

## Improved Rapid Slide Agglutination Test for Presumptive Diagnosis of Canine Brucellosis

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Received 8 May 1981/Accepted 11 September 1981

A modified rapid slide agglutination test for the presumptive identification of *Brucella canis* infection in dogs has been developed. The method required mixing 0.1 ml of canine serum with 0.1 ml of 0.2 M 2-mercaptoethanol solution. Equal volumes (0.05 ml) of the treated serum and the *B. canis* plate antigen were mixed. Agglutination results were read within 2 min. Clinical studies showed 100% agreement between this method and the conventional 2-mercaptoethanol tube agglutination test. Excellent correlation was shown between cultural isolation and the modified rapid slide agglutination test, using sera from experimentally infected dogs.

The basic procedures for canine brucellosis serodiagnosis are the 2-mercaptoethanol tube agglutination test (2ME-TAT) (1) and the rapid slide agglutination test (RSAT; Pitman-Moore, Inc., Washington Crossing, N.J.). The 2ME-TAT procedure has technical disadvantages that limit its widespread use in field evaluation of the disease: improper samples (dog blood hemolyzes readily, making it unsatisfactory for this test), inability to detect the low antibody titers of some chronically infected yet abacteremic dogs, failure of some infected bacteremic dogs to develop significant levels of agglutinins, and prozone phenomena (2). Furthermore, the procedure requires 48 h to obtain results. Hemolyzed specimens are acceptable for RSAT, and positive sera demonstrating prozone by the tube agglutination test give positive results by RSAT. This test has proven itself to be more acceptable for use by veterinarians. It is highly accurate for the identification of noninfected dogs because false-negative results have rarely been observed. We reported previously that RSAT is the superior procedure for the identification of the early stages of *Brucella canis* infection (3). The RSAT results were found to correlate well with those obtained by the 2ME-TAT technique in comparisons involving 2,300 canine sera (unpublished data from sera obtained from the National Veterinary Services Laboratory, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, Ames, Iowa). However, during several years of usage of the commercially distributed RSAT diagnostic kit, it was recognized that sera from healthy dogs that were culturally negative for *B. canis* occasionally react positively in the RSAT but not in the 2ME-TAT. In an attempt to eliminate the dis-

crepancy between the RSAT and 2ME-TAT, modification of the RSAT was explored.

The occurrence of nonspecific agglutinins in the sera of normal dogs has been reported before (P. A. Pickerill, Ph.D. thesis, Cornell University, Ithaca, N.Y., 1970). Pickerill and Carmichael (4) and Brown et al. (G. M. Brown, D. E. Pietz, and C. R. Ranger, Proc. U.S. Anim. Health. Assoc. 16:532, 1973) have shown that certain nonspecific agglutinins are removed from canine sera when the 2ME-TAT is employed. Therefore, the use of 2-mercaptoethanol in the RSAT procedure (2ME-RSAT) for the removal of nonspecific agglutinins was investigated in our laboratory.

### MATERIALS AND METHODS

**Sera.** Dog sera, stored at  $-20^{\circ}\text{C}$ , were from the following groups: (i) 27 random-source dogs experimentally infected with *B. canis*; (ii) 14 specific pathogen-free beagles experimentally infected with *B. canis*; (iii) 92 field serum samples from dogs of unknown history. (Contaminated sera were filtered through 0.2- $\mu\text{m}$  membrane filters before serological tests were performed.)

**Serological tests.** (i) The 2ME-TAT was performed by the recommended procedure of the U.S. Department of Agriculture Veterinary Diagnostic Laboratory, Ames, Iowa. The antigen for the test was obtained from Diagnostic Reagents Section of that laboratory. (ii) The RSAT procedure described in the package insert of the commercially distributed test kit (Pitman-Moore, Inc.) was performed on each serum. (iii) For the 2ME-RSAT, 0.1 ml of serum was mixed with 0.1 ml of 0.2 M 2-mercaptoethanol solution in a disposable plastic tube. After being mixed vigorously for 2 to 3 s, 0.05 ml of the 2-mercaptoethanol-treated serum was then used as in the RSAT procedure.

To determine the dilution effect of 2-mercaptoethanol diluent on RSAT results, aliquots of 39 sera (12

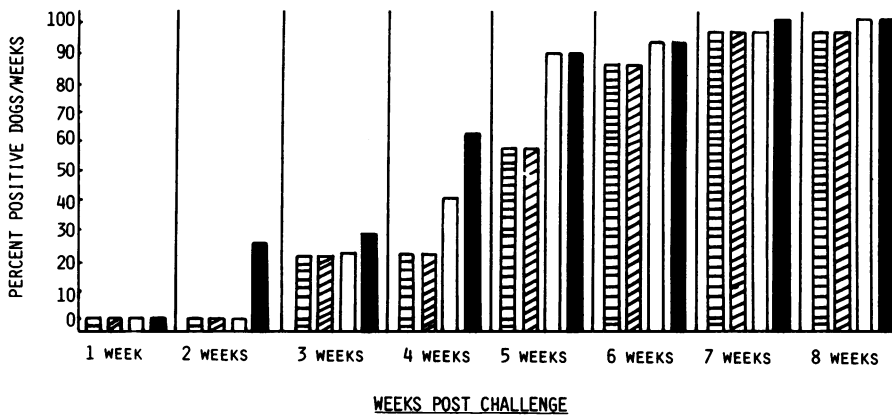


FIG. 1. Percent cumulative comparison of positive 2ME-TAT (▨), 2ME-RSAT (▩), cultural isolation (□), and RSAT (■) results on sera from 27 *B. canis*-infected dogs.

known *B. canis*-positive sera and 27 sera positive by RSAT but not by 2ME-TAT) were diluted with an equal volume of diluent-buffer with or without 2-mercaptoethanol and tested by the RSAT.

**RESULTS**

Serological and bacteriological findings for 27 experimentally infected dogs are presented in Fig. 1. *B. canis* infection was detected by the RSAT 2 weeks after challenge, which was 1 week before positive results were obtained by the 2ME-TAT, 2ME-RSAT, or blood culture techniques. By week 7, the differences between the four diagnostic procedures were slight. During weeks 3 through 5, the RSAT was either as likely as or more likely than blood culture to detect infection. Both were positive with greater frequency than the 2ME-TAT procedure. In all instances, when the 2ME-TAT was positive, the 2ME-RSAT also was positive for the same serum.

Serological evaluation of 14 experimentally infected specific pathogen-free dogs by the four diagnostic procedures is presented in Table 1. There was excellent correlation (12 of 14 [86%]) between cultural isolation and 2ME-RSAT results.

Table 2 presents the results of 92 field serum samples tested by the three serological procedures (RSAT, 2ME-RSAT, and 2ME-TAT). The 15 sera found positive by 2ME-TAT also were positive by 2ME-RSAT and RSAT. The 77 sera determined to be negative by 2ME-TAT also were negative by 2ME-RSAT; however, only 32 samples were negative by the RSAT procedure.

To determine the dilution effect of the 2-mercaptoethanol reagent on test results, several sera positive in both the RSAT and 2ME-TAT and all 27 sera positive only by the RSAT were diluted with an equal volume of buffer and tested

by the RSAT. The results compared with those obtained in the 2ME-RSAT are presented in Table 3. In the absence of 2-mercaptoethanol in the diluent, the 27 RSAT-positive and 2ME-TAT-negative sera remained positive. Addition of 2-mercaptoethanol to the diluent resulted in negative reactions of these 27 sera, whereas the sera previously found positive in both the RSAT and 2ME-TAT remained positive.

**DISCUSSION**

The 2ME-TAT and RSAT are two currently recommended procedures for serodiagnosis of canine brucellosis. The results of the RSAT and 2ME-TAT correlate well in experimentally infected dogs. In field situations, where little is

TABLE 1. Serological and bacteriological evaluations of 14 specific pathogen-free beagles experimentally infected with *B. canis*

Sex	Dog no.	RSAT <sup>a</sup>	2ME-RSAT <sup>a</sup>	Cultural isolation
M	34	4+	4+	+
	35	4+	4+	+
	36	±	-	-
	37	4+	4+	+
	38	2+	2+	-
	40	4+	3+	+
	41	4+	4+	+
F	34	4+	4+	+
	35	4+	4+	+
	36	±	-	-
	37	4+	4+	+
	38	±	-	-
	40	4+	4+	+
	41	4+	4+	-

<sup>a</sup> Decreasing slide agglutination scored from 4+ to -.

TABLE 2. Comparison of 2ME-TAT, RSAT, and 2ME-RSAT, using 92 selected canine sera<sup>a</sup>

2ME-TAT	(no.)	No. of sera			
		RSAT		2ME-RSAT	
		+	-	+	-
+	(15)	15	0	15	0
-	(77)	45	32	0	77

<sup>a</sup> The 92 sera were selected from field samples taken during several years.

known about the history of the animal, there are occasions when a clinically healthy dog culturally negative for *B. canis* will react positively in the RSAT but not in the 2ME-TAT. Such an animal is identified as a false-positive reactor.

Certain antibodies have been shown to cause nonspecific agglutination of *B. canis* antigens, and the use of 2-mercaptoethanol in the tube test aids in the removal of this type of nonspecific agglutination (2). Our results demonstrated clearly that the use of 0.2 M 2-mercaptoethanol solution as outlined in the modified RSAT procedure also was effective in the removal of such nonspecific antibodies. This procedure was further shown to produce results in agreement with those of the 2ME-TAT (Fig. 1).

In the study with experimentally infected dogs, all animals were negative by the RSAT, 2ME-RSAT, and 2ME-TAT at 1 week postinoculation with virulent *B. canis*. Only the RSAT detected agglutinins at 2 weeks postchallenge (7 of 27 [26%]) due to the presence of specific immunoglobulin M antibodies formed in the early stages of *B. canis* infection. This shows the greater sensitivity of the RSAT in the early diagnosis of *B. canis* infection over the other tests that utilize 2-mercaptoethanol. Figure 1 further shows that the results of both 2ME-TAT and 2ME-RSAT correlated closely with cultural isolation after 5 to 6 weeks postinoculation in the experimentally infected dogs. In experimentally infected specific pathogen-free beagles, excel-

lent correlation was demonstrated between the 2ME-RSAT and *B. canis* isolation. (Table 1).

When the 2ME-RSAT was compared with the RSAT and 2ME-TAT, using 92 selected field serum samples, there was excellent (100%) correlation between the 2ME-TAT and 2ME-RSAT (Table 2). However, the RSAT detected agglutinins more frequently than the 2ME-TAT or 2ME-RSAT (60 versus 15) (Table 2). Sera that were positive by the RSAT but negative by the 2ME-TAT were classified as false-positive. Due to the unfavorable condition of many sera (samples that were contaminated or samples from patients under antibiotic therapy) submitted to our laboratory, no attempts to isolate *B. canis* were made. It should be emphasized that the above sera were selected from field serum samples taken during several years for their discrepancies between the 2ME-TAT and RSAT results.

When the dilution effect of the 2-mercaptoethanol diluent was tested, the results showed that in all instances the serum samples from *B. canis*-infected dogs remained positive after dilution with buffer either with or without 2-mercaptoethanol. On the other hand, the false-positive sera remained positive by the modified RSAT procedure after dilution with the diluent alone, but became negative in the presence of diluent containing 2-mercaptoethanol. This indicates that 2-mercaptoethanol in the diluent is responsible for removal of the activity of nonspecific agglutinins (Table 3).

**Proposed test procedure for presumptive diagnosis of canine brucellosis.** The greater sensitivity of the RSAT for identification of *B. canis* infection earlier than serological procedures utilizing 2-mercaptoethanol and the accuracy of the RSAT in identifying noninfected animals make retention of the present RSAT protocol desirable as stage 1 of a testing procedure.

If the serum is negative by stage 1, no further testing is required. The animal is considered not to be infected with *B. canis*. Because of the occasional detection by RSAT of nonspecific agglutinins that are eliminated by treatment with 2-mercaptoethanol and the demonstrated high degree of correlation between the 2ME-TAT and 2ME-RSAT procedures, it is essential that a second stage of testing (2ME-RSAT) be conducted on RSAT-positive serum samples.

If the RSAT-positive sample also tests positive by 2ME-RSAT, the animal is presumptively diagnosed as being infected with *B. canis*. Blood should then be cultured for *B. canis* isolation.

If, on the other hand, the RSAT-positive sample tests negative by 2ME-RSAT, the animal may be in the early stage of *B. canis* infection (antibody predominantly of specific immunoglobulin M type), or alternatively, its serum may

TABLE 3. Effect of diluent with and without 2-mercaptoethanol on RSAT

Sera tested (no.)	No. positive/no. tested		
	RSAT	Modified RSAT <sup>a</sup>	Modified 2ME-RSAT <sup>b</sup>
<i>B. canis</i> positive (12)	12/12	12/12	12/12
False-positive (27)	27/27	27/27	0/27

<sup>a</sup> Serum diluted 1:2 (diluent containing no 2-mercaptoethanol).

<sup>b</sup> Serum diluted 1:2 (diluent containing 2-mercaptoethanol).

contain nonspecific macroglobulin agglutinins to *B. canis*. To distinguish between these two conditions, a second serum sample should be collected in approximately 30 days and retested by only the 2ME-RSAT procedure. If this sample tests positive, the animals should be presumptively diagnosed as having *B. canis* infection.

It should be noted that definitive diagnosis of canine brucellosis is based upon isolation of *B. canis* from the animal.

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