

## *Serological Activity of Brucella Abortus Antiserum Following Contamination and Storage<sup>1</sup>*

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**B**OVINE SERUM SAMPLES collected for a measurement of *Brucella abortus* agglutinins are often obtained under field conditions that may result in contamination of the blood samples. The effect of such contamination upon subsequent serological tests is not well known. Kahn (1925) states that the precipitin titer of contaminated sera did not change materially upon storage, but that the titer judged by complement fixation did. Kolle and Hetsch (1935) report a general decrease in agglutinin titer following storage of sterilized serum. The investigations reported below give additional information upon the effect of contamination and storage of antiserum upon specific agglutination tests.

### *Experimental Methods*

Test-tube and rapid-slide-agglutination tests were made according to the procedures of Huddleson (1943). In both instances the antigens were furnished by the U. S. Bureau of Animal Industry. Bovine serum samples, both positive and negative for agglutinins of *Brucella abortus*, were obtained from animals previously vaccinated with *B. abortus* strain 19.

Aliquots of positive and negative sera were contaminated with strains of *Streptococcus liquefaciens*, *Sarcina lutea*, *Proteus vulgaris*, *Micrococcus pyogenes* var. *aureus*, and *Escherichia coli* by adding approximately 1,000,000 bacteria of a single species for each ml. volume. Separate aliquots were contaminated with viable and heat-killed suspensions of each bacterial species. Viability or sterility, as the test required, was determined by streaking plates of Difco tryptose agar. Contaminated serum samples were centrifuged before preparing agglutination tests. Immediately after being harvested, all serum samples were filtered through a Seitz filter to assure sterility.

At the start, before aliquots were contaminated, test-tube and rapid-slide-agglutination tests were performed on positive and negative samples of serum. Both types of samples were tested for their content of heterologous agglutinins against each of the contaminating species. All homologous agglutination tests involving serum samples initially positive were done in replicates of five and,

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for samples contaminated with *E. coli* and *S. lutea*, in replicates of 20. Tests using negative serum samples were performed in replicates of 20. In most instances agglutinations were read after 48 hours incubation at 37°C without refrigeration. The greatest dilution of serum giving complete agglutination was recorded as the titer.

Following contamination, samples were stored at room temperature and tested during a three-day period. Duplicate specimens were stored at 4°C and tested over a two-month period.

### Experimental Results

Preliminary experiments were run to establish the incubation conditions required for maximum agglutination. For test-tube agglutinations, maximum titers were found regularly after 48 hours at 37°C (Table 1). Comparison

TABLE 1. INFLUENCE OF INCUBATION UPON AGGLUTINATION TITERS

Serum No.	Conditions of Incubation		
	37°C—48 hours	55°C— 4 hours 4°C—24 hours	55°C— 4 hours 4°C—32 hours
96	640 <sup>1</sup>	320	640
98	640	320	640
4c	2560	320	2560
5c	1280	160	1280
6c	2560	160	2560
7c	2560	320	2560
8c	2560	320	2560
9c	1280	160	1280
103a	1280	160	1280
104a	640	80	640

<sup>1</sup> Titer expressed as reciprocal of fraction of greatest dilution showing complete agglutination.

of the maximum titers found at 37° C with those found after incubation at 55°C showed that refrigeration for 24 hours (Huddleson, 1943) was not satisfactory for the 10 sera tested. Refrigeration for at least 32 hours was necessary.

TABLE 2. HETEROLOGOUS AGGLUTINATION BY BOVINE SERA POSITIVE AND NEGATIVE FOR *B. ABORTUS* AGGLUTININS

Antigen	Homolo- gous	<i>S.</i> <i>liquefaciens</i>	<i>S. lutea</i>	<i>M. pyogenes</i> var. <i>aureus</i>	<i>E. coli</i>	<i>P. vulgaris</i>
Titer <sup>1</sup>	50	80	0	40	10	40
	0	0	0	0	0	0

<sup>1</sup> Reciprocal of fraction of greatest dilution showing complete agglutination.

TABLE 3. INFLUENCE OF CONTAMINATION AND STORAGE UPON THE HOMOLOGOUS TITER OF BOVINE ANTI-B. ABORTUS SERA

Contaminant	Via- bility	Initial Titer <sup>1</sup>		Days stored at 4°C									
		(After con- tamination)		Days stored at 20-25°C		14		30		60			
		T.T.	R.S.	T.T.	R.S.	T.T.	R.S.	T.T.	R.S.	T.T.	R.S.		
Control (none)		40	50±	40	25	40	25	40	25	40	25	40	25
<i>E. coli</i>	+	40	50	40	25	40	25	40	25	80	25	80	25
<i>E. coli</i>	-	40	50	40	50	40	50	40	25	40	100	40	100
<i>M. pyogenes</i> var. <i>aureus</i>	+	40	50	40	50	40	50	40	25	80	50	80	50
<i>M. pyogenes</i> var. <i>aureus</i>	-	20	50	40	50	40	50	40	25	40	100	40	100
<i>P. vulgaris</i>	+	80±	50	40	50	40	50	40	50	80	50	80	50
<i>P. vulgaris</i>	-	40	40	40	50	40	50	40	50	50	100	80	100
<i>S. liquefaciens</i>	+	40	50	40	50	40	50	40	50	80	50	80	50
<i>S. liquefaciens</i>	-	40	50	40	50	40	50	40	50	80	100	80	100
<i>S. lutea</i>	+	40	50	40	50	40	50	40	50	40	—	40	—
<i>S. lutea</i>	-	40	50	40	50	40	50	40	50	40	100	40	100
Control (none)		0	0	0	0	0	0	10	0	10	0	10	0
<i>E. coli</i>	+	0	0	0	0	10	0	10	0	10	0	10	0
<i>E. coli</i>	-	0	0	0	0	0	0	10	0	10	0	10	0
<i>M. pyogenes</i> var. <i>aureus</i>	+	0	0	0	0	10	0	10	0	10	0	10	0
<i>M. pyogenes</i> var. <i>aureus</i>	-	0	0	0	0	0	0	10	0	10	0	10	0
<i>P. vulgaris</i>	+	0	0	20	0	20	0	20	0	20	0	20	0
<i>P. vulgaris</i>	-	0	0	0	0	0	0	10	0	10	0	10	0

<sup>1</sup> = reciprocal of fraction of greatest dilution showing complete agglutination.

T.T. = test-tube agglutination.

R.S. = rapid-slide agglutination.

Heterologous agglutination was found when each of the contaminating species was tested by the test-tube procedure with bovine anti-*B. abortus* serum, which had demonstrable homologous titer. No heterologous agglutination was observed when serum having no *B. abortus* agglutinins was tested against each of the contaminants. Heterologous titers were not observed in rapid-slide agglutinations. These data are shown in Table 2.

The titers obtained from homologous agglutination with contaminated serum samples are shown in Table 3. Samples in which a significant, though relatively low, titer of *B. abortus* agglutinins existed at the start showed no appreciable change in serological activity when they were contaminated and stored at room temperature for three days (Table 3). When duplicate portions of these contaminated serum samples were stored at 4°C, increases in agglutinin titers usually occurred after one month. Similar results were obtained irrespective of the species and whether the contaminating bacterial suspension was viable, the exception being *S. lutea* for which no changes were noticed. Such increases usually were not found in the uncontaminated control serum. Serum samples that at the start showed no titer of *B. abortus* agglutinins were not changed appreciably by contamination, except for those contaminated with *P. vulgaris*. No significance is attached to agglutinations occurring only at a titer of 1:10.

Additional experiments were run to determine the influence of storage upon uncontaminated high titered bovine anti-*B. abortus* serum. The filter-sterilized samples, each representing a different animal, were held at 4°C and tested over a five-month period. These data are shown in Table 4.

TABLE 4. INFLUENCE OF STORAGE AT 4°C ON HOMOLOGOUS TITER OF BOVINE ANTI-*B. ABORTUS* SERUM

Days from start	Titer <sup>1</sup> of sample number								
	4c	5c	6c	7c	8c	9c	96	103a	104a
1	2560	1280	2560	2560	2560	1280	640	1280	640
96							640		
100	2560	1280	2560	2560	2560	1280		1280	1280
147							640		

<sup>1</sup> Reciprocal of fraction of greatest serum dilution showing complete agglutination.

### Discussion

It is of seeming interest, though unexplainable, that a relationship existed between the occurrence of heterologous agglutination and the ability of contaminating bacterial species to alter homologous titers. This relationship is

summarized in Table 5. It may be seen that the increase in homologous titer following contamination of serum samples with either viable or nonviable bacterial cells varied generally as the heterologous titer. No increase in homologous titer occurred in the absence of heterologous agglutination of the contaminating species. Bovine serum negative for agglutinins of *B. abortus* was not altered in serological activity by contamination, except possibly in the instance of contamination by viable cells of *P. vulgaris*. Heterologous agglutination, *per se*, is commonly attributed to normal antibodies. While this concept could explain the heterologous titers observed here, it does not explain the apparent relationship to the homologous titer. The bovine sera used in the experiments were obtained from calves that had been immunized by the customary single-injection procedure of veterinarians. Single-injection immunizations usually result in more homogeneous antibody types than do multiple-injection immunizations (Landsteiner, 1945). On this basis one would not anticipate sufficient nonhomogeneity of antibody types to explain the results. It seems apparent that the homologous antibody types dealt with here were capable of broad serological activity.

Nonspecific cold agglutinins can hardly explain the increases in homologous titer since all of the test-tube agglutinations were incubated at 37°C and read without refrigeration. Increases in homologous titer were also observed in the rapid-slide procedure.

The element of chance variation, for these experiments, was considerably reduced through replication of the tests. It is apparent that heavy contamination of bovine anti-*B. abortus* serum would not alter the homologous titer if the

TABLE 5. RELATIONSHIP BETWEEN HETEROLOGOUS TITER AND INCREASES OF HOMOLOGOUS TITER, BOVINE ANTI-*B. ABORTUS* SERUM

Contaminant	Relative difference in homologous titer following 2 months in storage, 4°C				Heterologous titer at start
	Viable contaminant		Nonviable contaminant		
	T.T.	R.S.	T.T.	R.S.	
Control	0	0	0	0	0
<i>S. lutea</i>	0	0	0	1	0
<i>E. coli</i>	1	0	0	1	10
<i>M. pyogenes</i> var. <i>aureus</i>	1	0	0	1	40
<i>P. vulgaris</i>	1	0	1	1	80
<i>S. liquefaciens</i>	1	0	1	1	80

0 = no increase.  
1 = increase corresponds to 1 dilution.

T.T. = test-tube agglutination.  
R.S. = rapid-slide agglutination.

serum sample in question were tested for agglutinins within a three-day period, assuming a storage temperature of 25°C or less. Such results are of practical consequence.

### *Summary*

1. Storage for as long as 147 days at 4°C did not result in any detectable reduction in the homologous titer of sterile bovine anti-*Brucella abortus* serum.
2. Samples of bovine *B. abortus* antiserum having a homologous titer showed heterologous agglutination of *Streptococcus liquefaciens*, *Proteus vulgaris*, *Micrococcus pyogenes* var. *aureus*, and *Escherichia coli*. Samples having no measurable homologous titer failed to show heterologous agglutination.
3. Contamination of positive and negative bovine serum with viable or non-viable suspensions of bacteria did not alter the serological activity during storage for three days at room temperature.
4. Homologous titers of bovine anti-*B. abortus* serum were increased following contamination by the bacterial species shown above after storage for 1-2 months at 4°C. Serum samples negative at the start were not affected by contamination and storage.

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