Korean J. Vet. Res. Vol. 7, No. 2. (1967) 1—7

Studies on the Modified Complement Fixation Test of Swine Erysipelas*

Yun S. Jeon, D.V.M., M.S., Ph.D.

College of Agriculture, Seoul National University

Hyun J. Cho, D.V.M., M.S., Wha T. Oh, D.V.M., M.S.

Veterinary Research Laboratory, Office of Rural Development

I. INTRODUCTION

The detection of swine erysipelas antibody in swine may be possible by means of agglutination(Schoening et al. 1932), flourescent antibody technique(Dacres et al. 1959, Marshall et al. 1959) and hemagglutination inhibition test(Dinter 1958). An insensitivity of agglutination and unstability of the bacterial agglutinogen allowed the test to a limited use in the detection of chronic cases or herd infection of swine erysipelas in field (Stile et al. 1934, Schoening et al. 1935, Schoening et al. 1936, Rice et al. 1952). An application of fluorescent antibody technique (Dacres et al. 1959, Marchall et al. 1959) also possesses certain limitations such as time, relative cost, and laborious procedures. Hemagglutination inhibition test appears to show a least sensitivity.

The conventional complement fixation test is not applicable when the test system consisted with swine antiserum. This is due to a marked procomplementary activity of swine serum and causes false-negative direct complement fixation(Scherer et al. 1962). Although the chemical nature of the hemolytic compound is unknown, it contained in heat labile and stable fractions of swine serum (Lee. 1964).

Various procedures have been attempted for obviating the procomplementary activity of swine serum. These are the reduction of the amount of guinea pig complement in proportion to the procomplementary activity of each serum being tested(Bankowski et al. 1953, 1955), destruction of procomplementary activity by cold alcohol fractionation or ether extraction (Boulanger 1955), destruction by formalin, zymosan, cobravenom(Cowan 1961), and heat inactivation(Scherer et al. 1962). Though various procedures have been attempted to remove the procomplementary activity, these were not uniformly successful.

The supplementation of factor which consisted with C'1 and C'3,4 fractions of guinea pig or pig serum may inhibit the procomplementary activity of swine serum (Jeon 1965). The supplementation method of factor which is referred to modified complement fixation test may give some benefits such as a higher sensitivity and an undenaturation of antibody particle.

This article is to describe the modified complement fixation test of swine erysipelas by employing fresh rabbit serum and varum factors to the test system.

This studies were partially supported by the research grant of FG-Ko-103, USDA.

II. MATERIALS AND METHODS

1. Complement Fixation Test:

The procedure of the complement fixation test was based on Mayer's method(Mayer 1961), the test system as well as other systems were duplicated, the order of addition of reagents was followed as illustrated in each table, and the 100 per cent lytic unit was employed. The complete inhibition of the indicator system was recorded as 4, 3, 2, 1 and 0 for 0, 25, 50, 75 and

*The part of these studies was submitted to the Graduate School, Seoul National University for Dr. Cho's M.S. thesis

100 per cent hemolysis, respectively.

The diluent for all reagents of the serological test was Veronal-NaCl buffered solution, containing 0.145 M NaCl and 0.005 M Ca^{++} as calcium chloride hexahydrate(Mayer et al. 1948).

To prevent coagulation of blood and for its preservation Modifed Alsever's solution was used(Bukantz et al. 1946).

The stock sheep cells in Modified Alsever's solution were washed more than 3 times with Veronal-NaCl buffered solution by centrifugation at 1,000 r.p.m. for 10 minutes. A 2 per cent suspension of erythrocytes was made and sensitized two units of hemolysin. And more than two exact units of complement were used throughout the experiment.

2. Fresh Serum and Complement Components (C'1 and C'3,4)

In order to prepare various fresh serum and complement components of different animal sera, young healthy animals were employed. The blood was collected aseptically from the cardiac puncture, anterior venacava, and jugular vein for chicken and rabbit, pigs and bovine respectively. From 20 ml. to 50 ml. of the blood was slanted in large test tubes or bottles, allowed to clot and separated from the glass wall. It was then allowed to stand for two hours at room temperature and 2 hours at 2°C. before harvesting the serum. This serum was centrifuged at 1,500 r.p.m. for 20 minutes and used immediately or dispensed in 1,2 ml. aliquots and stored at −60°C. From these sera, C'1 fractions were made on the basis of the dilution method(Mayer 1961). Heat inactivated, at 56°C. for 30 minutes, sera were used as C' 3,4 fraction. In case of fresh serum supplementation, the stored serum was melted and used.

3. Bacterial Strains and Seed Culture

For the preparation of complement fixation antigen, three different strains, K—1, T—2, and J—9 were employed. They were obtained from the spleen of acute septicemic from of swine, from the tonsils of healthy swine, and from the skin lesions of the urticarial form of swine, respectively. They showed no hemagglutination activity except J—9 strain.

The strains of NL—11, Se—9, An—4, Cn—3461, R —6 and R—2 were obtained from Jensen Salsbery Laboratory, and NL—11 was used as the source of living vaccine, and the other were pooled and used for the preparation of gel absorbed bacterin. For the challenge, strain was employed.

Seed cultures of the above mentioned strains were prepared in serum agar medium. The components of medium except bovine serum were suspended in 1,000 ml. of beef infusion and heated to boiling to dissolve the medium completely. After autoclaving at 15 pounds (121, 6°C.) for 15 minutes, it was cooled to about 60 °C. and added sterile bovine serum. The final reaction of the medium was adjusted to pH 7.4 to 7.8. Three different strains of K—I, T—2 and J—9 were streaked on a serum agar plate and incubated five to seven days at 37°C. After the incubation, small, delicate, and smooth colonies were picked and cultured in serum broth for the preparation of bacterial antigen. The serum broth was prepared as serum agar medium except the addition of Bacto agar. The components of the medium is as follows:

Beef infusion(Beef meat infusion 25 and beef liver infusion 1)are combined). 1 liter

Bacto peptone	20 gm
Bacto lactose	5 gm
Bacto dextrose	5 gm
Sodium phosphate dibasic	11 gm
Potassium phosphate monobasic	1 gm
Bactor agar	15 gm
Bovine serum	100 ml

4. Antigen and Titration

A diluted seed culture was seeded to 500 ml. of serum broth and cultured for 48 hours at 37°C. The pure broth culture was added a final concentration of 0.5 per cent formalin, and collected bacteria by centrifugation at 20,000 r.p.m. by usingSharples centrifuge. The antigen was washed three times with 0.5 per cent formalin saline solution and the sediment was diluted with two volumes of 0.5 per cent formalin saline solution and stored at 2°C.

The antigen was titrated as illustrated in Table I. The maximum amount of antigen causing complete hemolysis was established as one unit of antigen, and a half or one-fourth doses of one unit of antigen were employed throughout the tests.

5. Antiserum and Titration

A number of antisera aginst swine erysipelas were prepared with three different vaccines or organism. These are NL. II living vaccine (E.V.A), aluminum hydroxide

TABLE I.

Titration of Antigen

Tube No.	1	2	3	4	5	6	7	8	9	10	11
Antigen Dilution	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	Control	
Antigen ml.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0	0
Complement 2EU ※ ml.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0
Diluent ml.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.4	0.6
Primary incubation at 37°C. for hour											
Sensitized RBC, 2U, 2% ml.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Secondary incubation at 37°C. for 30 minutes											

*Two exact units

gel adsorbed bacterin and a living virulent organism of 87184 strain.

About four months old pigs were immunized. A single dose of 2.0 ml. of E.V.A.(Gray et al. 1955) or 3 to 5 ml. of adsorbed bacterin(Callaway et al. 1955) were administered subcutaneously behind the ear. Fourteen days following the inoculation, the blood was withdrawn from anterior venacava aseptically and harvested the serum. The serum was immediately heat inactivated at 56°C. for thirty minutes and dispensed in amount of 2 ml. in screw capped test tubes and stored at −60°C.

The skin scarification method was employed for the virulent living bacteria of 87148 strain(Shuman 1951, Gray et al. 1955). At one week of the post inoculation, the blood was withdrawn and harvested serum. The serum was heat inactivated at 56°C. for 30 minutes and stored at −60°C. until to use. The antibody titration was carried out as illustrated in Table II.

In this study, the time requirement for complement fixation was 60 and 30 minutes for the primary and secondary incubation periods respectively. And each reagents in amount ml. were employed.

TABLE II

of Antibody

Tube No.	1	2	3	4	5	6	7	8	9	10	11
Atiserum Dilution	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	Controls		
Antiserum ml.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0	0.2**	0.2**
Antigen 1/2 U, ml.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0	0.2
Complement 2EU*, ml.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0
Diluent ml.	0	0	0	0	0	0	0	0	0.2	0.2	0.2
Primary incubation at 37°C. for 1 hour											
Sensitized RBC, 2U, 2%	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Secondary incubation at 37°C. for 30 minutes											

* two exact units

**1/8 diluted antiserum

In the antibody titration, 1st tube received 0.1 ml. of undiluted antiserum in addition to 0.04 ml. of factor or fresh unheated serum and 0.66 ml. of diluent. This gives 1/8 dilution of antiserum. From this, 0.4 ml. and 0.2 ml. were dispensed to second tube of the test system and first tube of serum control respectively. The same procedures were repeated for the further dilutions of antiserum.

III. EXPERIMETAL RESULTS AND DISCUSSION

It has been known that the complement fixation test with swine serum has generally been unsatisfactory because of its marked procomplementary activity, i.e., swine

servum generally enhances the hemolytic activity fo guinea pig serum used as a source of complement.

Howeuer, the supplementation of fresh unheated serum which is obtained from the normal healthy rabbit or bovine brought about the fixation of complement to the heated antibody antigen complex(Jeon 1965). Due to the facts that above mentioned, an application of the modified complement fixation test, that is, supplemetation of rabbit and bovine sera was attempted to the system of swine erysipelas.

1. Effect of dose of factor supplementations.

In the first experiment, an optimum amount of factor or serum of rabbt and bovine was studied. Antiserum used in this experiment was prepared with E.V.A., and J—9 antigen was employed. The results were illustrated in Tables III and IV. The results indicate followings:

TABLE III

Effect of Rabbit Factors

Supplement		Systems	Antiserum Dilution					Agn.
Type	Amount, ml.		1/8	1/6	1/32	1/64	1/128	Cont.
		T.S*	0	0	0	0	0	0
No	—	S.C.**	0	0	0	0	0	0
		T.S.	4	3	0	0	0	0
Serum	0.01	S.C.	0	0	0	0	0	0
		T.S.	4	4	4	0	0	0
Serum	0.02	S.C.	0	0	0	0	0	0
		T.S.	4	4	4	3	0	0
Serum	0.03	S.C.	0	0	0	0	0	0
		T.S.	4	4	4	4	3	0
Serum	0.04	S.C.	0	0	0	0	0	0
		T.S.	4	4	4	4	3	0
Serum	0.06	S.C.	4	0	0	0	0	0
		T.S.	4	4	4	4	4	0
Serum	0.08	S.C.	4	0	0	0	0	0
		T.S.	4	4	4	4	4	0
Serum	0.10	S.C.	4	4	0	0	0	0
		T.S.	4	4	4	4	4	0
C'1	0.04	S.C.	4	4	0	0	0	0
		T.S.	0	0	0	0	0	0
C'3,4	0.04	S.C.	0	0	0	0	0	0

*Test system **Serum control

1) Fresh or heat invactiated antiserum was unable to fix guinea pig complement in a conventional complement fixation test.

2) Supplementation of fresh rabbit or bovine serum to the heat inactivated antiserum was able to fix guinea pig complement and the degree of the fixation was enhanced.

3) Supplementation of heated rabbit or bovine serum at 56°C. for 30 minutes was unable to fix guinea pig complement.

4) Complement C'1 fraction, derived from leither of fresh rabbit or bovine serum, inhibited the procomplementary activity of pig serum and an excess amount resulted in anticomplementary effect.

An optimum quantity of serum factor required to the test system seemed to be a function of antibody titer. The higher the antibody titer showed the lower the procomplementary effect. However, an optimum amount of rabbit serum factor ranged 0.04 ml to 0.05 ml., and there were no significant differences on the range between rabbit and bovine serum factors.

2. Comparative Studies of Modified Compliment Fixation Tests Based on Different Factor and Antiserum Titer

TABLE IV

Effect of Dose of Bovine Factors

Supplement		Systems	Antiserum Dilution					Agn.
Type	Amount, ml.		1/8	1/16	1/32	1/64	1/128	Cont.
		T.S.*	0	0	0	0	0	0
No	—	S.C.**	0	0	0	0	0	0
		T.S.	2	2	2	1	0	0
Serum	0.01	S.C.	0	0	0	0	0	0
		T.S.	3	3	3	1	0	0
Serum	0.02	S.C.	0	0	0	0	0	0
		T.S.	4	3	3	2	1	0
Serum	0.03	S.C.	0	0	0	0	0	0
		T.S.	4	4	3	3	3	0
Serum	0.04	S.C.	0	0	0	0	0	0
		T.S.	4	4	4	3	3	0
Serum	0.06	S.C.	0	0	0	0	0	0
		T.S.	4	4	4	4	3	0
Serum	0.08	S.C.	2	1	0	0	0	0
		T.S.	4	4	4	4	3	0
Serum	0.10	S.C.	3	1	0	0	0	0
		T.S.	4	4	4	4	4	0
Serum	0.04	S.C.	4	4	0	0	0	0
		T.S.	4	4	4	4	4	0
C'1	0.04	S.C.	4	4	0	0	0	0
		T.S.	2	2	2	2	2	0
C'3, 4	0.04	S.C.	0	0	0	0	0	0

*Test system **Serum control

In this experiment, three different antisera totally 13 serum samples were tested, under the presence of optim umquantity of factor, for their serum titers and sensitivity of factor. The antisera were prepared by employing NL —11 living vaccine, aluminum hydroxide gel adsorbed bacterin and 87148 strain skin scarification. As the factor, two different fresh rabbit and bovine serum were used in an optimum amount of 0.04 m*l.* or 0.05 m*l.* Throughout the experiment, J—9 antigen was employed.

The results were illustrated in Table V. The results indicate followings:

1) No significant differences were observed in antibody titers, in the same factor group, among three differnet antisera.

2) Supplementation of rabbit serum showed a higher sensitivity on the antibody titer than that of bovine serum was supplemented.

In case of no factor supplementation, the negative control sera showed complete hemolysis, but the positive pig sera 2 out of 13 samples showed fixation. Bovine serum supplementation diminished negatively fixed serum, while the rabbit serum group brought about more than 1/32 titer of all serum samples. The detailed number and distribution of antibody titers in different supplementation were illustrated in Table VI.

As a serum factor, no significant individual differences of serum batches were experienced in case of rabbit. However, it was not seldom to encounter the individual differences on the bovine serum factors.

In order to prove the specific fixation demonstrated in the previous experiments, a number of swine pathogenic bacterial antigens were prepared and tested with swine erysipelas positive and negative sera. The heterogenous antigens tested with the pig sera were E. coli, Sal. pullorum, Sal. cholerasuis, Br. abortus and Swine origine PPLO. The results of the tests were negative.

IV. CONCLUSION

Throughout the studies the following experimental

Table V. Comparative Studies of Modified Complement Fixation Tests Based on Different Eactor and Antiserum Titer

Type of Serum*	Serum Dilution: No factor 1/8	1/16	1/32	1/64	Bovine Serum Factor 1/8	1/16	1/32	1/64	Rabbit Serum Factor 1/8	1/16	1/32	1/64
L—1	4	3	3	0	4	4	4	4	4	4	4	4
L—2	0	0	0	0	4	4	3	1	4	4	4	4
L—3	0	0	0	0	4	1	1	1	4	4	4	1
L—4	2	2	2	2	4	4	4	4	4	4	4	4
L—5	0	0	0	0	4	4	3	2	4	4	4	4
G—1	3	3	2	2	4	4	4	4	4	4	4	4
G—2	0	0	0	0	4	3	3	2	4	4	4	3
G—3	0	0	0	0	3	3	3	2	4	4	4	4
C—1	0	0	0	0	4	4	4	3	4	4	4	4
C—2	0	0	0	0	4	4	4	3	4	4	4	4
C—3	0	0	0	0	4	4	4	3	4	4	4	4
C—4	4	4	4	3	4	4	4	4	4	4	4	4
C—5	2	2	2	0	4	4	4	3	4	4	4	3
N—1	0	0	0	0	0	0	0	0	0	0	0	0
N—2	0	0	0	0	0	0	0	0	0	0	0	0
N—3	0	0	0	0	0	0	0	0	0	0	0	0
N—4	0	0	0	0	0	0	0	0	0	0	0	0

*L: Antiserum against living vaccine
G: Antiserum against gel vaccine
C: Antiserum against challenged with 87184 strain
N: Negative control serum

Table VI. Distribution of Antiserum Titer

Type of Supplementation	Antiserum Dilution: <1/8	1/8	1/16	1/32	1/64<	Total
No Supplementation	11	1	0	1	0	13
Bovine Serum Supplementation	1	2	2	4	4	13
Rabbit Serum Supplementation	0	0	0	3	10	13

results were obtained and are summarized here.

1) The methodology of the modified complement fixation test for swine erysipelas has been described.

2) The application of the above modified complement fixation test for the other major swine diseases has been suggested.

V. REFERENCES

Bankowski, R.A., and Kummer, B.A.: Vesicular Stomatitis and Vesicular Exanthema Differentiation by Complement Fixation. Amer. J. Vet. Res.,(1955): 16, 374

Bankowski, R.A., Wichmann, R., and Kummer. M.: A Complement Fixation Test for Identification and Differentiation of Immunological Types of the Virus of Vesicular Exanthema of Swine. Amer. J. Vet. Res., (1953): 14, 145

Boulanger, P.: Complement Fixation Tests of Swine Serum. 1. In the Diagnosis of Vesicular Stomatitis. Can ad. J. Comp. Med. & Vet. Sci., (1955): 19, 37

Bukantz, S.C., Rein, C.R., and Kent, J.F.: Studies in Complement Fixation. Preservation of Sheep's Blood in Citrate Dextrose Mixtures Modified Alsever's Solution) for Use in the Complement Fixation Reaction. J. Lab. Clin. Med., (1946): 31, 394

Culloway, E.E., Clark, R.S., Price, L.W., and Veney, S.A.: Field Use of an Adsorbed Swine erysipelas Bacterin. Vet. Med., (1955): 50, 39

Cowan, K.M.: Immunological Studies on African Swine Fever Virus. I. Elimination of the Procomplementary Activity of Swine Serum with Formalin. J. Immunol., (1961): 86, 465

Dacres, W.G., and Groth, A.H.: Identification of Erysipelothrix insidiosa with Fluoresent Antibody. J. Bact.,

(1959): 78, 298

Dinter, Z.: Uber den haemagglutinationshemmungstes beim Rotlauf. Berl. Munch. tierarzt. Wschr., (1959): 10 13

Gray, C.W., and Norden, C.J.: Erysipelas Vaccine Avirulent-A New Agent for Erysipelas Control. J. Amer. Vet. Med. Ass'n., (1955): 127, 506

Jeon, Y.S.: Modified Complement Fixation Test the Diagnosis of Hog Cholera. (1965): Unpublished.

Lee, Y.S.: Studies on the Procomplementary Effect of Swine Serum. The M.S. Thesis Submitted to the Graduate School of Seoul National University.(1964)

Mayer, M.M.: Kabat and Mayer's Experimental Immunoohemistry. C.C. Thomas Publisher, Springfield, Ill. (1961): 162

Mayer, M.M.. Osler, A.G., Bier, O.G., and Heidelberger, M.: Quantitative Studies of Complement Fixation. 1. A Method. J. Immunol., (1948): 59, 195

Rice, C.E., Connell, R., Byrne, J.L., and Boulanger, P.: Studies of Swine Erysipelas. IV. Serological Diagnosis in Swine. Canad. J. Comp. Med., (1952): 16, 209

Scherer, W.F., and Lewis. N.D.: Immunologic Studies of Japanese Encephalitis Virus in Japan. VI. An Evaluation of the Direct Complement Fixation Test for Detecting Infection of Swine. Amer. J. Vet. Res., (1962): 23, 1157

Schoening, H.W., and Creech, G.T.: Serological Studies of Swine Erysipelas with Particular Reference to Agglutination. J. Agr. Res., (1953): 50, 71

Schoening, H.W., and Creech, G.T.: Swine Erysipelas with Particular Reference to Serological Diagnosis. J. Amer. Vet. Med. Ass'n., (1936): 88, 310

Schoening, H.W., Creech, G.T., and Grey, C.G: A Laboratory Test for the Diagnosis of Swine Eryspelas. of Swine Erysipelas. Nor. Amer. Vet., (1932): 27,

Shuman, R.D.: Swine Erysipelas Induced by Skin Scarification. Proc. Amer. Vet. Med. Ass'n., (1951): p153

Stile, G.W., and Davis, C.L.: Swine Erysipelas and Its Economical Importance. J. Amer. Vet. Med. Ass'n., (1934): 85, 895

豚丹毒의 改良補體結合反應에 관한 硏究

서울大學校 農科大學 全 允 成
家畜衛生 硏究所 趙 顯 注
吳 和 鐸

豚丹毒에 대한 돼지 抗體를 檢出할 수 있는 實用的인 血淸學的 方法은 아직까지 없다. 그리고 補體結合反應은 가장 優秀하고 銳敏한 血淸學的反應이긴 하지만 돼지 血淸이 抗體이면 緬羊赤血球抗家兎血淸 및 기니픽 補體로 구성되는 溶血系下에서는 돼지 血淸의 親補體作用 때문에 補體結合反應이 불가능하다.

이 硏究에서는 正常家兎血淸이나 다른 正常素를 反應系에 첨가하여 親補體作用을 없애는 改良補體結合反應으로 豚丹毒 抗體 | 抗原의 特異的인 結合을 가능하게 하였다. 즉, 1/2 單位의 抗原, 2 正確單位의 기니픽補體, 2 單位, 2% 感作緬羊赤血球 그리고 0.04 ml의 家兎正常素는 豚丹毒 抗原-抗體結合物에 補體가 特異的으로 結合되게 하였다.