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Role of Rotavirus (Reo-Like) in Weanling Diarrhea of Pigs†

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Piglets weaned abruptly and precociously at 3 weeks of age and placed in a crowded nursery commenced diarrhea 3 to 5 days later. Death losses were low (approximately 6%), but weight gain ceased for 2 weeks. Large numbers of rotavirus (reo-like) particles were seen by electron microscopy in diarrhetic fluids. Sections of intestines showed a loss of adsorptive surface in that villi were shortened and fused with adjacent villi. Immunofluorescence revealed rotaviral antigens within damaged enterocytes. Rotavirus-containing gut fluid was harvested from sick, weaned piglets. This fluid, filtered free of bacteria, was used to inoculate per os colostrum-deprived piglets. These infected piglets developed diarrhea and dehydration, and large numbers of rotaviral particles were seen in their diarrhetic fluid. Also, rotaviral antigens were present in aberrant enterocytes, and the intestinal villi were shortened. Since the weaned piglets (3 weeks old) came from sows that were providing their piglet's intestine with passive antibody protection via milk, we concluded that the abrupt removal of the piglet from the gut-bathing antibody combined with the stress of weaning produced a neonate vulnerable to the ubiquitous rotavirus. Similar circumstances may prevail and operate in exacerbating rotaviral diarrhea in neonates of other species of mammals.

Weaning can be hazardous to neonates (J. G. Lecce and M. W. King, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, C114, p. 54; J. G. Lecce and M. W. King, Abstr. 69th Annu. Meet. Am. Soc. Anim. Sci., 1977, p. 46; see reference 8 for review of weanling diarrhea in human infants). At weaning, the neonate is shifted from an intimate association with mammary glands, and all the accompanying immunological, physiological, nutritional, and psychological benefits, to rearing regimens that may be less than ideal for health and growth. A combination of brutalizing management practices at weaning (see Discussion), which are dictated by the exigencies of economy, have produced in piglets the epitome of the weanling diarrhea syndrome. Because of the certainty that piglets will be subjected to severe stress at weaning, they make apt models for detecting the opportunistic pathogen(s) playing a role in this syndrome. For this purpose, newly weaned, 3- to 4-week-old piglets with diarrhea were studied. We report herein the causal association of rotavirus with weanling diarrhea in these early-weaned piglets.

MATERIALS AND METHODS

Experimental animals. Piglets came from two large commercial herds with similar weaning practices. In the first herd, piglets 17 to 21 days old were abruptly removed from their dam in a farrowing house, moved to a nursery, and regrouped. Here, the piglets were presented with a dry diet (mainly corn and soybean meal) and water. The drinking water contained 100 μg of oxytetracycline HCl and 70 μg of neomycin base (Pfizer Agricultural Division, New York) per ml for 10 days postweaning. About 600 pigs were weaned weekly. Piglets in the second herd were weaned similarly except that they were 24 to 28 days old.

Scanning and transmission electron microscopy. Techniques for detecting virus in gut fluid and for scanning villi from the midgut were the same as published elsewhere (15).

Phase and fluorescent microscopy. Enterocytes in gut sections were examined for the presence of rotavirus by direct and indirect fluorescent microscopy and for villus damage by phase microscopy (15).

Immune status. Both the immune status of the herd to rotavirus and the presence of antibody to rotavirus in the sow's mammary secretion were inferred from an examination of serum from 2-day-old nursing piglets for passively acquired antibody; i.e., immune sows have antibodies in colostrum which are absorbed at nursing by the agammaglobulinemic piglet. Fluorescent techniques and reagents for determining antibody to rotavirus in sow's serum were as previously described (15).

Infectivity and pathogenicity. Gut fluid, containing approximately 10^9 rotaviral particles/ml, from weaned piglets with diarrhea was centrifuged and filtered free of bacteria (0.45-μm membrane; Millipore

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Corp., Bedford, Mass.). This bacteria-free fluid was assessed for pathogenicity by infecting per os 5 newborn and 14 9- to 14-day-old, colostrum-deprived piglets reared in isolation. Techniques for farrowing, feeding, and infecting piglets in an isolated and sanitary environment were as described elsewhere (13, 15).

RESULTS

Invariably, about 3 days postweaning, piglets in both herds commenced diarrhea which lasted for 5 to 10 days. Weight gains were severely depressed in that piglets gained only 1 kg in the 2-week postweaning period. Piglets are capable of gaining 5 kg in this period (E. E. Jones, J. A. Coalson, and J. G. Lecce, J. Anim. Sci., in press). Six percent of the piglets died. Large numbers of rotavirus particles were observed by electron microscopy (Fig. 1A) in the gut fluid of 48% of the 50 pigs sampled. Rotavirus was more likely to be detected in gut fluids harvested within 3 days of the onset of diarrhea and less likely to be seen in the gut fluid from pigs that had had diarrhea for longer than a week.

Scanning electron microscopy showed that there was severe damage to the intestinal villi in that they were shortened, blunted, and often fused with neighboring villi (Fig. 2D, E). The morphology of the intestinal epithelial cell, as viewed by phase-contrast microscopy, was more cuboidal and squamous than columnar (Fig. 3B). Viral antigens were stained within enterocytes by direct immunofluorescence (Fig. 4A).

A pool of bacteria-free gut fluid containing rotavirus harvested from sick 26-day-old piglets (weaned for 5 days) produced vomiting and diarrhea about 24 h post-inoculation in 5 newborn and 14 9- to 14-day-old, colostrum-deprived piglets. The surviving sick pigs were killed 3 days post-inoculation. In all cases large numbers of rotavirus were observed in gut fluids (Fig. 1B), and many of the intestinal villi were blunted, fused (Fig. 2F), and covered with cuboidal-squamous epithelium (Fig. 3D). Rotaviral antigens were observed by indirect immunofluorescence within these aberrant enterocytes (Fig. 4B).

In the two herds under investigation, sera obtained from nursing 2-day-old pigs were uniformly positive for antibodies to rotavirus when tested by indirect immunofluorescence. Forty-four percent of the sera obtained from 52 piglets with weanling diarrhea (3 to 4 weeks of age) still had antibodies to rotavirus.

DISCUSSION

Recently, we reported that rotavirus was ubiquitous in swine and responsible for diarrhea, dehydration, and death in piglets reared artifi-

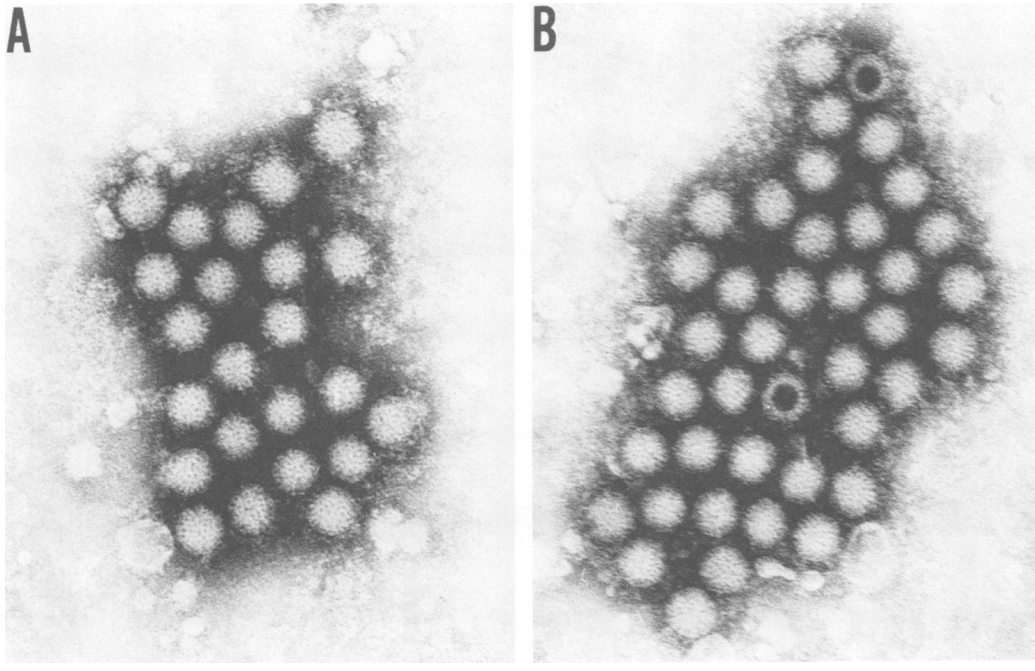
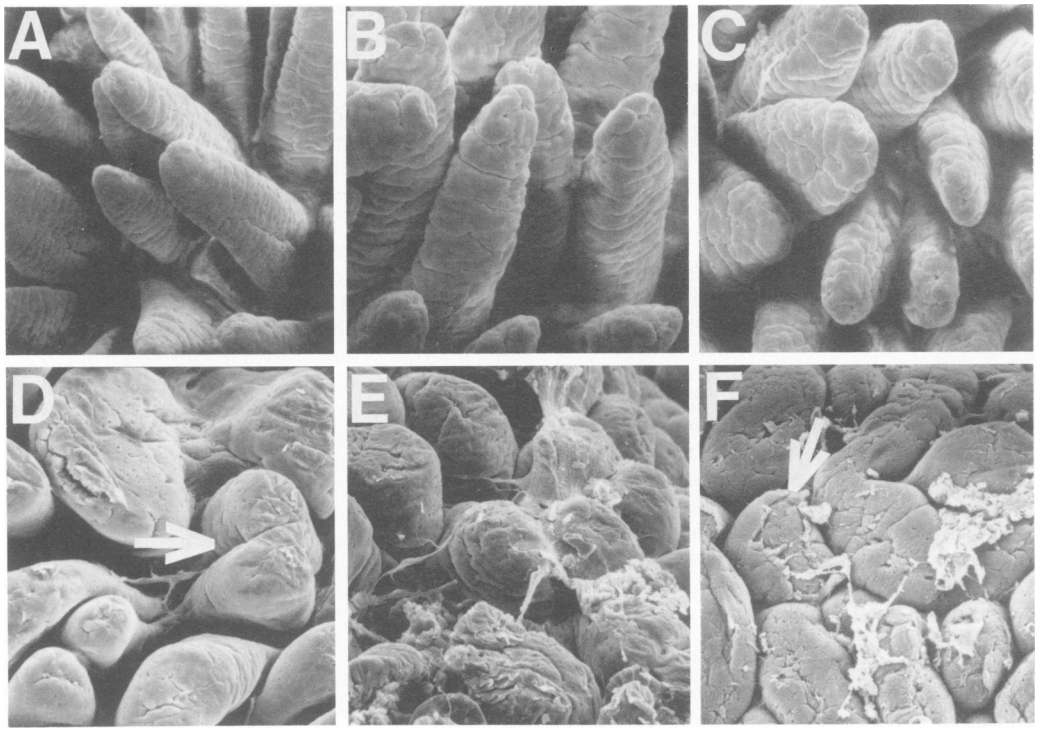


FIG. 1. *Electron micrographs of gut fluid from (A) a newly weaned, 24-day-old piglet and (B) a 12-day-old, colostrum-deprived piglet, 3 days after inoculation with a pool of bacteria-free gut fluid harvested from newly weaned piglets. (Viral particles approximately 70 nm; ×79,000).*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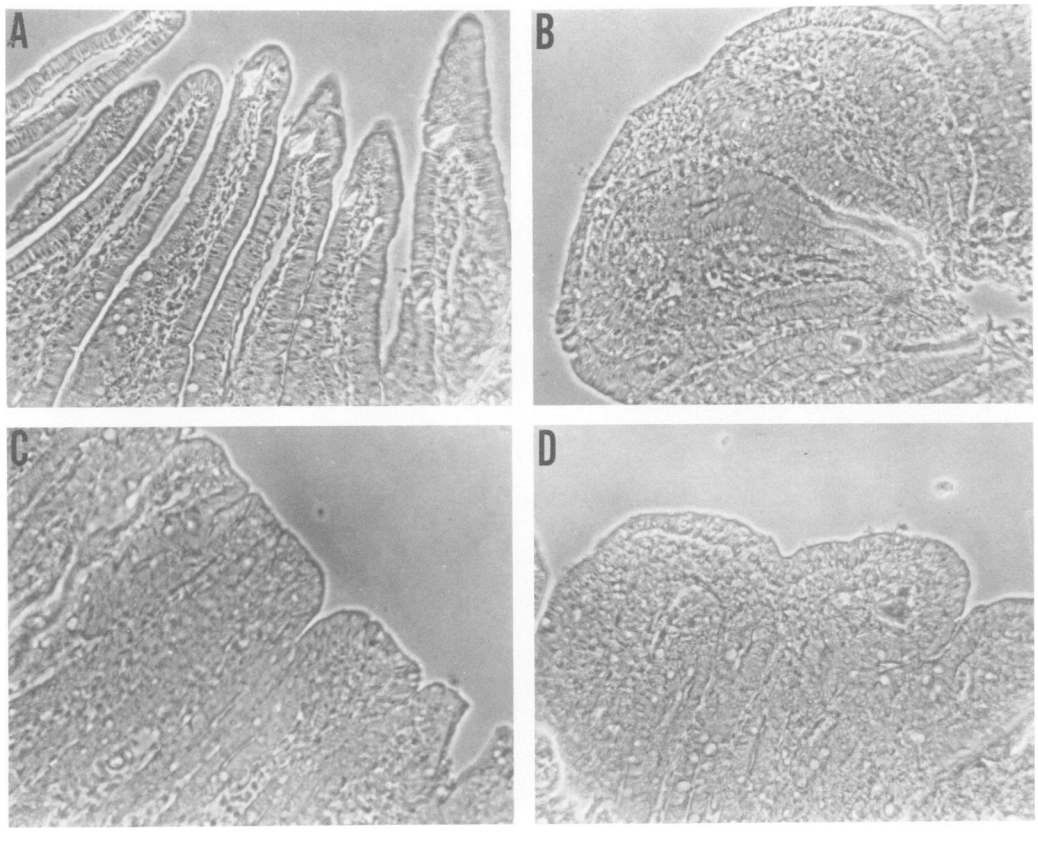
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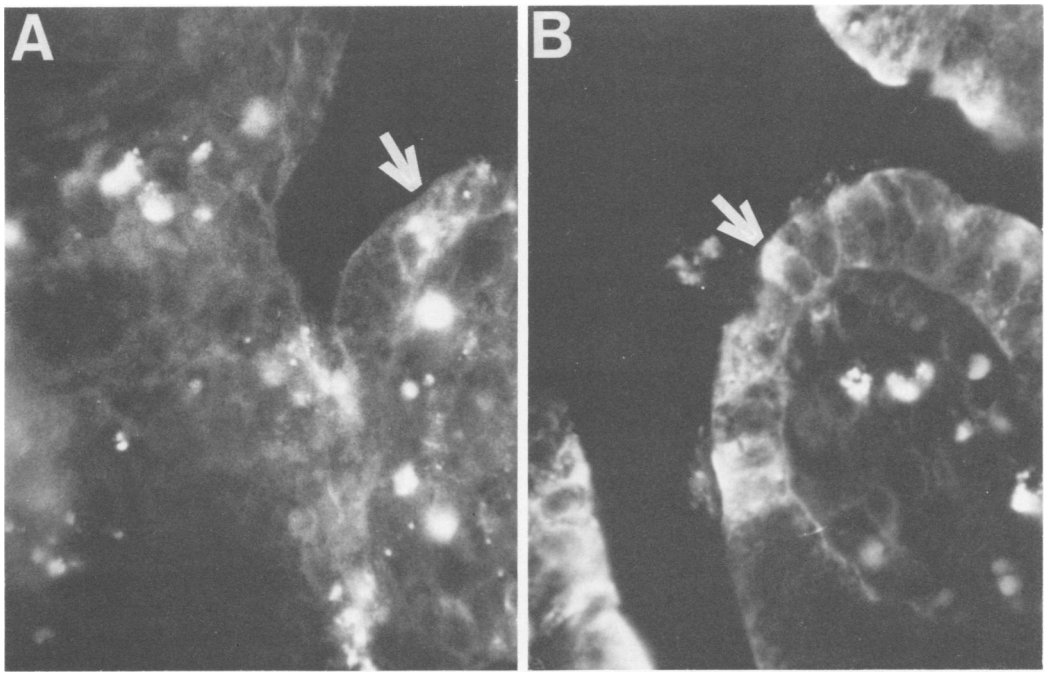

FIG. 4. *Fluorescent micrographs of midjejunum from: (A) 24-day-old, newly weaned piglet. Section stained directly with fluorescent antibody to piglet rotavirus. (B) 13-day-old, colostrum-deprived piglet 3 days after inoculation with rotavirus. Section treated with antiserum to porcine rotavirus (pig) and then stained with fluorescent antibody to porcine immunoglobulin. Arrows point to fluorescent enterocytes. (×237).*

cially from 1 day of age (6, 13–15). Others have noted the presence of rotavirus in nursing and weaned pigs, ranging in age from 8 days to 8 weeks of age (2, 26). We wondered how the above findings related to the diarrhea occurring in pigs being weaned early in modern management systems, i.e., at about 3 weeks of age as opposed to natural weaning at about 8 weeks of age. Diarrhea is a common problem in these early-weaned pigs and occurs with such regularity that it is considered normal by swine husbandrymen.

The syndrome occurring in these pigs at about 3 weeks of age has been called milk scours, colibacillosis, 3-week-enteritis, serum-modified TGE, l'enterite colibacillaire de la troisieme semaine, Drei-Wochen-Durchfall, nutritional scours, white scours, feed scours, and weaning diarrhea. Attempts to identify infectious agents have centered mainly on enterotoxigenic *Escherichia coli* (5, 9, 11, 12, 18, 21–23). However, data presented here demonstrate that there is a causal association of rotavirus with the diarrhea, dehydration, and depressed growth accompanying weaning in these kinds of piglets. That is, this virus can be seen in large numbers ($\sim 10^9$/ml) in gut fluids from sick but not from well pigs, and rotaviral antigens are detected within enterocytes on shortened, blunted, and often fused intestinal villi. Further, colostrum-deprived pigs inoculated with bacteria-free, rotavirus-containing gut fluid from the newly weaned sick pigs developed the same symptoms as seen in these weaned pigs, namely, diarrhea, dehydration, large numbers of rotavirus in diarrhetic fluids, rotaviral antigens in aberrant enterocytes, and shortened intestinal villi. This is also the syndrome produced in artificially reared

FIG. 2. *Scanning electron micrographs of the midjejunum from: (A–C) 3-week-old, normal colostrum-deprived piglets reared in isolation. Villi are thin and elongated. (D) Newly weaned 24-day-old piglet. Villi are blunted, shortened, and fused at the point of the arrow. (E) Newly weaned 24-day-old piglet showing more damage to villi. (F) Severely damaged villi from a 12-day-old, colostrum-deprived piglet, 3 days after inoculation with rotavirus. A villus is denuded at the point of the arrow. (×343).*

FIG. 3. *Phase contrast photomicrographs of midjejunum. (A) Elongated, thin villi from a normal 3-week-old, colostrum-deprived piglet reared in isolation. (B,C) Blunted, shortened, and fused villi from a newly weaned, 24-day-old piglet. (D) Blunted, shortened, and fused villi from 12-day-old, colostrum-deprived piglet 3 days after inoculation with rotavirus. (×47).*

piglets inoculated with serially passaged rotavirus (15). Recently, Bohl et al. published data also supporting the notion that rotavirus is the primary pathogen in weanling diarrhea and perhaps enterotoxigenic coli are secondary (2).

Thus, we propose that the etiology and pathogenesis of diarrhea occurring at weaning at 3 to 4 weeks of age and diarrhea of the pigs reared artificially from 1 day of age (6, 13–15) are the same. They differ only in timing and the fact that older pigs are clinically more resistant to infection. However, stress at weaning conspires against this resistance. Before weaning, piglets seem content with their siblings and surroundings, and their dam will provide them hourly with a nutritious liquid diet. Suddenly, they are removed from this environment, regrouped into a crowded nursery, surrounded by alien pigs, and presented with an unnatural dry diet; the disease described above ensues. These pigs are stressed in that they spend the next few days looking for food and water and fighting to establish a social order. Very little water and diet are consumed in this period. Added to this stress (and more important with respect to infectious diseases of the gut) is the fact that coincident with weaning, the antibody being supplied via the sow's milk will no longer be available to protect the piglet's enterocytes (3, 17). Without this passive protection from the sow's milk, the ubiquitous rotavirus, whose numbers have probably increased in the crowded, continuously used nursery, opportunistically overcomes the age resistance of the host (14, 15).

Others have found antigenically related rotavirus associated with diarrhea in mice, calves, foals, and infants. Also, antibody to rotavirus is present in the serum of most adolescents of these various species, demonstrating again a high prevalence of the virus in the community of mammals (1, 4, 7, 10, 16, 19, 20, 24–27). Thus it seems likely that the same kinds of stressing forces that exacerbate rotaviral diarrhea in newly weaned piglets could exacerbate rotaviral diarrhea in other species of mammals (14).

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