Seroepidemiological Investigation of *Brucella canis* Antibodies in Different Human Population Groups

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A study was conducted to determine the prevalence of *Brucella canis* antibodies in specified groups based on their exposure to dogs. The method used was a microtiter technique, and the presence of antibodies at a 1:12 or greater dilution of serum was considered a positive test. Eleven (5.7%) of the newborn infants had evidence of maternal antibodies, and 67.8% of the persons with an average exposure to dogs had *B. canis* antibodies, with a 62.1% prevalence in males and a 72.4% prevalence in females ($P < 0.001$). Veterinarians had a much higher rate of infection (72.6%) than male blood donors (56.9%) ($P < 0.01$). Patients with fevers of undetermined origin had significantly higher antibody titers to *B. canis* than all other patients ($P < 0.001$). This study presents evidence that the prevalence of *B. canis* antibodies in humans is high, and that the incidence of brucellosis may increase when physicians consider *B. canis* as a possible etiological agent in febrile illnesses.

Seroepidemiological surveys are undertaken when existing information relating to the interrelation between humans and a disease agent is inadequate. These types of surveys are also basic to the study of infectious diseases (19). Surveys for antibodies to a newly identified agent provide a direct way of determining whether the agent has made contact with humans. Essentially, these are exploratory or prevalence types of surveys which are designed to determine the magnitude of infection in a population (20) and 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.

Canine brucellosis due to *Brucella canis* has occurred in epidemic proportions throughout the United States since 1964 (3, 4, 13-16), and the prevalence of *B. canis* antibodies in dogs has been estimated to be approximately 77% (10). Little information is available concerning the incidence of *B. canis* infections in humans. Because of the dog's close association with humans, the dangers of the canine to human transmission of *B. canis*, either by occupational exposure or by natural means, have been discussed by a number of investigators (7, 11, 12, 17, 21, 23). The present study was undertaken to deter-

mine the prevalence of *B. canis* antibodies in different human populations based on their exposure to dogs. Not only would such information augment existing epidemiological knowledge of this newly recognized etiological agent of brucellosis in humans but its zoonotic significance is also important to public health.

MATERIALS AND METHODS

Specimens. Four populations were selected for the study, and three were selected on their exposure to dogs. The groups were placed in four categories: (i) no exposure, (ii) average exposure, (iii) potentially high exposure, and (iv) persons with fevers of undetermined origin compatible with illnesses of possible microbial origin.

The group with no exposure to dogs consisted of 193 newborn infants whose sera were collected from cord blood samples for routine serological determinations for syphilis. Sera from 59 mothers of these babies were also tested for the presence of *B. canis* antibodies.

The average exposure group was 2,026 individuals, consisting of hospitalized and nonhospitalized patients, hospital employees, and blood donors. The sera were obtained from the clinical serology laboratory. The exposure to dogs in this group was thought to represent the usual or everyday type of exposure to both infected and uninfected dogs.

The group with a potentially high exposure to dogs consisted of 73 practicing veterinarians. It was not known whether all of the veterinarians were exposed to dogs in their practices, but they would, as a group, have a more concentrated contact with sick dogs than the general population. Therefore, this group would have a greater prevalence of antibody

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ies. The subjects with fevers of undetermined origin 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 tests for febrile agglutinins be performed on their sera. Febrile agglutinins are serological tests used to aid in the diagnosis of typhoid, paratyphoid, brucellosis, tularemia, and various rickettsial diseases.

Antigen. *B. canis* antigen was obtained from the U.S. Department of Agriculture, Animal and Plant Service, Veterinary Service Laboratory, Ames, Iowa. Cross-reactivity of the antigen was investigated by absorbing sera positive for *B. canis* antibodies with other bacterial antigens [typhoid H (flagellar d), *Salmonella* group D (typhoid O, somatic 9, 12), paratyphoid B (flagella b, 12), *Brucella abortus*, *Francisella tularensis*, and *Proteus* O₁₉] and then retesting the same sera with *B. canis* antigen.

Procedure. A modified microtiter plate agglutination technique (MPAT) (5) was used for the serological determination of *B. canis* antibodies. Microtiter equipment manufactured by the Cooke Engineering Co., Alexandria, Va., was used throughout the study.

A drop of phosphate-buffered, physiological saline was added to each U-shaped well of a microtiter plate with a 25- μ l micropipette. Twenty-five microliters of undiluted serum was placed in the first well and diluted twofold serially using a 25- μ l microdilutor. Fifty microliters of *B. canis* antigen solution (4.4 ml of stock *B. canis* antigen diluted to 100 ml with phosphate-buffered, physiological saline) was added to each well, and the final dilutions were 1:6, 1:12, ... 1:768. The plates were covered with a plate sealer and incubated at 37 C for a total of 48 h. Reading the plates at 48 h occasionally yielded higher titers. Agglutination appeared as a circle with irregular edges or a folded-over circle when read with a test-reading mirror. Wells where no agglutination occurred had a smooth round button of antigen in the bottom of the well. Positive and negative controls were used with each series of test runs.

An antibody titer of 1:12 or greater was considered a positive test and was interpreted as the presence of *B. canis* antibodies. An antibody titer of 1:6 or less was regarded as a negative test, and the individual was considered to be without evidence of *B. canis* antibodies.

The MPAT was standardized by comparison tests using a standard macrotube test without knowledge of the MPAT test results.

RESULTS

The *B. canis* antigen appeared to be specific for *B. canis* antibodies. Although some samples exhibited agglutination reactions with other bacterial antigens, these same samples were again positive at the same dilution or within one dilution for *B. canis* antibodies when retested with *B. canis* antigen.

The MPAT compared favorably with the standard macrotube agglutination technique.

Of 40 samples tested, the sensitivity of the MPAT was 96.0% and the specificity was 86.7%. Two false-positive results occurred using the MPAT, and one false-negative occurred. 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 two methods.

Of the 193 newborn infants tested, only 11 (5.7%) had *B. canis* antibody titers of 1:12 or greater, with the highest being 1:24. Fifty-nine serum samples from mothers of these infants were also tested for the presence of *B. canis* antibodies. In all instances, infants who demonstrated the presence of antibodies had mothers with positive titers.

The distribution of *B. canis* antibodies in sera of persons with an average exposure to dogs is presented in Table 1. Of 2,026 sera tested, 67.8% exhibited antibodies to *B. canis*, and 58 (2.9%) subjects presented titers of 1:96 or greater.

The prevalence of antibodies, by sex, is shown in Table 2. Females had a 10.3% higher prevalence of *B. canis* antibodies than did males ($P < 0.001$).

The presence of antibodies in both sexes ranged from 78.4% in the 3- to 14-year age group to 48.2% in persons 65 years and older.

TABLE 1. Distribution of *B. canis* antibody titers among subjects with an average exposure to dogs

Antibody titer	No.	%
$\leq 1:6^a$	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
$\geq 1:768$	0	0.0

^a Negative for *B. canis* antibodies.

^b Titers of 1:12 or greater were positive for *B. canis* antibodies.

TABLE 2. *B. canis* antibodies in subjects with an average exposure to dogs, by sex^a

Subjects	Antibody status		Total	Prevalence (%)
	Positive ^b	Negative ^c		
Males	567	346	913	62.1
Females	806	307	1,113	72.4

^a $\chi^2 = 24.4$; $P < 0.001$.

^b 1:12 or greater antibody titer.

^c 1:6 or less antibody titer.

This inverse relationship between age and the presence of *B. canis* antibodies was especially pronounced in the females (Table 3). The overall chi-square value for the females, by age, was 50.3, which, with four degrees of freedom, was statistically significant ($P < 0.001$). This highly significant value was due almost entirely to a linear trend rather than a curvilinear trend, since departure from linear regression was not statistically significant (Table 3).

The prevalence of *B. canis* antibodies among the practicing veterinarians (72.6%) was significantly higher ($P < 0.001$) than that of their male blood donor counterparts (56.9%) in the average dog exposure group (Table 4). Male blood donors were used to compare with the practicing veterinarians because of the similarities in age, sex, and general health status. The veterinarians also had much higher antibody titers than the male blood donors. Among the veterinarians, 19.2% had titers of 1:96 and

greater, compared to only 1.8% for the donors (Table 5).

Table 6 presents the distribution of *B. canis* antibody titers in patients whose physicians requested that tests for febrile agglutinins be performed. Of 113 such patients, 91 (80.5%) were considered positive, with 10 (8.9%) having titers of 1:96 or greater. When these patients were compared to all other hospitalized patients, the difference in the prevalence of antibody titers was considered significant (Table 7). All other hospitalized patients were used to compare with the patients with fevers of undetermined origin because of their hospitalization status.

DISCUSSION

The presence of *B. canis* antibodies in the newborn infants was a result of transplacental antibody transfer, since they had no risk of antigenic stimuli. The results were similar to those found by Carmichael and Kenney (4), who reported that antibody tests from several

TABLE 3. *B. canis* antibodies among females, by age, with an average exposure to dogs^a

Age	Antibody status		Total	Prevalence (%)
	Positive ^b	Negative ^c		
3-14 ^d	26	6	32	81.3
15-24 ^d	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 ^e	44	32	76	57.9
65+ ^e	17	23	40	42.5

^a χ^2 (4 df) = 50.3; $P < 0.001$. Source of variation due to linear regression: df, 1; χ^2 , 48.6; $P < 0.001$. Source of variation due to departure from linear regression: df, 3; χ^2 , 1.7; $P > 0.050$.

^b 1:12 or greater antibody titer.

^c 1:6 or less antibody titer.

^d Age groups combined for statistical analyses.

^e Age groups combined for statistical analyses.

TABLE 4. *B. canis* antibodies among practicing veterinarians and male blood donors of corresponding ages (25 to 54 years old)^a

Subjects	Antibody status		Total	Prevalence (%)
	Positive ^b	Negative ^c		
Practicing veterinarians	53	20	73	72.6
Male blood donors	210	159	369	56.9

^a χ^2 = 6.2; $P \sim 0.01$.

^b 1:12 or greater antibody titer.

^c 1:6 or less antibody titer.

TABLE 5. Distribution of *B. canis* antibody titers among practicing veterinarians and male blood donors of the same age (25 to 54)

Antibody titer	Practicing veterinarians		Male blood donors	
	No.	%	No.	%
$\leq 1:6^a$	20	27.4	159	43.1
1:12 ^b	8	11.0	101	27.4
1:24	22	30.1	83	22.5
1:48	9	12.3	19	5.1
1:96	6	8.2	3	0.8
1:192	2	2.7	4	1.0
1:384	1	1.4	0	0.0
$\geq 1:768$	5	6.9	0	0.0

^a Negative for *B. canis* antibodies.

^b Titers of 1:12 or greater were positive for *B. canis* antibodies.

TABLE 6. Distribution of *B. canis* antibody titers among patients with fevers of undetermined origin

Antibody titer	No.	%
$\leq 1:6^a$	22	19.5
1:12 ^b	27	23.8
1:24	33	29.3
1:48	18	15.9
1:96	4	3.5
1:192	3	2.7
1:384	1	0.9
$\geq 1:768$	5	4.4

^a Negative for *B. canis* antibodies.

^b Titers of 1:12 or greater were positive for *B. canis* antibodies.

TABLE 7. Comparison of *B. canis* antibodies between patients with fevers of undetermined origin and all other hospitalized patients^a

Subjects	Antibody status		Total	Prevalence (%)
	Positive ^b	Negative ^c		
FUO patients ^d	91	22	113	80.5
Hospitalized patients	251	147	398	63.1

^a $\chi^2 = 12.1$; $P < 0.001$.

^b 1:12 or greater antibody titer.

^c 1:6 or less antibody titer.

^d FUO, Fevers of undetermined origin.

litters of pups from *B. canis*-infected bitches had antibodies to *B. canis*.

The higher prevalence of *B. canis* antibodies in females than in males was due either to the females' ability to produce a more efficient antibody response (26) or to an increased exposure to the agent. If females have a more efficient antibody response mechanism, the prevalence of *B. canis* antibodies would be higher in females if a sample of the general population (average exposure) was tested. However, population studies by other investigators (1, 6, 8, 25) concerning *B. abortus* indicate that females do not show a significantly higher prevalence of *B. abortus* antibodies than males. These studies suggest that significant differences in brucellae antibody titers between the sexes are primarily a result of exposure differences rather than biological differences. The fact that females had an almost identical *B. canis* antibody prevalence (72.4%) as the practicing veterinarians (72.6%) would also suggest that the difference in prevalence of titers was due to exposure.

Females have a higher exposure potential for contracting *B. canis* than males as a result of everyday tasks such as house cleaning and the handling of dog waste products. The portals of exit of the organism from diseased dogs are vaginal discharges and milk from females and semen and urine from males (2, 14, 24). 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 (22). Investigations have not been conducted to determine the diversity of environmental conditions under which this agent will remain viable; however, if *B. canis* has environmental characteristics similar to the other *Brucella* (9, 22), humans could readily become infected with this organism.

In the present study both sexes exhibited a definite linear trend in the prevalence of *B.*

canis antibodies by age. The differences in age were primarily due to a decrease in antibodies as age increased. The decrease in the development of antibodies with increasing age may have been due to the hosts' inability to produce humoral antibodies as age increases and not due to a decrease in exposure to the agent. To examine this possibility, a review of the literature was made to find a study that was performed under conditions similar to the present investigation. Harrison and Mauch (8) conducted such a study to determine the possible existence of disease due to infection with *B. abortus*. There was no statistical difference in the rates of antibody prevalence by age in either sex, nor was the chi-square value due to linear regression significant. These results indicated 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 a result of differences to exposure to *B. canis*. Very young persons would have a greater risk of infection because of their close contact with dogs. As the children mature and leave 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 decrease in the absence of antigenic stimuli.

The increased prevalence of *B. canis* antibodies in the veterinarians compared to that in the male blood donors was probably due to an increased frequency of exposure to infected dogs. Also, the titers of the veterinarians were much higher than those of the male blood donors, which might indicate that either the veterinarians were exposed more frequently to diseased dogs or they were exposed to dogs more heavily infected with *B. canis*.

Five persons (1.8%) with undiagnosed febrile illnesses had 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*.

The Center for Disease Control (18) reported recently 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 this investigation strongly support their supposition. When the medical community becomes more aware of this newly recognized etiological agent

of brucellosis in humans, its incidence will no doubt increase.

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