.

In a previous experiment at the University of Alaska Fairbanks, B. suis type 4 was isolated from only the retropharyngeal and mediastinal lymph nodes of a young grizzly bear 17 days following oral challenge with 10^9 CFU (R. Dieterich, pers. comm.). Neiland and Miller (1981) reported seroconversion and positive culture results in black bears challenged with 10^8 or 10^9 CFU B. suis type 4.

White foci observed on the livers of several bears were considered

unrelated to brucellosis.

Small Mammals

Exposures in two seropositive arctic ground squirrels were probably recent as one was positive on only the SP and the other on only the SP and BBA tests. Failure to isolate the organism may have been due to the infection being in the incubation stages or a relatively low exposure dose.

Exposure to Brucella organisms in small mammals would probably be through ingestion of bacteria shed on the ground by infected reindeer. In this study, 10^4 or 10^6 CFU B. suis type 4 were required to establish detectable infections in lemmings. Active infections in lemmings exposed indirectly to infected foxes were rare. Thus it is possible the same situation exists in the natural habitat; i.e. exposure doses are low and actual infections are rare. Lyamkin et al. (1983) sampled 2715 rodents from an area with infected reindeer in the Soviet Union and isolated organisms similar to B. suis type 4 from only 23. Pinigin and Zabrodin (1970) isolated organisms from none of 50 lemmings cultured.

TRANSMISSION OF BRUCELLA SUIS TYPE 4

FOX TO FOX

Housing

Only two rooms containing carnivore cages were available in the isolation area. In an effort to standardize housing conditions, four foxes receiving the lower challenge dose were housed in one room, and the four receiving the higher challenge dose as well as the non-challenged foxes intended as controls were housed in the other room. Even though the possibility of aerosol transmission of brucellosis from infected foxes to the non-challenged foxes was recognized, it was not considered likely at the time. Cleaning and handling procedures were designed to minimize the risk.

Although the two non-challenged foxes did become infected with brucellosis and could not serve as negative controls, they still had value in that their infections from aerosol exposure could be compared to the experimental infections induced by ingestion in the other foxes.

Source

Aerosols created during the cleaning of cages housing infected foxes, or aerosols disseminated with the saliva of infected foxes were considered the probable sources of exposure for the two non-challenged foxes that did become infected. Aerosol transmission of brucellosis is widely accepted as a biohazard in laboratories. The organism has been isolated from the air in abbatoirs, and brucellosis has been detected

in abbatoir workers who had exposure to the kill department by air flow but no contact with infected tissues. Guinea pigs can be infected by aerosols, the resultant lesions being generally comparable to those seen following subcutaneous challenge (Kaufman et al. 1980). Solid panels separated the sides of the individual fox cages, but the fronts of all cages consisted of metal bars open to the room. Jones (1984) reported transmission of B. canis was not prevented between susceptible dogs separated by partial walls. Detection of serologic titers in the non-challenged female and male at 5 and 7 weeks respectively following experimental challenge indicated transmission occurred in the early stages of challenge-infection which was concurrent with isolations of B. suis type 4 from saliva and feces of experimentally-infected foxes.

In natural conditions, aerosols from brucellosis-infected foxes could be an important source of infection for other foxes in situations of close contact in confined spaces such as would occur in a den.

Because serotiters were not detected until 13 weeks of age in one of three pups born to experimentally-infected foxes, it was felt this infection was acquired after birth rather than in utero.

Vertical transmission of an infectious agent occurs from mother to offspring through the placenta or milk; horizontal transmission occurs from animal to animal by such means as secretions or excretions. Transmission of B. abortus from coyote to coyote was mainly vertical (D. Davis, pers. comm). In these experiments, transmission of B. suis from fox to fox was horizontal.

Serology

Patterns of serotiters in the two foxes exposed via aerosol were not as well defined as those in experimentally-infected foxes. With the exception of the CF test, titers were intermittent in one, and only low titers persisted in the other. These results probably reflect a lower infective dose for these two foxes compared to those challenged experimentally. High titers were maintained on the CF test in both foxes suggesting the CF test may be the best indicator of infection.

Pups born to an experimentally-infected female were separated from the mother at 6 weeks of age. Detection of seropositive reactions 7 weeks later at 13 weeks of age on the SP and CF tests in one pup was consistent with an active acquired immune response to a brucellosis infection. Lack of a serologic response in the other two pups indicated the exposure dose was probably too low to consistently establish infection.

Bacteriology

B. suis type 4 was not isolated from hemocultures or oral or genital cultures of the two adults infected via aerosol suggesting the infective dose was low compared to experimentally infected foxes.

Lymph Nodes and Other Tissues

Isolation of B. suis type 4 from lymph nodes draining the head as well as more distant nodes (mediastinal, tracheobronchial, and supramammary) from the accidentally-infected adult control foxes indicated the organism probably gained access through the head by aerosols passing through the mucous membranes of the nasal or oral

cavities and was disseminated hematogenously as in experimentally infected foxes.

Likewise, isolation of the organism from the mandibular and retropharyngeal lymph nodes of one pup born to infected parents suggested an oropharynx or conjunctival route of exposure.

Pathology

Gross and microscopic lesions of lymph nodes in the adult control foxes were indicative of acute lymphadenitis similar to that seen in experimentally infected foxes. Three lymph nodes with gross or microscopic lesions that were also culture-positive were all from the female.

Follicular or paracortical hyperplasia were observed in the retropharyngeal lymph nodes of all three pups born to the experimentally-infected female. However, foci of neutrophils and necrosis were seen in only one, and that same node was culture-positive. Nodes draining body surfaces such as those in the pharynx, mediastinum, and mesenteric areas have, more or less, a constant degree of follicular hyperplasia (Jubb et al. 1985). Lesions observed in the lymph nodes of the culture-negative foxes were probably non-specific and not related to a brucellosis infection.

FOX TO LEMMINGS

Lemmings were intended to serve as sensitive biologic indicators of shedding of the organism from the foxes. Miller and Neiland (1980) reported as little as 20 CFU *B. suis* type 4 injected intraperitoneally

caused fatalities in D. rubricatus.

Foci of mononuclear cells were seen microscopically in the livers of 14 lemmings that were negative serologically and culturally and that had been exposed to foxes receiving 10^9 or 10^{11} CFU B. suis type 4. In previous experiments conducted at the University of Alaska, microscopic lesions in lemmings injected intraperitoneally with 10^4 CFU B. suis type 4 were characterized by focal areas of hepatic necrosis and mononuclear cell accumulations (R. Dieterich, pers. comm.). Similar lesions were not reported in 155 lemmings necropsied for other purposes (Dieterich 1975). It appears that although clinical infection was not observed in the above mentioned 14 lemmings, they were exposed to enough organisms to induce microscopic reactions in the reticuloendothelial system.

However, definite indications of brucellosis infection were demonstrated in only two lemmings exposed to two different foxes receiving the highest challenge dose. It was determined in a retrospective study that an oral infective dose on the order of 10^4 or 10^6 CFU was required to induce an infection in lemmings from which seropositive or culture-positive results could be obtained. Lemmings were only indirectly exposed to fox feces. Positive culture results were not obtained from fox urine samples. Under the conditions of these experiments, it appeared that foxes did not shed enough organisms in the urine to effectively produce generalized infections in lemmings.

Microscopic foci of mononuclear cells in the salivary glands of orally infected lemmings indicated the salivary glands were infected

with the Brucella organisms. Unfortunately, salivary glands of lemmings used as biologic monitors were not preserved for microscopic examination.

FOX TO REINDEER

Source

Infection in the reindeer could have occurred as a result of their ingesting infected fox feces. Workers conducting studies on transmission of brucellosis from coyotes to cattle felt this method was the most likely form of transmission. They theorized cattle ate carnivore feces in the spring for the added calcium the carnivores passed after eating rodents (D. Davis, pers. comm.). B. suis type 4 was cultured from one fecal sample of each of the two foxes involved. Very few fox fecal samples could be found in the room with the reindeer suggesting the reindeer did indeed eat them. Fox fecal material was not hard to find in either individual fox cages or in the room the mother fox and her young were kept after they were separated from the reindeer and the male fox.

Alternatively, B. suis type 4 organisms may have been transmitted to the reindeer by aerosols of fox saliva. Although oral samples from these two foxes were not culture-positive for B. suis type 4, oral samples collected from other foxes challenged with the same dose were culturepositive as long as 3 weeks PE.

Thirdly, one of the reindeer may have been infected through a bite in the jaw by one of the foxes. B. suis type 4 was isolated from the

abscess, and the reindeer halter appeared to have been chewed in that area.

Serology

Serologic responses, detected in two reindeer 1 week after foxes they were housed with were challenged, were typical of experimentally-induced brucellosis infections. However, antibody titers declined more rapidly than those observed in reindeer conjunctively challenged with 10^7 CFU B. suis type 4 in previous experiments (Dieterich et al. 1981) indicating the exposure dose from the foxes must have been lower.

Lymph Nodes and Other Tissues

Although B. suis type 4 was isolated from only one of the reindeer, culture-positive lymph nodes were typical of those seen in experimental brucellosis infections (Dieterich et al. 1981).

Isolation of the organism from the lung, jaw abscess and anorectal abscess of one reindeer was unusual. The halter on the reindeer had been chewed, and it was possible the jaw abscess resulted from a bite from one of the infected foxes. B. suis type 4 was not isolated from oral swabs from either of the two foxes, but it could have been missed in contaminating organisms (notably Proteus sp.). Infection in the lung suggested an aerosol route of infection, again probably originating from infected fox saliva. B. suis type 4 in the anorectal abscess could have resulted from external exposure from a bite, or from internal exposure after passage through the entire digestive tract. The organism has been isolated from abdominal abscesses in reindeer (Dieterich 1981).

ECOLOGY OF BRUCELLA SUIS TYPE 4 INFECTIONS

In ecologic terms, microparasitic diseases are of short duration, transient, tend to induce immunity, have their own reproduction in the host, usually have high growth rates in the host, are small in size, have a short generation time, low transmission efficiencies (e.g. 300,000 people are needed to maintain measles), require high host densities, and are usually associated with animals that herd or school or breed in large colonies (Anderson and May 1979; May 1983).

Brucellosis infections in foxes in these studies were shown to be of relatively short duration and induced at least a humoral immune response. B. suis type 4 organisms are certainly small in size and have their own reproduction in the fox host. Generation time in the fox was not determined.

However, foxes do not live in situations that fulfill the requirement of having a high host density to maintain brucellosis as a disease. Nor do foxes herd or school or breed in large colonies.

Normal activities of individuals or groups of red foxes tend to occur in areas of about 3.3 km in diameter. Home ranges do not tend to overlap and are affected by season, density of foxes, breeding activity, whelping, and age structure (Ables 1975; Follmann 1973; Hobgood 1984; Jones and Theberge 1982; Lloyd 1980; Longley 1962). Red foxes appear to be territorial in certain regions or habitats (Follmann 1973).

Arctic foxes tend to be solitary except for family units during

the breeding season. In winter they sometimes congregate at food sources such as carcasses and dumps (Burgess 1984; Chesemore 1967; Fine 1980). There is evidence that arctic foxes in northern Alaska are territorial (Burgess 1984; Fine 1980; Underwood and Mosher 1982). Thus, the social habits of neither red nor arctic foxes satisfy the requirements necessary to maintain brucellosis as a disease within their populations.

In contrast, reindeer are herd animals and tend to aggregate during the fawning season. Chances for exposure to contaminated abortion or birth products are then increased. Likewise, chances for ingesting organisms shed on the ground from draining abscesses would be increased. Brucellosis has been a major problem in elk in Wyoming that are congregated on feedgrounds during the calving season. In this regard, reindeer are a more suitable host for maintenance of brucellosis infections than foxes. The time of fox aggregation does not coincide with reindeer aggregations.

Microparasitic infections causing reproductive disease or high mortality relative to the growth rate of the host can impact the population growth of the host (Anderson and May 1979). Brucellosis in foxes caused neither reproductive disease as detected by lack of gross or microscopic lesions in wild and experimental animals nor mortality in experimental animals and thus would not be expected to significantly affect population growth.

Vertical transmission of a disease lowers the threshhold of introduction needed to maintain the disease (Anderson and May 1979).

Although the sample size was small (one female with three pups), vertical transmission of brucellosis in foxes was not demonstrated in experimental infections. Rather, transmission between foxes appeared to be mainly horizontal through the saliva.

Thus, in ecologic terms, foxes do not satisfy the requirements necessary to maintain brucellosis infections within their own species and thereby serve as a reservoir of infection for reindeer.

IMPLICATIONS OF TRANSMISSION OF BRUCELLA SUIS TYPE 4

Reindeer and caribou are the natural hosts for B. suis type 4. The most likely source of infection for foxes or other predators is through deliberate ingestion of reindeer abortion or birthing products contaminated with the Brucella organism. Reindeer are often herded more closely together during fawning time for protection, thus concentrating exposure sources. Caribou also aggregate during calving time.

In the current studies, red foxes were observed stalking newborn reindeer fawns. Several foxes collected had adult or reindeer fawn hair in their stomachs. Thus the opportunity for foxes in the area to be exposed to Brucella-containing material from the reindeer would be high during fawning time. Arctic foxes spend much of the late fall and early winter on the coast and sea ice and move inland to mate in the spring. They may follow caribou herds to feed on leftover wolf kills (Chesemore 1967, 1968a, and 1975).

Diets of both red and arctic foxes are extremely varied (Ables 1975; Chesemore 1967, 1968b, and 1975; Eberhardt 1977; Fine 1980; Garrott et al. 1983; Stephenson 1970; Underwood 1975). As scavengers and opportunists, both species of fox would be expected to eat reindeer or caribou abortion products. A diet high in infected material would be possible if such material were abundant.

Red fox denning reportedly takes place from late winter through early May (Ables 1975; Eberhardt 1977). If foxes are exposed during the early spring, reindeer abortion/fawning time, that would coincide

with the pregnancy period of red foxes in the area. If brucellosis were causing reproductive failures in female foxes, one would expect to find evidence of abortion in females that had been exposed to brucellosis. However, many females collected in this study in late April or early May were pregnant or showed signs of recent whelping. No trends were apparent between a female being pregnant or barren and having been exposed to brucellosis.

Mating in arctic foxes in northern Alaska begins in late February and peaks in early April. Gestation is 52 days, and young are born from May to July (Chesemore 1967). Thus, the two females collected in this study should have been bred by the time they were collected in late April or early May. However, although both were seropositive for brucellosis, neither was pregnant, lactating, nor showed signs of recent abortion. Neither gross nor microscopic lesions were seen in either uterus.

Salivary glands of foxes appeared to become readily infected with Brucella and to shed the organisms during experimental infections. Horizontal transmission from infected foxes to both non-challenged control foxes occurred in conditions conducive to aerosol formation (foxes housed in concrete-lined cages cleaned by hosing). Horizontal transmission between mother and offspring housed in an open room with wood shavings and not cleaned by hosing occurred in one of three pups. The potential for fox to fox transmission under natural conditions would be greatest from mother to offspring during the time of close confinement in the den. This would also coincide with the likely period

of recent exposure of the mother from infected reindeer.

Transmission of B. suis type 4 from foxes to reindeer occurred under the conditions of close confinement in experimental conditions. Under natural conditions, reindeer would be more likely to become infected from eating contaminated fox feces than from aerosols that would be sparsely dispersed. Rabies is known to be transmitted from foxes to reindeer by bites (R. Dieterich, pers. comm.), and it is possible brucellosis could also be transmitted from foxes to reindeer by bites.

Pregnant reindeer are most susceptible to brucellosis during mid-gestation (January-February), before foxes would become infected or several months afterwards. Thus, although it would be possible for reindeer in the wild to become infected by eating infected fox feces, the optimum timing for female reindeer exposure would probably not coincide with the shedding of the organism by the foxes. However, male reindeer would be equally susceptible to infection at all times of the year.

Rodents in the wild could become infected by ingesting contaminated reindeer abortion material, fox urine or feces, or vegetation around such material, or by aerosols from infected animals. Serotiters to brucellosis detected in arctic ground squirrels collected on the reindeer fawning grounds indicated they had been exposed. The low frequency of transmission from experimentally infected foxes to lemmings under conditions of very close confinement indicates transmission to rodents in the wild via infected urine is probably rare. Also, even though non-challenged foxes apparently became infected through aerosol exposure, lemmings

housed in the same room did not. Aerosol transmission from infected foxes to lemmings under natural conditions appears unlikely.

Lemmings or other small rodents and mammals have been considered potential reservoirs of brucellosis infection for reindeer and caribou by contaminating water and grasses with their excreta (Miller and Neiland 1980). They have also been considered potential reservoirs of brucellosis for predators by being an infected link in the food chain (Gorban and Grekova 1978). However, since a dose of 10^9 organisms for four consecutive days was required to consistently establish infection in foxes, the probability that a fox would become infected by eating infected lemmings does not appear great. Most workers indicate brucellosis travels to, not from, rodents (Boerr et al. 1980; Bosworth 1940; Meyer 1974; Verger 1972; Vest et al. 1965).

SUMMARY AND CONCLUSIONS

Field and laboratory studies were conducted to test the hypotheses that (1) the reindeer/caribou brucellosis organism, B. suis type 4, is incidentally transmitted to reindeer predators such as foxes but does not cause reproductive disease in them, and (2) infected predators such as foxes are terminal hosts and do not serve as reservoirs of infection for reindeer.

Reindeer and caribou are the natural hosts for B. suis type 4. Serologic and bacteriologic results from predators and small mammals collected on the fawning grounds of a known-infected reindeer herd indicated these animals had been exposed to the Brucella organism. Most results indicated exposures had been recent. Prevalence was low in species other than foxes.

In foxes collected on the fawning grounds of a known-infected reindeer herd, serologic prevalence for brucellosis was similar for males (50%) and females (30%), but all culture-positive animals were females. An abscessed lymph node from a female was the only pathologic lesion seen. No association between reproductive status of foxes and brucellosis infections was observed.

An infective dose of 10^7 CFU B. suis type 4 did not appear to be high enough to consistently establish infection in foxes challenged orally. Infections were established with doses of 10^9 or 10^{11}.

Serologic patterns in foxes experimentally infected with 10^9 or

10^{11} CFU B. suis type 4 by oral exposure were typical of brucellosis infections in other species. The standard agglutination tests (SP and ST) detected infections first and were followed by the BBA, Riv and CF tests. The CF test appeared to be the most reliable indicator of long-term infections.

Isolation of B. suis type 4 from hemocultures was intermittent. The organism was isolated from the feces during the second, third and fourth days of challenge in the foxes challenged with 10^9 CFU, and up to 6 days PE in foxes challenged with 10^{11} CFU. Oral cavity cultures were positive for as long as 3 weeks PE in foxes challenged with 10^9 or 10^{11} CFU.

Clinical effects of brucellosis infections were not observed in experimentally-infected foxes.

B. suis type 4 was frequently isolated from regional lymph nodes of the head up to 18 weeks PE, and from only more distant nodes at 22 and 66 weeks PE. Organisms did not tend to localize in the reproductive tracts of males or females.

Reproduction of captive foxes is very difficult and was successful in only one of seven pairs of foxes used for experimental infections. No gross or microscopic lesions in the non-gravid female reproductive tracts nor in the male reproductive tracts were observed.

Gross and microscopic pathology was limited to the lymph nodes and was typical of that described in dogs infected with other species of brucellae. Gross and microscopic lesions in the lymph nodes correlated with isolation of the organism. Lack of pathology in the reproductive

organs of field-collected male and female foxes and in experimentally-infected male foxes differentiated the disease in foxes from brucellosis in typical hosts. Lack of pathology in the non-gravid uterus was consistent with the nature of brucellosis infections in other species (Jubb et al. 1985). Pathology associated with B. canis infections in dogs such as anterior uveitis, discospondylitis, meningitis, encephalitis, osteomyelitis, dermatitis, and internal abscesses (Carmichael 1976; Meyer 1983) was not observed in naturally or experimentally infected foxes. Due to breeding failure, effects of B. suis type 4 on the pregnant fox reproductive tract were not determined in experimental infections.

Horizontal transmission occurred between foxes that were not in direct contact and also between a mother and one of three offspring that were in direct contact. The probable source of transmission was attributed to aerosols from salivary glands from infected foxes. Transmission among foxes under natural conditions would be most likely to occur during close associations found in the dens.

Transmission from foxes to lemmings that were exposed to infected fox urine occurred only rarely.

Transmission from infected foxes to two reindeer occurred under conditions of close confinement. Ingestion of organisms passed mechanically in the fox feces was considered the most probable source of infection for the reindeer. Culture-positive abscesses on the jaw and near the rectum of one of the reindeer suggested infections could be passed through contaminated saliva by the bite of an infected fox. Isolation of the organism from the lung of one of the reindeer also

implicated aerosols in transmission.

Possibilities for transmission from foxes to reindeer under natural conditions given the densities of these species would be less likely to occur. However, the potential adds to the difficulty of control measures in reindeer. Eberhardt and Hanson (1978) reported movements of arctic foxes tagged in Alaska of up to 24 km per day, 901 km in 81 days, and 945 km in a year. Red foxes may travel more widely in the North than in more southern parts of their range (E. Follmann, pers. comm.). Long distances covered within a few days by either species could be important in mechanically transmitting infective material. Even if vaccination helped reduce the sources of shedding of the organism and transmission of the disease within a herd, infected foxes could spread the organism to distant herds. The possibility of transmission of the disease from foxes to reindeer emphasizes the rationale for a control program rather than an eradication program for brucellosis in reindeer.

Field and experimental results supported the first hypothesis that the reindeer/caribou organism, B. suis type 4, is incidentally transmitted to predators such as foxes but does not cause reproductive disease as determined by lack of gross and microscopic lesions and in negative culture results from reproductive tracts.

The second hypothesis, that infected predators such as foxes are terminal hosts and serve as minor sources of infection for reindeer, was not supported. Foxes infected experimentally with B. suis type 4 shed the organism in feces and saliva. Brucellosis was transmitted from

infected foxes to reindeer under conditions of close confinement.

Understanding the epizootiology of brucellosis in foxes is vital to the development of the brucellosis control program for reindeer. It would be theoretically possible for one fox to serve as a vector carrying infectious material from an infected herd to a non-infected herd. Although foxes probably do not perpetuate the disease among themselves, they should be recognized as potential sources of infection for reindeer under certain conditions. Because these conditions are probably rare in natural situations, their role in the overall ecology of the disease in reindeer appears relatively minor.

APPENDIX

SEROLOGIC AND CULTURE RESULTS OF INDIVIDUAL ANIMALS

Appendix
Table 43. Hemoculture results and serologic response of fox 3402 following oral challenge with 8.34×10^7 CFU *B. suis* type 4 in 1982.

Weeks Post-Exposure	Hemoculture	SP[1]	BBA	RIV[1]	CF[2]	*B. canis*[1]
0	NC[3]	N25	-	N25	2	NT[4]
1	-	N25	-	N25	2	N25
2	-	N25	-	N25	1	NT
3	-	N25	-	N25	1	NT
4	-	N25	-	N25	2	NT
5	-	N25	-	N25	1	NT
6	-	N25	-	N25	1	NT
7	-	N25	-	N25	1	N25

[1] +=Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
[2] Numerical reaction on automated CF test
[3] Not cultured
[4] Not tested

Appendix
Table 44. Hemoculture results and serologic response of fox 3405 following oral challenge with 8.34×10^7 CFU B. suis type 4 in 1982.

Weeks Post-Exposure	Hemoculture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	B. canis[1]
0	NC[3]	NT[4]	N25	-	NT	N25	1	NT
1	-	NT	N25	-	NT	N25	NT	N25
2	+	NT	N25	-	NT	N25	1	NT
3	-	I50	N25	-	I25	N25	2	NT
4	-	NT	I100	+	NT	I25	88	NT
5	+	I400	I200	+	I200	+200	91	NT
7	-	NT	+200	+	NT	+200	93	N25
8	-	NT	+400	+	NT	+400	87	NT
9	-	NT	+400	+	NT	I400	86	NT
10	-	+400	+400	+	I400	+400	89	NT
12	-	NT	+400	+	NT	+400	88	NT
14	-	NT	+400	+	NT	+400	85	NT
16	-	+400	+400	+	I400	I400	89	NT

1 += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
2 Numerical reaction on automated CF test
3 Not cultured
4 Not tested

Appendix
Table 45. Hemoculture results and serologic response of fox 3404 following oral challenge with 4.9×10^9 CFU B. suis type 4 in 1982.

Weeks Post-Exposure	Hemoculture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	B. canis[1]
0	NC[3]	NT[4]	N25	-	NT	N25	4	NT
1	-	NT	N25	-	NT	N25	3	N25
2	-	I100	N25	WK[5]	N25	N25	5	NT
3	-	NT	I100	+	NT	+25	60	NT
4	-	+400	I200	+	+100	I200	86	NT
5	-	NT	+200	+	NT	+200	90	NT
7	-	+400	+200	+	+200	+200	91	N25
8	-	NT	+200	+	NT	+200	92	NT
9	-	NT	+400	+	NT	+400	91	NT

1 += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
2 Numerical reaction on automated CF test
3 Not cultured
4 Not tested
5 Weak reaction

Appendix
Table 46. Hemoculture results and serologic response of fox 3403 following oral challenge with 4.9×10^9 CFU B. suis type 4 in 1982.

Weeks Post-Exposure	Hemo-culture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	B. canis[1]
0	-	NT[3]	N25	-	NT	NT	2	NT
1	-	+25	N25	-	N25	N25	3	NT
2	-	NT	+100	+	NT	I100	79	N25
3	-	NT	+200	+	NT	+100	81	NT
4	-	NT	I200	+	I100	+400	88	NT
5	-	NT	+200	+	NT	+400	90	NT
7	-	NT	I400	+	NT	+400	90	NT
8	-	NT	I400	+	NT	I400	92	N25
9	-	NT	+400	+	NT	+400	90	NT
10	-	NT	+400	+	NT	+400	91	NT
12	-	NT	+400	+	NT	+400	94	NT
14	-	I400	+400	+	I200	+400	94	NT
16	-	NT	+100	+	NT	I400	94	NT
17	-	NT	+100	+	NT	I200	95	N25
19	-	NT	+200	+	NT	+400	94	NT
20	-	NT	+200	+	NT	+400	92	NT
22	-	+100	+200	+	+100	+400	94	NT

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
[2] Numerical reaction on automated CF test
[3] Not tested

Appendix
Table 47. Hemoculture results and serologic response of fox 3516 following oral challenge with 1.21×10^9 CFU B. suis type 4 in 1983.

Weeks Post-Exposure	Hemoculture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	B. canis[1]
-1	NC[3]	N25	N25	-	N25	N25	2	NT[4]
1	-	NT	N25	-	NT	N25	2	NT
2	+	NT	+25	-	NT	N25	2	N25
3	+	+100	+100	WK[5]	N25	+50	22	NT
4	NC	+50	+200	+	+25	+100	34	N25

1 += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
2 Numerical reaction on automated CF test
3 Not cultured
4 Not tested
5 Weak reaction

Appendix
Table 48. Hemoculture results and serologic response of fox 3517 following oral challenge with 1.21×10^9 CFU B. suis type 4 in 1983.

Weeks Post-Exposure	Hemoculture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	B. canis[1]
-12	NC[3]	N25	N25	-	N25	NT[4]	2	NT
-1	NC	NT	N25	-	NT	N25	2	N25
1	-	NT	N25	-	NT	N25	2	NT
2	-	NT	N25	-	NT	N25	1	NT
3	-	I50	+25	-	N25	N25	4	NT
4	-	NT	+100	+	NT	+50	28	N25

1 += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
2 Numerical reaction on automated CF test
3 Not cultured
4 Not tested

Appendix
Table 49. Hemoculture results and serologic response of fox 3537 following oral challenge with 1.21×10^9 CFU *B. suis* type 4 in 1983.

Weeks Post-Exposure	Hemoculture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	*B. canis*[1]
-12	NC[3]	NT	N25	-	NT[4]	N25	1	NT
-1	NC	NT	NT	NT	NT	NT	2	NT
1	-	NT	NT	NT	NT	NT	2	NT
2	+	NT	+400	+	NT	I100	82	N25
3	-	+100	+400	+	+50	+400	87	NT
5	-	NT	+100	+	NT	+400	86	N25
7	-	NT	+400	+	NT	I400	84	NT

1 += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
2 Numerical reaction on automated CF test
3 Not cultured
4 Not tested

Appendix
Table 50. Hemoculture results and serologic response of fox 3547 following oral challenge with 1.21×10^9 CFU B. suis type 4 in 1983.

Weeks Post-Exposure	Hemoculture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	B. canis[1]
-12	NC[3]	NT[4]	N25	-	NT	N25	1	NT
-1	NC	N25	N25	-	N25	N25	2	NT
1	-	NT	N25	-	NT	N25	1	NT
2	-	NT	+50	-	NT	N25	2	N25
3	-	I200	+200	+	NT	I100	76	NT
5	-	N25	+100	+	NT	+400	90	NT
7	-	N25	+400	+	NT	+400	89	N25
10	-	I200	+400	+	+25	+400	90	NT
13	-	NT	I200	+	NT	I400	90	NT

1 += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
2 Numerical reaction on automated CF test
3 Not cultured
4 Not tested

Appendix
Table 51. Hemoculture results and serologic response of fox 3548 following oral challenge with 1.06×10^{11} CFU *B. suis* type 4 in 1983.

Weeks Post-Exposure	Hemoculture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	*B. canis*[1]
-12	NC[3]	N25	N25	-	N25	N25	1	NT[4]
-1	NC	NT	N25	-	NT	N25	2	NT
1	C[5]	NT	+50	-	NT	N25	18	N25
2	-	+100	I400	+	I25	+25	75	I50
3	-	NT	+400	+	NT	+400	84	NT
5	-	NT	I200	+	NT	+400	88	NT
7	-	I400	+400	+	+100	+400	83	NT
10	-	NT	+400	+	NT	+400	90	N25
12	-	NT	+200	+	NT	I400	95	95
14	-	I400	+200	+	+200	+200	94	94

1 += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
2 Numeric value on automated CF test
3 Not cultured
4 Not tested
5 Contaminated

Appendix
Table 52. Hemoculture results and serologic response of fox 3549 following oral challenge with 1.06×10^{11} CFU *B. suis* type 4 in 1983.

Weeks Post-Exposure	Hemoculture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	*B. canis*[1]
-12	NC[3]	NT[4]	N25	-	NT	N25	1	NT
-1	NC	N25	N25	-	N25	N25	1	NT
1	+	NT	NT	NT	NT	NT	2	NT
3	-	NT	+400	-	NT	+400	82	I50
5	-	NT	+400	-	NT	+400	88	NT
7	-	I400	+400	-	+100	+400	89	NT
10	-	NT	+400	-	NT	+400	87	NT
13	-	+400	+200	-	I400	+400	91	NT
14	-	NT	I200	-	NT	+400	90	N25
15	-	NT	NT	-	NT	NT	NT	NT

1 += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
2 Numerical reaction on automated CF test
3 Not cultured
4 Not tested

Appendix
Table 53. Hemoculture results and serologic response of fox 3552 following oral challenge with 1.06×10^{11} CFU *B. suis* type 4 in 1983.

Weeks Post-Exposure	Hemoculture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	*B. canis*[1]
-12	NC[3]	NT[4]	N25	-	NT	N25	1	NT
-1	NC	N25	N25	-	N25	N25	1	NT
1	+	NT	+100	-	N25	N25	10	N25
2	+	+200	+200	+	NT	+50	79	N25
3	-	NT	+400	+	NT	+400	84	NT
5	-	NT	+400	+	NT	+400	90	NT
7	-	NT	+400	+	NT	+400	86	NT
10	-	+400	+400	+	I400	+400	90	NT
12	-	NT	+400	+	NT	I400	94	N25
14	-	NT	+200	+	NT	I400	93	NT
16	-	I400	100	+	I400	I400	44	NT

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
[2] Numerical reaction on automated CF test
[3] Not cultured
[4] Not tested

Appendix
Table 54. Hemoculture results and serologic response of fox 3559 following oral challenge with 1.06×10^{11} CFU B. suis type 4 in 1983.

Weeks Post-Exposure	Hemoculture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	B. canis[1]
-12	NC[3]	N25	N25	-	N25	N25	1	NT[4]
-1	NC	NT	N25	-	NT	N25	2	N25
1	C[5]	NT	+100	-	NT	N25	4	NT
2	C	NT	+400	+	NT	+50	76	N25
3	-	NT	+400	+	NT	I400	82	NT
5	-	I400	I200	+	+100	+400	84	NT
7	-	NT	+400	+	NT	+400	88	N25
10	-	NT	+400	+	NT	NT	NT	NT
12	-	NT	I400	+	NT	+400	95	NT
14	-	NT	I200	+	NT	I400	92	NT
16	-	NT	I200	+	NT	I200	89	NT
18	-	+400	+400	+	I400	I400	90	NT

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
[2] Numerical reaction on automated CF test
[3] Not cultured
[4] Not tested
[5] Contaminated

Appendix

Table 55. Culture of B. suis type 4 from tissues of two foxes orally challenged with 8.34×10^7 CFU in 1982.

Tissue	Sex	Pathology Number 3402[1] M	3405[2] F
Mandibular L.N.		-	+
Retropharyngeal L.N.		NC[3]	+
Superficial Cervical L.N.		NC	NC
Femoral L.N.		NC	+
Popliteal L.N.		-	+
Internal Iliac L.N.		-	+
Mesenteric L.N.		-	+
Tracheobronchial L.N.		-	NC
Supramammary/ Superficial Inguinal L.N.		NC	-
Epididymis		-	
Seminal Vesicle		-	
Testis		-	
Ovary			-
Uterus			-

Appendix
Table 55. (continued)

		Pathology Number	
		3402	3405
Tissue	Sex	M	F
Heart		-	-
Liver		-	-
Lung		-	-
Kidney		-	-
Spleen		-	-
Bladder		NC	-
Tonsils		NC	NC
Salivary Gland		NC	NC

[1] Sacrificed 7 weeks PE
[2] Sacrificed 16 weeks PE
[3] Not cultured

Appendix
Table 56. Culture of B. suis type 4 from tissues of two foxes orally challenged with 4.9×10^9 CFU in 1982.

Tissue	Sex	Pathology Number 3404[1] M	3403[2] M
Mandibular L.N.		+	NC[3]
Retropharyngeal L.N.		+	-
Superficial Cervical L.N.		NC	-
Femoral L.N.		NC	NC
Popliteal L.N.		+	-
Internal Iliac L.N.		+	+
Mesenteric L.N.		NC	NC
Tracheobronchial L.N.		NC	NC
Supramammary/ Superficial Inguinal L.N.		NC	NC
Epididymis		-	NC
Seminal Vesicle		-	NC
Prostate		-	NC
Testis		-	-

Appendix
Table 56. (continued)

Tissue	Sex	Pathology Number 3404[1] M	3403[2] M
Heart		-	-
Liver		-	-
Lung		-	-
Kidney		-	-
Spleen		-	-
Bladder		-	-
Tonsil		NC	NC
Salivary Gland		NC	NC

1 Sacrificed 9 weeks PE
2 Sacrificed 22 weeks PE
3 Not cultured

Appendix
Table 57. Culture of B. suis type 4 from tissues of four foxes held in the same room and orally challenged with 1.21 x 10^9 CFU in 1983.

Tissue	Sex	Pathology Number 3516[1] M	3517[2] F	3537[3] F	3547[4] M
Mandibular L.N.		+	-	+	+
Retropharyngeal L.N.		+	+	+	-
Superficial Cervical L.N.		NC[5]	-	+	NC
Femoral L.N.		NC	NC	NC	-
Popliteal L.N.		+	+	+	NC
Internal Iliac L.N.		+	-	+	+
Mesenteric L.N.		NC	NC	NC	NC
Tracheobronchial L.N.		+	+	NC	+
Supramammary/ Superficial Inguinal L.N.		-	NC	NC	NC
Epididymis		-			NC
Prostate		-			NC
Testis		-			-
Ovary			-	NC	
Uterus			-	-	

Appendix
Table 57. (continued)

Tissue	Sex	Pathology Number 3516[1] M	3517[2] F	3537[3] F	3547[4] M
Heart		-	-	-	-
Liver		+	-	-	+
Lung		-	-	-	-
Kidney		-	-	-	+
Spleen		+	+	-	+
Bladder		-	-	-	+
Tonsils		+	+	-	+
Salivary Gland		+	-	+	-
Thymus		-	NC	+	NC

[1] Sacrificed 3 weeks PE
[2] Sacrificed 4 weeks PE
[3] Sacrificed 7 weeks PE
[4] Sacrificed 13 weeks PE
[5] Not cultured

Appendix
Table 58. Culture of B. suis type 4 from tissues of four foxes held in the same room and orally challenged with 1.06×10^{11} CFU in 1983.

Tissue	Sex	Pathology Number 3548[1] M	3549[2] F	3552[3] M	3559[4] F
Mandibular L.N.		+	+	+	+
Retropharyngeal L.N.		+	+	+	+
Superficial Cervical L.N.		NC[5]	-	+	+
Femoral L.N.		NC	NC	NC	NC
Popliteal L.N.		-	-	+	NC
Internal Iliac L.N.		+	-	+	+
Mesenteric L.N.		+	-	+	+
Tracheobronchial L.N.		-	-	NC	NC
Supramammary/ Superficial Inguinal L.N.		NC	+	-	+
Epididymis		NC		NC	
Prostate		-		-	
Testis		-		-	
Ovary			-		-
Uterus			-		-

Appendix
Table 58. (continued)

Tissue	Sex	Pathology Number 3548[1] M	3549[2] F	3552[3] M	3559[4] F
Heart		-	-	-	-
Liver		-	-	-	-
Lung		-	-	-	-
Kidney		-	-	+	-
Spleen		-	-	-	-
Bladder		-	-	-	-
Tonsils		+	-	+	+
Salivary Gland		-	-	-	-
Thymus		-	-	+	-

[1] Sacrificed 14 weeks PE
[2] Sacrificed 15 weeks PE
[3] Sacrificed 16 weeks PE
[4] Sacrificed 18 weeks PE
[5] Not cultured

Appendix
Table 59. Hemoculture results and serologic response of non-challenged fox 3562 following oral challenge of four other foxes in the same room with 1.06×10^{11} CFU *B. suis* type 4.

Weeks Post-Exposure	Hemoculture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	*B. canis*[1]
-12	NC[3]	NT[4]	N25	-	NT	N25	1	NT
-1	NC	NT	N25	-	NT	N25	1	NT
1	NC	NT	NT	NT	NT	NT	NT	NT
2	NC	NT	N25	-	NT	N25	1	N25
3	-	N25	N25	-	N25	N25	2	NT
5	-	NT	I50	-	NT	+50	65	N25
7	-	+50	+100	+	I25	I50	80	NT
10	-	NT	I400	+	NT	I400	86	NT
12	-	I50	I50	WK[5]	+50	+25	87	NT
14	-	NT	+50	+	NT	+25	92	NT
16	-	NT	+50	+	NT	I50	86	NT
18	-	NT	+100	+	NT	I25	90	N25
20	-	I100	+25	+	N25	I25	77	NT

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
[2] Numerical reaction on automated CF test
[3] Not cultured
[4] Not tested
[5] Weak reaction

Appendix

Table 60. Hemoculture results and serologic response of non-challenged fox 3563 following oral challenge of four other foxes in the same room with 1.06×10^{11} CFU *B. suis* type 4.

Weeks Post-Exposure	Hemoculture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	*B. canis*[1]
-12	NC[3]	NT[4]	N25	-	NT	N25	1	NT
-1	NC	NT	N25	-	NT	N25	1	NT
1	NC	NT	N25	-	NT	N25	2	NT
2	NC	NT	N25	-	NT	N25	1	NT
3	-	NT	N25	-	NT	N25	2	NT
5	-	N25	N25	-	N25	N25	4	NT
7	-	NT	I100	-	NT	N25	31	N25
10	-	+25	I50	-	N25	I25	80	NT
12	-	NT	N25	-	NT	N25	78	NT
14	-	NT	N25	-	NT	N25	82	NT
16	-	NT	N25	-	NT	N25	80	N25
18	-	+25	+50	+	I25	N25	76	NT
20	-	NT	N25	-	NT	N25	72	NT

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25

[2] Numerical reaction on automated CF test

[3] Not cultured

[4] Not tested

Appendix

Table 61. Culture of B. suis type 4 from tissues of two non-challenged foxes held in the same room with four foxes orally challenged with 1.06×10^{11} CFU.

Tissue	Sex	Pathology Number 3562[1] F	3563[1] M
Mandibular L.N.		+	-
Retropharyngeal L.N.		+	-
Superficial Cervical L.N.		-	-
Femoral L.N.		NC[2]	NC
Popliteal L.N.		-	-
Internal Iliac L.N.		-	-
Mesenteric L.N.		-	+
Tracheobronchial L.N.		-	+
Supramammary/ Superficial Inguinal L.N.		+	NC
Epididymis			-
Prostate			-
Testis			-
Ovary		-	
Uterus		-	

Appendix
Table 61. (continued)

Tissue	Sex	Pathology Number 3562[1] F	3563[1] M
Heart		-	-
Liver		-	-
Lung		-	-
Kidney		-	-
Spleen		-	-
Tonsil		-	-
Salivary Gland		-	-
Bladder		-	-
Thymus		-	NC

[1] Sacrificed 20 weeks PE
[2] Not cultured

Appendix
Table 62. Hemoculture results and serologic response of fox 3629 following oral challenge with 1.06×10^{11} CFU *B. suis* type 4.

Weeks Post-Exposure	Hemoculture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF	*B. canis*[1]
-12	NC[2]	NT[3]	N25	-	NT	N25	NT	NT
-1	NC	N25	N25	-	N25	N25	24	NT
1	C[5]	+50	N25	-	N25	N25	2	N25
2	-	NT	I400	+	NT	N25	84	NT
3	-	NT	+400	+	NT	+400	86	N25
5	-	NT	+100	-	NT	+400	88	NT
7	-	NT	+400	+	NT	+400	84	N25
10	-	NT	+400	+	NT	+400	85	NT
12	-	+100	+100	WK[6]	I200	I400	91	NT
14	-	NT	+100	+	NT	I400	92	NT
16	-	NT	I100	+	NT	+200	93	NT
18	-	I200	+400	+	+100	+100	88	NT
20	-	NT	I100	+	NT	I200	87	NT
22	-	NT	+100	+	NT	+400	87	NT
23	-	I100	I100	+	+100	I400	4@640[7]	NT
25	-	NT	+50	+	NT	I400	4@320	NT
27	-	NT	+25	WK	NT	I400	4@160	NT
29	-	+100	+50	+	I200	I400	2@640	NT
33	-	N25	+50	+	NT	I100	NT	NT

Appendix
Table 62. (continued)

Weeks Post-Exposure	Hemo-culture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF	B. canis[1]
38	C	NT	I25	WK	NT	I400	4@640	NT
40	-	NT	+50	WK	NT	I100	4@320	NT
44	-	+100	I50	+	I100	+100	1@320	NT
46	-	NT	I50	+	NT	I400	4@320	NT
48	-	NT	+50	+	NT	I200	4@320	NT
51	-	NT	+25	+	NT	+100	1@640	NT
54	-	NT	+50	WK	NT	I200	1@640	NT
56	-	NT	+25	+	NT	I200	NT	NT
66	-	NT	+25	WK	NT	+200	NT	NT

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
[2] Not cultured
[3] Not tested
[4] Numerical reaction on automated CF test
[5] Contaminated
[6] Weak reaction
[7] Expressed as degree of reaction @ given dilution

Appendix
Table 63. Hemoculture results and serologic response of fox 3630 following oral challenge with 1.06×10^{11} CFU *B. suis* type 4.

Weeks Post-Exposure	Hemoculture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF	*B. canis*[1]
-12	NC[2]	NT[3]	N25	-	NT	N25	1[4]	NT
-1	NC	N25	N25	-	N25	N25	1	NT
1	C[5]	NT	+100	WK[6]	NT	N25	2	N25
2	C	NT	+400	+	NT	N25	72	NT
3	C	I400	+400	+	+50	I400	80	NT
5	-	NT	+400	+	NT	+400	86	NT
7	-	NT	+400	+	NT	+400	89	NT
14	-	NT	+200	+	NT	+400	91	NT
16	-	NT	I400	+	NT	+400	94	N25
18	-	NT	+400	+	NT	+400	90	NT
20	-	NT	+200	+	NT	+400	84	NT
22	-	+400	+200	+	+400	+400	84	NT
23	-	NT	I200	+	NT	+400	NT	NT
25	-	NT	I200	+	NT	+400	4@640[7]	NT
27	-	NT	I200	+	NT	+400	4@640	N25
29	-	NT	+200	+	NT	+400	4@640	NT
33	-	+400	+400	+	+400	+400	NT	NT
38	-	NT	I100	+	NT	+400	4@640	NT
40	-	NT	+100	+	NT	+400	NT	NT

Appendix
Table 63. (continued)

Weeks Post-Exposure	Hemo-culture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF	B. canis[1]
44	-	I400	I100	+	+400	+400	3@640	NT
46	-	NT	I50	+	NT	I400	3@160	NT
48	-	NT	I200	+	NT	I400	4@640	NT
51	-	NT	I200	+	NT	I400	4@640	NT
54	-	NT	I400	+	NT	+400	3@640	NT
56	-	NT	I200	+	NT	+400	2@640	NT
66	-	NT	+200	+	NT	+400	4@640	NT

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
[2] Not cultured
[3] Not tested
[4] Numerical reaction on automated CF test
[5] Contaminated
[6] Weak reaction
[7] Expressed as degree of reaction @ given dilution

Appendix
Table 64. Culture of B. suis type 4 from tissues of two foxes orally challenged with 1.06×10^{11} CFU and housed in the same room with two reindeer in 1983.

Tissue	Sex	Pathology Number 3629[1] M	3630[1] F
Mandibular L.N.		-	-
Retropharyngeal L.N.		-	-
Superficial Cervical L.N.		-	-
Femoral L.N.		NC[2]	NC
Popliteal L.N.		-	+
Internal Iliac L.N.		-	-
Mesenteric L.N.		-	-
Tracheobronchial L.N.		-	NC
Supramammary/ Superficial Inguinal L.N.		NC	NC
Epididymis		NC	
Prostate		-	
Testis		-	
Ovary			-
Uterus			-

Appendix
Table 64. (continued)

Tissue	Sex	Pathology Number 3629[1] M	3630[1] F
Heart		-	-
Liver		-	-
Lung		-	-
Kidney		-	-
Spleen		-	-
Bladder		-	-
Tonsils		-	-
Salivary Gland		-	-

[1] Sacrificed 66 weeks PE
[2] Not cultured

Appendix
Table 65. Hemoculture results and serologic response of fox 3565 born to female 3630 two months following oral challenge with 1.06×10^{11} CFU *B. suis* type 4.

Weeks of Age	Hemo-culture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	*B. canis*[1]
9	NC[3]	N25	N25	-	N25	N25	4	NT[4]
11	NC	N25	N25	-	N25	N25	3	NT
13	-	NT	I200	-	NT	I25	56	N25
14	-	I100	+50	+	N25	+25	66	NT

1 += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
2 Numerical reaction on automated CF test
3 Not cultured
4 Not tested

Appendix

Table 66. Hemoculture results and serologic response of fox 3577 born to female 3630 two months following oral challenge with 1.06×10^{11} CFU B. suis type 4.

Weeks of Age	Hemo-culture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]	B. canis[1]
9	NC[3]	N25	N25	-	N25	N25	1	NT[4]
11	NC	NT	N25	-	NT	N25	2	N25
13	-	NT	N25	-	NT	N25	1	NT
14	-	NT	N25	-	NT	N25	1	NT
15	-	N25	N25	-	N25	N25	NT	NT
16	-	NT	N25	-	NT	N25	NT	NT
18	-	NT	N25	-	NT	N25	0	N25
20	-	N25	N25	-	N25	N25	0	NT
21	-	NT	N25	-	NT	N25	0	NT

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25

[2] Numerical reaction on automated CF test

[3] Not cultured

[4] Not tested

Appendix
Table 67. Hemoculture results and serologic response of fox 3633 born to female 3630 two months following oral challenge with 1.06×10^{11} CFU B. suis type 4.

Weeks of Age	Hemo-culture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF	B. canis[1]
7	NC[2]	N25	N25	-	N25	N25	3[3]	NT[4]
9	NC	NT	N25	-	NT	N25	2	NT
11	NC	NT	N25	-	NT	N25	1	NT
13	-	NT	N25	-	NT	N25	1	N25
14	-	NT	N25	-	NT	N25	1	NT
15	-	NT	N25	-	NT	N25	0	NT
16	-	NT	N25	-	NT	N25	0	NT
18	-	NT	N25	-	NT	N25	0	NT
20	-	NT	N25	-	NT	N25	0	N25
22	-	NT	N25	-	NT	N25	0	NT
26	-	NT	N25	-	NT	N25	NT	NT
31	-	NT	N25	-	NT	N25	2@10[5]	NT
33	-	NT	N25	-	NT	N25	0	NT
37	-	N25	N25	-	N25	N25	NT	NT
39	-	N25	N25	-	N25	N25	1@10	NT
41	-	NT	N25	-	NT	N25	2@10	NT
44	-	NT	N25	-	NT	N25	3@10	NT
47	-	NT	N25	-	NT	N25	4@10	NT

Appendix
Table 67. (continued)

Weeks of Age	Hemo-culture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF	B. canis[1]
49	-	NT	N25	-	NT	N25	3@10	NT
60	-	NT	N25	-	NT	N25	2@10	NT

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25
[2] Not cultured
[3] Numerical reaction on automated CF test
[4] Not tested
[5] Expressed as degree of reaction @ given dilution

Appendix
Table 68. Culture of B. suis type 4 from tissues of three fox pups born to parents each previously orally challenged with 1.06×10^{11} CFU.

Tissue	Sex	Pathology Number 3565[1] F	3577[2] M	3633[3] F
Mandibular L.N.		+	-	-
Retropharyngeal L.N.		+	-	-
Superficial Cervical L.N.		+	-	-
Femoral L.N.		NC[4]	NC	NC
Popliteal L.N.		NC	-	NC
Internal Iliac L.N.		+	-	-
Mesenteric L.N.		-	-	-
Tracheobronchial L.N.		-	NC	NC
Supramammary/ Superficial Inguinal L.N.		NC	-	-
Epididymis			NC	
Testis			-	
Ovary		-		-
Uterus		-		-

Appendix
Table 68. (continued)

Tissue	Sex	Pathology Number 3565[1] F	3577[2] M	3633[3] F
Heart		-	-	-
Liver		-	-	-
Lung		-	-	-
Kidney		-	-	-
Spleen		+	-	-
Bladder		-	-	NC
Tonsils		-	-	-
Salivary Gland		-	-	-
Thymus		+	-	-

[1] Sacrificed at 14 weeks of age
[2] Sacrificed at 20 weeks of age
[3] Sacrificed at 60 weeks of age
[4] Not cultured

Appendix

Table 69. Hemoculture results and serologic response of reindeer #3230 housed in the same room with two foxes challenged orally with 1.06×10^{11} CFU *B. suis* type 4.

Weeks Post-Challenge of Foxes	Hemo-Culture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]
0	-	NT[3]	N25	-	NT	N25	2
1	-	N25	I50	-	N25	N25	2
2	C[4]	NT	+200	+	NT	N25	41
3	+	NT	+400	+	NT	+400	88
5	-	NT	+400	+	NT	+400	88
7	-	I400	+400	+	I200	+200	91
10	-	NT	+400	+	NT	+400	94
12	-	NT	I400	+	NT	I200	94
14	-	+400	I400	+	I400	I200	95
16	-	NT	I400	+	NT	I400	93
17	-	+400	+50	+	+400	+200	88

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25

[2] Numerical reaction on automated CF test

[3] Not tested

[4] Contaminated

Appendix

Table 70. Hemoculture results and serologic response of reindeer #3554 housed in the same room with two foxes challenged orally with 1.06×10^{11} CFU *B. suis* type 4.

Weeks Post-Challenge of Foxes	Hemo-Culture	ST[1]	SP[1]	BBA	ME[1]	RIV[1]	CF[2]
0	-	NT[2]	N25	-	NT	N25	2
1	-	NT	+50	-	NT	N25	2
2	-	N25	I25	-	N25	N25	3
3	-	N25	+50	-	N25	N25	9
5	-	NT	I100	+	NT	+400	76
7	-	NT	+400	+	NT	+400	90
10	-	I200	+400	+	I200	I400	84
12	-	NT	+25	+	NT	+25	94
14	-	NT	I100	+	NT	I25	94
16	-	NT	+100	+	NT	N25	87
17	-	NT	I50	+	NT	N25	86

[1] += Positive reaction at given dilution; I= Incomplete reaction at given dilution; N25= Negative at 1:25

[2] Numerical reaction on automated CF test

[3] Not tested

Appendix

Table 71. Culture of B. suis type 4 from tissues of two reindeer housed four months in the same room with two foxes orally challenged with 1.06×10^{11} CFU.

Tissue	Pathology Number 32301	35541
Mandibular L.N.	+	-
Retropharyngeal L.N.	+	-
Parotid L.N.	+	-
Superficial Cervical L.N.	+	-
Subiliac L.N.	-	-
Popliteal L.N.	+	-
Medial Iliac L.N.	-	-
Mesenteric L.N.	-	-
Mediastinal L.N.	-	-
Tracheobronchial L.N.	-	-
Superficial Inguinal L.N.	-	-
Epididymis	-	-
Testis	-	-
Seminal Vesicle	-	-
Heart	-	-
Liver	-	-
Lung	+	-
Kidney	-	-

Appendix
Table 71. (continued)

Tissue	Pathology Number 3230[1]	3554[1]
Spleen	-	-
Biceps Femoris	-	-
Salivary Gland	NC	-
Urine	-	-
Jaw Abscess	+	
Rectal Abscess	+	

[1] Sacrificed after 18 weeks of exposure to challenged foxes

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