Comparative studies of human rotavirus serotype G8 strains recovered in South Africa and the United Kingdom

A. D. Steele,1 S. P. Parker,2 I. Peenze,1 C. T. Pager,1 M. B. Taylor3 and W. D. Cubitt2

1 MRC/MEDUNSA Diarrhoeal Pathogens Research Unit, PO Box 173, Medunsa 0204, Pretoria, South Africa
2 Department of Virology, Camelia Botnar Laboratories, Great Ormond Street Hospital, London, UK
3 Department of Medical Virology, Institute of Pathology, University of Pretoria, Pretoria, South Africa

Epidemiological studies on the VP7 serotype prevalence of human rotaviruses in South Africa and the United Kingdom identified several strains which could not be serotyped as G1–G4 by monoclonal antibodies. Further analysis of these strains with a G8-specific monoclonal antibody and with probes for human rotaviruses confirmed them as G8 rotaviruses. These G8 strains exhibited a high degree of sequence identity when compared with each other and with other rotavirus G8 strains. Five South African strains were further characterized as VP6 subgroup I, but with a long RNA electropherotype, which is similar to the G8 strains previously isolated in Finland. In the UK strains, one was VP6 subgroup II with a long RNA electropherotype (similar to the Italian G8 strain). The other two were subgroup I with a short RNA electropherotype. None of these strains exhibited the super-short RNA electropherotype described in the prototype G8 strains recovered from Indonesia (69M).

Rotavirus is the most common cause of severe diarrhoea worldwide, affecting over 125 million young children every year in developing countries, and is believed to account for one-quarter of all the deaths due to diarrhoea among children less than 5 years old (Cook et al., 1990). The magnitude of rotavirus disease has stimulated much research which has culminated in the release of a rotavirus vaccine (RotaShield).

Several rotavirus vaccine studies have indicated the need for a polyvalent vaccine candidate encompassing the four epidemiologically important human rotavirus VP7 serotypes, G1–G4 (Hoshino & Kapikian, 1994). However, other VP7 serotypes have been recovered from human infants including G6 (Gerna et al., 1992), G9 (Clark et al., 1987), G10 (Dunn et al., 1993) and G12 (Taniguchi et al., 1990).

Serotype G8 strains were first recovered from young children with gastroenteritis in Indonesia (Matsuno et al., 1985). The prototype strain (69M) is VP6 subgroup I and has a ‘super-short’ RNA electropherotype. Additional G8 strains were identified in Finland with a long RNA electropherotype and subgroup I specificity (Gerna et al., 1990a, b) and in Italy (subgroup II; long). A bovine G8 strain, (subgroup I, long RNA pattern) was identified in Scotland (Snodgrass et al., 1990).

Epidemiological surveys have investigated the antigenic characteristics of human rotaviruses circulating in London, UK (Noel et al., 1991, 1994) and Ga-Rankuwa, South Africa (Steele et al., 1995). In both regions untypeable rotavirus strains were identified which could not be VP7 serotyped.

In this study, rotavirus G8 strains recovered from five young children in South Africa and three in England were characterized further (Table 1). The children were identified in the Emergency or Outpatient Clinic of the hospitals, treated with oral rehydration therapy and discharged. The hospitals included Queen Elizabeth Hospital, London, where G8 rotaviruses were previously identified from six patients (Noel et al., 1994); Pretoria Academic Hospital, Pretoria (two G8 strains were detected) and Ga-Rankuwa Hospital, Ga-Rankuwa (seven specimens).

In London, VP7 serotype-specific monoclonal antibodies were utilized to serotype strains recovered from Queen Elizabeth Hospital and a small sample from Pretoria Academic Hospital (Coulson et al., 1987; Noel et al., 1991). A VP7 G8 serotype-specific monoclonal antibody (B:37) was obtained from Ruth Bishop, Melbourne, Australia and utilized in further antigenic analysis (Tursi et al., 1987; Noel et al., 1994).

Rotavirus strains from infants at Ga-Rankuwa Hospital were characterized with a panel of VP7 serotype-specific monoclonal antibodies including those described above and a separate set whose use is described elsewhere (Coulson et al., 1987; Padilla-Noriega et al., 1990; Shaw et al., 1985). The panel of monoclonals was used as described in detail elsewhere (Padilla-Noriega et al., 1990). The Ga-Rankuwa strains were

Author for correspondence: Duncan Steele.
Fax +27 12 521 5794. e-mail adsteele@medunsa.ac.za
The VP7 gene sequences of three of the rotavirus isolates in this study have been deposited in GenBank (accession numbers AF143688–AF143690).
determined to be G8-specific by molecular techniques including hybridization with VP7-specific PCR-generated probes (Flores et al., 1989, 1990) and a nested, multiplex PCR technique (Gouveia et al., 1990).

In brief, the VP7 serotype-specific probes were generated by reverse transcription of the whole VP7 gene of reference rotavirus strains as described previously (Flores et al., 1990). A slightly smaller PCR product was generated for strain 69M (G8) using the common 5' end primer with a specific primer for strain 69M (5' ACACTGATTCTGTGACG 3'). The VP7 serotype-specific probes were generated by nested PCR using common primers bordering the hyperdivergent region as described previously (Flores et al., 1989). The amplified products were purified by electrophoresis through low melting point agarose gels and labelled with $^{32}$P as described previously (Steele et al., 1993).

The viral dsRNA was extracted from the faecal specimens, diluted 1:10 in distilled water and denatured in 1 M NaOH at room temperature for 15 min before neutralization in 3 M NaCl and 0.5 M Tris. Nitrocellulose membranes were pre-wetted in $6 \times$ SSC (0.9 M NaCl, 90 mM sodium citrate) and applied to a Hybond apparatus (Nytran; Schleicher and Schuell). The wells were washed with $6 \times$ SSC before 100 ml of each sample was loaded and the RNA was applied to the membrane by vacuum suction. After the samples had filtered through, the wells were washed with $6 \times$ SSC, the filters removed, air-dried and baked at 80 °C for 1 h. Hybridization was performed overnight at 54 °C as described in detail elsewhere (Flores et al., 1990; Steele et al., 1993).

In addition, VP6 subgroup-specific monoclonal antibodies developed by Greenberg et al. (1983) were utilized. These monoclonal antibodies against subgroup I rotaviruses (clone 255/60) and subgroup II rotaviruses (631/9) have been extensively used in studies worldwide. The methods for their use have been described in detail elsewhere (Greenberg et al., 1983; Steele & Alexander, 1988). For PAGE, viral dsRNA was extracted by phenol–chloroform treatment of the 10% stool suspensions and ethanol precipitation overnight at –20 °C. Electrophoresis was performed through 10% polyacrylamide gels at 100 V for 18 h at ambient temperature and the dsRNA segments were visualized by silver staining techniques (Steele & Alexander, 1987).

Finally, the PCR-generated cDNA of the VP7 genes of three strains was sequenced by the dideoxynucleotide chain termination method with the Sequenase 2.0 kit (USB), using a selection of primers derived from the sequence of strain 69M.

In this study, human rotavirus G8 serotypes were identified in England and South Africa either by their reactivity with a G8-specific monoclonal antibody or by hybridization to G8-specific probes (Table 1). Further antigenic and molecular analyses of the viruses revealed distinct constellations of the well known epidemiological markers of rotaviruses (RNA electropherotype, VP6 subgroup and VP7 serotype) (Table 1).

Rotavirus G8 strains have a wide geographical distribution, having been recovered in Indonesia, Finland, Italy, Nigeria, Brazil and recently Malawi (Matsumoto et al., 1985; Gerna et al., 1990a, b; Adah et al., 1997; Santos et al., 1998; Cunliffe et al., 1999). Furthermore, G8 strains have been recovered from at least two species – humans and calves – although they do not seem to be epidemiologically significant in either. Bovine rotaviruses were identified in Scotland and Thailand (Snodgrass et al., 1990; Taniguchi et al., 1991). In this study, G8 strains were identified in England and South Africa, extending their global distribution.

It has been suggested that G8 strains are reassortant viruses between human and bovine rotaviruses (Ohshima et al.,

### Table 1. Description of the patients shedding VP7 serotype G8 rotavirus strains in South Africa and England and characteristics of the virus strains recovered

An additional three strains from England and four from South Africa were typed as serotype G8 by monoclonal antibody (UK) and VP7 probe (South Africa). Insufficient material was available for further study.

<table>
<thead>
<tr>
<th>Rotavirus strain</th>
<th>Year</th>
<th>Region</th>
<th>Age</th>
<th>Sex</th>
<th>Admission</th>
<th>MAb studies</th>
<th>Genetic analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>QEH14262</td>
<td>1990</td>
<td>London, UK</td>
<td>16 months</td>
<td>M</td>
<td>Casualty</td>
<td>VP7</td>
<td>VP6 VP7 RNA profile VP7 probe</td>
</tr>
<tr>
<td>QEH25964</td>
<td>1992</td>
<td>London, UK</td>
<td>13 months</td>
<td>M</td>
<td>Casualty</td>
<td>VP7</td>
<td>VP6 VP7 RNA profile VP7 probe</td>
</tr>
<tr>
<td>QEH27389</td>
<td>1992</td>
<td>London, UK</td>
<td>9 months</td>
<td>M</td>
<td>Casualty</td>
<td>VP7</td>
<td>VP6 VP7 RNA profile VP7 probe</td>
</tr>
<tr>
<td>UP1</td>
<td>1992</td>
<td>Bronkhorstspruit, RSA</td>
<td>12 months</td>
<td>F</td>
<td>Casualty</td>
<td>VP7</td>
<td>VP6 VP7 RNA profile VP7 probe</td>
</tr>
<tr>
<td>UP30</td>
<td>1993</td>
<td>Kwaggafontein, RSA</td>
<td>5 months</td>
<td>M</td>
<td>Casualty</td>
<td>VP7</td>
<td>VP6 VP7 RNA profile VP7 probe</td>
</tr>
<tr>
<td>GR438</td>
<td>1985</td>
<td>Ga-Rankuwa, RSA</td>
<td>6 months</td>
<td>M</td>
<td>POPD*</td>
<td>VP7</td>
<td>VP6 VP7 RNA profile VP7 probe</td>
</tr>
<tr>
<td>GR352</td>
<td>1986</td>
<td>Ga-Rankuwa, RSA</td>
<td>4 months</td>
<td>F</td>
<td>POPD</td>
<td>VP7</td>
<td>VP6 VP7 RNA profile VP7 probe</td>
</tr>
<tr>
<td>GR570</td>
<td>1987</td>
<td>Ga-Rankuwa, RSA</td>
<td>6 months</td>
<td>F</td>
<td>POPD</td>
<td>VP7</td>
<td>VP6 VP7 RNA profile VP7 probe</td>
</tr>
</tbody>
</table>

* POPD, Paediatric Outpatient Department.
Rotavirus G8 strains in South Africa and UK

Fig. 1. Comparison of the deduced VP7 amino acid sequence of the serotype 8 strains.
Table 2. Comparison of the nucleic acid and amino acid sequence identity of the G8 strains detected in South Africa and the United Kingdom with other reported G8 strains

<table>
<thead>
<tr>
<th></th>
<th>69M</th>
<th>B37</th>
<th>HAL1166</th>
<th>QEH14262</th>
<th>UP30</th>
<th>GR570</th>
<th>678</th>
<th>A5</th>
<th>HGM89</th>
</tr>
</thead>
<tbody>
<tr>
<td>69M</td>
<td>96</td>
<td>97</td>
<td>92</td>
<td>90</td>
<td>90</td>
<td>95</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>B37</td>
<td>97</td>
<td>90</td>
<td>92</td>
<td>86</td>
<td>86</td>
<td>89</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>HAL1166</td>
<td>98</td>
<td>96</td>
<td>95</td>
<td>89</td>
<td>86</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>QEH14262</td>
<td>95</td>
<td>90</td>
<td>94</td>
<td>89</td>
<td>86</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>UP30</td>
<td>91</td>
<td>92</td>
<td>92</td>
<td>94</td>
<td>96</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>GR570</td>
<td>92</td>
<td>91</td>
<td>93</td>
<td>94</td>
<td>94</td>
<td>96</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>678</td>
<td>98</td>
<td>95</td>
<td>96</td>
<td>94</td>
<td>95</td>
<td>95</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>A5</td>
<td>95</td>
<td>94</td>
<td>94</td>
<td>92</td>
<td>93</td>
<td>93</td>
<td>93</td>
<td>93</td>
<td>93</td>
</tr>
<tr>
<td>HGM89</td>
<td>93</td>
<td>91</td>
<td>92</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
</tbody>
</table>

Amino acid sequence identity (%)

1990; Browning et al., 1992). In this study, as in Finland (Gerna et al., 1990b), most of the G8 rotaviruses superficially resemble animal strains with subgroup I specificity and a long RNA electropherotype, which is similar to the AU-1 genogroup of human rotaviruses (Nakagomi et al., 1989).

However, one strain each from the UK and South Africa exhibited a short RNA profile and VP6 subgroup I, which is more typical of human rotaviruses belonging to the DS-1 genogroup (Nakagomi et al., 1989). Human G8 strains have some homology to viruses from the DS-1 genogroup (subgroup I, G2, short RNA pattern) (Ohshima et al., 1990). Surprisingly, similar homology was noted between 69M and bovine rotaviruses, suggesting the possibility of a bovine–human reassortant.

Browning et al. (1992), attempting to address this question, described three possible origins of the G8 strains. Finnish human G8 strains (represented by HAL1166) appeared to be derived from a bovine origin, whilst the Indonesian human G8 strain (69M) may have resulted from a reassortment event between bovine and DS-1-like viruses. The Scottish bovine G8 rotavirus (678) appeared to be distinct from both human and bovine rotavirus genogroups. Due to the limited distribution of these viruses in cattle and humans, it was suggested that there may be a third host which acts as the natural reservoir for the G8 strains (Browning et al., 1992).

In this study, viruses with different constellations of the epidemiological markers were detected. Several strains with a long RNA electropherotype and subgroup I antigen were found, two strains with subgroup I and a short RNA pattern, and one UK strain (QEH25964) had a subgroup II specificity with a long RNA profile similar to strain PA171 from Italy. These data support the suggestion of different and probably reassortant origins for the serotype G8 strains.

The VP7 gene sequences of three of the isolates in this study were completed and have been submitted to GenBank (accession numbers AF143688–AF143690). The VP7 gene consisted of 1062 bp with an AUG codon at bases 49–51, which initiates a 978 nucleotide open reading frame. Eight cysteines and nine proline residues are conserved amongst the strains sequenced, in common with most other VP7 gene sequences published. Potential glycosylation sites are present at positions 69–71 and 238–240 in common with other rotavirus VP7 serotypes.

The VP7 gene sequences in this study are clearly that of a serotype G8 rotavirus, although the level of identity is less than is usual for strains of the same serotype specificity. The two South African strains showed a high degree of identity at the nucleic acid and amino acid level and were remarkably conserved when compared to the UK isolate (Fig. 1 and Table 2). This was surprising, as the two South African strains were subgroup I with a long RNA pattern whereas the UK strain was subgroup I and had a short RNA pattern.

Human G8 rotaviruses shared an unexpectedly high level of identity with G3 strains (Hum et al., 1989), which was interpreted to suggest a possible common ancestral strain. Continual genetic drift would account for the lack of obvious identity (Hum et al., 1989). Interestingly, the deduced amino acid sequence of antigenic region C, previously described to be similar to that of G3 rotaviruses, was completely conserved amongst the strains identified in this study, possibly offering further support for this idea.

Serotype G1–4 strains are considered to be the predominant rotaviruses causing human disease worldwide. However, unusual strains are described as prevalent in some locations. For instance, in Brazil one-third of infections were with uncommon serotypes, e.g. G5 strains (Gouvea et al., 1994). In the Indian subcontinent, unusual combinations and rare human strains predominated (Bern et al., 1992; Ramachandran et al., 1996). In Europe, between 2 and 14% of strains could not be typed (Gerna et al., 1990a; Noel et al., 1991) and serotype G8 strains were detected (Noel et al., 1994). Recently, serotype G8 strains were detected in Nigeria.
(Adah et al., 1997), in Brazil (Santos et al., 1998) and in Malawi (Cunliffe et al., 1999).

The VP4 type of the Malawi strains was examined by a reverse transcriptase method using a nested, multiplex PCR with a cocktail of primers for the common human VP4 genotypes (Cunliffe et al., 1999). Surprisingly, P[6] types predominated in the rotavirus strains recovered and this was associated with G8 strains as well as G1, G3 and G9 strains. The VP4 genotyping of the three G8 strains in this study was performed using the same methods and primers as Cunliffe et al. The two South African strains with subgroup I and long RNA profiles were also typed as having the P[6] VP4 genotype. The English strain with a short RNA profile was typed as P[4]. Further characterization of these genes is planned.

The serotype G8 strains in this study were recovered from infants in the recognized target age-group for rotavirus infection. Interestingly, none of the infants were hospitalized, but were examined as out-patients. This may indicate that serotype G8 rotaviruses are less virulent than the more commonly identified serotypes. Nevertheless, human serotype G8 rotavirus infection is widespread. In a study to investigate the prevalence of antibodies to serotype G8 strains, young Ecuadorian children exhibited a sero-prevalence of 27%, while 3–5% of German children had antibodies (Brussouw & Sidoti, 1991).

The diversity of strains circulating in different regions may be important for vaccine administration and development. Since reassortant vaccines were engineered to contain the prevalent G antigens (i.e. G1–G4), these vaccines may protect less well against unusual strains circulating in countries planning to implement a rotavirus vaccine strategy. The lower efficacy of rotavirus vaccines in some countries is not fully understood and is likely to be due to many factors. One explanation may be that disease is caused by VP7 serotypes not covered by current vaccine candidates, whereas an alternative suggestion is that this may be due to the lack of incorporation of a human P type in the tetravalent rhesus rotavirus vaccine (Hoshino & Kapikian, 1994).

We would like to thank the South African Medical Research Council, Tygerberg, and the Office of Science and Technology, United Kingdom, for the research funds for this study.

References


Received 5 January 1999; Accepted 13 July 1999