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Complement Fixation Test for the Diagnosis of Hog Cholera

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INTRODUCTION

Hog cholera is an acute, highly contagious disease of swine, and it occurs in most areas of the world where swines are raised in considerable numbers. The diagnosis of the disease may be made on the basis either by clinical observation, pathological findings and transmission of the disease to susceptible pigs with and without adding known antiserum.

Recently, a number of methods for the detection of hog cholera antigen in swine have been reported. These are neutralization test on cell culture, gel diffusion technique, fluorescent antibody technique, END method, and heterologous tests such as amylase and hemolysis tests. However, these methods possess certain limitations such as time, relative cost, laborious procedures or low sensitivity.

Due to the facts above it has been always worthwhile to find out a complement fixation test which is highly flexible, simple and reliable. However, it has been known that the conventional complement fixation test is unable to detect antigen-antibody complex when the pig antiserum is incorporated. In fact, pig antiserum as well as normal serum, possesses a hemolytic activity to the hemolytic system which is consisted with guinea pig complement, sheep erythrocytes and anti-sheep erythrocyte rabbit serum.

The purpose of this study is to describe a complement fixation test for hog cholera on the emphasis of preparations of anti-hog cholera rabbit serum and porcine origin pancreas antigens and its titration.

MATERIALS AND METHODS

Distilled water, used in these studies had almost neutral pH and had less than 10 p.p.m. of ions (NaCl based). The glassware was cleaned by immersing in neutral detergent, washing with plain tap water, soaking in distilled water and rinsing with renewed distilled water before drying. In the titration procedure the test system as well as others were duplicated and the 100 per cent lytic unit was employed. The complete inhibition of hemolysis of the indicator system was recored as 4, 3, 2, 1 and 0 for 25, 50, 75 and 100 per cent hemolysis, respectively. The delivery of undiluted hemolysin and complement was accomplished by 0.1 or 0.2 ml. Kahn pipettes subdivided to 1/100 or 1/50 ml.

To allow for decay, with an exception of hemolysin titration, a primary and secondary incubation periods were used. All of titrations were accomplished at 37°C. water bath and allowed 60 minutes for the primary and 30 minutes for the secondary incubation period.

1. Diluent for Complement Fixation Test

The diluent for all reagents of the serological test, except for the pancreas antigen preparation, was Veronal-NaCl buffered solution, containing 0.145 M NaCl and MCa^{++} as calcium chloride, and 0.0005 M Mg^{++} as magnesium chloride (Mayer et. al. 1961). The diluent was prepared as follows:

Sodium chloride .....48.75 gm
5.5-diethyl barbituric acid (Merck).....6.40 gm
Sodium 5.5-diethyl barbiturate(Merck).....10.28 gm
Calcium chloride, dihydrate.....0.22 gm
Magnecium chloride, hexahydrate .....1.02 gm

The acid was dissolved in 500 ml. of hot water (approx. 703°C) and the others added to 1000 ml of water. The solution was cooled to approximately 25°C. and made up to 2000 ml. with distilled water and kept in 2°C. refrigerator as the stock buffer solution.

The final dilution, one part of the stock buffer solution was added into previously cooled 4 parts of distilled water. The stock solution was stored at 2°C. refrigeration

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while the diluted solution was used only for one day.

2, Sheep Erythrocytes

An equal amount of sheep blood was collected in a bottle in which Modified Alsever's solution (Bukantz et al. 1946) was contained. Modified Alsever's solution was prepared as following.

Glucose	2.05 gm
Sodium citrate	0.80 gm
Sodium chloride	0.42 gm
Distilled water, made up to	100 ml

The components were mixed and sterilized by employing filtration procedure instead of autoclaving. The solution was stored at 2°C. for the future use. The stock sheep cells were washed more than 3 times with Veronal-NaCl buffered solution by centrifugation at 1000 r.p.m. for 10 minutes. A 2 or 4 per cent suspension of erythrocytes was made and used.

3. Homolysin and Titration

Anti-sheep erythrocytes rabbit serum was prepared by using chemically treated sheep erythrocytes (Rhee et al. 1966). Hemolysin was titrated as illustrated in Table I. After incubation at 37°C. for 30 minutes the result

Table I Titration of Hemolysin

Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Hemolysin dilution	1* / 1T	1 / 2T	1 / 3T	1 / 4T	1 / 5T	1 / 6T	1 / 7T	1 / 8T	1 / 9T	1 / 10T	CONTROL		
Hemolysin, ml.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1*	0	0
Sheep RBC, 4%, ml.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Complement, 1/30, ml.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0	0.2	0
Diluent, ml.	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.6	0.5	0.7
37°C., 30 minutes incubation													

* 1/1000 dilution of hemolysin

was read. The minimum amount of hemolysin which causes complete hemolysis of the sheep erythrocytes was called one unit. In this study two units of hemolysin were used.

If the end point of 100 per cent hemolysis is observed at the dilution of 1/5,000 under the experimental condition, the quantity of the 10^{-2} diluted stock hemolysin required for the preparation of 10 ml. of the diluted hemolysin yielding a strength of two units in a final sensitized cell suspension was calculated by solving X of the following equation:

$$\frac{0.1 \times 2 \times \frac{10}{0.1}}{5000} = \frac{X}{100}$$

Therefore, 0.8 ml. of 10^{-2} sub-diluted hemolysin contained in 10 ml. is mixed with an equal amount of four per cent sheep erythrocytes.

4. Complement and Titration

From more than five mature guinea pigs, blood was collected by cardiac puncture and serum was harvested, pooled and centrifuged at 1,500 r.p.m. for ten minutes. Aliquots of 1.2 ml. were dispensed in test tube and stored at −60°C. until to use.

Complement was titrated as illustrated in Table II. A 1/25 dilution of complement was made and dispensed using 0.1 ml. Kahn pipette. The least amount of complement causing complete hemolysis of two units two per cent sensitized cells was established as one exact unit. In this experiment more than 1.1 full units of the complement were employed.

Table II Titration of Complement

Tube No.	1	2	3	4	5	6	7	8	9	10
Complement, 1/25, ml.	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1	0
Diluent, ml.	0.58	0.57	0.56	0.55	0.54	0.53	0.52	0.51	0.50	0.60
Primary incubation at 37°C. for 1 hour										
Sensitized Rbc. 2 units, 2% ml.	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Secondary incubation at 37°C. for 30minutes										

If 0.06 ml. of 1/25 diluted complement showed one exact unit, the quantity of the complement which gives 1.1 full units was calculated by solving X of the following equation:

$$\frac{25 \times 02}{1.2 \times 0.07} = X$$

Therefore, 0.2 ml. of a 1/65 dilution of complement gave 1.1 full units.

5. Tissue Culture Antigen for Positive Antiserum:

Tissue culture methods employed in this experiment was largely based on Dulbecco's method (Dulbecco et al. 1954). Two kidney cortexes were obtained from baby pig, less than 10 days old, and minced into small pieces, washed 3 or 4 times in PBS until it shows clear supernatant, and transferred into a small flask containing 60 ml. of a prewarmed (37°C.) 0.25 per cent trypsin (1 : 250)—PBS. Separation of kidney cells into single cell was hastened by means of a magnetic stirrer for 10 minutes at 37°C. Dulbecco's PBS was made as following: A solution; NaCl 0.8 gm, KCl 0.2 gm, Na_2HP_4 1.15 gm, KH_2PO_4 0.2 gm, distilled water 800 ml. B solution; $MgCl_2$. $6H_2O$ 0.1 gm, distilled water 100 ml. C solution; $CaCl_2$ 0.1 gm, distilled water 100 ml Autoclaved A, B and C solution separately and mixed when cooled.

A turbid supernatant containing dispersed cells was collected into centrifuge tube immerced ice cold water and about 20 ml. of fresh prewarmed trypsin was added to the fragments left in the flask. The same procedure was repeated 15 to 20 times until the parenchymatous kidney tissue had been entirely converted into cell clusters or single cells. All supernatants were centrifuged at 600 r.p.m. for 2 to 3 minutes, and the pellets were collected and washed 2 times with growth medium. The growth medium was made with nine parts of Earles BSS containing 0.5 per cent lactalbumin, one part of bovine serum (in a later part of experiments, bovine diarrhea antibody free serum was employed) and 200 unites of penicilline G and 10 micro grams of dihyrostreptomycin per ml. of the growth medium. The pellets were stored at 5°C. refrigerator for overnight and centrifuged at 600 r.p.m. for 2 to 3 minutes. This was diluted with growth medium so that each tube contained 500,000 cells in 2.0 ml.

The cells were cultured at 37°C, incubator for 4 to 5 days and changed the growth medium prior to 0.2 ml. of virus inoculation. Cultivation continued at 37°C. for one week, harvested the supernatant and kept at −60°C. deep freezer. This was used as an antigen for the preparation of anti-hog cholera rabbit serum.

6. Anti-hog Cholera Rabbit Serum:

Healthy adult rabbits were immunized with tissue culture antigen, prepared in a porcine kidney cell culture infected with ALD virulent strain of hog cholera virus, via ear vein in amounts of 1.0 ml, 2.0 ml, 3.0 ml. and 4.0 ml. at one week intervals, and immune serum was collected after one week from the last inoculation. On the immunization of rabbits, antigens were neither treated with antibiotics nor antiseptic but kept in −60°C. deep freezer and handled aseptically. The antisera prepared from rabbit were heat inactivated at 56°C. for 30 minutes and kept in a deep freezer until to use.

7. Swine Pancreas Antigens:

Swine pancreas were obtained from hog cholera infected and normal swines. In order to secure the infected pancreas, pigs were artificially infected with 2.0 ml. of AID splenic virus material via intramuscular route. After virus inoculation, body temperature of swine was checked 2 times in the morning and evening. Swines, showing a typical temperature curve, were ceased prior to die. It required 6 to 10 days after virus inoculation to cease the infected pig. An anterior part of the pancreas, where a lesser amount of fat is contained, was collected and stored at −60°C. deep freezer until to use. From slaughter house, on the other hand, clinically healthy pigs were selected and from these normal pancreass were obtained and used as non infected antigens.

A 20 per cent of pancreas suspension was made in physiological saline under the presence of a small amount of abrasive. Coarse tissue particles and abrasive were eliminated by a preliminary centrifugation at 2000 r.p.m. for 20 minutes, and the supernatant was heated at 50°C. for 30 minutes, and centrifuged again at 3000 r.p.m. for 30 minutes. These procedures resulted in a clear supernatant which gave a less anticomplementary and procomplementary effects but a specific reaction. A few cases of pancreas antigens which showed a cloudy or milky appearance were discarded mainly due to the procomplementary effects. The antigen, stored at 2°C. refrigerator, was used within three days after preparation.

EXPERIMENTAL RESULTS

1. Formation of Anti-hog Cholera Rabbit Serum with Various Antigens:

Formation of anti-hog cholera rabbit serum with various antigens was studied.

Four different tissue culture origin antigens were prepared accordance with a slight modification of Dolveco's tissue culture method as mentioned previously. The antigens were a fresh and heated ALD strain of hog cholera virus (HCV) antigens, and a fresh and heated ROVAC strain of HCV tissue culture antigns. A detailed proceduere of the preparation of the tissue culture origin antigens were described previously. Besides the tissue culture antigens, pig and rabbit tissue antigens were prepared and tested their immunogenicity.

In the preparation of pig tissue origin ALD HCV antigens, pigs were inoculated with ALD HCV infected whole blood in amount of 2.0 ml. via subcutanous route and the pigs showing a typical hog cholera symptom were ceased. This required seven to ten days after the virus inoculation. From the ceased pigs, spleen, liver, lymph node, parotid gland, submaxillary gland and erythrocytes were collected and used as antigens. All tissues except the erythrocytes, a 10 per cent suspension was made in a partially purified state and used as antigen for the preparation of hog cholera antiserum in rabbits.

Tissue origin ROVAC HCV antigens were prepared by using an infected rabbit spleen in a 10 per cent susension in saline solution. Rabbits were inoculoted 2.0 ml. of the virus material via subcutaneous route. The rabbits, showing a typical febrile reaction that was checked two times every day were used as a source of rabbit tissue antigens for the preparation of rabbit tissue origin antiserum in rabbits, were ceased, and from these rabis, spleen, lymph node, parotid gland, supmaxillary gland and erythrocytes were collected. The tisisue was emulsified and centrifuged at 3,000 r.p.m. for 30 minutes after it was suspended being 10 per cent in saline. The supernatant was divided into fresh and hated group and used as antigens.

All tissue antigen, except tissue culture antigens, were added 0.5 per cent phenol as a preservative and frozen until to use.

For each type of antigens, at least four rabbits were used and inoculated the antigen via ear vein in amounts of 1.0, 2.0, 3.0 and 4.0 ml. at one week interval. After one week from the last inoculation of the antigen, antisera were collected and heat inactivated at 56°C. for 30 minutes before the titration of CF antibody of the individual serum. In the antibody titration, ALD HCV infected swine pancreas antigen was used and other procedures were followed as described previously.

The results were illustrated in Table I and the results indicats that the tissue origin antigens prepared from both of swine and rabbit resulted in no antibody formation while the tissue culture origin antigens were positive.

TABLE 1. Formation of Anti-hog Cholera Rabbit Serum with Various Antigens

Types of Antibody*	Mean Antibody Ttiter
Fresh Tissue Culture Origin ALD HCV Antibody	64
Heated Tissue Culture Origin ALD HCV Antibody	32
Fresh Tissue Culture Origin ROVAC HCV Antibody	32
Heated Tissue Culture Origin ROVAC HCV Antibody	16
Fresh Pig Tissue Origin ALD HCV Antibodies**	<8
Heated Pig Tissue Origin ALD HCV Antibodies	<8
Fresh Rabbit Tissue Origin ROVAC HCV Antibodies	<8
HeatedRabbit Tissue Origin ROVAC HCV Antibodies	<8

* Tested with pig pancreas antigen infected with ALD strain of hog cholera virus

** Antibodies includ an individual antiserum of spleen, liver, lymph node, parotid gland, submaxillary gland and erythrocytes antigens prepared from ALD HCV infected pigs

2. Formation of Anti-hog Cholera Rabbit Serum with Tissue Culture Origin ALD Strain of Hog Cholera Virus Antigen:

In the previous experiment it has been shown that the tissue culture origin hog cholera virus was adequate in its immunogenicity. In shis experiment, the antibody formation of hog cholera virus in rabbit was studied on the emphasis of the rate of antibody formation by means of tissue culture origin ALD HCV antigen.

Two different batches of ALD HCV antigens were prepared accordance with a slight modication of Dolveco's tissue culture method as descried previously. The antigens were used without heat inactivation as well as without adding preservatives. During the immunization, the antigens were kept at −60°C.

For each of two batches of the antigen, five adult rabbits were used and immunied via ear vein in amounts

of 1.0, 2.0, 3.0 and 4.0 ml. at one week interval. After one week from the last injection, the rabbits were bled and collected serum. The individual serum was heat inactivated at 56°C. for 30 minutes and titrated their CF antibody. In the antibody titration, a positive antigen of swine pancreas infected with ALD HCV was used and other procedures were followed as described previously.

The results were illustrated in Table II and the results indicate that the antigenic responses appeared at 2 nd week of the virus inoculation, and at 4 th week the antibody titer was reached 32 to 128.

TABLE II Formation of Hog Cholera Antibody in Rabbits by Tissue Cultufe Origin ALD HC Virus Antigen

	ATIBODY TITER WEEK AFTER IMMUNIZATION			
RABBIT NO.	1	2	3	4
1—1	<8	8	16	32
1—2	<8	8	16	64
1—3	<8	32	32	64
1—4	<8	16	32	64
1—5	<8	8	16	32
2—1	<8	16	32	128
2—2	<8	16	32	64
2—3	<8	16	32	64
2—4	<8	8	16	32
2—5	<8	8	16	32

* Titrated with 2 units of swine pancreas antigen prepared by the infection of ALD strain of hog cholera virus

3. Antigenicity of Varius Tissue Antigens Prepared from Hog Cholera Infected Swine:

It has been shown, in the previous experiments, the tissue culture origin ALD HCV antigen resulted in a formation of HCV specific antibody in rabbit, and the rate of antibody formation was also demonstrated. In this experiment, the antigencity of various tissue antigens prepared from hog cholera infected swine was tested with a specific antiserum prepared in rabbits. The procedure of anti-hog cholera rabbit serum prepation was followed as described previously.

In the pig tissue antigen preparation, healthy swines were inoculated 2.0 ml. of a virulent blood, obtained by the inoculation of ALD HCV via subcutaneous route. Swines, showing a typical febrile reaction were ceased just before die or after showing a fatal febrile reaction. From the ceased swines, a portion of pancreas in which less lipid was contained, spleen, congested lymph node, parotid gland, liver, kidney and a whole blood in anticoadulant were collected separately.

Pancreas antigen was prepared as described previously. For the erythrocytes antigen, washed and serum free erythrocytes were allowed to hemolysis in acidic cold distilled water and made a fresh and heated stromrata antigens Other tissue antigens were made by centrifugation for two times at 1,000 r.p.m. for 10 minutes and 4,5000 r.p.m. for 30 minutes after the tissue was suspended and emulsified in a 10 per cent

Table III Antigenicity of Various Tissue Antigens Prepared from Hog Cholera Infected Swines

TYPES OF ANTIGEN	MEAN ANTIGEN TITER
Pancreas antigen	> 32
Spleen antigen	< 8
Lymph node antigen	< 8
Parotid gland antigen	< 8
Liver antigen	< 8
Kidney	< 8
Erythrocytes antigen	< 8

* Tested with anti-hog cholera rabbit serum prepared with tissue culture origin ALD HCV antigen

concentration in saline. The partially purified tissue suspensions were tested their CF antigenicity with or without heat inactivation at 50°C. for 20 minutes. In the cases of heated antigen, they were again centrifuged at 3,000 r.p.m. for 20 minutes and the supernatants were used as antigen. More than 10 infected swines were tested for the studies.

In the antigen titration, a 1/15 diluted anti-hog cholera rabbit serum was used that was prepared by tissue culture origin ALD HCV antigen. Other proceures were followed as described previously.

The results were illustrated in Table III and the results indicate that the pancreas antigen showed antigenicity in a titer of above 32 while the others were negative.

4. Effect of Temperature on the Antigenicity of Swine Pancreas Antigen:

It has been experienced that an adequate heating of pancreas antigen brought about an increased antigenicity

while a fresh or over heated antigen unable to show a higher antigenicity. In this experiment, an optimum temperature required to the purification of the antigen was determined.

Three of positive and negative pancreas antigens were prepared as described previously. Each batch of antigen was divided into four groups and tested their antigenicity as a fresh state, heated states of 50°C, 56°C, and 60°C. Other procedures were followed as described previously.

Table IV Effect of Temperature on the Antigenicity of Swine Pancreas Antigen

	DEGREE OF HEMOLYSIS											
	HEATED FOR 30 MINUTES AT											
	50°C			56°C			60°C			UNHEATED		
TYPES OF	ANTIGEN DILUTION											
ANTIGEN	1/8	1/16	1/32	1/8	1/16	1/32	1/8	1/16	1/32	1/8	1/16	1/32
Positive No. 3	4*	4	4	4	1	0	2	0	0	0	0	0
Positive No. 9	4	4	4	4	2	1	3	1	0	AC	0	0
Positive No. 13	4	4	4	4	0	0	0	0	0	0	0	0
Negative No. 12	0	0	0	0	0	0	0	0	0	0	0	0
Negative No. 23	0	0	0	0	0	0	0	0	0	0	0	0
Negative No. 25	0	0	0	0	0	0	0	0	0	0	0	0

* Titrated with anti-hog cholera rabbit serum
AC: Anticomplementary effect

The results were illustrated in Table IV and the results indicate that an unheated and 60°C. 30 minutes heated antigens were unable to fix complement. On the other hand, 50°C. 30 minutes heated antigen showed a titer of more than 1/32.

5. Antigenicity of Swine Pancreas Antigens With or Without Hog Cholera Infected

Antigenicity of three different groups of pancreas antigens were tested. These were 23 samples of positive antigens obtained from hog cholera infected pigs, 21 samples of negative antigens obtained from pigs that showing no clinical and pathological abnormality, and 14 samples of antigens obtained from the vaccinated and challenged pigs.

The vaccinated and challenged samples were made by vaccination of pigs with ROVAC vaccine and challenged with ALD strain of HC virus after three weeks of the vaccination. All samples were stored at —60°C. until to test, and liquified antigens were tested on the same day of the preparation or not exceed more than 2 days in refrigerator. Other procedures were followed as described previously.

The results were illustrated in Tables V and VI. The results indicate followings. In case of the positive antigens, one and two out of 24 samples showed procomplementary and anticomplementary effects respectively, and the others possessed their antigen titer more than 1/16. In case of the negative antigens, one out of 21 samples showed anticomplementary effect ant 2 out of 21 samples showed a slight non specific fixation. In case of the vaccinated and challenged antigens, five out of 14 samples showed fixation at the dilution of 1/8 and others werenegative.

TABLE V Antigenicity of Swine Pancreas Antigens with or without Hog Cholera Infected

Source of Antigen	Pancreas Antigen Dilution 1/8	1/16	1/32
Infected Pig No. 1	4	4	4
Infected Pig No. 2	4	4	4
Infected Pig No. 3	4	4	4
Infected Pig No. 4	4	4	4
Infected Pig No. 5	4	4	4
Infected Pig No. 6	4	4	4
Infected Pig No. 7	0	0	0
Infected Pig No. 8	4	4	4
Infected Pig No. 9	4	4	4
Infected Pig No. 10	4	4	4
Infected Pig No. 11	4	4	4
Infected Pig No. 12	4	4	4
Infected Pig No. 13	AC	4	3
Infected Pig No. 14	4	4	4
Infected Pig No. 15	4	4	4
Infected Pig No. 16	4	4	4

Infected Pig No. 17	4	4	4
Infected Pig No. 18	4	4	4
Infected Pig No. 19	4	4	4
Infected Pig No. 20	AC	AC	AC
Infected Pig No. 21	4	4	4
Infected Pig No. 22	4	4	4
Infected Pig No. 23	4	3	0
Non-infected Pig No. 1	0	0	0
Non-infected Pig No. 2	0	0	0
Non-infected Pig No. 3	0	0	0
Non-infected Pig No. 4	0	0	0
Non-infected Pig No. 5	0	0	0
Non-infected Pig No. 6	2	0	0
Non-infected Pig No. 7	0	0	0
Non-infected Pig No. 8	0	0	0
Non-infected Pig No. 9	0	0	0
Non-infected Pig No. 10	0	0	0
Non-infected Pig No. 11	3	0	0
Non-infected Pig No. 12	0	0	0
Non-infected Pig No. 13	0	0	0
Non-infected Pig No. 14	0	0	0
Non-infected Pig No. 15		0	0
Non-infected Pig No. 16	0	0	0
Non-infected Pig No. 17	0	0	0
Non-infected Pig No. 18	0	0	0
Non-infected Pig No. 19	0	0	0
Non-infected Pig No. 20	0	0	0
Non-infected Pig No. 21	0	0	0
Vaccinated-challenged Pig No. 1	0	0	0
Vaccinated-challenged Pig No. 2	0	0	0
Vaccinated-challenged Pig No. 3	4	0	0
Vaccinated-challenged Pig No. 4	0	0	0
Vaccinated-challenged Pig No. 5	0	0	0
Vaccinated-challenged Pig No. 6	4	0	0
Vaccinated-challenged Pig No. 7	0	0	0
Vaccinated-challenged Pig No. 8	4	0	0
Vaccinated-challenged Pig No. 9	4	0	0
Vaccinated-challenged Pig No.10	0	0	0
Vaccinated-challenged Pig No.11	0	0	0
Vaccinated-challenged Pig No.12	0	0	0
Vaccinated-challenged Pig No.13	0	0	0
Vaccinated-challenged Pig No.14	4	0	0

AD: Anticomplementary Effect.

TABLE VI Distribution of Antigen Titers of Various Swine Pancreas with or without Hog Cholera Infected

Source of Pancreas Antigen	Number of Swine					
	Antigen Dilution					Total Pig Tested
	1/8	1/16	>1/32	>1/8	AC*	
Infected Pig	0	1	19	1	2	23
Non-infected Pig	0	0	0	20	1	21
Vaccinated and Challenged Pig	4	1	0	9	0	14

*AC : Anticomplementary Effect

DISCUSSION

It has been experienced that, in the complement fixation test of hog cholera, swine antiserum resulted in hemolysis of the sensitized sheep erythrocytes of guinea pig complement. Furthermore, most of swine tissue antigens tested in these studies showed a tendency of hemolytic activity unless the antigens were partially purified and heat treated. Such characteristics of swine serum and tissues brought about an attempt on the development of complement fixation, for hog cholera diagnosis, in which rabbit origin hog cholera antiserum, swine tissue antigen and hemolytic system were incorporated.

1. Preparation of Hog Cholera Antiserum in Rabbits:

Antigens prepered from hog cholera infected, tissue culture material was adequte for the preperation of hog cholera antiserumin in rabbits. Although an unheated antigen caused the formation of higher titer of antibody in rabbits it may bring about a hypersensitive state in rabbits were inoculated more than four times via intravenous route. The side reaction was removable if the antigen was heated at 50°C. for 30 minutes or inoculation of decreased amount of antigen. The reaction may due to bovine serum which was contained in the tissue culture material. A number of tissues obtained from swines and rabbits that were infected with virulent strain and rapinized strain of hog cholera virus respectively, were not immunogenic in rabbits. This may due to the presence of a low concentration of antigenic materials and excess amount of tissue marials in the antigens.

2. Preparation of Complement Fixing Antigens:

A number of hog cholera infected tissue antigens, namely pancreas, spleen, liver, kidney, lymph node, parotid gland and erythrocytes stromata were tested and it has

been shown that the pancreas was only organ that possesses antigenicity. Other tissue antigens, except stromata and pencreas, obtained and made from the infected pig rather showed a tendency of a enhanced hemolytic activity compared to that of normal. Such hemolytic activity may due to the excess amount of serum contained in the infected tissue materials.

Even the positive pencreas antigen, unheated fresh state, a hemolytic activity was observed, and the activity was removed by heating the antigen at 50°C. for 30 minutes. Certain pancreas antgien showing a milky appearance, showed both of hemolytic and anticomplementary effects even the antigen was heated. Heating the antigen at above 60°C. for 30 minutes caused the diminishing antigenicity of the supernatant and increasing the precipitate. This may indicate that the milky appearance antigen is hemolytic and anticomplement due to the excess amount of lipidic compounds present in the antigen.

It is noteworthy, due to the facts that above mentioned, in the sampling the test pancreas and in the heating process, that the sample must be selected from a portion of pancreas where less lipid is contained, and heating the pancreas should be maintained at 50°C. for 30 minutes. The optimum heating temperature and time may remove maximum quantity of pancreas lipid and give maximum antigenicity.

Pancreas antigen, in a state of tissue, can be storeed at 60°C. for several months without lossing the antigenicity. In the liquified state is suspended, heated and centrifuged supernatant antigen could be stored in fefrigerator for 2 to 3 days without heving defect on the antigenicity. However freezing the antigen may remove antigenicity and increased formation of precipitate. Slightly autolyzed pancreas may not influence on the antigenicity.

In these studies the positive pancreas were obtained from pigs that were died or just prior to die. Therefore, the relation between the appearance of detectable antign in the infected pancreas and time of the course must be studied further. Pancreas antigens, prepared from hog cholera infected pigs that were died or killed to just prior to die, were highly specfic. According to the accumulated results, not less than 95 per cent of the positive panreas and more than 97 percent of the negative pancreas were detectable by means of this method. About 30 per cent of antigens prepared from pigs that were challenged after 2 to 3 weeks of vaccination showed 1/8 serum titer, and others showed no serum titer. Furtherinore, vaccinated but not challenged pigs showed negative serum titer. These results may suggest the method is appl icable for the detection of hog cholera infected pigs in the diagnostic purpose.

SUMMARY

From thse studies, the methodology of the complement fixation test for hog cholera has been described, and the application of the test for the diagnosis of hog cholera has been suggested.

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豚콜레라의 診斷을 위한 補體結合反應에 關한 硏究

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全 允 成

豚콜레라는 世界各處에서 널리 流行되는 돼지의 重要한 疾病이다. 그러나 이 疾病을 血淸學的으로 診斷할 수 있는 簡單하고 高度로 特異한 方法은 아직 없다. 이 硏究에서는 補體結合反應을 利用한 보다 우수한 血淸的診斷法을 實驗하여 좋은 成績을 얻었다.

檢出抗原은 感染豚의 膵臟乳劑이며 陽性例에서의 力價는 1/16 이상이였다. 抗血淸은 組織培養源 바이러스材料를 家兎에 接種하여 만들었고 이의 力價는 1/32 이상이었다. 補體는 1.1~1.2 充分單位를 사용하였고 感作赤血球는 2單位 2%로 하였다.

이 方法으로써 感染豚의 95% 이상이 特異的으로 檢出될 수 있었고, 非感染豚은 97% 이상이 陰性으로 檢出될 수 있었다. 따라서 이 方法은 豚콜레라를 診斷하는데 利用될 수 있는 보다 낳은 血淸學的 方法로으 믿어진다.