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THE COLOSTRUM-DEPRIVED, ARTIFICIALLY-REARED, NEONATAL PIG AS A MODEL ANIMAL FOR STUDYING ROTAVIRUS GASTROENTERITIS

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1. ABSTRACT

Rotavirus gastroenteritis is one of the main causes of acute diarrhea in young humans and animals worldwide. The colostrum-deprived, artificially-reared, neonatal pig has been extensively used in our laboratory as a model animal for studying an experimentally-induced rotaviral gastroenteritis. Details on procurement of newborn pigs, immunological characteristics and artificial rearing conditions of colostrum-deprived neonatal pigs as well as on rotavirus inoculation, clinical manifestations and evaluation of intestinal damage caused by rotavirus infection are described. Our experimentally-induced rotavirus gastroenteritis model has been characterized clinically by anorexia, diarrhea, ocassional vomiting and high titers of rotavirus shedding in feces. Data reported herein provides additional information, particularly on feeding regimens of pigs before rotavirus inoculation, extent of anorexia, severity of diarrhea and extent of fecal virus shedding, as well as on the effect of rotavirus infection and size of rotavirus inocula on intestinal damage, growth and mortality during the post-infection period. On the basis of these results and others previously reported by us and by other researchers, and because of the intestinalanatomy and physiology similarities to that of human infants, the colostrum-deprived, artificially-reared, neonatal pig is the most suitable and useful model animal for studies designed to evaluate prevention and treatment of rotaviral gastroenteritis.

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2. INTRODUCTION

Gastrointestinal viral infections are common in young animals and humans and often are associated with enteritis and diarrhea (1, 2). Rotavirus infection is a major cause of severe diarrhea in children (3) as well as of neonatal diarrhea in several animal species worldwide (4). The main characteristics of rotavirus infections have been reviewed extensively (5, 6). The need for an animal model in which to study the pathogenesis, as well as the prevention and treatment of rotavirus gastroenteritis, has long been recognized (1). Because the porcine gastrointestinal tract and digestive physiology are very similar to that of humans, gnotobiotic (7 - 16) as well as conventional (17 - 22) pigs have been extensively used as model animals for their relevance to acute infantile diarrhea. Although rabbits (23, 24) and mice (25, 26) also have been used as model animals for studies related to rotaviral gastroenteritis, neonatal pigs appear to be the most suitable species for this type of research.

Our involvement in artificial rearing of pigs as well as in gastrointestinal research has led to the use of the colostrum-deprived, artificially-reared, neonatal pig as a model animal for studying rotaviral gastroenteritis in human infants. This paper is a review of the literature on

the use of neonatal pigs in rotaviral gastroenteritis research along with a description of most of the experimental procedures which have been used in our model during the last decade including aspects related to procurement of colostrum-deprived neonatal pigs, immunological considerations of newborn pigs, feeding programs for artificial rearing of colostrum-deprived pigs with emphasis on their early post-natal period, rotavirus inoculation, assessment of clinical manifestations, and evaluation of intestinal damage during the post-inoculation period. The protocols of the research carried out at the NCSU Piglet Core, which are described herein, were approved by the NCSU Institutional Animal Care and Use Committee.

3. PROCUREMENT OF COLOSTRUM-DEPRIVED NEONATAL PIGS

Although germ-free, gnotobiotic or specific-pathogen free (SPF), pigs have been extensively used as a model animal for rotaviral gastroenteritis research (7 - 16), the required surgical procedures (hysterectomy, hysterotomy or cesarean section) which are performed on or near the 112th day of gestation (27, 28) are expensive because of the specialized equipment, facilities and personnel needed to undertake them. Since facilities to procure SPF pigs are not readily available, we have been using naturally-farrowed, full-term gestation (114 - 115 days), neonatal pigs (18 - 22) which are obtained under conditions that prevent or minimize sow-acquired, particularly rotavirus, infections. Due to the ubiquitous distribution of rotavirus in swine farms, early research in our laboratory has shown that conventionally-farrowed, colostrum-free pigs are infected, at or shortly after birth, with enough rotavirus so as to result in diarrhea, dehydration and death within 5 to 6 days of age (29). This problem is aggravated in neonatal pigs farrowed during fall and winter when the highest natural incidence of rotavirus has been observed (Gomez, personal communication). A similar winter seasonality of rotavirus gastroenteritis has been reported in epidemiological studies with humans (30 - 32).

To prevent sow-acquired infections, pregnant sows obtained from the NCSU Swine Farm are transferred to an isolated farrowing facility, 5 days before farrowing, so that neonatal pigs carrying no known or defined pathogens are farrowed in an antiseptically clean stall after repeating bathing and sanitizing of sows with an iodinated detergent (Wescodyne®, American Sterilizer Company, Medical Products Division, Erie, PA) before delivery. In most of our studies, between 40 and 50 newborn pigs farrowed by five sows have been used in each trial. Usually, the estimated and actual farrowing dates of five sows occur within a 1- to 3-day interval. In order to minimize this interval and synchronize farrowings, as soon as presence of milk (indication that farrowing should occur within 24 hours) is detected in the teats of one sow, an intramuscular injection of 1.5 to 2.0 mL (5 mg/mL of dinoprost tromethamine) of prostaglandins F_{2alpha} (Lutalyse®, The Upjohn Co., Kalamazoo, MI) per sow is administered to the remaining sows. Using this procedure, experimental pigs are born on the same day or have a difference in age of 1 day.

Implementation of a high level of sanitation during farrowing is the most critical aspect to obtain "sanitary" or "pathogen free-like" newborn pigs. To achieve this, sows are attendant-farrowed so that pigs are caught as they are being born, the umbilical cord is clamped with a navel cord clamp (NASCO, Fort Atkinson, WI) to help prevent navel infection, and gently detached from the sow. Each pig is immediately moved from the farrowing crate into a clean plastic container, sprayed with a disinfectant solution, freed from adhering membranes and dried with disposable paper towels, and transferred to an isolation room where its umbilical cord is cut at about 10 cm from the body and disinfected with tincture of iodine (2%). We have been using a 70% ethanol solution as a disinfectant solution because rotaviruses have been shown to be easily inactivated with high concentrations (70% to 90%) of ethanol (33, 34). To minimize the exposure of newborn pigs to the sow environment, newborn pigs are moved out of the farrowing room into the isolation room within 1 to 2 minutes after they are born. Pigs are kept warm, with a heating lamp, in either a disinfected cage or cardboard box until the end of farrowing when they are identified by ear notches and weighed. Pigs are then transferred into a separate room containing an automatic feeding device (Autosow). When simultaneous observations of non-infected and rotavirus-infected pigs are needed, two separate Autosows must be used.

Crossbred newborn pigs obtained in our laboratory during the period January 1992 - December 1996 were farrowed by either white line (½ Yorkshire, ½ Landrace) or crossbred (¼ Yorkshire, ¼ Landrace, ¼ Hampshire, ¼ Duroc) sows which were bred by Hampshire or Duroc boars. There was no difference ($p > .05$) in the average number (10.2 ± 2.7 vs. 11.0 ± 2.3) and weight (1.36 ± .23 vs. 1.38 ± .19 kg) of newborn pigs per litter as well as in the proportion (%) of males and females (51:49 vs. 48:52) farrowed by either white line (n = 96) or crossbred (n = 42) sows. Values are means ± standard deviation. Parity of the sows varied from 1st (12%), 2nd to 4th (58%), and higher than 5th (30%), but the aforementioned parameters were not affected ($p > .05$) by parity. On the average, 12% of the newborn pigs per litter weighed less than 1.0 kg and in 44% (61/138) of the litters

all newborn pigs weighed more than 1 kg. Newborn pigs weighing less than 1.0 kg are seldom used for experimental purposes.

4. IMMUNOLOGICAL CONSIDERATIONS OF NEWBORN PIGS

The pig is born void of circulating immunoglobulins and belongs to a group of neonatal mammals which acquire passive immunity from their dam exclusively postpartum. Furthermore, the intestinal absorption during its early postnatal period is qualitatively non-selective, henceforth, the neonatal pig is able to absorb a wide variety of unaltered or undigested macromolecules (35, 36). The cessation of this non-selective absorption process (closure) seems to be dependent on the feeding regimen rather than on the age of the pig (36). Thus, closure in nursing pigs occurs between 24 and 36 hours of age, while pigs that were starved from the time of birth continued absorbing large molecules for at least 86 hours (37).

Extensive research (36 - 43) has demonstrated the importance of sow's colostral immunoglobulins in conferring the newborn pig with passive immunity to infectious disease that is essential to pig survival. Henceforth, colostrum-free pigs have been shown to be very susceptible to microbial pathogens, particularly to enterotoxigenic *Escherichia coli*. Early attempts to artificially rear newborn pigs showed that colostrum-deprived pigs died very shortly after birth because of severe diarrhea and dehydration (44, 45).

5. FEEDING PROGRAMS FOR ARTIFICIAL REARING OF COLOSTRUM-DEPRIVED PIGS

Due to the aforementioned considerations, feeding regimens for artificial rearing of colostrum-deprived pigs are critical for their survival and adequate growth, particularly during their early post-natal period. In our laboratory, pigs are reared with an automatic feeding device and the details of the characteristics and functioning of this device as well as the environmental rearing conditions have been previously reported (46, 47).

To overcome the lack of passive immunity conferred to newborn pigs by sow's colostral immunoglobulins, colostrum-deprived pigs have been fed milk replacers containing porcine (48 - 52), or bovine (51, 52) serum-derived immunoglobulins or fed bovine colostrum (42, 43, 53). Porcine serum-derived immunoglobulins did not provide effective passive immunity when fed to colostrum-deprived pigs for only 1 day post-partum (48, 49). However, when porcine (50 - 52) or bovine (51, 52) serum-derived immunoglobulins were fed for at least a 5- to 10-day period after birth as an integral part of milk replacers, survival was improved. Apparently more passive immunity was conferred to pigs when immunoglobulins were fed for an extended period after birth, probably as a consequence of a delayed closure time resulting from the feeding regimen used. In these studies, colostrum-deprived pigs were reared in non-isolated environments and fed diets fortified with antibiotics.

The results of several studies carried out in our laboratory suggest that porcine serum-derived immunoglobulins or bovine colostrum can be satisfactorily used as immunoglobulin sources to provide sufficient passive immunity to colostrum-deprived pigs which are artificially-reared in an isolated environment, without antibiotic supplementation, so as to support growth comparable to those of pigs nursed by their dams (Gomez, unpublished results). At present, porcine serum-derived immunoglobulins are commercially available (American Protein Corporation, Ames, IA) but they are mainly used in dry diets for early-weaned (10 to 14 days of age) pigs. Unfortunately, the variability in their IgG content as well as their limited solubility are two major current constraints to expand their use in artificial rearing of colostrum-deprived pigs. Henceforth, in order to provide passive immunity to newborn colostrum-deprived pigs we have been using bovine colostrum which is obtained from dairy cattle maintained at the NCSU dairy herd and consists of pools of the first day's secretions from cows. Bovine colostrum contains antibodies to rotavirus (54) and protects neonatal pigs from the clinical effects of porcine rotavirus (55). Bovine milk concentrate prepared from milk of cows hyperimmunized with human rotavirus serotypes has also been used to induce passive immunity to infantile rotavirus gastroenteritis (56, 57). Passive immunity has also been provided to newborn pigs obtained within 12 hours of birth by inclusion of a commercially available bovine IgG product (Colostrum-Plus, LaBelle Associates, Inc., Bellingham, WA) in the formula for the first 72 hours after birth (58). The Colostrum-Plus dry powder containing bovine IgG and supplemented with essential vitamins, minerals and live microbial cultures appears to be a promising replacement of conventional bovine colostrum.

Details of feeding regimens to artificially rear colostrum-free pigs with automatic feeding devices have been previously described (53, 59, 60). Their initial approach was to feed diets in volumes and at frequencies similar to pigs nursing naturally. A semiautomatic feeding system using a gravity-flow enteral feeding bag has been described by McClead *et al.* (61) to artificially rear pigs that were allowed to nurse for 4 to 10 hours after birth and

Table 1. Effects of feeding bovine colostrum (BC) to sow's colostrum-deprived, artificially-reared, pigs on body growth and incidence of diarrhea during the first 5 days of life[1]

				Avg weight/pig (kg) at			Avg daily gain (g)	Diarrhea
Trial	% BC[2]	% DM diet[3]	No. pigs	Birth	3rd day	5th day	Birth to 5th day[4]	5th day[5]
1	20	20	30	1.38 ± .19	1.65 ± .29	1.87 ± .34[b6]	123 ± 43[b]	43
2	50	20	24	1.47 ± .28	1.71 ± .34	2.05 ± .38[a]	146 ± 37[a]	30
3	50	15	24	1.57 ± .13	1.75 ± .12	1.97 ± .17[ab]	100 ± 38[c]	13

[1]:Pigs were fed the bovine colostrum/basal diet mix for 3 days after birth and switched to basal di*et al*one thereafter. Number of feedings and feeding intervals were 8 and 2 h, 10 and 1½ h, and 12 and 1¼ h for trials 1, 2 and 3, respectively. [2]:Percentage (v/v) of BC in mix; 20 = 20 parts BC + 80 parts diet; 50 = 50 BC/50 diet. [3]:Dry matter content in basal liquid diet. [4]:Average daily gains during first 4 days of life since pigs were weighed on their 5th day of life before the first feeding. [5]:Percentage of pigs having non-pathological diarrhea or liquid feces on 5th day. [6 abc]:Values are means ± SD; values in a column with unlike superscripts are significantly different ($p < .05$) from each other. Avg: Average.

it has also been used to rear colostrum-deprived neonatal pigs (16). The regimen developed in our laboratory (41) consisted of feeding pigs a daily volume of diet that was ~30% of their body weight; e.g., a 1,200 g pig was fed 360 mL/day and feedings were divided into 24 equal increments. In order to minimize labor expenses, to prevent and correct mechanical problems with the feeding device's functioning, particularly those happening overnight, as well as to supervise every feeding so as to measure and record the actual diet intake, the number of feedings have been reduced from 24 to either 12, 10 or 8 feedings per day with intervals between feedings of 1¼, 1½ or 2 hours, respectively, starting at 0800 h and finishing between 2130 - 2200 h, every day.

High survival rate, adequate growth and absence or low incidence of diarrhea during the first 5 to 7 days after birth are essential to successfully used colostrum-deprived, artificially-reared, neonatal pigs in studies related to gastrointestinal research. The daily volume of diet, the number and frequency of feedings as well as the dry matter (DM) content of the basal liquid diet are factors that affect the survival, performance and health status of neonatal pigs. Table 1 presents data on growth from birth to 5 days of age and incidence of diarrhea from 3 trials in which bovine colostrum (20% or 50% v/v) provided passive immunity to colostrum-deprived pigs when included into liquid diets containing either 20% or 15% DM and fed for 3 consecutive days after birth; thereafter, pigs were fed the liquid di*et al*one. Pigs were fed at the same daily rate (300 mL/kg body weight) but at different feeding intervals and number of feedings per day. Pigs were weighed at birth, 3rd and 5th day, and volume of diet for each pig was adjusted according to its body weight. At 5 days of age, the average weight and daily gain of pigs fed 50% (v/v) bovine colostrum mixed with a 20% DM liquid diet (trial 2) were higher ($p < .05$) than those of pigs in trials 1 and 3, but 30% of the pigs in that group had non-pathological diarrhea compared to 43% and 13% of the pigs in trials 1 and 3, respectively. Rectal swabs taken from diarrheic pigs in all 3 trials were negative to hemolytic *E. coli* and porcine rotavirus. The results of trial 3 further indicate that lowering the DM content of the basal diet and increasing the number of feedings per day, by reducing the interval between feedings, resulted in an appreciable decrease in the incidence of non-pathological diarrhea but also produced the lowest ($p < .05$) body weight gain during the first 4 days of life (Table 1).

In most of our studies, between 20% and 30% of colostrum-deprived, artificially-reared, neonatal pigs have shown non-pathological or "nutritional" diarrhea around 5 to 7 days of age which subsided or disappeared thereafter. The immunological differences between colostrum-fed and colostrum-deprived neonatal pigs, the differences in composition between sow's colostrum and milk for naturally-reared pigs and the milk replacers used for artificial-reared pigs and the differences in the amount and number of feedings per day for naturally- vs. artificially-reared neonatal pigs are some of the factors which may contribute to the incidence of "nutritional" diarrhea in colostrum-deprived, artificially-reared, neonatal pigs. Mortality due to this problem has been practically nil or very low, unless newborn pigs had pathological diarrhea, particularly caused by rotavirus, which could happen when strict sanitary conditions at birth were not followed. In general, artificially-reared neonatal pigs fed to appetite or overfed are prone to diarrhea and this has been reported with colostrum-deprived pigs (41, 53, 60, 62) as well as with newborn pigs allowed to nurse their dams for their

first 2 days of life (62, 63). Therefore, limited or restricted feeding scales and/or a decrease in the DM content of the liquid diet has helped in eliminating or minimizing the magnitude of non-pathological diarrhea, but has also reduced the growth rate of pigs (60; Gomez, unpublished data).

6. ROTAVIRUS INOCULATION

In studies of experimentally-induced rotaviral gastroenteritis, the age of pigs at infection varied from 12 to 14 hours (11) to 28 days of age (7); in the majority of studies, pigs were infected between 2 and 8 days of age (8, 10, 14, 15, 17, 20 - 22). In our most recent studies, pigs have been inoculated with rotavirus at 5 or 6 days of age.

Human rotavirus isolates obtained from human infants with acute gastroenteritis (7, 8, 10, 11, 13, 15, 17), porcine rotavirus isolates (12, 14, 17, 19 - 22) as well as calf and foal rotaviruses (15) have been used as inocula to infect pigs. The source of rotavirus inocula that we have been using is a stock of bacteria-free fecal supernatant which was obtained from intestinal contents collected from rotavirus infected pigs as described by Lecce *et al*. (29), and belongs to rotaviruses of group A which have been established as the cause of significant diarrheal disease in both the human and animal young.

In several of the reports mentioned above, the concentration of rotavirus in the inocula was not always given but in those in which concentration was mentioned (8, 13, 17, 19 - 22), it varied between 10^6 and 10^8 rotavirus particles per pig. In a couple of studies in which rotaviruses were propagated by cell culture, the titer of the inocula used was 3.2 x 10^4 $TCID_{50}$/mL (tisssue culture infective dose)(12) or 10^7 PFU/mL (plaque-forming units)(16). The minimal infective dose of rotavirus to induce clinical illness in colostrum-deprived, cesarean-derived, newborn pigs (inoculated at approximately 2 hours after birth) was 1 PFU (64). In our most recent experiments, we have been using inocula supplying between 10^4 and 10^5 rotavirus particles per pig and all pigs have shown the characteristic clinical manifestations of rotaviral-induced gastroenteritis (Gomez, unpublished results).

Oral administration has been the most common route of rotavirus inoculation but in a few studies, intranasal (7, 17) or nasogastric (13) administration has been used. In order to ensure total consumption of the rotavirus-containing preparation, particularly when neonatal pigs have been either fed throughout the day and/or allocated into the experimental groups the same day of infection, pigs have been inoculated, per os, after a 4- to 5-hour fasting period with 10 mL of the diet containing the specified rotavirus concentration followed by ~15 mL of regular diet. When pigs are not fed overnight and are distributed into the experimental groups 1 or 2 days before infection, rotavirus inoculation is carried out before the first feeding of the day.

7. CLINICAL MANIFESTATIONS OF ROTAVIRUS INFECTION

A detailed overview of the rotavirus replication cycle as studied in continuous cell cultures from monkey kidneys has been described by Estes (65) and it appears that a similar replication process may happen in the enterocytes of the small intestine. The pathogenesis of diarrhea caused by rotavirus infection as studied in miniature swine piglet has been described by Graham *et al*. (66).

Despite the variability of experimental conditions described above, induced rotavirus gastroenteritis in neonatal pigs, particularly when porcine rotavirus isolates were used (12, 14, 16, 17, 20, 22), has been characterized clinically by anorexia, diarrhea, ocassional vomiting, loss of weight and high titers of rotavirus shedding in feces.

The onset of diarrhea in practically all studies occurred within 2 to 3 days after rotavirus inoculation. The duration of diarrhea, however, was quite variable. Middleton *et al*. (8), using conventional pigs which were removed from the sow at 2 to 3 days of age and inoculated per os at 6 days of age with human rotavirus, reported that diarrhea began 32 hours after virus ingestion and lasted for only 24 hours. In another study (7), none of the gnotobiotic pigs intranasally inoculated with human rotavirus developed any clinical signs of infection during the following 3 to 4 weeks; however, pigs infected with porcine rotavirus had profuse diarrhea as early as 18 hours after viral infection. Others reported diarrhea duration varying from 3 to 6 days (20) to 10 to 18 days (11, 17) after rotavirus inoculation. In all cases, severity of diarrhea diminished with time after rotavirus inoculation.

Large amounts of virus in feces were found shortly before or at the onset of diarrhea (7, 8, 10, 11, 14, 21, 22); the amount of virus shed in infected pigs feces exceeded the quantity of virus ingested (8). Rotavirus was detected in infected pigs feces regardless of whether or not they had diarrhea (15).

Refined experimental procedures used in our rotaviral gastroenteritis research during the last 2 years have provided additional detailed information, particularly on the extent of anorexia, severity of diarrhea and magnitude of fecal virus shedding, as well as on the effect

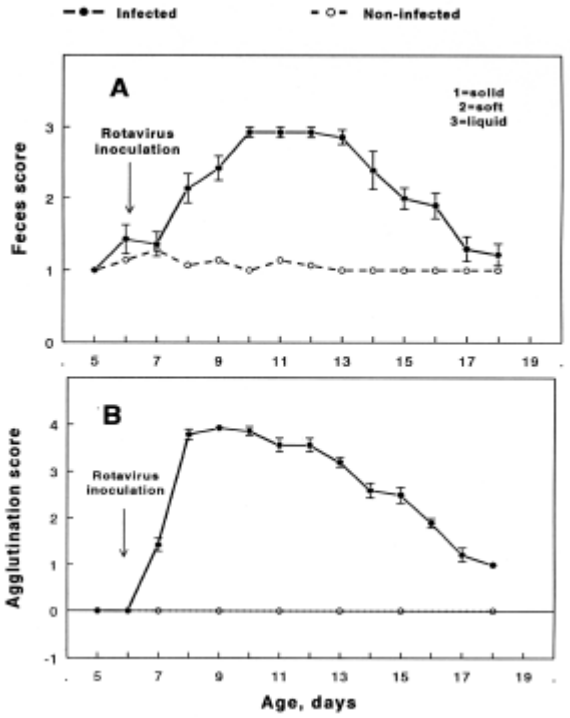

Figure 1. Effect of rotavirus infection on feces consistency (**A**) and fecal rotavirus shedding (**B**). Each value is the mean ± SEM of 10, except for day 19 when n = 9, pigs per group.

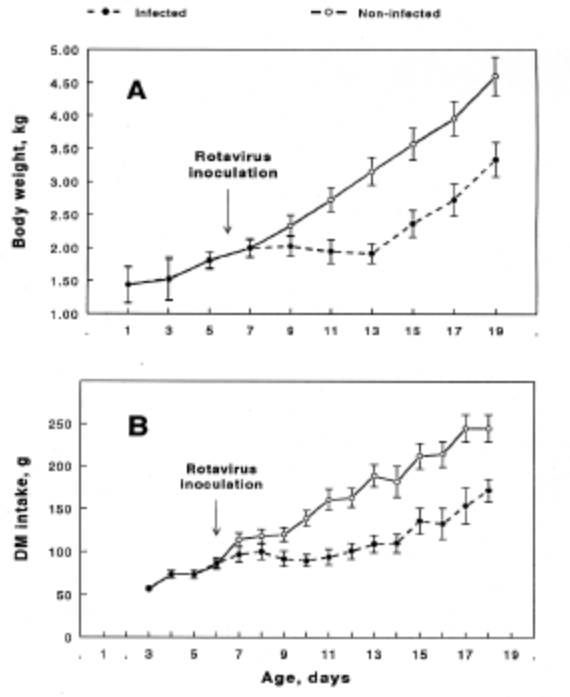

Figure 2. Effect of rotavirus infection on body growth (**A**) and diet intake (**B**).

of rotaviral infection on growth and mortality rate during the post-inoculation period. The detailed experimental protocol including a description of the scales used for feces scores and agglutination scores used to assess fecal rotavirus shedding has been previously reported (22). Under our experimental conditions, the onset of diarrhea occurred within 48 hours after virus ingestion, profuse diarrhea lasted for at least 5 days (from 2 to 7 days post-inoculation) and diarrhea progressively ceased thereafter (Figure 1A). Furthermore, high levels of rotavirus shedding were found in feces of infected pigs from 2 until 7 days after virus ingestion and declined thereafter (Figure 1B). In this particular study, infected pigs were inoculated with 7.8 x 10^4 rotavirus particles per pig at 6 days of age (Gomez *et al*., unpublished results).

The inoculation of rotavirus produced vomiting in ~40% of pigs within 24 to 48 hours after rotavirus ingestion and before the onset of diarrhea. Because of the vomiting and the relatively long period, practically one week after virus ingestion, of anorexia (Figure 2B) along with the diarrhea produced by rotavirus infection, infected pigs lost or gained little body weight during the week following rotavirus inoculation (period from 6 to 13 days of age, Figure 2A). Thereafter, infected pigs resumed growth to the extent that their average daily gain during the period from 13 to 19 days of age (Figure 2A) was similar ($p > .05$) to that of non-infected pigs (228 ± 12 vs. 232 ± 17 g per day, respectively). Values are means ± standard error. However, the average body weight of infected pigs at the end of the trial (19 days of age) was significantly lower ($p < .01$) than that of non-infected pigs (3.34 ± .26 vs. 4.61 ± .29 kg). Except for the rotavirus inoculation, non-infected pigs were otherwise simultaneously but separately reared under similar conditions (Gomez *et al*., unpublished results).

In a few studies (10, 14), 25% to 30% of gnotobiotic rotavirus infected pigs died by 4 to 5 days after virus ingestion. However, in most of the reports, the effects of rotavirus infection on mortality of pigs have been seldom studied mainly because of the short duration of the experimental periods required to ascertain intestinal damage caused by rotavirus infection (7 - 10, 12, 13, 15) rather than the long term effect on pigs' survival. The dose and infectivity of rotavirus inocula, the age and condition of pigs at time of inoculation and the duration of the experimental periods appear to be the main factors responsible for the variable cumulative mortality caused by rotaviral enteritis. In several experiments carried out in our laboratory, colostrum-deprived pigs reared under similar conditions and infected by 5 to 6 days of age with the same source of rotavirus at doses between 10^6 and 10^7 rotavirus particles have shown mortality rates between 40% and 50%

Table 2. Jejunal and ileal villi height (VH in micrometers), crypt depth (CD in micrometers) and lactase activity[1] in non-infected and rotavirus-infected neonatal pigs at different infective stages.

		Mid-jejunum[2]			Mid-ileum[2]		
Infective stage	n	VH	CD	Lactase	VH	CD	Lactase
6 days of age - before rotavirus inoculation							
Non-infected	5	563 ± 79	129 ± 4	94 ± 8	597 ± 50	157 ± 18	86 ± 14
13 days of age or 7 days post-inoculation							
Non-infected	4	637 ± 70^a	136 ± 17^b	77 ± 8^a	1029 ± 128^a	173 ± 18	109 ± 14^a
Infected	4	442 ± 41^b	218 ± 22^a	46 ± 8^b	437 ± 11^b	172 ± 21	37 ± 4^b
19 days of age or 13 days post-inoculation							
Non-infected	7	625 ± 50^a	148 ± 9^b	56 ± 5^a	1169 ± 134^a	148 ± 9^b	52 ± 3^a
Infected	7	498 ± 32^b	210 ± 16^a	39 ± 3^b	533 ± 54^b	190 ± 8^a	34 ± 3^b

[1]:Expressed as micromoles of lactose hydrolyzed per minute per g of protein. [2]:Values are means ± SE. Values in a column, within each infective stage, with unlike superscripts are different ($p < .05$).

by the end of the experimental periods (10 to 12 days post-inoculation)(21; Gomez, unpublished results); lowering the rotavirus dose to 10^5 or 10^4 has reduced the mortality to 10% and 20% (Gomez, unpublished results). Increasing the dose of rotavirus to 8.4 x 10^8 resulted in a cumulative mortality of 70%, with most of the mortality (45% from 15% to 60%) occurring between 6 and 8 days after virus ingestion (22).

8. EVALUATION OF INTESTINAL DAMAGE

Young conventional pigs infected with human rotavirus have shown viral invasion of villous epithelium, lesions on jejunal mucosa and depression in mucosal disaccharidase activity (13). Viral induced histopathological changes have been restricted to the small intestine and were more pronounced within the jejunum and the ileum (12). Severe stunting of villi has been reported in studies with gnotobiotic pigs infected with either porcine rotavirus (12, 17) or neonatal calf rotavirus (9), as well as with conventional pigs infected with human rotavirus (13) and colostrum-deprived pigs infected with porcine rotavirus (20 - 22). As a consequence of these changes, villi surface area was significantly reduced (21).

Villous atrophy in pigs with clinical signs of rotaviral enteritis appeared to be more severe between 24 and 48 hours after the onset of diarrhea (12). In addition, crypt hyperplasia has been found in most studies (12, 13, 17, 22) but crypt depth was not affected in infected pigs studied by Rhoads *et al*. (21).

Table 2 presents data on villi height, crypt depth and lactase activity at the mid-jejunum and mid-ileum of non-infected and rotavirus-infected pigs at different infective stages (Gomez *et al*., unpublished results). Jejunal and ileal villi height in infected pigs at 7 days post-inoculation were reduced ($p < .05$) to ~70% and 42%, respectively, while jejunal crypt depth was 60% higher than that of non-infected control pigs of similar age. By the end of the trial or 13 days after inoculation, jejunal and ileal villi were still shorter ($p < .05$) than villi of non-infected pigs as well as of villi of pigs before rotavirus inoculation. Our results indicate that the shortening of ileal villi was more pronounced than that observed in jejunal villi, in part due to the fact that ileal villi in non-infected pigs grew taller than jejunal villi from 6 to 13 or 19 days of age. Neonatal pigs inoculated at 8 days of age with a higher dose of rotavirus inoculum (8.4 x 10^8 particles per pig) than that used in this study (7.8 x 10^4 particles administered at 6 days of age) and evaluated at 4 rather than 7 days post-inoculation had a more dramatic reduction of both jejunal and ileal villi height (22).

Rotaviral-induced villous atrophy appears to be due to a rapid and extensive infection of the differentiated epithelial cells that subsequently leads to their accelerated desquamation from the villi (12) and the resulting diarrhea in rotavirus-infected animals seems to be caused by the repopulation of the damaged mucosa with immature cells that cannot absorb nutrients (13). The decrease or loss in body weight following rotavirus infection and the mortality of pigs presumably are the result of gut damage as evidenced by the depression in the activity of intestinal enzymes and the subsequent impaired or abnormal villous-digestive processes. Lactase has been extensively studied as the main marker enzyme in rotavirus gastroenteritis because it is localized on the brush border of differentiated epithelial cells and not in the crypts or the lower portions of the villi of the small intestine, it occurs at maximum concentrations in neonatal mammals and gradually

decreases with age (67), and it appears to function as a combined receptor and uncoating enzyme for rotaviruses (68).

Rotavirus infection depressed lactase-specific activity in the duodenal mucosa of children (69) as well as in the jejunal and ileal mucosa of pigs (13, 16, 20 - 22, 66). The decrease in jejunal and ileal lactase-specific activity at 7 and even 13 days post-inoculation found in our recent study (Table 2, Gomez *et al*., unpublished results) confirms these reports. The magnitude in the depression of lactase-specific activity appears to be associated, at least to some extent, with the dose of rotavirus used as evidenced by a larger reduction of lactase activity in pigs infected with inoculum containing a high concentration of rotavirus (22). Our data on jejunal and ileal lactase activity (21, 22; Gomez, unpublished results) clearly indicate that even when pigs have recovered from diarrhea induced by rotaviral infection, their gut is still not fully recovered as evidenced by shorter villi and lower lactase activity than that found in non-infected pigs of similar age (Table 2).

Activities of other intestinal enzymes such as sucrase (66), alkaline phosphatase (16, 20, 66), leucine aminopeptidase (16) and Na^+,K^+-ATPase (20, 66) have also been depressed by rotavirus infection in pigs. Absorption of Na^+ and water in jejunum and ileum (66) as well as active glucose transport in the mid-jejunum (Gomez & Black, unpublished data) have been significantly decreased in rotavirus-infected pigs. On the other hand, activity of thymidine kinase, a crypt cell enzyme involved in mucosal regeneration, has been shown to increase threefold in rotavirus-infected pigs as compared to values of control, non-infected pigs (20).

9. PERSPECTIVE

The results presented herein as well as those reported in the literature clearly indicate that the neonatal pig is an useful model animal to study rotaviral gastroenteritis for young mammals in general, and human infants in particular. Under the conditions described above, colostrum-deprived, artificially-reared, neonatal pigs are susceptible to viral invasion which leads to clinical signs and intestinal injury that are consistent with those reported in rotavirus gastroenteritis in human infants as well as in gnotobiotic pigs. Because of the importance of rotaviral gastroenteritis as one of the main causes of morbidity and mortality in infants and young animals in developed and in developing countries, our well-defined and reproducible neonatal pig research model appears to be a very suitable model animal to further study the pathogenesis as well as the prevention and treatment of rotavirus gastroenteritis.

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