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PORCINE ROTAVIRUSES FROM TWO MALAYSIAN PIGGERIES II. ROTAVIRUS ELECTROPHEROTYPES

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SUMMARY

Electropherotypes (strains) of porcine rotaviruses from 2 piggeries were determined by polyacrylamide gel electrophoresis of extracted genomic virus RNA. Nineteen electropherotypes were identified during a 3-month period: 9, 7 and 3 were from group A, presumptive group B or E, and C rotaviruses respectively. Analysis of electropherotype distribution based on piggery revealed that 16 electropherotypes were detected in one piggery and 9 in another. More groups A and B or E electropherotypes were detected in one of the 2 piggeries while group C electropherotypes were evenly distributed in both. Common electropherotypes of all the rotavirus groups were present in both piggeries. Our data showed that relatively high degree of genetic variation occurred in rotaviruses, especially non-group A viruses, infecting Malaysian piglets. The extent of the variation differed in different piggeries and non-group A rotavirus strains were as numerous as group A strains.

Keywords: Porcine rotavirus, electropherotypes

INTRODUCTION

Rotaviruses are a cause of gastroenteritis in human and a variety of animals (Holmes, 1979). The viruses are classified into several groups based on distinct non-cross-reacting antigens (Pedley *et al.*, 1986; Bridger, 1987). Group A rotaviruses are called typical rotaviruses while viruses of other groups are labeled as atypical rotaviruses. In addition to the use of serological methods in group classification, the segmented genome consisting of 11 double-stranded molecules produces distinct electropherotypic migration pattern which allows identification of rotavirus groups (Pedley *et al.*, 1986) and variations (electropherotypes or strains) within groups. Electropherotypes of group A, B, C and E porcine rotaviruses has been reported previously (Bridger and Brown, 1985; Chasey *et al.*, 1986; Pedley *et al.*, 1986; Terrett *et al.*, 1987; Nagesha *et al.*, 1988).

The electropherotypes of typical and atypical rotaviruses in Malaysian piglets are described in this paper.

MATERIALS AND METHODS

Faeces

Piglets 1 to 5 weeks old were sampled from April diarrhoeic and 28 normal piglets from a piggery in Melaka and from 247 diarrhoeic and 46 normal piglets from a piggery situated 150 km away in Sepang.

Rotavirus detection

The procedures for viral RNA extraction from faecal samples, RNA polyacrylamide gel electrophoresis (PAGE) and silver staining were as described previously (Yap *et al.*, 1992). Briefly, double-stranded RNAs were extracted from faeces using a chloroform-phenol mixture and then electrophoresed using Laemmli's discontinuous PAGE system (Laemmli, 1970) without sodium dodecyl sulfate in all buffers. Electrophoresis was done at 8°C using a 10 cm long 7% separating gel with a 3% stacking gel and a current of 20 mA per slab gel for the first hour followed by 10mA for the next 18 hours. After electrophoresis, the gels were stained with silver nitrate. All samples were screened using 20 µL sample volume and negative samples were retested using 80 µL.

Labelling of rotavirus electropherotypes

As all the rotaviruses detected had 'long' RNA migration pattern, they were labelled 'L' followed by the alphabet denoting the particular rotavirus group and a number to identify a particular rotavirus

RESULTS

Group A electropherotypes

Figure 1 shows the RNA profiles of the 9 electropherotypes identified from a total of 128 group A rotaviruses (recognized by the tight migration of gene segments 7 to 9). Differences in mobility involved mostly genomic RNA segments 2 and 3, 4 and the 7, 8, 9 triplets. Electropherotypes LA5 to LA7 consistently demonstrated separate migration of segments 2 and 3 while in LA8 and LA9 the 2 segments co-migrated. However, eletropherotypes LA1 to LA5 have very close migration of segments 2 and 3 which appeared as either 2 separate bands or a single band in different runs under the same electrophoresis conditions. In this gel the 2 genomic segments co-migrated. Segment 4 has 3 clearly different migration positions. Migration of segment 6 was relatively similar among electropherotypes except one (LA7) which migrated slower. The triplet segments of 7, 8 and 9 of most electropherotypes showed co-migration of segments 8 and 9. However, all 3 segments co-migrated in LA3, LA4 and LA7.

The electropherotype LA5 was the numerically dominant strain and accounted for 75% of the total group A viruses detected.

Group C electropherotypes

Nineteen rotaviruses with a 4-3-2-2 electropherotic migration pattern of the 11 genome segments typical of group C rotaviruses were identified. Figure 2 shows the RNA profile and schematic representation of the 3 group C rotavirus electropherotypes : LC1 had 14 members (74%); LC2, 4 (21%) and LC3, 1 (5%). The biggest difference in mobility was observed for segments 8 and 9: migration of segment 9 was much slower in LC3 than LC1, and it co-migrated with segment 8 in LC2. Segments 10 and 11 of LC2 migrated faster compared with the 2 other electropherotypes.

Presumptive groups B or E electropherotypes

Seven electropherotypes were identified from 16 viruses which have the 4-2-2-2-1 genomic migration pattern suggestive of group B or E rotaviruses (Figure 3). Among the electropherotypes, differences in mobility were detected in most of the segments. Six electropherotypes showed separate migration of all 11 segments while one (LB/E6) demonstrated co-migration of segments 7 and 8. LB/E3 was numerically dominant with 6 members (38% of the presumptive groups B or E viruses) while the other electropherotypes have 1 to 2 members.

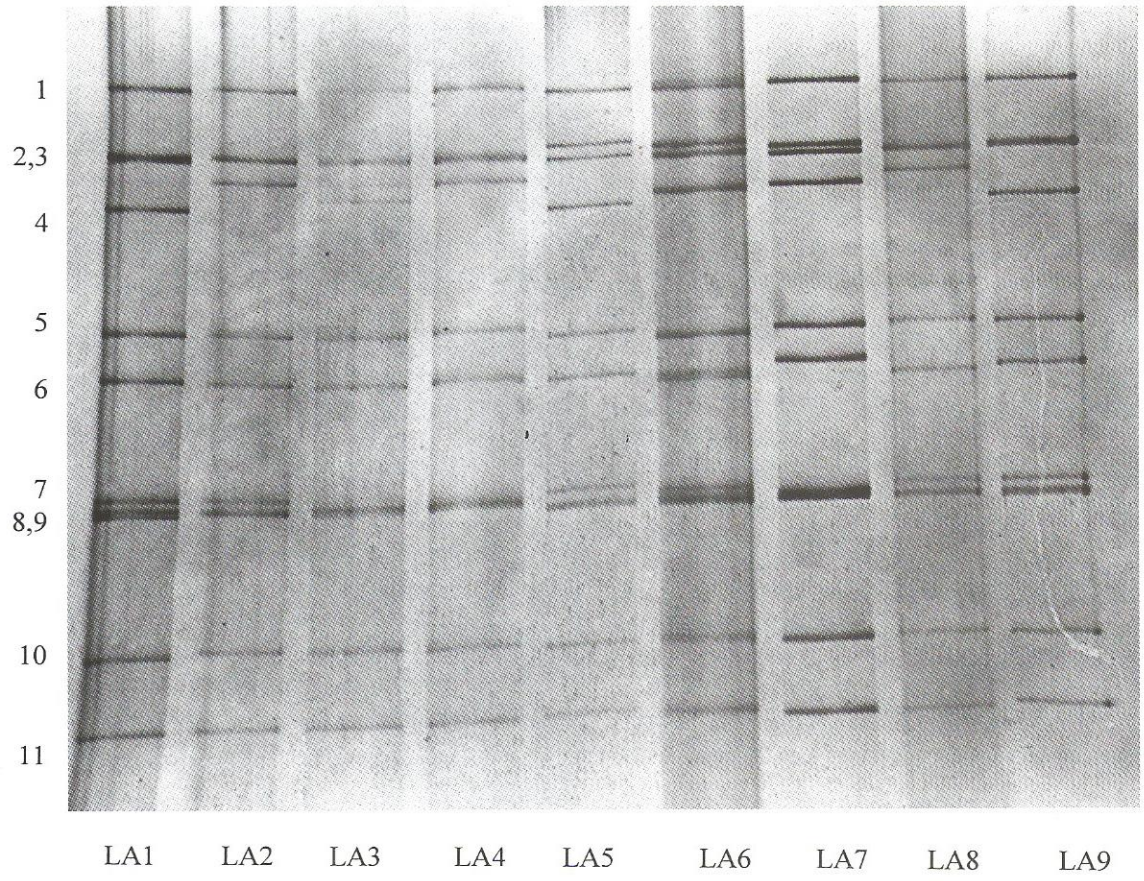

Figure 1. Genomic RNA profiles of group A rotavirus electropherotypes detected from Malaysian pigs.

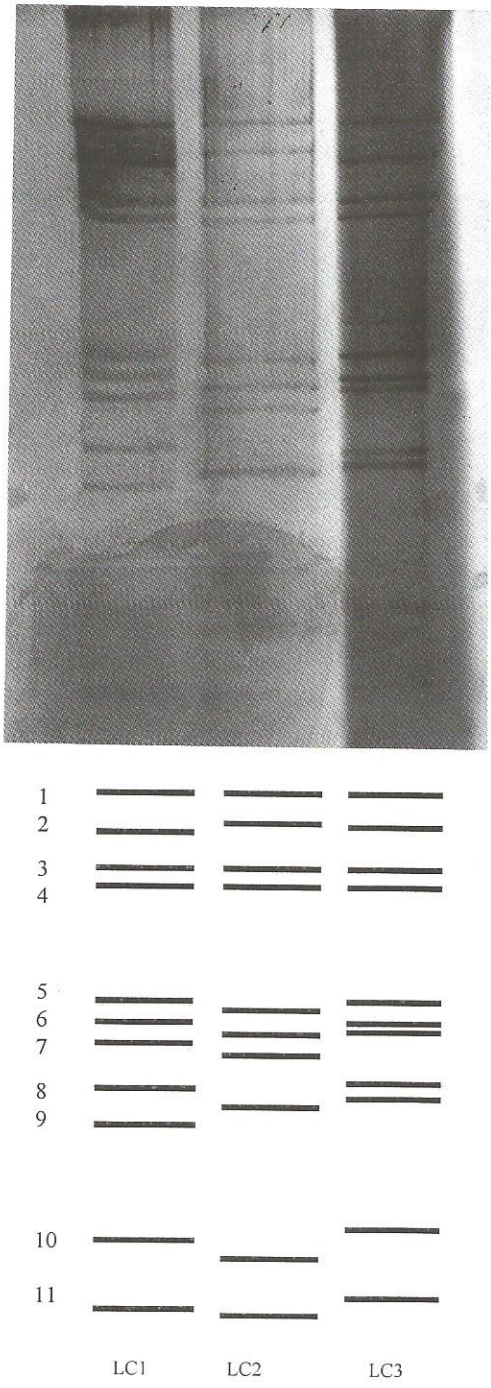

Figure 2. Genomic RNA profiles (upper panel) and schematic representation (lower panel) of group C rotavirus electropherotypes detected from Malaysian pigs. The schematic representation also gave the positions of very fade bands that did not appear in the photograph.

Distribution of electropherotypes according to piggery

Four electropherotypes (LA1, 3, 5, 9) were common to both piggeries while 4 others (LA2, 4, 7, 8) occurred only in the Melaka piggery and 1, LA6, was restricted to the Sepang piggery. Thus a total of 8 and 5 group A rotavirus electropherotypes were detected in the Melaka and Sepang piggery respectively.

The dominant group C rotavirus electropherotype, LC1, was found in both piggeries while LC2 and LC3 only in Melaka and Sepang piggeries respectively.

The dominant electropherotype of the presumptive group B or E rotaviruses (LB/EA3) occurred in both piggeries. Five other electropherotypes (LB/E1, 2, 4, 5, 6) occurred only in the Melaka piggery while 1 electropherotype, LB/E 7, was found only in the Sepang piggery .

Overall, there were 16 and 9 electropherotypes in the Melaka and Sepang piggery respectively during the period of study.

DISCUSSION

Distinct variations in genomic pattern of porcine group A rotaviruses have been detected by PAGE (De San Juan *et al.*, 1986; Liprandi *et al.*, 1987; Fu *et al.*, 1989). At least 8 group A electropherotypes were detected from 6 piggeries in New Zealand (Fu *et al* 1988) and 11 electropherotypes from 9 swine herds in Venezuela (Liprandi *et al.*,1987). The 9 group A electropherotypes identified from 2 piggeries in this country fell within the range of the previous studies.

This study revealed that genetic variation, expressed as different electropherotypes, was greatest among the group B or E rotaviruses and the least among group A rotaviruses even though the former was numerically less than the latter. Proportionally, for every 10 rotavirus of each group they were 4.3, 1.6 and 0.7 group B or E, C and A electropherotypes respectively.

A numerically dominant strain was present in each of the 3 rotavirus groups detected in this study. In group A and C rotaviruses they formed the majority of the viruses. Group B or E rotaviruses have considerably fewer members in the dominant electropherotype perhaps due the presence of more electropherotypes.

Our results showed distinct patterns in the distribution of electropherotypes in different piggeries. The number of electropherotypes circulating in the Melaka piggery was nearly 2 fold higher than in the Sepang piggery (16 compared with 9 electropherotypes). While group C electropherotypes were evenly distributed among the 2 piggeries, group A and B or E electropherotypes were more dominant in the Melaka piggery. More electropherotypes were found in piggeries in this country compared to a previous study in New Zealand (Fu *el al.*, 1989). In this regard the 5 and 8 group A electropherotypes present in the Sepang and Melaka piggeries were much higher than the 2 to 3 detected in different piggeries in New Zealand.

Common electropherotypes of all the rotavirus groups detected were present in the 2 piggeries in contrast to previous studies which reported none among different swine herds and piggeries (Liprandi *et al.*,

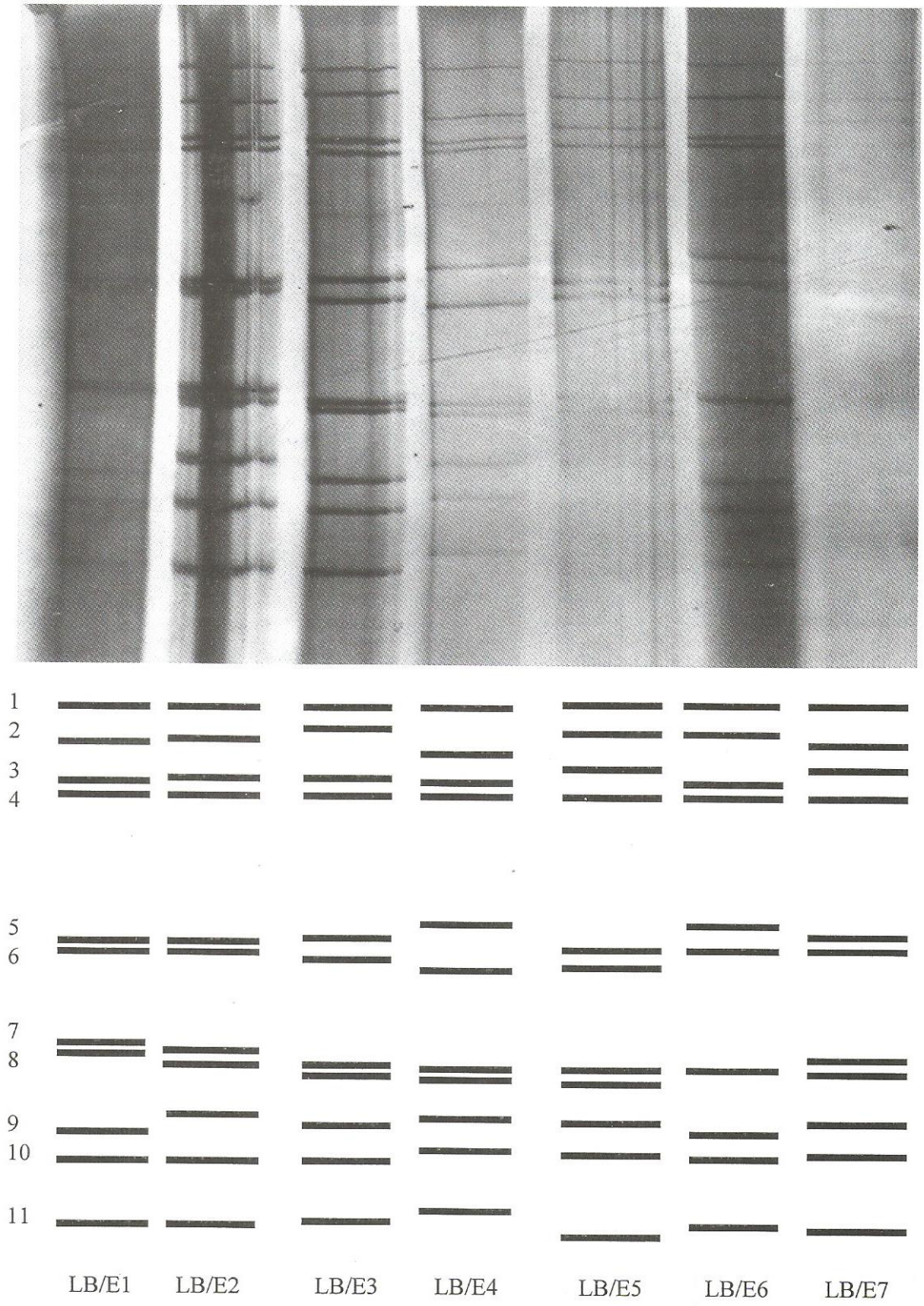

Figure 3. Genomic RNA profiles (upper panel) and schematic representation (lower panel) of presumptive group B or E rotavirus electropherotypes detected from Malaysian pigs.
The schematic representation also gave the positions of very fade bands that did not appear in the photograph.

rotavirus electropherotypes were common to both piggeries. However, only single common electropherotype was detected for group C and the presumptive group B or E rotaviruses. This may be due to a lower number of these viruses. The numerically dominant electropherotypes were represented among the common electropherotypes in all the rotavirus groups; in the atypical rotavirus groups they were in fact the common electropherotypes.

In conclusion, we have shown that relatively high degree of genetic variation, expressed in the number of electropherotypes present, occurred in rotaviruses

infecting Malaysian piglets. Genetic variation was greater in atypical rotaviruses although they were numerically less than typical rotaviruses. The extent of genetic variation was influenced by different piggeries although there were common variants. Numerically, non-group A rotavirus strains were as common as group A rotavirus strains.

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RINGKASAN

ROTAVIRUS PORSIN DARIPADA DUA LADANG TERNAKAN KHINZIR.
II. ELEKTROFORETOTIP ROTAVIRUS

Elektroforetotip (strain) rotavirus porsin daripada dua ladang ternakan khinzir telah ditentukan melalui elektroforesis gel poliakrilamida RNA virus genom yang diekstrak. Sembilan belas elektroforetotip telah dikenalpasti dalam suatu tempoh tiga bulan: 9, 7, dan 3 masing-masing daripada rotavirus kumpulan A, kumpulan B dan E andai, dan kumpulan C. Analisis taburan elektroforetotip berasaskan ladang ternakan khinzir menunjukkan 16 elektroforetotip telah dikesan dalam satu ladang dan 9 ladang kedua. Lebih banyak elektroforetotip kumpulan A dan B atau E telah dikesan dalam satu daripada ladang khinzir dikaji, sambil elektroforetotip kumpulan C sama banyak taburannya dalam dua ladang tersebut. Elektroforetotip sepunya untuk kesemua kumpulan rotavirus wujud dalam kedua-dua ladang khinzir ini. Data kami menunjukkan kepelbagaian genetik agak tinggi tahapnya berlaku pada rotavirus, terutama sekali virus bukan kumpulan A, yang menjangkiti anak babi Malaysia. Kadar kepelbagaian ini berbeza mengikut ladang ternakan khinzir dan strain rotavirus bukan kumpulan A banyaknya sama dengan strain kumpulan A.