Isolation of Reovirus Type 2 from Diarrheal Feces of Pigs

Toyoko FUKUTOMI, Takeshi SANEKATA[1], and Hiroomi AKASHI[2]

Okayama Prefectural Veterinary Diagnostic Laboratory, Mitsu-cho, Okayama 709–21, [1]Department of Veterinary Microbiology, Tottori University, Tottori 680, and [2]National Institute of Animal Health, Kannondai, Tsukuba, Ibaraki 305, Japan

(Received 20 November 1995/Accepted 16 January 1996)

ABSTRACT. Reoviruses designated as OS-320 to OS-324 were isolated from a total of 5 fecal specimens from 3 with diarrhea and 2 apparently healthy pigs aged 3 months. The serotype of these isolates was determined as reovirus type 2 by cross neutralizing tests. Furthermore, the result of hemagglutination test suggested the isolates were different from the other reoviruses. A survey of neutralizing antibody against reoviruses showed type 2 virus was widely prevalent among pigs in Okayama Prefecture. This paper is the first report of the isolation of reovirus type 2 from pigs. — KEY WORDS: reovirus, serotype 2, swine.

J. Vet. Med. Sci. 58(6): 555–557, 1996

Reovirus has been isolated from many species of mammals [6, 9, 12, 15, 16] and birds [1, 8]. The mammalian reoviruses have been shown to have 3 serotypes [14, 16]. Isolation of reovirus type 1 from pigs was reported by Kasza [7] and McFerran and Connor. [11], and reovirus type 3 by Roble *et al.* [13]. In Japan, Hirahara *et al.* [4] isolated reovirus type 1 from respiratory tract of pigs with a respiratory disease. Although serological surveys reveal that all the serotypes of reovirus are spreading among pigs in the field, no type 2 reovirus has been isolated from pigs so far. This paper describes the isolation of reoviruses from pigs with or without diarrhea and characterization of one of the isolates as reovirus serotype 2.

A total of 5 fecal specimens from 3 diseased and 2 apparently healthy pigs aged 3 months reared in the same pig house were collected from a farm in January 1994. Virus isolation was performed according to the method described for rotaviruses by Sanekata *et al.* [17]. Briefly, a 10% emulsion of the feces was centrifuged to pellet debris and a supernatant fluid was filtrated through a 450 nm pore sized membrane, and treated with 10 μg/m*l* of trypsin at 37°C for 30 min, and the suspension was inoculated onto Vero cells. Incubation was performed in the presence of 2 μg/m*l* of trypsin.

Electron microscopic observation and physicochemical properties of an isolate were performed by the method described in the previous report [4].

The Lang strain (type 1), the Amy strain, the BN-77 strain and the 39 strain (type 2) and the Abney strain (type 3) were used as the reference strains of reovirus in this study. The Amy strain was kindly supplied by Toyama Institute of Health. Polyacrylamide gel electrophoresis (PAGE) was performed on viral RNA extracted from virus infected culture fluid by the method described elsewhere [2].

A hyperimmune antiserum to the isolate was prepared in chickens according to the method described by Sanekata *et al.* [17]. An immune serum to each reference strain of reovirus was kindly supplied by Division of Veterinary Microbiology, Kyoto-Biken Laboratories.

Hemagglutination (HA) antigens were prepared from infected Vero or primary bovine kidney cell cultured fluid. The procedure of HA was performed by the method described by Hirahara *et al.* [4]. Paired sera collected from the pigs positive for virus isolation were tested for neutralizing (NT) antibodies to the isolate and the reference strains of reovirus to confirm the identity of the virus. A survey of NT antibodies to reoviruses was performed using sera collected from pigs in farms in 5 areas in Okayama Prefecture.

Five cytopathic agents designated as OS-320 to OS-324 were recovered from each fecal specimen. CPE of all samples appeared at the first passage level, 4 to 6 days after inoculation. The isolates were purified by the plaque formation method described by Matsuno *et al.* [10]. All 5 viruses were not distinguished serologically by NT test using the antiserum to the OS-320 strain. Thus, the OS-320 strain was used for further characterization. Electron microscopic observation of the virus partially purified from the infected cell culture fluid revealed spherical virus particles, about 70 to 72 nm in diameter, with double layered capsid.

The virus replication was not inhibited by 5-iodo-2'-deoxyuridine, showing the nucleic acid type is RNA. The virus was not affected by ethyl ether, chloroform, or pH 3.0. And the virus was stable against heating at 50°C for 1 hr in the presence of 1 M $MgCl_2$. By PAGE analysis of viral RNA extracted from the OS-320 strain and the reference strains, it was cleared that the OS-320 strain as well as the reference strains had 10 segments which had similar electrophoretic mobility (Fig. 1). These results reveals that the OS-320 strain belongs to reovirus group.

Although the reference viruses of 3 serotypes were found to agglutinate human A, O and swine erythrocytes at 4°C, 25°C, and 37°C, the OS-320 strain which has a titer of $10^{7.3}$ $TCID_{50}$/m*l* showed no agglutinating activity with these erythrocytes (data not shown).

Cross NT tests were performed with antisera to the OS-320 strain, the Lang strain (type 1), the Amy strain and the BN-77 strain (type 2), and the Abney strain (type 3). The OS-320 strain was neutralized as same titer as to the homologous antiserum by the antiserum to the Amy strain of type 2, but not to the other strains including the BN-77 strain of type 2. In addition, the antiserum to the OS-320 strain neutralized the BN-77 strain as well as the homologous strain, and neutralized the Amy strain very weakly (Table 1). Thus, the OS-320 strain was identified

as reovirus type 2. Antigenic variety of reovirus type 2 has been reported by Hartley *et al.* [3]. Moreover, they classified reovirus type 2 into 4 subtypes. Although we could not determine the subtype of the isolates in this study, the antigenic variety of reovirus type 2 was also observed by the cross NT tests. In Table 1, the OS-320 strain, the Amy strain, and the BN-77 and the 39 strains isolated from pig, human, and cattle, respectively, also showed antigenic differences among these strains. There was a one-way antigenic relationship between the Amy and the OS-320 strain, between the Amy and the BN-77 strain, and between the OS-320 and the BN-77 strain.

The paired sera collected from the reovirus-isolated pigs were tested for NT antibodies to the OS-320 strain and the reference strains of reovirus. Four to eight times NT antibodies increase to the OS-320 strain was observed on the paired sera from 3 pigs with diarrhea and an apparently healthy pig, showing the infection of the virus (data not shown).

As shown in Table 2, positive rate of NT antibody to reovirus type 1 was 82%, 36%, type 2 was 100%, 77%, and type 3 was 56%, 27% in farms and heads, respectively. Positive rate of NT antibody to the OS-320 strain was 100%, 92%. There was very little difference in the positive rate to the isolate between 5 areas in Okayama Prefecture. Hirahara *et al.* [5] reported that reovirus had widely spread among pigs in Japan. Although the seropositive rate to reovirus type 2 was higher than those of reovirus type 1 and 3 in this study, pathogenicity of reovirus type 2 has not so clearly been verified as yet. In the present study, the virus was also isolated from apparently healthy pigs reared in the same pig house.

This paper is the first report of the isolation of reovirus type 2 from pigs. The result of HA test with human and swine erythrocytes suggested that the OS-320 strain has different characteristics from the other reoviruses. Further virological and pathological studies of this isolate should be carried out.

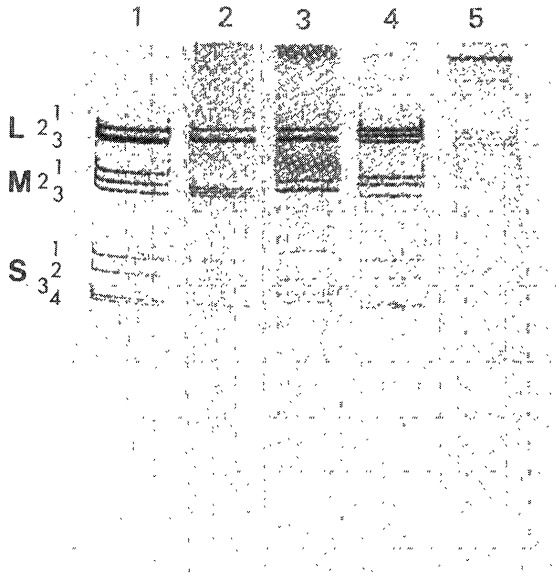

Fig. 1. The electrophoretic analysis of genomic RNAs from each serotype of reovirus. Lane 1: the Lang strain of serotype 1, Lane 2: the 39 strain of serotype 2, Lane 3: the BN-77 strain of serotype 2, Lane 4: the Abney strain of serotype 3, Lane 5: the OS-320 strain.

Table 1. Cross neutralizing (NT) tests with antisera to reovirus OS-320 strain and the reference viruses

Virus strain	NT antibody titer to				
	OS-320	Lang (type 1)	Amy (type 2)	BN-77 (type 2)	Abney (type 3)
OS-320	128	4	128	<8	<4
Lang (type 1)	<4	128	<8	<8	<8
Amy (type 2)	8	<8	512	8	<8
BN-77 (type 2)	64	<8	128	64	<8
39 (type 2)	<4	8	128	<8	8
Abney (type 3)	<4	<8	16	<8	256

Table 2. Survey of neutralizing antibody against reovirus in Okayama Prefecture in Japan

Area	Serotype							
	Lang (type 1)		BN-77 (type 2)		Abney (type 3)		OS-320 (type 2)	
	Positive[a] number (%) of							
	farm	head	farm	head	farm	head	farm	head
A	3 (75)	4 (16)	4 (100)	21 (84)	3 (75)	8(32)	4 (100)	22 (88)
B	3 (75)	8 (35)	4 (100)	16 (70)	2 (50)	4(17)	4 (100)	18 (82)
C	4 (100)	10 (40)	4 (100)	15 (60)	2 (50)	5(20)	4 (100)	22 (88)
D	3 (100)	14 (58)	3 (100)	24(100)	2 (67)	10(42)	3 (100)	24 (100)
E	9 (75)	16 (33)	12 (100)	37 (76)	6 (50)	13(27)	12 (100)	45 (96)
Total	22 (82)	52 (36)	27 (100)	113 (77)	15 (56)	40(27)	27 (100)	131 (92)

Survey was conducted on 146 pigs in 27 farms.
a) Antibody titer over 1:2 was considered positive.

REFERENCES

1. Fujisaki, Y., Kawamura, H., and Anderson, D. P. 1969. *Am. J. Vet. Res.* 30: 1035–1043.
2. Fukutomi, T., Fujiwara, M., Sanekata, T., and Akashi, H. 1995. *J. Vet. Med. Sci.* 57: 739–741.
3. Hartley, J. W., Rowe, W. P., and Austin, J. B. 1962. *Virology* 16: 94–96.
4. Hirahara, T., Yasuhara, H., Matsui, O., Kodama, K., Nakai, M., and Sasaki, N. 1988. *J. Vet. Med. Sci.* 50: 353–361.
5. Hirahara, T., Yasuhara, H., Matsui, O., Kodama, K., Nakai, M., and Sasaki, N. 1989. *J. Jpn. Vet. Med. Assoc.* 42: 481–484 (in Japanese with English summary).
6. Hull, R. N., Minner, J. R., and Mascoli, C. C. 1958. *Am. J. Hyg.* 68: 31–44.
7. Kasza, L. 1970. *Vet. Rec.* 87: 681–686.
8. Kawamura, H., Shimizu, F., Maeda, M., and Tubahara, H. 1965. *Natl. Inst. Anim. Health Q.* 5: 115–124.
9. Low, T. Y. and Wenner, H. A. 1963. *Am. J. Hyg.* 77: 293–304.
10. Matsuno, S., Inouye, S., and Kono, R. 1977. *J. Clin. Microbiol.* 5: 1–4.
11. McFerran, J. B. and Connor, T. J. 1970. *Res. Vet. Sci.* 11: 388–390.
12. McFerran, J. B., Nelson, R., and Clarke, J. K. 1973. *Arch. Ges. Virusforsch.* 40: 72–81.
13. Roble, M. G., McAdaragh, J. P., Phillips, C. S., and Bicknell, F. J. 1971. *Vet. Med. Small Anim. Clin.* 66: 903–909.
14. Rosen, L. 1960. *Am. J. Hyg.* 71: 242–249.
15. Rosen, L., Abinanti, F. R., and Hovis, J. F. 1963. *Am. J. Hyg.* 77: 38–48.
16. Sabin, A. B. 1959. *Science* 130: 1387–1389.
17. Sanekata, T., Yoshida, Y., and Okada, H. 1981. *J. Immunol. Methods* 41: 377–385.