Stability of Rotavirus in Human Stool at Different Temperatures

B. Kurmi*, K.N. Bhilegaonkar, H.V. Murugkar, A. Kumar, Z.B. Dubal, M. Dixit, S. Rawat, Anjay and A. Patiyal

Division of Veterinary Public Health
Indian Veterinary Research Institute, Bareilly – 243122

(Received 12.12.2011; accepted 05.11.2012)

ABSTRACT

The study was intended to test the stability of rotavirus in human stools at high temperatures corresponding to ambient temperatures in tropical countries like India. Results indicated that Rotavirus RNA could be detected by RNA-PAGE and RT PCR up to 15 days at temperatures of 37°C and 42°C which was the total period of this study, thus implying that the rotaviral RNA possesses a high degree of robustness which may allow it to remain infectious during high ambient temperatures that they may encounter during the subsequent faeco-oral spread and also enables it to be diagnosed through molecular techniques even if the samples are not maintained at optimum transport conditions.

Keywords: Rotavirus, stability, temperature

Introduction

Rotavirus has been recognized as the most common cause of severe gastroenteritis in a wide variety of mammalian species, mostly young ones including calves, piglets, sheep, kittens, puppies, mice, horses, rabbits, children and birds worldwide and is one of several viruses that cause infections often called stomach flu. Rotaviruses cause an estimated 130 million cases of gastroenteritis and 800,000 deaths in children between the ages of 6 month to 2 years in developing countries (Das et al., 2003). It has been reported that approximately 1.2 lakh deaths per year comprising of 20.8% of all the reported diarrhoeal cases in India are caused by rotavirus and account for 17% of world’s estimated rotavirus associated deaths (Jain et al., 2001). Rotavirus infection mainly spreads through faeco-oral route, respiratory route, person-person contact or contaminated food, water, surfaces, utensils etc. The infectious dose is presumed to be 10-100 virus particles. There is evidence that animal rotaviruses can infect humans and during co-infection of different rotaviruses, recombinants can arise by genetic exchange as one or several RNA segments may reassort with different strains. Maintenance of rotavirus infectivity in natural conditions is mainly attributed to its physical resistance, but varies with climatic factors, such as temperature, humidity, pH, etc. The incidence is higher during winter season in temperate climate whereas, no seasonal variations are documented for tropical countries. Rotaviruses may remain infectious on inanimate surfaces for prolonged periods (Sattar et al., 1986) and the roles of fomites and hands in its transmission are complimentary and may be synergistic (Ansari et al., 1988). Rotaviruses can retain their infectivity for several hours on the skin and the transfer of infectious rotavirus can occur readily between animate surfaces as well as between animate and nonporous inanimate surfaces (Samadi et al., 1983).

The knowledge of the stability of rotaviruses is a pre-requisite to know the epidemiology and risk factor of the virus and to develop precautionary
measures. It may also be helpful in the development of methods for the detection and concentration of virus from sewage-contaminated water supplies and to develop heat treatment standards to control its transmission. Thus present study was carried out to investigate the stability of rotavirus in faecal samples at different temperatures and to study the effect of these temperatures on the integrity of rotavirus RNA.

Materials and Methods

A human stool sample which was confirmed positive by both RNA-PAGE and RT-PCR for rotavirus, maintained in the Division of Veterinary Public Health, IVRI, Izatnagar was taken as the base study material. Aliquots of 0.1 g each of sample was taken in 30 microcentrifuge tubes. Two sets of 15 tubes each were kept at temperatures of 37°C and 42°C, respectively. One set of the sample was removed at every 24 h interval and processed for RNA extraction. The dsRNA was extracted by phenol chloroform method (Sambrook and Russell, 2001) and used for the detection of rotavirus by RNA PAGE and RT PCR techniques.

For detection of the 11 segmented dsRNA of rotavirus, the extracted RNA was subjected to RNA-PAGE using 7.5% separating gel and 5% stacking gel. The RNA samples with loading dye were loaded in gel. The gel was stained by silver staining for visualisation of the bands (Svensson, 1986).

The dsRNA was subjected to reverse transcription to produce cDNA using specific primers and M-MLV reverse transcriptase enzyme. The synthesised cDNA was amplified in thermocycler using Rota 1 and Rota 2 oligonucleotide generic primers (Forward- GAT CCG AAT GGT TGT GTA ATC CAA T and Reverse- AAT TCG CTA CGT TTT CTC TTG G ) targeting the sequence of VP7 gene which is conserved in all group A rotaviruses (Husain et al., 1995). After an initial denaturation of 5 min at 94°C, 30 cycles each of 1 min denaturation at 94°C, annealing of 1 min at 55°C and 2 min extension at 72°C was carried out, followed by a final extension for 7 min at 72°C. PCR products were stored at -20°C until further use. Agarose gel electrophoresis was carried out on an ethidium bromide-stained agarose gel for visualization of a 304 bp band and compared with a standard molecular weight marker of 1 kbp DNA ladder.

Results and Discussion

Rotavirus is a virus of immense public health importance. Transmission of rotavirus is dependent not only on its interaction with the host, but on its interaction with the environment outside of the host. The longer a virus can survive outside a host, the greater are its chances for transmission. In countries like India, high temperatures during the summer months are known to act as a natural biosecurity barrier against a large number of infectious viruses. However the stability of rotaviruses at high temperature plays an important role in persistence of these viruses in the environment and consequently pose the risk of infecting a large population. Survivability of the virus in the environment, particularly in the faeces, therefore is an important area of study to determine its infectious potential. In view of the seriousness of rotavirus infections, rapid and accurate diagnosis of the infection is of paramount importance. Amongst the various diagnostic tests employed for the diagnosis of rotavirus from faecal samples, SDS-PAGE following silver staining and RT-PCR are now being extensively used as a confirmatory method for detection of rotavirus (Hussain et al., 1995).

The accurate diagnosis of rotaviral infection with these methods, however, depends upon the integrity of rotavirus RNA and the stability of the RNA at higher temperatures. In our study, rotavirus RNA could be detected by RNA-PAGE (Fig. 1) and RT-PCR (Fig. 2) for up to 15 days which was the total period of this study (Table 1). A positive RT-PCR assay most likely indicates that intact virus particles, not naked RNA, are present in the sample (Abbaszadegan et al., 1999). In the present study, all the RNA segments of the treated rotavirus could be demonstrated in the RNA-PAGE indicating the likelihood of the virus retaining its viability even at higher temperatures. The study carried out indicated that the integrity of the rotaviral RNA was maintained even up to 15 days at temperatures of 37°C and 42°C, implying that rotaviruses are highly stable at high temperatures which allows them to remain infectious during the
Table 1. Effect of temperatures (37°C and 42°C) on the stability of Rotaviral RNA

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Time of exposure (days)</th>
<th>37°C RNA PAGE</th>
<th>RT-PCR</th>
<th>42°C RNA PAGE</th>
<th>RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>NC</td>
<td>+</td>
<td>+</td>
<td>NC</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>NC</td>
<td>NC</td>
<td>+</td>
<td>NC</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>NC</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>NC</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>NC</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>NC</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>13</td>
<td>NC</td>
<td>+</td>
<td>NC</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+ positive, - negative)

Fig. 1. Detection of rotaviral dsRNA in extracted faecal samples by RNA-PAGE showing 11 RNA segments of group 1 rotavirus
Lane 1-9: Samples; Lane 10: Positive control

Stability of Rotavirus in human stool

various conditions that they may encounter between one host and another. Many workers have reported that rotavirus maintains its infectivity for 7-9 months at room temperature (25°C) in faeces (Woode and Bridger, 1975; Woode, 1978). Another study demonstrated the infectivity of porcine rotavirus maintained for 32 months at approximately 10°C in the original stool specimens (Ramos et al., 2000). In view of these observations, it is suggested that a more comprehensive study with longer storage durations needs to be carried out to know the exact period of rotaviral RNA stability.

Fig. 2. Detection of rotavirus by RT-PCR. PCR product (304 bp) of VP7 gene of human group A rotavirus
Lane M: DNA marker of 100 bp; Lane 1-7: PCR product of 304 bp
References


