Chapter 2

Review of literature
2.1 Discovery

During 1950-1960, virologists discovered a number of enteric viruses with the advent of tissue culture technology, but none of them was found to be an important etiological agent of infectious diarrhea. In the 1960s, virus like particle were visualized in the intestinal tissue of diarrheic mice, rectal swab of healthy vervet monkey and stool of sample from diarrheic calves (Adam and Kraft, 1963; Malherbe et al., 1963; Mebus et al., 1969). These were designated as EDIM (Epizootic Diarrhea of Infant Mice), SA-11 (Simian strain) and NCDV (Nebraska Calf Diarrhea Virus) respectively in due course of time. The discovery of 27nm Norwalk virus and its association with epidemic viral gastroenteritis in older children and adults in 1972 by Kapikian et al. was followed by the discovery of the 70nm human rotavirus and its association with severe endemic diarrhea in infants and young children in 1973 by Bishop et al. In the past three decades with the advances in molecular diagnostic technology several different groups / types of rotaviruses have been reported in sporadic infections and epidemics of gastroenteritis.

2.2 Rotavirus morphology and structure

Rotavirus, a member of the family Reoviridae is an icosahedral virus with a distinctive wheel-like shape as visualized by negative-stain electron microscopy (Figure. 2.1). Complete particles have a double-layered capsid and measure about 70 nm in diameter. Single layered particles measure about 55 nm. Within the inner capsid is the 37-nm core, which contains the RNA genome comprising of 11 segments of double stranded RNA, each coding for structural viral proteins (VP) or nonstructural proteins (NSP). The viral genome is located within the inner layer (core) that is formed by three viral proteins VP1,VP2 and VP3. The middle layer consists of trimerized VP6 (coded by gene segment 6), which makes up over 51% of the virion. The outermost layer of the virus particle is composed of two surface viral proteins, VP4 and VP7. VP7 is a glycoprotein coded by gene segment 7, 8 or 9. It forms the smooth outer capsid surface that is perforated by 132 channels.
penetrating the virion and reaching the viral core. VP4 is a protease-sensitive protein encoded by gene segment 4. It forms 60 spike-like structures that protrude from the viral surface. The spikes of VP4 are about 10-12 nm in length with wide knob like structure at distal end (Flewett, 1976; Elias, 1977) (Figure 2.2).

Fig 2.1: Electron micrograph of rotavirus particles from the filtrate of a stool collected from a child with acute gastroenteritis. 70-nm particles possess distinctive double-shelled outer capsid. Bar = 100 nm.


Fig 2.2: Schematic diagram depicting rotavirus structure
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2.3 Genome organization

The rotavirus genome consists of 11 segments of double stranded RNA enclosed in three concentric layers of capsid proteins, the outer, intermediate, and inner capsids (Prasad et al., 1988; Shaw et al., 1993; Estes, 2001) that range in size from 0.6 to 3.3 kilo base pairs with total genomic size of approximately 18 kilo base pairs. Each positive-sense RNA segment starts with a 5'-guanidine followed by a set of conserved sequences that are a part of 5' noncoding sequences. An open reading frame coding for the protein product and ending with the stop codon follows, and then another set of noncoding sequences, which contains a subset of conserved terminal 3' sequences and which ends with two 3'-terminal cytidines is found. The lengths of the 3' and 5' noncoding sequences vary for different genes. No poly (A) signal is found at the 3' end of the genes. All the rotavirus gene sequences are A+T rich ranging from 58 to 68% (Estes and Cohen, 1989). Rotavirus RNA segments fall into 4 size classes, which is evident by polyacrylamide gel electrophoresis (PAGE) of RNA. The RNA segments are numbered in the order of migration during polyacrylamide gel electrophoresis with the slowest RNA segment designated gene 1 and so on. In general, Group A has 4 large segments, 2 medium sized segments, three smaller and 2 smallest segments (referred to as 4,2,3,2 pattern). This migration pattern is called as Long pattern and is a characteristic of all subgroups II rotaviruses i.e. serotypes G1, G3, G4, G9 (Figure 2.3a). Some group A rotaviruses do not display this characteristic migration pattern. In such strains, the 11th RNA segment migrates more slowly than usual, yielding a Short pattern which is a characteristic of all subgroups I human rotavirus i.e. serotypes G2 (Sajal et al., 1989) (Figure 2.3a). In case of mixed infections, more than 11 segments are expected (Figure 2.3b).
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Fig 2.3: Electrophoretic migration of Rotavirus ds RNA depicting
(a) Long and Short Pattern   (b) Mixed infection

The genome of rotavirus is highly diverse and three primary sources of this diversity have been proposed: point mutations, reassortment and rearrangement of the viral genome. The genome segments encode for 6 structural proteins, designated as viral particles (VP1, VP2, VP3, VP4, VP6, and VP7) and 6 non-structural proteins (NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6). The details of genome length, protein product, size, location, the functions and properties of each gene are all summarized in Table 2.1.
### Table 2.1: Rotavirus genome and proteins

<table>
<thead>
<tr>
<th>Genome Segment Size (bp)</th>
<th>Length of noncoding sequences</th>
<th>Protein Product(s)</th>
<th>Protein Size No. of AA / Mole Wt (Daltons)</th>
<th>Location in Virus Particle</th>
<th>Functions and Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (3302)</td>
<td>18 17</td>
<td>VP1</td>
<td>1088 / 125,005</td>
<td>Core</td>
<td>RNA-dependent RNA polymerase, ssRNA binding, complex with VP3</td>
</tr>
<tr>
<td>2 (2690)</td>
<td>16 28</td>
<td>VP2</td>
<td>880 / 102,431</td>
<td>Core</td>
<td>RNA binding, required for replicase activity of VP1</td>
</tr>
<tr>
<td>3 (2591)</td>
<td>49 34</td>
<td>VP3</td>
<td>835 / 98,120</td>
<td>Core</td>
<td>Guanylyltransferase, Methyltransferase, ss-RNA binding, complex with VP1</td>
</tr>
<tr>
<td>4 (2362)</td>
<td>9 22</td>
<td>VP4 (VP5* VP8*)</td>
<td>770 / 86,782 529 / 60,000 247 / 28,000</td>
<td>Outer capsid</td>
<td>Hemagglutinin, neutralization antigen, protease-, enhanced activity, virulence, putative fusion region, cell attachment, protection</td>
</tr>
<tr>
<td>5 (1611)</td>
<td>30 93</td>
<td>NSP1</td>
<td>495 / 56,654</td>
<td>Nonstructural</td>
<td>Basic, zinc finger, RNA binding</td>
</tr>
<tr>
<td>6 (1356)</td>
<td>23 139</td>
<td>VP6</td>
<td>397 / 44,816</td>
<td>Inner capsid</td>
<td>Hydrophobic, inner subgroup antigen, protection (?mechanism)</td>
</tr>
<tr>
<td>7 (1104)</td>
<td>25 131</td>
<td>NSP3</td>
<td>315 / 34,600 Da</td>
<td>Nonstructural</td>
<td>Acidic dimer, binds 3' end of viral mRNAs, interacts with eIF4G1, analog of cellular poly(A)-binding protein, inhibits host translation</td>
</tr>
<tr>
<td>8 (1059)</td>
<td>46 59</td>
<td>NSP2</td>
<td>317 / 36,700</td>
<td>Nonstructural</td>
<td>Basic, RNA binding, NTPase activity</td>
</tr>
<tr>
<td>9 (1062)</td>
<td>48 33</td>
<td>VP7</td>
<td>326 / 37,368</td>
<td>Outer capsid glycoprotein</td>
<td>RER integral membrane glycoprotein, inner, neutralization antigen, two hydrophobic NH2 terminal regions, Ca2+ binding, protection</td>
</tr>
<tr>
<td>10 (751)</td>
<td>41 182</td>
<td>NSP4</td>
<td>175 / 20,290</td>
<td>Nonstructural</td>
<td>RER transmembrane glycoprotein, role in morphogenesis, enterotoxin, secreted cleavage product, protection</td>
</tr>
<tr>
<td>11 (667)</td>
<td>20 49</td>
<td>NSP5</td>
<td>198 / 21,725</td>
<td>Nonstructural</td>
<td>Basic, phosphoprotein, RNA-binding, protein kinase, interacts with NSP2 and NSP6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NSP6</td>
<td>92 / 11,012</td>
<td>Nonstructural</td>
<td>Interacts with NSP5, present in viroplasms and most virus strains</td>
</tr>
</tbody>
</table>

Key:
- mRNA: messenger RNA
- tRNA: transfer RNA
- 5' 3': 5' to 3' direction
- Core: viral core protein
- Outer capsid: outer capsid protein
- Inner capsid: inner capsid protein
- RER: rough endoplasmic reticulum
- NTPase: nucleoside triphosphatase
2.3.1 Structural Proteins

The Core Proteins

The core proteins VP1, VP2 and VP3 function as structural proteins. Each of the core protein plays a role in the process of RNA transcription and replication.

VP1

VP1 forms the sub core part of virus particle having MW of 125,000 and 1088 amino acids. This protein is the viral RNA polymerase and functions as both the viral transcriptase and replicase. VP1 gene is well conserved and shows 63% nucleotide similarity between the more distant rotaviruses. This protein does not react in immunoprecipitation and immuno-blot with many hyperimmune antisera of purified virus particles or with serum of infected children or animal. VP1 reactive antiserum can be obtained by using VP1 protein synthesized in baculovirus expression system. Thus, this protein is immunogenic but this nature depends on the degree to which the protein is accessible to the immune system (Estes and Cohen, 1989; Estes, 2001).

VP2

VP2 is major core protein of 880 amino acids having molecular weight 102,431. It binds to ds RNA and DNA, however binding activity of this protein to RNA is not sequence specific. Transcriptase activity of VP1 is functional only in presence of VP2 protein (Patton et al., 1997). This protein is partially exposed on the single shelled particle and is highly immunogenic. It is involved in mRNA binding. Periodic array of Leucines at every seventh residue in between amino acid 536 and 665 is seen. This region represents the part of protein that mold it to interact with target site on DNA or dsRNA (Estes and Cohen, 1989; Estes, 2001).

VP3

VP3 is minor sub core protein of 835 amino acid and having MW 98120 and. This protein has RNA polymerase activity and guanyltransferase
activity that caps viral and non-viral RNAs in vitro. Thus, this protein is a multifunctional capping enzyme (Chen et al., 1999).

**The Capsid Proteins**

**VP4**

VP4 is present on the outer capsid as a series of 60 short spikes about 10-12 nm in length, with a knob-like structure at the distal end. VP4 is a non-glycosylated protein, has a molecular weight of 86,732 and constitutes 1.5% of virion protein. VP4 is the viral attachment protein both in vivo and in vitro (Ludert et al., 1996) and is a determinant of viral growth in vitro and virulence factor in vivo (Hoshino et al., 1995; Bridger et al., 1998). Trypsin treatment cleaves this protein into VP8 (amino-terminal, molecular weight about 28,000) and VP5 (molecular weight about 60,000) proteins which enhances viral infectivity in vitro (Kapikian et al., 2001). There are two-trypsin cleavage sites arginine 241 and arginine 247 in SA11 4fm strain. In several human rotaviruses additional trypsin cleavage site either by lysine or arginine at 246 is found. VP8 contains the most sequence variations in VP4 and determines the viral P genotypes (Kapikian et al., 2001). Antibodies against VP8 neutralize the virus by inhibiting viral attachment (Ruggeri et al., 1991). VP8 is responsible for binding to sialic acid and hemagglutinating activity in many animal strains but absent in most human rotavirus strains (Fiore et al., 1991). VP5 functions as a rotavirus attachment protein and plays a role in cellular entry of rotavirus (Denisova et al., 1999).

**VP6**

VP6 protein is located on the inner capsid of the virus. It has a molecular weight of 44,816 and constitutes 51% of the virion protein. It is the major structural component of virions and plays a key role in virion structure due to its interactions with both; the outer capsid proteins VP4 and VP7 and the core protein VP2. VP6 spontaneously forms trimers and and there are 780 molecules of VP6 per virion. It is extremely stable. Due to these characteristics and presence of conserved epitopes among many virus
strains, this antigen is targeted in diagnostic assays. VP6 is a hydrophobic protein that is highly antigenic and immunogenic and may play a role in inducing viral immunity. VP6 specifies subgroup reactivity, whereas a separate domain (s) of this protein contains the major determinant of group A rotavirus antigenic reactivity (Mattion et al., 1994). The amino acid composition of the VP6 protein of several group A, B, and C rotavirus strains has been determined. Immunodominant sites of VP6 that contain group-specific epitopes for the group A rotaviruses have been localized in four regions on VP6 (amino acid residues 32 to 64, 155 to 167, 208 to 294 and 380 to 397) (Kohli et al., 1983). In addition, amino acid residues 172, 305 and 315, and region 296 to 299 were reported as contributing to subgroup epitope (Lopez et al., 1994; Tang et al., 1997). It was assumed until recently that all human and animal rotavirus share common antigenic determinants present predominantly on VP6. However, several human and animal rotavirus strains do not share these determinants. Rotaviruses that share the group antigen are now classified in group A, whereas rotaviruses that lack this antigen are classified as non-group A viruses (Bridger, 1994; Saif and Jiang, 1994; Tao, 1988).

**VP7**

VP7 protein is encoded by gene segment 7, 8 or 9 depending on the viral strain. This protein has a MW of 37,368 and is located on the outer capsid forming the smooth external surface of the outer shell. It constitutes 30% of the virion protein, thus making it the second most abundant rotavirus protein as well as the major constituent of the outer capsid. There are 780 molecules of VP7 arranged around 132 aqueous holes on the outer capsid. The VP7 glycoprotein is the major neutralization antigen of rotaviruses detected by hyperimmune antiserum and has served as the basis for identification of serotypes (Hoshino and Kapikian, 1996). Three major antigenic regions (A, B and C) have been identified on VP7. All three regions can be the target of cross reactive or serotype specific antibodies (Kapikian et al., 2001). VP7 is a glycoprotein that contains one to three potential sites for N linked glycosylation.
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The amino acid sequence of the VP7 of strains belonging to each of the 14 serotypes has been deducted from the nucleotide sequence of the VP7 gene. A comparison of the deduced amino acid sequences for all 14 serotypes indicates that there are nine regions of the linear amino acid sequence of VP7 that are highly divergent (Green et al., 1989; Nishikawa et al., 1989). Each of these regions is highly conserved in rotavirus strains within the same serotype. There is sufficient conservation of sequence of human rotavirus strains within a serotype that it is possible to predict the serotype of an isolate by direct sequence analysis of two of these variable regions (VR5 or A and VR8 or C) of VP7 (Green et al., 1988).

2.3.2 Nonstructural proteins

The rotavirus genome codes for six nonstructural proteins (NSP1,2,3,4,5,6), which are involved in replication (NSP1,2,3,5,6) and morphogenesis (NSP4). All of these nonstructural proteins except for NSP4 interact with nucleic acid. Most of the nonstructural proteins possess a basic charge and they function as a part of the replicating complex, as chaperons to transport RNA or proteins to the sites of RNA replication, translation, assembly or to gather the genome segments for packing.

**NSP1**

The amino acid sequence of this protein is highly variable of all rotavirus proteins. It binds to zinc and RNA.

**NSP2**

NSP2 is present at high concentration in the viroplasm, where replication occurs. NSP2 helps in packaging the viral mRNA into core like replication intermediates through the NTPase activity.

**NSP3**

NSP3 is a sequence specific RNA binding protein that binds the nonpolyadenylated 3’ end of the rotavirus mRNAs (Piron et al., 1999). NSP3 binds to cellular translation initiation factor (eIF4G1) and enhances the translation of viral mRNA (Vende et al., 2000).
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**NSP4**

NSP4 is an endoplasmic reticulum (ER) specific glycoprotein encoded by gene segment 10 of group A rotavirus and has a molecular weight of 20,290. This protein plays an important role in rotavirus morphogenesis and functions as an intracellular receptor for converting double layered particles in cytoplasm to triple layered particles in ER.

NSP4 (glycoprotein), an enterotoxin having cytosolic domain is produced while replication. It interacts with enterocytes of duodenum and small intestine to initiate budding. NSP4 is retained within endoplasmic reticulum membrane with a C terminal domain projected into cytosol and two N linked high mannose oligosaccharide residues within a short luminal region. It alters intracellular calcium homeostasis and reduces cell viability. A peptide derived from cytoplasmic domain of NSP4 plays a major role in pathogenesis of rotavirus. This binding of NSP4 induces Ca\(^{2+}\) mobilization, which changes the ionic balance and leads to fluid secretion that makes NSP4 an attractive target for the development of vaccine/drug against all rotaviruses infections. However, [Ca\(^{2+}\)] mobilization by NSP4 has not been demonstrated in human intestinal cells, and the cellular mechanisms by which NSP4 may alter intracellular calcium homeostasis within enterocytes remain unknown (Morris and Estes, 2001).

**NSP5**

NSP5 protein is encoded by segment 11 of rotavirus genome. It is a phosphoprotein with autocatalytic kinase activity and is present in infected cells a various isoforms (Poncet et al., 1997; Torres Vega et al., 2000). NSP5 plays a role in viral replication by forming oligomers that are localized in the viroplasm and associated with NSP2 protein.

**NSP6** It interacts with NSP5 and plays a regulatory role in viral replication (Torres Vega et al., 2000).
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2.4 Classification of rotaviruses

Rotaviruses are classified as a genus in the family Reoviridae, members of this family are found to inhabit both the respiratory and enteric tracts of man and animals. Initially these genera were not associated with any known diseases and hence called “orphan” (Sabin, 1959). Out of nine reoviridae genera, four genera, Reovirus, Orbivirus, Rotavirus and Coltivirus infect humans and remaining genera include viruses that infect fish, insects and plants.

On the basis of the cross reactive antigenic epitopes located on the major structural protein VP6, rotaviruses are classified into groups (A to G) (Bridger, 1994; Saif and Jiang, 1994). Group A rotaviruses are major pathogens in humans and animals. Of the non-group A rotaviruses (groups B to G) only groups B and C have been detected in humans so far. Group B viruses have been recovered from humans, pigs, cattle, sheep, goats, and rats; group C viruses from humans, pigs, cattle, dogs, and ferrets; group D viruses from chickens, pheasants, and turkeys; group E viruses from pigs; groups G virus from chickens (Devitt and Reynolds, 1993; Bridger, 1994; Saif and Jiang, 1994; Gueguen et al., 1996; Otto et al., 1999).

2.5 Group A rotaviruses

2.5.1 Groups, subgroups, electrophoretotypes and serotypes / genotypes in group A rotaviruses

Rotaviruses have three important antigenic specificities, based on group, subgroup and serotype that are determined by the viral proteins (Coluchi et al., 2002). Group specificity is determined by the inner capsid protein VP6, that divides rotaviruses into seven groups (A-G) (Kapikian et al., 2001; Coluchi et al., 2002). Group A rotaviruses can be further divided into two sub groups (I and II) based on the specificity of the epitopes that are present on VP6 (Coluchi et al., 2002).

The identification of rotaviruses according to their G and P serotypes is based on both VP7 (G-type) and VP4 (P-type) antigenic specificities. Both VP7 and VP4 proteins induce neutralizing antibodies and are involved in
protective immunity. Based on these proteins, a binary system of classification has been developed. Genotypes of VP4 and VP7 are determined by sequence / hybridization analysis, whereas serotypes are determined by reactivity with polyclonal or monoclonal antisera (Estes, 2001). A correlation between genotype and serotype has been established for VP7, however, not for VP4 though sequence variation between amino acids 84 and 180 has been suggested for this purpose (Larralde et al., 1991). These proteins are important targets for vaccine development (Parashar et al., 1998).

Based on the diversity of the VP7 and VP4 encoding genes, rotaviruses are classified into 27G and 35P types respectively (Esona et al., 2010; Abe et al., 2011; Matthijnssens et al., 2011). Out of these, 11 are known to cause human infections. Further, classification of these genotypes into lineages and sublineages has been also described on the basis of point mutations occurring naturally in the corresponding genes. Currently, a more complete classification system based on all 11 genome RNA segments has been proposed by Matthijnessens et al (2008).

Rotavirus strains are classified into electropherotypes (E types) on the basis of differences in the relative migration pattern of RNA segments in polyacrylamide gel electrophoresis (PAGE), thereby creating more opportunities for strain diversification. The most common major E-type patterns are designated "long" and "short" based on the fact that short-E-type strains have a striking reduction in the migration rate of segment 11 due to duplication within the 3' untranslated region of this segment (Matsui et al., 1990).

2.5.2 Replication

Rotaviruses replicate exclusively in the cytoplasm. The virion enters the cell by endocytosis (or direct membrane penetration if activated by protease), and the outer shell of the double capsid is removed in lysosomes with the liberation of 50-nm subviral particles, thus activating the viral RNA polymerase (transcriptase). RNA positive-sense transcripts induce the production of proteins and are a template for the production of antisense
strands, which remain associated with the positive-sense strand. About 8 hours after infection, viroplasmic inclusions of dense granular material, representing newly synthesized proteins and RNA, accumulate in the cytoplasm. Viral RNA is packaged into core particles, and viral capsid proteins assemble around the cores. These particles accumulate in vesicles of the endoplasmic reticulum and leave the viroplasm by budding through membrane of the endoplasmic reticulum, where they acquire the outer capsid protein. The budding process (plus transient acquisition of an envelope) is unique to rotaviruses among members of the family Reoviridae. Particles are released by cell lysis.

2.5.3 Transmission

Rotavirus is transmitted by the faecal-oral route (Fig 2.4), via contact with contaminated hands, surfaces and objects (Butz et al., 1993) and possibly by the respiratory route (Dennehy, 2000). The faeces of an infected person can contain more than 10 trillion infectious particles per gram (Bishop, 1996) only 10–100 of these are required to transmit infection to another person (Graham et al., 1987). Rotaviruses are stable in the environment and have been found in estuary samples at levels as high as 1–5 infectious particles per US gallon (Rao et al., 1984).

Fig 2.4: Route of rotavirus entry
2.5.4 Clinical Manifestations

Incubation period of rotavirus infection is about 1-3 days (Davidson et al., 1975). The illness caused by rotavirus infection, vary in severity from subclinical infection, mild diarrhoea to chronic, occasionally fatal dehydration. The onset of illness is abrupt, with watery diarrhoea, fever, vomiting, dehydration and electrolyte imbalance. The duration of symptoms can vary widely.

Rotavirus gastroenteritis is a mild to severe disease characterized by vomiting, watery diarrhoea, and low-grade fever. There is an incubation period of about two days before symptoms appear (Maldonado and Yolken, 1990). Symptoms often start with vomiting followed by four to eight days of profuse diarrhoea. Dehydration is more common in rotavirus infection than in most of those caused by bacterial pathogens, and is the most common cause of death related to rotavirus infection (Bernstein et al., 1991).

Rotavirus A infections can occur throughout life: the first usually produces symptoms, but subsequent infections are typically asymptomatic (Bishop, 1996) as the immune system provides some protection (Velázquez et al., 1996). Consequently, symptomatic infection rates are highest in children under two years of age and decrease progressively towards 45 years of age (Cameron et al., 1978; Koopman et al., 1989). Infection in newborn children, although common, is often associated with mild or asymptomatic disease (Grillner et al., 1985; Hrdy, 1987). The most severe symptoms tend to occur in children six months to two years of age, the elderly, and those with compromised or absent immune system functions. Due to immunity acquired in childhood, most adults are not susceptible to rotavirus; gastroenteritis in adults usually has a cause other than rotavirus, but asymptomatic infections in adults may maintain the transmission of infection in the community (De Champs et al., 1991). Symptomatic reinfections are often due to a different rotavirus A serotype (Linhares et al., 1988; Butz et al., 1993).
2.5.5 Pathogenesis

Rotavirus infect mature enterocytes in the mid and upper part of the villi in the small intestine, resulting in cell lysis and stripping of the superficial layer of epithelial cells from the underlying lamina propria (Fig 2.5). The incubation in young children is 24-48 hrs but may be >3 days in infected adults. In humans, rotavirus infections appear to be restricted to small intestine. Rotavirus replication occurs within the mature epithelial cells, lining the upper portion of villi, resulting in the shortening and atrophy of villi, mononuclear cell infiltration in the lamina propria, distended cisternae of the endoplasmic reticulum, mitochondrial swelling and irregular microvilli (Moon, 1994). Rotavirus attachment and entry into cells constitute a multistep process. It has been suggested that rotaviruses interact first with a sialic acid receptor and then with a sialic acid -independent receptor (Jolly et al., 2000) part of lipid micro domains (Guerrero et al., 2002) and recognize integrins (Coulson et al., 1997). Two hypothesis have been proposed for entry of rotavirus into target cell: through direct entry or fusion (Kaljoit et al., 1999) and through Ca^{2+}-dependent endocytosis (Ruiz et al., 1997). In the Ca^{2+}-dependent endocytosis process infectious rotavirus is endocytosed into the cytoplasm with a very low Ca^{2+} concentration that leads to Ca^{2+} afflux from the vesicles to the cytoplasm. Once the endosome Ca^{2+} concentration equilibrates with the cytoplasm, below the level for stability of outer capsid, the virus loses its outer proteins and then lyses with the vesicle membrane, permitting the virus to escape to the cytoplasm (Ruiz et al., 2000).

Rotavirus infection is associated with the development of chronic diarrhea, abnormal gastric motor function, malabsorption of disaccharidases (maltase, sucrase and lactase) and post enteritis weight loss (Khoshoo et al., 1990). Rotavirus infection also results in mildly raised levels of serum aminoaspartate and aminotransferase resulting from damage to gut (Kovacs et al., 1986; Grimwood et al., 1988). Histologic studies have also shown that diarrhea may occur in the absence of mucosal epithelial lesions, suggesting that other mechanisms may be involved in the induction of rotavirus gastroenteritis (Kohler et al., 1990). The NSP4 protein encoded by gene
segment 10 may act as a viral enterotoxin. Production of NSP4 leads to elevations in calcium levels and induces secretory diarrhea in a similar way to bacterial gut infections (Ball et al., 1996; Morris et al., 1999). It has been hypothesized that rotavirus evokes intestinal fluid and electrolyte secretion by activation of the enteric nervous system located in the intestinal wall (Lundgren et al., 2000).

Rotavirus infections rarely cause other complications (Haffejee, 1991; Ramig, 2007). There are rare reports of complications involving the central nervous system (CNS) where rotavirus was detected in the fluid of the CNS in cases of encephalitis and meningitis (Pager et al., 2000a; Kehle et al., 2003; Goto et al., 2007). Recent studies have confirmed that rotavirus infection is not always confined to the gut, but can cause viremia (Widdowson et al., 2005; Chitambar et al., 2008).

Fig 2.5 Rotavirus Pathogenesis

2.5.6 Diagnosis

Diagnosis of infection with rotavirus normally follows diagnosis of gastroenteritis as the cause of severe diarrhoea. Most children admitted to hospital with gastroenteritis are tested for rotavirus A (Patel et al., 2007). Specific diagnosis of infection with rotavirus A is made by identification of the virus in the patient's stool by enzyme immunoassay. There are several licensed test kits in the market which are sensitive, specific and detect all serotypes of rotavirus A (Beards et al., 1984; Smith et al., 1993). Other
methods, electron microscopy and polyacrylamide gel electrophoresis, are used in research laboratories (Beards et al., 1988). Reverse transcription-polymerase chain reaction (RT-PCR) can detect and identify all species and serotypes of human rotavirus (Fischer and Gentsch, 2004).

2.5.7 Propagation in cell culture

Initial attempts to propagate human rotaviruses in tissue culture were without success. Various scientists have tried the isolation of rotaviruses. In 1963, Malherbe and Harwin (1963) described the isolation of a 70-nm virus designated SA11 (simian agent 11) in Vervet monkey kidney cell culture. In 1967, Malherbe and Strickland-Cholmeley (1967) described another virus similar to SA11, the O (offal) agent, which was isolated in vervet monkey kidney cell culture from the mixed washing of intestines of cattle and sheep. In 1969, Mebus et al. (1969) demonstrated the presence of 70-nm virus particles in stools from calves. In 1971, Mebus et al. (1971) reported successful cultivation of the Nebraska calf diarrhoea virus (NCDV) in primary fetal bovine cell cultures. Wyatt et al. (1974; 1976) succeeded in propagating the human rotavirus in organ cultures of human foetal intestine. Banatvala et al. (1975) and Bryden et al. (1977) showed rotaviruses to replicate to some extent in particular cells (IB-RS2 pig kidney, continuous monkey kidney LLC-MK2).

The first human rotavirus to be grown efficiently and reproducible in cell culture was a mutant of the Wa human rotavirus (VP7 serotype 1) that emerged during 11 serial passages of this strain in gnotobiotic piglets (Wyatt et al., 1980). Efficient growth of the mutant required pretreatment of virus with trypsin. Later, efficient techniques were developed for the direct cultivation of most human rotaviruses in cell cultures. Sato et al. (1981) without serial passage in animals, succeeded in in-vitro cultivation of human rotavirus using the roller tube cultures of MA 104 cells. In the year 1982, Urasawa et al. (1982) succeeded in propagation of the human rotavirus (HRV) by serial passage in tissue culture. Authors also made a successful adaptation of HRV to stationary cultures and subsequently, demonstrated three human rotavirus
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serotypes by plaque neutralization. The development of techniques for cultivation of human rotaviruses in cell culture made it possible to identify virus serotype by conventional assays, which include (a) neutralization of cytopathic effect (CPE), (b) reduction of virus yield in roller-tube or multi-well plate cultures, (c) plaque or focus reduction, and (d) viral interference (Hoshino and Kapikian, 1996).

2.6 Epidemiology of group A rotavirus infections in children

2.6.1 International Status

Group A rotaviruses, are the major etiological agents of acute gastroenteritis in infants and young children (Figure 2.6), cause 35% to 50% of hospitalizations during the first two years of life, producing a significant disease burden (Kapikian et al., 2001).

Fig 2.6: Infections causing gastroenteritis in the developed and developing world (adapted from Kapikian 2001)

The global mortality rate of gastroenteritis due to rotavirus has been estimated to be 527,000 deaths annually (Parashar et al., 2009). Worldwide, it is estimated that rotavirus cause 114 million episodes of infantile gastroenteritis requiring only home care, 24 million cases requiring a visit to a clinic and 2.4 million hospitalizations (Glass et al., 2006). Thus, by the age of 5 years almost all children will require a clinic visit and 1 in 65 children requires hospitalization. In developing countries 1205 children die from
rotavirus disease each day and 82% of these deaths are in children in the poorest countries (Parashar et al., 2003).

High incidence of rotavirus disease exists in both developed and developing countries (Kapikian et al., 2001), this suggests that improvements in water supply, hygiene practices and sanitation will not lower the transmission of the virus. This underscores the urgent need for intervention measures such as vaccination, particularly to prevent childhood deaths in developing nations (Glass et al., 2005). Although the incidence of rotavirus in developed and developing countries is similar there are significant differences in terms of age, seasonality, serotype prevalence, mortality and access to medical care.

The global distribution of rotavirus serotypes till date, have shown G1-G4 to be the majority of the strains detected. However, recently G9 have emerged and become the important serotype in various parts of the world such as Australia, Ghana, India and Brazil (Kirkwood et al., 2002; Armah et al., 2003, Gentsch et al., 2005, Santos and Hoshino, 2005). G8 strains have also emerged as important strains in Africa (Gentsch et al., 2005, Santos and Hoshino, 2005).

A recent review encompassing 124 studies published on the global distribution of rotavirus serotypes and genotypes during 1989-2004 in 52 countries from 5 continents, included 45,571 typed specimens (Santos and Hoshino, 2005). The most common G-P combinations detected were G1P[8] (64.7%), G2P[4] (12%), G3P[8] (3.3%) and G4P[8] (8.5%) that made up 88.5% of the total. The distribution among the 5 continents however varied. G1-G4 accounted for >90% of the rotavirus infections in North America, Europe and Australia. However, in South America and Asia they represented only 68% and in Africa 50%. G5, G8 and G9 strains appeared focally. Unusual strains were higher in Africa (27%), Asia (14%) and South America (11%), than in North America (5%), Europe (1.4%) and Australia (0.1%).

WHO sentinel hospitalized rotavirus surveillance networks from 2001-2008 showed 40% of hospitalizations among children aged <5 years due to diarrhea worldwide (WHO, 2008). The most common strains were G1-G4 and G9, and the distribution of these strains varied markedly across regions. A
total of 62,584 hospitalized patients aged <5 years were tested for rotavirus. The median rotavirus detection rate was 40%, which was high in the South East Asian and Western Pacific Regions (45%) but lowest in the Regions of the Americans (34%). The positivity reported in this surveillance study was greater than those percentages reported in two literature reviews earlier, one during 1986-1999 and other during 1990-2004, which indicated a median rotavirus detection rate of 22% and 29% respectively (Parashar et al., 2003; Parashar et al., 2009). Recent studies published during 2000-2004 reported a median detection rate of rotaviruses as 39% (Parashar et al., 2006b). A similar report from Dhaka Hospital in Bangladesh during 1993-2004 also indicated that the percentage of childhood diarrhea hospitalization attributed to rotavirus had increased from 22% during 1993-1995 to 42% during 2002-2004 (Tanaka, et al., 2007).

Review of rotavirus prevalence in Africa carried out from 1975-2006 involved 189 studies with children <5 years of age. Average prevalence rate was 30% (17-38%). The data showed presence of the most common genotypes G1(25%), G4(16%), G2(13%), G3(12%), P[8](37%), P[6](35%) and P[4](11%) in all these studies with variation in the distribution of common G and P serotypes from 1996-2006 (Waggle et al., 2008). Another such review of last 30 years (1975-2005) in Kenyan children showed rotavirus prevalence of 6-65% throughout these years. G1 was the most predominant type till the year 2002 with recent emergence of G9 and G8 to a less extent (Kiulia et al., 2008). Molecular epidemiological studies in north eastern and middle belt regions of Nigeria detected unusual strains like G2P[6] and G8P[1] more often along with common strains G1, G2 and G3 (Adah et al., 2001). Recent studies carried out in North Africa and Nigeria during 1999-2009 have shown rotavirus prevalence of 16-61% in children less than 5 years. Predominance of G1P[8] (23-56%) and presence of mixed infections (37%) was noted (Aminu et al., 2010; Khoury et al., 2011).

The Euro Rota Net has been established to gather comprehensive information on rotavirus types co-circulating throughout Europe. This infrastructure has been formed so that it serves as a platform for future surveillance network. During a surveillance network carried out between
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2004-2006 in Czech Republic, Slovenia, Croatia, Albania and Bulgaria, 1500 rotavirus stool samples were collected and studied by molecular characterization of G and P genes. The common G/P combinations ranged between 50-85%. G9 was identified in 2-35%. Significant differences were found in the geographical distribution of rotavirus genotypes between countries included in the study (Olga et al., 2007). Another study conducted between February 2005 and August 2006 in 12 hospitals in France, Germany, Italy Spain and United Kingdom studied 3734 samples from children <5 years of age. The rotavirus positivity was noted in 43.4%. Genotyping of 1217 rotavirus positive samples showed presence of G1P[8] (40.3%), G9P[8] (31.2%), G4P[8] (13.5%) and G3P[8] (7.1%). The data indicated that rotavirus gastroenteritis places high demands on European health care systems, accounting for 56.2% hospitalizations and 32.8% emergency department visits due to community acquired gastroenteritis in children <5 years (Forster et al., 2009). A multicenter prospective study carried out in Spain during 2006-2007 to assess the incidence of nosocomial rotavirus infections showed rotavirus positivity in 59% with G9P[8] (66%) as the commonly found genotype (Gutirrrez-Gimeno et al., 2010).

Several studies in various Asian countries have estimated the disease burden of childhood diarrhea, to be considerably high ranging from about 20% in India (Jain et al 2001) and Bangladesh (Unicomb et al., 1997) to nearly 70% in the Philippines (Paje-Villar et al., 1994). The Asian Rotavirus Surveillance Network (ARSN) founded in 2001 surveys for the rotavirus incidence in various Asian countries. The data gathered by the ARSN have shown rotavirus to be more common than previously estimated (Bresee et al., 2005). Recent hospital based surveillance studies carried out in China during 2006-2008, have shown rotavirus infections in 37.2% of clinic visits, 48.1% of ward based admissions and 58.1% of nosocomial diarrhea. G3P[8] was the most predominant genotype followed by G1P[8] (Zeng et al., 2010). Studies carried out in Japan during the last 28 years (1981-2008) have shown rotavirus infections in 18.8%, with G1P[8] as the predominant genotype in circulation (Oey et al., 2010). Similar studies carried out during the years 2000-2011 in different Asian countries like Thailand, South Korea,
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Bangladesh, Hong Kong and Japan have shown rotavirus prevalence from 15.5-40.0% with predominance of G1P[8] (36.0-62.8%) (Chan et al., 2011; Khananurak et al., 2010; Shim et al., 2011). Followed by G2P[4] and / or G9P[8] and / or G3P[8] (Ahmed et al., 2010; Mitui et al., 2011; Paul et al., 2011; Kawai et al., 2012).

Reports on the Australian rotavirus surveillance program since June 1999 have shown the re emergence of G1(40%) serotype as the dominant strain during the years 2003-2004. Initially, G1 was dominant in surveys conducted in Australia from 1993 to 1996 (Bishop et al 2001), and during 1999-2001 (Masendyez et al., 2000; 2001). This serotype was then replaced by serotype G9 which became the dominant strain nationally in 2001/2003 comprising 40% of the strains (Kirkwood et al., 2002) and 2002/2003 comprising 74.7% (Kirkwood et al., 2003). However, there was a decline in the prevalence of G9 strains in Australia during 2003/2004 (Kirkwood et al., 2004). Remarkable increase in the prevalence of G3 (25.7%) during 2003/2004 as compared to the earlier years was noted, where G3 represented less than 2% of all the strains. Serotype G2 was responsible for outbreaks in central Australia during 2003/2004. Studies during 2004-2005 showed predominance of G1 (48.3%) followed by G3 (36.7%) and G9 (6.9%) (Kirkwood et al., 2006). In a surveillance carried out during 2007-2008, 419 specimens out of 600 (69.8%) were positive for group A rotavirus. G1 was the dominant serotype nationally, representing 52% of specimens, followed by serotype G2 (19.8%), serotype G9 (12.2%), and serotype G3 (11%). No serotype G4 strains were identified (Kirkwood et al., 2008). Similar studies carried out during 2008-2009 on 445 specimens of 592 showed G2P[4] as the dominant type (50.3%), followed by genotype G1P[8] (22.5%). Genotypes G3P[8], G4P[8] and G9P[8] each represented less than 5% of circulating strains. Uncommon rotavirus genotype combinations, including G1P[4], G4P[4], G1P[6] and G3P[6] were identified during this study period (Kirkwood et al., 2009). These studies showed geographic variations as in the previous years.

Overall 5% of the circulating strains in the world constituted unusual strains, with varying frequency in different continents like Africa (27%), Asia-
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(14%), South America (11%), North America (5.5%), Europe (1.4%) and
Australia (0.1%). These unusual strains include P[1] associated with G4/G6,

The diversity of rotaviruses noted worldwide thus pose a challenge for
vaccine development.

2.6.2 Indian Status

Considerable research has been carried out on rotavirus disease in
India in different settings. These studies have documented the
circulating in India as well as
strains (Broor et al., 2003). It has been esti
1,00,000-1,50,000 annual deaths in India are due
children born in India die from rotavirus by t
accounting for 17% of the world’s estimated rota
2003). These viruses have been detected in the s
almost throughout the year, although a marked
noted, with a larger number of cases occurring in
year, November to February.

Surveillance studies in 22 Indian cities from 1982-2001 showed
rotavirus positivity ranging from 6 to 45% in a total of 15,476 samples
examined. These studies reported G1(24.7%) and G2(23.4%) to be the most
prevalent strains (Broor et al., 2003). Similar studies carried out in 18 different
cities from India during the years 1996-2001 detected rotavirus in 23.4% of
patients with diarrhea. G1 was found to be the predominant genotype in
circulation (Kang, 2005). During 2005-2007 surveillance carried out in 7
different regions showed rotavirus positivity in 39% of 4243 enrolled patients.
Common types detected were G2P[4] (25.7%), G1P[8] (22.1%) and G9P[8]
(8.5%). G12 in combination with P[4], P[6] and P[8] were detected in 6.5%
(Kang et al., 2009).

In north India, rotavirus diarrhoea showed a seasonal variation with a
high incidence of the disease in winter months at low relative humidity (Broor
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et al., 1993). In a study from Dehli during 1990-2001, 286 specimens could be G-typed. These included 80 G1, 43G2, 31G3, 9G4, 21G9 and 15 with multiple G types (Husain et al., 1996, Bahl et al., 2005, Chakravati et al., 2005). Of the 192 specimens that were P typed 55 were P[8], 30 were P[4], 33 were P[6], 10 had multiple P types and 64 remained P untypeable (Husain et al., 1996, Bahl et al., 2005).

In an extensive study in different cities like Simla, Bhopal, Lucknow, Nagpur, Davengere, Dehli, Hyderabad 287 strains were G and P genotyped by RT-PCR. The four common strains G1P[8], G2P[4], G3P[8], and G4P[8] were found in 43% of samples where as G9P[8] strains made up 17% of the total. Serotype G2 strains were often detected in mixed infections (Jain et al., 2001). In another study from 5 Indian cities, 63 rotavirus strains were analyzed. The common strains accounted for 33%, whereas P[6] strains were found in 43%. Eleven percent of the strains were non-typeable, and another 11% of the specimens had mixed infections (Ramachandran et al., 1996). Characterization of 465 rotavirus strains from north India during 2000-2007, common G types G1, G2 and G9 comprised 65% of the strains and common P types P[4],P[8] and P[6] comprised 76% of the total P types. High percentage of unusual (17%) strains and fecal specimens with mixed G(12%) and P(15%) rotavirus strains were noted. Two novel rotavirus strains with unusual G/P combinations G2P[11] and G3P[11] were detected in patients with diarrhea. Emergence of G12 with high prevalence (14.4%) was noted for the first time (Sharma et al., 2008). Recent studies carried out in New Dehli on the characterization of nontypeable strains showed G1 (54.5%) and P[8] (77.8%) genotypes as the predominant types. G8P[6] strain belonging to DS-1 genogroup was detected for the first time. These studies highlighted the importance of characterizing multiple genes of nontypeable strains to detect novel strains (Sharma et al., 2009). Surveillance and molecular characterization of rotavirus strains circulating in Manipur, north-eastern India showed G1P[8] and G2P[4] to constitute 58% of the total positive strains with increased prevalence of emerging G12 strains (Mukherjee et al., 2010). Similar studies on the circulating genotypes of rotavirus strains in Lucknow, India showed G1 (38.0%), G2 (15.2%), G3 (16.5%), G9 (10.9%), G4 (5.1%)
and mixed G types (10.1%) indicating strain diversity in northern India (Mishra et al., 2010).

In a study conducted during 1998-2000 in Kolkata, rotavirus positivity was noted in 12.6%. The predominant genotype was G1P[8] (20%), followed by G2P[4] (15%) and G4P[8] (6%). A number of uncommon genotypes, G1P[4] (4%), G2P[8] (2.5%), G2P[6] (0.6%), G4P[4] (2.5%), and G4P[6] (1.25%), were observed. Twenty-two percent of specimens showed mixed infections, and 24% of the total samples remained nontypeable (Das et al., 2002). In a similar study conducted in eastern and northern India during 2001, G1P[8] was the predominant (49.2%) type. Mixed infections were noted in 30% specimens, while 16.7% remained nontypeable (Das et al., 2004). Novel rotavirus strain G4P[8] was also reported among children with acute diarrhoea during a study in Kolkata (Khetawat et al., 2001). Rotavirus surveillance study carried out in two leading hospitals from eastern India during the period 2003-2005 detected G1 (53.8%) as the predominant strain followed by G2 (22.5%), G12 (17.1%) and G9 (2.1%). These results were remarkably different from earlier studies carried out during 1998-2001 that detected infections with mixed P types in 35.2% of the specimens (Samajdar et al., 2006).

The studies on serotyping and genotyping of rotaviruses in different cities from South India showed variations among the rotavirus strains in circulation. In Chennai during December 1997-March 1999 G2 (68.8%) was predominant followed by G1 (14.6%). Mixed infections were seen in 14.6%. SGI was found in 60% and SG II in 20% (Ananthan and Saravan, 2000). In another study in 1995-1999 rotavirus positivity was noted in 22.5%. Serotype G2 was detected in 66.1% and mixed infections like G1, G2 P[4],P[8] and G2P[4],P[8] were noted. Strains with SGI were found predominantly in circulation (Saravaran et al., 2004). In a study between 1994-1998 in Mysore and Bangalore, serotype G3 was predominant in Bangalore and G1 in Mysore (Aijaz et al., 1996). A study from Hyderabad during January 1998 to March 1999 detected rotavirus by RNA PAGE in 16.2%. Subgroup II was found to be predominant than subgroup I. Serotyping showed serotype G1 (55.2%), G4 (27.8%) and mixed infections in 17.2% of the strains (Anand et
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al., 2000). In a community and hospital based study from Vellore G1(36.5%), G10(17.1%), G2(15.9%), G9(7.3%) and mixed infections (7.3%) were detected in the community. Infections with these genotypes differed from those found in hospitalized children where G1(46.8%), G9(19.1%), G2(8.5%), G10(1.1%) and mixed infections (4.3%) were detected. G10P[11] strains caused disease in the community indicating the need for careful epidemiological studies (Banerjee et al., 2006). Similar studies carried out during 1995-1999 showed rotavirus positivity in 18-24%. G1 was predominant followed by G4(23.8%) and G2(19%). G3 and G8 strains were detected during the years 1997-2002 (Kang et al., 2002).

From western India, studies from Pune showed G2 (45%) to be the commonest followed by G1(13.8%), G4(8.3%) and G3(0.1%). Mixed infections were noted in 22.9% (Kelkar et al., 1997). Another such study in Pune showed G1 and G2 to be predominant types among children in the years 1990-1994 and 2002 respectively (Zade et al., 2009). Molecular characterization of G1 rotavirus strains from Pune, at two time points showed circulation of multiple lineages of G1 genotype indicating diversity (Arora et al., 2009).

Studies on neonatal rotavirus infections have shown region specific asymptomatic infections with unusual strains. These included G9P[11] in Dehli (Circirello et al., 1994), G10P[11] in Bangalore and Mysore (Vethanayagan et al., 2004). Asymptomatic and symptomatic infection with G10P[11] strain have been reported from Vellore (Itturiza- Gomara et al., 2004b). In studies carried out in Calcutta and Vellore, testing of 3530 children for nosocomial enteric infections showed rotavirus in 22.5% of the cases (Dutta et al., 1992; Desikan et al., 1996). Multicentric study conducted in India, during 2005-2007 has reported prevalence rate of rotavirus as 39%. The most common strains detected were G2P[4] (25.7%), G1P[8] (22.1%), G4P[8] (8.5%) and G12 strains in combinations with P[4], P[8] and P[6] (6.5%). Mixed infections ranged from 8-38.5%. A number of unusual combinations such as G1P[4], G1P[6], G2P[8], G9P[4] and G9P[6] were reported (Kang et al., 2009).
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Rotavirus shows genetic and antigenic diversity in terms of subgroup, electropherotype and G and P genotypes. Subgroup distribution studies in North India in cities like Chandigarh and Delhi have shown higher prevalence of subgroup II (50-70%) compared to subgroup I (30%) (Chakravarti et al., 1992; Singh et al., 1989). In a few cases in Delhi Non I Non II as well as subgroup I and II have been detected (Chakravarti et al., 1992). In all these studies, subgroup II was found responsible for the clinical severity of the disease. In south India studies in different cities like Vellore, Chennai and Hyderabad have shown subgroup II (63-66%) to be predominant followed by subgroup I (6-37%), non I non II (6%) and dual specificity of subgroup I and II in 20-37% (Brown et al., 1988; Ananthan et al., 2000). In Manipur, strains with unusual electropherotype were detected (Krishnan et al., 1994). Similar study in Mysore detected strains with subgroup I and long electropherotype to be of animal origin (Jagannathan et al., 2000).

The diversity of rotavirus strains and the high prevalence of mixed infections in India are unique feature of rotavirus epidemiology in India and emphasizes that vaccines should be formulated against a broad range of strains.

2.7 Epidemiology of group A rotavirus infections in adults

2.7.1 International Status

Rotavirus infections in adults are seen in five settings:
1. Epidemic spread.
2. Endemic spread.
3. Travel related infection.
4. Child to Adult transmission.
5. Water borne outbreaks.

The infections typically manifest nausea, malaise, head ache, abdominal cramping, diarrhea and fever. Although infections in adults are milder than those in children, deaths due to rotavirus have been reported (Hrdy 1987).
2.7.1.1 Epidemic Spread

Among adults epidemics of rotavirus gastroenteritis occur in two settings:

1. Infections in closed communities with no contact with pediatric cases.
2. Infections in geographically isolated populations characterized by infections of both children and adults.

In the early 1980s and 1990s rotavirus infections in adults have been described in closed communities using serology basis. One of the largest outbreaks caused by rotavirus was reported from Micronesia involving nearly 3500 people in 1964 (Foster et al., 1980). Since then several outbreaks have occurred in closed communities like Finnish military base (Meurman et al., 1977), an Israeli Kibbutz (Friedman et al., 1988) and South American Indian community (Linhares et al., 1981). Outbreaks of rotavirus have also occurred in long term health care facilities, in patients that are immuno compromised, with multiple comorid disorders, and in hospital settings (Cubitt et al., 1980, Halvorsrud et al 1980, Marrie et al., 1982; Abbas et al., 1987; Lewis et al., 1989; Dupuis et al., 1995). Rotavirus has also been reported as a pathogen in 5% of diarrhea outbreaks in home for elderly (Ryan et al., 1997). Among adults, rotavirus outbreaks are not only confined to geriatric population, but outbreaks of rotavirus among college students (MMWR 2000), in preschool children and adults of Truk district in South Pacific (Foster et al., 1980) and James Bay Cree in Quebec (Robinson et al., 1985) have also been reported. Epidemics in such isolated population were due to the susceptibility of the population to attack by agents introduced from outside thus facilitating epidemic spread (Black, 1975; Hrdy, 1984).

During the 21st century, studies on outbreaks of gastroenteritis due to rotaviruses in adults have been focused on characterization of the strains involved in these outbreaks. Study of such outbreak in Japan during a school trip detected rotavirus in 45 school children out of a total of 107 (aged 11-12 years). Rotavirus genotype G2 was the causative agent of infection (Mikami et al., 2004). Samples screened from 263 outbreaks of gastroenteritis in the US during 1998-2000 identified three outbreaks in adults to be due to
rotavirus G2 serotype (Griffin et al., 2002). Characterization of outbreaks in aged care facilities in Australia during 1997-2000 detected G2 in three, G4 in two, G1 in one and G9 in one outbreak (Marshall et al., 2003). Similar finding of association of G2 strains (11-70%) in 4 geographically and temporally distinct outbreaks of gastroenteritis in school children and adults 30-80 years old in Japan during 2000, have been reported (National Institute of Infectious Diseases, Surveillance Report 2000 a-d). Many outbreaks of rotavirus infection caused by G2P[4] rotavirus strains in adults in a closed community have been reported from Japan involving 55% of adults in a mental care facility (Yan et al., 2005) and in an institute for rehabilitation of adults (Iijima et al., 2006). Yamagami et al also reported two cases with rotavirus infection in outbreaks caused by G1 and G2 strains among adult residents at welfare facilities in Japan (Yamagami et al., 2007).

Outbreaks due to rotavirus serotypes other than G2 have also been documented. Banyai et al reported 20% of attack rate in adults from a psychiatric nursing home to be due to G1 serotype (Banyai et al., 2002). An outbreak with serotype G1 in a day care nursery in Rio de Janeiro, Brazil, infecting 58.8% of children and 6.7% adults has been reported (de Castro et al., 1994). Feeney and colleagues have identified G4P[8], G1P[8] and G9P[8] in two outbreaks studied in homes for the elderly (Feeney et al., 2006). Similarly, G4P[8] was found to be the causative agent of infection in adults from Nicaragua (Bucardo et al., 2007). Two epidemic outbreak studies in Sao Paulo, Brazil during 2003-2004 showed G3P[8] as the predominant type, followed by G9P[8] and G1P[8] (Martini et al., 2008).

Reports on lack of G2 specific neutralizing antibodies in adults support the hypothesis that adults could be more susceptible to G2 infection (Brussow et al., 1991). Thus the predominance of G2 strains in outbreaks that have occurred in adults suggest that natural immunity to more common strains (G1) does not always provide adequate heterotypic immunity to infections with G2 strains. Patients over 60 years have higher attack rate and clinical illness than younger adults (Hozel et al., 1980). The possible explanation for severe disease could be because of increased susceptibility to dehydration and lowered antibody levels.
Recently, a food borne outbreak has been reported in a mother and child sanatorium. Rotavirus was detected by RT-PCR in potato stew (Mayr et al., 2009). In another waterborne outbreak reported from Turkey during 2005, rotavirus antigen was detected in 52.7% of the patients aged 0-91 years (Koroglu et al., 2011).

2.7.1.2 Endemic Spread

Rotavirus infection in children is seasonal with peak activity during the months of November to December (winter months) in temperate climates. Several findings however suggest that rotavirus infections in adults are not season specific. Cox and Medley (2003) have shown the presence of anti Rotavirus IgM in the serum samples throughout the year. The high rates of IgM were attributed to IgM persistence, IgM cross reactivity or possibly to non seasonal high infection rates in adults. This hypothesis has been supported by other researchers who found that infections in adults do not mirror winter seasonality (Nakajima et al., 2001) as in children. Thus, these studies have suggested that endemic infections in adults do not arise solely due to the transmission of rotavirus from children to adults.

Endemic rotaviral infections in adults vary according to the geographical distribution and characteristics of patients. Low rates of rotavirus infection have been noted in earlier studies from Bangladesh (9%) (Black et al., 1980), England (4%) (Jewkes et al., 1981), Thailand (5%) (Escheverria, 1983), Switzerland (3%) (Loosli et al., 1985), Sweden (3%) (Svenungsson et al., 2000), UK (3%) (Itturiza-Gomara et al., 2000a), Netherlands (2-4%) (de Wit et al., 2001) and Michigan (4%) (Koopman et al., 1989). However, in studies conducted in different geographical areas higher rates of infection have been noted. In Japan rotavirus was found in 14% of patients with diarrhea (Nakajima et al., 2001), in Australia 17% of all cases had diarrhea due to rotavirus (Pryor et al 1987). 20.5% of adults in day care centers were infected with rotavirus (Baron Romera et al., 1985). In Indonesia 42% of patients had diarrhea due to rotavirus (Oyofo et al., 2002). In Mexico 63% of adults with acute gastroenteritis were positive for rotavirus (del
Refugo Gonzalez et al., 2001). In Paraguay, Martinez et al showed that 17.3% of patients over 18 years of age were positive for rotavirus. The infections in adults occurred year round with the same frequency (Martinez et al., 2005). In Sao Paulo, Brazil 10% rotavirus infection rate was noted in adults during a survey conducted in 2003-2005 (Carraro et al., 2008). A study from five hospitals in Saudi Arabia during 2004-2005 reported 12% rotavirus positivity rate in children and adults (Kheyami et al., 2008). Similar study carried out by Faruque et al detected rotavirus in 40% of the patients (Faruque et al., 2004).

Studies on the characterization of strains from adults infected with rotavirus have also been reported worldwide. In Japan, rotavirus strain isolated from an adult with severe diarrhea was studied genetically. Characterization revealed G2P[4] as the causative agent which was similar to the strain circulating in children (Kaga et al., 1994). Study of rotavirus in Swedish adults showed 3.2% positivity, with G9P[8] as the predominant (42.9%) genotype causing infections in adults. This report of G9 in adults was the first one to be documented from Europe (Rubilar-Abreu et al., 2005). Characterization of 162 specimens positive for rotavirus from Denmark identified rotavirus strains like G2P[4] and G4P[8] as the causative agents. High frequency of mixed infections was detected, indicating chances for rotavirus diversity (Fischer et al., 2005). In an eight year (1996-2003) surveillance programme in Brazil rotavirus positivity among adults was noted to in 7.1%. G1P[8] was the predominant genotype followed by G9P[8] (Carmona et al., 2006). In another study from Brazil in 2003, 22.5% of individuals aged 6-72 years were found to be infected with rotavirus. The most common G-P genotype combination detected was G1P[8] (86.9%) followed by G9P[8] and G12P[9] (Pietrunchinski et al., 2006). The reason speculated for such high percentage of rotavirus diarrhea among adults was strain variation in the G1 genotypes (Pietrunchinski et al., 2006). Study in Nepal among children and adults during 2003-2004 showed 5% of rotavirus infections in adolescents and adults. G1P[8] genotype was predominant (Uchida et al 2006). Surveillance of rotavirus in the Paraguayan population during 2004-2005 showed rotavirus incidence in 19.4% of adults. Emergence
of rotavirus with short electropherotype - G2P[4] was detected in these adults (Amarilla et al., 2007). Studies conducted in China during 2000-2006, documented 9% rate of rotavirus infection in adults. Winter seasonality was observed with G3P[8] as the predominant genotype causing infections (Wang et al., 2007). Studies on the aetiology of community-acquired acute gastroenteritis in hospitalized adult patients during 2005-2007 from Germany showed rotavirus positivity in 15% (Jansen et al., 2008). Similar studies carried out in Paraguay during 2006-2007 showed G2P[4] as the predominant genotype in circulation (Martinez et al., 2010). Similar studies carried out in Bangladesh during 2004-2006 detected rotavirus in 11% of individuals aged >15-76 years. G2 was the predominant genotype followed by G1 and G9. Common P-types were P[4], P[8] and P[6] (Paul et al., 2010).

2.7.1.3 Traveler’s Diarrhoea

Rotaviruses have been implicated as an important contributor to traveler’s diarrhea among adults. In two studies conducted among US students travelling in Mexico, rotavirus was identified in about 25% of patients with diarrhea by electron microscopy (Bolivar, 1978; Vollet, 1979). In a third study among two students groups travelling to Mexico, 17% showed substantial rise in antibodies titres to rotavirus (Keswick et al., 1982). Ryder et al found rotavirus in 26% of Panamanian travellers to Mexico who had diarrhea (Ryder et al., 1981). Steffen et al found rotavirus in 9% of travelers with diarrhea returning from Jamaica (Steffen et al., 1999). No information on traveler’s diarrhea caused by rotavirus has been reported recently.

2.7.1.4 Transmissions from children to adults

Rotaviral infections commonly occur among adults in families which have young children with diarrhea. These adults are at high risk of infection. Wenman et al showed that 36 of 102 adults caring for children were infected with rotavirus. In contrast to 4 of 86 adults whose children had no rotaviral infections became infected (1979). This was supported by another finding by Grimwood et al in a family study in New Zealand (1983). Kim et al detected
rotavirus infections in 55% of adults whose children were admitted to hospitals compared with 17% of adult contacts whose children were not infected (Kim et al., 1977). Rodriguez et al reported 70% of rotavirus attack rate in adults after exposure to children infected with rotavirus in a playgroup (Rodriguez et al., 1979). Variations in clinical illness following rotavirus infections have been seen which may be due to the differences in virulence among strains. Infection among pediatric nurses has also been described (Von Bonsdorff et al., 1976). This has been supported by another case report from a hospital study (Orstavik et al., 1976). The presumed sources of secondary spread of rotavirus are fecal oral inoculation and transmission from contaminated environmental surfaces. Although no direct evidence exists for respiratory spread, 30% of rotavirus infections characterized by respiratory symptoms alone have been reported (Guruwith et al., 1981). Hence respiratory spread may be important in intra family transmission.

It is generally believed that serotype specific immunity plays a role in protection against disease, so the epidemiology of G and P serotypes of circulating strains forms a critical knowledge base for the development and implementation of rotavirus vaccines (Gentsch et al., 1992, Santos and Hoshino 2005). As natural infection with rotavirus does not provide complete protection against subsequent infections, older children and adults may repeatedly get infected with some of these resulting in symptomatic disease. Hence, characterization and comparison of rotaviruses circulating in different age groups may provide a key role for understanding the spread of rotavirus in the community (Uchida et al., 2006). Recently, studies on asymptomatic rotavirus infections in England have shown adults in contact with children to be at high risk for asymptomatic infections (Phillips et al., 2010).

### 2.7.1.5 Water Borne outbreak

Waterborne rotaviral infections are common in areas of the world where sanitation and purification treatments are in adequate. Studies in countries like Hungary, Brazil, US, Japan have shown different rotavirus serotypes (G1, G2) to be the causative agents of outbreaks in adults
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(Timenetsky et al., 1996; Banyai et al., 2002). Numbers of outbreaks of waterborne rotaviral infections have been reported (Escheverria et al., 1983; Timenetsky et al., 1996, Marshall et al. 2003). In all cases significant proportion of adults have been symptomatically infected and in many cases attack rate has been higher in adults than children.

In 1982-83 two epidemics of diarrhea occurred in coal mining districts of China (Hung et al., 1983, 1984). The etiologic agent was shown to be non-group A rotavirus. The source of epidemic was later traced out to be water contaminated with feces containing rotavirus. The attack rate was highest among persons 10-20 yrs old, with peak rates of 28.1% in 30-40 yrs age group. Outbreaks of rotaviral gastroenteritis in countries like Vail, Colorado USA have shown attack rate of 43.8% in adults (Hopkins et al., 1984).

2.7.2 National Status

A study to detect rotaviruses in fecal samples of children and adults suffering from acute diarrhea was carried out in Bombay during 1984-86, using ELISA, Latex agglutination and electron microscopy. Rotavirus was detected in feces of children while no fecal sample from adults showed positivity to rotavirus. (Desai et al., 1993)

Studies in adults mostly carried out in India have been done to study the serology of rotavirus infection. Saha et al (1995) have shown that rotavirus antibodies are present in all age groups and that antibodies decline with increasing age. In another study prevalence rate of anti rota IgM in healthy children (10-15yrs) and adults (20-25yrs) has been shown to be 10%, while prevalence of anti rota IgA to be more in adults (60%) as compared to children (19.6%) (Ray and Kelkar, 2004a). Presence of 38.6% anti rota IgM in mothers whose child was infected with rotavirus indicated subclinical infection among mothers. (Ray and Kelkar, 2004a).

Investigations of neutralizing antibodies (NAbs) in convalescent phase sera from mothers whose children were hospitalized for rotavirus infection showed higher NAb positivity to animal rotavirus serotypes like G3, G6 and G10 (Ray and Kelkar, 2004b). Similar findings were also reported in a study
carried out in adults who experienced natural rotavirus infections (Kelkar et al., 1996). High NAbs against rotavirus were detected in mothers from high socio economic group as compared to those from low socio economic group (Ray et al., 2007). In a family outbreak study same rotavirus genotype was found to infect both child and adults (Awachat et al., 2006).

Early reports on group B rotavirus in Pune, India (Kelkar and Zade, 2004) and CAL-1 strain from Kolkata (Krishnan et al., 1999) showing 90% homology to the Chinese ADRV strain highlight the need to survey for non-group A rotaviruses which are contributing towards emerging diarrhoeal infections. With efforts now being done to develop rotavirus vaccine in India, it is imperative to know the prevalence, type of rotavirus strains infecting adults.

2.8 Immunity

Several studies have been conducted on the immune response to rotavirus infections in humans and animals, however, the mechanisms underlying protection against rotavirus disease remain poorly understood. It is currently accepted that clinical protection may involve local (mucosal) and systemic antibodies, and / or cell-mediated immunity (Bishop et al., 1983; Offit et al., 1986; Ward, 1996). Although VP6 has been recognized as the most immunogenic rotaviral protein, genetic studies have demonstrated that antibodies directed at either VP4 or VP7 are also able to neutralize rotavirus and protect susceptible hosts.

Both primary and secondary rotavirus infections in humans elicit antibodies of the IgM, IgG and IgA classes in intestinal, saliva and serum secretions. Early animal studies suggested that the presence of rotavirus antibodies in the intestinal lumen correlated with protection against disease, and oral administration of preparations containing rotavirus antibodies has successfully treated chronic rotavirus infections in immunocompromised children (Ebina et al., 1983; Hilpert et al., 1987). In addition, orally administered gamma globulin has been shown to reduce the duration of illness and shedding of rotavirus in hospitalized infants (Parashar et al., 1998). These observations indicated that intestinal immunity protects against rotavirus diarrhea and that the success of vaccine will partly depend upon its
ability to induce mucosal immune responses. Furthermore, some authors have indicated that immunologic responses occurring at the intestinal mucosal surfaces (rotavirus specific secretory IgA) are predictors of clinical immunity (Coulson et al., 1992; Matson et al., 1993; Ward, 1996).

2.9 Treatment

Treatment of acute rotavirus infection is nonspecific and involves management of symptoms and, most importantly, maintenance of hydration (Light and Hodes, 1943). If untreated, children can die from the resulting severe dehydration (Alam and Ashraf, 2003). Depending on the severity of diarrhoea, treatment consists of oral rehydration with plain water, water plus salts, or water plus salts and sugar (Sachdev, 1996). Some infections are serious enough to warrant hospitalisation where fluids are given by intravenous drip or nasogastric tube, and the child’s electrolytes and blood sugar are monitored (Smith et al., 1993).

2.10 Prevention and control

As improved sanitation does not decrease the prevalence of rotaviral disease, and the rate of hospitalisations remains high, despite the use or oral rehydrating medicines, the primary public health intervention is vaccination (Bernstein 2009). The aim of a rotavirus vaccine should be to prevent severe rotavirus gastroenteritis during the first 2 years of life, the period when this disease is most serious and takes its greatest toll (Bishop, 1994, Kapikian, 1996b). A realistic goal for a rotavirus vaccine is to duplicate the degree of protection against disease that follows natural infection. An effective rotavirus vaccine will clearly decrease the number of children admitted to the hospital with dehydration or seen in emergency departments but it shall also decrease the burden on the practicing primary care practitioner by reducing the number of office visits or telephone calls due to rotavirus gastroenteritis. Finally, effective rotavirus vaccines are most needed in resource poor countries, where mortality associated with rotavirus is high.
Initial approaches for rotavirus vaccines were based on the classical “Jennerian” approach and utilized simian and bovine rotavirus strains, which provided cross-protection against human rotavirus strains but did not cause illness in infants and young children because of their species-specific tropism. These included three nonhuman rotavirus vaccines, two bovine rotavirus strains, RIT 4237 (P6[1]G6) and WC3 (P7[5]G6), and a simian (rhesus) rotavirus reassortant vaccine (RRV) strain (P[3]G3). These vaccines demonstrated variable efficacy in field trials and gave particularly disappointing results in developing countries (Hanlon et al., 1987; Lanata et al., 1989). Hence next generation of vaccines was formulated to include more than one rotavirus G serotype to provide heterotypic as well as homotypic immunity. The ability of rotaviruses to reassort during mixed infections in vitro allowed the production of reassortant vaccines, termed the “modified Jennerian” approach (Kapikian et al., 1996a). The first multivalent live oral reassortant vaccine developed was RotaShield (a rhesus rotavirus tetravalent [RRV-TV] vaccine). This tetravalent vaccine contained a mixture of four virus strains representing the most commonly seen G types, G1 to G4: three rhesus-human reassortant strains containing the VP7 genes of human serotypes G1, G2, and G4 strains were substituted for the VP7 gene of the parent RRV, and the fourth strain comprised serotype G3 of rhesus RRV (Kapikian et al., 1996b). But it was withdrawn from the market because of intussusceptions among some of the infants who received the rotavirus vaccine.

2.10.1 Currently licensed rotavirus vaccines
2.10.1.1 Rotateq

Current human-animal reassortant rotaviruses for use as vaccines include either human VP7 or VP4 genes. A pentavalent human-bovine (WC3) reassortant live-attenuated, oral vaccine (RotaTeq) has been developed by Merck Research Co. This vaccine contains five live reassortant rotaviruses. Four reassortant rotaviruses express the VP7 protein (G1, G2, G3, or G4) from the human rotavirus parent strain and the attachment protein (P7[5])
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from bovine rotavirus parent strain WC3. The fifth reassortant virus expresses
the attachment protein (P1A[8]) from the human rotavirus parent strain and
the outer capsid protein G6 from the bovine rotavirus parent strain. RotaTeq
is administered in three oral doses at 1- to 2-month intervals beginning at 6 to
12 weeks of age. The efficacy of RotaTeq was evaluated in two phase III
trials (Vesikari et al., 2006b; Block et al., 2007). In these trials, the efficacy of
RotaTeq against rotavirus gastroenteritis of any severity after completion of a
three dose regimen was 74%, and that against severe rotavirus
gastroenteritis was 98%. RotaTeq also proved to be strongly efficacious in
preventing rotavirus gastroenteritis of any severity caused by the predominant
G1 serotype (75% efficacy) and the G2 serotype (63% efficacy). RotaTeq
reduced the incidence of office visits by 86%, emergency department visits by
94%, and hospitalizations for rotavirus gastroenteritis by 96%. Efficacy
against all gastroenteritis hospitalizations of any etiology was 59%. RotaTeq
was licensed in February 2006 by the Food and Drug Administration (FDA)
for use among infants in the United States and is routinely recommended as
a three-dose schedule at 2, 4, and 6 months of age (Parashar et al., 2006a)
As of May 2007, applications for licensure of RotaTeq have been filed in more
than 100 countries, including Australia, Canada, the European Union, Asia,
and Latin America. Through its partnership with the Rotavirus Vaccine
Program at the Program for Appropriate Technology in Health (PATH), Merck
plans to conduct clinical trials in Africa and Asia.

2.10.1.2 Rotarix

Rotarix (RIX4414) is an oral, live-attenuated, human rotavirus vaccine
(GlaxoSmithKline Biologicals, Rixensart, Belgium) derived from the most
common circulating wild-type strain G1P[8]. Rotarix is based on a rotavirus of
entirely human origin, and is administered to infants in two oral doses with an
interval of at least four weeks between doses. Rotarix was approved first in
Mexico (2004) and has since been approved in over 100 countries, including
the USA and European Union and has been included in National
Immunization Programmes in Brazil, El Salvador, Mexico, Panama, and
Venezuela. In 2008, it was included in the World Health Organization’s list of vaccines for purchase by United Nations agencies (Weiser et al., 2010). Protection rate of 85% against severe rotaviral gastroenteritis and 100% protection against the most severe dehydrating rotaviral gastroenteritis episodes was noted in trials conducted in Latin America and Finland (Ruiz-Palacios et al., 2006). The vaccine also proved to be strongly efficacious in preventing rotavirus gastroenteritis of any severity caused by the predominant G1 serotype (92% efficacy) and serotypes G3, G4, or G9 (88% efficacy).

2.10.1.3 LLR

LLR is a live-attenuated, monovalent (G10 P[12]) vaccine derived from a lamb. This oral, three-dose vaccine was developed by the Lanzhou Institute of Biomedical Products in China and was licensed in China in 2000 (Fu et al., 2007).

2.10.2 Rotavirus vaccines in development

There are four vaccines in various stages of development: RV3 is a live, G3 P[6] rotavirus vaccine candidate developed from a rotavirus strain that caused asymptomatic infection in healthy newborns in obstetric hospital nurseries in Melbourne, Australia (Bishop, 1993); RV-UK (also called BRVT-V) is a live-attenuated tetravalent (G1, G2, G3, G4) human-bovine (UK) reassortant vaccine with licenses granted to several vaccine manufacturers in developing countries (Nakagomi and Cunliffe, 2007) and 116E and I321 are both naturally occurring bovine-human reassortant vaccines developed from two rotavirus strains identified in India (Glass et al., 2005).

2.11 Group B rotaviruses

2.11.1 History

The importance of serogroup A rotaviruses in causing widespread infantile diarrhea is well established. In contrast, there is lack of understanding of the epidemiology and biology of the non group A rotaviruses causing disease in humans. Group B rotaviruses (GBR) are called adult
diarrhea rotavirus (ADRV) because they mostly infect adults and older children (Hung et al., 1983; Mackow et al., 1995). The human group B rotaviruses were first detected in Mainland China during the year 1982, in large epidemics affecting nearly one million people (Hung et al., 1983). Since then they have been causing large water borne epidemics infecting thousands of people (Chen et al., 1985, Hung et al., 1984, Fang et al., 1989, Yang et al., 1998).

Nakata and coworkers developed an enzyme linked immuno sorbant assay (ELISA) for detecting this virus in stool specimens (Nakata et al., 1986; 1987). Penaranda and coworkers used this ELISA to study ADRV (Penaranda et al., 1989) and found that gamma globulin pools from Chinese population collected from 1977 to 1987 were positive for anti ADRV antibodies. These findings indicated that there were factors which prompted the large outbreak of disease. Sera analyzed from countries like China, Hong Kong, Australia, United States, Canada, Kenya and United Kingdom where group B rotavirus infections in human have not been reported to date were found to be positive, indicating wide spread infection with group B rotaviruses (Penaranda et al., 1989, Nakata et al., 1987, Krishnan et al., 1999, Mackow, 1995, Ushijima et al., 1992). Antibodies were also found in rats and pigs leading to a tentative hypothesis that the natural reservoir for this virus was likely to be animal in nature (Hung, 1988). For many years these viruses were restricted to China, but recently they have been isolated sporadically in India since 1998 and Bangladesh since 2001 (Ahmed et al., 2004, Kobayashi et al., 2001, Sanekata et al., 2003, Kelkar et al., 2004). The animal group B rotaviruses were isolated from lambs (UK and US), pigs (USA) cows (US, Japan and India) goat (China) and rats (US) (Chasey and Banks, 1984, Theil et al., 1985, 1995; Eiden et al., 1991; Parwani et al., 1996; Sanekata et al., 1996; Tsunemitsu et al., 1999; Barman et al., 2004). The human and animal group B rotaviruses were mostly isolated from adult animals. Because of the inability to serially propagate human group B rotaviruses in cell culture with the exception of one porcine strain (Bridger, 1994) the epidemiology and characterization of group B rotaviruses remain poorly understood and no
diagnostic method for detecting group B rotaviruses is available commercially to date.

2.11.2 Classification

Group B rotaviruses belong to the family Reoviridae and have a ds RNA genome made up of 11 independent gene segments. They can be differentiated from groups A and C rotaviruses by the migration pattern of their segmented RNA (4-2-1-1-1-1-1-1-1-1) on PAGE (Saif and Jiang, 1994). No serotyping or genotyping system has been described for group B rotaviruses due to the difficulties in adapting these viruses to cell culture and the availability of adequate amount of gene sequence data.

GBR is genetically and antigenically distinct from group A rotavirus and known to cause severe cholera-like diarrhea mostly in adults (Mackow, 1995). Despite the limited number of sequence data, human group B rotaviruses were classified genetically into two lineages, with the Chinese lineage represented by the ADRV and WH-1 strains, and the Indian-Bangladeshi lineage represented by the CAL-1 and Bang373 strains (Ahmed et al., 2004; Yang et al., 2004). It has been suggested that group B rotaviruses in these two lineages diverged from a common ancestral strain several decades ago (Yang et al., 2004). Although group B rotaviruses have also been detected in mice, calves, pigs, and sheep, they are distinct genetically from human group B rotaviruses (Shen et al., 1999; Ghosh et al., 2007). Recently, a novel rotavirus group represented by J19 strain from China and B219 strain from Bangladesh was found to be related genetically to group B rotaviruses, suggesting that these groups may have been derived from the same evolutionary origin (Jiang et al., 2008; Nagashima et al., 2008). GBRs are tentatively divided into five genotypes on the basis of nucleotide sequences of the outer capsid glycoprotein (VP7) genes:

- Genotype 1 - the murine strain
- Genotype 2 - human strains
- Genotype 3 - bovine and some porcine strains
- Genotype 4 and 5 - other porcine strains (Kuga et al., 2009).
Antigenic differences between group B rotaviruses from different species in their reactivity in immunoelectron microscopy and enzyme-linked immunosorbent assay (ELISA) have been reported (Nakata et al., 1986; Snodgrass et al., 1984).

2.11.3 Genome organization

The nucleotide sequences as well as the encoded polypeptides of the group B rotaviruses are totally different from that of group A and C rotaviruses. Overall, the group B rotaviruses exhibit 18% to 28% amino acid identity to the equivalent proteins from group A rotaviruses (Mackow et al., 1995). This low level of gene homology clearly indicates the evolutionary distance between the group A and B rotaviruses.

2.11.3.1 Structural Proteins

Viral Core Proteins

VP1

VP1 is coded by gene segment 1 and is 3509 bp in length, with an uninterrupted open reading frame (ORF) encoding a 1159 aa polypeptide (Eiden and Hirshon, 1993). The molecular weight of this protein is 131.6 kDa. The aa region of 643-689 in the IDIR protein contains the conserved RNA polymerase domains. Most of the aa differences in GBR VP1 have been found in the N-terminal region (376 aa) and C-terminal region (317 aa) (Yamamoto et al., 2010).

VP2

VP2 is encoded by gene segment 2. This gene encodes 933 aa protein in ADRV and 934 aa in CAL and IDIR. Within the group B rotaviruses, there is a high degree of sequence conservation in this protein (the CAL and ADRV proteins are 97% identical at the amino acid level, while both CAL and ADRV proteins are 83-85% identical to the IDIR strain VP2 protein). Recent studies have shown sequence divergence in the VP2 protein among GBRs in the 80 aa sequence from the N-terminus (Yamamoto et al., 2010).
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**VP3**

VP3 is encoded by the gene segment 3 from CAL strain has been sequenced and was found to be 2341 nucleotide long encoding 763 aa polypeptide. Amino acid differences in VP3 sequences among GBR strains have been noted throughout the gene. However a conserved motif (ALYSLSNXXN) (Ito et al., 2001) was found in all GBRs as well as GAR and GCR. Some sequences similar to the active sites of guanylyltransferase in VP3 were conserved among GBRs (Cook and McCrae, 2004).

**Viral Outer Capsid proteins**

**VP4 protein**

The VP4 protein is encoded by gene segment 4, the protein varies in sizes among different strains of group B. In CAL, VP4 gene comprises 2306 nucleotides encoding a protein of 750 aa. In ADRV this protein is 749 aa long, while in IDIR it is 751 aa (Kobayashi et al., 2001). The difference in lengths of the VP4 proteins from CAL and ADRV is due to three nucleotide insertions in the CAL gene sequence in the region corresponding to the amino acid residues 99-102 of ADRV (Kobayashi et al., 2001). Overall however, the CAL and ADRV strains share highly similar VP4 proteins (94% identity at the amino acid level) (Sen et al., 2003). The VP4 protein carries two regions, the putative VP5 region (aa 215-750) and the putative VP8 region (aa 1-207). The VP5 is better conserved in all the proteins compared to VP8. The sequence in the N-terminal hydrophilic region in the VP8* portion have more amino acid diversity than any other region in VP4 (Yamamoto et al., 2010). This is similar to that reported for group A rotaviruses.

**VP7 protein**

The VP7 protein-encoding gene segment 9 has been sequenced from a number of strains (Chang et al., 1997; Kobayashi et al., 2001). In ADRV strain, VP7 is encoded by the ninth segment and has a length of 814 nucleotide (Chen et al., 1990). The single ORF in this segment encodes a 249 aa polypeptide with a molecular weight of 28.5 kDa. The VP7 protein of
CAL strain is closely related to ADRV (95% identity aa) (Kobayashi et al. 2001). The identities of both human viral proteins with the murine IDIR and with several different bovine strains (WD653, ATI, Mebus, Nemuro) (Chang et al., 1997; Tsunemitsu et al., 1999) is extremely low (approximately 48-63% identity at the aa level), indicating extreme variation in this outer capsid antigen for GBRs.

**VP6 protein**

It is the major inner capsid protein encoded by gene segment 5. VP6 has been sequenced from ADRV and CAL strains. It was found to be 1269 nucleotides long encoding 391 aa long polypeptide with a molecular weight of 44 kDa (Mackow et al., 1993) The CAL VP6 gene shows close identity to the ADRV gene (94% nucleotide and 95% AA identity) (Kobayashi et al., 2001).

### 2.11.3.2 Non-Structural Proteins

**NSP1**

The gene segment 6 in ADRV, CAL and KB63 (Mackow et al., 1995; Kobayashi et al., 2001; Sen et al., 2001) encode NSP1 protein. This gene segment is 1276 nucleotides in length. In all the GBRs, this gene segment possesses two overlapping reading frames (Mackow et al., 1995; Sen et al., 2001). The first of these ORFs is 101 aa in length and the second is 321 aa in length. A cysteine rich region was found in the peptide 1 of NSP1 protein in GBRs while a cysteine and histidine rich region was highly conserved in peptide 2.

**NSP2**

The gene segment 8 of GBRs encodes the NSP2 protein. In CAL, this gene segment has a total length of 1007 nucleotides. NSP2 sequences from GBRs show much less diversity however they are considerably distinct from those of GARs and GCRs (Yamamoto et al., 2010). In CAL, the gene segment possesses untranslated regions (UTRs) at the 5’ and 3’ ends that are 55 and 46 nucleotides long, respectively. These UTRs may be important
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for packaging of the eleven genomic segments during virus assembly in infected cells as proposed for the group A rotaviruses (Estes, 1996). However, unlike in the group A rotaviruses, the UTRs of the GBRs ADRV, IDIR and CAL do not show absolute conservation of the termini and there are heterogenous sites, especially at the 3’-terminus where 12 sites out of 46 are heterogenous. If there is a role for UTRs in packaging of RNA segments during rotavirus replication, then this lack of conservation at the termini in GBRs suggests that such functions are likely to be dependent on secondary structure elements than on the absolute nucleotide sequences. The folded mRNA structures from all these three strains show common conserved structural motifs inspite of the variation at the UTRs (Sen et al., 2003). The open reading frame in the CAL gene is 906 bp long (nt 56-nt 961) and encodes a 302 aa long protein. The CAL and ADRV genes are 98% identical at the nucleotide level while the CAL gene is 77% identical to the murine IDIR strain. Thus, genotypically, the CAL and ADRV sequences are almost identical while the CAL strain is relatively distant to the IDIR strain.

**NSP3**

The gene segment 7 of GBRs encodes the NSP3 protein. This gene segment in CAL and ADRV strains is 1178 and 1179 nucleotides long, respectively. It encodes for a 347 aa long polypeptide. Among the NSP3 sequences of the GBRs, the N-terminal 120 aa region is highly conserved and corresponds to the RNA binding region revealed for GAR NSP3 (Deo et al., 2002). Remarkably, amino acids in a motif RNXXW in the alpha helix 4 (H4) which are essential for RNA binding are conserved in NSP3 of GBR. In contrast, the remaining two-thirds portion is divergent (Yamamoto et al., 2010).

**NSP4**

NSP4 is an integral membrane Endoplasmic Reticulum (ER) retained protein, which binds double shelled particles and facilitates their budding into the lumen of the ER. NSP4 has been associated with the unique feature of rotavirus morphogenesis, the maturation of particles by budding through the
membranes of the ER. NSP4 is suggested to be a viral enterotoxin. NSP4 is a nonstructural, transmembrane glycoprotein encoded by gene segment 10 of group B rotaviruses and was found to be 751 nucleotides in length (219 aa) in CAL strain. Two hydrophobic regions and two putative enterotoxin regions in NSP4 (Ishino et al., 2006) are highly conserved among GBR strains (Yamamoto et al., 2010).

NSP5

The rotavirus NSP5 protein is encoded by gene segment 11 and has been sequenced from ADRV, CAL, IDIR, and KB63 strains (Chen et al., 1990; Shen et al., 1999; Sen et al., 2001). The CAL and ADRV gene 11 is 631 nucleotides long encoding 170 aa long polypeptide which is four aa shorter and longer than the proteins from KB63 and IDIR, respectively. In the NSP5 gene sequence, the C-terminal 60 aa sequence was more conserved than the remaining N-terminal portion comprising 110 aa (Yamamoto et al., 2010).

2.11.4. Epidemiology of group B rotavirus

Epidemics of severe rotavirus gastroenteritis have been reported from several areas of China (Hung et al., 1984, Wang et al., 1985). These epidemics were noteworthy because of all age groups (particularly adults) were infected. The virus associated with these outbreaks was called as Adult Diarrhea rotavirus (ADRV). Infections with this virus were severe (cholera like) in all age groups, and mortality resulting from severe dehydration has been reported in elderly patients in China (Su et al., 1986).

ADRV was first identified in two large outbreaks of diarrhea throughout the People’s Republic of China in 1982. To study the prevalence of this virus ELISA was developed by Nakata and coworkers to detect virus in stool specimens (Nakata et al., 1986, 1987). A total of 219 human sera and 18 immunoglobulin pools collected from eight countries were tested. Low prevalence of 4.2% was detected in convalescent phase sera from different countries like China, US, Kenya, Thailand and Canada, indicating
requirements of continuous surveillance (Nakata et al., 1987). Many studies have been carried out thereafter to study prevalence of group B rotavirus (Brown et al., 1987; Hung et al., 1987; Hung et al., 1988; Fang et al., 1989; Penaranda et al., 1989; Ushijima et al., 1992; Mackow et al., 1995). These studies have shown that 5-15% of the global adult population may be exposed to group B rotaviruses. In another study, Baculo virus expressed group B rotavirus VP6 antigen from murine group B rotavirus IDIR strain was used in solid phase ELISA to detect anti VP6 antibodies in serum samples (Eiden et al., 1994). This assay was used to assess 423 serum samples collected from residents of Maryland, USA and residents of a nursing home in Switzerland. Only 4 individuals, 54 to 95 years of age were tested positive (Eiden et al., 1994). Similar studies were carried out using theT7 promotor construct to assess antibodies against the product of gene segment 8 from IDIR strain (Eiden et al., 1992).

The availability of RT-PCR methods for detecting virus from stool specimens made the detection of group B rotavirus more straightforward than before (Eiden et al., 1991; Gouvea et al., 1991; Sen et al., 2000). Several studies using this technique have been carried out in different countries like India and Bangladesh (Krishnan et al., 1999; Sanekata et al., 2003; Kelkar and Zade, 2004; Barman et al., 2006; Kelkar et al., 2007; Rahman et al., 2007; Chitambar et al., 2011). Majority of these studies used the NSP2 gene for the detection of group B rotaviruses. Using this technique, group B prevalence as high as 18.5% was noted in children (Barman et al., 2006) and 5.5% in adults (Sanekata et al., 2003). In addition to this several oligonucleotide primers have been validated for the use in such assays, and it is now possible to design RT-PCR assays targeting one or more gene segments simultaneously (Sen et al., 2000).

To date, only four group B rotaviruses have been characterized, these include human ADRV and CAL strains, the murine IDIR strain and the ovine KB63 strain. Overall, group B rotavirus genomes have been characterized using hybridization technique (Chen et al., 1985; Eiden et al., 1986; Mc Crae, 1987), oligonucleotide finger printing of individual gene segments (Pedley et al., 1983; Desselberger et al., 1986, Eiden et al., 1986; McCrae 1987; Eiden
et al., 1988) and nucleotide sequencing of different gene segments (Mackow, 1995; Shen et al., 1999; Kobayashi et al., 2001; Sen et al., 2001; Ahmed et al., 2004; Rahman et al., 2007; Aung et al., 2009).

In the early 2000s, nucleotide sequencing of the CAL strains from India was carried out. To study the evolution of group B, genes VP4, VP7, VP6, NSP1, NSP2, NSP3 and NSP5 were sequenced (Kobayashi et al., 2001; Sen et al., 2001). High sequence identities to human GBR strain ADRV was noted in these studies. GBR strains detected recently during the years 2000-2001 / 2008 and 2007 from Bangladesh and Myanmar respectively were analyzed genetically (Ahmed et al., 2004, Rahman et al., 2007; Saiada et al., 2011). Sequence analysis of structural and non structural genes showed close identity with strains from Indian-Bangladeshi lineage. Similar studies were also carried out in Pune, India (Lahon and Chitambar, 2011).

Recently, studies based on genetic characterization have focused on whole genome analysis of GBR strains (Yamamoto et al., 2010). Sequence analysis of all the 11 segments of human GBR strains detected from India (IDH-0841 and IC-008), Bangladesh (Bang 117) and Myanmar (MMR-B1) was carried out. Highest nucleotide identity (>98%) was noted in the VP6 and NSP2 genes, while lowest identities were observed in NSP4 (96.1%), NSP5 (95.6%) and VP8 encoding region of the VP4 gene (95.9%).

All these studies indicate that extensive epidemiologic studies on GBRs are necessary to understand the status of distribution and epidemiologic features.

2.12 Group C rotaviruses

Presence of group C rotavirus was first described in piglets in 1980 (Saif et al., 1980). These have been reported to cause sporadic cases or clustered outbreaks of acute gastroenteritis in humans affecting both adults and children (Espejo et al., 1984; Peneranda et al., 1989). Sequence analysis of group C VP7 gene obtained from human isolates from distant parts of the world has shown a remarkable conservation in contrast to group A rotavirus (Rasool et al., 1994; Jiang et al., 1995). They have been recognized in
humans and animals, both in industrialized countries including Australia, United States, United Kingdom, Japan and Italy and in developing countries or regions such as India, China and Malaysia (Brown et al., 1988; Nagesha et al. 1988; Ushijima et al., 1989; Chen et al., 1991; Rasoni et al., 1994; Jiang et al. 1995; James et al., 1998). Thus, group C rotavirus strains are globally distributed and are thought to be one of the emerging pathogens in humans. Thereafter from the 2000s, studies from different countries on group C have been focused on the detection and characterization of these viruses (Banyai et al., 2006; Khamrin et al., 2008; Medici et al., 2009; Araujo et al., 2011).