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Comparison of the Direct Agglutination and Indirect Hemagglutination Tests in the Determination of Blood Serum Titers to *Escherichia coli* Organisms¹

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A comparison of the direct agglutination test and the indirect hemagglutination test for the detection of blood serum antibodies to *Escherichia coli* organisms indicated that these serological tests were comparable. In some instances the indirect hemagglutination test provided higher endpoint readings. Preparation of the antigens for the indirect hemagglutination test was more time consuming than for the direct agglutination test. Crude extract and purified polysaccharides were comparable as red blood cell sensitizing agents.

Colibacillosis is one of the major digestive disturbances of the neonate of several species (1, 3, 6, 9, 13, 16). Attempts to utilize natural passive immunity as a means of protection have been reported (5, 7, 8). Evaluation of the effectiveness of vaccination must be determined in part through blood serum titers (11, 12). The indirect hemagglutination test for somatic (O) *Escherichia coli* antibody has been advocated as a more sensitive method of serum titer determination than the direct conventional agglutination test (9, 10).

These investigations were initiated to compare the efficacy of the indirect hemagglutination test versus the conventional tube agglutination test, by utilizing blood serum of adult swine and their piglets. The adult swine had been vaccinated with heat-inactivated antigens of specific serotypes of *E. coli*.

MATERIALS AND METHODS

Experimental animals. Eight pregnant second-litter sows, of Duroc or Chester White breeding, and their piglets were used as investigational animals. The sows were obtained from two farm herds and housed in a conventional swine housing facility for at least 30 days prior to farrowing. The sows weighed between 140 and 160 kg each. Piglets of the sows remained with the sows for at least 4 weeks postfarrowing.

Inoculation. Bacterins were prepared from *E. coli* O55:B5 (Difco) grown on veal infusion agar. After incubation (18 h), the bacterial growth was removed, placed in sterile phosphate-buffered saline (PBS), and heated for 2.5 h at 121 C. This was then diluted to a nephelometer density of tube three (3). Each sow received 10 cm³ of the heat-killed bacterin intramuscularly at least 3 weeks pre-farrowing. The O55:B5 serotype was selected because of its infrequent existence in the average swine environment, avoiding existence of blood titers previous to vaccination, and because purified lipopolysaccharides of this type were readily available. This serotype is also noted for its minimum of reciprocal antigen relationship to other *E. coli* organisms.

Blood was collected from the anterior vena cava prior to vaccination, immediately following farrowing, and 1- and 4-week postfarrowing. Serum was obtained by the conventional method of coagulation, cooling, and centrifugation. Collected serum was frozen and stored at -20 C until use.

Antigens. Crude antigens of *E. coli* O55:B5 were prepared according to the procedures of Vosti (14) with modifications. After incubation (18 h) in brain heart infusion broth, the organisms were recovered by centrifugation and were suspended in sterile PBS at 1 ml of sediment per 10 ml of PBS. They were then boiled for 2.5 h. After centrifugation, the supernatant fluid (crude extract) was stored at -20 C.

Purified lipopolysaccharides of *E. coli* O55:B5 (Difco) were prepared according to the technique of Westphal (15).

Serological tests. All serum samples were heat inactivated at 56 C for 30 min. The indirect hemagglutination test was one of the tests used to detect the O antibody (9, 10, 14). A 3% suspension of sheep red blood cells (RBC) was washed 3 times in sterile PBS

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by centrifugation, and then was added to a 10% suspension of the respective crude or purified polysaccharide. The suspension was incubated at 37 C in a water bath for 30 min. This was then washed 3 times in sterile PBS, and suspended in 2.5% PBS.

Serums to be evaluated were diluted by a twofold serological dilution in sterile PBS (pH 7.3), and 0.5 ml of each dilution was added to a test tube (10 by 75 mm). Sensitized sheep RBC (0.05 ml) was also added to each tube. The tubes were well shaken and incubated at 37 C for 2 h. Controls consisted of PBS, negative and inactivated serum with the sensitized sheep RBC. All tubes were read by gross observation by using a Quebec bacterial colony counter and reread after 12 h of refrigeration at 4 C. A flocculent covering of uniform thickness which exhibited agglutination when suspended and which nearly covered the entire tube bottom was considered positive. A test was designated as negative when the RBC was in a compact disk-like formation and formed a uniform suspension when suspended.

The direct tube agglutination test was performed with antigen made from smooth colonies grown on tryptose agar. The colonies were removed from the agar with sterile saline, centrifuged, and diluted to 1 ml of cells to 9 ml of sterile PBS (pH 7.3). The diluted cell suspension was heated to 121 C for 2 h (2). Serums to be investigated were diluted twofold. A 0.5-ml sample of each dilution was added to the test tubes (10 by 75 mm) in addition to 0.05 ml of the antigen suspension. The tubes were well shaken and then incubated in a water bath at 48 C for 18 h (4). Titers for all tests were recorded as the reciprocal of the highest serum dilution exhibiting a positive agglutination.

RESULTS

Comparisons of the direct agglutination test to indirect hemagglutination test with 44 serum samples indicated that 36.3% of the serum samples had identical titers as was determined by both methods of titration. Titers of 70.5% of the samples were either identical or within one dilution of being identical. A greater number of the indirect hemagglutination tests had higher dilution end points than were detected for the direct agglutination test (Table 1). The indirect hemagglutination test provided a more readily detectable end point, but considerable time and effort were required to prepare the antigens and RBC indicator fraction.

Comparative tests were made for RBC sensitivity by using RBC of individual species including swine, human (O group), and sheep. The sheep RBC provided the greater detectable reactions of those investigated.

Comparisons were made of purified lipopolysaccharides and crude extracts of *E. coli* test organisms for sensitization of the RBC indicator fraction for the indirect hemagglutination

test. Of the 32 antiserums investigated, 37.5% had equal endpoint readings, and 34.4% had a 1-dilution difference. Tests made with crude extracts provided 15.7% more endpoint readings of two dilutions greater than the purified lipopolysaccharides preparations (Table 2). There were no observable differences in the utilization of either testing procedure with the purified or crude extracts of the test organism.

DISCUSSION

The direct agglutination and indirect hemagglutination tests provided very similar (two dilutions) end-point readings for 90% or more of the serums evaluated. This is in contradiction to previous investigations (6, 9, 10, 11). These investigations indicate a greater number of indirect hemagglutination tests had higher endpoints than the direct agglutination test. This is not necessarily indicative of greater sensitivity; however, it is possibly indicative of more readily detectable endpoint reactions. The indirect hemagglutination test provided a more readily determined endpoint, but a much greater effort was necessary in preparing the antigens for hemagglutination than that required for the direct agglutination test. These investigations concur with the work of Kunin

TABLE 1. Comparison of direct agglutination to indirect hemagglutination tests in the detection of *E. coli* antibodies

End-point readings	Direct agglutination		Indirect hemagglutination	
	No. of tests	Percentage of total tests	No. of tests	Percentage of total tests
Equal	16	36.4	16	36.4
1-Dilution greater	7	15.9	8	18.2
2-Dilutions greater	2	4.5	8	18.2
3-Dilutions greater	0	0	3	6.8

TABLE 2. Comparison of crude extracts to purified polysaccharides of *E. coli* organisms for the sensitization of RBC indicator cells

End-point readings	Direct agglutination		Indirect hemagglutination	
	No. of tests	Percentage of total tests	No. of tests	Percentage of total tests
Equal	12	37.5	12	37.5
1-Dilution greater	5	15.6	6	18.8
2-Dilutions greater	1	3.1	6	18.8
3-Dilutions greater	0	0	2	6.3

(7), who suggested that the problem of heterogenic reactions deterred from the effectiveness of this test procedure.

Other investigators utilized RBC of various species including human (9), sheep (11), and swine (6). Comparative tests utilizing these species of RBC indicated that the sheep RBC provided the most readily detectable hemagglutination under the condition of this investigation.

Comparisons of the crude *E. coli* extracts to the purified lipopolysaccharides extracts indicated that they were comparable RBC sensitizers, the crude extract being preferable in most laboratories. These results concur with those of Vosti (14).

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