A serological survey of 1,208 male military recruits ages 18-26, was made to detect titers to *Brucella canis*, a known canine and occasional human pathogen. Five (0.4%) men had titers of 1:100 or greater to this antigen. The clinical condition produced by this organism is discussed as well as the role of this pathogen in the differential diagnosis of febrile illness. Although the incidence of significant titers reported herein is low, the mere fact of their detection may be of epidemiological importance.

The Incidence of Brucella Canis Antibodies in Sera of Military Recruits

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Introduction

Brucellosis has been known to occur in dogs since 1931 but was considered a medical rarity until 1966 when a major concern developed over a widespread outbreak of canine abortions. The causative organism of this infectious abortion, *Brucella canis*, was isolated in 1967 by Carmichael[1] and others.[2-4] Since this report, many episodes of canine abortion from this gram-negative coccobaciilus have been reported in the United States. The disease, first recognized in breeding kennels of beagles, has been reported in field trial dogs as well as in dogs raised for research.

The dangers of canine to human transmission of brucellosis have been discussed by Nicholetti, Quinn, Minor[5] and Faigel.[6] Although the only animal thus far found to be naturally infected with *Br. canis* is the dog, the disease may have zoonotic potentials because of the dog's close association with man.

Little information is available concerning the incidence of *Br. canis* infections in humans, thus a study was initiated to survey a population for demonstrable *Br. canis* antibodies.

Materials and Methods

Fifteen milliliters of whole blood was collected by venipuncture from 1,208 presumably healthy men, age range of 18-26 years. After centrifugation, the serum was pipetted into screw-topped glass tubes and stored at–20°C until used.

Brucella canis stock antigen was prepared and antigen concentration adjusted according to the method of Carmichael.[7] A culture of *Br. canis* was grown on tryptose agar (Difco) in Roux flasks. After 60 hours sterile phosphate-buffered saline (pH7.2 0.15M) was added to the flasks and the growth harvested. The cell suspension was filtered through eight layers of gauze, washed twice in phosphate-buffered saline (PBS) and resuspended in PBS. Merthiolate (0.01%) was added as a preservative and the suspension was placed in a water bath at 60°C for one and a half to two hours to kill the organisms. This stock antigen, diluted with PBS to an optical density of 0.19 at 420 NM on a Coleman Junior spectrophotometer, was used as the test antigen.

The sera were initially screened for antibodies to *Br. canis* according to the method of Carmichael[7] at dilutions of 1:10 and 1:50 using the tube agglutination method.

Sera which were positive at 1:10 or 1:50 were further diluted with PBS to yield final dilutions of 1:100, 1:200, 1:400, 1:800. The serum dilutions were incubated at 50°C and read for completeness of agglutination at 48 hours.

A known positive control serum and a known negative control serum were tested with each group of sera tested.

Results

The agglutination test procedure used proved reliable in the detection of *Br. canis* antibody. A titer of 1:100 or greater was considered "positive" and of significance.

There were 1,208 sera tested of which five were positive for *Br. canis* agglutinins. Two specimens demonstrated 1:100 titers and two demonstrated 1:200 titers for *Br. canis* agglutinins. One sample was positive at a 1:400 dilution for *Br. canis*. Twelve hundred and three of the sera tested were negative for *Br. canis* agglutinins.

Discussion

The first recognized human case of brucellosis originating in the United States occurred in 1906,[8] and in 1911, Ferenbaugh[9] traced human cases of brucellosis in the United States to goats. Three years later, Traum[10] isolated and identified brucella organisms from aborting sows. To date, human brucellosis has been attributed to the following four species: 1) *Brucella melintensis* (a pathogen of goats); 2) *Brucella abortus* (cattle); 3) *Brucella suis* (hogs); and 4) within the past three years, *Brucella canis* (dogs).

A sporadic disease, human brucellosis exhibits signs and symptoms simulating other conditions. Identification of suspects is often made only on epidemiological information or the pattern of illness. The early stages of the acute form of brucellosis are often characterized by chills and fever. The chronic form produces fever, weakness, and chronic vague symptomatology. Incubation periods vary between 5 and 21 days. Often months may elapse between the time of infection and the first apparent symptoms. The natural course of the disease in most patients is characterized by

permanent remission of fever and symptoms within 3 to 6 months. Agglutinins against brucella usually appear during the second and third weeks of illness—and persist for months to years.

Brucellosis, although not commonly associated with coryza or pharyngitis, must be differentiated from other acute febrile illnesses such as upper respiratory disease (viral), typhoid fever, mononucleosis, malaria, and tuberculosis.

Active brucellosis, due to *Br. melintensis, Br. suis,* or *Br. abortus,* is usually associated with titers of 1:100 or greater. Agglutinations at dilutions less than 1:100 are often very difficult to interpret and are usually not considered significant. Cross-reactions or non-specific agglutination at 1:25-50 serum dilutions are not uncommon. Prozones have been known to occur in some sera.

In the 1968 Brucellosis report, three cases of human brucellosis due to *Br. canis* were reported.[11] Morisset and Spink recently reported two additional cases in laboratory personnel, making a total of five reported human cases.[12] The portal of entry of these two latest cases could not be determined but both were exposed to large quantities of viable organisms used in preparing antigens. One patient, a 53-year-old woman, exhibited only mild signs of fatigue. Agglutination titer was 1:320 against *Br. canis*. The other patient, a 33-year-old man, had severe fatigue accompanied by headache, chills, nausea, enlarged cervical, epitrocheal, and maxillary lymph nodes, and an enlarged, soft, tender spleen. His agglutination titer was 1:320 against *Br. canis*. The causative organism was isolated from the blood.

In March 1970, a case of human brucellosis was reported in which seven of ten blood cultures grew *Br. canis*[3] The patient's serum was found to have a 1:250 titer against *Br. canis*. *Brucella canis* was isolated from the blood of a male German shepard dog in the same household. The dog's serum had a titer of 1:500 against *Br. canis*.

Only five human sera in this study demonstrated titers of 1:100 or greater to *Br. canis*. Since the clinical records of these five individuals were not available at the time of this study, no statement can be made as to the presence of clinical illness.

This study is the first to survey a human population for the presence of *Br. canis* agglutinins.

The fact that 0.4% of the population selected had significant demonstrable (i. e., 1:100 or greater) titers to *Br. canis* suggests that the incidence of clinical and subclinical human brucellosis due to this organism is very low. Though the incidence of significant titers reported here is low, the mere fact of their detection may be of epidemiological importance.

We do not know the significance of a titer in the absence of blood culture, nor have we been able to establish the fact that these men were exposed to *Brucella canis*. They may have been exposed to some as yet unknown agent that would produce a cross-reaction with the *Brucella canis* antigen used. Other, as yet not thoroughly researched, organisms may cause cross-reactions with the *Brucella canis* antigen, thus producing a false positive on the sero-agglutination test. The recruits tested were not Army dog handlers, nor had they been exposed to dogs since their induction to the Army.

Meyer[14] examined ten strains of canine abortion organism and found these strains to have the same characteristics which identify and define the species *Brucella suis*. Meyer recommended that the canine abortion organism be considered an additional bio-type and be classified as *Brucella suis,* Type V.

Carmichael and Bruner[1] have reported significant reactions between *Brucella canis* antigen and *Brucella ovis* antiserum.

If the dog is capable of serving as a mechanical or biological vector of brucella organisms, an infected dog might transmit the disease to humans sharing the same environment.

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APHA ANNUAL MEETING SCHEDULE

1973	San Francisco	November 4-8
1974	New Orleans	October 20-24
1975	Anaheim	October 12-16
1976	Miami Beach	October 10-14
1977	Washington, D.C.	October 30-November 3