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AN IMMUNODIFFUSION TEST FOR DETECTION OF HOG CHOLERA VIRUS ANTIBODIES IN SWINE SERUM

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An immunodiffusion test (IDT) was developed for detecting hog cholera virus (HCV) antibodies in swine serum. The antigen used in the IDT was prepared from the LPC-Chinese strain HC vaccine virus infected rabbit spleens. Results of the IDT were obtained within 24 hours and well correlated with the END method for antibody titration, capable for detection of HCV antibodies as low as an END titer of 1:2.

The END method[3], interference and indirect immunofluorescent test (IIFT) are currently used to detect HCV antibodies in swine serum. For the detection of HCV antibodies, American and European workers mainly used IIFT. However, Japanese workers preferred the END method. The END method has been also used successfully for detecting HCV antibodies in Taiwan. Recently the END method has been modified by using microtiter procedure to detect HCV antibodies, which makes the test simpler and more rapid[4]. These tests are accurate and sensitive; however they require a laboratory equipped for cell culture procedures up to 7 to 9 days for completion and considerable expertise to conduct. In addition, some serums are cytotoxic to indicator cell cultures which prevents the detection of neutralization antibodies in the tests. Therefore, there is a need for a rapid, accurate, sensitive and economical diagnostic test to detect HCV antibodies. Utilization of IDT correlated with virus neutralization test (VNT) has been reported for many viral diseases[1,2,5], but not reported for HC.

The IDT described in the present report is the result of our efforts to develop a simple, rapid and inexpensive technique for diagnosing HC.

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MATERIALS AND METHODS

Calls: Primary swine testicle (ST) cells were prepared by trypsinization of testes obtained from 4 to 6 weeks old healthy piglets. The procedures for preparing the cells were previously described[4].

Viruses: LPC-Chinese (LPC) strain HC vaccine virus, 816 rabbit passage was used for antigen preparation. A_{76} strain of HCV with a titer of 100 $TCID_{50}$ was used for the END method. Miyadela strain of Newcastle disease virus cultivated in allantoic fluid of 10 days old chick embryos was used for challenge in the END method.

Animal: Large white New Zealand rabbits weighed about 2 kgs were used for producing LPC strain of HC vaccine viral antigens. Rabbit were intravenously inoculated with 1 ml of LPC strain of HC vaccine virus containing 10^6 rabbit infective dose. Seventy-two hours after inoculation, rabbits showing a fever response up to 41.5°C were sacrified. The spleen was collected for antigen preparation.

Serum samples: 132 serum samples collected from 6 to 8 weeks old piglets were used for IDT and END tests. All sera were inactivated at 56°C for 30 minutes.

END method: A simple and rapid microtiter procedures for END method previously described[4] was used for titration of HCV antibodies.

Antigen preparation: Ten grams of

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LPC vaccine virus infected rabbit spleen were ground in a mortar and pestle, and a 20% emulsion (W/V) was made in 0.02 M Tris buffer (pH 7.5). The emulsion was sonicated for 5 minutes at 200 W in an ice bath. The sonicate was sedimented 100,000 × g for 2 hour. The resulting pellet was discarded and the supernatant fluid was concentrated to approximately 5% of the original volume by dialysis of the antigen against 0.02 M Tris buffer (pH 7.5) in the negative pressure.

Immunodiffusine test: Ouchterlony's method[6] was used, substituting agarose in 0.02 M Tris buffer containing an additional 0.15 M NaCl and 0.05% sodium azide was used. An amount of 16 ml of warm 0.65% agarose was poured into a plastic Petri disches (8.4 mm diameter) and allowed to stand at 25°C for 1 hour. A template with five peripheral wells and one central well (4 mm diameter) and 2 mm apart in all directions was used to make wells in the agar. The agar plngs were removed by suction. The central well was filled with HCV antigen (20 μl) and the alternated two outer wells were filled with reference serum (20 μl). The remaining three outer wells were filled with test serums. The Petri dishes were inocubated in a humidified chamber at 37°C and examined for 24–72 hours over a oblique beam of light. The concentration of HCV antigen was adjusted to a concentration of precipitin line formed clearly in the middle of wells filled with antigen and reference serum. The precipitin lines or deviation of the reference serum precipitin lines near the wells of the test serum were recorded as weak positive for HCV antibodies. If the reference serum precipitin lines extended to the test serum well without deviation, the test serum was recorded as a negative.

RESULTS

The experimental data indicated that an extractable, soluble antigen was present in rabbit spleen infected with LPC strain of HC vaccine virus. The antigen was not detected in noninfected rabbit spleen. The optimal concentration of HCV antigen to form a clear precipitin line in the middle of antigen and reference serum wells filled with serum of an END titer of 1:128 was between 1.5 and 2 ml/10 gms of rabbit infected spleen. The concentration of the antigen could detect HCV antibodies as low as an END titer of 1:2 through 1:256 in swine serum. As shown in Fig. 1, positive reference serum, designated Ab, had an END titer of 1:128. The END titers of test serums in wells 1, 2, and 3 were 1:128, 1:256 and $<$1:2 respectively. The reference serum precipitin lines on each side of the serum negative for HCV extended into well 3 without deviation. The reference serum precipitin lines form precipitin lines of identity with serum positive for HCV in well 1 and 2. The precipitin reactions were readily interpreted after 24 hours incubation.

In order to test the correlation between the IDT and the END methods for detecting HCV antibodies, 132 serum samples were tested independently by both diagnostic tests. The results are presented in Table 1. The results of the IDT correlated 100% with those of the END method when serum titers were 1:8 or greater. The IDT could detect 92.3% of serum samples as positive when their titers were as low as 1:2. Ten serum samples had an END titer of less than 1:2 gave negative precipitin reactions for HCV antibodies.

DISCUSSION

The END method, interference and IIFT are currently available techniques for detection of HCV antibodies. Although these techniques are accurate and sensitive, they have several undesirable features which include the length of time required to complete the test, use of aseptic techniques and expensive facilities. The results indicated that IDT well correlated with the END method, capable of detecting HCV antibodies titers as low as 1:2, and did not have the undesirable features as mentioned.

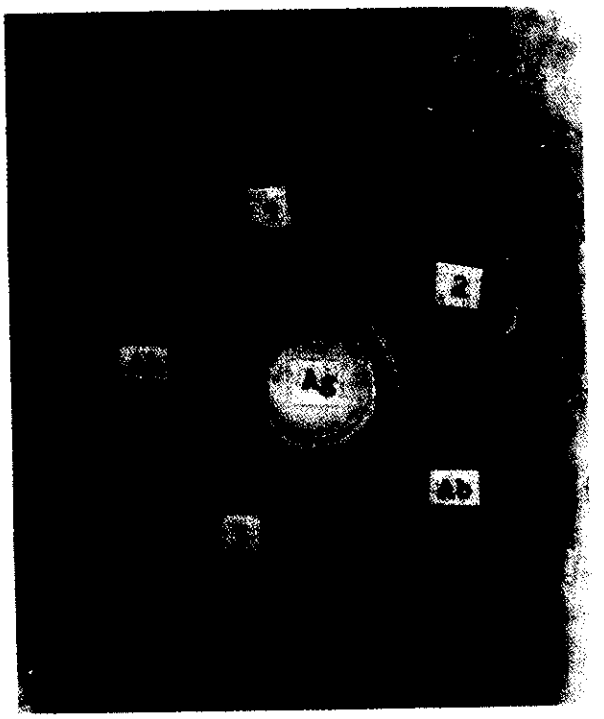

Fig. 1. The IDT test reactions between HCV antigen and HC reference serum or test serum.
Ag=HCV antigen; Ab=HC reference serum (END titer 1:128); 1=test serum (END titer 1:128); 2=test serum (END titer 1:256); 3=test serum (END titer less than 1:2)

Table 1. Correlation of END Method and IDT for Detection of HCV Antibodies

No. of Samples	END titer	Results of IDT			
		No. Positive	No. weak Positive	%	No. Negative
10	<1:2	0	(0)*	0	10
14	1:2	13	(7)	92.3	1
31	1:4	29	(4)	93.6	2
31	1:8	31	(5)	100	0
15	1:16	15	(1)	100	0
10	1:32	10	(0)	100	0
9	1:64	9	(0)	100	0
5	1:128	5	(0)	100	0
7	1:256	7	(0)	100	0

* No of positive serums include No. of weak positive serums.

Unlike the viral neutralization test (VNT), the IDT can not titrate serum samples. However, an estimate titer of the tested serum in the IDT could be made by comparing the resulting precipitin line of a reference serum known neutralization titer with those which develop with test serums by the location and the intensity of the

precipitin line. When the constant concentration of antigens was used, serums with low VNT titers develop precipitin lines which increase in intensity and closer of the precipitin lines to the central well of the antigens as the VNT titers increase. The experimental results indicated that the titer of serum in the IDT could best be estimated in serum with a titer of 1:2 through 1:128. Three out of 45 serums with VNT titers between 1:2 and 1:4 were negative in the IDT. Therefore, the IDT is apparently less sensitive in detecting HCV antibodies than is the END method.

Conclusively, the IDT described in the present report appears to be a useful method in assaying swine serum for HCV antibodies.

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應用免疫擴散法測定猪瘟抗體

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以兎化猪瘟毒感染之兎脾臟抽取可溶性猪瘟抗原供免疫擴散法試驗，其效果良好。本免疫擴散法從猪血清檢出猪瘟抗體之敏感度高，血清抗體價僅爲 2 倍之血清樣本，有 92.3% 可以免疫擴散法檢出猪瘟抗體。本法在 24 小時卽可判定。

臺灣省家畜衛生試驗所。