Evidence of Intrafamilial Transmission of Rotavirus in a Birth Cohort in South India

Indrani Banerjee,1 Beryl Primrose Gladstone,1 Miren Iturriza-Gomara,2 James J. Gray,2 David W. Brown,2 and Gagandeep Kang1*

1Department of Gastrointestinal Sciences, Christian Medical College, Vellore, India
2Virus Reference Department, Centre for Infection, Health Protection Agency, London, United Kingdom

Transmission of rotavirus infection was studied in a birth cohort of children based in an urban slum in Vellore and their familial contacts. Contemporaneous samples from index patients and their familial contacts were collected for analysis in three different settings. Firstly, samples were collected from familial contacts during a period of rotavirus infection in children from the cohort. Secondly, on occasions when a family member had rotavirus diarrhea, samples from the cohort child were taken for analysis. Lastly, asymptomatic surveillance samples collected at predetermined time points from both the cohort child and familial contacts were analyzed. From 560 samples collected from family members during symptomatic and asymptomatic rotavirus infections in these children, three rotavirus transmissions were identified, accounting for a secondary attack rate of 0.54%. In four instances of rotavirus diarrhea in a family member, one infection was transmitted to the cohort child. Nucleotide sequence and phylogenetic analysis demonstrated a high degree of similarity in all these pairs ranging between 99% and 100% at both the nucleotide and the deduced amino acid levels, highly suggestive of person-to-person transmission of rotavirus infection. There was complete concordance of rotavirus genotyping between these pairs. No transmission events were noted from 14 asymptomatic rotavirus infections identified during routine surveillance of family members. This study is the first to use phylogenetic analysis to study the intrafamilial spread of rotavirus infection.

INTRODUCTION

Rotavirus belonging to the Reoviridae family has been recognized for more than 30 years as the most common cause of infectious gastroenteritis in infants and young children worldwide. Globally, group A rotavirus diarrhea results in an estimated 611,000 childhood deaths each year, 80% of these occurring in the developing world [Parashar et al., 2006]. However, rotavirus illness in adults is often under appreciated. While Group B and C rotavirus infection have been reported as widespread epidemics and sporadic illness in adults [Chen et al., 1985; Oishi et al., 1993], infection with group A rotavirus in adults has been reported in a few studies as endemic disease, epidemic outbreaks, travel related gastroenteritis or infections transmitted from children to adults [Anderson and Weber, 2004]. The commonest setting for this infection in adults is among familial contacts of infected children and health-care professionals caring for these children [von Bonsdorff et al., 1976; Wenman et al., 1979].

Transmission of rotavirus is generally known to occur through the oro-fecal route. The virus can survive in the environment and has been detected in drinking water, fomites and even air samples from rooms housing infected children [Keswick et al., 1983; Pickering et al., 1986; Ansari et al., 1991; Butz et al., 1993]. The virus is extremely difficult to eradicate and relatively resistant to inactivation by chemical disinfectants and antiseptics commonly used in hospitals and other institutions [Ansari et al., 1991]. Hand washing with soap is ineffective and may actually spread the virus over a greater surface area [Ansari et al., 1991]. Only a disinfectant spray comprising of 79% ethyl alcohol and 0.1% o-phenylphenol successfully blocked transmission of rotavirus infection to humans [Ward et al., 1991].

Symptomatic children excrete large quantities of virus when symptomatic and shedding of lower concentrations...
even after resolution of symptoms [Melnick and Ren- nick, 1980; Flewett, 1983; Richardson et al., 1998]. Additionally, asymptomatic children have also been reported to shed virus in their stool [Eiden et al., 1988; Kang et al., 2004; Amar et al., 2007]. These factors are critical in determining introduction and spread of rotavirus infection within the community. Volunteer studies in adults have estimated that only ten infectious particles of rotavirus can cause clinical disease [Ward et al., 1986]. A combination of the fact that infection with rotavirus is possible with a small infectious dose, that the virus survives for prolonged periods in the environment and cannot be easily inactivated by cleansing agents facilitates transmission of this infection.

Most studies reporting transmission from infected children to family members were prior to the advent of molecular methods that accurately characterized viral strains [Wenman et al., 1979; Grimwood et al., 1983; Koopman et al., 1989]. Viral transmission was assumed to have taken place if rotavirus was detected in the index patient and the contact in the same time period. In this study, person-to-person transmission of rotavirus in a community setting in southern India has been investigated by comparing the nucleotide sequences of index strains with those causing secondary infection in familial contacts.

MATERIALS AND METHODS

Epidemiological Methods

A birth cohort of four hundred and fifty two children, resident in an urban slum area in Vellore, was recruited between April 2002 and July 2003 and was monitored for a total of 3 years with the primary aim of studying the natural history of rotavirus infection and to assess the protective effect of natural rotavirus infection on subsequent homotypic and heterotypic exposure. The details of the monitoring of this cohort has been published previously [Banerjee et al., 2006, 2007]. Briefly, during the follow-up period each child was visited at home, twice a week, to record any morbidity in the child, or any diarrhea in the family. Fecal samples were collected every two weeks from each child for surveillance. Samples were also collected when a child had diarrhea.

Surveillance was also established for the families of the cohort children with the collection of stool samples from a subset of the mothers and siblings under 5 years of age during family episodes of gastroenteritis. In order to study transmission of rotavirus infection, samples were collected in three different scenarios. Samples were collected from familial contacts if the child of the cohort had rotavirus infection. Samples were collected from the cohort child if one of the family members had diarrhea. Additional samples were also collected from children of the cohort and family members as asymptomatic surveillance samples every 3 months.

Ethical approval was obtained for the study from the CMC Research Committee and recruitment depended on parents having given informed written consent.

Laboratory Methods

All fecal samples were screened for rotavirus using an ELISA, based on detection of the VP6 protein and using the manufacturer’s instructions (Rota IDEIA, Thermo Fischer Scientific, Ely, UK). The EIA-positive specimens were processed for extraction of viral RNA using the guanidium isothiocyanate-silica method [Boom et al., 1990]. Complementary DNA was synthesized from the extracted viral RNA through reverse transcription in the presence of random hexamers. G and P typing was performed using VP7- and VP4-specific multiplex heminested RT-PCRs as described previously [Gouvea et al., 1990; Gentsch et al., 1992; Iturriza-Gomara et al., 2004].

Sequencing was performed using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). The same primers as in the first round PCR reaction were used for sequencing of the VP7 and VP4 genes. The PCR amplicons obtained by the RT-PCR were purified and sequenced in both directions. Sequences were resolved in an automated DNA sequencer (ABI PRISM 310 Genetic Analyzer [Applied Biosystems]), and electropherograms were analyzed using the sequence analysis software (Sequence Navigator, version 1.01, Applied Biosystems). Multiple alignments and phylogenetic analyses were performed using Bioedit (version 7.0.5.3) (http://www.mbio.ncsu.edu/bioedit/bioedit.html) (Version 1.6.6). The dendrograms were confirmed by at least two of the following methods; neighbor joining, maximum parsimony, and/or maximum likelihood. Genetic clusters were defined when bootstrap values (generated from 1,000 pseudoreplicates) were >85% (data not shown). Partial nucleotide sequences of VP7 and VP4 genes were submitted to the GenBank database and the accession numbers are EU221259–EU221272.

RESULTS

Family Surveillance During Rotavirus Infection in Children of the Cohort

Stool samples were collected as soon as possible after identification of the index case from family members during 235 episodes of infection in the cohort children. A total of 560 stool samples from family members (more than one sample was collected per infection) were obtained, with 71 from fathers, 241 from mothers, 205 from siblings and 46 samples from other members of the family (including grandparents, uncles, aunts, etc.). The median time (IQR) of collection of the stool sample from all the contacts was 12 (8–21) days after the onset of diarrhea in the index case.

Of the samples collected from familial contacts, six samples were positive for rotavirus by VP6 PCR but only three could be successfully genotyped. Therefore, there were three definitive instances when rotavirus was documented to have spread to a family contact. The secondary attack rate of definitive rotavirus

infection (calculated as the number of definite rotavirus transmission divided by the total number of susceptible individuals) in the population studied was 0.54%. On two of these occasions, the primary infection was transmitted from the index children to their mothers and in the third; the virus was transmitted to a brother. In two episodes, the index child had asymptomatic rotavirus infection and in the third episode, infection was transmitted from a child with diarrhea (Table I). All secondary infections were asymptomatic. The genotype of the rotavirus infection transmitted to the mothers were G2P[4] and G9P[8] respectively, whereas it was the G10P[11] strain which was transmitted to the sibling. There was complete concordance of genotyping among the pairs of samples collected.

**Rotavirus Positive Diarrheal Episodes in Familial Contacts**

During the period of surveillance, samples were also collected from 60 diarrheal episodes in familial contacts and simultaneous samples were collected from the study children. Samples were not collected from family members other than the study child. Four of these samples from the family were positive for rotavirus, accounting for 6.7% of all samples from familial contacts and study children. The genotypes detected were two G2P[4] and one each of G9P[8] and G9P[untyped]. Only one parallel sample from an asymptomatic study child was positive for rotavirus (Table I). The index case was the sister of the cohort child and both samples were typed as G9P[8]. This pair of samples was subjected to further genetic analysis.

**Routine Surveillance Samples From Familial Contacts**

In addition, surveillance samples were collected from family members. Of the total of 452 families, 406 families gave at least one surveillance sample during the 3-year monitoring period and 208 families were compliant for more than 1 year. Stool samples were collected at 67% of the scheduled collection in these 208 families. A total of 2,805 surveillance samples were collected from family members of which 1,070 samples were collected from under five siblings of the children of the cohort and 1,735 were mother’s samples. There were 14 samples positive for rotavirus by VP6 PCR among the family contacts (0.43%). Three were mother’s surveillance stool samples (1 G2P[4] and 2 untyped) and 11 were from sibling surveillance samples (2 G10P[11], 3 G1, 1 P[4], and 5 untyped). However, none of the samples collected from children in the cohort at the same time were positive for rotavirus.

**Transmission Genetics**

Four pairs of samples (index cases and contacts) were available for comparison of the respective nucleotide sequences of the VP7 and VP4 genes. There was significant homology at the nucleotide level among the
pairs studied, ranging between 99% and 100% at the nucleotide level and between 99% and 100% at the deduced amino acid levels confirming that the strains infecting the index cases were identical to that detected in the contacts (Table II). Phylogenetic trees of the four pairs were also constructed and showed close clustering of the pair of samples with each other (Figs. 1 and 2).

**DISCUSSION**

This report documents evidence of intrafamilial transmission of rotavirus infection. Nucleic acid sequencing and phylogenetic analysis of strains from four pairs of concurrent index cases and their familial contacts demonstrated a high degree of homology. Transmission of rotavirus infection has been previously studied in day-care-centers, nurseries, hospitals, and nursing-homes, where there is an increased incidence of rotavirus infection and a high chance of cross contamination [Ryder et al., 1977; Halvorsrud and Orstavik, 1980; Rodriguez et al., 1982; Pickering et al., 1986]. When rotavirus infection is introduced by an index child into a closed group, about 50% of exposed children and 15–30% of exposed adults have been reported to have become infected [Wenman et al., 1979; Pickering et al., 1986; Rodriguez et al., 1987; Koopman et al., 1989]. The

![Fig. 1. Phylogenetic tree constructed from sequences of the VP7 gene of the four child-family pair of rotavirus strains and with other representative G-types using the Maximum Likelihood method. The tree was rooted using VP7 sequence of human Group C rotavirus Bristol strain. The bar indicates the variation scale.](image)

**TABLE II. Nucleotide Sequence Analysis of Cases and Contacts**

<table>
<thead>
<tr>
<th>Index case</th>
<th>Family contact case</th>
<th>VP7 identity %</th>
<th>VP4 similarity %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nucleotide</td>
<td>Amino acid</td>
</tr>
<tr>
<td><strong>Pair 1</strong></td>
<td>CRI 1444</td>
<td>CRI 1480</td>
<td>99.6</td>
</tr>
<tr>
<td><strong>Pair 2</strong></td>
<td>CRI 21113</td>
<td>CRI 21162</td>
<td>99.8</td>
</tr>
<tr>
<td><strong>Pair 3</strong></td>
<td>CRI 21428</td>
<td>CRI 21528</td>
<td>99.8</td>
</tr>
<tr>
<td><strong>Pair 4</strong></td>
<td>CRI 27226</td>
<td>CRI 27548</td>
<td>99.2</td>
</tr>
</tbody>
</table>

—, Sample not sufficient for sequencing of the VP4 gene.
data presented showed much lower transmission events, possibly because of the difference in the setting and higher exposure rates in the population which may result in constant boosting and possibly higher rates of protection from infection.

Earlier community based transmission studies have been variable in their results. The first report used detection of rotavirus in stools utilizing electron microscopy for screening adults in contact with infected children and documented a low attack rate of 0.17 per adult per year [Wenman et al., 1979]. However, another study documenting rise in complement fixing antibody titers, showed that up to 68% of members of the family of index cases with RV diarrhea had evidence of infection [Grimwood et al., 1983]. A proportion of these patients was negative for rotavirus antigen in the stool and showed only a positive antibody response and this could be a result of immunological boosting rather than definite infection.

The earlier studies were based on the epidemiological supposition that identification of rotavirus in the contact represented transmission of rotavirus from the index patient as they were temporally associated. No study has, so far, utilized newer molecular methods to prove that the strains were identical. A recent study documented that a similar genotype infected grandparents of a child with rotavirus diarrhea suggesting transmission of the infection [Awachat and Kelkar, 2006]. We have carefully monitored a group of children as part of a birth cohort and it is important to note that spread from these children were noted whilst they had either symptomatic or asymptomatic rotavirus infection. The viral load in asymptomatic infection is considerably less than those with diarrhea [Kang et al., 2004]. Despite this, asymptomatic children did spread the infection supporting the low infective dose of rotavirus evidenced by volunteer studies in the past [Kapikian et al., 1983; Ward et al., 1986]. This is of epidemiological significance as it means that infected asymptomatic individuals can perpetuate this infection in the community.

The other important finding that has emerged from this analysis is that the secondary attack rate of rotavirus infection is only 0.54% over 3 years of follow up or 0.18% per year, which is very similar to that reported previously [Wenman et al., 1979]. This value may be an underestimate because of the delay in collection of samples for familial contact screening.

Genotyping of the four transmitted strains showed that intrafamilial transmission was not restricted to any particular strain. Interestingly, the so-called neonatal G10P[11] strain was found to spread to an older child. Phylogenetic analysis of all the four pairs showed close similarity between the primary and secondary infecting strains (Figs. 1 and 2). This type of analysis has been

Fig. 2. Phylogenetic analysis of the VP4 genes of the three child-family pair of rotavirus strains and with other representative P-types using Maximum Likelihood Method. The bar indicates the variation scale.

Intrafamilial Rotavirus Transmission

used to document nosocomial and iatrogenic spread of other viruses like hepatitis C [Kozarek, 1997; Tallis et al., 2003]. To our knowledge this is the first instance wherein this form of analysis has been used to study the epidemiology of rotavirus infection. Recent efficacy of live attenuated rotavirus vaccines has been confirmed in large clinical trials [De Vos et al., 2004; Ruiz-Palacios et al., 2006]. Strain characterization and phylogenetic analysis could be used to confirm whether these “vaccine strains” can also spread to familial contacts including adults and other children.

REFERENCES


