Canine Brucellosis: Bacteriological and Serological Investigation of Naturally Infected Dogs in Mexico City

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Bacteriological investigation of canine brucellosis in Mexico City revealed a high rate (11.8%) of *Brucella canis* infection in a sampling of 59 stray dogs. When conservative criteria were employed in the interpretation of serological test results, there was general agreement between the serological and bacteriological findings; however, some animals with localized male genital tract infections could not be judged as infected solely by serological tests. All Mexican field isolates were identified as *B. canis*; however, some diversity was observed as regards nitrate reduction, growth in the presence of basic fuchsin, and the degree of mucoidness. The seemingly high prevalence of *B. canis* infection in Mexico City dogs suggests the need for further inquiry into the possible public health significance.

Canine brucellosis caused by *Brucella canis* was first recognized in colonies of beagles in the United States (9, 17, 23, 29), but now it is known to occur in various breeds and in several countries (8). The disease has been diagnosed by both serological and bacteriological methods in Japan, West Germany, Brazil, Czechoslovakia, Mexico, and Madagascar (8, 13, 16, 30, 31, 34), but seroepidemiological studies suggest that it is present elsewhere (8, 12). Human infections have been reported (3, 16, 22, 24, 28).

Several serological procedures devised for serodiagnosis of *B. canis* infection in dogs and humans have been applied to epidemiological studies, generally without concurrent cultures of blood or tissues. The commonly used serological methods include the rapid slide agglutination test (SAT) (15, 19), the tube agglutination test (TAT) (1, 5), and the 2-mercaptoethanol tube agglutination test (ME-TAT) (1). The TAT is the only published method that has been evaluated by extensive bacteriological study (27). Additional methods also have been employed, including complement fixation (1), immunodiffusion in agar gel (GD) (11, 25, 33), and immunofluorescence (32). Nevertheless, there is a dearth of information regarding the relative sensitivity and specificity of the various test procedures, especially as applied to diagnosis of canine brucellosis in the field. It is recognized that the sensitive SAT incorrectly classifies as infected many animals subsequently found to be noninfected by other, more definitive, serological methods (4, 6) and, in some cases, by

cultural studies (6). However, the extent to which false positive SAT reactions occur is not known.

Seroprevalence rates in dogs that ranged from approximately 1 through 6% have been reported in different regions of the United States (4, 6, 14, 21); in Peru and Mexico, however, they were found to be approximately 25% (8, 13). Reasons for the disparate prevalences have not been explained, but they may reflect unique characteristics of the sample population, for example, population density and the extent to which dogs are permitted to interact and breed; failures of serological procedures to correctly identify infected animals also may give inaccurate assessments of the actual infection rates. Heterospecific reactions are known to occur between surface antigens of *B. canis*, a naturally nonsmooth (mucoid) organism, and antibodies to a variety of other bacterial species, including *Brucella ovis*, *Actinobacillus equuli*, *B. melitensis* 115(R), *Bordetella bronchiseptica*, and certain other nonsmooth *Brucella* species (7, 11). Actual infection rates still remain uncertain, for comparative serological and bacteriological studies on populations with naturally infected dogs are wanting.

Because the isolation of *B. canis* is the only method to establish a definitive diagnosis (1, 6), advantage was taken of an opportunity to make a prefatory evaluation of several serological methods in a Mexico City, Mexico, population of dogs previously reported (13) to have an exceptionally high seroprevalence of the disease.

Positive cultures of blood and selected tissues were used in this study as the basis for designating *B. canis* infection. An additional objective was to characterize all isolates to obtain further insight into possible biochemical or serological diversity of *B. canis* strains, as well as to identify any heterotypic organisms that cross-react with *B. canis* in the serological tests employed. The results of this study corroborate the unusually high prevalence of *B. canis* in Mexico City dogs, provide preliminary information on the relative efficiency of four serological methods in detecting the disease, present data on certain atypical biochemical features of some of the isolates, and suggest that the Mexico City dog population would be a useful one for additional field evaluations of diagnostic methods and epidemiological studies.

MATERIALS AND METHODS

Specimen collection. Venous blood samples (10 ml) were obtained from a total of 59 randomly assembled stray dogs provided by two dog collection centers in the Mexico City area (Federal District and Ecatepec de Morelos, Mexico). A portion (3 to 4 ml) of each blood sample was inoculated into broth medium (see below). Sera then were harvested at the collection centers by centrifugation of clotted blood, and each sample, in 0.04-, 0.02-, 0.01-, and 0.005-ml amounts, was tested immediately by the SAT, using a commercially available (Pitman-Moore, Inc.) rose bengal-stained *B. ovis* antigen. Animals whose sera had any degree of agglutinating activity at the greatest serum amount tested (0.04 ml) were transported to a laboratory (Instituto Nacional de Investigaciones Pecuarias, Palo Alto, Mexico) equipped for performing necropsy and bacteriological examinations. Dogs then were euthanized with an overdose of pentobarbital sodium and selected tissues were collected for bacteriological culturing. Tissues for culturing included spleen, lymph nodes (retropharyngeal, internal inguinal, and mesenteric), and, from males, epididymides and the prostate gland. Macroscopic lesions were recorded.

Serological methods. The SAT (19) was used for preliminary screening, since the test is highly sensitive and false negative reactions are rare (4, 19). The commercially available Canine Brucellosis Diagnostic Test Kit (Pitman-Moore, Inc.) utilizes a plastic pipette that delivers approximately 0.05 ml; however, we used a special graded 0.2-ml pipette (Bellco) that delivers 0.08-, 0.04-, 0.02-, 0.01-, and 0.005-ml amounts of sera. Degrees of agglutination from complete (plus-4) to no agglutination were recorded, using as controls a standard positive *B. canis* antiserum prepared in specific pathogen-free (SPF) beagles. Additional tests, described previously, included the TAT (5), the ME-TAT (1), and a GD test that utilized a soluble antigen prepared by the extraction of *B. canis* organisms with sodium deoxycholate (SDC). The method used was a modification of a procedure originally described for extraction of cell wall antigens from smooth brucellae

(2). Briefly, packed *B. canis* cells were suspended in 5 volumes (vol/vol) of 1% SDC in 0.1 M phosphate-buffered saline, pH 7.4. After mixing, the suspension was homogenized by brief (30 s) sonic treatment and incubated in a water bath for 30 min at 85°C. Bacterial debris was removed by centrifugation for 20 min at $10,000 \times g$. The sediment was discarded, and the supernatant fluid was then centrifuged for 6 h at $30,000 \times g$ (Beckman model L ultracentrifuge). The supernatant was the GD test antigen. When this antigen was reacted against an antiserum (TAT = 1:800) prepared in SPF beagles, two precipitin lines appeared within 24 h. Tests were performed essentially as described by Myers and Siniuk (26); however, the gel was 0.8% agarose in pH 7.2 borate-buffered saline, with 0.1% sodium azide added as preservation, and microslides (Hyland) were used. Well patterns consisted of a central well (2.5-mm diameter) surrounded by six equally spaced peripheral wells (1.5-mm diameter), each being separated by a distance of 2 mm. Antigen was placed in the larger central well, and aliquots (20 μ l) of serum were introduced into the peripheral wells. Each set of six peripheral wells was routinely inoculated with positive, unknown, and negative serum samples, using alternate wells. Recently, a similar GD method was reported (33) for diagnosis of *B. canis* infection. To test for antigenic relatedness of the isolated bacterial strains, each strain was colony purified, and antigenic extracts were prepared as described above. In such instances, antigen preparations were placed in the peripheral wells; the center well was charged with *B. canis* antiserum.

Cultural methods. Cultures of blood were performed by described methods (1, 5). Tissue samples, obtained aseptically at necropsy, were divided into two portions and one was streaked directly on Thayer-Martin medium (GIBCO), using a heavy inoculum. Care was taken to avoid contamination. This selective medium was reported to support the luxuriant growth of *B. ovis* (1) and has been found equally valuable for the isolation and growth of *B. canis*, a less fastidious organism. The second tissue portion (ca. 1 g) was placed in Albimi brucella broth (BBL) that contained cycloheximide, polymyxin B, and bacitracin at appropriate concentrations (1). All cultures were incubated aerobically for 5 to 7 days at 37°C. Isolated strains recovered on solid medium then were tested for agglutinability with a *B. canis* antiserum, and those showing any degree of agglutination were subcultured and stored for further identification and study. Subcultures from broth onto solid Thayer-Martin medium were made at 2- to 3-day intervals for a period of 7 days and processed as above. Representative isolates from each animal then were colony purified and stored at 4°C as lyophilized cultures.

Bacteriological examinations. Isolates were identified by their morphological, biochemical, and serological properties according to described methods for *Brucella* species (1, 22). Strains presumptively identified as *B. canis* were further characterized by oxidative metabolic tests (1). *B. canis* (RM 6-66) (7) was used as the standard organism for comparisons.

Experimental infections. Isolated strains with characteristics of *B. canis* were imported to the United States for dog inoculation studies. Beagle dogs, main-

tained in separate isolation units at the Baker Institute, were given inocula (10^8 living organisms) by the oral-conjunctival route, with each dog receiving a separate colony-purified isolate. Inoculated animals were observed for the development of bacteremia, and their serological responses were followed for 14 weeks by weekly cultures of blood and tests of sera for *B. canis* antibodies.

RESULTS

Serological and cultural findings. Negative SAT and hemoculture results were recorded for 37 of the 59 dogs in the sample population. Of the 22 animals that were studied further in detail, sera from 15 caused partial (50% or less) agglutination of the SAT antigen only in the 0.04- and 0.02-ml amounts; sera from 7 animals caused complete agglutination in the 0.02-ml, or less, amounts. Results are summarized in Table 1 where it is seen that 5 of the 7 dogs (no. 2, 3, 11, 25, and 51) with SAT agglutinins in the 0.02-ml serum amounts, or less, also had high titers (1:200 or greater) by the TAT and the ME-TAT procedures. These animals also had precipitating antibodies that gave two lines of identity in GD tests with the SDC-extracted *B. canis* antigen. Pure cultures of *B. canis* were isolated from the blood and several tissues of all dogs with homologous precipitating antibodies and titers $\geq 1:200$ by the TAT and ME-TAT. Direct and indirect (broth-to-solid medium) culture methods gave identical results. Eleven animals whose sera caused incomplete agglutination of the SAT antigen (0.02-ml amounts or greater) were found negative (titers $< 1:25$) by the TAT, ME-TAT, and GD tests, and *B. canis* was not isolated. However, two bacteriologically negative animals (no. 48 and 49) had TAT and ME-TAT titers of 1:100, but the agglutination was incomplete in all dilutions and GD results were negative. A weak GD reaction of partial identity was observed in the serum from one culturally negative animal (no. 27) that had significant levels of agglutinins. Unexpectedly, two males that did not have convincing serological evidence of canine brucellosis (no. 38 and 45) were found infected, for small numbers of *B. canis* organisms were isolated in pure cultures from the epididymides and/or prostate gland, but not from the splenic or lymph node tissues. Several unidentified organisms were isolated from tissues of *B. canis*-negative animals, but none were found to cross-react serologically with *B. canis*.

Macroscopic lesions typical of canine brucellosis (9) were observed only in the dogs from which *B. canis* was isolated. All animals with systemic infections had markedly enlarged lymph nodes, some being soft and edematous with areas of hemorrhage, and others firm. Spleens were enlarged, firm, and with nodular

surfaces. Enlarged and firm epididymides were prominent lesions in the infected males, with the exception of the two dogs (no. 38 and 45) with localized infections. The exceptional animals did not have macroscopic lesions.

Properties of the isolates. Results are summarized in Table 2. The isolated strains were nonsmooth (rough) and, with one exception (Mex. 38-76), had growth and colonial characteristics typical of *B. canis*. The exceptional strain, though rough by acriflavine agglutination and crystal violet staining tests, was less mucoid than typical *B. canis*, resembling more closely a less-mucoid variant (7) previously found to be of reduced antigenicity and pathogenicity for dogs (10). With the exception of strain Mex. 38-76, all isolates produced typical ropy growth in broth after several days of incubation.

All strains were non-glucose-fermenting, gram-negative coccobacilli that did not require CO_2 and failed to produce H_2S . They produced catalase, oxidase, and abundant amounts of urease. None were agglutinated by anti-smooth *B. abortus* sera, but they were strongly agglutinated by anti-rough *B. canis* and *B. ovis* sera. However, differences were observed between the reference strain (RM 6-66) and several Mexican strains with regard to nitrate reduction and dye-inhibition characteristics. Three strains (Mex. 25-76, Mex. 51-76, and Mex. 38-76) failed to reduce nitrate, and one (Mex. 11-76) was found unusually active. Diversity also was noted in bacterial growth in the presence of basic fuchsin, usually found strongly inhibitory to *B. canis* at dye concentrations of 1:25,000 to 1:50,000 (1, 7), for five strains (Mex. 2-76, Mex. 3-76, Mex. 25-76, Mex. 51-76, and Mex. 38-76) were not completely suppressed at these concentrations.

Because of these results, further examination of the isolates was done by oxidative-metabolic tests. The identification of all Mexican isolates as *B. canis* was strongly supported by their oxidative-metabolic patterns, which were found similar to those described previously for *B. canis* (1, 22).

Response of dogs to the Mexican isolates. All SPF dogs inoculated with the various strains had a bacteremia within 3 weeks that persisted for at least 14 weeks, when they then were used for other studies. None had febrile responses, although superficial lymph nodes became enlarged after 4 weeks of infection. All animals developed *B. canis* agglutinating and precipitating antibodies within 3 weeks after the inoculations. Immunodiffusion tests of sera from the infected dogs revealed two precipitation lines of identity with the reference strain (RM 6-66) antigen, with the exception of the dog inoculated

TABLE 1. Serological and cultural findings on 22 dogs with presumptive (SAT) evidence of *B. canis* infection

Dog (sex)	SAT serum amt (ml)				Cultural results ^b									
					Serological test ^a									
	0.04	0.02	0.01	0.005	TAT	ME-TAT	GD	Blood	Spleen	Retropharyngeal LN	Inguinal LN	Mesenteric LN	Epididymides	Prostate gland
2(M)	4	4	4	4	1/2,000	1/1,000	ID ^c	+	+4	+4	+4	+4	+4	+4
3(M)	4	4	4	4	1/1,000	1/500	ID	+	+4	+4	+4	+4	+4	+4
11(F)	4	4	4	4	1/500	1/500	ID	+	+4	+4	+4	+4	NT	NT
25(M)	4	4	3	3	1/500	1/500	ID	+	+3	+3	+3	+3	+3	+3
51(F)	4	4	3	1	1/200	1/200	ID	+	+3	+3	+3	+3	+3	+3
38(M)	2	2	2	2	1/100(I)	1/100(I)	Neg.	-	-	-	-	-	+1	+1
45(M)	2	2	0	0	1/100(I)	1/100(I)	Neg.	-	-	-	-	-	-	+1
48(M)	2	1	0	0	1/100(I)	1/100(I)	Neg.	-	-	-	-	-	-	-
49(M)	4	4	2	2	1/100(I)	1/100(I)	Neg.	-	-	-	-	-	-	-
10(M)	2	2	1	0	Neg.	Neg.	Neg.	-	NT	NT	NT	NT	-	-
12(F)	2	2	0	0	Neg.	Neg.	Neg.	-	NT	NT	NT	NT	-	-
13(M)	3	2	1	0	Neg.	Neg.	Neg.	-	-	-	-	-	-	-
15(M)	2	2	1	0	Neg.	Neg.	Neg.	-	-	-	-	-	-	-
17(F)	1	1	0	0	Neg.	Neg.	Neg.	-	-	-	-	-	-	-
20(M)	2	1	0	0	Neg.	Neg.	Neg.	-	-	-	-	-	-	-
27(M)	2	2	1	0	1/100	1/200	ID(P)	-	-	-	-	-	-	-
31(M)	3	2	1	0	Neg.	Neg.	Neg.	-	-	-	-	-	-	-
33(M)	3	2	1	0	Neg.	Neg.	Neg.	-	-	-	-	-	-	-
44(M)	2	1	0	0	Neg.	Neg.	Neg.	-	-	-	-	-	-	-
47(F)	4	4	2	1	Neg.	Neg.	Neg.	-	-	-	-	-	-	-
50(M)	2	2	0	0	Neg.	Neg.	Neg.	-	-	-	-	-	-	-
56(F)	1	1	0	0	Neg.	Neg.	Neg.	-	-	-	-	-	-	-

^a Serological tests used were SAT, TAT, ME-TAT, and GD tests. Results of the SAT recorded as degrees of agglutination, graded from complete (4) to no agglutination (0). TAT and ME-TAT results were recorded as negative (Neg.) when there was no agglutination in serum dilutions of 1:25. Incomplete agglutination (1) is noted where all dilutions failed to completely agglutinate the *B. canis* antigen.

^b Cultural results include only *B. canis* isolations. Plus-four (+4), confluent growth; negative (-), isolation not made. NT, Not tested. LN, Lymph nodes.

^c ID, Two precipitin lines of identity with *B. canis* antigen (RM 6-66), except dog 27, where there was one line of partial identity (P).

TABLE 2. *Properties of the isolates*

Strain	Colony characteristics					Urease ^a activity	Growth on dyes ^b			Agglutination by antisera	
	Acridavine agglutination	Crystal violet stain	Catalase	Oxidase	CO ₂ requirement		H ₂ S production	Nitrate reduction	Thionine (1/25,000)	Basic fuchsin	
										(1:25,000)	(1:50,000)
RM 6-66	+	+	+	+	—	—	+	+	—	+	+
Mex. 2-76	+	+	+	+	—	—	+	+	—	+	+
Mex. 3-76	+	+	+	+	—	—	+	+	±	+	+
Mex. 11-76	+	+	+	+	—	—	+	+	—	+	+
Mex. 25-76	+	+	+	+	—	—	+	+	±	+	+
Mex. 45-76	NT ^c	NT	+	+	—	—	NT	NT	NT	+	+
Mex. 51-76	+	+	+	+	—	—	+	+	±	+	+
Mex. 38-76	+	+	+	+	—	—	+	+	+	+	+

^a Less than 15 min.^b Dye-inhibition tests were done three times, using different dye lots, with identical results.^c NT, Not tested.

with strain Mex. 38-76. Only one precipitation line of identity was observed in the serum from this animal.

DISCUSSION

An unusually high prevalence of canine brucellosis in the Mexico City area is indicated by the isolation of pure cultures of *B. canis* from the blood or tissues from 7 of 59 stray dogs, thus corroborating previous serological findings (13). Although there was a general concordance between the serological and cultural results, when consideration was given to serological values, it is clear that the two methods are not in complete agreement. This is not unexpected, for brucellosis in dogs, as in other species, presents many questions for which answers are not available, as discussed elsewhere (6, 8).

With the relatively small sample represented in this study, it is inappropriate to evaluate critically the performance of the test procedures, but certain inferences seem justified. Several explanations might account for the higher frequency (34%) of "seropositive" animals, as identified by the SAT, if 50% (2-plus) agglutination in the 0.04-ml serum amount were used as the criterion for "positive" status. As noted earlier, the 0.04-ml serum amount approximates the volume delivered by the Canine Brucellosis Diagnostic Kit pipette. The SAT is a presumptive (screening) test (19), having the advantages of speed and sensitivity; however, a recent study (4) indicated that approximately 50% of SAT-positive stray animals did not have diagnostically significant ($\geq 1:200$) TAT titers. In this study we also observed a high rate of reactors when 2-plus or greater agglutination in the 0.04-ml serum amount was scored as positive. Results of the SAT and positive infection status were better correlated, however, when complete agglutinations of the SAT antigen with 0.02-ml serum amounts, or less, were classified as seropositive. Nevertheless, two male animals (no. 38 and 45) that were found to harbor *B. canis* organisms in the epididymides and prostate gland (presumed as chronic infections) did not show significant serological evidence of infection by any of the tests employed, including the SAT.

There appeared to be good correspondence between the TAT, ME-TAT, and GD results, for the animals with systemic infections were readily identified; but all tests failed to indict the male dogs with localized genital tract infections. One animal (no. 27) classified as "seropositive" by the TAT and ME-TAT, but not by the SAT, was found bacteriologically negative, despite repeated isolation attempts; however, a weak precipitin line of partial identity

was observed in GD tests. Immunodiffusion reactions of partial identity also have been observed in sera from North American dogs that had inconclusive *B. canis* agglutinin titers (Flores-Castro and Carmichael, unpublished data). Such findings emphasize the limitations of available diagnostic methods, especially as applied to individual samples which commonly are presented in the United States. Results indicate further the need for more specific test antigens and their extensive field evaluation. The Mexico City dog population would seem a model one for this purpose, since dogs are abundant and *B. canis* appears to have the opportunity for natural "herd" spread because of limited control measures. The close association of dogs with humans also may explain the remarkably high seroprevalence (13.3%) of *B. canis* antibodies in human sera from Mexico City hospitals and clinical laboratories (13). Limited serological surveys of U.S. human populations revealed prevalence rates less than 0.5% (18, 20).

The overall serological results reported in this paper further emphasize that the SAT, though valuable as a screening procedure, must be interpreted cautiously and should be used along with additional confirmatory tests. The data also suggest that the diagnostic value of the SAT would be improved by using smaller test volumes of serum. For example, inclusion in the commercial SAT diagnostic kit of a pipette delivering 0.01 ml would readily allow semiquantitative determinations by varying the number of drops added to the standard volume of test antigen. Gel-diffusion tests appeared to correlate well with infection, especially in animals with TAT or ME-TAT titers $\geq 1:200$. Similar results, using a *B. canis* GD antigen, were obtained by Weber and Krauss (33) and by Myers et al. (25), who employed GD antigen prepared from *B. ovis*. Others (11), however, have found that GD reactions between *B. canis* and *B. ovis* antigens and *B. ovis* antiserum are not entirely homologous. Reactions of identity were observed, however, between the *B. ovis* antigen and *B. canis*, *B. melitensis* 115(R), and *B. ovis* antisera, raising the possibility of false positive GD reactions in field animals infected with nonsmooth bacteria that share antigenic determinants with *B. canis*. This possibility did not occur in the present study, since *B. canis* was isolated from all animals whose sera gave GD reactions of identity with the *B. canis* antigen.

The Mexican isolates all were identified as *B. canis* and characterized by several methods, including oxidative-metabolic activities and the production of brucellosis in dogs by experimental infections with each strain. Although most

isolates corresponded in their properties to *B. canis*, differences were noted between the type strain (RM 6-66) and certain of the isolates with respect to their ability to reduce nitrate and the degree of growth inhibition by basic fuchsin. Differences in the growth of *B. canis* in the presence of basic fuchsin and safranin O also have been observed for a strain (D 519) recovered from a dog in Madagascar (30). Despite these strain differences, pathogenicity of the isolates for dogs were similar.

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LITERATURE CITED

1. Alton, G. C., L. M. Jones, and D. E. Pietz. 1975. Laboratory techniques in brucellosis, 2nd ed., p. 149-153. World Health Organization, Geneva.
2. Barber, D., O. Dimitriu, T. Vasilescu, and A. Cerbu. 1961. Contribution à l'étude de la structure antigénique des *Brucella*. I. Séparation de l'antigène M et de quelques complexes qui le contiennent. Arch. Roum. Pathol. Exp. 20:202-213.
3. Blankenship, R. M., and J. P. Sanford. 1975. *Brucella canis*—a cause of undulant fever. Am. J. Med. 59:424-426.
4. Brown, J., J. Blue, R. E. Wooley, O. W. Dreesen, and L. E. Carmichael. 1976. A sero-survey of a population of Georgia dogs for *Brucella canis* and an evaluation of the slide agglutination test. J. Am. Vet. Med. Assoc. 169:1214-1216.
5. Carmichael, L. E. 1968. Canine brucellosis: isolation, diagnosis, transmission. Proc. U.S. Livestock San. Assoc. 71:517-527.
6. Carmichael, L. E. 1976. Canine brucellosis: an annotated review with selected cautionary comments. Theriogenology 6:105-116.
7. Carmichael, L. E., and D. W. Bruner. 1968. Characteristics of a newly recognized species of *Brucella* responsible for infectious canine abortions. Cornell Vet. 58:579-592.
8. Carmichael, L. E., and L. W. George. 1975. Canine brucellosis: newer knowledge. Dev. Biol. Stand. 31:237-250.
9. Carmichael, L. E., and R. M. Kenney. 1968. Canine abortion caused by *Brucella canis*. J. Am. Vet. Med. Assoc. 152:605-616.
10. Carmichael, L. E., and R. M. Kenney. 1970. Canine brucellosis: the clinical disease, pathogenesis, and immune response. J. Am. Vet. Med. Assoc. 156:1726-1734.
11. Diaz, R., and N. Bosseray. 1973. Identification d'un composé antigénique spécifique de la phase rugueuse (R) des *Brucella*. Ann. Rech. Vet. 4:283-292.
12. El Bahri, L., F. Ben Osman, and A. Chadli. 1970. Serological survey for canine brucellosis in Tunis. Arch. Inst. Pasteur Tunis 47:315-331.
13. Flores-Castro, R., and R. Segura. 1976. A serological and bacteriological survey of canine brucellosis in Mexico. Cornell Vet. 66:347-352.

14. Fredrickson, L. E., and C. E. Barton. 1974. A serological survey for canine brucellosis in a metropolitan area. *J. Am. Vet. Med. Assoc.* **165**:987-989.
15. George, L. W., and L. E. Carmichael. 1974. A plate agglutination test for the rapid diagnosis of canine brucellosis. *Am. J. Vet. Res.* **35**:905-909.
16. Godoy, A. M. 1974. *Brucella canis* in Brasil. *Proc. Natl. Meet. Microbiol. (Brasil)* **5**:77-78.
17. Hill, W. A., G. L. Van Hoosier, and N. McCormick. 1970. Epizootic abortion in a canine production colony. I. Epizootiology, clinical features, and control procedures. *Lab. Anim. Care* **20**:205-208.
18. Hoff, G. L., and N. J. Schneider. 1975. Serologic survey of agglutinins to *Brucella canis* in Florida residents. *Am. J. Trop. Med. Hyg.* **24**:157-159.
19. House, C., F. F. Badakhsh, and L. E. Carmichael. 1975. Review of current aspects of canine brucellosis testing. *Proc. Am. Assoc. Vet. Lab. Diag.* **18**:121-134.
20. Lewis, G. E., and J. K. Anderson. 1973. The incidence of *Brucella canis* antibodies in sera of military recruits. *Am. J. Public Health* **63**:204-205.
21. Lovejoy, G. S., H. D. Carver, I. K. Moseley, and M. Hicks. 1976. Sero-survey of dogs for *Brucella canis* infection in Memphis, Tennessee. *Am. J. Public Health* **66**:175-176.
22. Meyer, M. 1969. *Brucella* organisms isolated from dogs: comparison of characteristics of members of the genus *Brucella*. *Am. J. Vet. Res.* **30**:1751-1756.
23. Moore, J. A. 1969. *Brucella canis* infection in dogs. *J. Am. Vet. Med. Assoc.* **155**:2034-2037.
24. Munford, R. S., R. G. Weaver, C. Patton, J. A. Feeley, and R. Feldman. 1975. Human disease caused by *Brucella canis*. *J. Am. Med. Assoc.* **231**:1267-1269.
25. Myers, D. M., V. M. Varela-Diaz, and E. A. Coltorti. 1974. Comparative sensitivity of gel diffusion and tube agglutination tests for the detection of *Brucella canis* antibodies in experimentally infected dogs. *Appl. Microbiol.* **28**:1-4.
26. Myers, D. M., and A. A. Siniuk. 1970. Preliminary report on the development of a diffusion-in-gel method for the diagnosis of ram epididymitis. *Appl. Microbiol.* **19**:335-337.
27. Pickerill, P. A., and L. E. Carmichael. 1972. Canine brucellosis: control programs in commercial kennels and effect of reproduction. *J. Am. Vet. Med. Assoc.* **160**:1607-1615.
28. Swenson, R. M., L. E. Carmichael, and K. R. Cundy. 1972. Human infection with *Brucella canis*. *Ann. Intern. Med.* **76**:435-438.
29. Taul, L. K., H. S. Powell, and O. E. Baker. 1967. Canine abortion due to an unclassified gram-negative bacterium. *Vet. Med. Small Anim. Clin.* **73**:543-544.
30. Verger, J.-M., M. Gâté, M. Piéchaud, R. Chatelain, J. Ramisse, and J. Blancou. 1975. Isolément de *Brucella suis* biotype 5 à Madagascar, chez une chienne. Validité du nom d'espèce *Brucella canis*. *Ann. Microbiol. (Paris)* **126**:57-74.
31. von Kruedener, R. B. 1974. Isolierung und Bestimmung von *Brucella canis* aus einem Beaglebestand. *Zentralbl. Veterinaarmed.* **21**:307-310.
32. Weber, A., and N. A. Hussein. 1976. Der indirekte immunofluoreszenz Test zum Nachweis von *Brucella canis*-Infektionen. *Zentralbl. Veterinaarmed.* **23**:151-154.
33. Weber, A., and H. Krauss. Über die Brauchbarkeit des Agargelpräzipitationstestes zum Nachweis von *Brucella canis* Infektionen bei Beagle-Hunden. *Berl. Muench. Tieraerztl. Wochenschr.* **88**:425-437.
34. Yamauchi, C., T. Suzuki, T. Nomura, Y. Kukita, T. Iwaki, Y. Kazuno, and A. Ghoda. 1974. Canine brucellosis in a beagle breeding colony. *Jpn. J. Vet. Sci.* **36**:175-182.