THE MICROAGGLUTINATION TEST VERSUS THE TUBE AGGLUTINATION TEST IN THE DETECTION OF ANTIBODY AGAINST *SALMONELLA PULLORUM* AND *SALMONELLA TYPHIMURIUM* IN TURKEYS

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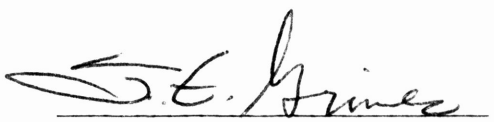

J. E. Grimes

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ABSTRACT

Microagglutination tests conducted on field collected sera resulted in 32.7% agreement with the tube agglutination test for *Salmonella pullorum*. A 36.2% agreement resulted in similar testing for *S. typhimurium*.

Agreement obtained using both stained and unstained antigens in microagglutination test was 22.6% and 32.8% for *S. pullorum* and *S. typhimurium* respectively.

The microtest was sufficiently sensitive to detect infected turkeys. However, further comparison should be done before use of the microtest in place of the tube test.

Results of tests on sera from turkeys inoculated with killed antigens of *S. pullorum* and *S. typhimurium* were inconclusive. The experiment should be repeated using live organisms in order to obtain better antibody production.

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INTRODUCTION

The tube agglutination test is currently the most widely used test for the detection of *Salmonella pullorum* and *S. typhimurium* in turkeys. The test is conducted by procedures approved by The National Poultry Improvment Plan (NPIP) (1). The microagglutination test also has been approved by the NPIP for use; however, it is not currently being used as the routine procedure in the Pullorum Testing Laboratory at Texas A&M University.

This research was conducted to compare results obtained by the microagglutination test with those obtained by the tube agglutination test. This comparison was made in order to determine whether or not the microagglutination test could be used routinely at Texas A&M University in place of the tube agglutination test.

MATERIALS AND METHODS

This study was conducted in two phases: 1) learning techniques and procedures and 2) data collection through performance of tests on turkey sera. For both phases, field collected turkey sera which had been submitted to the Pullorum Testing Laboratory were used. These specimens were

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first tested by Pullorum Testing Laboratory personnel using the tube agglutination procedure. The results were recorded as 4+, or complete agglutination, which indicated that a serum was positive for antibodies against S. pullorum or S. typhimurium. Turkeys giving these reactions are called reactors. A 3+ and 2+, or partial agglutination, was considered a "suspicious" reaction; and no agglutination, a negative reaction, indicated that no antibodies were detectable.

Reactor turkeys chosen at random are called in for attempts to culture salmonellae from the birds. Culturing is done because cross-reactive antibodies may give positive results. The culturing procedure is more definitive. Culture results were made available to this investigator for informational and comparative purposes.

PHASE 1. Sera tested by the microagglutination test for S. pullorum included 18 reactors, 15 suspicious, and 66 negative specimens. For S. typhimurium 68 reactors, 16 suspicious, and 27 negative sera were tested. The suspicious and negative sera were chosen randomly from the sera submitted. Reactor sera tested represented all 4+ sera submitted.

The microagglutination test was conducted as outlined by Williams (2). For this procedure, 250 µl of antigen was placed in each well of a rigid plastic microplate. To this 10 µl of serum to be tested was added to the corresponding well. The plates were then covered with a plastic sealer and incubated for 18 to 24 hours at 37C in a water bath. The results were read using the same designations, 4+, 3+, 2+, and negative as was used for the tube test. Reading of the reactions was facillitated by using a specially designed mirror.

The antigen used in the test was stained S. pullorum and S. typhimurium antigen provided by Dr. J. E. Williams at The United Stated Depart-

ment of Agriculture, Southeast Poultry Research Laboratory, Athens, Georgia. These stained antigens were designated USDA. A 1:12.5 dilution of each antigen was made in saline containing 0.5% phenol as per instructions sent with the antigens.

PHASE 2. Field collected reactor sera, as determined by the tube agglutination test, were used for the microagglutination tests which were conducted as outlined by Williams (2).

The USDA antigens and the unstained antigens used in the tube agglutination test were used in the microtests. The unstained antigens were designated TAMU.

The proper dilution of the TAMU antigens were determined by conducting a series of tests on reactor and negative sera until the results obtained using the microtest were in approximately 90% agreement with the results obtained using the tube agglutination test. The final dilutions used were: 1:218 for S. pullorum TAMU and 1:9 for S. typhimurium TAMU.

In order to compare results of tests with field collected sera and sera from turkeys inoculated with antigens, 27 10-week-old Broad Breasted White turkeys were acquired. The turkeys were bled and preinoculation sera were collected and stored frozen prior to being tested. Five turkeys were inoculated intravenously with S. pullorum and 5 were inoculated with S. typhimurium. The bacteria which had been grown in the laboratory from pre-existing cultures, were killed with 3% formaldehyde. Also, 5 turkeys were inoculated intravenously with the S. pullorum TAMU test antigen and 5 turkeys were inoculated with the S. typhimurium TAMU test antigen. The turkeys were kept in outdoor pens and fed commercial feed.

Three weeks following inoculation, blood samples were collected from the turkeys and allowed to clot. The serum was then removed for

testing. Microagglutination tests were conducted on the preinoculation and postinoculation sera from the turkeys using both USDA and TAMU S. pullorum and S. typhimurium antigens previously mentioned.

RESULTS AND DISCUSSION

PHASE 1. When the S. pullorum USDA antigen was used at a dilution of 1:12.5, it was found that the antigen was too concentrated and the tests were difficult to read. Therefore, the S. pullorum USDA antigen was diluted to 1:25 for all subsequent tests. It also was found that the reading of microtest results with sera designated as suspicious by the tube test were extremely difficult since they were subject to ones judgement. Therefore, for data collection purposes, only reactor sera as determined by the tube agglutination method were used.

PHASE 2. Results of microtests conducted on reactor sera are shown in Tables 1a and 1b. The tube test results are also shown for comparison. A 32.7% agreement was obtained using the 2 test methods for S. pullorum testing and the agreement was 36.2% for S. typhimurium.

Results obtained by tests using both the stained USDA and unstained TAMU antigens are presented in Table 2. The percent agreement was low, 22.6% and 32.8% for S. pullorum and S. typhimurium respectively. The high sensitivity of the unstained TAMU antigen is not desirable. It is probable that the antigen is too sensitive and is reacting with antibodies to closely related salmonellae which could have infected the turkeys. S. pullorum contains antigens 9 and 12 and S. typhimurium contains antigens 1, 4, 5, and 12. These antigens are shared by other serotypes of salmonella.

Only 1 isolation of S. pullorum was made from the turkeys cultured.

The serum from this bird gave a 4+ reaction in both the microtest and the tube test. It is felt that the microtest is sufficiently sensitive to detect infected turkeys. However, further comparison should be done before use of the microtest in place of the tube test.

Results of tests on sera from turkeys inoculated with killed antigens of S. pullorum are shown in Table 3. It is apparent that antibody response in these birds was poor. Only 5 of 8 birds responded. Some of these sera also cross reacted with S. typhimurium antigen (Table 4). In addition, reactions were obtained with sera from control birds.

Somewhat similar results were obtained with tests on turkeys inoculated with S. typhimurium. These results are given in Table 4.

The turkeys used for inoculation may have become infected with salmonella prior to their use. Hence the reactions obtained cannot be attributed solely to the antigens inoculated. Therefore, it is concluded that this experiment should be repeated. It is felt that it should be repeated with actual infection of the turkeys with live organisms since this would cause better antibody production than killed antigens. Culturing of the organisms would then be possible as well. It would be preferable to control extraneous infections in so far as possible.

LITERATURE CITED

1. Anon. 1976. The national poultry improvement plan and auxiliary provisions. Agricultural Research Service, USDA, Beltsville, Maryland.

2. Williams, J. E., and A. D. Whittemore. 1971. Serological diagnosis of pullorum disease with the microagglutination system. Appl. Microbiol. 21:394-399.

Table 1a. Microtest results on field collected sera having positive (4+) reactions to *Salmonella pullorum* by the tube test.

Test Results With *S. pullorum*		
	Tube Test	Microtest
No. tested	52	52
No. positive	52	17
Percent positive	100	32.7
Percent negative	0	67.3

Table 1b. Microtest results on field collected sera having positive (4+) reactions to *Salmonella typhimurium* by the tube test.

Test Results With *S. typhimurium*		
	Tube Test	Microtest
No. tested	144	144
No. positive	144	52
Percent positive	100	36.2
Percent negative	0	63.8

Table 2. Microtest results of field collected turkey sera having positive (4+) reactions by the tube test using USDA stained and TAMU unstained antigen to test Salmonella pullorum and Salmonella typhimurium.

Antigen	No. tested	Percent positive	Percent negative
Pullorum USDA[A]	31	22.6	77.4
Pullorum TAMU[B]	31	80.6	19.4
Typhimurium USDA[A]	70	32.8	67.2
Typhimurium TAMU[B]	70	92.8	7.2

A stained antigen

B unstained antigen

Table 3. Microtest results using Salmonella pullorum antigen in testing preinoculation and postinoculation turkey sera which was inoculated with killed Salmonella pullorum and Salmonella typhimurium antigens and uninoculated control turkey sera.

Antigen inoculated and method of killing	No. tested	No. of positive reactions with sera and type of test antigen indicated			
		Preinoculation		Postinoculation	
		USDA[A]	TAMU[B]	USDA[A]	TAMU[B]
S. pullorum					
formaldehyde killed	5	0	0	3	5
phenol killed	3	0	0	2	2
S. typhimurium					
formaldehyde killed	5	0	0	1	3
phenol killed	5	0	0	0	2
Controls (uninoculated)	9	0	0	4	6

A stained antigen

B unstained antigen

Table 4. Microtest results using Salmonella typhimurium antigen in testing preinoculation and postinoculation turkey sera which was inoculated with killed Salmonella pullorum and Salmonella typhimurium antigens and uninoculated control turkey sera.

Antigen inoculated and method of killing	No. tested	No. of positive reactions with sera and type of test antigen indicated			
		Preinoculation		Postinoculation	
		USDA[A]	TAMU[B]	USDA[A]	TAMU[B]
S. typhimurium					
formaldehyde killed	5	2	0	1	4
phenol killed	5	1	0	2	4
S. pullorum					
formaldehyde killed	5	1	0	1	4
phenol killed	3	1	0	1	2
Control (uninoculated)	9	4	0	3	4

A stained antigen

B unstained antigen