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Detection of porcine rotavirus in stools by a latex agglutination test

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ABSTRACT

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We developed a simple agglutination test for the detection of porcine rotavirus in stools from pigs with diarrhea. The virus was detected with high sensitivity and specificity by a slide agglutination test using latex particles coated with antibody against the porcine rotavirus strain OSU (LA-antiOSU). The agglutination of LA-antiOSU with OSU on a glass slide was evident macroscopically within 2 min. The sensitivity of this latex agglutination (LA) test was four times higher than that of the electron microscope method. The LA test is available for the rapid diagnosis of porcine rotavirus infections.

INTRODUCTION

Rotavirus (Saif et al., 1980; Bohl et al., 1982; Bridger et al., 1982), porcine transmissible gastroenteritis (TGE) virus (Sasahara et al., 1958) and porcine epidemic diarrhea (PE) virus (Takahashi et al., 1983) are very important causative agents of viral gastroenteritis in pigs. Although many rotaviruses are present in the stools of pigs infected with rotavirus, there is no simple method for the detection of the virus. Although electron microscopy (Bishop, 1974) and the tissue culture method (Fukusho et al., 1981) can detect rotavirus, these methods are very complicated and need a lot of equipment. In the case of human rotavirus, many methods have been developed for the direct detection of the virus in stools by immunoassay, such as enzyme immunoassay (Sarrkinenn, 1981), radioimmunoassay (Kalika et al., 1977), reverse passive hemagglutination (RPHA) (Sanekata et al., 1979) and latex agglutination (LA; Sanekata et al., 1981). In this paper, we report the development of a new LA test for the rapid diagnosis of porcine rotavirus infections.

MATERIALS AND METHODS

Stool samples

Stool samples were collected from pigs with gastroenteritis. The age of the pigs ranged from 3 to 15 days. The stool samples were collected 1–5 days after the onset of the disease.

Viruses

OSU (Theil, 1977), Wa, NCDV, and SA11 strains were used to represent porcine, human, calf, and simian rotaviruses respectively. These viruses were prepared in MA-104 cells and purified as described previously (Sanekata, 1981). This procedure involved rate zonal centrifugation using sucrose and metrizamide. The resulting virus-containing bands were further purified by flotation in a metrizamide gradient at an ultracentrifugation speed of 36 000 rpm for 16 h. The high purity of the virus fraction was confirmed by electron microscopy.

Preparation of anti-OSU, anti-Wa, anti-NCDV and anti-SA11 serum

Anti-OSU, anti-Wa, anti-NCDV and anti-SA11 sera were prepared as previously described (Sanekata, 1981). Anti-OSU IgG fraction (anti-OSU IgG) for the preparation of latex particles coated with antibody was purified with 33% saturated ammonium sulfate

Preparation of latex particles coated with anti-OSU IgG (LA-antiOSU)

LA-antiOSU was prepared as described previously (Sanekata, 1981). Latex particles (diameter 236 nm, Dow Chemical Company, USA) were washed with 0.07 M phosphate-buffered saline (PBS, pH 7.2) by centrifugation at 10 000 rpm for 4 min at 4°C in a Hitachi SRT15AA rotor, and then suspended in PBS at a concentration of 1.0% (w/w). An equal volume of anti-OSU (1.0 mg/ml) was added to the latex suspension and the mixture was shaken at room temperature for 2 h. To eliminate unconjugated antibodies, the mixture was centrifuged at 10 000 rpm for 5 min at 4°C, and the supernatant was discarded. After washing the pellet three times with PBS by centrifugation, the LA-antiOSU was suspended at 0.7% in PBS and kept at 4°C.

Detection of porcine rotavirus by the LA method

A 10% stool suspension in PBS was homogenized for 1 min using a Teflon tissue grinder. The homogenate was centrifuged at 15 000 rpm for 5 min at 4°C and the supernatant was subjected to the LA test. Ten μ l of the stool specimen was mixed with an equal volume of PBS on a glass slide. For the inhibition test, 10 μ l of the same stool specimen was preincubated with 10 μ l of anti-OSU (immune adherence hemagglutination titer (IAHA): 1:160) for 6 min at room temperature. Then 20 μ l of LA-antiOSU was added to each

specimen. After shaking for 2 min, the agglutination patterns were examined macroscopically. Specimens developing agglutination in the LA test but not in the inhibition test were regarded as positive.

IAHA test

The IAHA test was carried out according to Sanekata et al. (1982). To each well of serial twofold dilutions of the sera on a U-shaped microtitration plate was added 4 units of virus in 25 μ l, and the plate was incubated at 37°C for 1 h. Then 25 μ l of complement was added, and the culture was incubated at 37°C for 40 min. Dithiothreitol (DTT; 3 mg/ml) and human erythrocytes were then added.

Electron microscopy

The examination of virus by electron microscopy was carried out as described previously (Sanekata, 1983). Briefly, a 10% stool suspension in double-distilled water (ddw) was mixed with an equal volume of trifluoroethane, and centrifuged at 3000 rpm for 30 min. The supernatant was layered on a 20% sucrose solution, then centrifuged at 40 000 rpm for 2 h. The pellet was suspended in 0.2 ml of ddw, stained with 2% uranyl acetate, and examined with a JEOL JEM-100CX electron microscope.

RESULTS

Cross-reactivity of anti-OSU antibody

The reactivities of OSU, Wa, NCDV and SA11 with anti-OSU, anti-Wa, anti-NCDV and anti-SA11 were examined by IAHA (Table 1). All four antisera showed cross-reaction with heterologous viruses, but the reactions were not as strong as with homologous viruses. OSU reacted strongly with anti-OSU and anti-SA11.

TABLE 1

Antigenic relationships of porcine, human, bovine and simian rotavirus by IAHA tests

Rotavirus	IAHA antibody titers of antisera			
	Porcine (OSU)	Human (Wa)	Bovine (NCDV)	Simian (SA11)
Porcine (OSU)	102 400	51 200	12 800	25 600
Human (Wa)	12 800	204 800	12 800	12 800
Bovine (NCDV)	12 800	12 800	204 800	6 400
Simian (SA11)	102 400	25 600	6 400	25 600

Specificity of the LA test

LA-antiOSU caused agglutination with OSU within 2 min on a glass slide. To confirm that the agglutination of LA-antiOSU was really due to a specific reaction between OSU and LA-antiOSU, the agglutinated specimens were examined under the electron microscope. Rosette formation and bridge formation of the viruses on and between the LA-antiOSU particles were observed (Fig. 1). Agglutination of LA-antiOSU with OSU was completely inhibited by preincubation of OSU with anti-OSU at room temperature for 6 min (Fig. 2).



Fig. 1. Agglutination of latex particles coated with anti-OSU IgG (LA-antiOSU) with OSU ($\times 72\,000$). LA-antiOSU was mixed with OSU for 2 min at room temperature; the mixture was stained with 3% uranyl acetate and then observed under the electron microscope.

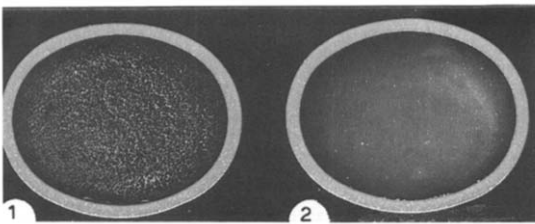


Fig. 2. Specific agglutination of LA-antiOSU with OSU. 1. OSU was mixed with LA-antiOSU for 2 min. 2. OSU was preincubated with anti-OSU at room temperature for 6 min and mixed with LA-antiOSU for 2 min.

Sensitivity of the LA test

The sensitivities of the LA and electron microscope methods were estimated and compared (Table 2). An OSU suspension was used as an indicator. The highest dilutions of the virus detected as positive by the LA and electron microscope methods were 1:1024 and 1:256, respectively. To test whether the LA method detects OSU in stool extract with the same sensitivity as OSU diluted in PBS, as described above, OSU was diluted in PBS and in a 10% extract of normal stools of pigs. The LA method detected OSU in the stool extract with the same sensitivity as in PBS.

Stability of LA-antiOSU

Samples of LA-antiOSU were kept at 4°C for 6 months and their reactivity was examined monthly. No reduction in sensitivity and no tendency to develop nonspecific agglutination were observed during a period of 6 months.

Detection of porcine rotavirus in stool samples by the LA test and electron microscopy

Stool samples were prepared from pigs with diarrhea. Detection of rotavirus by the LA test was carried out and compared with the results of electron microscopy (Table 3). All stool samples in which rotavirus was detected by electron microscopy showed a strong positive agglutination, while the other samples were negative. But one of the four samples negative by electron mi-

TABLE 2

Comparison of the sensitivity of latex agglutination (LA) and electron microscope (EM) tests

Rotavirus	Maximum reciprocal dilution detected by	
	LA	EM
Porcine OSU	1024	256

TABLE 3

Detection of porcine rotavirus in pigs with diarrhea by LA test

Viruses from stool samples detected by electron microscopy	Number of samples	LA test	
		Positive	Negative
Rotavirus	17	17	0
Coronavirus	4	0	0
Negative	4	1 ^a	0

^aConsidered to be positive for rotavirus from the results of inhibition test.

crosscopy was positive by the LA test. Samples containing coronavirus or enterovirus-like particles were all negative by the LA test.

DISCUSSION

Viral gastroenteritis is a very important disease in pigs. The main causative agents are rotavirus, TGE virus and PE virus. Hence it is very important to develop a simple method to diagnose rotavirus infections. Several methods have been reported for detecting human rotavirus in stool specimens, such as the enzyme-linked immunosorbent assay, radioimmunoassay and electron microscopy. However, these methods seem to be inappropriate for routine diagnosis. Electron microscopy is complicated and needs expert technicians. Radioimmunoassay requires radioisotopes. The tissue culture method employing MA-104 cells takes a considerable time to perform. Enzyme immunoassay is not economical for testing a small number of samples. The agglutination method is thought to be useful, however, since it is rapid, simple and sensitive enough to be employed as a routine test (Sanekata et al., 1979, 1981, 1990). In this study, we developed the LA test for the detection of porcine rotavirus directly from stools, and compared the sensitivity of the LA test with that of the electron microscope test.

Many kits for the detection of human rotavirus using immunological methods are commercially available. These kits are prepared using anti-human rotavirus, anti-simian rotavirus or anti-calf rotavirus. Since these viruses differ from porcine rotavirus in antigenicity, the anti-Wa or anti-NCDV antisera did not cross-react with porcine rotavirus-like homologous antibodies (Table 1). Hence we consider that these antibodies are not suitable for the detection of porcine rotavirus. A kit for the detection of human rotavirus using anti-NCDV antibody could in fact detect only 12 of 17 rotavirus-positive samples, while LA-antiOSU could detect all of these porcine rotaviruses.

Since binding of rotavirus with LA-antiOSU occurred within 2 min on a glass slide, an LA test result can be obtained within 8 min, including the inhibition test. Especially in the case of liquid samples, rotavirus could be detected directly: 10 μ l of liquid stool was layered on a glass slide, and an equal volume of PBS was added and mixed gently using a stick. Then 20 μ l of LA-antiOSU was added and mixed for 2 min until complete agglutination occurred.

Although the number of stool samples examined was small, LA-antiOSU could specifically detect porcine rotavirus in pig stools. This LA-antiOSU did not react with stools containing coronavirus or enterovirus-like particles. The LA method was thus found to be useful and practical for the detection of porcine rotavirus in stools. It is rapid and simple, and is sensitive enough for diagnostic and epidemiological investigation.

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