Reduction of Brucella Species and Francisella tularensis Cross-Reacting Agglutinins by Dithiothreitol

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Cross-reaction agglutinin titers to Brucella abortus antigen were found in 42 of 128 tularemia serum specimens, and cross-reaction titers to Francisella tularensis antigen were found in 8 of 34 brucellosis serum specimens. The cross-reaction titers were reduced to 10 or less by dithiothreitol, suggesting that the titers are due to immunoglobulin M antibody.

Francis and Evans (4) demonstrated that Brucella species and Francisella tularensis contain common antigens which can result in serological cross-reactions. The cross-reactions were not considered to be a problem because of the clinical differences between the two diseases (6).

Anderson et al. (1) were able to determine the immunoglobulin G (IgG) portion of Brucella agglutination titers by the use of 2-mercaptoethanol, which inactivates the IgM agglutinin. We recently reported on the use of dithiothreitol (DTT) in place of 2-mercaptoethanol for the determination of IgG Brucella agglutinins (5). The purpose of this investigation was to determine whether IgM or IgG agglutinins are responsible for Brucella and F. tularensis cross-reactions by the use of DTT.

Serum specimens. The sera used in this study were sent to the Centers for Disease Control through various state health departments for the determination of F. tularensis and Brucella agglutinin titers. Of the specimens, 128 were from patients suspected of having tularemia and had elevated (≥160) F. tularensis agglutinin titers, and 34 were from suspected cases of brucellosis and had elevated (≥160) Brucella agglutinin titers.

Reference sera. Reference (control) sera of known high and low F. tularensis and Brucella agglutinin titers were obtained from the Biological Products Division of the Centers for Disease Control.

Antigen suspensions. The F. tularensis stock suspension of antigen was obtained from the Biological Products Division of the Centers for Disease Control. The working suspension used in the tests was a 1:10 dilution of the stock suspension in phosphate-buffered saline, pH 7.2, containing a final concentration of 0.005% safranin O. The Brucella abortus (strain 1119-3) stock suspension of antigen was prepared and standardized by the National Animal Disease Laboratory, U.S. Department of Agriculture, Ames, Iowa. The working suspension was a 1:50 dilution of the stock suspension in phosphate-buffered saline, pH 7.2, containing a final concentration of 0.005% safranin O.

Routine F. tularensis microagglutination test (MAT). The MAT for F. tularensis has been described in detail previously (3).

Routine Brucella MAT. The routine MAT for the detection of Brucella agglutinins has been described previously (2).

DTT MAT. The DTT MAT tests for the determination of F. tularensis and Brucella IgG agglutinin titers followed the same procedure as the routine MAT, except that 0.005 M DTT was included in the phosphate-buffered saline serum diluent. The final concentration of DTT after the addition of antigen to the tests was 0.0025 M. Reference sera of known low and high titer were included in each run as controls. Phenol must not be present in the test system because it interferes with the action of the DTT.

The serum specimens were screened for serological cross-reactions at a 1:20 dilution of the specimen. Forty-eight of the 128 tularemia specimens with elevated (≥160) F. tularensis agglutinin titers had cross-reaction titers ≥ 20 with Brucella antigen. Eight of the 34 brucellosis specimens with elevated (≥160) Brucella agglutinin titers had cross-reaction titers ≥ 20 with F. tularensis antigen. Six of the 48 tularemia specimens with cross-reaction titers did not have a sufficient volume to do additional tests. Thus, there were 42 tularemia and 8 brucellosis specimens available for the determination of cross-reaction endpoints with the routine agglutination test and the DTT agglutination test.

The distribution of the routine agglutination test cross-reaction titers <160 and their DTT test titers are shown in Table 1. The cross-
that the specimens tularemia. The results reduced to <10 dtu.

titers of the 35 tularemia specimens ranged from 20 to 80, and the cross-reaction titers of the 7 brucellosis specimens ranged from 20 to 40. These titers were reduced to 10 or less in the DTT test for all but one specimen.

The routine agglutination test cross-reaction titers ≥160 and their DTT test titers are shown in Table 2. Seven (16.7%) of the 42 tularemia specimens had Brucella cross-reaction titers ≥160. Two of the tularemia specimens, T-33 and T-74, had Brucella agglutinin titers that were higher than the standard MAT F. tularensis titers. The Brucella agglutinin titers of these specimens dropped to <10 and 10, respectively, in the DTT tests. The cross-reaction titers of the remaining five tularemia specimens were reduced to <10 in the DTT test for all but one specimen. One of the eight brucellosis specimens had an F. tularensis cross-reaction titer of 320, which was reduced to 20 in the DTT test. The results obtained with the DTT test on the 42 tularemia specimens and the 8 brucellosis specimens which had cross-reaction titers suggest that the cross-reaction agglutinin titers are due primarily to IgM antibody. We do not recommend the routine use of DTT in the F. tularensis MAT because it also reduces specific F. tularensis IgM antibody.

**LITERATURE CITED**


