

Isolation of Group A Swine Rotaviruses Displaying Atypical Electropherotypes

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Swine rotaviruses displaying distinctive electropherotypes were isolated from the feces of diarrheic piglets in two swine herds in the province of Buenos Aires, Argentina. In one case all samples isolated showed abnormal electropherotypes. All samples were classified as group A reactive when assayed by an enzyme-linked immunosorbent assay. Three samples from this herd were adapted to grow in tissue culture. The electrophoretic pattern of the genomic RNA as well as the group A reactivity of one of these viruses was retained after cloning in MA-104 cells. In the other pig unit were found samples displaying both classical and abnormal electropherotypes. These viruses were also positive in the enzyme-linked immunosorbent assay; however, since they could not be adapted to grow in tissue culture, this classification must be considered tentative. The abnormal electropherotype exhibited by these pig viruses strongly resembles those of human origin called super short.

The characteristic electrophoretic pattern (electropherotypes) of the segmented double-stranded (ds) rotavirus RNA genome has been widely used as a diagnostic method and as a tool for the identification and characterization of different virus strains. Up to 1980 just one general electropherotype was recognized for these viruses, and the genomic ds RNA segments were grouped in four different size classes containing 4, 2, 3, and 2 segments, respectively. Viruses displaying this standard electropherotype have been identified in different mammalian species and found to share a common group-specific antigen which is now known as group A. However rotaviruses carrying unusual genomic ds RNA electrophoretic patterns have been reported (since 1980) in avian species and in different mammalian species (3-7, 9, 15, 16, 20, 22, 23, 25-30). It was later found that there was no antigenic relationship between viruses carrying atypical and standard electropherotypes, although cross-reactivity was found among viruses sharing atypical genomic RNA patterns regardless of the species they infected (17). These observations favor the idea that the rotavirus genus is composed of different groups of viruses displaying characteristic electropherotypes that are not antigenically related (17, 26). However, Pedley et al. (18) and Dolan et al. (8) reported abnormal RNA migration profiles among samples belonging to group A viruses isolated from immunodeficient, infected children. A similar finding was reported for viruses isolated from fecal samples of healthy neonates (2). In addition, atypical genomic RNA patterns can be generated in tissue culture after serial passages of group A bovine rotaviruses at a high multiplicity of infection (12). Up to now two types of these abnormal electropherotypes were observed. Some abnormal electropherotypes contain more than the usual 11 segments, whereas the second type of abnormal electropherotypes contains 11 segments with an unusual distribution.

In this communication we report the finding of group A rotaviruses of swine origin displaying unusual electropherotypic RNA patterns belonging to that second type. These

strains were detected in two different swine herds, during a survey programmed to study the incidence of rotaviruses in swine herds of the province of Buenos Aires, Argentina.

RNA extraction for analysis by polyacrylamide gel electrophoresis (PAGE) of fecal or tissue culture samples was performed as previously described by Sorrentino et al. (27); briefly, 10 to 20% stool suspensions were prepared in 0.05 M Tris hydrochloride buffer (pH 7.5) and clarified by centrifugation (5,000 × g, 5 min). When viral RNA was prepared from MA-104-infected cells, cultures were frozen and thawed twice and then clarified as above.

Samples (200 μl) of each clarified suspension were mixed with an equal volume of extraction cocktail containing 1 M LiCl-0.02 M EDTA-sodium dodecyl sulfate, incubated for 10 min at 50°C, and then extracted twice with phenol-chloroform (21) at the same temperature; RNA was precipitated from the aqueous phase by adding 2.5 volumes of cold ethanol. After at least 2 h at -70°C, RNA was pelleted, dried, suspended in 10 μl of Laemmli sample buffer, and loaded on top of 10% polyacrylamide gels by the method of Laemmli (13), except that bisacrylamide was decreased to 0.13% to achieve better resolution of ds RNA segments. Gels were run for 16 h at 14 mA (constant current); RNA was visualized by silver staining by the method of Herring et al. (11).

Enzyme-linked immunosorbent assays (ELISAs) from two different sources were used. The combined enzyme immunoassay for rotavirus and adenovirus was kindly provided by H. G. Pereira of the Instituto Oswaldo Cruz, Rio de Janeiro (19). Samples found to contain atypical viruses were additionally assayed with an ELISA kit from the World Health Organization provided by T. H. Flewett from Birmingham to the Buenos Aires Children's Hospital of Argentina.

The following coating sera were used: for the first test, hyperimmune goat serum raised against simian rotavirus SA-11 and for the World Health Organization test, hyperimmune rabbit serum raised against the three human rotavirus serotypes. Typing sera were hyperimmune guinea pig sera raised against Wa and DB human strains in the first test and

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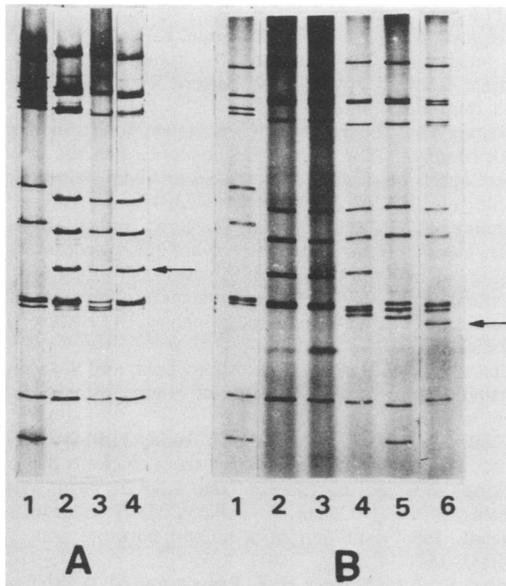


FIG. 1. Analysis by PAGE (10% acrylamide) of atypical swine rotavirus ds RNA obtained from fecal samples (A) or tissue culture fluids (B). (A) Lanes: 1, standard simian rotavirus (SA-11); 2 to 4, RNA profiles obtained from feces collected at the first pig unit surveyed (see the text). (B) Lanes: 1, simian virus SA-11; 2 to 5, RNA profiles of tissue culture-adapted viruses (see the text); 6, atypical RNA profile obtained by analysis of a fecal sample collected at the second pig unit. Extraction of ds RNA from fecal samples and from tissue culture fluids was as described in the text.

against human strain D in the World Health Organization test.

Case 1. A scour problem on an intensive pig breeding unit in Buenos Aires province was investigated in September 1985. The unit consisted of 60 sows, farrowed in crates, with piglets on a solid concrete creep area. Piglets were weaned at 35 to 40 days of age. This unit has been experiencing scour problems for three years. In September 1985 an important outbreak took place, affecting 20% of the litters with a morbidity of 80 to 100% and mortality rates ranging between 2 and 5%. Samples from seven diarrheic piglets (between 4 and 10 days old) were analyzed and in all cases were ELISA and PAGE positive.

Two different electropherotypes were found. One displayed an unusual grouping of the genomic ds RNA segments; in this case segments 7 and 11 showed atypical positions for a group A rotavirus electropherotype (Fig. 1, lane 3). When observed by electron microscopy these samples showed large numbers of particles with classical rotaviral morphology (data not shown). The other patterns are those shown in Fig. 1A, lanes 2 and 4. They are composed of several bands, with the more prominent ones belonging to a profile similar to that shown in lane 3; these observations are in agreement with the idea that the original fecal samples contained more than one virus strain. This pig unit was sampled again in October, November, and December 1985 and in June 1986. Up to December 1985 the sanitary situation of the place was similar to that found in September, and rotaviruses were isolated from 100% of the diarrheic piglets sampled. A total of 28 positive samples were analyzed, and in all cases the abnormal RNA patterns reported above were found. In June 1986 out of 12 diarrheic piglets only one was PAGE positive and ELISA negative and displayed an electropherotype characteristic of group B

rotaviruses (data not shown). On the other hand the three samples collected during September 1985 were adapted to grow in MA-104 cells (24). Samples shown in Fig. 1A, lanes 2 and 4, retained their original electropherotype after adaptation to MA-104 cells (Fig. 1B, lanes 2 and 3, respectively). The electrophoretic pattern of the other sample (Fig. 1A, lane 3) changed after its adaptation, giving rise to an electropherotype characteristic of group A rotaviruses (data not shown). It is likely that the original virus sample contained at least two virus populations; one had the atypical RNA pattern, and the other had the pattern of standard (group A) virus not visualized after silver staining of the gel and was selected after adaptation to grow in MA-104 cells. A fourth sample, collected in December 1985, was adapted to tissue culture. In this case, the original sample presented an atypical ds RNA pattern which was only slightly different from the other two described above. Due to the low concentration of virus particles in the original sample, the RNA segments gave very faint bands after silver staining of the gel (data not shown). After adaptation, the original atypical electropherotype was also retained (Fig. 1B, lane 4). This strain was cloned twice by limited dilution in MA-104 cells, indicating that, at least in this case, the virus with the atypical RNA pattern was not defective; in addition this strain was ELISA positive and retained its original virulence for piglets (Bellinzoni et al., manuscript in preparation).

Recently, Pedley et al. (18) reported abnormal rotavirus RNA profiles in viruses isolated from multiple fecal specimens obtained from immunodeficient children; in one case, additional bands were found migrating between segments 1 and 7 to 9. These strains grew very slowly when propagated individually in tissue culture; however, during mixed infections they readily reassorted with bovine rotaviruses (1). One of these reassortants had a ds RNA genome composed of all the bovine segments except segment 11, which was replaced by a rearranged segment 11 of human origin that migrated between segments 6 and 7. The electropherotypic pattern of this strain strongly resembles the atypical RNA patterns found in viruses isolated from this pig unit. It will be interesting to establish whether, in the viruses described herein, band 7 also has sequences derived from segment 11.

Case 2. The swine herd was also located in the province of Buenos Aires (100 km from the other). The unit consisted of 160 sows; management conditions were similar to those for the first herd, except for poorer sanitary conditions, and very frequent outbreaks of diarrhea had been occurring for several years. A scour problem was first investigated in July 1986. Approximately 20% of the litters were affected, with morbidity and mortality rates within litters of ca. 100 and 80%, respectively. Of 12 samples collected from 4- to 10-day-old diarrheic piglets, 4 were ELISA positive, and PAGE analysis revealed two different electropherotypes. Three of the samples showed a standard group A ds RNA pattern (identical to that shown in Fig. 1B, lane 5), and one showed an abnormal ds RNA profile also displaying ds RNA segments at a position unexpected for group A rotaviruses. Also in this case electron microscopic visualization of the samples showed a high number of particles morphologically indistinguishable from standard rotaviruses (data not shown) (Fig. 1B, lane 6). Samples were collected again 15 days later. From a total of 15 diarrheic piglets, 2 samples were ELISA and PAGE positive. In both cases the same abnormal electropherotype described above was observed. However, upon adaptation of one of these viruses to tissue culture a virus strain displaying the standard group A ds RNA profile was recovered (Fig. 1B, lane 5), suggesting the existence of a mixture of viruses in the original sample.

Since it has not yet been possible to grow and clone viruses carrying this abnormal electropherotype, it cannot be established whether they belong to group A viruses. However, it should be pointed out that this abnormal dsRNA pattern strongly resembles that of a human rotavirus strain isolated in Indonesia (10, 14); this electropherotype was designated super short and lately has been considered to be a new human serotype. Short electropherotypes were always associated with human isolates; however viruses carrying genomes similar to those shown in Fig. 1B lane 6 should also be considered as swine super short electropherotypes.

Viruses with atypical RNA patterns and belonging to group A have only been occasionally found in children. At least one (and probably two) of the porcine viruses described here fall into this category. Electrophoretic patterns like those described herein have been not reported, to date, in swine isolates. Similar RNA patterns were found in samples of human origin (18) or in reassortants generated between bovine and atypical human viruses (1).

The abnormal viruses described in this paper pose interesting questions regarding their origin, behavior, and fate. We found them to be persistent in the field for several months and to retain their virulence, even after cloning in MA-104 cells, suggesting that the real incidence, in pigs, of these viruses could be underestimated.

In this regard we emphasize the importance of PAGE analysis during our survey. The unique characteristics of these viruses would not have been apparent with only electron microscopy or the ELISA for analysis of the isolates.

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