Porcine Pararotavirus: Detection, Differentiation from Rotavirus, and Pathogenesis in Gnotobiotic Pigs†

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Some characteristics of a newly recognized porcine enteric virus are described. Tentatively, the virus was referred to as porcine pararotavirus (PaRV) because it resembled rotaviruses in respect to size, morphology, and tropism for villous enterocytes of the small intestine. However, it was antigenically distinct from porcine, human, and bovine rotaviruses and reoviruses 1, 2, and 3, and the electrophoretic migration pattern of PaRV double-stranded RNA was distinct from the electrophoretic migration patterns of the rotaviral and reoviral genomes. By passage in gnotobiotic pigs, PaRV was isolated from two suckling diarrheic pigs originating from two herds. After oral exposure of gnotobiotic pigs, villous enterocytes of the small intestines became infected as judged by immunofluorescence, resulting in villous atrophy and diarrhea. Mortality was high when gnotobiotic pigs less than 5 days old were infected. The C strain of this virus was serially passed 10 times in gnotobiotic pigs, and electron microscopy, immunofluorescence, and serological tests indicated no extraneous agents. The virus was serially passed five times in cell cultures which contained pancreatin in the medium, but replication was negligible or absent, as the number of immunofluorescent cells decreased with each passage. Since rotaviral infections are frequently diagnosed by direct electron microscopy of fecal specimens, the presence of other morphologically similar viruses, such as PaRV, should be considered. The use of immune electron microscopy is suggested as a means of helping recognize this situation.

We have previously reported the detection of a rotavirus-like virus from the intestinal tract of a suckling pig with diarrhea (15). Tentatively, we are referring to this virus as porcine pararotavirus (PaRV). However, the virus was in association with two other viruses, a calicivirus-like virus and a 23-nm virus. All three viruses were serially passed on gnotobiotic pigs, resulting in their replication in the intestinal tract and the occurrence of diarrhea.

Morphologically, the PaRV was indistinguishable from rotavirus by electron microscopy. However, immunofluorescence (IF) and immune electron microscopy (IEM) indicated that the virus was different from rotavirus or reovirus (15).

This report describes studies on PaRV in regard to separation from a mixed viral population, methods for detection, differentiation from rotavirus and reovirus, and pathogenicity for gnotobiotic pigs.

MATERIALS AND METHODS

Experimental infection of pigs. Gnotobiotic pigs were used in studies on transmission and experimental infections so as to minimize the presence of extraneous pathogens and guarantee a highly susceptible animal, using procedures previously described (12). Specimens used for oral exposure usually consisted of a 1:10 dilution of large-intestine contents, having been collected aseptically from the donor pig by aspiration with syringe and needle. This latter procedure tends to minimize contamination with extraneous microorganisms and is considered highly important. As indicated in the text, some specimens were centrifuged and filtered through membrane filters.

Electron microscopy. Intestinal contents were examined by electron microscopy and IEM for detection and identification of viruses as previously described (14). Briefly, 20% suspensions of sonicated and filtered large intestinal contents from PaRV-infected gnotobiotic pigs were incubated overnight at 4°C with 1:25 dilutions of gnotobiotic pig convalescent antisera. Samples were then centrifuged at $31,000 \times g$ for 1 h and negatively stained. Grids were examined at 80 kV in a 201 electron microscope (Philips Electronic Instruments, Inc., Mahwah, N.J.).

IF and histology. Two methods were used for preparing intestinal epithelial cells, or enterocytes, from

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pigs for IF staining. The routine and more commonly used method consisted of making mucosal smears on microscope slides from the duodenum, jejunum, and ileum as previously described (3). By the second method, small segments from various portions of the small intestine were fixed in acetone for 30 min, dehydrated in additional acetone for 1 h or more, and then placed directly in melted paraffin in a vacuum oven at 60°C and 25 in. (63.5 cm) of Hg for 10 min (6). Specimens were then embedded in paraffin blocks and sectioned in the usual way. Sections were deparaffinized by rinsing in xylene, followed by a 5-min rinse in acetone. Smears or sections were stained for viral antigens by direct or indirect IF procedures.

For histological examinations, segments (2.5 cm) from the duodenum, jejunum, and ileum were fixed in 10% Formalin, embedded in paraffin, sectioned, and stained by azure and eosin as described previously (7).

Electrophoresis of ds RNA. Rotaviral and porcine PaRV double-stranded (ds) RNA were extracted from the intestinal contents of infected gnotobiotic pigs by CF-11 cellulose chromatography by a previously described procedure (18). Reovirus 3 ds RNA was extracted from infected L-929 cell cultures in a similar manner. The ds RNA preparations were subjected to electrophoresis in composite 2.5% polyacrylamide-0.5% agarose vertical slab gels prepared with Peacock Tris-borate-EDTA buffer, pH 8.3, and then stained with ethidium bromide as described previously (18).

Cell cultures and media. Monolayers of primary pig kidney cells and the following cell lines were used for viral studies: fetal rhesus monkey kidney (MA104), swine testes (ST), and porcine kidney (PK15). Growth medium was Eagle minimal essential medium supplemented with 10% fetal bovine serum (Sterile Systems, Logan, Utah), penicillin (100 IU/ml), streptomycin (100 µg/ml), and mycostatin (25 U/ml). Maintenance medium was the same but did not contain serum. All cell cultures were rinsed and fed with maintenance medium at least 3 h before and rinsed again just before viral inoculation. Agar overlay for plaque detection was maintenance medium supplemented with 0.8% Noble agar (Difco Laboratories, Detroit, Mich.), 0.0007% neutral red, and pancreatin as indicated below.

Pancreatin in cell culture media. Pancreatin was added to agar and liquid media to facilitate replication, plaque formation, or cytopathic effect (1, 10, 16). A stock solution composed of 1 volume of pancreatin (4 × NF, catalogue no. 610-5720; GIBCO Laboratories, Grand Island, N.Y.) and 9 volumes of phosphate-buffered saline (pH 7.2) was added to media at a level which was slightly less than toxic for the cellular monolayers. This amount varied slightly, depending on the cell type and age of cells, and was predetermined for each cell system. With MA104 cells, about 1.2% of the stock solution was added to agar or liquid media just before overlaying the viral inoculated monolayers. In the case of 96- or 24-well plates, about 30 µl (1 drop) of 1:200 or 1:40, respectively, of the stock solution was added to the liquid medium in each well after viral inoculation.

Detection of virus in cell cultures. Large-intestine contents were diluted 1:25 with maintenance medium containing 100 µg of gentamicin per ml, centrifuged at 700 × g for 30 min, and stored at -20°C. Supernatant fluids were inoculated on cell cultures grown in 96- or

24-well microtiter plates or 2-ounce (60-ml) bottles.

A cell culture immunofluorescence test was used for detecting PaRV and rotaviruses by a method similar to procedures previously described (2, 5). Cells were grown for 6 to 10 days in flat-bottomed, 96-well microtiter plates (Costar, Cambridge, Mass.), wells were rinsed as described above, and 0.15 ml of diluted viral specimens were inoculated into each well. Routinely, intestinal contents were tested at 1:25, 10⁻², and 10⁻³ dilutions, using maintenance medium as diluting fluid. After viral inoculation, the plates were placed in centrifuge plate carriers (Dynatech Laboratories, Inc., Alexandria, Va.) and centrifuged at 2,800 rpm (1,300 × g) in a GLC-1 centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.), using a type HL-4 head. In some tests, one drop (30 µl) of a 1:40 dilution of stock pancreatin solution was added to each well. After the plates were incubated at 37°C for 16 to 24 h in a 5% CO₂ atmosphere, the medium was aspirated from individual wells and the wells were rinsed with phosphate-buffered saline (pH 7.2). The cells were fixed by adding to each well about 0.2 ml of a solution containing 80% acetone and 20% distilled water for a period of 10 min at room temperature (13). The fixative was aspirated and replaced with phosphate-buffered saline and, after 5 min, aspirated and replaced with 30 µl of fluorescein isothiocyanate-conjugated viral antisera, if the direct IF staining procedure was used. Thereafter, the IF staining procedure was conventional. For indirect IF staining, the procedure was similar, except porcine antiviral sera were initially applied to the fixed monolayers, rinsed thoroughly with phosphate-buffered saline, and stained with fluorescein isothiocyanate-conjugated rabbit anti-porcine immunoglobulin G (Miles Laboratories, Inc., Elkhart, Ind.). Microtiter plates were inverted on the stage of a Dialux fluorescent microscope (Leitz/Opto-Metric Div. of E. Leitz Inc., Rockleigh, N.J.) equipped with a Ploem illuminator and a 200-W mercury arc lamp, and the monolayer in each well was viewed through 12.5× eyepieces and a 10× objective.

The viral antisera used in the indirect IF procedures and in preparing fluorescein isothiocyanate-conjugated antisera (17) were from gnotobiotic pigs which had convalesced from infection or had been hyperimmunized with the strains of viruses as listed below.

Serology. Plaque reduction neutralization tests were conducted in MA104 cells grown in 2-ounce (60-ml) bottles similar to a method previously described (10). The viral strains used for these tests included: porcine rotavirus (OSU) (3, 16), bovine rotavirus (NCDV) obtained from C. A. Mebus (Plum Island Animal Disease Center, Greenport, N.Y.), human rotavirus type 2 (WA) obtained from R. G. Wyatt (National Institute of Allergy and Infectious Diseases, Bethesda, Md.), reovirus type 1 (Lang), reovirus type 2 (D5, Jones), and reovirus type 3 (Abney). All reoviruses were obtained from the American Type Culture Collection, Rockville, Md. Fourfold dilutions of heat-inactivated sera, beginning with 1:4, were mixed with equal volumes of virus diluted so as to give about 50 PFU per 0.1 ml. Virus-serum mixtures were incubated at 37°C for 1 h, and 0.2 ml was inoculated onto the monolayers of 2-ounce (60-ml) culture bottles which had been previously rinsed as described above. After a viral adsorption period of 1 h, the monolayers were rinsed with about 10 ml of maintenance medium to remove

residual serum and overlaid with an agar medium containing pancreatin as described above. Plaques were counted after 4 to 8 days. Neutralizing antibody titers were expressed as a reciprocal of the initial serum dilution that resulted in an 80% reduction in plaques.

Antibodies for PaRV were assayed by an indirect IF test. The antigen substrate consisted of intestinal mucosal smears, made on microscope slides, from gnotobiotic pigs infected with PaRV. Smears were fixed with acetone. Test sera in fourfold dilutions were applied to the smears. After 30 min, the slides were rinsed and stained for 30 min with rabbit fluorescein isothiocyanate-conjugated anti-porcine immunoglobulin G. Antibody titers were expressed as the reciprocal of the highest dilution which produced fluorescing cells.

RESULTS

Isolation of PaRV in gnotobiotic pigs. A mixture of three viruses—PaRV, calicivirus-like virus, and a 23-nm virus—was detected in an intestinal specimen from a 27-day-old diarrheic suckling pig, as previously described (15). After three serial passages of this specimen in gnotobiotic pigs, diarrhea still occurred, but only a few PaRV and many calicivirus-like particles were observed by IEM in the intestinal specimen from one of the pigs. This specimen was diluted 1:30, centrifuged at $1,200 \times g$ for 30 min, and passed through a series of membrane filters (Millipore Corp., Bedford, Mass.), ending with a 0.05- μ m filter. The object was to remove the larger PaRV particles (55 to 70 nm) from the smaller calicivirus-like particles (28 to 38 nm) in the specimen. One milliliter of this filtered specimen was orally administered to each of two 12-day-old gnotobiotic pigs. No diarrhea occurred, but 3 days postexposure, pig 1 was euthanatized, and by IEM only calicivirus-like virus was observed in the intestinal specimen. At 27 days postexposure, pig 2 was orally exposed to a viral preparation which contained a mixture of the three viral agents. Theoretically, pig 2 would be immune to the two smaller viral agents but susceptible to PaRV, as subsequently proved to be the case. The pig developed diarrhea and was euthanatized 68 h after viral exposure. By IEM, intestinal specimens from this pig revealed (i) rotavirus-like particles which were agglutinated by antiserum prepared against the composite of the three viruses but not agglutinated by anti-porcine rotavirus serum and (ii) an absence of the other two viral agents.

Subsequently, 10 serial passages were made in gnotobiotic pigs. With each passage, diarrhea occurred, and enterocyte smears stained by direct IF revealed infection with PaRV but not with rotavirus. Enterocyte smears from several passage levels were examined by direct IF, and all were negative for transmissible gastroenter-

itic virus, porcine enteric calicivirus, and reovirus 3. Convalescent serum samples from several passage levels were examined, and all were negative for porcine rotavirus antibodies, as is later described. This virus is referred to as the C strain of PaRV.

More recently, a second isolate (S strain) of PaRV from a different herd was made in gnotobiotic pigs. The S strain originated from a 27-day-old suckling pig which had a profuse liquid diarrhea. The pig was euthanatized. IF staining of enterocytes were positive for PaRV but negative for rotavirus, transmissible gastroenteritic virus, and porcine enteric calicivirus. The S strain has been serially passed five times in gnotobiotic pigs and has remained free of rotavirus, transmissible gastroenteritic virus, porcine enteric calicivirus, and reovirus, as judged by IEM, IF, or serology. Diarrhea occurred at each passage level.

Clinical signs and pathogenicity. After separation from extraneous viruses, the C strain was serially passed 10 times, using a total of 56 gnotobiotic pigs ranging in age from 7 h to 19 days. Diarrhea occurred in pigs at each passage level but was most severe and consistently produced in pigs less than 5 days old. Of 33 pigs exposed at 5 days of age or less, diarrhea was observed in 32, usually beginning at 17 to 30 h after exposure. In these diarrheic pigs, feces were usually tan, liquid to creamy in consistency, and often contained yellow flecks of undigested curd.

Since most pigs were euthanatized between 16 and 72 h after exposure, limited data are available on morbidity and mortality at different ages of viral exposure. Observations were made on 16 pigs which were not euthanatized. Of 10 pigs exposed when less than 72 h old, all developed diarrhea, and 9 died. Of six pigs exposed when more than 5 days old, diarrhea was observed in five, and none died.

Gross lesions were evident only in the intestinal tract. The small and large intestines contained abnormal amounts of fluid, and the wall of the small intestines, especially ileum, was thin.

Histologically, the small intestines were characterized by shortening of the villi, especially in the ileum. The crypts were elongated in those segments where villi shortening occurred. These lesions were not usually evident in pigs which were examined at the onset of diarrhea but were seen in pigs several hours after the onset of diarrhea.

Detection of viral antigens in enterocytes by IF. After infection of gnotobiotic pigs with either strain of PaRV, villous enterocytes along the entire small intestines became infected as determined by direct or indirect IF. Fluorescence was observed in villous but not crypt enterocytes, as

shown in IF-stained, paraffin-embedded sections (Fig. 1). Mucosal smear impressions proved to be a simple and effective means for demonstrating infected enterocytes (Fig. 2). Fluorescence was observed only in cytoplasm. The highest number of fluorescing cells were detected at the onset of diarrhea, usually 14 to 20 h after viral exposure, and the number decreased rapidly within 12 to 24 h after the onset of diarrhea. In some specimens, 100% of the enterocytes on the tips of villi were infected, with the numbers decreasing to the base of the villi (Figs. 1 and 2). Villous enterocytes of the ileum were initially most severely infected, followed by those of the jejunum and duodenum.

EM and IEM. Intestinal contents from gnotobiotic pigs infected with PaRV revealed viral particles which were indistinguishable in size and morphology from those of porcine rotavirus (Fig. 3), concurring with our previous report (15). As with porcine rotaviruses, the large PaRV particles of both the C and S strains averaged 70 nm in diameter and had a double outer capsid layer with a smooth periphery, whereas the smaller particles averaged 55 nm in diameter and had a single outer capsid layer.

By IEM, both strains of PaRV were agglutinated with serum from convalescent gnotobiotic pigs previously infected with either the C or S

FIG. 1. IF-stained, paraffin-embedded section of the ileum of a pig 20 h after infection with PaRV. Enterocytes of the villi but not crypts were stained. Magnification, $\times 120$.

FIG. 2. IF-stained mucosal smear of the duodenum of a pig 20 h after infection with PaRV. One villus is shown, with the majority of fluorescing cells on the villous tip. $\times 300$.

strains of PaRV. However, neither viral strain was agglutinated with serum from convalescent gnotobiotic pigs previously infected with porcine rotavirus (OSU).

Cell culture studies. Exposure of MA104, PK15, primary pig kidney, or ST cell cultures with intestinal contents from PaRV (C strain)-infected gnotobiotic pigs resulted in infected cells, as shown by IF staining. Inoculation of MA104 and PK15 cells in 96-well plates yielded 10^7 fluorescing foci per ml by the cell culture immunofluorescence test with some intestinal specimens. The presence of pancreatin in the maintenance medium enhanced the sensitivity of the test by about 5- to 10-fold. IF occurred only in the cytoplasm. IF cells have been detected through five serial passages on MA104 cells containing pancreatin in the maintenance medium. However, the number of infected cells declined in successive passages, suggesting little, if any, viral replications. IF was not observed when cells were stained for rotavirus or reovirus 3 antigens. Attempts to demonstrate plaques on monolayers grown in 2-ounce (60-ml) bottles were negative, even when pancreatin was incorporated in the agar overlay.

FIG. 3. IEM of PaRV incubated with hyperimmune gnotobiotic pig PaRV serum (diluted 1:500). (A) Particles (55 nm) with a single outer capsid layer; (B) particles (70 nm) with a double outer capsid layer.

The ds RNA preparations derived from the intestinal contents of PaRV-infected gnotobiotic pigs produced electrophoretic migration patterns with 11 resolved segments that differed significantly from the characteristic rotaviral genome electrophoretic migration pattern (Fig. 4). The electrophoretic migration patterns of the ds RNA preparations from gnotobiotic pigs infected with the C and S strains, although similar, did differ in the electrophoretic mobility of their third-largest ds RNA segment. The electrophoretic migration patterns of the PaRV ds RNA preparations were distinctly different from the electrophoretic migration pattern produced by the reoviral genome (data not shown).

Serology. Neutralizing antibodies for porcine, bovine, or human rotaviruses or for reoviruses 1, 2, or 3 were not detected in sera diluted 1:4 from gnotobiotic pigs convalescing from infection with either the C or S strains of PaRV (Table 1). As expected, there was some cross-reactivity among the three rotaviruses, especially with the hyperimmune bovine rotavirus antiserum, and among the three reoviruses. However, no cross-reactivity was observed among the rotaviruses, the reoviruses, and PaRV. Antibodies for PaRV were detected in

convalescent pigs by an indirect IF test, using gut smears from PaRV-infected pigs as an antigen substrate (Table 2). Antibody titers from convalescent gnotobiotic pigs ranged from 16 to 1,024, and those from hyperimmunized gnotobiotic pigs ranged from 1,024 to 4,096. Antibodies for PaRV were not detected in pigs which had recovered from infection with porcine or human rotavirus or with reovirus 3 (Table 2).

Cross protection. Gnotobiotic pigs that recovered from infection with either the C or S strain of PaRV were susceptible to infection with porcine rotavirus (OSU). For example, two 8-day-old gnotobiotic pigs were exposed to PaRV (C). Diarrhea occurred in both pigs. At 28 days after exposure, both were serologically positive with PaRV but negative with porcine rotavirus. At this time, they were challenged with porcine rotavirus (OSU), and severe diarrhea occurred. One pig was euthanatized 48 h after exposure, and IF tests on mucosal scrapings indicated many enterocytes infected with rotavirus but none with PaRV. The second pig was serologically positive with porcine rotavirus 14 days

FIG. 4. Comparison of the electrophoretic migration patterns of ds RNA preparations extracted from the intestinal contents of gnotobiotic pigs infected with (A) porcine rotavirus (OSU strain), (B) PaRV (C strain), and (C) PaRV (S strain). Size of ds RNA segments decrease from top to bottom. Direct comparison of the electrophoretic migration patterns in lanes B and C cannot be made because these are from different electrophoretic runs. It can be noted, however, that the electrophoretic mobility of the third-largest ds RNA segment relative to that of the fourth segment is different for the C and S strains of PaRV.

TABLE 1. Plaque reduction neutralizing antibody titers

Virus	Antibody titer obtained with antisera to ^a :					Reovirus type 3
	PaRV (C strain)	PaRV (S strain)	Rotaviruses			
			Porcine	Human	Bovine	
Porcine rotavirus	<4 ^b	<4	580	<4	120	<4
Human rotavirus	<4	<4	5	720	105	<4
Bovine rotavirus	<4	<4	28	8	6,000	<4
Reovirus 1	<4	<4	<4	<4	<4	350
Reovirus 2	<4	<4	<4	<4	<4	78
Reovirus 3	<4	<4	<4	<4	<4	1,150

^a Antisera were obtained from gnotobiotic pigs about 21 days after oral infection, except for the bovine rotavirus antiserum, which was obtained from a hyperimmunized gnotobiotic pig. Antibody titers are expressed as reciprocal of the initial serum dilution resulting in an 80% reduction in PFU.

after being challenged with porcine rotavirus. A similar cross-protection test was conducted with the S strain of PaRV and porcine rotavirus, and similar results occurred.

DISCUSSION

We have tentatively given the name porcine pararotavirus to the enteric virus described in this report. It resembles rotavirus in respect to size, morphology, and tropism for enterocytes but is antigenically distinct. We previously described the detection and some characteristics of this virus, but it was associated with two other viruses (15). This report describes its separation from a mixed viral population, methods for detection, differentiation from the other viruses, and pathogenicity in gnotobiotic pigs.

PaRV and rotaviruses are indistinguishable in regard to size and morphology as viewed by direct EM. However, by IEM, PaRV agglutinates with homologous antiserum but not with rotaviral antisera; likewise, porcine rotavirus agglutinates with homologous antiserum but not with PaRV antiserum. If fecal samples are examined by direct EM, no distinction can be made between rotavirus and PaRV. Since the diagnosis of rotaviral infections is frequently made by direct EM of fecal specimens, the presence of other morphologically similar viruses, such as PaRV, should be considered.

Debouck and Pensaert (8) and Bridger (4) have also reported the presence of rotavirus-like particles in intestinal contents from diarrheic pigs which did not react as rotaviruses as judged by IEM or IF, suggesting similarities to the PaRV we are describing. To our knowledge, viruses with characteristics of PaRV have not been described in animals other than swine.

All known mammalian and avian rotaviruses share a group antigen which can be demonstrated by IF, complement fixation, IEM, or gel diffusion (9, 11). The two isolates of PaRV did not contain this group rotaviral antigen as determined by IF or IEM. For example, enterocytes or cell cultures infected with PaRV did not fluoresce when stained by direct or indirect IF with rotaviral reagents, but did fluoresce when stained with the homologous reagents.

We have also shown by neutralization tests that there are marked antigenic differences among PaRV, rotaviruses, and reoviruses. Gnotobiotic pigs which had recovered from PaRV infections were negative for neutralizing antibodies at serum dilutions of 1:4 against porcine, human, or bovine rotaviruses or against reoviruses 1, 2, or 3. As anticipated, some cross-reactivity occurred within the rotavirus and reovirus groups (Table 1).

At present, the *Reoviridae* family of viruses are composed of three genera; reoviruses, rota-

TABLE 2. Assay of antibodies to PaRV or porcine rotavirus by indirect IF, using gut smears from infected gnotobiotic pigs as antigen substrate

Virus (strain) ^a	Antibody titer obtained with antisera to ^b :				
	PaRV (C strain)	PaRV (S strain)	Porcine rotavirus	Human rotavirus	Reovirus 3
PaRV (C)	1,024 ^c	64	<1	<1	<1
PaRV (S)	256	64	<1	<1	<1
Porcine rotavirus	<4	<4	256	64	<1

^a Enterocyte smears from infected pigs.

^b Antisera were obtained from gnotobiotic pigs about 21 days after oral infection. Neutralizing antibody titers for the antisera against rotaviruses and reovirus are given in Table 1.

^c Reciprocal of the highest fourfold dilution of serum resulting in fluorescing cells.

viruses, and orbiviruses (9). Morphologically, complete orbiviruses are described as having a fuzzy periphery (19), whereas complete rotaviruses and the PaRV, as described in this report, have a smooth periphery. Incomplete orbiviruses can also morphologically resemble incomplete rotaviruses (19). To our knowledge, orbiviruses and reoviruses have not been reported to infect enterocytes. Previously, we reported that PaRV particles were not agglutinated with pooled antibodies prepared against arboviruses (orbiviruses) of the Kemerovo and palyam groups (15).

As a further distinction, the electrophoretic migration patterns of the PaRV dsRNA preparations differed significantly from the characteristic electrophoretic migration patterns produced by the rotaviral and reoviral genomes. Moreover, these PaRV electrophoretic migration patterns differ from the electrophoretic migration patterns formed by the 10 segments of the orbivirus genomes (19). These differences provide additional evidence that PaRV is unique and distinct from other known members of the *Reoviridae* family.

Infection of gnotobiotic pigs with PaRV results in infection of villous enterocytes along the entire small intestines, resulting in villous atrophy and diarrhea. In these respects, PaRV resembles porcine rotavirus and transmissible gastroenteric virus, a coronavirus. Mortality was 90% in 10 gnotobiotic pigs exposed when less than 72 h old, but was 0% in 6 pigs exposed when more than 5 days old. No information is yet available on the prevalence of this infection in swine.

PaRV infection in pigs can be diagnosed by direct or indirect IF on mucosal smears from the small intestines and by IEM on intestinal contents, using anti-PaRV serum. Specimens should be collected as soon after onset of diarrhea as possible. The virus will infect certain cell lines, such as Ma104 and PK15, as judged by IF. However, effective viral replication has not been established in serial cell culture passages, even when enzyme treatments similar to those previously reported for the successful propagation of rotaviruses are used (1, 11, 16, 20).

IF examinations of gut specimens collected from infected pigs at various intervals after viral exposure suggest the following sequence of events. Mature enterocytes, as found on the tips of villi, are initially and rapidly infected. These cells are sloughed and replaced by immature enterocytes which are much less susceptible to the virus, resulting in a somewhat self-limiting infection. Thus, for detecting optimal numbers of infected cells by IF, best results were obtained when intestinal specimens were collected at the onset or shortly before the anticipated

occurrence of diarrhea, before the majority of infected cells were sloughed into the lumen of the gut.

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