Journal of Clinical Microbiology, July 1982, p. 186–190
0095-1137/82/070186-05$02.00/0

Vol. 16, No. 1

Shedding of Rotavirus in Feces of Sows Before and After Farrowing†

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Received 4 February 1982/Accepted 12 April 1982

The purpose of this study was to determine whether sows shed rotavirus near the time of farrowing. Twelve sows purchased from a common source and seropositive for rotavirus were housed in isolation in farrowing crates from 5 days before to 3 weeks after farrowing. Fecal samples were collected at 3- to 4-day intervals and examined for the presence of rotavirus by direct electron microscopy. Samples were also treated with pancreatin and inoculated onto monkey kidney cells. Rotaviral antigens were detected by a direct immunofluorescence technique, and selected positive cultures were examined by immunoelectron microscopy. Rotavirus was detected in the feces of 5 of 12 sows as early as 5 days before to 2 weeks after farrowing. Diarrhea related to rotavirus developed in 4 of 12 litters. Two of these four litters were farrowed by sows which shed rotavirus at 7 and 10 and 14 days after farrowing. The results of this study indicate that sows immune to rotavirus can shed virus in their feces at a time when piglets are particularly susceptible to infection and that adult swine are of primary importance in the epidemiology of rotavirus as initiators of infection.

Rotavirus (RV) has been studied extensively in mammalian neonates because the virus is a severe and frequent cause of nonbacterial gastroenteritis in this age group (2, 5–7, 10, 13, 14, 22). The role of adult animals in the epidemiology of RV, however, is poorly understood. There is no apparent age resistance to RV, as infections have been reported in adult cattle (22), swine (12), and humans (7, 15, 16, 19, 20). Asymptomatic adult humans shed RV in their feces for a limited period of time, but in most of these cases the adults had contact with clinically ill children (15, 16, 19, 20). This suggested that RV shedding by adult humans was a result of reinfection. Rotaviral infections among adult swine and cattle are predominantly subclinical as indicated by the high number of seropositive animals (1, 3, 21). There is little information, however, as to whether adult animals may be carriers or intermittent shedders of RV.

Several observations by ourselves (Benfield and McAdaragh, unpublished data) and others (8, 9) indicate that clinically "normal" sows may be the source of RV for susceptible pigs. These observations included: (i) colostrum-deprived and conventionally farrowed pigs raised in fumigated isolation rooms were often infected with RV shortly after birth; (ii) pigs weaned from sows and placed in fumigated isolation rooms often developed rotaviral diarrhea within 3 to 4 days after weaning; and (iii) pigs farrowed to sows in fumigated isolation rooms often developed rotaviral diarrhea within 2 weeks after birth. The present study was designed to determine whether sows shed RV in their feces during the time they were confined to farrowing crates. The results indicate that sows immune to RV shed virus in their feces at a time when their piglets are particularly susceptible to infection.

† Published as journal article no. 1816 of the South Dakota State University Agricultural Experiment Station, Brookings, S. Dak.

MATERIALS AND METHODS

Sows. Twelve crossbred, open sows were obtained from a commercial producer and were in direct contact with other sows in the group after breeding. Approximately 5 days before the expected date of farrowing, sows were placed in commercial farrowing crates and housed in individual isolation rooms. Sows remained in the farrowing crates until their pigs were weaned, 3 weeks after farrowing. Blood samples were collected from sows 30 days before and on the day of farrowing. Pigs farrowed by these sows were observed for clinical signs of diarrhea until weaned.

Cell culture. Monkey kidney (MK) cells were grown in minimal essential medium with Earle balanced salt solution (MEME) supplemented with 10% (vol/vol) fetal bovine serum and antibiotics (100 U of penicillin, 100 μg of streptomycin, 100 μg of gentamicin sulfate, and 2.5 μg of amphotericin B per ml). Cells were seeded in 25-cm^2 plastic tissue culture flasks and used for virus isolation 24 to 48 h later.

Fecal samples. Fresh feces (100 g) were collected from sows 1 to 5 days before farrowing, on the day of

TABLE 1. Serum titer to RV, RV shedding in feces of individual sows, and RV diarrhea in litters of pigs

Sow no.	Serum titers		RV shedding in sow feces[a]		RV diarrhea in litters[b]
	Before farrowing	After farrowing	Before farrowing	After farrowing	
2-3	160	160	ND[c]	Pos (7, 10)	Pos (13)
12-3	160	160	Neg	Neg	Pos (8)
20-5	80	160	Neg	Neg	Neg
21-6	ND	80	Neg	Neg	Neg
21-8	160	160	Pos (4)	Neg	Neg
22-1	40	160	Neg	Neg	Neg
22-3	40	40	Pos (1, 5)	Neg	Neg
22-4	160	160	Neg	Pos (14)	Pos (14)
22-5D	40	80	Neg	Neg	Neg
22-5H	320	160	Neg	Pos (7)[d]	Neg
22-12	160	80	Neg	Neg	Neg
33-7	160	160	Neg	Neg	Pos (13)

[a] Neg, Negative; no RV isolated in MK cells. Pos, Positive; RV isolated in MK cells and confirmed by direct immunofluorescence and immunoelectron microscopy. Parentheses indicate time in days before or after farrowing.

[b] Neg, No diarrhea in litter; Pos, diarrhea in litters and RV observed in feces by direct electron microscopy. Parentheses indicate age in days when pigs began scouring.

[c] ND, Not done.

[d] RV particles were observed in this fecal sample by direct electron microscopy.

farrowing, and 3, 7, 10, 14, 17, and 21 days after farrowing. Feces were homogenized in distilled water and centrifuged for 20 min at 10,000 × g. Supernatant fluids were collected and centrifuged at 5,000 × g to further clarify the suspension. The resulting supernatant fluid was then divided into two portions and centrifuged for 30 min at 40,000 × g. The pellet of one portion was then resuspended in 0.5 ml of distilled water for direct electron microscopy (11). The other pellet was resuspended in 2 ml of Hanks balanced salt solution (HBSS) containing 25 μg of pancreatin per ml and antibiotics as described for the MK cells. Samples containing pancreatin were then incubated for 30 min in a 37°C water bath.

Virus isolation. The fecal-pancreatin mixture (1.0 ml) was inoculated onto MK cells, which were previously washed three times with HBSS. Adsorption was for 1 h at 37°C, after which unadsorbed inoculum was removed and the MK monolayer was washed once with HBSS. Control MK cells were treated similarly except that HBSS containing 25 μg of pancreatin per ml was used in place of inoculum. Cells were maintained on MEME without fetal bovine serum. At 24 and 48 h after inoculation, scrapings of the cell monolayer were removed with a sterile inoculating loop. The scraping was transferred to a glass slide, allowed to air dry, and fixed for 10 min in acetone at room temperature. The cells were stained with a fluorescein-conjugated porcine–anti-porcine RV conjugate and a type 3 reovirus conjugate.

Samples negative for rotaviral antigens after 48 h were frozen and thawed, sonicated for 10 s to disrupt the cells, and centrifuged for 30 min at 40,000 × g. The pellet was suspended in 1 ml of HBSS containing pancreatin and antibiotics as described. Uninoculated MK control cells were passaged and treated as described for the inoculated cells. Inoculation of monolayers, direct immunofluorescence, and passage after 48 h were done as described for the initial passage. If no rotaviral antigens could be demonstrated in MK cells after three passages, the original sample was considered negative.

Immunoelectron microscopy. Tissue culture fluids from samples positive for rotaviral antigens by direct immunofluorescence were examined by immunoelectron microscopy as described by Saif et al. (17). The convalescent-phase antiserum was used at a 1:10 dilution and was previously prepared in gnotobiotic pigs (11).

Serology. Antibody titers were determined by a viral neutralization immunofluorescence test. A porcine RV isolated from sow 22-4 in this study was used as the standard virus. The virus was treated with 25 μg of pancreatin per ml for 30 min at 37°C and diluted in MEME without fetal bovine serum to approximately 100 to 300 median tissue culture infectious doses. Sera were inactivated for 30 min at 56°C and diluted in twofold increments from 1:5 to 1:640 in 96-well flat-bottomed microtiter plates. Equal volumes of the virus were added to the serum dilutions, and the serum-virus mixtures were allowed to react for 1 h at 37°C. At this time, 100 μl of a suspension containing 10^4 MK cells in MEME with 2% fetal bovine serum was added to each well.

Plates were incubated for 48 h at 37°C in 5% CO_2. After 48 h, the medium was removed and the plates were submerged in a mixture of 60% acetone (vol/vol) in water. Plates were fixed for 15 min at room temperature, the acetone was decanted, and the plates were allowed to air dry. A fluorescein-labeled porcine–anti-porcine RV conjugate (50 μl) was added to each well and incubated at 37°C for 30 min. Excess conjugate was removed by washing twice with phosphate-buffered saline (pH 7.2). After the last wash approximately 0.1 ml of the phosphate-buffered saline was left on each well to prevent drying. Plates were examined on an inverted microscope equipped with epi-fluorescence and bright-field optics. Titers were expressed as the reciprocal of the highest serum dilution that inhibited 90% of the fluorescing cells. Known positive RV

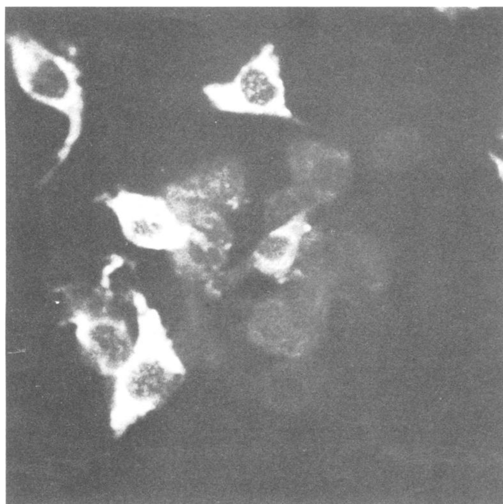

FIG. 1. Direct immunofluorescence of MK cells inoculated with fecal sample from sow 2-3, 24 h postinoculation of MK cells. ×250.

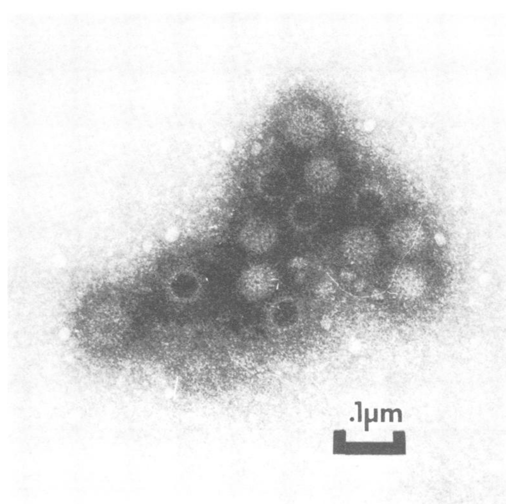

FIG. 3. Virus-antibody aggregates observed on immunoelectron microscopy.

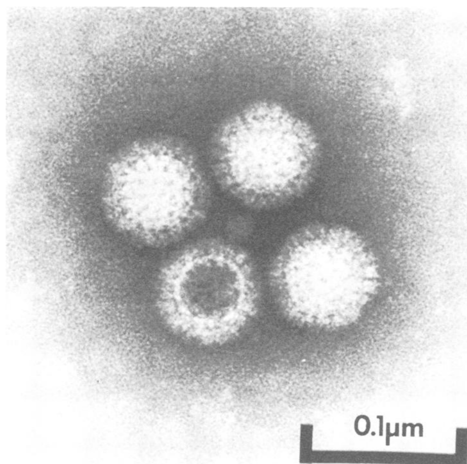

FIG. 2. Negative stain of RV in cell culture fluids harvested from MK cells 24 h after inoculation with fecal sample from sow 22-4.

antiserum and virus-infected and noninfected MK cells served as controls.

RESULTS

Diarrhea was not observed in sows during the course of this study. Except for sow 22-1, which had a fourfold increase in serum titer, antibody titers did not significantly change (Table 1).

RV was isolated from the feces of 5 of 12 (42%) sows (Table 1). There was no difference in the number of sows shedding RV before and after farrowing. Direct electron microscopy detected a few RV particles in feces of sow 22-5 at 7 days after farrowing, and virus was subsequently isolated in MK cells. Fecal samples collected on the day of farrowing (not shown in Table 1) from sows 2-3, 12-3, 21-8, 22-1, and 33-7 were negative for RV. Samples were not obtained from the remaining seven sows because these animals were constipated.

In all cases RV was isolated in MK cells on the first passage and there was no evidence of a cytopathic effect. Rotaviral antigens detected by direct immunofluorescence were limited to the cell cytoplasm, and staining was most intense 24 h after inoculation of the MK cells (Fig. 1). There was no positive reaction with the reovirus type 3 conjugate. Electron microscopy revealed particles with a morphology typical of RV (Fig. 2). On immunoelectron microscopy, RV particles were observed as single particles coated with antibody or, more frequently, as virus-antibody aggregates (Fig. 3).

Piglets from 4 of 12 (33%) litters developed a diarrhea of 3 to 4 days in duration, and RV was identified in the feces by electron microscopy (Table 1). No mortalities resulted, but all piglets in each of these four litters scoured.

DISCUSSION

The role of adult animals in the epidemiology of RV infections has not been thoroughly investigated. Results of this study demonstrate that sows seropositive to RV naturally shed the virus at a time (from 5 days before to 2 weeks after farrowing) when piglets are particularly susceptible to infection. The ability of sows to shed RV in their feces is important from an epidemiologi-

cal standpoint because it demonstrates that adult sows shed RV at a time they are in close proximity with their litters and at a time when pigs are most susceptible to infection. It further demonstrates that "immune" adults may be shedding RV as a result of either a transient reinfection or a carrier state.

RV infection of susceptible humans or animals occurs through contact either with individuals shedding RV in their feces or with a contaminated environment. In this study, the virus shed in the feces of sows could provide for both means of contact. Since RVs are stable in feces for up to 7 to 9 months at 18 to 20°C (21) and are relatively resistant to common disinfectants (18), it is difficult to prevent contamination of animal housing once infection has been introduced. The quantity of RV shed in the feces of the adult sows was small, since amplification in tissue culture was required to detect the virus. Sow 22-H apparently excreted large quantities of RV 7 days after farrowing, since it has been estimated that 10^7 to 10^9 particles per ml of sample are necessary for detection by direct electron microscopy.

Although the amount of RV shed in the feces of sows was small, the close contact that litters had with the contaminated feces was sufficient to infect the litters of sows 2-3 and 22-4. The failure to observe diarrhea in the piglets from litters of sows 21-8, 22-3, and 22-5H indicates a general lack of correlation between litter infection and sow shedding of RV. In our experimental design, however, we examined only those litters that developed diarrhea. Since the sows were seropositive to RV, the presence of antibody in the sows' milk may have been sufficient to prevent development of diarrhea in these litters. Lecce et al. (9) have postulated that RV is prevalent in pigs, that a small amount of virus or a virus of low infectivity is transmitted postpartum from dam to young, and that colostral antibody causes an asymptomatic infection of little consequence. Bohl (4) has suggested that there may be a built-in defensive mechanism whereby the mother sheds RV at a time (most likely near or after parturition) when the neonate has a great deal of passive immunity. This would allow the young to develop active immunity as a result of a limited viral infection. The ability of seropositive sows to shed RV at various times before or after farrowing as demonstrated in this study could provide for such a mechanism.

Fecal shedding by sows seropositive to RV could result from reinfection with RV through contact with other swine shedding RV or with a contaminated environment, or from the fact that sows were persistently infected with RV and intermittently shed virus in their feces. There is apparently no age resistance to bovine RV, and repeated infection and excretion in immune animals probably occurs (22). Reinfection of the seropositive sows in this study seems unlikely since all sows were purchased from the same farm and had similar clinical histories including prior exposure to RV. Except for sow 22-1, no increase in antibody titers to RV was detected from the time sows were purchased to when they were placed in isolation rooms for farrowing. Orstavik et al. (15) have described an RV-associated gastroenteritis in two adult humans related to RV reinfection. In both cases, the subjects showed a 16-fold increase in serum immunoglobulin G titer after infection and had prior exposure to RV-infected children. On the basis of the fourfold increase in serum antibody titer, sow 22-1 may have been reinfected during the time it had contact with the other 11 sows. Environmental contamination also seemed unlikely since the isolation rooms used in this experiment were thoroughly cleaned and disinfected before the sows were housed. However, reinfection of the sows cannot be excluded since at least two serotypes of RV exist in pigs (4), an observation that may account for reinfections. It is also known that secretory immunoglobulin A antibody in the intestinal tract is more important in preventing intestinal infections than are serum antibody levels as measured in this study. Since serum antibody levels are not indicative of the amount of protection, sows could be reinfected with the same or a different serotype of RV regardless of their serum antibody levels.

Although there is no evidence to support a carrier state for RV in sows, the possibility that sows in this experiment were carriers of RV and intermittently shed the virus at varying intervals should be considered as an alternative to reinfection. Lecce and co-workers (8, 9) have proposed the existence of carrier sows but were unable to detect RV in occasional samples of feces from sows. However, their method of detection, immunoelectron microscopy, is not as sensitive as the technique employed in our study. In the present study, fecal samples were concentrated, treated with pancreatin, and amplified on a susceptible cell culture to maximize RV detection.

Recurrent RV infections in swine herds are not uncommon, and it has been difficult to explain the source of infection. The ability of RV-"immune" sows to shed this virus in their feces, whether as a result of a transient reinfection or exacerbation of a carrier state, provides an important reservoir of RV to contaminate conventional farrowing facilities. It also provides a direct source of RV to piglets at a time they are most susceptible to RV infections. An important analogy exists between the shedding of RV by sows at parturition in this study and a

report in humans that 1 of 20 enema samples collected from mothers during labor contained RV (R. Bishop, personal communication). Thus, asymptomatic adult animals which shed virus at parturition may be most important in the epidemiology of RV as initiators of infections. Whether shedding of RV is most likely to occur as a result of hormonal influences of pregnancy or lactation remains an area for further study.

ACKNOWLEDGMENTS

We thank Rita Miller for typing the manuscript.

This work was supported in part by research agreement PL-95-113-Section 1433 of the United States Department of Agriculture.

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