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PORCINE ROTAVIRUS FROM TWO MALAYSIAN PIGGERIES. I. EPIDEMIOLOGY OF TYPICAL AND ATYPICAL ROTAVIRUSES

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SUMMARY

Porcine rotavirus infection patterns from 2 distantly situated piggeries were studied. Faecal samples collected over a period of 3 months were analyzed by RNA electrophoresis and silver staining. Rotaviruses were detected from 157 piglets (33%) from a total of 481 diarrhoeic animals aged 1 to 5 weeks. Six of 74 non-diarrhoeic animals (8%) were rotavirus-positive. Based on electropherotic patterns, 78% (23% of total animals tested) and 12% (3.4) of the viruses were identified as group A and C rotaviruses respectively while 10% (2.8) were probable group B or E rotaviruses. In the two piggeries, group A rotavirus infections were rather similar in prevalence and age distribution but non-group A rotavirus infections were dissimilar in these 2 aspects and also in the proportion of the rotavirus groups detected. Piglets from 9 out of the 11 serial sow parity groups examined conformed to a pattern in which the prevalence of rotavirus-associated diarrhoea in piglets showed a decreasing trend with increasing sow parity number until the sixth parity, thereupon high prevalence was maintained with increasing parities.

Keywords: Porcine rotavirus, prevalence

INTRODUCTION

Porcine rotavirus, detected since 1975 (Rodger *et al.*, 1975), is considered to be a cause or contributory agent of diarrhoea and other gastrointestinal disorders in pigs (Rodger *et al.*, 1975; Tzipori and Williams, 1978; Pospischil & Guscetti, 1989).

There are considerable differences in reports on rotavirus infection of piglets. Prevalence of rotavirus infections in diarrhoeic piglets from various parts of the world ranged from 22 to 52% (Askaa and Bloch 1981; Svensmark, 1983; Utrera et al., 1984; de San Juan *et al.*, 1986; Liprandi *et al.*, 1987; Mattion *et al.*, 1989; Lim *et al.*, 1990; Joergensen *et al.*, 1990; Atii *et al.*, 1990). Although most studies reported higher prevalence of rotavirus in older piglets (Svensmark, 1983; Liprandi *et al.*, 1987; Utrera, 1984), high prevalence was also observed in younger animals (Atii *et al.*, 1990) including piglets a few days old (Debouck *et al.*, 1984). Differences were also seen in reports of subclinical rotavirus infection: rotavirus was detected in normal piglets in some studies (Debouck and Pensaert, 1983; Joergensen *et al.*, 1990; Lim *et al.*, 1990) but not in others (Utrera *et al.*, 1984; Atii *et al.*, 1990). Reports on the prevalence of non-group A rotaviruses in piglets ranged from none detected (Liprandi *et al.*, 1987) to 6.6% of the animals tested (Fu *et al.*, 1989).

In view of the differences in rotavirus infection reported, the epidemiology of typical and atypical rotaviruses in Malaysian piglets was investigated to determine its pattern of infection.

MATERIALS AND METHODS

Faecal samples

Single faecal sample was collected from each of 559 newborn to 5-week-old piglets between April and June 1994. Of these, 267 (239 diarrhoeic and 28 normal faeces) were from a piggery in Melaka ('Melaka piggery') and 293 (247 diarrhoeic and 46 normal faeces) from a piggery situated 150 km away in Sepang ('Sepang piggery'). Piglets from both farms were weaned 4 weeks after birth

Detection of rotavirus

The procedures for viral RNA extraction from faecal samples, RNA polyacrylamide gel electrophoresis (PAGE) and silver staining were described previously (Yap *et al.*, 1992). Briefly, the samples were electrophoresed in slab gels 16 cm wide and 1 mm thick consisting of a 7% separating gel 10 cm long and a 3% stacking gel 3 cm long using Laemmli's discontinuous PAGE system (Laemmli,

1970) without sodium dodecyl sulfate in all buffers. Processed faecal extracts were run at a constant current, 20 mA per slab gel for the 1st h and 10 mA for the next 18 h, at 8°C. RNA bands then were stained with 0.011M silver nitrate. A sample volume of 20 µl was used in screening and negative samples were retested using 80 µl. Four samples from the Sepang Piggery were excluded from the data analyzed as the backgrounds were too dark for any bands to be observed.

Identification of rotavirus groups

Group A rotaviruses were identified by the close migration of the 7-8-9 triplet gene segments and group C rotaviruses by the genome segments distributed in the typical 4-3-2-2 pattern. For viruses with a genome distribution pattern of 4-2-2-2-1 (sometimes described as 4-2-2-3), we were unable to make a definitive group identification as the pattern was reported for group B (Pedley *et al.*, 1983, 1986; Snodgrass *et al.*, 1984; Nagesha *et al.*, 1988) as well as group E rotaviruses (Pedley *et al.*, 1983, 1986). Therefore, these viruses were labeled as presumptive groups B or E rotaviruses.

RESULTS

Rotavirus distribution in diarrhoeic piglets according to piggery and age

The prevalence figures of group A and non-group A rotaviruses in diarrhoeic piglets from the 2 piggeries combined were 26 and 7% respectively (Table 1). Of the rotaviruses detected, group A rotaviruses were numerically dominant (80%) while groups C and B- or E-like rotaviruses were about equally distributed at 11 and 9% respectively.

Analysis of individual piggery shows prevalence of group A rotaviruses was essentially the same. However, the prevalence of non-group A rotaviruses in the Melaka piggery (9%; 27% of the total virus identified) was nearly twice of the Sepang piggery (5%; 14%). Distribution based on rotavirus group revealed

Table 1. Rotavirus distribution in diarrhoeic piglets according to piggery and age

Animals		Rotavirus groups identified			
Age	No. tested	A (%)	C	B/E	Total (%)
Location: Sepang & Melaka piggeries combined					
Week 1	97	9 [9]	5	2	16 [17]
Week 2	110	13 [12]	0	4	17 [15]
Week 3	104	32 [31]	3	6	41 [39]
Week 4	101	57 [56]	2	0	59 [58]
Week 5	69	14 [20]	8	2	24 [35]
Total	481	125 [26]	18	14	157 [33]
Location: Sepang piggery					
Week 1	52	6 [12]	5	2	13 [25]
Week 2	52	5 [10]	0	1	6 [12]
Week 3	53	15 [28]	1	0	16 [30]
Week 4	54	36 [65]	1	0	37 [69]
Week 5	31	7 [23]	1	0	8 [26]
Total	242	69 [29]	8	3	80 [33]
Location: Melaka piggery					
Week 1	45	3 [7]	0	0	3 [7]
Week 2	58	8 [14]	0	3	11 [19]
Week 3	51	17 [33]	2	6	25 [49]
Week 4	47	21 [45]	1	0	22 [47]
Week 5	38	7 [18]	7	2	16 [43]
Total	239	56 [23]	10	11	77 [32]

that group C rotaviruses were about equally distributed in the 2 piggeries but groups B- or E-like rotaviruses were 3.5 times more prevalent in the Melaka piggery.

Age distribution of group A rotavirus infection was essentially similar in both piggeries. Infection occurred in all age groups studied. Prevalence rose to a maximum in 4-week-old piglets before declining sharply in older animals. Nevertheless, prevalence in 1-week-old piglets and the maximum attained were higher in the Sepang piggery.

Age distribution pattern of non group A rotavirus infection differed in the Melaka and Sepang piggeries. In the Melaka piggery, group C rotaviruses infected mostly 5-week-old piglets (73%) and none of 1 to 2 weeks old animals. In contrast, most infections occurred in 1-week-old animals (63%) in the Sepang piggery. Even though infection of group B or E rotaviruses occurred mostly in younger animals in both piggeries, the trend towards infection of older animals in the Melaka piggery was observed. In this piggery, the majority of those infected (82%) were 2 to 3 weeks old age piglets with peak infection (58%) occurring in 3-week-old piglets. In contrast, all the viruses were detected in 1 to 2 weeks old animals in the Sepang piggery.

Rotaviruses distribution in non-diarrhoeic piglets

Table 2 shows that a small percentage of non-diarrhoeic piglets from the 2 piggeries was rotavirus-positive. Infections were detected in all age groups, except 2-year-old animals. There was no preponderance of infection in any age group. All the 3 group A rotaviruses detected, together with a single group C rotavirus, were from the Melaka piggery. One rotavirus suggestive of groups B or E was detected from each of the 2 piggeries.

Table 2. Rotavirus distribution in non-diarrhoeic piglets

Animals		Rotavirus groups identified		
Age	No. tested	A	Non A	Total
Week 1	13	0	1[a]	1
Week 2	16	0	0	0
Week 3	20	1[b]	1[c]	2
Week 4	20	1[b]	0	1
Week 5	5	1[b]	1[d]	2
Total	74	3	3	6

[a]Group B or E rotavirus from Sepang piggery
[b]Group A rotavirus from Melaka piggery
[c]Group B or E rotavirus from Melaka piggery
[d]Group C rotavirus from Melaka piggery

Rotavirus distribution in diarrhoeic piglets according to sow parity

The trend of decreasing prevalence of rotavirus-associated diarrhoea in piglets from first to sixth parity sows was broken by a sharp increase in piglets from fourth parity sows (Figure 1). Prevalence then rose again sharply in piglets from seventh parity sows and remained around this level with increasing sow parity, except for a deep decline in prevalence involving piglets from ninth parity sows.

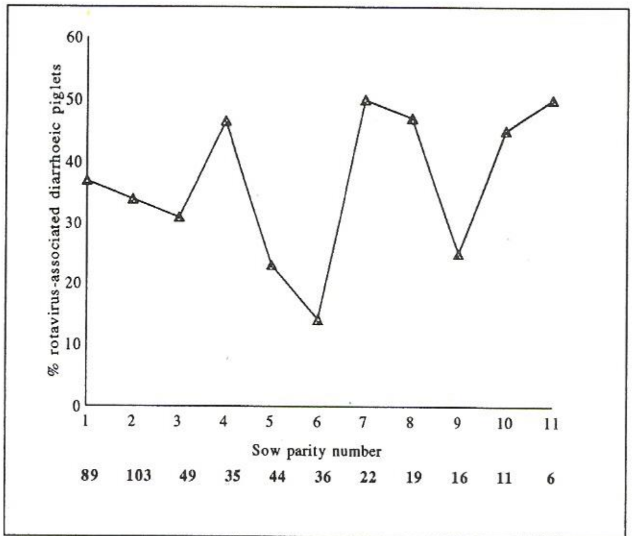

Figure 1. Rotavirus distribution in diarrhoeic piglets according to sow parity. Figure below each sow parity number represented the number of animals tested

The prevalence of rotavirus-associated diarrhoea in piglets from sixth parity sows was significantly different from piglets from sows of first parity (χ^2) [corrected] = 7.20; P=0.0194) and fourth parity (χ^2) [corrected] = 7.17; P=0.0074). It was also significantly different from sows of seventh (Fisher exact test; P=0.0038), eight (P=0.0094), and ten (P=0.0394) parities.

DISCUSSION

Group A rotavirus infection in diarrhoiec piglets is generally more prevalent in tropical than temperate countries. In the former, prevalence figures of 21 to 55% (average 50%) were reported (Utrera *et al.*, 1984; de San Juan *et al.*, 1986; Liprandi *et al.*, 1987; Lim *et al.*, 1990; Joergensen *et al.*, 1990; Atii *et al.*, 1990) compared to 18 to 35% (average 27%) for temperate countries (Svensmark, 1983; Nagesha *et al.*, 1988; Fu *et al.*, 1989; Mattion *et al.*, 1989). The 26% prevalence in Malaysian piglets was at the lower range for tropical countries.

In previous studies non-group A (atypical) rotavirus infection was not detected in piglets (Liprandi *et al.*, 1987) or in only a small proportion of animals (1 to 7%) (de San Juan *et al.*, 1986; Nagesha *et al.*, 1988; Mattion *et al.*, 1989; Fu *et al.*, 1989). In these studies atypical rotaviruses accounted for between 6 to 26% of the total rotavirus detected. The prevalence of non-group A rotaviruses in Malaysian piglets (7% of animals tested; 20% of the total rotaviruses identified) was at the high end of the range previously noted in other countries. Thus atypical rotaviruses accounted for a sizable proportion of rotavirus-associated diarrhoea in Malaysian piglets.

Asymptomatic rotavirus infection occurred in Malaysian piglets but prevalence was relatively low (8%) compared with figures of 13 (Lim *et al.*, 1990) and 21% (Joergensen *et al.*, 1990) in other tropical countries.

The age distribution of group A rotavirus infection in Malaysian piglets was similar to previous studies. Infection was more prevalent in older piglets as previously reported (Bohl *et al.*, 1978; Svensmark, 1983; Utrera, 1984; Liprandi *et al.*, 1987) and declined sharply in 5-week-old animals in agreement with previous studies (Debouck & Pensaert, 1983; Svensmark, 1983). Analysis of rotavirus infection in 2 different piggeries revealed that both have essentially similar pattern of group A rotavirus infection in terms of prevalence and age distribution. In contrast, each piggery has its own pattern of atypical rotavirus infection (prevalence, distribution according to rotavirus groups and age). The reason for the dichotomy in the pattern of rotavirus infection based on rotavirus groups is not clear.

Subsequent sow parities were reported to increase colostrum and serum anti-rotavirus IgA titers in sows (Gelberg *et al.*, 1991). In addition, it is probable that antibodies against a wider range of rotavirus groups and strains are produced by older sows as they are more likely to be exposed to them. In this situation the prevalence of rotavirus infection in piglets should decrease with subsequent sow parities as maternal antibodies of this nature would enhanced passive protection to suckling piglets. Support for this came from a previous study which showed rotavirus infection was more prevalent in piglets from gilts than sows (Svensmarks, 1983) and also from our results which revealed a general trend in which prevalence of rotavirus-associated diarrhoea in piglets decreased with increasing sow parities. In addition, our results also suggested that sows above a particular parity were more likely to produce litters more predisposed to rotavirus infection. However, such interpretations need confirmation since among the sow parity groups examined there were 2 with prevalence of rotavirus-associated diarrhoea at variant with the patterns described. In addition, sample sizes were small in some of the groups.

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RINGKASAN

ROTAVIRUS PORSIN DARIPADA DUA LADANG TERNAKAN KHINZIR DI MALAYSIA.
I. EPIDEMIOLOGI ROTAVIRUS TIPIKAL DAN ATIPIKAL

Pola jangkitan rotavirus daripada dua ladang ternakan khinzir yang letaknya berjauhan dikaji. Sampel tinja yang dikumpul dalam tempoh 3 bulan dianalisiskan melalui elektroforesis RNA dan perwarnaan argentum. Rotavirus telah dikesan dalam 157 ekor (33%) daripada 481 ekor anak khinzir bercirit birit berumur 1 hingga 5 minggu. Enam daripada 74 ekor haiwan tiada cirit birit (8%) menunjukkan rotavirus-positif. Berasaskan pola elektroferosis, 78% (23% daripada jumlah haiwan diuji) dan 12% (3.4) daripada virus telah masing-masing dikenalpasti sebagai rotavirus kumpulan A dan C sambil 10% (2.8) mungkin daripada rotavirus kumpulan B atau E. Dalam dua ladang ini, jangkitan rotavirus kumpulan A adalah serupa prevalens dan taburan umurnya tetapi kumpulan jangkitan rotavirus bukan kumpulan A, tidak serupa dalam dua aspek tersebut dan juga dalam kadar kumpulan rotavirus yang dikesan. Anak-anak khinzir daripada sembilan kumpulan pariti ibu khinzir bersiri daripada 11 kumpulan dikaji mematuhi pola di mana prevalens cirit birit terkait rotavirus dalam anak khinzir itu menunjukkan trend menurun dengan tertingkatnya nombor pariti ibu sehingga pariti keenam, justeru itu prevalens tinggi dikekal dengan peningkatan pariti.