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Porcine Rotavirus-Like Virus (Group B Rotavirus): Characterization and Pathogenicity for Gnotobiotic Pigs†

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A rotavirus-like virus (RVLV) was isolated from a diarrheic pig from an Ohio swine herd. This virus infected villous enterocytes throughout the small intestine of gnotobiotic pigs and induced an acute, transitory diarrhea. Complete virions were rarely observed in the intestinal contents of infected animals; the predominant particle detected by immune electron microscopy was a corelike particle 52 nm in diameter. The genome of the porcine RVLV was composed of 11 discrete segments of double-stranded RNA that produced an electropherotype distinct from the genome electropherotypes of reovirus, rotavirus, and porcine pararotavirus. Porcine RVLV was antigenically unrelated to rotavirus, porcine pararotavirus, or reovirus but was antigenically related to a bovine RVLV.

Rotaviral infections are an important cause of diarrhea in young animals and children (7). Early serological studies indicated that rotaviruses recovered from different species shared a common group antigen (19, 22). However, we recently isolated a virus from a diarrheic pig that was morphologically indistinguishable from rotaviruses but lacked the common group antigen (14) and proposed that this virus tentatively be referred to as porcine pararotavirus (2). Shortly thereafter, Bridger (4) described another rotavirus, also lacking the rotavirus group antigen, that was recovered from diarrheic pigs in England. Additional studies demonstrated that not only was this virus antigenically distinct from the rotaviruses, it was also antigenically and biochemically unrelated to the porcine pararotavirus isolated in the United States (5, 11, 12). At present, the only report of this type of porcine rotavirus-like virus (RVLV) is the original one from England.

This report describes the isolation of a RVLV from a diarrheic pig in the United States that is antigenically unrelated to rotaviruses and porcine pararotavirus but is similar to the porcine RVLV isolated in England. The pathogenicity of this virus for gnotobiotic pigs is also described.

MATERIALS AND METHODS

Clinical specimen. The original specimen containing the RVLV was the intestinal contents of a diarrheic 27-day-old conventional suckling pig from an Ohio swine herd. The RVLV was initially detected, along with a rotavirus, in the intestinal contents of a gnotobiotic pig orally inoculated with a bacteria-free filtrate of the original specimen. A mixed infection was suspected when genome electropherotyping (18) of the rotavirus present in the gnotobiotic pig intestinal contents revealed numerous unexpected additional genome segments. This RVLV, designated the Ohio isolate, was recovered free of the contaminating rotavirus by two sequential passages in rotavirus-immune gnotobiotic pigs (gnotobiotic pig passages 2 and 3). Three additional sequential passages were then made in rotavirus-susceptible gnotobiotic pigs (gnotobiotic pig passages 4, 5, and 6). Rotavirus could not be detected by genome electropherotyping of intestinal contents or by immunofluorescent staining of small intestinal mucosal smears derived from the gnotobiotic pigs during these latter three passages.

Viruses. Porcine rotavirus (G isolate) was propagated in monolayers of MA104 cell cultures on a roller drum as described previously (3). Reovirus type 3 (Abney isolate) was propagated in stationary MA104 monolayers. Porcine pararotavirus (Cowden isolate) was passaged in gnotobiotic pigs as described previously (2). Bovine RVLV was passaged in a gnotobiotic calf. A preliminary description of this virus has been reported (L. J. Saif, K. W. Theil, and D. R. Redman, Abstr. 63rd Annu. Meet. Conf. Res. Work. Anim. Dis., abstr. no. 98, 1982; manuscript in preparation).

Hyperimmune and fluorescein-conjugated antisera. Hyperimmune antiserum to the Ohio porcine RVLV was prepared as follows. A gnotobiotic pig, convalescent from oral exposure to virus from gnotobiotic pig passage 4, was given an intramuscular injection of virus-laden gnotobiotic pig intestinal contents combined with an equal volume of Freund complete adjuvant. A second intramuscular injection without adjuvant was given 5 weeks later. Serum was collected 1 week after the last injection.

Hyperimmune antiserum to the bovine RVLV was prepared in a gnotobiotic calf (L. J. Saif, K. W. Theil, and D. R. Redman, manuscript in preparation).

Fluorescein-conjugated antisera to porcine rotavirus, porcine pararotavirus, and transmissible gastroenteritis virus were prepared as previously described (2, 17). Fluorescein-conjugated rabbit antisera to porcine immunoglobulin G (IgG) and to bovine IgG used in the indirect immunofluorescent stains were obtained from Miles Laboratories, Inc., Elkhart, Ind.

Experimental infection of gnotobiotic pigs. Gnotobiotic pigs were procured and maintained by previously described methods (10). The inoculum was a bacteria-free filtrate of a 1:10 dilution (prepared in minimum essential medium) of the intestinal contents from gnotobiotic pig passage 4 of the RVLV. Eleven gnotobiotic pigs, 5 to 6 days of age and obtained from four different litters, were each orally inoculated with ca. 2 ml of inoculum. A noninoculated littermate in one experiment, kept in an isolator separate from the inoculated pigs, served as a control. Eight of the inoculated

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TABLE 1. Clinical signs and changes in the small intestines of gnotobiotic pigs inoculated orally with porcine RVLV

Pig no.	Diarrhea[a]	Time[b] of sacrifice (h)	Histopathological changes[c] in small intestine			Infected enterocytes detected by immunofluorescence		
			Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
1	Yes (5)	NS	NA	NA	NA	NA	NA	NA
2	Yes (6)	NS	NA	NA	NA	NA	NA	NA
3	Yes (6)	NS	NA	NA	NA	NA	NA	NA
4	Yes	48	VA	None	None	−	+	+
5	Yes	18	VA	AS	AS	−	+	+
6	No	18	VA	AS	AS	−	+	−
7	Yes	27	None	AS	VA	+	+	+
8	Yes	46	AS	AS	VA	+	−	+
9	No	15	None	None	None	+	+	+
10	Yes	15	None	None	None	+	+	+
11	Yes	24	None	None	None	+	+	−
12	No	Control	None	None	None	−	−	−

[a] Number in parentheses indicates the number of days that diarrhea was detected in pigs not sacrificed.
[b] Times post-inoculation were measured. NS, Not sacrificed; control, 6-day-old gnotobiotic pig sacrificed 24 h after start of experiment.
[c] NA, Not applicable; VA, villous atrophy; AS, sloughing of enterocytes from villous apex.

animals and the control were sacrificed, and specimens were collected from their small intestines for histopathology, immunofluorescent staining, and scanning electron microscopy.

Histopathology. Formalin-fixed segments from the duodenum, jejunum, and ileum were stained with azure and eosin (6).

Immunofluorescent staining. Mucosal smears and paraffin-embedded sections of the duodenum, jejunum, and ileum were prepared and examined by immunofluorescence microscopy as described previously (2, 17).

Scanning electron microscopy. Segments of the duodenum, jejunum, and ileum were rinsed with saline to remove adherent mucus and residual intestinal contents and fixed in a solution containing 3% glutaraldehyde, 2% paraformaldehyde, and 1.5% acrolein in 0.1 M collidine buffer at pH 7.3. Fixed tissues were then dehydrated in an ethanol-Freon series and gently vacuum dried (8). Dried specimens were sputter coated with ca. 15 nm of platinum, viewed, and photographed with an ISI-40 scanning electron microscope.

Immune electron microscopy. Intestinal contents collected from gnotobiotic pigs infected with the RVLV were examined by immune electron microscopy by previously described procedures (13). Specimens were reacted with hyperimmune anti-porcine RVLV serum or hyperimmune anti-porcine rotavirus serum, and negatively stained grids were then examined at 80 kV in a Philips 201 electron microscope.

Electrophoresis of viral dsRNA. Viral double-stranded (ds) RNA was extracted from the intestinal contents of infected gnotobiotic pigs or from infected cell culture fluid by CF11 cellulose chromatography (18). Viral dsRNA was subjected to electrophoresis in 7.5% Laemmli polyacrylamide gels and stained with silver as described previously (3).

Cell culture. Monolayers of primary porcine kidney cells and MA104 cells were prepared in screw-cap tubes or in Leighton tubes containing cover slips, using Eagle minimum essential medium supplemented with 10% fetal bovine serum and antibiotics as described previously (3). Monolayers were washed three times, inoculated with 5% gnotobiotic pig intestinal contents containing porcine RVLV, and then refed with serum-free growth medium containing either pancreatin (GIBCO Laboratories, Grand Island, N.Y.) or trypsin (type IX; Sigma Chemical Co., St. Louis, Mo.) at concentrations just less than toxic for the monolayers. Inoculated monolayers in screw-cap tubes were incubated for 3 days on a roller drum, whereas those in Leighton tubes were incubated in a stationary position.

After incubation, the cell cultures were frozen, and two additional subpassages were performed as above with undiluted cell culture fluid as the inoculum. Inoculated monolayers were examined for cytopathic effect, and at each passage, cover slip monolayers were examined for infected cells by an indirect immunofluorescent stain with hyperimmune anti-porcine RVLV serum.

RESULTS

Experimental infection of gnotobiotic pigs. All gnotobiotic pigs inoculated with the porcine RVLV became infected, as indicated by either the manifestation of clinical signs or the demonstration of infected enterocytes (Table 1). Diarrhea, accompanied by anorexia, was first observed between 15 and 24 h after inoculation and was initially characterized by profuse, watery, yellow feces. Those gnotobiotic pigs that were not sacrificed (pigs 1 through 3) developed profuse, watery, yellow diarrhea that persisted for ca. 3 days and then became progressively less severe, so that by day 5 or 6 after inoculation the feces were again normal. Two inoculated gnotobiotic pigs (pigs 6 and 9) were clinically normal when sacrificed but were infected as determined by immunofluorescent staining.

Immunofluorescent staining. Villous enterocytes throughout the small intestine were infected with the porcine RVLV as determined by an indirect immunofluorescent stain with hyperimmune anti-porcine RVLV serum (Table 1). The two procedures employed to prepare intestinal specimens for immunofluorescent staining gave similar results. Mucosal smears allowed observations to be made on numerous villi from an intestinal region, whereas sections prepared from paraffin-embedded tissues permitted a more precise determination of the location of infected enterocytes on a more limited number of villi. The greatest numbers of infected enterocytes were detected near the onset of diarrhea (15 to 18 h post-inoculation) and decreased rapidly thereafter. Immunofluorescence was observed only in the cytoplasm of villous enterocytes and was never detected in the epithelial cells lining the crypts. Generally, the greatest number of infected villous enterocytes were detected in the ileum; fewer enterocytes were infected in the duodenum and jejunum. In these latter two regions particularly, infection

was often restricted to only those enterocytes covering the villous tip or to very discrete foci of enterocytes situated along the sides of the villi (Fig. 1). Very few infected enterocytes were observed in the small intestinal mucosa later than 24 h post-inoculation.

Mucosal smears containing enterocytes infected with porcine RVLV gave negative immunofluorescent reactions when stained for rotaviral, pararotaviral, and transmissible gastroenteritis viral antigens. However, these smears did give a positive immunofluorescent reaction when stained by the indirect method with hyperimmune anti-bovine RVLV serum. Further, MA104 cell monolayers infected with porcine rotavirus or reovirus gave negative immunofluorescence reactions when stained by the indirect method with hyperimmune anti-porcine RVLV serum.

Histopathology. The extent of histopathological changes in the small intestine of infected gnotobiotic pigs was variable, but some changes were apparent in most animals sacrificed at or after 18 h post-inoculation (Table 1). Most often, these changes were restricted to the extreme tips of villi of normal length and consisted of the sloughing of enterocytes from the apex. The microvilli of the enterocytes remaining on the villous tips were either sloughed or shortened with focal sloughing. Occasionally, within the duodenum and ileum, the loss of enterocytes was extensive enough to cause a reduction in villous length (villous atrophy).

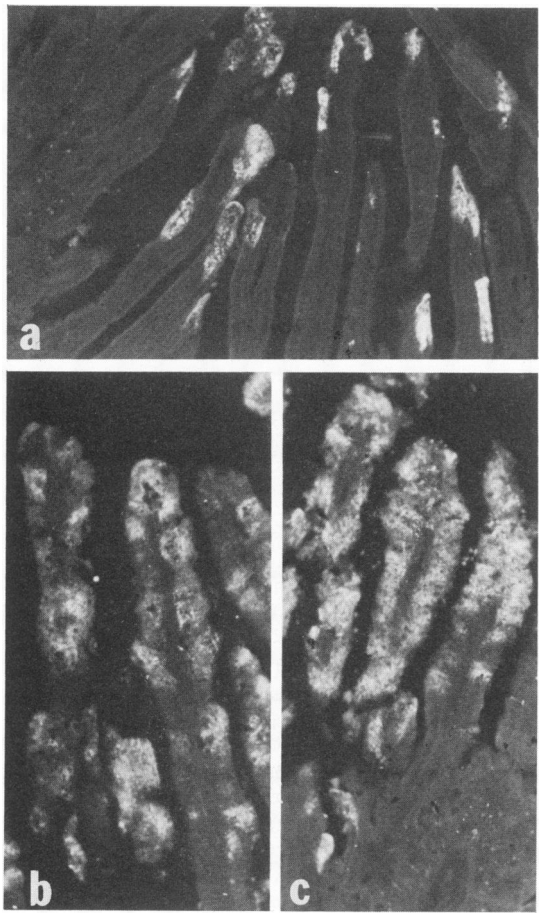

FIG. 1. Porcine RVLV immunofluorescent stain of paraffin-embedded small intestine sections from gnotobiotic pig 9 (15 h after infection). Infected enterocytes are present on the villi, but not in the crypts, of the duodenum (a), jejunum (b), and ileum (c). Magnification, ×120.

Scanning electron microscopy. Morphological alterations in the small intestine could be detected by scanning electron microscopy before they were detectable by histological examination. The first changes were observed in pigs sacrificed at 15 h post-inoculation and consisted of rounded, swollen enterocytes located on the extreme tips of the villi throughout the small intestine (Fig. 2). These swollen enterocytes often occluded the nearby goblet cell openings. Many of these enterocytes were sparsely covered with microvilli, and those microvilli that remained were short and irregular. Usually, the severity of intestinal damage was greater in pigs sacrificed several or more hours after the onset of diarrhea and was characterized by shortened villi with partially denuded tips.

The noninoculated control pig remained normal, was not infected with RVLV, and had long, slender finger-like villi throughout the small intestine (Table 1 and Fig. 2). These villous surfaces were smooth but interrupted by numerous transverse furrows. The apical regions of the villi were covered with tightly packed, irregularly shaped polygonal enterocytes interspersed with occasional goblet cells. The enterocytes were covered by a dense array of microvilli that gave the villi a distinct velvet-like appearance.

Immune electron microscopy. Particles resembling complete rotavirus particles were extremely rare and could be detected in the intestinal contents of only one infected gnotobiotic pig (Fig. 3). However, the intestinal contents of infected gnotobiotic pigs did contain numerous hexagonal corelike particles that were aggregated by hyperimmune anti-porcine RVLV serum (Fig. 3) but not by hyperimmune anti-porcine rotavirus serum. Corelike particles measured 52 nm in diameter and were often penetrated by stain.

Electrophoresis of viral dsRNA. The porcine RVLV genome produced an electropherotype with 10 resolved bands (Fig. 4), but the staining intensity of the third largest band indicated that it was actually two comigrating segments (segments 3 and 4). Therefore, the porcine RVLV genome is composed of 11 discrete segments of dsRNA. The porcine RVLV electropherotype was distinctly different from the electropherotypes produced by the rotaviral, reoviral, and porcine pararotaviral genomes. The most apparent differences between the porcine RVLV and the rotavirus electropherotypes were in the migration distances of segments 5, 6, and 9. Porcine RVLV segments 5 and 6 formed a tight couplet that migrated further than the corresponding segments 5 and 6 of the rotaviral genome. Similarly, porcine RVLV genome segment 9 migrated further than segment 9 of the rotavirus genome. The porcine RVLV electropherotype was similar, but not identical, to the bovine RVLV genome electropherotype.

Cell culture. Attempts to adapt porcine RVLV to serial passage in MA104 or primary porcine kidney cell cultures by using procedures suitable for porcine rotavirus isolation were unsuccessful. Cytopathic effects were not observed in inoculated monolayers maintained on the roller drum, and infected cells were not detected in inoculated cover slip monolayers.

DISCUSSION

A virus morphologically similar to, but antigenically distinct from, porcine rotaviruses and pararotaviruses was isolated from a diarrheic pig in Ohio. This virus induced an acute, transitory diarrhea in experimentally infected gno-

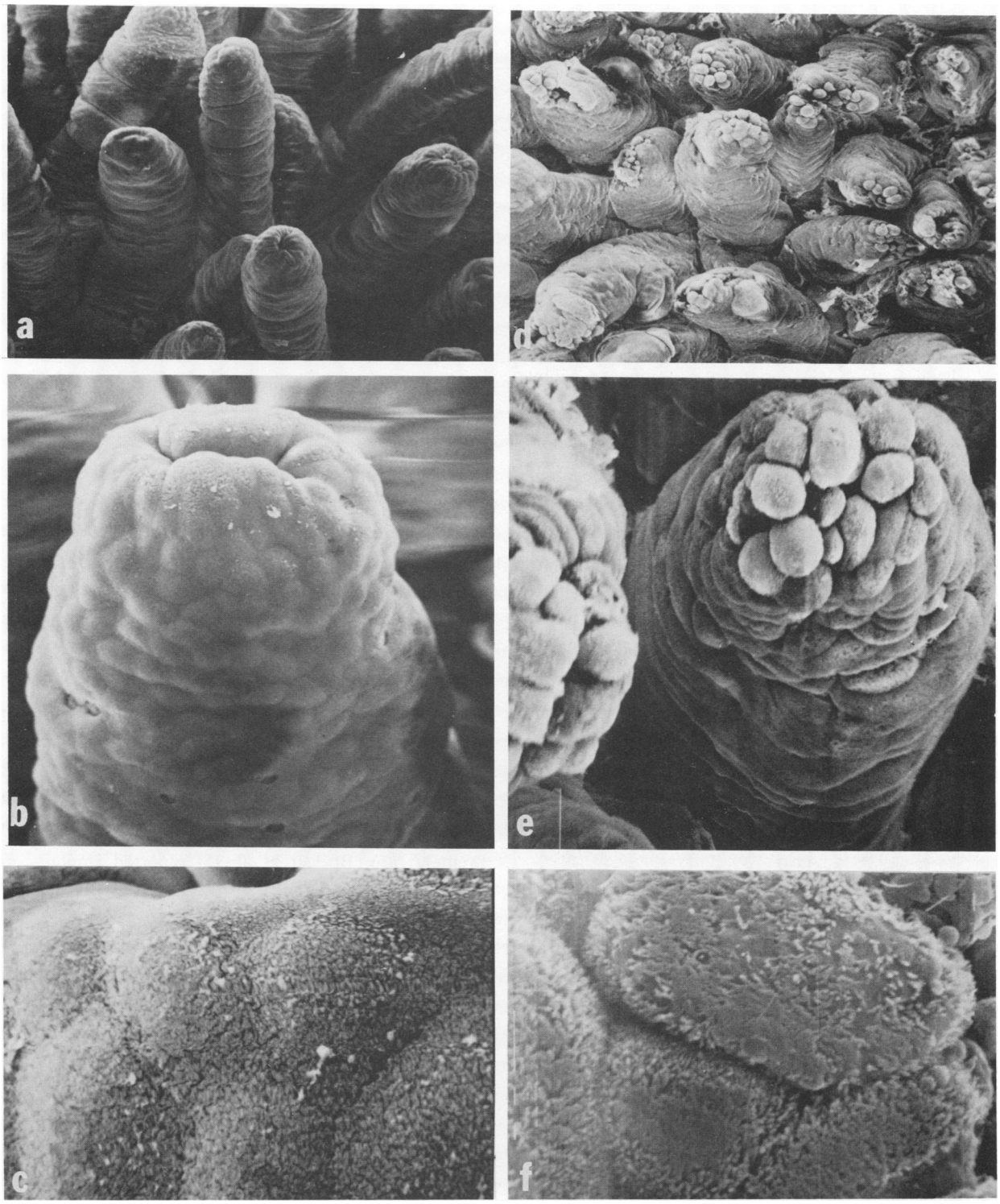

FIG. 2. Scanning electron micrographs of jejunum segments from gnotobiotic pig 10 (15 h after inoculation with porcine RVLV) and gnotobiotic pig 12 (control). (a) Long, slender, finger-like villi containing transverse furrows from the control pig. Magnification, ×190. (b) Extrusion zone at villus apex from control pig. Polygonal enterocytes are evenly distributed over the surface of the apex. Randomly distributed goblet cell openings are readily apparent. Magnification, ×900. (c) Dense microvilli coat of the enterocytes from the control pig. Magnification, ×4,500. (d) Numerous villi, from the infected pig, with swollen enterocytes on the tips. Magnification, ×190. (e) Higher magnification of villus in (d). The tip of the villus is covered with swollen enterocytes possessing distinct borders. The swollen enterocytes are separating from one another and occluding goblet cell openings. Magnification, ×900. (f) Microvilli coat of enterocytes from the infected pig. Microvilli are sparse and are often short and irregular. Magnification, ×4,500.

tobiotic pigs and thus must be included in the growing list of primary etiological agents of porcine diarrhea.

Pedley et al. (12) have proposed that rotaviruses be subdivided into three groups, with members of each group sharing their own distinctive common group antigen. According to their nomenclature, the original rotaviruses, the porcine RVLV isolated in England, and the porcine pararotaviruses would belong to groups A, B, and C, respectively. The Ohio isolate of porcine RVLV was not antigenically related to either group A or group C rotaviruses but did share antigens with a bovine RVLV. As the electropherotypes of the Ohio isolate and the antigenically related bovine RVLV were similar and these electropherotypes were in turn similar to the electropherotype of the porcine RVLV isolated in England (5), it was concluded that the Ohio isolate should be tentatively considered to be a group B rotavirus.

Little is known concerning the pathogenesis of porcine RVLV. Pedley et al. (11) briefly noted infected villous enterocytes throughout the small intestine of a gnotobiotic pig at 18 h after inoculation with the porcine RVLV isolated in England. Our studies with the Ohio isolate also demonstrated that villous enterocytes throughout the small intestine were infected, but the infection was often restricted to discrete foci of enterocytes located at or near the villous tip; extensive foci of infected enterocytes were seldom observed. Our observations suggest that the following sequence of events occurs within the small intestinal mucosa of gnotobiotic pigs infected with the Ohio isolate. Enterocytes, usually on the villous tips, are infected, and by 15 h after infection have lost many of their microvilli. The vast majority of these damaged enterocytes desquamate from the tips by 24 h after infection, occasionally in numbers sufficient to induce a shortening of the villi. These lost enterocytes are apparently replaced within 5 days, and the small intestine regains its normal function. Although similar to group A porcine rotaviral infections (9, 17, 20), the group B porcine rotaviral infection is less extensive and consequently induces considerably fewer histopathological changes within the small intestine.

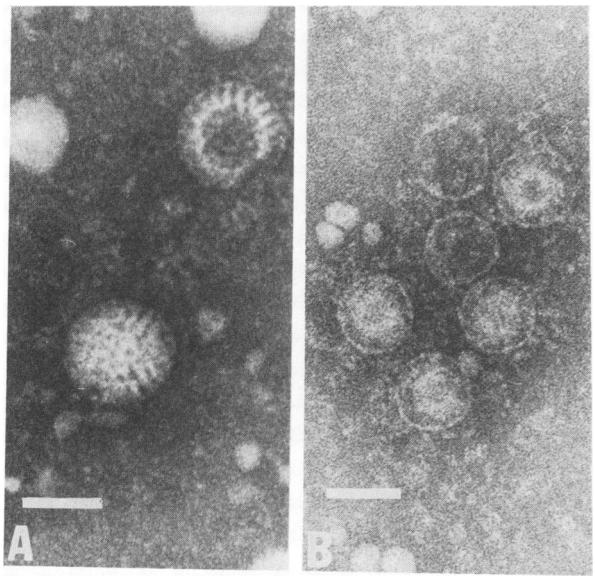

FIG. 3. Negatively stained porcine RVLV from intestinal contents of experimentally infected gnotobiotic pigs. (A) Complete virus particles ca. 70 nm in diameter; (B) 52-nm corelike particles aggregated with anti-porcine RVLV serum. Bar, 50 nm.

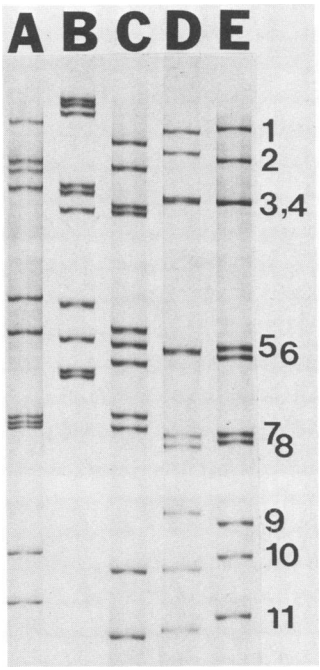

FIG. 4. Comparison of the porcine RVLV (Ohio isolate) genome electropherotype with the genome electropherotypes of other dsRNA viruses. Migration is from top to bottom, and numbers on the right designate segments of the porcine RVLV genome. Lanes: A, porcine rotavirus, G isolate; B, reovirus type 3, Abney isolate; C, porcine pararotavirus, Cowden isolate; D, bovine RVLV; and E, porcine RVLV, Ohio isolate.

A very striking characteristic of the Ohio isolate was that the predominant morphological form of the virus was a corelike particle 52 nm in diameter that closely resembled rotavirus cores generated by treatment with thiocyanate (1). Particles resembling complete rotavirus virions were extremely rare. Bridger et al. (5) reported similar corelike particles in their preparations of the group B porcine rotavirus isolated from England. It may be that these corelike particles represent the common morphological form of group B porcine rotaviruses, as we have also found them to be the predominant particle in specimens containing another isolate of porcine RVLV (unpublished data). Furthermore, a novel rotavirus recently was identified as an etiological agent of human diarrhea in China (15, 16), and corelike particles ca. 50 nm in diameter were often encountered in stool specimens collected from infected patients; this novel rotavirus possesses an electropherotype quite similar to those of group B porcine rotaviruses. Although it is not known whether this novel human rotavirus is actually a group B rotavirus, the frequency with which the corelike particles were detected in the specimens is of considerable interest.

Although group A rotaviruses are clearly an important cause of diarrhea in young pigs (3, 21), group B rotaviral infections are rarely identified. In fact, this is only the second report of the isolation of group B rotaviruses from pigs. This situation is perplexing, given the similarities between group A and group B rotaviral infections. Our studies with the Ohio isolate of group B porcine rotavirus suggest several possible explanations for this difference. First, as infection with the group B porcine rotavirus did not

induce extensive histopathological changes within the small intestine, it is possible that many naturally occurring group B rotaviral infections are either subclinical or very mild. Second, if the common morphological form of most group B porcine rotavirus isolates is the corelike particle, it is likely that these infections will often be missed by electron microscopic examination of stool specimens, unless immune electron microscopy with monospecific antiserum is used.

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