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A SERO-EPIDEMIOLOGIC INVESTIGATION OF *BRUCELLA CANIS* ANTIBODIES IN SPECIFIED HUMAN POPULATIONS IN OKLAHOMA

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A SERO-EPIDEMIOLOGIC INVESTIGATION OF *BRUCELLA CANIS* ANTIBODIES IN SPECIFIED HUMAN POPULATIONS IN OKLAHOMA

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TO MY MOTHER

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CHAPTER I

INTRODUCTION

The epizootiology of canine brucellosis is worthy of consideration from both the veterinary and the human health standpoints. The dog, if capable of serving as a mechanical or biologic vector of *Brucella* organisms, could present a serious problem in the control and eradication of brucellosis. An infected dog might transmit the disease to humans who come in contact with the animal. Brucellae-infected dogs could be of serious consequence to the livestock industry as well as to human health.

Classical Brucellosis in Dogs

Numerous reports from many countries have shown that dogs may occasionally become infected with classical strains of *Brucella* if the dogs run with infected sheep (*Brucella melitensis*), swine (*Brucella suis*), or cattle (*Brucella abortus*). A review of the literature on canine brucellosis through 1951 [1], and additional reports [2,3], clearly indicate a natural susceptibility of dogs to *Brucella*. In contrast with the severe manifestations of brucellosis often seen in cattle,

swine, and sheep, naturally occurring canine brucellosis is usually a mild disease with virtually no mortality; however, abortions, orchitis, epididymitis, lymphadenopathy, arthritis, and bacteremia can occur [4].

The dog, like man, appears to be a terminal host for the classical strains of Brucella. The spread of Brucella from diseased dogs to other animals by contact has been reported, but direct transmission usually could not be demonstrated [5]. Dogs have been infected experimentally with either Brucella suis or Brucella abortus by intravenous or intraperitoneal inoculation [1].

Canine Brucellosis Due to Brucella Canis

History

Prior to 1964, canine abortions of epizootic proportions were considered of little concern to dog breeders and veterinarians. About 1962, according to breeders, an unusually high frequency of abortion began to be observed in kennels of Beagles in the Midwest and South. During 1964 and 1965, breeders throughout the country began to notice abortions in their dogs (reviewed by Carmichael and Kenney [5]). In October, 1966, Carmichael called attention to this widespread abortion, apparently due to Gram-negative coccobacilli [6]. By 1968, the disease had been diagnosed in more than 800 dogs in 38 states [5].

The etiologic agent was quickly identified as a species of Brucella, and was later called Brucella canis by Carmichael and Bruner [7], Jones and co-workers [8], and Moore and Bennett [9]. Meyer [10], however, argued for its designation as Brucella suis, biotype 5. In contrast to previous reports of brucellosis in dogs caused from the

classical Brucella, the microorganism responsible for this new disease was highly contagious, and transmission was shown to readily occur by ingestion of infected tissues and by contact with infective discharges [5]. Since 1968, other epizootics of canine abortions and infertility due to B. canis have been described [11, 12, 13, 14, 15, 16, 17].

Natural Disease and Symptomatology

The dog is the only species in which the natural disease has been diagnosed [15]. The Beagle accounts for the vast majority of infections. In addition, Weimaraners, Foxhounds, Old English Sheepdogs, American Pointers, and Greyhounds occasionally have been found diseased [7]. The disease in dogs is characterized by abortions, infertility, endometritis, prostatitis, epididymitis, testicular degeneration, lymphocytic infiltration of the urogenital system, and lymph node hyperplasia [5, 11, 14, 15, 18, 19].

Entry, Escape, and Transmission of the Organism

The sites which may serve as portals of entry are the oral cavity, vagina, and conjunctivae, which suggests that the infection can take place on all mucous membranes [7]. Intravenous and subcutaneous inoculation can also produce infections [15]. Portals of exit in infected dogs are vaginal discharges, milk, semen, and urine from males only [7, 13, 20]. From a clinical viewpoint, transmission is known to occur by the following means [15]: a) contact with aborted fetal tissue, b) contact with a vaginal discharge from an infected bitch, c) venereal transmission, d) congenital transmission, and e) by urine contamination from male dogs.

Experimental Species Susceptibility

Species of animals which have been experimentally infected with Brucella canis include the mouse, rabbit, and guinea pig [21]. Three of 14 cats inoculated orally with B. canis developed bacteremias but did not develop agglutinating titers to the organism [22]. Nonpregnant sheep, swine, and cattle are highly resistant to infection by the oral-conjunctivae route [22]. There are two instances where nonhuman primates have been experimentally infected by the B. canis organisms [15, 23]. No published works have been conducted in which a systematic study of species susceptibility has been performed.

Detection of Disease in Dogs

It has been established that diseased dogs develop specific humoral agglutinating antibody for Brucella canis [5, 6, 7, 9, 22]. The antigen employed in the detection of these agglutinins must be prepared from B. canis since the commercially available classical brucellae antigens will not react with sera that is positive for B. canis antibodies [24].

Moore [14] observed that, of six suspect male dogs with no B. canis bacteremia and 12 male dogs with bacteremia, all 18 dogs had agglutinating titers to the corresponding organism. The dogs without bacteremia never produced a titer greater than 1:200, while those with the condition demonstrated titers of 1:200 to 1:800. From pathological, cultural, and serological studies, Moore showed that all the dogs exhibiting agglutinating titers above 1:200 possessed lesions consistent with those seen in infected dogs. This investigator postulated that a titer of less than 1:200 was indicative of previous infections and that

some, if not all, dogs with agglutinating titers of 1:100 or 1:200 had apparently been infected previously.

In a report in which B. canis was eradicated from a colony of 265 beagles by the removal of 63 culture positive dogs, the 202 (71%) remaining dogs had titers of 1:100 to 1:200 [13]. Carmichael [21] regards agglutination tests of less than a 1:100 dilution as negative. He found that organisms were isolated from the blood of animals with titers of 1:100 or greater. Moore [13] states that dogs demonstrating titers of 1:100 or greater possibly reflect one or a combination of the following: a) exposure and actively acquired immunity, b) infection with recovery, and c) subclinical infection.

Prevalence of Brucella canis Infection in Dogs

It has been difficult to ascertain the degree of infection with Brucella canis in the canine population for which there may be several explanations. Since the organism can cause serious economic losses for dog breeders and kennel owners, the concern of diagnosing clinical disease is of prime importance because it is thought that only dogs who are clinically ill can transmit the disease; thus, subclinical disease or infection is of secondary importance. As a result, usually only clinically ill dogs that are part of an epidemic are tested and at high dilutions of serum. Therefore, isolated cases of disease are missed as well as those who may have antibodies at low dilutions.

B. canis disease is still in its embryonic development as a cause of canine disease and is not included as part of a differential diagnosis by many veterinarians. Therefore, dog sera are not routinely tested for B. canis antibodies, except possibly, in instances when

epidemics of abortions occur.

As with most newly recognized diseases, studies involving B. canis have occurred in a research environment with no cooperative studies for standardization of methods and procedures. As a consequence, it has been difficult to compare results of different investigators or to combine their results in order to arrive at general conclusions.

There has been only one study to assess the prevalence of B. canis antibodies in a population of dogs at low serum dilutions [24]. Blood samples were collected from 650 dogs, consisting of 345 mixed breed dogs, 214 pointers and setters, and 91 other purebred dogs. The dogs included in the survey were randomly selected from three sources: a) dogs hospitalized in the Auburn University Small Animal Clinic, b) pound dogs obtained for instructional and research purposes by Auburn University, Alabama, and c) various kennels in Alabama and Georgia.

The sera were tested at dilutions of 1:25, 1:50, 1:100, and 1:200, and the results are shown in Table 1. The interesting part of this study was that 77.4 percent of the dogs had a titer of 1:25 or greater. The author stated that the occurrence of agglutinating titers for B. canis antibodies was apparently much greater in the canine population than had been previously thought. He concluded that there was a need for further work in determining just what was a significant titer for B. canis and what, if any, was the significance of a titer of less than 1:100.

Dogs as a Vector in the Transmission of Classical Brucellosis to Man

The possibility of transmission of classical brucellosis from

TABLE 1

BRUCELLA CANIS ANTIBODIES IN 650 DOGS[a]

Antibody Titer	Number	Percent
Negative	147	22.6
1:25	101	15.5
1:50	207	31.9
1:100	151	23.3
≥1:200	44	6.7
Total	650	100.0

[a]Lewis [24].

dogs to human beings has been considered by investigators; however, few suspected cases have been reported in the literature [25]. Dargein and Plazy observed a bitch which whelped three dead young pups and whose serum contained Brucella agglutinins [1]. The dog shared quarters with 14 naval officers, seven of whom were hospitalized with brucellosis. The dog was destroyed, and further cases of brucellosis were not observed among naval personnel. The authors concluded that the infection had been contracted from the dog.

Nenzani assumed that a bitch which aborted had served as the source of infection for a human patient [25]. The owner of the dog had assisted in removing fetal membranes after the animal aborted. Prior to this time, the dog had been seen eating aborted material from a cow. The dog reacted at the 1:5,000 dilution to the serum agglutination test.

Ostertag and Mayer reported an infection in two individuals which was traced to a dog used in an infected flock of sheep [26]. Numerous isolations of Brucella melitensis were from dogs which gave positive reactions to one or more tests.

Berman observed two dogs living on a dairy farm where a number of brucellae-infected cows were kept [1]. Of the seven people on the farm, five were hospitalized with brucellosis. The serum-agglutination test of the dog was positive at 1:1,600, and three weeks prior to these observations, the animal had been seen eating aborted material from a cow.

Nicoletti and co-workers reported a case of probable transmission of Brucella suis infection to a suburban housewife from a dog which had aborted. The woman had had no contact with domesticated farm

animals [25]. She had removed two dead fetuses from the dog without protection to her skin. The blood serum of the dog was positive for brucellae agglutinins at a 1:800 dilution, and B. suis Type I was recovered at autopsy. It was discovered later that swine from a herd located one mile away were also infected with the same organism.

Transmission of Brucella canis from Dogs to Man

Occupational Exposure

To date, only 12 cases of human infection with Brucella canis have been reported and are presented in Table 2. In the 1968 Annual Brucellosis Summary for the United States, the Center for Disease Control of the United States Public Health Service briefly cited three infections caused by this organism [27]. One animal caretaker had only serological evidence of an infection. Two laboratory technicians had been infected by the oral route while pipetting viable organisms. Both exhibited serological evidence of infection, and B. canis was isolated from the blood of one.

Morissett and Spink observed and treated two laboratory personnel with B. canis infection [16]. Both had been exposed to large quantities of viable organisms in the preparation of antigen. Although the portal of entry could not be definitely determined, it was likely that contamination of the hands with skin abrasions resulted in entry of the organisms. High antibody titers were observed in both individuals, and Brucella canis organisms were isolated from the blood in one.

Two cases of canine brucellosis were reported in the 1970 Center for Disease Control United States Brucellosis Summary [28]. These cases

TABLE 2

REPORTED HUMAN BRUCELLOSIS DUE TO *BRUCELLA CANIS*

Case	Year	Symptomatic	Titer	Bacteriologically Proven
Animal Caretaker[a]	1968	No	"Positive"	No
Lab Technician[a]	1968	Yes	1:250	Yes
Lab Technician[a]	1968	Yes	1:500	No
Lab Technician[b]	1969	No	1:320	No
Lab Technician[b]	1969	Yes	1:320	Yes
Animal Caretaker[c]	1970	No	1:100	No
Lab Technician[d]	1970	Yes	1:320	No
Lab Technician[d]	1970	Yes	1:640	Yes
Housewife[e]	1972	Yes	1:250	Yes
Laborman[f]	1972	Yes	1:250	Yes
Arkansas Man[f]	1972	No	Not Done	Yes
Tennessee Man[g]	1973	Yes	Unknown	Yes

[a]National Brucellosis Committee [27].

[b]Morissett and Spink [16].

[c]McCormick *et al.* [29].

[d]National Brucellosis Committee [28].

[e]Swenson *et al.* [30].

[f]Munford *et al.* [31].

[g]Personal Communication [32].

involved technicians who worked in a research laboratory. A culture of B. canis was dropped, and exposure probably occurred for both at that time. One had onset one month later and had antibody titers of 1:320 and 1:640,and cultures were negative. The second case had onset two months after exposure with antibody titers of 1:640, and B. canis was isolated from a blood specimen.

In addition, McCormick and co-workers reported the presence of agglutinins in the serum of an asymptomatic animal caretaker [29]. The blood cultures were negative, although he was not cultured until two months after the initial serologic response. Within five months, agglutinins to B. canis had completely disappeared.

Naturally Acquired Brucellosis

Until recently, it appeared that disease due to Brucella canis was a possibility only to those people who had continuous exposure experience to either infected dogs or in laboratory personnel working with large quantities of viable bacteria and posed little or no hazard to the general population. Recently there have been three reports in the literature and one by personal communication of naturally acquired brucellosis caused by this organism.

A twenty-three year old woman was hospitalized with a diagnosis of bacterial endocarditis [30]. Seven of 12 blood cultures drawn over the first 7 days of hospitalization yielded a Gram-negative bacillus that was identified as B. canis by the Special Bacteriology Laboratory of the Bacterial Reference Unit, National Center for Disease Control. Serum obtained from the patient agglutinated B. canis at a titer of 1:250. Titers against Brucella abortus were less than 1:20. One year

after completion of antibiotic therapy, the patient remained well. Blood cultures were negative, and the agglutination titer against B. canis was 1:25. The patient's family had two dogs, a male German Shepherd and a smaller mongrel female, both of whom appeared in good health. Blood cultures from the male dog were culture positive for B. canis, and the serum had an agglutination titer of 1:500. Blood cultures and agglutination titers from the female dog were persistently negative. Sera obtained from 12 additional family members did not show any titer to B. canis. Even though the dog was treated, blood cultures remained positive and agglutination titers were 1:200. The family refused further evaluation or therapy for the dog, and one year later they reported that he continued to appear well. Bacteremic male dogs are known to excrete B. canis in their urine [17], so it would appear likely that infection occurred through direct contact with materials contaminated by the dog. The fact that no other family member had serologic evidence of infection of B. canis is in agreement with the previous suggestion that this organism does not readily infect the human host [16], although there has been only one sero-epidemiologic population study [33] to prove or disprove this suggestion. Canine infections caused by B. canis are found almost exclusively in animals confined in kennels [30]; thus, the finding of a diseased dog in the community at large in this particular case report was somewhat unusual. Since the family knew little of the dog's past history, it was possible that the dog had become infected while in a kennel.

In August, 1972, a case of human infection by B. canis occurred in a Jacksonville, Florida, air conditioning mechanic [31]. This case

is of epidemiologic interest because the patient had minimal animal contact. During the first 3 days of hospitalization, he had shaking chills twice each day, and each chill was followed by a rise in temperature to 102 F. On the third hospital day his temperature became normal, and it remained normal throughout his hospital course. Seven blood cultures grew an organism which was tentatively identified as Mima polymorpha, but was later identified as a Brucella species by the Florida State Laboratory. Identification of the organism as B. canis was done by the Laboratory Division of the Center for Disease Control one month later. At this time serum was tested for agglutinating antibodies against B. canis, and the resulting titer was 1:250. One month later no demonstrable titer was observed at a 1:50 dilution of serum. The epidemiologic investigation was designed to define the source of the patient's infection and to discover if other persons exposed were also infected. Prior to his illness the patient had multiple direct and indirect exposures to dogs. As an air conditioning mechanic, his work which required crawling underneath houses, resulted in contact with dogs and feces contaminated soil. His work had not taken him to a veterinarian clinic or kennel in the preceding year, however. The family owned an apparently healthy 4 1/2 year old spayed dog. The serum titer of the dog for B. canis was a 3+ at 1:50 and 2+ at 1:100 which was lower than those usually seen in actively infected animals (a 4+ titer of 1:100 dilution was considered "positive"). Among the patient's immediate family (wife and three children), only one son, age 11, had been ill during the six months preceding the investigation. This son was reported to be the most active of the children, frequently playing

outdoors with neighborhood dogs. Three of the patient's co-workers had also been ill in the preceding months. Two had illnesses which were diagnosed as viral in origin, and the third did not seek medical aid. A survey of neighbors who owned dogs failed to reveal any illness among the neighborhood dogs, and no animals were found with known histories of abortion or exposure to aborting dogs. The source of infection in this patient was unknown; only a suspicious serologic titer in the family dog incriminated this otherwise innocent bitch. None of the patient's family contacts had serologic evidence of infection, although his 11 year old son may have had a milder illness with seronegativity at the time the specimen was obtained more than one month later. The possibility remained that the patient may have acquired the disease while at work, and even the absence of serologic evidence of B. canis illness in his co-workers did not exclude this possibility.

B. canis has also been isolated at time of surgery from a clotted aortic bifurcation graft [31]. This 60 year old patient also had a retroperitoneal abscess, and the isolate may possibly have come from this source. The patient, an Arkansas man who owned three hunting hounds, died three years later; no autopsy was performed, nor were postmortem cultures obtained.

Recently there has been one additional case of B. canis disease in man [32]. A young man in Memphis, Tennessee, had several positive blood cultures. His clinical illness was quite similar to that of the Florida case.

Human Symptomatology

A sporadic disease, human Brucella canis disease exhibits signs and symptoms simulating other diseases. Identification of suspects is sometimes made only on epidemiological information or the pattern of illness. The illness has been characterized by fever, chills, posterior cervical, epitrochlear, axillary lymphadenopathy, and splenomegaly [30]. Definitive diagnosis is based upon the isolation of the causative agent or by detection of antibodies by serologic means.

Serological Epidemiology

Background

The immune status of the host is of prime importance in nearly all epidemiological situations where an infectious disease is concerned. This can be tested and demonstrated experimentally in animals by actually exposing them to infectious agents and observing the results. In man, however, this demonstration has not been so easy. Prior to about 1900, the information regarding human immunity was limited to reports by individuals concerning whether they had been vaccinated or had had a disease, or to the presence of actual physical signs [34]. These were very limited measures because they did not take into account the fact that much of man's immunity is acquired subclinically through mild, unrecognized, or inapparent infection.

Around the beginning of the twentieth century, measurements relating to the immune status of a given population to such infectious diseases as tuberculosis, diphtheria, and syphilis began to be obtained [34]. Such measurements were used mostly to diagnose individual cases.

The use of serological tests for disease surveys may be said to have begun with attempts to map out the distribution of syphilis in segments of urban populations by Williams in 1916 [35].

Epidemiologic Surveys of Infectious Diseases

Epidemiologic surveys are undertaken when existing information concerning the interrelation between man and a disease agent is inadequate. The reasons for such inadequacy are many and include the following examples: a) the lack of sensitive or specific diagnostic methods, b) the disease rarely requires medical attention, c) the lack of official requirement for disease reporting, and d) when newly encountered infectious agents are found. Surveys may be performed to reflect the current activity of the agent such as disease or the presence of the agent, for prior activity such as persisting antibody, or the presence of a characteristic disability such as paralysis in poliomyelitis [36].

Surveys of seroimmunity (serologic epidemiology) are basic to the study of infectious diseases [37]. Poliomyelitis provides an important example. Because of the great frequency with which poliovirus infection leads to inapparent disease, the occurrence of paralysis provides a highly misleading picture of the extent of human infection with polioviruses. A far different picture is revealed by serologic surveys which consist of determining the presence of poliovirus antibodies in the sera of an appropriately selected sample of the population.

Serologic surveys have also contributed to understanding the distribution of many diseases. For instance, such studies revealed antibodies to yellow fever virus in residents of large areas where the disease had not been recognized clinically and where the only vector

then known did not exist. These observations led to the recognition that yellow fever had a sylvatic cycle [36].

A very important use of serologic surveys relates to newly encountered agents potentially pathogenic for man. Surveys for antibodies to a newly identified agent provide a direct way for determining whether the agent has made contact with man. Essentially, these are exploratory or prevalence types of surveys which are designed to determine the magnitude of infection in a population. The extent of the problem is reflected in the calculation of crude rates [34].

Human Immunoglobulins

The gamma globulins were first recognized and designated as a distinct group by Tiselius in 1936 [38]. Approximately one year later, Tiselius and Kabat demonstrated that the antibodies of serum were present in the gamma globulin fraction [39]. This fraction of serum is composed of at least five distinct globulins with antibody activity. These globulins are designated as classes of "immunoglobulins" abbreviated as follows: IgG, IgM, IgA, IgD, and IgE; however, only the first two classes are important as part of the humoral defense system which acts against microorganisms (Reviewed by Weisser, Myrvick and Pearsall [40]). Some properties of these immunoglobulins are presented in Table 3.

IgG Immunoglobulins

The IgG globulins constitutes the largest part of the immunoglobulin system, with an average adult serum concentration of approximately 1200 mg per 100 ml. Many of the antibodies in normal human serum,

TABLE 3

PROPERTIES OF HUMAN IMMUNOGLOBULINS

Class	Sedimentation Constant	Approx. Mol. Wt.	Placental Transfer	Antibody Activity	Synthesis Begins at:	Adult Level Reached at:
IgG	7S	160,000	Yes	Yes	4-6 wks.	8 yrs.
IgM	19S	900,000	No	Yes	Early after Birth	1-2 yrs.
IgA	7S, 11S, 18S	170,000-600,000	No	Yes (Cellular)	2-3 wks.	12 yrs.
IgD	7S	160,000	No	Unknown	Unknown	Unknown
IgE	8S	160,000	No	Yes (Reaginic)	Unknown	Unknown

such as bacterial and viral antibodies, are IgG globulins. These alone confer passive immunity to the newborn child, since only that molecule is chemically constructed to cross the placenta. The long half life of the IgG globulin also allows it to provide long lasting immunity. On the other hand, IgG globulin shows a relatively slow response to antigenic stimulation,and its small size makes it a poor agglutinator of bacteria.

IgM Immunoglobulins

The IgM globulin represents about 5.8 percent of the normal serum immunoglobulins. In the average adult, this concentration is approximately 100 mg per 100 ml. The molecular weight of this molecule is approximately 900,000 and, by the virtue of its large, bulky shape, it is much more efficient than the IgG globulin in cellular agglutination. These antibodies are usually the first detected antibodies after antigenic challenge.

Passage of Antibodies Between Mother and Offspring

Prior to birth, animals of most species form little or no antibody, presumably for two reasons, namely, because they are immunologically immature and because exposure to stimulation by foreign antigens is limited by the barriers of investing membranes (Reviewed by Weisser, et al. [40]). When the fetus encounters strong antigen stimulation, the principal antibodies found are IgM. Antibodies of the IgM class are formed most readily and are often present in small amounts in cord blood and indicates previous infection of the fetus.

The membranes surrounding the embryo serve as a barrier to the

passage of many substances such as harmful toxins and microorganisms. Thus, it may be assumed that the embryo does not normally require the protection of antibodies so essential to the adult. Following birth, however, prompt exposure to many potentially harmful agents in the external environment demands that the young possess protective antibodies in order to have a chance for survival. This is accomplished by the ability of certain maternal antibodies to cross the placenta.

Essentially all antibodies that reach the human infant are acquired before birth by way of the placenta. Whereas IgM, IgA, IgD, and IgE do not pass the human placenta in significant amounts, IgG passes beginning at the third to fourth month of fetal life. Maternal antimicrobial antibodies passively acquired during fetal life serve to defend the newborn infant against infections during the first few weeks of life and before the infant's body is competent to form protective antibodies. Passive protection by maternal antibodies can be effective only against those agents to which the mother is immune and to which she possesses substantial quantities of specific protective antibodies. The low point in serum levels of IgG and, consequently, the least resistance to infection occurs at approximately three months of age.

Interaction of Antigens and Antibodies in vitro

Antibodies of the molecular classes IgA, IgG, IgM, and IgE are known to participate in various types of *in vitro* antigen-antibody reactions in a highly specific manner. Antibodies which react with soluble antigens to form precipitates are called "precipitins", and antibodies which agglutinate particulate antigens such as bacteria are

called "agglutinins". Antibodies reacting with antigens to produce other effects have been named in like manner according to the effects produced.

Agglutination Reaction

The agglutination procedure has been widely used for such purposes as the quantitation of antibodies in serum (Reviewed by Bennett [41]). This test determines the greatest (highest) dilution of the serum that will cause agglutination of a known bacterial antigen. The process of agglutination occurs in two steps: a) the primary stage, in which antibody is absorbed onto the surface of the antigen, and b) the secondary stage, in which the antibody-coated antigen particles agglutinate. Agglutination by antibody is influenced by pH and salt concentration and is usually optimal at pH values near neutrality and salt concentrations in the physiological range.

Serial Dilutions

Dilution is the act of making a weaker solution from a stronger one. This is usually done by adding a diluent, such as saline, to the material in the amount required to make a certain dilution. Dilutions are usually expressed as 1 unit of the original solution to the total number of units of final dilution. Therefore, a 1:10 dilution calls for 1 unit of the concentrated solution to be diluted to a total of 10 units.

The systematic redilution of a fluid a number of times is called a "serial dilution". An example of a serial dilution is as follows:

Into each of five test tubes is measured 1 ml of saline. One ml of serum is placed in the first tube and mixed. Since there is 1 ml of serum in a total of 2 ml, a 1:2 dilution exists in the first tube. One ml of this 1:2 dilution is removed and mixed with the 1 ml of saline in the second tube. The dilution of serum in the second tube is one-half that of the first tube or 1/2 x 1/2 = 1/4 or 1:4. This operation is repeated serially with the three remaining tubes and the final dilution of serum in each tube would be 1:2, 1:4, 1:8, 1:16 and 1:32.

Whether or not to include the antigen which is added to the serum dilutions as part of the final dilution, usually depends upon the volume that is added and/or the concentration used. If only a small amount of antigen is added in relationship to the total volume, it is usually not included as part of the final dilution. However, whenever dilute antigen is added in an amount that would greatly increase the total volume, the antigen is usually included as part of the dilution. For instance, in the above example:

The final volume in the first tube is 0.5 ml of serum and 0.5 ml of saline (1 ml total). If 2 ml of dilute antigen were added, the final dilution of serum would be 0.5/3.0 or 1:6. Therefore, the final dilution of serum in each tube would be 1:6, 1:12, 1:24, 1:48, and 1:96.

Titers

The titer may be defined as the quantity of a substance required to produce a reaction with a given volume of another substance. It is usually referred to as a measure of the number of antibody

molecules per unit volume of the original serum and gives an indication of the antibody concentration in a serum sample. The antibody titer of a serum is the highest dilution which will give reaction with antigen.

Detection of Antibodies by the Microtiter Technique

The tube agglutination technique is the standard test for the detection of Brucella antibodies; however, in recent years the microtiter technique with its many advantages has been applied to several viral and bacterial serological tests [42, 43, 44]. The original microtiter system was introduced in 1950 and first described in 1955 (reviewed by Witlin [45]). The system was further refined by Sever in 1962 [46]. Application of the microtechnique to serologic tests is of practical importance; it saves time, space, antigen, and sera. It has an additional advantage over the rapid slide agglutination test of not only providing a screening method to detect positive sera but also providing titers which correlate highly with the more laborious tube agglutination test [47]. The technique utilizes precalibrated micropipettes and microdilutor loops, titration plates, and a test reading mirror.

Serologic Surveys of Brucella canis Antibodies in Human Populations

Little information is available concerning the incidence of Brucella canis infections in humans. The dangers of canine to human transmission of this organism, either by occupational exposure or by natural means, have been discussed by a number of investigators [4, 12, 16, 23, 29, 30, 33].

To date, there has been only one published report of a population survey for the prevalence of B. canis antibodies in humans [33].

Lewis conducted a serological survey on 1,208 apparently healthy, male, military recruits, ages 18-26, and a titer of 1:100 or greater was considered positive and of significance. This particular titer was chosen as significant because the investigator inferred that bacteremia usually occurred at this level. Only five (0.4%) sera in this study demonstrated titers of 1:100 or greater. The author stated that although the incidence of significant titers was low, the mere fact of their detection may be of epidemiological significance.

One of the principal purposes of an exploratory sero-epidemiologic survey is to be sensitive enough to detect a particular antibody when present. This purpose was not achieved in the above-mentioned study because B. canis antibodies had to be present at a 1:100 dilution before the serum was considered positive. Antibody surveys that relate to newly encountered agents that may be potentially pathogenic for man, such as B. canis, should be sensitive enough to detect subclinical infection as well as clinical disease; therefore, antibodies present in sera, even at low titers, may be of epidemiological significance. Not only would such information augment existing epidemiological knowledge of this newly recognized disease in man, but its zoonotic significance could also be of public health importance.

Purpose of the Present Investigation

Since canine and human brucellosis due to B. canis may be underdiagnosed, the extent of infection in both man and dog must be established to determine the potential importance of this disease as a public health problem. Although the occurrence of agglutinating B. canis antibodies is apparently much greater in the canine population

than has been previously thought, it is not known to what degree man has become infected.

The object of this study, then, was to determine the prevalence of B. canis infection in selected human population groups in Oklahoma. For this purpose, a microtiter plate agglutination technique was developed as an epidemiological tool for the detection of B. canis antibodies.

CHAPTER II

MATERIALS AND METHODS

Brucella canis Antigen

Brucella canis antigen was not available from a commercial source; therefore, it was necessary to contact various investigators who were engaged in similar studies. Jack Oakes, D.V.M., of the United States Department of Agriculture (U.S.D.A.) was contacted, and the problem of obtaining the antigen was discussed. As a result of this meeting, B. canis antigen was obtained from the United States Department of Agriculture, Animal and Plant Service, Veterinary Service Diagnostic Laboratory, Ames, Iowa.

Antigen Specificity

Many investigators have demonstrated that Brucella canis does not exhibit patterns of possessing a common antigen with Brucella abortus, Brucella suis, or Brucella meletensis [24, 28, 29, 30, 48]. B. canis is a rough Type-R[1] organism, and antigens prepared from this type of cell may have similar surface antigens with Type-R strains of the other Brucella [4], although it lacks the O antigens[2] present in

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[1]Rough Type-R colonies are associated with the loss of the surface gluco-lipid-protein complexes. Virulence is commonly, but not always, associated with the smooth Type-S colony.

[2]Heat stable somatic antigens.

smooth Type-S strains [10].

Spink and Morrissett [48] and Lewis [49] have reported that B. canis will cross react with another newly recognized species of Brucella, B. ovis, a cause of epididymitis in rams. B. canis has also been shown to cross react with animal strains of Bordetella bronchiseptica [22] and Pasturella multocida [50]. There have been no reports of cross-reactions of B. canis with organisms outside the family Brucellaceae.

Experiments were conducted to verify the specificity of the U.S.D.A. B. canis antigen. Agglutination-absorption, fluorescent immunoglobulin, and paired serum tests using B. canis and B. abortus antigen were performed.

Agglutination-absorption. Non-specific agglutinins to B. canis antigen were investigated by the absorption of sera with B. canis antigen and then by performing agglutination tests on the sera using the same antigen. Cross-reactivity of the antigen was investigated by absorbing sera positive for B. canis antibodies with other bacterial antigens[3] and then retesting the same sera with B. canis antigens.

Fluorescent immunoglobulins. These tests were performed by the Department of Microbiology, University of Oklahoma Health Sciences Center, under the direction of Richard M. Hyde, Ph.D., Professor of Immunology. This procedure determined if B. canis antigen, after incubation with serum, was coated with human immunoglobulins, and if so,

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[3]Typhoid H (flagellar d), Salmonella Grp. D. (typhoid O, somatic 9, 12), Paratyphoid B (flagellar b, 12), Brucella abortus, Francisella tularensis, and Proteus OX19.

what classes of immunoglobulins were present. The procedure for this study is presented in APPENDIX A.

Paired serum tests. Over one-half of the sera tested for B. canis antibodies were also tested for B. abortus antibodies in order to determine the possibility of cross-reactions between the two antigens. Both procedures were performed at the same time using the same methods, and both procedures were performed under the same conditions.

Microtiter Plate Agglutination Technique

A microtiter plate agglutination technique was used for the serologic determination of agglutinating antibodies which were specific for Brucella canis antigen. The tests were performed utilizing microtiter equipment manufactured by the Cooke Engineering Company, Alexandria, Virginia.

Microtiter Equipment

Microdropper pipettes. The microdropper pipettes were made of molded propylene and possessed a working capacity of six milliliters. They had a stainless steel needle tubing fused into the delivery end and were calibrated so that each drop delivered 0.025 ml of isotonic physiological saline at 25 C with a plus or minus 2 percent tolerance.

Microdiluting loops. The microdiluting loops were made of precision slotted stainless steel which employed capillary action in taking up measured quantities of solutions. They were standardized to draw up 0.025 ml of isotonic physiological saline at 25 C with an accuracy of plus or minus 0.02 milligrams. The diluting loops were attached to slender metal handles which were tapered at the top to

provide loop-spacing[4] when more than one loop was used.

Titration plates. Each standard plate was 128.6 cm × 82.5 mm × 11 mm containing 8 rows of 12 cups, each possessing a 0.125 ml capacity. The cups were U-shaped.

Test reading mirror. For convenient, accurate reading of test patterns, the titration plates were placed on a support, the center of which was removed to expose the complete underside of all the wells; a 2X magnifying mirror was used to examine the wells.

Reagents

Antigen. Brucella canis antigen was supplied by the United States Department of Agriculture, Animal and Plant Service, Veterinary Service Diagnostic Laboratory, Ames, Iowa. The antigen was prepared from killed whole cells.

Diluent. The method used by the U.S.D.A. at Ames, Iowa, employed 2-mercaptoethanol-3.5% saline as a diluent in the tube agglutination test for B. canis. Mercaptoethanol splits the disulfide bonds of IgM globulins and inhibits the agglutination of the IgM class of brucellae antibodies without seriously interfering with the agglutinating activity of the IgG class of brucellae antibodies. Reddin and co-workers [51] demonstrated that humans who had bacteriologically proven Brucella abortus disease consistently had a mercaptoethanol resistant or IgG class of brucellae antibody. The presence of the IgG immunoglobulin appeared to be a distinguishing feature in differentiating active from

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[4]The handles are designed with a special taper to give the same microdilutor spacing as the plate wells, permitting easy insertion for accurate, simultaneous titrations.

inactive disease in persons having ill-defined complaints, sterile blood cultures, and low titers of brucellae antibodies. Consequently, mercaptoethanol is used when there is interest only in the diagnosis of active disease. Since this study was concerned with identifying persons infected with B. canis regardless of disease state, 2-mercaptoethanol was omitted from the test system.

The U.S.D.A. method also involved the use of 3.5 percent saline instead of buffered, physiological (0.85%) or normal (0.0875%) saline. Test results were determined by clearing of the supernate in a tube, and the increased concentration of saline tended to hold the antigen in suspension to prevent autoagglutination [52]. The volume of diluent used in the microtiter plate agglutination procedure was extremely small, and clearing of the supernate could not be observed. Therefore, buffered, physiological saline was used as the diluent in the microtiter procedure, and the test results were determined by the occurrence of strict agglutination in the bottom of the well.

Microtiter Procedure

A drop of phosphate buffered, physiological saline[5] was added to each U-shaped well of a microtiter plate with a 25 µl (0.025 ml) micropipette. Twenty-five lambda of undiluted serum was placed in the first well and diluted twofold serially using a 25 µl microdilutor. The resulting serum dilutions were 1:2, 1:4, ... 1:256. Fifty lambda (0.05 ml) of working [6] Brucella canis antigen was then added to each

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[5] 0.15 M KH_2PO_4 + Na_2HPO_4, 0.85% NaCl, pH 7.2.

[6] 4.4 ml stock B. canis antigen diluted to 100 ml with phosphate buffered, physiological saline.

well. The final dilutions were 1:6[7], 1:12, ... 1:768. The microtiter plates were covered with a plate sealer[8] and incubated at 37 C for a total of 48 hours. Reading the plates at 48 hrs occasionally yielded higher titers. Using a test reading mirror, agglutination appeared as an irregular edged circle or a folded-over circle. Serum samples in which there was no agglutination had a smooth round button of antigen in the bottom of the well. Positive[9] and negative[10] controls were used with each series of test runs. A summary of the microtiter procedure is presented in Table 4.

Interpretation of Test Results

It was the purpose of this investigation to ascertain the prevalence of Brucella canis infections in various selected population groups by determining the presence of specific antibodies in serum. Interpretation of the test results on the basis of the different antibody titers was considered unnecessary for purposes of this study. Since a one tube difference is allowable as experimental error [46], an antibody titer of 1:12 or greater was considered a positive test and was interpreted as evidence of B. canis infection in an individual. An antibody titer of 1:6 or less was regarded as a negative test, and the individual was considered to be without evidence of infection.

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[7] 12.5 µl serum in 75 µl total volume.

[8] Precut plastic tape used to prevent evaporation.

[9] Positive control consisted of a high titered dog serum provided by the Veterinary Services Diagnostic Laboratory, U.S.D.A., Ames, Iowa.

[10] Negative control consisted of phosphate buffered, physiological saline.

TABLE 4

MICROTITER PLATE AGGLUTINATION TEST PROCEDURE FOR DETERMINING THE PRESENCE OF BRUCELLA CANIS ANTIBODIES

Procedure	Well Number								Positive Control	Negative Control
	1	2	3	4	5	6	7	8		
Volume of saline (ml)	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.050
Volume of serum (ml)	0.025 (mix)	0.025 of Well 1	0.025 of Well 2	0.025 of Well 3	0.025 of Well 4	0.025 of Well 5	0.025 of Well 6	0.025 of[a] Well 7	0.025	0
Initial serum dilution	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	-	-
Volume of antigen (ml)	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Final serum dilution	1:6	1:12	1:24	1:48	1:96	1:192	1:384	1:768	-	-

[a]0.025 ml from tube 8 is removed and discarded.

Standardization of the Microtiter Plate Agglutination Test

The microtiter plate agglutination test was standardized by comparison tests using a standard macro tube test performed by the State and Federal Brucellosis Laboratory, Oklahoma City, Oklahoma,under the supervision of Brian Espe, D.V.M. The procedure is shown in APPENDIX B, Table 1. The similarities and differences between the two methods are presented in APPENDIX B, Table 2. The standard macro tube tests were performed without knowledge of the microtiter test results. In both procedures, a test was considered negative when an antigen-antibody reaction occurred only in the first dilution, and positive when the reaction occurred in any dilution greater than the first. The usually accepted policy of recognizing a one tube dilution difference as the range of procedural error was observed in determining the comparability of results between the standard macro tube agglutination test and the microtiter plate agglutination test.

Selection of Human Population Groups

The subjects whose sera were tested for Brucella canis antibodies were divided into three categories on the basis of their possible exposure to dogs, the only known natural reservoir for B. canis. The categories were: a) no exposure[11] to dogs, b) an average exposure[12] to dogs, and c) a potentially high exposure[13] to dogs. The study also

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[11] Newborn infants.

[12] The exposure to dogs in this group of persons was thought to represent the usual or everyday type of exposure to both healthy and sick dogs. The hospital population was considered to be representative of this type of exposure and would not have more contact with dogs or less contact with dogs than the general population.

[13] Practicing veterinarians and veterinary students.

included a group of persons with febrile illnesses of possible microbial origin. An authorization for access to medical records was granted by the Administrator of The University Hospital, Oklahoma City, Oklahoma (APPENDIX C).

Subjects with No Exposure to Dogs

This group consisted of newborn infants born during August and September, 1973, at The University Hospital. Sera were collected from cord blood samples sent to the Serology Laboratory for a routine serological determination for syphilis. These persons would have had no exposure to dogs. Thus, the presence of Brucella canis antibodies in these newborns would be the result of an in utero infection or from placental transfer. To resolve these possibilities, sera from mothers of a group of these newborn infants were also tested for B. canis antibodies.

Subjects with an Average Exposure to Dogs

These subjects, who had sera submitted to the Serology Laboratory for syphilis determinations, consisted of hospitalized and non-hospitalized patients, hospital employees and students, and blood donors.

Hospitalized and non-hospitalized patients. These serum samples included all Medical Services such as Medicine, Surgery, Obstetrics-Gynecology, and Pediatrics. The samples were collected daily without regard to whether the patient was or was not hospitalized or to which Medical Service initiated the routine serological determination for syphilis. Serum collection began on July 15, 1973, and ended on October 31, 1973. The samples were identified according to the patients'

medical records number, hospitalization status, age and sex, and medical service.

Hospital employees and students. These were a small group of samples consisting primarily of new employees and students in which a routine syphilis serology was performed for either employment or school. The collection of samples was initiated on July 15, 1973, and was terminated on September 30, 1973. The samples were identified according to the subjects' medical records number and age and sex.

Blood donors. The University Hospital is unique among all other hospitals in the Oklahoma City area because it maintains its own blood acquisition program. From July 15, 1973, to October 31, 1973, sera were collected from this source. There were no sera collected from persons below age 18 or above age 65 because these ages are prohibited from donating blood. The samples were identified according to the subjects donor number and age and sex.

Subjects with a Potentially High Exposure to Dogs

Since dogs are the natural and possibly the only reservoir for Brucella canis, persons assumed to have a more intimate or concentrated contact with sick dogs than the general population would possibly have a higher infection rate due to B. canis and, thus, a greater prevalence of antibodies. In order to examine this hypothesis, serum samples were collected from people in the Veterinary Sciences. Sera were collected December 11, 1973, from veterinary students and faculty at the School of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma. The sera were identified according to the subjects' name, age and sex, and year in school. Sera were also collected on January 27 and 28, 1974,

from practicing veterinarians who were present at the Oklahoma Veterinary Medicine Association convention in Oklahoma City, Oklahoma. These sera were identified according to name, age and sex, and residence.

Subjects with Fevers of Undetermined Origin

These persons were not grouped together on the basis of their exposure to dogs, but because they had similar illnesses. These were patients whose physicians had requested that febrile agglutinins[14] be performed on their sera. Serum samples were collected from July 1, 1973, to January 31, 1974. They were identified according to the patients' medical records number and age and sex.

Use of a Questionnaire to Associate Brucella canis Infection to Dog Exposure

Before a questionnaire could be used in the study, it was necessary to gain the approval of the Human Experimentation Committee (HEC) of the University of Oklahoma Health Sciences Center. Copies of the protocol and a cover letter were sent to the Chairman of the committee. The cover letter had to provide the following: a) identification of the project, b) a statement in which written informed consent would be obtained from each individual in the the study, c) a copy of the consent form to be used, and d) a statement regarding the potential risks to the subjects and the potential benefits from the proposed research. A copy of the cover letter is presented in APPENDIX D.

In addition to the questionnaire, a method of obtaining informed consent of each individual was necessary. The basic elements of informed

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[14]Serology tests used to aid in the diagnosis of typhoid, paratyphoid, brucellosis, tularemia, and various rickettsial diseases.

consent were: a) a fair explanation of the procedures to be followed, b) a description of the risks and benefits to be expected, c) an offer to answer any inquiries concerning the procedures, d) an instruction that the subject was free to withdraw his consent and to discontinue participation in the project at any time, and e) a statement indicating that any grievances which the subject had in regard to his participation in the study could, at any time, be taken to the Office of Research Administration. The subject was also instructed that he had not waived any of his legal rights or released the institution from liability for negligence. All materials were submitted to the HEC, and on January 28, 1974, the study was approved. A copy of the approval letter is presented in APPENDIX E.

Purpose and Design of the Questionnaire

The questionnaire was designed to help explain the presence of Brucella canis antibodies in relationship to a person's exposure to dogs. The questionnaire consisted of three parts: a) a cover letter designed to obtain written informed consent, b) a consent statement form, and c) questions pertaining to the subject's exposure to dogs. The questions were designed to elicit information regarding the type, duration, and frequency of dog contact. The complete questionnaire is shown in APPENDIX F.

Selecting the Sample

A sample of persons thought to have an average (everyday) exposure to dogs was used in this phase of the study. Through a random

sampling procedure[15], a test group of 150 persons who were considered infected with Brucella canis was selected. This group was stratified equally between low titers (1:12) and higher titers (1:48 or greater). A control group of 150 persons was selected to represent those who were considered not to be infected with B. canis. In this situation, non-infection consisted of persons with no detectable antibodies. The test group and control group represented a 15 percent sample size. A period of six weeks was allowed for the questionnaires to be returned before the data were analyzed.

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[15]The random sample was selected with the assistance of Donald E. Parker, Ph.D., Assistant Professor, Department of Biostatistics and Epidemiology.

CHAPTER III

RESULTS

Brucella canis Antigen Specificity

Agglutination--Absorption

Seventeen serum samples were tested for nonspecific agglutination, and the detailed results are presented in APPENDIX B, Table 3. Agglutination with *Brucella canis* antigen after the serum was absorbed with *B. canis* antigen did not occur. Table 4, APPENDIX B, shows results of 14 serum samples that were examined for cross-reactivity with other bacterial antigens. Although some of the samples exhibited agglutination reactions with the other antigens, these same samples were again positive at the same serum dilution or within one dilution for *B. canis* antibodies when retested with *B. canis* antigen.

Fluorescent Immunoglobulins

Fluorescent immunoglobulin studies were performed on *Brucella canis* antigens that had agglutinated in serum samples of different antibody titers. An untreated antigen sample was included for a background control. The complete results of these studies are shown in APPENDIX B, Table 5.

Antigen from the high titered samples had significant amounts of IgM globulin, and the low titer serum contained a questionable amount.

The untreated antigen, antigen from the negative titer adult serum, and antigen from the newborn infant serum had nondetectable amounts of IgM immunoglobulin present.

Antigen from all serum samples had questionable amounts of IgG globulin with the exception of the untreated antigen and of the low titer adult serum sample which was negative for detectable amounts of IgG immunoglobulins. All of the samples were negative for detectable amounts of IgA immunoglobulins.

Brucella canis and Brucella abortus Antibody Determinations on Paired Sera

Of 1,172 sera tested, 787 (67.1%) reacted with Brucella canis antigen and 64 (5.5%) were positive for Brucella abortus antibodies (APPENDIX B, Table 6). Only 55 (7.0%) of the positive B. canis sera reacted with B. abortus antigen also. Of these 55 sera, 19 had higher B. canis antibody titers, 18 had higher B. abortus titers, and 18 had equal antibody titers to both antigens.

Evaluation of the Microtiter Plate Agglutination Technique

Thirty-nine serum samples were tested for Brucella canis antibodies using the microtiter technique and a standard tube technique. The standard tube test was referred to as the standard or accepted method, and the microtiter test was compared to it (Table 7, APPENDIX B). The sensitivity of the microtiter method was 96.0 percent and the specificity was 85.7 percent. Two false positive results occurred using the microtiter technique, and one false negative occurred.

Brucella canis Antibodies in Newborn Infants and Their Mothers

Microtiter agglutination tests for Brucella canis antibodies were performed on 193 sera from newborn infants. Only 11 (5.7%) had antibody titers of 1:12 or greater with the highest titer being 1:24 (Table 5).

Fifty-nine serum samples from mothers of these infants were also tested for the presence of B. canis antibodies and compared to their infants (Table 6). In all instances, a mother's serum contained antibodies when the serum of her newborn infant contained antibodies, and a newborn's serum was negative for B. canis antibodies when its mother's serum was negative. However, of 42 newborn infants whose sera were negative for B. canis antibodies, only 12 of their mothers were also negative (28.6 percent). Also, a mother whose serum contained B. canis antibodies could have a newborn whose serum was without antibodies; a result that occurred 63.8 percent of the time (30/47).

Prevalence of Brucella canis Infection Among Subjects with an Average Exposure to Dogs

The distribution of Brucella canis antibodies in sera of persons with an average exposure to dogs is presented in Table 7. Of 2,026 sera tested, 67.8 percent exhibited evidence of infection. There were 58 (2.9%) subjects with antibody titers of 1:96 or greater.

The prevalence of B. canis infection, by sex, is shown in Table 8. Females had a 10.3 percent higher prevalence rate of infection than did males. This difference was statistically significant ($p < 0.001$).

TABLE 5

DISTRIBUTION OF *BRUCELLA CANIS* ANTIBODY TITERS AMONG NEWBORN INFANTS

Antibody Titer	Number	Percent
0	157	81.3
1:6	25	13.0
1:12	9	4.7
1:24	2	1.0
Total	193	100.0

TABLE 6

BRUCELLA CANIS ANTIBODIES IN NEWBORN INFANTS AND CORRESPONDING MOTHERS

	Newborn Infants		
Mothers	Positive[a]	Negative[b]	Total
Positive[a]	17	30	47
Negative[b]	0	12	12
Total	17	42	59

[a] 1:6 or greater antibody titer.

[b] 0 antibody titer.

TABLE 7

DISTRIBUTION OF BRUCELLA CANIS ANTIBODY TITERS AMONG SUBJECTS WITH AN AVERAGE EXPOSURE TO DOGS

Antibody Titer	Number	Percent
≤ 1:6[a]	653	32.2
1:12[b]	538	26.5
1:24	559	27.6
1:48	218	10.8
1:96	45	2.3
1:192	12	0.6
1:384	1	0.0
≥ 1:768	0	0.0
Total	2,026	100.0

[a] 1:6 or less antibody titer - Not infected.

[b] 1:12 or greater antibody titer - Infected.

TABLE 8

BRUCELLA CANIS INFECTION IN SUBJECTS WITH AN AVERAGE EXPOSURE TO DOGS, BY SEX

Subjects	Infection Status		Total	Prevalence of Infection (%)
	Positive[a]	Negative[b]		
Males	567	346	913	62.1
Females	806	307	1,113	72.4
Total	1,373	653	2,026	67.8

$\chi^2 = 24.4270$ $p < 0.001$

[a] 1:12 or greater antibody titer.

[b] 1:6 or less antibody titer.

The largest proportion of subjects in this population were the 15-24 year and the 25-34 year age group. Together, these age groups contributed 64.0 percent of the total population (Table 9). The two extreme ages of life (below age 15 and above age 64) consisted of only 6.4 percent of the total population.

Most age groups according to sex were comparable (Table 9). However, the 15-24 year age group consisted of approximately 10 percent more females which was due to the large Obstetric Clinic at The University Hospital. The donating of blood by more males accounted for the difference, by sex, in the 35-44 year age group.

The distribution of B. canis antibody titers, by age, is presented in Table 10. The prevalence of B. canis infection ranged from 78.4 percent in the 3-14 year age group to 48.2 percent in persons 65 years and older. There was an apparent tendency for the prevalence of infection to decrease as age increased. This inverse relationship between age and the prevalence of B. canis infection was especially pronounced in the females (Table 11) when compared to the males (Table 12).

The overall chi-square value for the females, by age, was 50.3015, which, with four degrees of freedom, was statistically significant ($p < 0.001$). This highly significant value was due almost completely to a linear trend rather than a curvilinear trend since departure from linear regression was not statistically significant (Table 11). Even though the chi-square value for the males, by age, was less than for the females, it was also statistically significant with a probability less than 0.001 (Table 12). However, the data did not conform to a straight line linear relationship as did the females.

TABLE 9

DISTRIBUTION OF SUBJECTS WITH AN AVERAGE EXPOSURE TO DOGS, BY AGE AND SEX

Age	Males		Females		Total	Percent
	Number	Percent	Number	Percent		
3-14	19	2.1	32	2.9	51	2.5
15-24	298	32.6	466	41.9	764	37.7
25-34	256	28.0	277	24.8	533	26.3
35-44	157	17.2	122	11.0	279	13.8
45-54	97	10.6	100	9.0	197	9.7
55-64	48	5.3	75	6.7	123	6.1
65+	38	4.2	41	3.7	79	3.9
Total	913	100.0	1,113	100.0	2,026	100.0

TABLE 10

DISTRIBUTION OF BRUCELLA CANIS ANTIBODY TITERS AMONG SUBJECTS WITH AN AVERAGE EXPOSURE TO DOGS, BY AGE

	Antibody Titers														Total	
	1:6[a]		1:12[b]		1:24		1:48		1:96		1:192		1:384			
Age	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
3-14	11	21.6	13	25.5	17	33.3	8	15.7	2	3.9	0	0	0	0	51	100.0
15-24	172	22.5	212	27.7	251	32.9	102	13.4	24	3.1	3	0.4	0	0	764	100.0
25-34	191	35.8	129	24.2	129	24.2	70	13.1	9	1.7	4	0.8	1	0.2	533	100.0
35-44	102	36.5	90	32.3	64	22.9	18	6.5	2	0.7	3	1.1	0	0	279	100.0
45-54	85	43.1	47	23.9	51	25.9	10	5.1	4	2.0	0	0	0	0	197	100.0
55-64	51	41.6	26	21.1	34	27.6	8	6.5	3	2.4	1	0.8	0	0	123	100.0
65+	41	51.8	21	26.6	13	16.5	2	2.5	1	1.3	1	1.3	0	0	79	100.0
Total	653	32.2	538	26.5	559	27.6	218	10.8	45	2.3	12	0.6	1	0	2,026	100.0

[a] 1:6 or less antibody titer - Not infected.

[b] 1:12 or greater antibody titer - Infected.

TABLE 11

BRUCELLA CANIS INFECTION AMONG FEMALES WITH AN AVERAGE EXPOSURE TO DOGS, BY AGE

Age	Infection Status		Total	Prevalence of Infection (%)
	Positive[a]	Negative[b]		
3-14	26	6	32	81.3
	>404[c]	>94	>498	>81.1
15-24	378	88	466	81.1
25-34	196	81	277	70.8
35-44	85	37	122	69.7
45-54	60	40	100	60.0
55-64	44	32	76	57.9
	>61	>55	>116	>52.6
65+	17	23	40	42.5
Total	806	307	1,113	72.4

$$\chi^2_{(4df)}{}^{d} = 50.3015 \qquad p < 0.001$$

[a] 1:12 or greater antibody titer.

[b] 1:6 or less antibody titer.

[c] Age groups combined for statistical analyses.

[d]

Source of Variation	d.f.	χ^2	Probability
Due to linear regression	1	48.5715	$p < 0.001$
Departure from regression line	3	1.7300	Not Significant

TABLE 12

BRUCELLA CANIS INFECTION AMONG MALES WITH AN AVERAGE EXPOSURE TO DOGS, BY AGE

Age	Infection Status		Total	Prevalence of Infection (%)
	Positive[a]	Negative[b]		
3-14	14	5	19	73.7
	228[c]	89	317	71.9
15-24	214	84	298	71.8
25-34	146	110	256	57.0
35-44	92	65	157	58.6
45-54	52	45	97	53.6
55-64	28	20	48	58.3
	49	37	86	57.0
65+	21	17	38	53.3
Total	567	346	913	62.1

$X^2_{(4df)} = 20.5435$ $p < 0.001$

[a] 1:12 or greater antibody titer.

[b] 1:6 or less antibody titer.

[c] Age groups combined for statistical analyses.

Tables 13 and 14 present data concerning the prevalence of infection with B. canis among subjects, by sex, grouped according to hospitalization status, hospital employees, students, and blood donors. Among the males (Table 13), B. canis infection ranged from 55.6 percent in the employees and students to 65.9 percent in the non-hospitalized patients. The rates of infection in the various groups of males did not differ significantly. Within the female groups (Table 14), however, there was a significant difference ($p < 0.010$) in the prevalence of infection. B. canis infection was from 64.0 percent in the hospitalized patients to 78.5 percent in the female employees and students. Except for the hospitalized females, all the female groups had a higher prevalence of B. canis infections than the male groups, respectively.

Prevalence of Brucella canis Infection Among Subjects with a Potentially High Exposure to Dogs

The distribution of Brucella canis antibodies in the sera of 73 practicing veterinarians and 170 veterinary students is shown in Table 15. The veterinarians had an infection rate of 72.6 percent while the veterinary students exhibited a rate of 63.5 percent (Table 16). The difference in the prevalence of B. canis infections in the two groups was not statistically significant. The practicing veterinarians, however, had higher titers of B. canis antibodies than did the veterinary students (Table 15). Fourteen (19.4%) veterinarians had antibody titers of 1:96 or greater compared to 6 (3.6%) in the students. Five veterinarians demonstrated B. canis antibody titers of 1:768 or greater. None of the veterinary students exhibited comparable levels of antibodies.

B. canis infection among veterinary students, by academic class,

TABLE 13

BRUCELLA CANIS INFECTION IN MALES ACCORDING TO HOSPITALIZATION STATUS, EMPLOYEES AND STUDENTS AND BLOOD DONORS

Subjects	Infection Status		Total	Prevalence of Infection (%)
	Positive[a]	Negative[b]		
Hospitalized Patients	98	61	159	61.6
Non-hospitalized Patients	87	45	132	65.9
Employees and Students	25	20	45	55.6
Blood Donors	357	220	577	61.9
Total	567	346	913	62.1

$\chi^2_{(3df)} = 1.6600$ p = Not Significant

[a] 1:12 or greater antibody titer.

[b] 1:6 or less antibody titer.

TABLE 14

BRUCELLA CANIS INFECTION IN FEMALES ACCORDING TO HOSPITALIZATION STATUS, EMPLOYEES AND STUDENTS, AND BLOOD DONORS

Subjects	Infection Status		Total	Prevalence of Infection (%)
	Positive[a]	Negative[b]		
Hospitalized Patients	153	86	239	64.0
Non-hospitalized Patients	433	148	581	74.5
Employees and Students	106	29	135	78.5
Blood Donors	114	44	158	72.1
Total	806	307	1,113	72.4

$$\chi^2_{(3df)} = 12.2592 \qquad p < 0.01$$

[a] 1:12 or greater antibody titer.

[b] 1:6 or less antibody titer.

TABLE 15

DISTRIBUTION OF BRUCELLA CANIS ANTIBODY TITERS IN PRACTICING VETERINARIANS AND VETERINARY STUDENTS

Antibody Titer	Practicing Veterinarian		Veterinary Students	
	Number	Percent	Number	Percent
≤ 1:6[a]	20	27.4	62	36.5
1:12[b]	8	11.0	32	18.8
1:24	22	30.1	53	31.1
1:48	9	12.3	17	10.0
1:96	6	8.2	3	1.8
1:192	2	2.7	1	0.6
1:384	1	1.4	2	1.2
≥ 1:768	5	6.9	0	0
Total	73	100.0	170	100.0

[a] 1:6 or less antibody titer - Not infected.

[b] 1:12 or greater antibody titer - Infected.

TABLE 16

BRUCELLA CANIS INFECTION BETWEEN PRACTICING VETERINARIANS AND VETERINARY STUDENTS

Subjects	Infection Status		Total	Prevalence of Infection (%)
	Positive[a]	Negative[b]		
Practicing Veterinarians	53	20	73	72.6
Veterinary Students	108	62	170	63.5
Total	159	84	243	66.3

$\chi^2 = 1.8805$ $p < 0.20$

[a] 1:12 or greater antibody titer.

[b] 1:6 or less antibody titer.

is presented in Table 17. The upper-classmen (third and fourth year) had a higher infection rate than the lower-classmen (first and second year). The difference in the prevalence of infection was considered significant (χ^2 = 3.0855, p ~ 0.10). All fourth year students had evidence of infection compared to 65.4 percent in the first year students. Antibody titers tended to increase as the students progressed in their education and training (Table 18). Two fourth year students had B. canis antibody titers of 1:384 which were higher than those of any other class.

There was no important difference in the prevalence of B. canis infection between the veterinary students (36.5%) and the male blood donors[16] (36.1%) of a similar age (15-34 years old). The veterinary students did reveal, however, the presence of higher levels of B. canis antibodies in their sera (Table 19).

The infection rate among the practicing veterinarians was found to be significantly higher (p ~ 0.01) than that of their male blood donor[17] counterparts (Table 20). Of 210 male blood donors who had serological evidence of B. canis infection, only 7 (3.3%) had antibody titers of 1:96 or greater as compared to 14 (26.4%) among the practicing veterinarians (Table 21).

Prevalence of Brucella canis Infection Among Patients with Fevers of Undetermined Origin

The distribution of Brucella canis antibodies in sera from

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[16]This group was used to compare with the veterinary students because of its similarities in age, sex, and general health status.

[17]This group was used to compare with the practicing veterinarians because of its similarities in age, sex, and general health status.

TABLE 17

BRUCELLA CANIS INFECTION AMONG VETERINARY STUDENTS ACCORDING TO ACADEMIC CLASS

Academic Class	Infection Status		Total	Prevalence of Infection (%)
	Positive[a]	Negative[b]		
First	34	18	52	65.4
Second	28	26	54	51.9
Third	30	18	48	70.0
Fourth	16	0	16	100.0
Total	108	62	170	63.6

$\chi^2_{(3df)} = 12.4625 \qquad p < 0.01$

[a] 1:12 or greater antibody titer.

[b] 1:6 or less antibody titer.

TABLE 18

DISTRIBUTION OF *BRUCELLA CANIS* ANTIBODIES IN INFECTED VETERINARY STUDENTS BY ACADEMIC CLASS

Academic Class	Antibody Titer												Total	
	1:12		1:24		1:48		1:96		1:192		1:384			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
First	10	29.4	21	61.8	2	5.9	0	0	1	2.9	0	0	34	100.0
Second	12	42.8	11	39.3	4	14.3	1	3.6	0	0	0	0	28	100.0
Third	7	23.3	14	46.7	7	23.3	2	6.7	0	0	0	0	30	100.0
Fourth	3	18.7	7	43.7	4	25.1	0	0	0	0	2	12.5	16	100.0

TABLE 19

DISTRIBUTION OF BRUCELLA CANIS ANTIBODY TITERS IN INFECTED VETERINARY STUDENTS AND MALE BLOOD DONORS OF THE SAME AGE (15-34)

Antibody Titers	Veterinary Students		Male Blood Donors	
	Number	Percent	Number	Percent
1:12	32	29.6	124	48.2
1:24	53	49.1	97	37.8
1:48	17	15.7	29	11.3
1:96	3	2.8	5	1.9
1:192	3	2.8	2	0.8
Total	108	100.0	257	100.0

TABLE 20

BRUCELLA CANIS INFECTION BETWEEN PRACTICING VETERINARIANS AND MALE BLOOD DONORS OF CORRESPONDING AGES (25-54 YEARS OLD)

Subjects	Infection Status		Total	Prevalence of Infection (%)
	Positive[a]	Negative[b]		
Practicing Veterinarians	53	20	73	72.6
Male Blood Donors	210	159	369	56.9
Total	263	179	442	59.5

$\chi^2 = 6.2277$ $p \sim 0.01$

[a] 1:12 or greater antibody titer.

[b] 1:6 or less antibody titer.

TABLE 21

DISTRIBUTION OF BRUCELLA CANIS ANTIBODY TITERS AMONG INFECTED PRACTICING VETERINARIANS AND MALE BLOOD DONORS OF THE SAME AGE (25-54)

Antibody Titers	Practicing Veterinarians		Male Blood Donors	
	Number	Percent	Number	Percent
1:12	8	15.1	101	48.1
1:24	22	41.5	83	39.5
1:48	9	17.0	19	9.1
1:96	6	11.3	3	1.4
1:192	2	3.8	4	1.9
1:384	1	1.9	0	0
≥ 1.:768	5	9.4	0	0
Total	53	100.0	210	100.0

patients with febrile illnesses is presented in Table 22. This group of persons had a B. canis infection rate of 80.5 percent compared with 63.1 percent for the total hospitalized[18] population (Table 23). The difference in infection rates was statistically significant ($p < 0.001$).

Relationship Between Brucella canis Infection and Exposure to Dogs

The random sample selected among Brucella canis infected (cases) and non-infected (controls) persons with an average exposure to dogs, by age and sex, is shown in APPENDIX B, Table 8. There was no significant difference between the random sample and the population from which it was selected. Three hundred questionnaires, equally divided between cases and controls, were mailed (15 percent sample size), and 101 (33.7%) were answered (APPENDIX B, Table 9). The control group had a 38.7 percent response rate, while the B. canis infected group responded at a rate of 28.7 percent, $p < 0.10$ (APPENDIX B, Table 10).

The data concerning the relationship between present exposure[19] to dogs and the prevalence of B. canis infection is presented in Table 24. Persons having serological evidence of infection had an exposure rate of 69.8 percent to one or more dogs compared to 51.7 percent for the control group. The observed difference had a probability of approximately 0.10 and was considered of borderline significance.

The control group had a mean of 1.63 dogs per person and a

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[18]This group was used to compare with the F.U.O. patients because of its hospitalization status. It consisted of persons with an average exposure to dogs.

[19]Subject having a dog at the time of blood collection.

TABLE 22

DISTRIBUTION OF BRUCELLA CANIS ANTIBODY TITERS AMONG PATIENTS WITH FEVERS OF UNDETERMINED ORIGIN

Antibody Titer	Number	Percent
≤ 1:6[a]	22	19.5
1:12[b]	27	23.8
1:24	35	31.0
1:48	19	16.8
1:96	4	3.5
1:192	3	2.7
1:384	1	0.9
≥ 1:768	2	1.8
Total	113	100.0

[a] 1:6 or less antibody titer - Not infected.

[b] 1:12 or greater antibody titer - Infected.

TABLE 23

COMPARISON OF BRUCELLA CANIS INFECTION BETWEEN PATIENTS WITH FEVERS OF UNDETERMINED ORIGIN AND ALL OTHER HOSPITALIZED PATIENTS

Subjects	Infection Status		Total	Prevalence of Infection (%)
	Positive[a]	Negative[b]		
F.U.O. Patients	91	22	113	80.5
Hospitalized Patients	251	147	398	63.1
Total	342	169	511	66.9

$\chi^2 = 12.1322 \qquad p < 0.001$

[a] 1:12 or greater antibody titer.

[b] 1:6 or less antibody titer.

TABLE 24

RELATIONSHIP BETWEEN PRESENT EXPOSURE[a] TO DOGS AND BRUCELLA CANIS INFECTION

Exposure to Dogs	Subjects		Total
	Cases[b]	Controls[c]	
Yes	30 (69.8)[d]	30 (51.7)	60
No	13 (30.2)	28 (48.3)	41
Total	43 (100.0)	58 (100.0)	101

$\chi^2_c = 2.6274$ $p \sim 0.10$

[a]Subject having a dog at time blood was collected.

[b]1:12 and 1:48 or greater antibody titers - Infected.

[c]0 antibody titer - Not infected.

[d]Percent of total.

median of 1.88 (Table 25). Subjects who had evidence of B. canis infection had a mean and median of more than two dogs per person. The data also revealed that the mean and median number of dogs increased as the B. canis antibody titer increased.

Of the subjects (cases and controls) who had dogs at the time blood was collected, 59.8 percent considered them to be primarily outside dogs[20] compared to 40.2 percent as primarily inside dogs[21] (Table 26). However, 46.8 percent of the cases regarded their dogs to be primarily inside dogs compared to 32.0 percent for the controls. Even though the chi-square value was not interpreted as highly significant ($p < 0.20$),an apparent trend was observed. Persons who had serological evidence of B. canis infection appeared to have more inside dogs than those without B. canis infection. There were no apparent differences in breed or sex of dog among the cases and controls. Also, occupation and the length of time a person had a dog appeared to be unimportant factors in the development of B. canis infections.

Of the 41 subjects (cases and controls) who did not have a dog when their blood was collected for B. canis antibody determinations, 61.0 percent had a dog previously (Table 27). Only three (23.1%) of the persons who had serological evidence of B. canis infection never had a dog compared to 13 (46.4%) in the control group. However, this observed difference was not considered statistically significant.

Data in Table 28 demonstrates both present and past exposure to dogs and the association of B. canis infections in the cases and controls.

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[20]Dog stays primarily outside of house.

[21]Dog stays primarily inside of house.

TABLE 25

NUMBER OF DOGS OWNED AMONG *BRUCELLA CANIS* INFECTED SUBJECTS AND CONTROLS

Subjects	Number of Dogs								Total Dogs	Total Subjects	Mean	Median
	1	2	3	4	5	6	7	8				
Controls[a]	17	9	3	0	1	0	0	0	49	30	1.63	1.88
Infected[b] (Low titers)	7	5	2	1	0	0	0	0	27	15	1.80	2.10
Infected[c] (High titers)	6	4	3	0	1	0	0	1	36	15	2.40	2.38
Total Infected	13	9	5	1	1	0	0	1	63	30	2.30	2.22

[a] 0 antibody titer - Not infected.

[b] 1:12 antibody titer - Infected.

[c] 1:48 or greater antibody titer - Infected.

TABLE 26

RELATIONSHIP BETWEEN BRUCELLA CANIS INFECTION AND PRIMARY HABITAT OF DOG

Habitat of Dog	Subjects		Total
	Cases[a]	Controls[b]	
Inside[c]	29 (46.8)[e]	16 (32.0)	45 (40.2)
Outside[d]	33 (53.2)	34 (68.0)	67 (59.8)
Total	62 (100.0)	50 (100.0	112 (100.0)

$\chi^2_c = 1.9386$ $p < 0.20$

[a] 1:12 and 1:48 or greater antibody titer - Infected.

[b] 0 antibody titer - Not infected.

[c] Dog stays primarily inside of house.

[d] Dog stays primarily outside of house.

[e] Percent of total.

TABLE 27

RELATIONSHIP BETWEEN PAST EXPOSURE[a] TO DOGS AND *BRUCELLA CANIS* INFECTION

Exposure to Dogs	Subjects		Total
	Cases[b]	Controls[c]	
Yes	10 (76.9)[d]	15 (53.6)	25 (61.0)
No	3 (23.1)	13 (46.4)	16 (39.0)
Total	13 (100.0)	28 (100.0)	41 (100.0)

$\chi^2_c = 1.1715$ p = Not Significant

[a]Subject having a dog prior to, but not at the time of, blood collection.

[b]1:12 and 1:48 or greater antibody titer.

[c]0 antibody titer.

[d]Percent of total.

TABLE 28

RELATIONSHIP BETWEEN PRESENT AND PAST EXPOSURE[a] TO DOGS AND BRUCELLA CANIS INFECTIONS

Exposure to Dogs	Subjects		Total
	Cases[b]	Controls[c]	
Yes	40 (93.0)[d]	45 (77.6)	85
No	3 (7.0)	13 (22.4)	16
Total	43 (100.0)	58 (100.0)	101

$\chi^2_c = 3.3970$ $p \sim 0.05$

[a]Ever had a dog.

[b]1:12 and 1:48 or greater antibody titer.

[c]0 antibody titer.

[d]Percent of total.

When both types of exposure to dogs were combined, the observed difference approached definite statistical significance ($p \sim 0.05$).

CHAPTER IV

DISCUSSION

Brucella canis antigen obtained from the U.S.D.A. appeared to be specific for B. canis antibodies [53]. The antigen did not react with non-specific serum proteins. If the sera contained non-specific protein that would react with B. canis antigen, agglutination should have resulted after absorption. B. canis antigen did not react with antibodies specific for other bacterial antigens which included micro-organisms within the same family as B. canis. Many of the sera did, however, agglutinate the pooled antigen. This was probably due to the fact that an OX19 antigen of Proteus vulgaris was included in the pooled antigen. This organism is often isolated from the gastrointestinal tract of man and is considered a normal commensal organism. Therefore, man could normally have low antibody titers to this organism in his serum.

Studies have not been performed on human sera to determine the classes of antibodies (immunoglobulins) produced by B. canis infections, and only one such study has been attempted in dogs. Spink [54] reported that immunoglobulins found in Beagles with B. canis disease were classified as IgM agglutinating antibody in pure form and three different forms of IgG antibodies. The results of the fluorescent immunoglobulin studies on human sera were similar to those performed in dogs by Spink. In order to confirm the presence of IgG antibodies in the sera, mercaptoethanol

agglutination tests were performed on some high titered samples by the State and Federal Brucellosis Laboratory, and the results demonstrated that some of the sera contained mercaptoethanol-resistant or IgG antibodies specific for B. canis [55]. The newborn serum sample (APPENDIX B, Table 5) did not contain IgM antibodies and questionable fluorescence for IgG antibody. These results were consistent with the fact that IgM antibody cannot cross the placenta and can be present only from the result of an in utero infection.

In order to further determine the specificity of the B. canis antigen, cross-reactions to B. abortus antigen were studied. Of the sera tested, 4.7 percent showed the presence of B. abortus antibodies. This rate was similar to that reported by other investigators [56, 57]. Among the positive B. canis sera (67.1%), 7 percent were also positive to B. abortus antigen (Table 6, APPENDIX B). These reactions could have been due to either cross-reactions or to dual infections. However, on the basis of the results from the other specificity studies performed, it would appear that the latter explanation is more plausible.

The microtiter plate agglutination technique compared favorably with a standard macro tube agglutination technique; other investigators [45, 47, 58, 59] have reported similar findings. There were some differences in the results between the two methods, but they were not considered significant. The microtiter plate agglutination technique was a valuable tool in this investigation because a large number of samples were involved. This method of determining infection by the presence of antibodies can be very accurate and precise in the hands of competent personnel.

The results of the studies performed on newborn infants' sera indicated that although B. canis antibodies were able to cross the placenta, none of the infants had high antibody titers. Only 5.7 percent of the newborns had antibody titers of 1:12 or greater compared to 69.2 percent for the other subjects in the study. These newborn subjects had no risk of antigenic stimuli; therefore, the presence of B. canis antibodies were most likely a result of transplacental antibody transfer. In addition to the evidence of the transplacental passage of maternal B. canis antibodies from mother to offspring, this phase of the investigation further supported the concept that B. canis antigen was specific for B. canis antibodies. There were no mothers who were negative for B. canis antibodies when their newborn infants were positive, and no newborns were positive when their mothers were negative for B. canis antibodies. The results showed, however, that some of the mothers had B. canis antibodies even though their newborn infants appeared negative. There may be two explanations for these findings. The newborns would not have received antibodies from their mothers if the antibodies were only of the IgM class. Also, if IgG antibodies were present in minute amounts, they may not have been detectable by the present method. The results from these studies were similar to those found by Carmichael and Kenney [11]. These investigators reported that antibody tests from several litters of pups from bitches that had apparently recovered from B. canis disease suggested that they had not acquired infection in utero. They did state, however, that low levels of maternal antibody (IgG) were detected in several pups.

A total of 1,373 (67.8%) subjects having an average exposure to

dogs exhibited serological evidence of infection. Females had a significantly higher prevalence rate of infection than did males. This apparent difference in rates may be due to either the female's ability to produce a more efficient antibody response (biological) [60] or to an increased exposure to dogs by the females.

That the human male is more susceptible than the female to most infectious diseases is familiar knowledge. This is due in part to the different social, cultural, and occupational exposure of the two sexes, but it may also be a result of, as mentioned previously, biological differences. The biological differences have been hypothesized by Washburn and co-workers [60] to be a result of a greater quantity of genetic material possessed by the female X chromosone resulting in a higher antibody response. Good and co-workers [61] have shown that the familial distribution of some patients with reduced blood levels of immunoglobulins (hypogammaglobulinemia) suggests that a gene on the female X chromosone has some role in the production of antibodies. It has also been demonstrated, under experimentally controlled conditions, that female animals have a higher resistance to disease than male animals [62].

It is well established that brucellosis due to B. abortus occurs more frequently in males than in females. This difference is generally assumed to be a result of a higher degree of infection due to increased occupational exposure in males (Reviewed by Spink [63]). Assuming females had a more efficient antibody response than males, one might expect the prevalence of B. abortus infections to appear higher in females than in males if a sample of the general population (average exposure)

was tested for B. abortus antibodies. However, studies by other investigators [64, 65, 66, 67] indicate that females do not show a significantly higher prevalence of B. abortus antibodies than that of males. This suggests that when significant differences in brucellae infection rates occur between the sexes, based on the presence of specific antibodies, these differences are primarily a result of increased exposure to the agent rather than biological differences. The fact that females had an almost identical infection rate (72.4%) with B. canis as the practicing veterinarians (72.6%) would also suggest that the difference in infection between males and females was due to exposure differences and not to biological differences.

There are several reasons why females may have more exposure to B. canis than males. Females have the major responsibility of housekeeping, which usually includes taking care of the family dog. It has been documented previously that the portals of exit of the organism from diseased dogs are vaginal discharges, milk, semen, and urine. There have been no reports of attempts to isolate B. canis from dog feces, although feces is regarded as a possible route of transmission for the other brucellae [63]. Females, consequently, would have a much higher exposure potential of acquiring B. canis infection than males as a result of every day tasks, such as housecleaning (respiratory tract transmission) and the handling of dog waste products (indirect transmission). Examples of direct transmission might include bathing the dog, assisting in the delivery of pups, and usual physical contact in a close dog - master relationship.

Investigations have not been conducted to determine the

diversity of environmental conditions under which B. canis will remain viable; however, this has been studied in the other species of Brucella. These organisms will remain viable in soil dried under natural conditions for 43 days and in damp soil for 72 days, but exposure to the sun reduces the survival time to only a few hours [63]. Kuzdas and Morse [68] reported that brucellae have been detected in tap water for 10 days at 25 C, but water kept at 8 C permitted survival for 57 days. These same investigators determined that brucellae would survive for 385 days in untreated manure kept at 8 C. Brucellae organisms can also survive on cloth and fabric for 5 to 78 days [63].

The apparent high resistance of these organisms to environmental conditions is rather unique in respect to the difficulties encountered when attempts are made to culture them in vitro from clinical specimens. Nevertheless, if B. canis has similar environmental characteristics as the other Brucella and is capable of surviving outside the definitive host for long periods of time, then it would be reasonable to assume that man could readily become infected with this organism.

Age is a variable that should be considered in epidemiologic studies. The age of the subject may influence the type of disease occurring. Knowledge of age associations is important for two reasons [70]: a) the study of variation in the frequency of a disease may assist in understanding the factors responsible for its development and b) associations between age and disease frequency may be so strong that age may produce indirect effects that must be considered in the examination of differences in disease rates related to other variables.

Factors governing the age incidence of disease may be divided

into environmental factors, host factors, and factors related to the agent of disease [71]. Environmental factors are conditions which may alter the likelihood of exposure to infection. Of the host factors which may affect the age incidence of disease, specific immunity to infection, endocrine factors, and tissue susceptibility play important roles. The age incidence of disease is also affected by the infectiousness of the agent. Different microorganisms are known to vary in their infectivity for various age groups. None of these factors alone, however,can explain the age incidence of any disease. Aging involves the passage of time which is accompanied by exposure to various environmental conditions and infectious agents that can alter body chemistry and physiology.

In the present study, both sexes exhibited differences in the prevalence of B. canis infection, by age. A definite linear trend [69] was observed. The differences in age were primarily due to a decrease in infection as age increased, particularly in females. It may be argued that the decrease in B. canis infection with increasing age is due to the host's inability to produce humoral antibodies as age increases and not due to a decrease in exposure. In order to examine these possibilities, a search of the literature was made to find a study that was performed under similar conditions as the present investigation. These conditions would include: a) the selection of subjects with an average exposure to an infectious agent similar to B. canis, b) the subjects stratified by age and sex, and c) the use of a serologic test to detect infection. Harrison and Manch [64] conducted such a study in 1928. They were concerned with the possible existence of disease due to

infection with Brucella abortus in England. The subjects were blood donors stratified by age and sex. Infection with B. abortus was interpreted as the presence of B. abortus antibodies. There was no statistical difference in the rates of B. abortus infection by age in either sex, nor was the chi-square value due to linear regression siginficant. From the results of this study, it appeared that the antibody response to B. abortus antigen did not decrease as age increased. If this type of antibody response is similar for all species of Brucella, then age differences observed in the present investigation are most likely a result in differences in exposure to B. canis.

There are several explanations, based upon exposure to dogs, that would account for the decrease in B. canis infections with increasing age. Very young persons would have a higher risk of infection than young adults because of their closer contact with dogs. Young adults would have a higher infection rate than older persons because their children would have dogs. As the children mature and leave the home, the family dog may die from old age or the parents may have no further need to keep the dog; therefore, exposure potential would be less in older persons, and antibodies would wane in the absence of antigenic stimuli. Other evidence that would support this age - exposure hypothesis is that females in this study have a higher infection rate than males in almost every age category which, as previously discussed, is very likely a result of greater contact with dogs.

The practicing veterinarians had higher titers of B. canis antibodies than the veterinary students. This would imply that veterinarians had an increased frequency of exposure to dogs (small, but

continuous doses of the organism) or contact with more, severely ill dogs (large doses of the organism). The veterinarians had significantly higher B. canis infection rates than did male blood donors of the same age, which was probably due to an increased frequency of exposure to infected dogs.

There were significant differences in the prevalence and degree (high antibody titers) of infection among the veterinary students according to academic class. Upper-classmen had a 13.4 percent higher infection rate than lower-classmen ($p < 0.10$), and the antibody titers were also much higher in the upper-classmen than in the lower-classmen. These differences provide evidence that a higher degree of exposure to dogs (quantitative and qualitative) occurred in the upper-classmen.

The subjects with undiagnosed febrile illnesses were a group selected on the basis of type of illness and not on their exposure to dogs. The prevalence of B. canis infection in this group of persons was highly significant when compared to all other patients. Five persons (1.8%) had B. canis antibody titers of 1:768 or greater, and two of these had an admitting diagnosis of non-bacteriologically proven brucellosis. It may be that a number of persons who have fevers of undetermined origin have brucellosis due to B. canis.

In order to test the hypothesis that B. canis infection was a result of exposure to dogs, a case (infected) - control (not infected) study was conducted. This type of study would also lend support to the specificity of the B. canis antigen. The case - control type of epidemiological study has been reviewed extensively by MacMahon and Pugh [70] in relation to the selection of cases and controls, information on

exposure, analysis, and interpretation.

Approximately one-third of the total mailed questionnaires were answered. The response rate was low in comparison to other studies [72, 73, 74]. Factors which may have accounted for the low response were: a) the style in which the accompanying letter was written, b) the population involved, and c) the lack of funds available for follow-up on those who did not respond.

The style of the introductory letter was admittedly impersonal. Certain guidelines were required by the Human Experimentation Committee and the Surgeon General's directive of February 8, 1966. Unless read very carefully, the letter and consent statement might have been misinterpreted by the subjects involved in the study. Because of these new rulings, such letters may tend to frighten people from responding.

Perhaps one of the most important considerations for the investigator who wishes to obtain high response rates is the selection of a study population whose members are likely to have some personal interest in the study. Because the present investigation did not directly affect the persons involved, they may not have been motivated to answer and return the questionnaire. Also, the study groups were selected from an institution whose primary obligation is the health care of indigents and, therefore, of a low socioeconomic background. For this reason, response rates might have been affected due to a lack of understanding the purpose of the questionnaire and the technical language involved. It must be remembered, however, that factors of lower socioeconomics would have affected both the infected and uninfected persons as far as total response was concerned; therefore, response bias due to this factor was

thought to affect both groups (cases and controls) equally.

The effects of nonresponse on such studies are many and have been discussed by several authors [70, 73, 74, 75]. It is often difficult to know whether the loss in nonresponse is selective with respect to the cases or the controls. To settle this question, a more vigorous study of the nonresponders would be necessary. However, this was not within the main scope of the present investigation nor were funds available for conducting such a study on the nonresponse variable. In retrospect, the nonresponse may have affected the outcome of the study in favor of the controls rather than the cases. The cases had a 10 percent lower response rate than the controls. This might have been due to the fact that the cases had more dogs than the controls. Thus, the cases might have been reluctant to respond to the questionnaire because they were afraid that their dogs might be seized.

The results of the case - control study demonstrated that the dog was certainly "man's best friend". Approximately 60 percent of the total subjects had a dog at the time their blood was collected, and 85 percent had a dog at some time in their lives. These facts would tend to make a study of this type difficult to interpret because the controls as well as the cases had a high exposure rate to dogs. However, definite trends were observed. Even though both groups were exposed to dogs, the infected group had a higher exposure rate to dogs than did the controls ($p \sim 0.05$). This suggested that exposure to dogs (cause) was necessary for B. canis infection (effect), but the association would be regarded as indirect evidence. In order for infection to have occurred, the agent must have been present in the dog. This factor was not determined.

Since the infected persons had more dogs than the controls, this would suggest that the more dogs a person had, the more likely the person would be infected with B. canis. Furthermore, the cases had more inside dogs than the controls. Although the difference was of borderline significance, it might imply that close contact was necessary for infection to occur.

Future studies concerning the epidemiology of B. canis infection in man should focus directly on the natural reservoir, the dog, and its relationship to man. One type of study that could be done would be a prospective study involving both serologically and bacteriologically proven infected and uninfected dogs. Serial bloods could then be collected on their owners in order to determine if and when serological evidence of infection or disease occurs. This type of study would also aid in determining if dogs with B. canis antibodies, in the absence of positive cultures, could transmit the infection to man.

Studies need to be conducted to attempt to isolate B. canis from dog feces. This would be a difficult task because of the normal commensal organisms which are present in the gastrointestinal tract. However, if it could be shown that B. canis is transmitted through the feces of diseased dogs, the high prevalence of B. canis infection in man could be more easily explained.

Lastly, investigations concerning the resistance of B. canis to environmental exposure would be very helpful in understanding the route of transmission of the organism from dogs to man. If the organism can remain viable for long periods of time outside the host as can the other brucellae, infection may occur both directly or indirectly.

In February, 1974, the Center for Disease Control [76] reported that the incidence of B. canis disease may be higher than the number of reported cases indicate. Furthermore, it was reported that a specific B. canis antigen must be used because the standard brucellae antigen used in febrile agglutinin testing would not react with B. canis antibodies. The results of the present investigation strongly support their suppositions.

Although the incidence of canine brucellosis due to B. canis is not known, the prevalence of infection has been estimated to be about 77 percent [24]. This figure approximates that of human infection (67.8%) found in the present study. Since man is closely associated with dogs, this disease could become a potential public health problem for physicians as well as veterinarians. When the medical community becomes more aware of this newly recognized disease, its incidence will no doubt increase. However, before a reasonable estimate can be made of the magnitude of the problem, B. canis will have to be considered as a possible cause of disease, especially in patients with fevers of undetermined origin, with B. canis antigen used as a diagnostic tool for detecting B. canis antibodies instead of the usual commercially available B. abortus antigen.

CHAPTER V

SUMMARY

A study was conducted to determine the prevalence of Brucella canis infection in specified human population groups in Oklahoma. Infection was interpreted as the presence of specific antibodies to B. canis antigen at titers of 1:12 or greater. The epidemiological tool used to detect these antibodies was the microtiter plate agglutination technique.

Preliminary studies were designed to determine the specificity of the antigen using agglutination-absorption techniques, immunofluorescent studies, and paired sera tests using Brucella abortus and B. canis antigen. The results of these studies exhibited strong evidence that the antigen was specific for B. canis antibodies. It was also necessary to determine the reliability of the microtiter plate agglutination test. It was found to be both sensitive and specific when compared to the standard macro tube agglutination technique.

Three groups of subjects were selected to be tested for the presence of B. canis antibodies based on their exposure to dogs: a) newborn infants (no exposure), b) blood donors, patients, and hospital employees (average exposure), and c) practicing veterinarians and veterinary students (high exposure). A fourth group was also examined for the prevalence of B. canis infection. These were ill patients with

signs and symptoms that would be compatible with those caused by the brucellae (fevers of undetermined origin).

Some of the newborn infants exhibited B. canis antibodies in cord blood samples taken at delivery. This indicated the presence of maternal antibodies. There were no B. canis antibodies in infants whose mothers were negative for B. canis antibodies.

Among 2,026 subjects considered to have an average exposure to dogs, 67.8 percent had serological evidence of B. canis infection. Females had a significantly higher prevalence of infection than males ($p < 0.001$). There was also a significant difference ($p < 0.001$), by age, in both sexes, with the infection rate decreasing with increasing age. It was suggested that the differences in B. canis infection between sexes and among the various age groups were due to differences in exposure to dogs.

The infection rates of B. canis among upper-classmen veterinary students were higher ($p < 0.10$) than those of lower-classmen. The difference was apparently due to greater exposure to dogs in the upper-classmen.

The prevalence of B. canis infection in practicing veterinarians was statistically higher ($p < 0.01$) than that of male blood donors. Also, the degree of infection (high antibody titers) was much higher among the veterinarians than among the male blood donors. These results indicated that either the veterinarians were exposed more frequently to diseased dogs (anamnestic response), or they were exposed to dogs which were more heavily infected with B. canis.

Patients who had fevers of undetermined origin had a

significantly higher ($p < 0.001$) antibody response to B. canis antigen than all other patients. It was suggested that some of these patients might have had undiagnosed brucellosis due to B. canis.

A case (infected) - control (uninfected) study was conducted to test the hypothesis that persons infected with B. canis have more exposure to dogs than persons not infected. The population consisted of a random sample of subjects who were considered to have an average exposure to dogs. Information concerning a history of dog exposure was obtained by the use of mailed questionnaires. The results of this study further supported the concept that B. canis infection in humans was associated with exposure to dogs. The cases (infected) had a higher mean number of dogs than the controls (uninfected), and persons with a history of ever having dogs were significantly higher ($p \sim 0.05$) in the cases than in the controls.

Brucellosis due to B. canis is thought to be infrequent in humans (12 reported cases), but little published information is available concerning the prevalence of infection. The results of the present investigation strongly indicate that B. canis infection is very prevalent in specified human population groups in Oklahoma. Indirect evidence is presented that would suggest that human infection with B. canis is associated with exposure to dogs.

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APPENDICES

APPENDIX A

FLUORESCENT IMMUNOGLOBULIN PROCEDURE FOR ANTIGEN SPECIFICITY

An experiment was conducted using fluorescent antibodies to determine if human immunoglobulin was sensitizing Brucella canis antigen. B. canis antigen was incubated in human sera that had previously agglutinated the antigen. Sera with different agglutination titers were used. Antigen without incubation with serum was used as a control.

Heavy chain specific fluorescein labeled human anti-IgG, anti-IgM, and anti-IgA were used to determine which gamma globulin might be coating the B. canis antigen. Two antisera were used -- one positive and one negative. The negative antisera was absorbed with red blood cells coated with IgG and IgM gamma globulin. For anti-IgG absorption, Rh positive red blood cells were coated with anti-D, and for anti-IgM absorption, type A red blood cells were coated with anti-A. The positive antisera was centrifuged and absorbed with untreated B. canis antigen to eliminate unwanted specific staining.

Three antigen forms were used: a) untreated antigen, b) antigen treated with non-agglutinating serum, and c) antigen treated with agglutinating serum. Incubation of B. canis antigen in human serum was for 1 hr at 37 C, and incubation of the antigen in the antiserum was for 30 min at 37 C.

APPENDIX B

TABLE 1

STANDARD MACRO TUBE AGGLUTINATION TEST PROCEDURE FOR DETERMINING THE PRESENCE OF *BRUCELLA CANIS* ANTIBODIES

Procedure	Tube Number							
	1	2	3	4	5	6	7	8
Volume of saline (ml)	1.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Volume of serum (ml)	0.5 (mix)	1.0 of tube 1	1.0 of tube 2	1.0 of tube 3	1.0 of tube 4	1.0 of tube 5	1.0 of tube 6	1.0 of tube 7[a]
Initial serum dilution	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Volume of antigen (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Final serum dilution	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024

[a] 1 ml from tube 8 is removed and discarded.

TABLE 2

COMPARISONS OF THE MICROTITER PLATE AGGLUTINATION TEST AND THE STANDARD MACRO TUBE AGGLUTINATION TEST IN THE DETERMINATION OF *BRUCELLA CANIS* ANTIBODIES

Comparison	Test	
	Microtiter	Standard Tube
Antigen	Same	Same
Calculation of final serum dilution	Same	Same
Diluent	0.85% saline	3.5% saline
Titer determination	Agglutination	Clearing of supernate
Total volume per tube	0.075 ml	1.0 ml
Final dilution of serum	1:6,1:12...1:768	1:8,1:16...1:1024

TABLE 3

AGGLUTINATION-ABSORPTION TESTS TO DETERMINE SPECIFICITY OF *BRUCELLA CANIS* ANTIGEN

Serum No.	*B. canis* titer before absorption	*B. canis* titer after absorption[a]
345 (8)	0[b]	0[b]
377 (8)	0	0
210 (8)	1:6	0
214 (8)	1:6	0
233 (8)	1:6	0
305 (8)	1:6	0
328 (8)	1:6	0
342 (8)	1:12	0
344 (8)	1:12	0
271 (8)	1:24	0
306 (8)	1:24	0
16 (8)	1:48	0
170 (8)	1:48	0
168 (8)	1:96	0
329 (8)	1:96	0
24 (8)	1:192	0
77 (8)	1:192	0

[a]Sera absorbed with *B. canis* antigen.

[b]No agglutination.

TABLE 4

AGGLUTINATION-ABSORPTION TESTS TO DETERMINE SPECIFICITY OF *BRUCELLA CANIS* ANTIGEN USING OTHER BACTERIAL ANTIGENS[a]

Serum No.	*B. canis* titer before absorption	Reaction with other antigens	*B. Canis* titer after absorption
210 (8)	1:6	+[b]	1:6
214 (8)	1:6	+	1:6
233 (8)	1:6	0[c]	1:6
305 (8)	1:6	+	0
342 (8)	1:12	0	1:12
344 (8)	1:12	+	1:12
271 (8)	1:24	+	1:24
306 (8)	1:24	+	1:24
16 (8)	1:48	0	1:48
170 (8)	1:48	+	1:24
168 (8)	1:96	+	1:96
329 (8)	1:96	+	1:96
24 (8)	1:192	+	1:96
77 (8)	1:192	+	1:192

[a]Pooled Typhoid H (flagellar d), *Salmonella* Grp. D (typhoid O, somatic 9, 12), Paratyphoid B (flagellar b, 12), *Brucella abortus*, *Francisella tularensis*, *Proteus* OX19.

[b]Agglutination.

[c]No agglutination.

TABLE 5

CLASSES OF BRUCELLA CANIS IMMUNOGLOBULINS IN HUMAN SERA USING FLUORESCENT TECHNIQUES

Antigen	Antisera				
	IgM	IgM Control	IgG	IgG Control	IgA
Untreated[a]	0[b]	0	0	0	0
Adult - 1:192 titer	+[c]	0	+/0[d]	0	0
Adult - 1:96 titer	+	0	+/0	0	0
Adult - 1:6 titer	+/0	0	0	0	0
Adult - 0 titer	0	0	÷/0	0	0
Newborn - 1:124 titer	0	0	+/0	0	0

[a]Not treated with serum.

[b]No fluorescence.

[c]Positive fluorescence.

[d]Questionable fluorescence.

TABLE 6

COMPARISON OF PAIRED SERA REACTED WITH BRUCELLA CANIS AND BRUCELLA ABORTUS ANTIGEN

B. canis Antigen	B. abortus Antigen		Total
	Positive[a]	Negative[b]	
Positive[a]	55	732	787
Negative[b]	9	376	385
Total	64	1,108	1,172

[a]1:12 or greater antibody titer.

[b]1:6 or less antibody titer.

TABLE 7

MICROTITER PLATE AGGLUTINATION TECHNIQUE COMPARED TO A STANDARD MACRO TUBE AGGLUTINATION TECHNIQUE

Microtiter Test	Standard Test		Total
	Positive[a]	Negative[b]	
Positive[c]	24	2	26
Negative[d]	1	12	13
Total	25	14	39

[a]1:16 or greater antibody titer.

[b]1:8 or less antibody titer.

[c]1:12 or greater antibody titer.

[d]1:6 or less antibody titer.

Sensitivity = 96.0 percent

Specificity = 85.7 percent

TABLE 8

RANDOM SAMPLE OF *BRUCELLA CANIS* INFECTED[a] AND NON-INFECTED[b] SUBJECTS WITH AN AVERAGE EXPOSURE TO DOGS, BY AGE AND SEX

Age	Infected						Total Population								Total Population[c]	
	Males		Females		Total				Males		Females		Total			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
3-14	2	3.1	3	3.5	5	-	23	-	[illegible]					-	8	-
15-24	24	37.5	43	50.0	67	-	341	-						-	116	-
25-34	18	28.1	20	23.3	38	25.3	213	26.						31.3	131	29.8
35-44	11	17.2	9	10.5	20	13.3	113	13.						16.0	64	14.5
45-54	5	7.8	6	6.9	11	7.3	61	7.5						15.3	54	12.3
55-64	4	6.3	2	2.3	6	-	-	-						-	35	-
65+	0	0	3	3.5	3	-	-	-	4				10	-	32	-
Total	64	100.0	86	100.0	150	100.0	814	100.0	78	100.0	72	100.0	150	100.0	440	100.0

$\chi^2_{(4df)} = 0.8161$ p = Not Significant $\chi^2_{(4df)} = 2.3457$ p = Not Significant

[a] 1:12 and 1:48 or greater antibody titer.

[b] 0 antibody titer.

[c] Population from which random sample was selected.

TABLE 8

RANDOM SAMPLE OF BRUCELLA CANIS INFECTED[a] AND NON-INFECTED[b] SUBJECTS WITH AN AVERAGE EXPOSURE TO DOGS, BY AGE AND SEX

	Infected						Total Population		Non-Infected						Total Population[c]	
	Males		Females		Total				Males		Females		Total			
Age	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
3-14	2	3.1	3	3.5	5	-	23	-	2	2.6	1	1.4	3	-	8	-
15-24	24	37.5	43	50.0	67	-	341	-	13	16.7	18	25.0	31	-	116	-
25-34	18	28.1	20	23.3	38	25.3	213	26.3	28	35.9	19	26.4	47	31.3	131	29.8
35-44	11	17.2	9	10.5	20	13.3	113	13.9	14	17.9	10	13.9	24	16.0	64	14.5
45-54	5	7.8	6	6.9	11	7.3	61	7.5	11	14.1	12	16.7	23	15.3	54	12.3
55-64	4	6.3	2	2.3	6	-	-	-	6	7.7	6	8.3	12	-	35	-
65+	0	0	3	3.5	3	-	-	-	4	5.1	6	8.3	10	-	32	-
Total	64	100.0	86	100.0	150	100.0	814	100.0	78	100.0	72	100.0	150	100.0	440	100.0

$\chi^2_{(4df)} = 0.8161$ p = Not Significant $\chi^2_{(4df)} = 2.3457$ p = Not Significant

[a] 1:12 and 1:48 or greater antibody titer.

[b] 0 antibody titer.

[c] Population from which random sample was selected.

TABLE 9

QUESTIONNAIRE RESPONSE OF *BRUCELLA CANIS* INFECTED[a] AND NON-INFECTED[b] SUBJECTS WITH AN AVERAGE EXPOSURE TO DOGS, BY AGE AND SEX

Age	Infected						Non-Infected					
	Males		Females		Total		Males		Females		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
3-14	0	0	2	8.3	2	4.7	0	0	0	0	0	0
15-24	5	26.3	7	29.2	12	27.8	3	9.7	4	14.8	7	12.1
25-34	7	36.8	7	29.2	14	32.5	14	45.1	7	25.9	21	36.2
35-44	3	15.8	2	8.2	5	11.7	10	32.3	7	25.9	17	29.3
45-54	3	15.8	4	16.7	7	16.3	4	12.9	5	18.6	9	15.6
55-64	1	5.3	1	4.2	2	4.7	0	0	2	7.4	2	3.4
65+	0	0	1	4.2	1	2.3	0	0	2	7.4	2	3.4
Total	19	100.0	24	100.0	43	100.0	31	100.0	27	100.0	58	100.0

[a] 1:12 and 1:48 or greater antibody titer.

[b] 0 antibody titer.

TABLE 10

QUESTIONNAIRE RESPONSE ACCORDING TO *BRUCELLA CANIS* INFECTED SUBJECTS (CASES)[a] AND CONTROLS[b]

Questionnaire Response	Subjects		Total
	Cases	Controls	
Yes	43 (28.7)[c]	58 (38.7)	101
No	107 (71.3)	92 (61.3)	199
Total	150 (100.0)	150 (100.0)	300

$\chi^2 = 3.3584$ $p < 0.10$

[a] 1:12 and 1:48 or greater antibody titer.

[b] 0 antibody titer - Not infected.

[c] Percent of total.

APPENDIX C

THE UNIVERSITY HOSPITAL • 800 N.E. 13th Street Oklahoma City, Oklahoma 73190

M E M O R A N D U M

TO: Whom it May Concern

FROM: Jeptha W. Dalston, Ph.D.
Hospital Administrator

DATE: August 10, 1973

SUBJECT: Authorization for Access to Medical Records

This is an authorization to allow Mr. Patrick Monroe, a candidate for the Doctor of Public Health degree in the Department of Biostatistics and Epidemiology, access to medical charts for the purpose of gathering basic epidemiologic data; such as age, sex, race, occupation, and place of residence. This information will be used in the strictest confidence and will form the basis of his dissertation topic for the doctorate degree.

Your cooperation will be appreciated.

JWD:sn

cc: Mr. Pat Monroe
Stan Silberg, Ph.D.

APPENDIX D

The
University of Oklahoma Health Sciences Center

Post Office Box 26901 Oklahoma City, Oklahoma 73190

DEPARTMENT OF
BIOSTATISTICS AND EPIDEMIOLOGY

January 16, 1974

Edward Frohlich, M.D., Chairman
Human Experimentation Committee
Office of Research Administration
Room 120, Medical School Building

Dear Doctor Frohlich:

I am a candidate for the Doctor of Public Health Degree in the Department of Biostatistics and Epidemiology. The title of my dissertation topic is "A Sero-epidemiologic Investigation of Brucella canis Antibodies in Specified Human Populations in Oklahoma". The principal investigators are myself, Dr. Stanley Silberg, Professor of Biostatistics and Epidemiology, and Dr. Patrick Morgan, Commissioner's Office, State Health Department.

The study consists of obtaining discarded sera that were submitted to the Clinical Serology Laboratory of University Hospital for routine syphilis serologies and using these same sera for the determination of antibodies to Brucella canis.

At the time the study was initiated it was not known that there had to be an approval by the HEC to do additional tests on sera that was to be discarded. Since it was not known whether the results would be encouraging when the project was started, a decision to send questionnaires to individuals in the study was not decided upon. However, the results have been quite encouraging, and it was concluded that questionnaires would be beneficial in order to gather data as to the degree of exposure to dogs of a random sample of those people with no antibodies to the organism compared to those with antibodies. A written consent form will be obtained from everyone who is sent a questionnaire.

There will be no risk to the subjects involved because the blood has already been drawn for other tests that were requested by the physician and no additional blood will be drawn specifically for the proposed investigation. The potential benefits from the proposed research study is discussed in the protocol and is summarized on page 17.

The approval of this investigation by the Human Experimentation Committee would be greatly appreciated.

Respectfully submitted,

Patrick Monroe
Doctor of Public Health Candidate
Department of Biostatistics and
Epidemiology Tel: 271-5521

PM:nba

APPENDIX E

The
University of Oklahoma Health Sciences Center

Post Office Box 26901 Oklahoma City, Oklahoma 73190

HUMAN EXPERIMENTATION COMMITTEE
Research Affairs Office
Medical School Building
800 N.E. 13th Street

January 31, 1974

Patrick Monroe
A Sero-epidemiologic Investigation of Brucella canis Antibodies in Specified Human Populations in Oklahoma
APPROVED - January 28, 1974 by the Human Experimentation Committee

Patrick Monroe
Department of Biostatistics
and Epidemiology

Dear Mr. Monroe:

The Human Experimentation Committee reviewed and approved the captioned application which will involve human subjects in accordance with the assurance approved by the Public Health Service.

It is the opinion of this Committee that the rights and welfare of the individuals who are to be studied will be completely respected; that informed consent will be obtained in a manner consonant with the Surgeon General's directive of February 8, 1966, and that the risks to the individual as well as the potential medical benefits from the proposal fully warrant its being carried out.

Sincerely yours,

Edward D. Frohlich, M.D.
Chairman
Human Experimentation Committee

mn

cc: Dr. William E. Brown, Jr.
Dr. John R. Sokatch

APPENDIX F

The
University of Oklahoma Health Sciences Center

DEPARTMENT OF
BIOSTATISTICS AND EPIDEMIOLOGY

Post Office Box 26901 Oklahoma City, Oklahoma 73190

Enclosed is a short questionnaire concerning your exposure to dogs. The questionnaire will be strictly confidential and will be used solely for the purpose of gathering data for a research study at the University of Oklahoma Health Sciences Center.

Blood was withdrawn from you while you were a patient or a blood donor at the University Hospital or Children's Hospital for tests requested by your physician. This blood, before being discarded, was used for additional testing as part of a research study. The results of this research test in no way affect your health status or that of your pets. As a result of your cooperation in this study, it may be possible in the future to develop a new test in order to diagnose disease.

The questionnaire should take only a few minutes to complete, and the information gathered will be of great value. Please answer all of the questions and feel free to comment on any that seem unclear.

However, you are not required to complete the questionnaire, and are free to withdraw from the study at any time. If you do wish to participate in the research study, please read and sign the consent form statement, and then please return the completed questionnaire and the signed consent form in the pre-addressed and stamped envelope provided.

Any inquiries concerning the study may be taken to the Office of Research Administration in Room 120 of the Medical School Building, telephone 271-4690.

Your cooperation will be greatly appreciated.

Sincerely,

Patrick Monroe
(Doctor of Public Health Candidate)
Department of Biostatistics
and Epidemiology

PM:ba

Consent Form

By signing this consent form, I voluntarily consent to participate in the following investigation, "A Sero-epidemiologic Investigation of Brucella canis Antibodies in Specified Human Populations in Oklahoma". I understand my participation is of no risk to me and may prove beneficial in advancing medical knowledge. By signing this consent form, I have not waived any of my legal rights or released this institution from liability or negligence. I may revoke my consent and withdraw from this study at any time.

_____
(Signature of patient, subject or guardian

---

1. Name: (Last) _____ (First) _____
2. Date of birth: ____ ____ ____ (mo. day yr.) 3. Male ____ Female ____
4. Usual occupation: _____
5. Do you now have a dog(s)? Yes ____ No ____ 6. How many? ____
7. If yes, for how long have you had a dog(s)? _____
8. Is the dog(s) primarily an inside house dog? Yes ____ No ____
9. Sex of dog: male ____ female ____ 10. Breed of dog: _____
11. Is dog(s) primarily an outside dog? Yes ____ No ____
12. Sex of dog: male ____ female ____ 13. Breed of dog: _____
14. If any of dogs are female, have they ever aborted (had dead puppies)? Yes ____ No ____
15. If you do not now have a dog(s), have you ever had one? Yes__ No__
16. If yes, how long ago? _____ 17. For how long did you have the dog? _____
18. If the dog was female, did she ever abort (have dead puppies)? Yes ____ No ____
19. If you do not now have a dog, how often are you now in close contact with other peoples' dogs?

None _____ Rarely _____ Occasionally _____ Daily _____