New Porcine Rotavirus Serotype Antigenically Related to Human Rotavirus Serotype 3

HADYA S. NAGESHA* AND IAN H. HOLMES

School of Microbiology, University of Melbourne, Parkville, Victoria 3052, Australia

Received 10 August 1987/Accepted 15 October 1987

Serotyping of porcine rotaviruses isolated in MA104 cells from Australian piglets with diarrhea showed that two strains belonged to serotype 3 and one strain was antigenically similar to the OSU strain of porcine rotavirus (serotype 5). In addition, neutralizing antibodies to human rotavirus serotype 4 (ST-3 strain) were detected in serum samples from sows in one area, and so it seems probable that porcine rotaviruses of at least three serotypes occur in Australia.

Rotaviruses have been recognized as an important cause of diarrhea in many species of young animals and in children (12, 17, 22). It has been reported that rotavirus gastroenteritis is very prevalent in piglets in the first week of their life and immediately after weaning (7, 23, 29, 30).

In Australia, porcine rotavirus has been detected during outbreaks of diarrhea in piglets (24, 26), and propagation of one strain in gnotobiotic piglets and in cell culture has been well established (2).

Studies in the United States and Europe have identified two serotypes of porcine rotavirus. The OSU strain has been found to be antigenically distinct from human and other animal rotavirus serotypes and was reported to be widely distributed in the pig population (8). The Gottfried strain of porcine rotavirus has been found only in the United States and is antigenically similar to human rotavirus serotype 4 (20).

It was of interest to study whether similar or different porcine rotavirus serotypes were present in Australian pigs. Australia is well isolated from the rest of the world, and rigorous quarantine rules have restricted the entry of pigs from most countries, but periodic imports from Canada and Ireland do occur.

In this paper, we describe the isolation of porcine rotaviruses in MA104 cells and make a serological comparison of these isolates with each other and with well-characterized human and animal rotaviruses.

MATERIALS AND METHODS

Reference viruses and antisera. The following rotavirus strains and rabbit hyperimmune antisera to these strains were obtained from M. J. Albert and B. S. Coulson, Department of Gastroenterology, Royal Children’s Hospital, Parkville, Victoria, Australia: human rotavirus RV-4 (serotype 1), RV-5 (serotype 2), RV-3 (serotype 3), and B37 (new serotype) and porcine rotavirus AT/76 (1, 2, 11).

Virus strains ST-3 (serotype 4), UK bovine (serotype 6), simian rotavirus SA-11 (serotype 3), rhesus rotavirus (serotype 3), and canine rotavirus (K9; serotype 3) were included in this study as reference serotypes (11, 20).

Hyperimmune antisera to three strains of porcine rotavirus (CRW-8, TFR-41, MDR-13), SA-11, and UK bovine rotavirus were produced in seronegative rabbits by using the immunization schedule described previously (3). Guinea pig hyperimmune antiserum to the OSU strain of porcine rotavirus was obtained from Y. Hoshino, National Institutes of Health, Bethesda, Md. (20). Neutralizing monoclonal antibody (5B8) to the OSU strain of porcine rotavirus and monoclonal antibodies specific for subgroup I and II antigens were produced by H. Greenberg, Veterans Administration Medical Center, Palo Alto, Calif.

Source of fecal samples and detection of rotavirus by enzyme-linked immunosorbent assay. Fecal samples were collected from piglets with diarrhea in three different piggeries, one situated in New South Wales and two in Victoria, Australia. Approximately 10% (vol/vol) of clarified fecal suspension was used for detection of rotavirus group A antigens by enzyme-linked immunosorbent assay with hyperimmune NIC rotavirus antisera by the method of Beards et al. (4). Strong positive samples giving optical density OD values of ≥2 were selected for virus isolation. In addition, all samples were screened by polyacrylamide gel electrophoresis as described below to identify their RNA migration patterns.

Viruses. The porcine rotaviruses were adapted to grow in MA104 cells in roller tubes (1). The cells were grown in Eagle minimal essential medium (with nonessential amino acids) supplemented with 0.02 M N-2-hydroxyethylpipera- zine-N'-2-ethanesulfonic acid (HEPES) buffer, gentamicin, and 10% fetal calf serum. The cells were washed with maintenance medium to remove fetal calf serum before being infected. Virus inocula were pretreated with porcine trypsin (10 μg/ml for 30 min at 37°C) (type IX; Sigma Chemical Co., St. Louis, Mo.), and viruses were serially passaged in serum-free tissue culture medium containing antibiotics and 0.5 μg of trypsin per ml.

Immunofluorescence and neutralization tests. Immunofluorescence and neutralization tests were performed with microtiter plates by the method described by Thouless et al. (27), except that MA104 cells were used and virus was not centrifuged onto cell monolayers. Immunofluorescence tests were used to monitor virus growth at each passage. The fluorescent focus neutralization test was used for serotyping porcine rotavirus isolates. The neutralizing antibody titer was expressed as the reciprocal of the highest antibody dilution causing 50% reduction in the number of fluorescent foci (5, 11, 27). Two viruses were considered serotypically distinct if the ratio between homologous and heterologous reciprocal neutralizing antibody titer was ≥20-fold, whereas if this ratio was <20-fold, the viruses were considered to be antigenically related and to belong to the same serotype (20).

Electrophoretotyping of rotavirus RNA. The technique used
for electrophoretotyping of rotavirus RNA is similar to the method described by Rodger and Holmes (25) and Herring et al. (16). In brief, RNA was extracted from preparations of purified virus (or directly from cell culture fluids), electrophoresed in 10% polyacrylamide gels (0.75 mm thick) with 3.5% stacking gels at constant current of 12 mA for 16 h with a Laemml discontinuous buffer system, and stained with silver (16).

Subgrouping of porcine rotavirus strains. Porcine rotavirus strains were subgrouped by enzyme immunoassay by the method described by Greenberg et al. (14).

RESULTS

Virus isolation. The porcine rotavirus strains designated CRW-8 and TFR-41 showed cytopathic effect in their first passage, whereas the strain designated MDR-13 showed clear cytopathic effect only after its second passage. The cytopathic effects included increased cytoplasmic granularity, rounding of cells, cell clumps with “flagging” (C. A. Mebus, cited in reference 17), obscure cell boundaries, and cytoplasmic extensions. By 48 to 72 h, most of the cells had detached from the surface.

The virus strains designated above were from three different places: CRW-8 was from Corowa, New South Wales, Australia, TFR-41 was from Trafalgar, Victoria, Australia, and MDR-13 was from Mount Derrimut, Victoria, Australia. The growth of these three strains of viruses at each passage was monitored by immunofluorescence as described above. Strains CRW-8 and TFR-41 grew to titers of about $5 \times 10^4$ to $5 \times 10^7$ fluorescent focus units (FFU)/ml, whereas strain MDR-13 showed low titers, of about $2 \times 10^4$ to $5 \times 10^6$ FFU/ml.

Electropherotypes of porcine rotavirus RNA. Electropherotypes of cell culture-adapted porcine rotavirus strains were determined after electrophoresis of their double-stranded RNA in 10% polyacrylamide slab gels and silver staining. RNA patterns of these virus strains differed from each other and from the laboratory strains in current use (Fig. 1). They had migration patterns typical of group A rotaviruses.

Porcine rotavirus subgroups. Subgrouping enzyme immunoassays with monoclonal antibodies showed that these three porcine rotavirus strains belonged to subgroup I.

Serotyping. Results of cross-neutralization tests (Table 1) revealed that two porcine rotaviruses belonged to serotype 3 and one strain was antigenically closely related to an American porcine rotavirus, strain OSU. Strain MDR-13 showed a two-way cross-relation with a human serotype 3 strain (RV-3) and a one-way cross with SA-11, which is the prototype for serotype 3. Strain CRW-8 was neutralized by antiserum to RV-3 (serotype 3), but antiserum to CRW-8 strain neutralized RV-3 and SA-11 to low titers (62-fold less). Thus, there was a one-way antigenic relation between the human rotavirus serotype 3 strain (RV-3) and strain CRW-8. There was also a one-way cross between strains CRW-8 and MDR-13. The other members of serotype 3 (rhesus rotavirus and canine rotavirus [K9]) were neutralized by antiserum to CRW-8 and MDR-13 to high titers. From these results it was concluded that both these two porcine rotavirus strains (CRW-8 and MDR-13) should be classified as serotype 3.

Reciprocal cross-neutralization tests also showed that porcine rotavirus TFR-41 was not related to either strain CRW-8 or strain MDR-13. Guinea pig anti-OSU hyperimmune serum and a monoclonal antibody (5B8) against OSU neutralized strain TFR-41 to very high titers ($3.2 \times 10^5$ and $2 \times 10^5$, respectively) and showed a much weaker reaction with strain CRW-8 and negligible reactions with strain MDR-13. This suggested that strain TFR-41 was antigenically closely related to strain OSU. Ten different strains with similar RNA patterns, isolated from the Trafalgar herd, were neutralized by antiserum to TFR-41, which indicated that viruses belonging to this serotype were common pathogens in this pig population (data not shown).

Porcine rotavirus AT/76 was not neutralized by antiserum to strain TFR-41, nor was it neutralized by guinea pig anti-OSU hyperimmune serum or by monoclonal antibody 5B8 (anti-OSU). However, it showed a one-way antigenic relation with strains MDR-13 and CRW-8.

To gauge the prevalence of infection with viruses belonging to serotypes 5 (TFR-41), 3 (CRW-8), and 4 (ST-3), serum samples from 12 sows of the Trafalgar herd were examined for neutralizing antibodies to strains TFR-41, CRW-8, and ST-3. Reciprocal titers of between 200 and 6,400 were observed in all cases.

DISCUSSION

Porcine rotaviruses have been adapted to cell culture and previously shown to belong to two different serotypes (8, 20). Porcine rotavirus OSU is distinct from all other rotavirus serotypes and represents serotype 5 (subgroup I), whereas strain Gottfried is serologically similar to the human rotavirus serotype 4, subgroup II (14, 20). Our results show that two Australian strains (CRW-8 and MDR-13) are distinct from the above-mentioned porcine rotavirus serotypes and represent a new porcine rotavirus serotype. Strain MDR-13 clearly belongs to rotavirus serotype 3, whereas strain CRW-8 shows a one-way antigenic cross with human serotype 3. Our results do not support the previous assignment of strain AT/76 to serotype 5 (2), which was based only on a one-way test, but they do confirm that antiserum to human serotype 3 (RV-3) neutralizes strain AT/76 (Table 1). The previous report also showed that antiserum to equine rotavirus H-1 (serotype 5) did not neutralize AT/76, whereas
TABLE 1. Serotyping of porcine rotaviruses by fluorescent focus neutralization test

<table>
<thead>
<tr>
<th>Rotavirus (strain)</th>
<th>Serotype</th>
<th>CRW-8</th>
<th>MDR-13</th>
<th>AT/76</th>
<th>TFR-41</th>
<th>OSU</th>
<th>RV-4</th>
<th>RV-5</th>
<th>RV-3</th>
<th>SA-11</th>
<th>ST-3</th>
<th>B37</th>
<th>UK bovine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine (CRW-8)</td>
<td>3</td>
<td>200,000</td>
<td>20,000</td>
<td>2,880</td>
<td>1,280</td>
<td>20,000</td>
<td>300</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>800,000</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>&lt;200</td>
</tr>
<tr>
<td>Porcine (MDR-13)</td>
<td>3</td>
<td>1,200</td>
<td>80,000</td>
<td>1,040</td>
<td>800</td>
<td>4,160</td>
<td>&lt;200</td>
<td>200</td>
<td>&lt;200</td>
<td>200,000</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>&lt;200</td>
</tr>
<tr>
<td>Porcine (AT/76)</td>
<td>3</td>
<td>125,000</td>
<td>16,400</td>
<td>50,000</td>
<td>800</td>
<td>260</td>
<td>&lt;100</td>
<td>1,600</td>
<td>51,200</td>
<td>800</td>
<td>&lt;100</td>
<td>3,900</td>
<td>250</td>
</tr>
<tr>
<td>Porcine (TFR-41)</td>
<td>5</td>
<td>3,200</td>
<td>3,200</td>
<td>200</td>
<td>20,000</td>
<td>320,000</td>
<td>&lt;200</td>
<td>200</td>
<td>&lt;200</td>
<td>12,800</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>&lt;200</td>
</tr>
<tr>
<td>Porcine (OSU)</td>
<td>5</td>
<td>1,100 a</td>
<td>740</td>
<td>&lt;100 b</td>
<td>&lt;200</td>
<td>200,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human (RV-4)</td>
<td>1</td>
<td>4,050</td>
<td>740</td>
<td>&lt;100 b</td>
<td>&lt;200</td>
<td>200,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human (RV-5)</td>
<td>2</td>
<td>1,600</td>
<td>1,040</td>
<td>1,500 b</td>
<td>&lt;200</td>
<td>50,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human (RV-3)</td>
<td>3</td>
<td>3,200</td>
<td>11,540</td>
<td>&lt;200 b</td>
<td>&lt;200</td>
<td>800,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simian (SA-11)</td>
<td>3</td>
<td>3,200</td>
<td>16,640</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>800,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simian (RRV)</td>
<td>3</td>
<td>12,800</td>
<td>12,800</td>
<td>&lt;200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canine (K9)</td>
<td>3</td>
<td>12,800</td>
<td>16,640</td>
<td>&lt;200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human (ST-3)</td>
<td>4</td>
<td>200</td>
<td>800</td>
<td>&lt;200 b</td>
<td>&lt;200</td>
<td>200,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human (B37)</td>
<td>N d</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>300 b</td>
<td>&lt;200</td>
<td>50,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine (UK bovine)</td>
<td>6</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>&lt;200 b</td>
<td>&lt;200</td>
<td>740,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a The numbers are the reciprocals of serum dilutions giving a 50% reduction in the numbers of fluorescent foci. Titers are the average of four to six determinations. Homologous values are in boldface type.

b Data extracted from previously published article (2).

Reciprocal of 50% plaque reduction neutralization titer. This test was kindly done by L. J. Saif, Food Animal Health Research Program, Ohio Agricultural Research and Development Centre, The Ohio State University, Wooster, Ohio.

d N, New serotype of human rotavirus (2).

antiserum to equine rotavirus H-2 (serotype 3) did (2). Therefore, it appears that this strain of porcine rotavirus (AT/76) is related to serotype 3 rather than to OSU (serotype 5).

Our results have identified porcine rotavirus strains belonging to serotype 3, which includes not only human and simian rotaviruses but also canine (Cu-1 and K9), feline (Taka), equine (H-2), lapine, and murine rotaviruses (6, 15, 20–22, 28). None of our porcine rotavirus strains are antigenically related to rotavirus serotypes 1, 2, 4 or a “super-short” human rotavirus (2) or to UK bovine rotavirus (serotype 6). There are no published reports showing serological relationships of porcine rotaviruses with human serotypes 1 or 2. However, two rotavirus strains, Gottfried and SB-2, are antigenically identical to St. Thomas no. 4 rotavirus, which belongs to serotype 4 (20). We have found neutralizing antibodies to St. Thomas no. 3 (serotype 4) in serum samples from sows in Trafalgar, Victoria, Australia (data not shown), which suggests that Gottfried-like strains may also occur in Australian swine herds.

In serotyping of rotaviruses, one-way antigenic relationships have previously been documented (10, 20). In our studies we have found one-way crosses within serotype 3. Among three strains of porcine rotaviruses (CRW-8, MDR-13, and AT/76), MDR-13 shows a two-way antigenic relation with serotype 3 rotaviruses, whereas CRW-8 and AT/76 show one-way relationships with serotype 3 strains and with each other. Such one-way crosses within a serotype could be due to antigenic drift in the neutralizing antigenic epitope. Coulson et al. (11) found that a neutralizing monoclonal antibody directed against VP7 of RV-3 (serotype 3) did not neutralize RV-1 (serotype 3). This suggests some variation in VP7 within a serotype. From the results of Caust et al. (10a), a clear candidate for this serological variability is the carbohydrate attached to VP7. Not only do the number of potential glycosylation sites vary within a serotype (C. Hum, unpublished data), but mutations that add carbohydrate moieties to amino acids at or near an antigenic region have been shown to dramatically reduce the binding of monoclonal or polyclonal antibodies to VP7. The situation appears similar to that of the well-studied haemagglutinin protein of influenza virus, in which antigenic changes (i.e., drift) occur both by amino acid substitutions and carbohydrate addition (13, 31). However, the rate of change in VP7 appears to be much lower than that of influenza virus. It is hoped that VP7 sequence comparisons will eventually lead to an explanation of one-way crosses within rotavirus serotypes.

Another problem being faced in serotyping of rotaviruses is antigenic bridging between two different serotypes (19, 20). This has been shown to be due to the minor neutralizing antigen, VP3, which is the fourth gene product (18, 19). In our studies we have noted that strain CRW-8 is neutralized to homologous titer by antiserum to RV-3 virus (serotype 3), as well as being significantly neutralized by guinea pig anti-OSU serum. Rabbit anti-CRW-8 serum also neutralizes OSU (serotype 5), although only to low titer. We believe that
this cross-reaction is due to VP3, because serotype 3 relatedness of CRW-8 has been confirmed by using anti-VP7 monoclonal antibodies, and the cross-reactivity has segregated with VP3 in reassortant studies (manuscript in preparation). We agree with Hoshino et al. (18, 19) that it is essential to define the serotypes of future rotavirus isolates in terms of both their surface proteins, VP3 and VP7.

ACKNOWLEDGMENTS

We thank the managements of Bunge Meat Industries Ltd., Corowa, New South Wales, Australia; Melbourne University piggy, Mount Derrimut, Victoria, Australia; and Willow Grove Piggy, Petersville, Trafalgar, Victoria, Australia for their cooperation and generous supply of clinical samples. Chris Mum’s help in collecting samples is gratefully acknowledged. We thank Ingrid Jahnke and Paula Kelly for their excellent technical assistance and Linda Mercer and Phillip Kantharidis for their help in photography. We thank Niall Pugh for typing the manuscript neatly. We thank T. H. Flewett for providing ST-3 strain, UK bovine strain of rotavirus; H. Melherbe for providing SA-11; N. Schmidt for providing rhesus rotavirus; and G. Woode for supplying rotavirus K9. We also thank M. L. Dyall-Smith for valuable advice and criticism in preparation of this manuscript.

LITERATURE CITED