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Epidemiological Studies of Piglet Diarrhoea in Intensively Managed Danish Sow Herds

IV. Pathogenicity of porcine rotavirus

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Svensmark, B., J. Askaa, C. Wolstrup and K. Nielsen: Epidemiological studies of piglet diarrhoea in intensively managed Danish sow herds. IV. Pathogenicity of porcine rotavirus. Acta vet. scand. 1989, 30, 71–76. – Colostrum-deprived piglets inoculated with rotavirus 24 h after birth developed a profuse diarrhoea that spread to non-inoculated, colostrum-deprived litter mates and, occassionally, to colostrum-fed piglets. Case fatality rates in these 3 categories of piglets were 63.2 %, 35.7 % and 8.3 %, respectively. Surviving piglets recovered in 1–2 weeks, but shedded virus via the faeces for up to 3 weeks p. i. The D-xylose test revealed severe malabsorption, with extremely flat absorption curves for up to 3–4 weeks p. i. Malabsorption was more marked in piglets with a long-lasting faecal virus excretion than in piglets where virus disappeared from the faeces within 10 days p. i. Infected piglets (colostrum-fed and colostrum-deprived) had decreased weight gains and were 5 days older at a bodyweight of 25 kg than non-inoculated controls. It is concluded that rotavirus is probably of significance in diarrhoeal syndromes in suckling piglets, alone or in combination with *E coli* or other pathogens.

colostrum; challenge; infectivity.

Introduction

Experimental inoculation of rotavirus isolates into colostrum-deprived piglets is regularly followed by clinical symptoms of diarrhoea and, occassionally, vomiting (*Bohl et al.* 1978, *McAdaragh et al.* 1980, *Askaa & Bloch* 1984). On the other hand, experimental inoculations of conventionally reared piglets with rotavirus have often failed to cause clinical signs. Similar to rotavirus infection in other species, villous atrophy is a prominent finding at post mortem, and this is probably of significance for the clinical syndrome due to a marked decrease in absorptive area of the small intestine (*Torres-Medina & Underdahl* 1980, *McAdaragh et al.* 1980).

The epidemiology of rotavirus infection in Danish swine herds has been described in a previous paper (*Svensmark et al.* 1989). The results suggested that rotavirus could play a role as an etiological agent in so-called steatorrhoea, i. e. a syndrome of mild diarrhoea occurring at 3–5 weeks of age.

The present paper describes clinical and pathophysiological findings in colostrum-deprived and colostrum-fed piglets inoculated with a rotavirus strain isolated from Danish piglets.

Material and methods

Animals

Fifty-seven piglets from 5 litters were used in the experiments. Thirty three piglets were removed from the dam immediately after delivery and reared without colostrum on

cow's milk and ad libitum access to a balanced electrolyte/glucose solution. The piglets were kept on sterilized wood shavings in a room with accurate climate control. Of the 33 piglets, 19 were inoculated orally with rotavirus 24 h after birth and kept isolated from the remaining 14 colostrum-deprived piglets until all 33 piglets were returned to the dams 48 h after birth to suckle with their 24 colostrum-fed littermates that had remained with the sows.

Virus inoculation

The rotavirus strain was isolated from a field outbreak of steatorrhoea. A 20 % faecal suspension in Eagle's essential medium was made and centrifuged at 3000 × G for 30 min at 4° C. The supernatant was filtered through a 0.45 micrometer filter and inoculated orally at a dose of 1 ml per piglet. The suspension contained between 10^5 and $10^{5\,5}$ infective doses (TCID 50) per ml and 10^5 plaque forming units per ml.

Following inoculation, faecal samples from all inoculated and non-inoculated in contact controls were taken daily and screened for rotavirus by the ELISA test as described by *Askaa & Bloch* (1981). The litters were observed clinically and when diarrhoea occurred, faecal samples were obtained for bacteriology.

Malabsorption test

The D-xylose absorption test was used as described by *Dietz* (1981). Piglets were studied during the acute diarrhoeal phase (age 5–6 days, 19 piglets), during remission (10–11 days of age, 8 piglets) and after clinical recovery (3–4 weeks of age, 17 piglets). Thus, each piglet was studied on several occassions to examine the sequential changes occurring in the individual animal. Moreover, piglets of comparable age were included as controls. These piglets were derived from non-inoculated litters kept separate from the experimental litters. Before the D-xylose test, it was confirmed that the piglets did not shed rotavirus in their faeces. Prior to the D-xylose test the younger piglets were starved for 4–5 h, the older piglets for 12 h. D-xylose was given orally in a 50 % solution at a dose rate of 0.5 g per kg body weight. Blood samples were taken before drenching and at 30, 60, 90, 120 and 180 min and analyzed for D-xylose at 520 nm according to the procedure described by *Roe & Rice* (1948) with minor modifications.

Results

Clinical findings

All virus-inoculated, colostrum-deprived piglets developed diarrhoea 1–2 days post inoculation (p. i.). The condition was serious, stools were watery and the piglets became weak and dehydrated. Surviving piglets recovered after 1–2 weeks. Also, non-inoculated, colostrum-deprived in-contact piglets developed diarrhoea 1–3 days after they were returned to the sows together with their virus-inoculated litter mates. Clinical symptoms were similar to those of the inoculated piglets. In 3 litters, diarrhoea was observed in colostrum-fed piglets. Symptoms varied from mild to severe, profuse diarrhoea in these piglets.

Case fatality rates

Twelve of the 19 inoculated piglets, i. e. 63.2 %, died after a course of 1–7 days p. i. Five of the 14 colostrum-deprived, non-inoculated in-contact piglets died (35.7 %). Case fatality rate in the colostrum-fed, non-inoculated piglets in contact was 8.3 % (2 deaths among 24 piglets).

Post mortem

The stomach was empty and in some piglets

filled with gas. The small intestine was pale, dilated and contained watery ingesta. The mucosa was pale, appeared thin. Haemorrhages or necroses of the mucosa were not seen. All other organs were normal. Histopathology was not performed.

Virus excretion

The sows and the piglets remaining with them were not excreting rotavirus prior to the returning of the inoculated piglets. In 4 of the 5 litters, all piglets – inoculated and non-inoculated – shed virus, starting 1–2 days after inoculation or contact, resp. Virus shedding persisted for 4–21 days. In the fifth litter, only inoculated piglets were excretors. Three of the 5 sows excreted virus during the first week after the inoculated piglets were returned to suckle. No symptoms were observed in the sows. Bacteriological culture from faeces of diarrhoeic piglets or from intestine and internal organs at post mortem revealed non-hemolytic *E coli* in a few piglets. They could not be assigned to serotypes known to be pathogenic for swine. Other bacteria were not found.

Malabsorption tests

Absorption curves in normal control piglets varied with age. In 5-days-old piglets, blood xylose rose to ab. 1.5–2 mmol/l (average 1.8 mmol/l) 30 min after D-xylose application (Fig. 1). The curve remained at this level through the rest of the test period (180 min). In 3–5-week-old piglets, the curve was steeper and reached peak levels of 3.5–4 mmol/l (av. 3.7 mmol/l) in 30–60 min, followed by a decline over the next 2 h (Fig. 2). In 5–6 day-old piglets with acute diarrhoea, the absorption curves were extremely flat, with peak levels of ab. 0.75 mmol/l. When the piglets were re-tested during remission, i. e. at an age of 10–11 days, absorption cur-

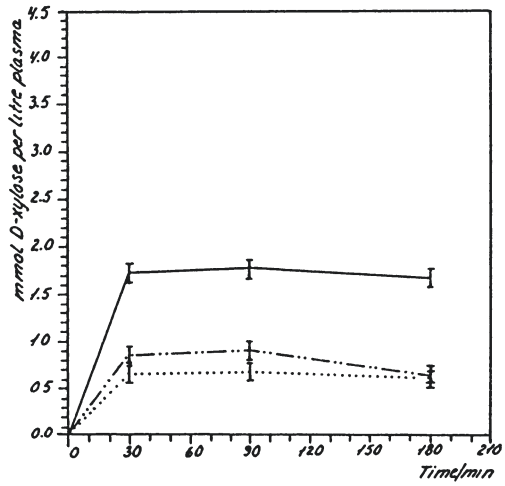

Figure 1. D-xylose absorption. Mean and standard deviation.
—— Control piglets, age 5 days (n = 5)
····· Piglets with acute diarrhoea, age 5–6 days (n = 14)
·-·-· Piglets during remission, age 10–11 days (n = 8)

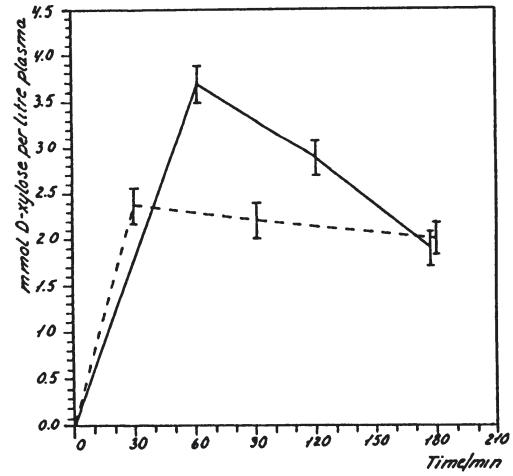

Figure 2. D-xylose absorption curves, mean and standard deviation.
—— Normal control pigs, age 3–5 weeks (n = 4)
– – – – Piglets in convalescence from rotavirus diarrhoea, age 3–4 weeks (n = 13)

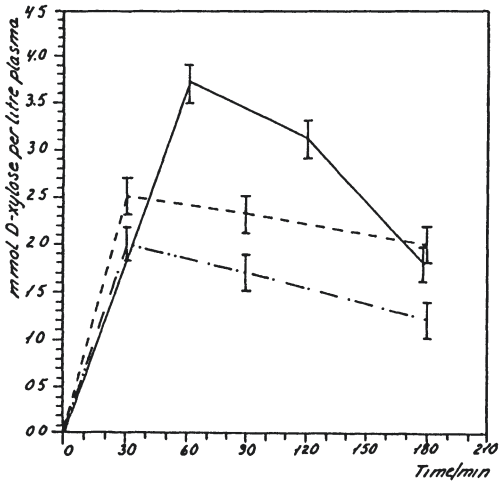

Figure 3. D-xylose absorption curves related to the duration of virus excretion. Absorption tests were done 3–4 weeks after rotavirus inoculation. Mean standard diviation.

—— Normal controls, age 3–5 weeks (n = 4)
- - - - Piglets in convalescence, virus excretion for 4–10 days (n = 9)
·-·-· Piglets in convalescence, virus excretion for more than 10 days (n = 4)

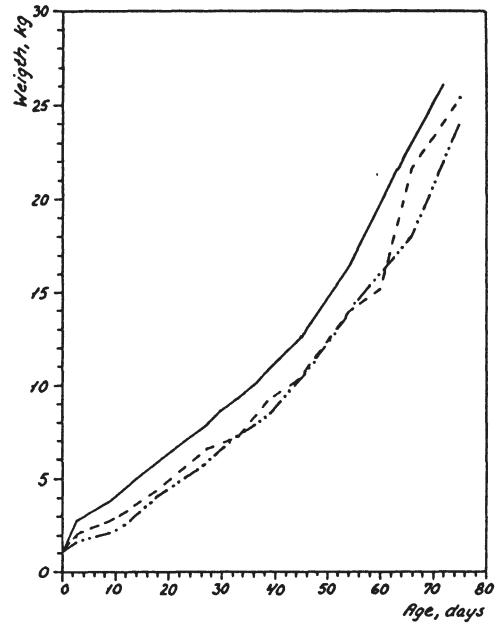

Figure 4. Weight curves.

—— Control piglets (n = 11)
- - - - Inoculated, colostrum-fed piglets (n = 2)
·-·-· Inoculated, colostrum-deprived piglets (n = 7)

ves were still very flat, with peak levels below 1 mmol D-xylose/l (Fig. 1).

In 13 piglets re-tested after clinical recovery – at 3–4 weeks of age – absorption curves were still abnormal when compared to normal control piglets of similar age. Curves were flat and peak levels were below 2.5 mmol/l (av. 2.4 mmol/l), see Fig. 2. Four of the 13 piglets has excreted rotavirus for more than 10 days after inoculation, whereas the other 9 piglets had excreted virus for 4–10 days. As shown in Fig. 3, malabsorption was more marked in piglets with a long-lasting virus infection in the gut than in piglets with a virus excretion of shorter duration.

Weight gains

Rotavirus infection was associated with a decreased weight gain in both colostrum-deprived and colostrum-fed piglets (Fig. 4). At 25 kg, the inoculated piglets were 5 days older than non-inoculated, non virus-excreting controls.

Discussion

Colostrum-deprived piglets inoculated with rotavirus 24 h after birth developed profuse diarrhoea with a high case fatality rate (63 %). When these piglets were mixed with their colostrum-fed litter mates, the virus spread to the latter and they developed diarrhoea with a case fatality rate of 8 %. Likewise, colostrum-deprived in-contact piglets became infected and showed symptoms of diarrhoea, with a case fatality rate of 36 %. In comparison, colostrum-deprived

piglets reared for purposes other than virus inoculation suffer a mortality rate of 15–20 %.

Three out of 5 sows excreted rotavirus via the faeces following contact with their inoculated offspring but did not show signs of diarrhoea.

Thus, rotavirus appears capable of inducing severe diarrhoea in neonatal piglets deprived of colostrum for 24 h after birth and then allowed to suckle. This finding is in keeping with results obtained by others. E. g., *Askaa & Bloch* (1984) produced severe diarrhoea in neonatal, colostrum-deprived piglets by intranasal or oral inoculation. Similar findings were reported recently by *Janke* (1985) with 3 different virus isolates. Early studies by *Theil et al.* (1978) and *McAdaragh et al.* (1980) revealed that the severity of the infection is age-dependent. Thus, piglets inoculated at 5–14 days of age developed diarrhoea but suffered a low mortality rate. Our study has, furthermore, revealed that sows are easily infected by contact with an infected litter. *Benfield et al.* (1982) reported that sows are often subclinically infected with rotavirus and it seems reasonable to assume that sows are an important source of infection to their offspring in field outbreaks. Establishment of a rotavirus infection by increasing infection pressure might, in future work, be an important model in the evaluation of potential rotavirus vaccine candidates.

The piglets in the present experiment excreted rotavirus for up to 21 days p. i. which is similar to findings by *de Leeuw et al.* (1979) and *Debouk & Pensaert* (1982). The diarrhoeal phase was considerably shorter than the duration of virus shedding, but the malabsorption associated with the infection and caused by villous atrophy lasted for at least 3 weeks as revealed by abnormal (flat) D-xylose absorption curves in piglets recovering from the experimental infection. This was also reflected by decreased weight gains. D-xylose absorption studies in calves and children with rotavirus infection have yielded similar results, although absoption tests were normal approx. 10 days p. i. (*Woode et al* 1978, *Mavromichalis et al.* 1977). The present study has further revealed that there was an association between the longevity of virus infection and malabsorption, since piglets with the most long-lasting virus excretion were the last to normalize their D-xylose absorption.

Obviously, the effect of rotavirus infection is more serious and long-lasting than the clinical phase would indicate, and the results obtained here strongly indicate that rotavirus is a gastrointestinal pathogen in suckling piglets. A previous study (*Svensmark et al* 1989) revealed a herd prevalence rate of approx. 75 % and a litter prevalence rate of approx. 30 % in Danish sow herds. This wide occurrence and the capability to induce diarrhoea as demonstrated here and in other reports indicate that rotavirus alone or in combination with other pathogens (*E. coli* a. o.) is of significance in diarrhoeal syndromes occurring so often in suckling litters.

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Sammendrag

Epidemiologiske undersøgelser af diarre hos grise i intensivt drevne sobesætninger.

IV Pathogenicitet af porcin rotavirus

Grise, der ikke fik colostrum, udviklede profus diarre efter podning med rotavirus 24 timer efter fødslen. Diarreen spredtes til ikke-podede, colostrum-depriverede kuldfæller og i enkelte kuld desuden til colostrumforsynede grise. Lethaliteten i de 3 grupper af grise var hhv. 63,2 %, 35,7 % og 8,3 %. Overlevende grise restitueredes efter 1–2 uger, men udskilte virus med fæces i op til 3 uger efter podningen. D-xylosetest afslørede svær malabsorption med meget flade absorptionskurver i op til 3–4 uger p. i. Malabsorptionen var mere udpræget hos grise med langvarig fækal virusudskillelse end hos grise, der hurtigt rensede sig for virus. Tilvæksten hos inficerede grise – såvel colostrum-depriverede som colostrumforsynede – var nedsat, og disse grise var 5 dage længere om at nå en legemsvægt på 25 kg end ikke-inokulerede kontrolgrise. Det konkluderedes, at rotavirus sandsynligvis spiller en rolle som diarreårsag hos diende grise, alene eller i kombination med *E coli* og andre agentia.

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