A Serological Survey for *Brucella canis* in Dogs in the Province of Quebec

R. HIGGINS, F. HOQUET, R. BOURQUE AND Y. GOSSELIN

SUMMARY

A serological survey for canine brucellosis has been conducted on 341 dogs from different regions of the province of Quebec. A significant titer was found in six sera (1.6%) with the 2-mercaptoethanol tube agglutination test. Only two dogs presented titers ⩾1:200 which is considered as indicative of active infection.

RÉSUMÉ

Une enquête sérologique relative à *Brucella canis*, chez des chiens de la province de Québec

Les auteurs ont effectué une enquête sérologique sur la brucellose canine, chez 341 chiens provenant de différentes régions de la province de Québec. Au total, six sérums (1,6%) se sont avérés positifs (⩾1:100), à l'épreuve d'agglutination en tubes avec le 2-mercaptoéthanol. Par contre,deux chiens seulement ont présenté des titres de 1:200 et plus, ce qui est considéré comme l'indice d'une infection active.

INTRODUCTION

Brucella canis infection in dogs was first reported in 1966 (3). The disease is recognized now as a serious economic problem in breeding kennels and among dogs used in research (11). Canine brucellosis is not readily apparent in immature dogs or nonpregnant females and the clinical manifestations vary in susceptible animals. Abortions and infertility in bitches and epididymitis with scrotal dermatitis in males are the common signs (7). Infected dogs are afebrile and commonly have prolonged bacteremia (ten to 36 months) occurring concurrently with a specific agglutinin response (12). Natural transmission among dogs is most commonly associated with oral or genital contact with infected vaginal discharge, semen, or aborted fetuses and placental tissues (6). The disease is very difficult to eliminate from an individual animal, even with various combinations of drugs (6).

Brucella canis infection in dogs is widespread in the United States (6, 7, 10). It is also known to occur in Japan, West Germany, Brazil, Czechoslovakia, Mexico and Madagascar (9). However, seroepidemiological studies suggest that it is present elsewhere (5). Natural *B. canis* infections have been reported in man (15). Agglutinins against this microorganism have also been detected in cat, racoon, bobcat, red fox and coyote sera (14).

The prevalence of canine brucellosis in Canada is unknown. In this serological study, the natural occurrence of *B. canis* agglutinins in pet dogs in the province of Quebec is reported.

MATERIALS AND METHODS

Blood Specimen Collection — Blood was obtained from 341 dogs of various ages and of both sexes, taken on a random basis from September 1978 through February 1979. Veterinarians in the different clinics and in the veterinary diagnostic laboratories from the areas of Quebec, Montreal, Rimouski, Sherbrooke, Alma, Nicolet and St. Hyacinthe, were invited to collect blood. All blood samples were allowed to clot and were centrifuged at 200 g for 15 minutes. The serum was stored at -20° C. Culture of blood from dogs was not attempted.

Rapid Slide Agglutination Test (RSAT) — Sera were first tested by the rapid slide agglutination test. Canine brucellosis agglutination antigen[1] and a reagent serum kit were obtained from a commercial source. The test was performed as recommended by the manufacturer. Positive canine reference serum[1] was used as a control.

2-Mercaptoethanol (ME) Tube Agglutination Test (ME-TAT) — *Brucella canis* concen-

*Département de Pathologie et de Microbiologie (Higgins, Hoquet, Bourque) et Département de Médecine (Gosselin), Faculté de Médecine vétérinaire de l'Université de Montréal, C.P. 5000, Saint-Hyacinthe, Québec J2S 7C6.

[1]Canine Brucellosis Diagnostic Test, Pitman-Moore Inc., Washington Crossing, N.J. 08560.

TABLE I

SEROPOSITIVE REACTIONS TO *BRUCELLA CANIS* IN DOGS DETECTED BY THE RAPID SLIDE AGGLUTINATION TEST (RSAT) AND THE 2-MERCAPTOETHANOL TUBE AGGLUTINATION TEST (ME-TAT)

Region	No. of animals	Seropositive sera		Titers		
		RSAT	ME-TAT	1:50	1:100	⩾1:200
Alma	24	5(20.8)[a]	1(4. 1)	1	0	0
Montreal	33	9(27.2)	3(9.09)	3	0	0
Nicolet	52	7(13.4)	0(0. 0)	0	0	0
Quebec	145	23(15.8)	4(2. 7)	1	2	1
Rimouski	26	7(26.9)	4(15. 3)	2	2	0
St. Hyacinthe	55	16(29.1)	3(5. 4)	2	0	1
Sherbrooke	6	2(33.3)	0(0. 0)	0	0	0
Total	341	69(20.2)	15(4. 3)	9(2.6)	4(1.1)	2(0.5)

[a]Percentage are indicated in parentheses.

trated antigen[2] and control sera[3] were obtained from the US Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services Laboratory (VSL), Ames, Iowa[4]. The ME-TAT was performed as recommended by VSL and as reported previously (14). All sera were tested in volumes of 0.04, 0.02 and 0.01 mL; 1 mL of formalized 2-ME and 1 mL of formalized *B. canis* test-antigen were added to each of these volumes. The final dilutions of each serum thus tested were 1:50, 1:100 and 1:200. Results were interpreted as complete, incomplete or negative, based on the amount of clearing, as well as the typical pattern of agglutination. Positive and negative reference sera were used as control.

RESULTS

The percentages of reactors for RSAT and ME-TAT were respectively of 20.2 and 4.3. Results are presented in Table I and the prevalence in each region is indicated. A total of nine sera had a titer of 1:50, four sera had a titer of 1:100 and two sera a titer of ⩾1:200, or respectively 2.6%, 1.1% and 0.5%. If titers ⩾1:100 are considered significant, the percentage of seropositive animals is 1.6. Titers ⩾1:200 occurred in the two regions of Quebec and St. Hyacinthe. The regions of Rimouski and Montreal had a higher number of reactors, 15.3% and 9.09% positive respectively, with the ME-TAT. Two sera reacting negatively with the RSAT and had suspicious reactions with the ME-TAT (⩽1:50).

DISCUSSION

The purpose of the present study was to determine the prevalence of agglutinins to *B. canis* in dogs in the province of Quebec. Bacterial isolation was not attempted in the seropositive animals. However, among the 341 dogs tested, only two are likely to have a positive blood culture, since dogs with titers of 1:200 or greater generally are bacteremic whereas dogs with titers of 1:100 to 1:200 are not (13).

A total of 54 false-positive reactions were found with the RSAT. Two sera were negative with the RSAT, whereas they were suspicious (⩽1:50) with the ME-TAT. Similar findings have been already reported (1, 8). In spite of those two reactions, the present results indicate that negative RSAT results do not appear to require confirmation, as mentionned by other authors (8, 11).

Titers ⩾1:200 are considered indicative of active *B. canis* infection in dogs (10). But utilizing the criterion of the 1:100 titer to determine if a serum sample is positive or not (2), the present data demonstrated that six of the 341 dogs (1.6%) were positive for *B. canis* antibodies. Titers 1:50 are not considered as positive and some authors have mentionned

[2]Serial No. 17701, US Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services Laboratory, Ames, Iowa.

[3]Serial No. 12512, 12711, 12510, US Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services Laboratory, Ames, Iowa.

[4]Dr. G.M. Brown, US Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services Laboratory, Ames, Iowa.

that cross-reactions between *B. canis* and other microorganisms as *Bordetella bronchiseptica* (7), *Pasteurella multocida* (12) and *Moraxella* spp (9) could be responsible for such low titers.

The animals tested in the present work are almost all pet dogs. Their infection rate in Quebec is relatively low, but the stray dog population is probably another matter. In other studies, the percentage of positive reactions is higher in stray dogs than in pet dogs (2, 10). Studies should be undertaken to determine the prevalence of the disease in stray dogs in Canada. There was no correlation between seropositivity and sex or age.

Brucella canis infections are endemic and can give rise to the suspicion that the zoonotic potential may be greater than suspected. Veterinarians must be informed about the problem and control measures are essential to prevent the emergence of the disease in Canada.

ACKNOWLEDGMENTS

The authors wish to acknowledge the collaboration of Drs. R. Letarte, G. Marsolais and veterinarians of the Ministry of Agriculture of Quebec for collecting the sera.

REFERENCES

1. BROWN, J., J.L. BLUE, R.E. WOOLEY and D.W. DREESEN. *Brucella canis* infectivity rates in stray and pet dogs populations. Am. J. publ. Hlth 66:889-892. 1966.
2. BROWN, J., J.L. BLUE, R.E. WOOLEY, D.W. DREESEN and L.E. CARMICHAEL. A serologic survey of a population of Georgia dogs for *Brucella canis* and an evaluation of the slide agglutination test. J. Am. vet. med. Ass. 169:214-216. 1979.
3. CARMICHAEL, L.E. Abortion in 200 beagles (News report). J. Am. vet. med. Ass. 149:1126. 1966.
4. CARMICHAEL, L.E. Canine brucellosis: An annotated review with selected cautionnary comments. Theriogenology 6:105-116. 1976.
5. CARMICHAEL, L.E. and L.W. GEORGE. Canine brucellosis: newer knowledge. Dev. Biol. Stand. 31:237-250. 1976.
6. CARMICHAEL, L.E. and R.M. KENNEY. Canine abortion caused by *Brucella canis*. J. Am. vet. med. Ass. 152:605-616. 1968.
7. CARMICHAEL, L.E. and R.M. KENNEY. Canine brucellosis: The clinical disease pathogenesis, and immune response. J. Am. vet. med. Ass. 156:1726-1734. 1970.
8. FLORES-CASTRO, R. and L.E. CARMICHAEL. Canine brucellosis. VI. Current status of methods for diagnosis. Cornell Vet. 68:76-88. 1978.
9. FLORES-CASTRO, R., F. SUAREZ, C. RAMIREZ-PFEIFFER and L.E. CARMICHAEL. Canine brucellosis: Bacteriological and serological investigation of naturally infected dogs in Mexico city. J. clin. Microbiol. 6:591-597. 1977.
10. FREDERICKSON, L.E. and C.E. BARTON. A serologic survey for canine brucellosis in a metropolitan area. J. Am. vet. med. Ass. 165:987-989. 1974.
11. GALPHIN, S.P. A serologic survey for *Brucella canis* in dogs on a military base. J. Am. vet. med. Ass. 171:728-729. 1977.
12. HOFF, G.L., W.J. BIGLER, D.O. TRAINER, J.G. DEBBIE, G.M. BROWN, W.G. WINKLER, S.H. RICHARDS and M. REARDON. Survey of selected carnivore and opossum serums for agglutinins to *Brucella canis*. J. Am. vet. med. Ass. 165:830-831. 1974.
13. LEWIS, G.E. A serological survey of 650 dogs to detect titers for *Brucella canis* (*Brucella suis*, type 5). J. Am. anim. hosp. Ass. 8:102-107. 1972.
14. RANDHAWA, A.S., W.H. DIETERICK, C.C. HUNTER, V.P. KELLY, T.C. JOHNSON, B. SVOBODA and D.F. WILSON. Prevalence of seropositive reactions to *Brucella canis* in a limited survey of domestic cats. J. Am. vet. med. Ass. 171:267-268. 1977.
15. SWENSON, R.M., L.E. CARMICHAEL and K.R. CUNDY. Human infection with *Brucella canis*. Ann. Intern. Med. 76:435-438. 1962.