Chapter 2
Review of Literature

2.1. History

Despite the magnitude of the problem of infantile diarrhea, the search for important etiological agents – bacterial, virus or parasite was unrewarding until 1970s. Human rotavirus was first identified by Bishop et al. in 1973 by electron microscopy of thin sections of duodenal mucosa from infants suffering from diarrhea. This 70 nm particle was subsequently designated as rotavirus. This virus was detected in feces of pediatric patients with diarrhea and was found to be the most important etiologic agent in both developed and developing countries (Wyatt et al. 1978). Though rotavirus was discovered in 1973, Adams and Kraft reported virus-like particles in intestinal sections of mice infected with epizootic diarrhea of infant mice (EDIM) virus (Adams and Kraft 1963), which were similar to those observed by Bishop et al. (1973). Malherbe et al. in 1963 too described a similar virus designated as Simian Agent 11 (SA11). Mebus et al. in 1969 demonstrated the presence of 70 nm virus particles in stools from calves with diarrheal illness and showed that this agent could be passaged serially in calves with the production of disease. In 1971, Mebus et al. reported successful cultivation of Nebraska Calf diarrhea virus (NCDV) in primary fetal bovine cell cultures. Later studies revealed that the murine, simian, O and bovine agents shared morphological characteristics and group antigen with other rotaviruses (Flewett et al. 1974; Kapikian et al. 1974; Lecatsas, 1972).

2.2. Morphology

Rotavirus particles have a distinct morphology when examined under electron microscope and resemble a wheel with short spokes, which was originated from the Latin word ‘rota’, meaning ‘wheel’ (Flewett et al. 1974). Three distinct particles have been observed, the complete infectious particle, which is triple-layered (previously referred to as double-shelled; TLP), the
double-layered rough particles (referred previously as single-shelled particles; DLP), lacking the outer shell and the core or single layered particles which encapsulate the genome. Three dimensional cryoelectron microscopy (cryo-EM) reconstructions revealed that particles possess icosahedral symmetry and have triangulation number of T=13 (Prasad et al. 1988; 1990). The outer most layer is made up of 780 copies of VP7 as 260 trimers and 120 copies of VP4 as 60 bilobed dimer spikes (Prasad et al. 1990). The spike like structures on the virion is made up of VP4. VP4 undergoes proteolytic cleavage near the tip of the spike that increases viral infectivity (Lopez et al. 2005). The intermediate layer is composed of 780 molecules of VP6 protein exclusively and it is believed that this layer also exhibits a T=13 arrangement (Prasad et al. 1990). Cryoelectron microscopy has revealed that the innermost layer of the virion is made up of the core lattice protein VP2, arranged with T = 1 symmetry (Lawton et al. 1997).

A distinctive feature of the virion structure is the presence of large channels linking inner core through the outer two layers. Three types of channels can be distinguished based on their position and size i.e., type I, II and III. Twelve type I channels run down the icosahedral five fold axes, 60 type II channels are those on the six coordinated positions surrounding the five fold axes and 60 type III channels are those on the six-coordinating positions around the icosahedral three fold axes. 120 channels are along the 6-coordinated centers and 12 are along the 5-coordinated centers (Prasad et al. 1990). Cryoelectron microscopy studies have indicated that nascent mRNA transcripts exit via type I channels on the five-fold axis in the double layered particles (DLPs) (Lawton et al. 1997). Positioned at the inner surface at each of the 12 vertices of the core is an enzyme complex consisting of one copy each of the viral RNA polymerase, VP1 and the capping enzyme, VP3 (Chen et al. 1999; Liu et al. 1992). Anchored to each core enzyme complex is believed to be one of the 11 double-stranded RNA (dsRNA) genome segments organized in a highly condensed ordered form (Pesavento et al. 2006).
2.3. Physicochemical Properties

The RV infectivity depends on the integrity of the outer capsid which is stabilized by calcium ions (Gajardo et al. 1997). For this reason, treatment with EDTA or any other divalent ionchelating agent leads to destabilization of the outer capsid and loss of infectivity. Calcium has been detected in triple layered particles and potential calcium binding sites have been identified in the structure of VP7 (Dormitzer et al. 1992; Gajardo et al. 1997). For the generation of core particles, the inner layer can be disrupted by treatment with chaotropic agents such as sodium thiocyanate or high concentrations of calcium chloride (Cohen et al. 1979, Almeida et al. 1979). Treatment of triple layered particles with chloroform or deoxycholate disrupts VP7-VP4 integrity and abrogates infectivity (Estes et al. 1979). The RV are relatively resistant to inactivation, they are stable from pH 3.0 to pH 9.0 and they can retain infectivity after months of storage at 4°C or even at -20°C with CaCl₂ for stabilization of the outer capsid (Estes et al. 1979). Infectivity and hemagglutination are abolished by repeated freezing and thawing (Bastardo et al. 1980; Bishai et al. 1978). Removal of surface protein can be accomplished by treatment of the particles with basic solutions, fixatives and ethanol (Sattar et al. 1983). However, among all RV, human RV are somehow more difficult to cultivate and more susceptible to inactivation by decapsidation (Estes, 2001).

2.4. Genome Structure

The mRNAs are monocistronic except for gene 11 (Matton et al. 1991) that encodes for NSP5 and NSP6. The RVs are dsRNA viruses with a segmented genome and they have their own RNA dependent RNA polymerase (RdRp) that transcribes each viral RNA segment to its corresponding mRNA (Kapikian, 2001). The genomic RNA is highly packed within the core and actively interacts with proteins from the core, mainly VP2 (Labbe et al. 1994). Each mRNA has a 5’-methylated guanidine cap followed by several non-coding sequences, an open reading frame and a stop codon. The 3’ portion of each mRNA is composed of
non-coding sequences, terminal cytidines and no poly-adenylation sequences. The NSP2 and NSP5 proteins are involved with packaging of RNA (Patton et al. 2006). The NSP2 protein has non-sequence specific affinity for nucleotides and single-stranded RNA (ssRNA). It forms an octamer that undergoes a conformational shift when bound to ssRNA which is a typical feature of packaging proteins (Schuck et al. 2001). The NSP5 protein undergoes phosphorylation which has been proven indispensable for virus replication. Also, NSP5 has affinity for ssRNA, dsRNA and VP2 but the functionality of these interactions is unknown (Vende et al. 2002).

2.5. Pathogenesis

After entrance orally into gastrointestinal tract, propagation of rotavirus occurs in epithelial cells of villi of small intestine. Cell lysis occurs finally by the viral propagation, causing curtailment of the villi. Diarrhea due to rotavirus infection is considered to be caused by some different mechanisms (Ramig, 2004). Destruction of intestinal epithelial cells directly hinders absorption of water and sugars. Rotavirus infection in the cells also causes changes of metabolism of disaccharides, decreasing absorption of water, salt and sugar. Diarrhea observed remarkably before destruction of epithelial cells is considered to be implicated in activation of enteric nervous system via neurotransmitters. In addition, rotavirus non-structural protein NSP4 or its peptide fragment has a enterotoxin-like activity which induces high concentration of intracellular calcium, causing various effects including excretion of water from the cells. NSP4 which is produced in the infected cells is released outside cells and reach neighbouring noninfected cells, exhibiting the enterotoxin activity. To prevent rotavirus infection in intestine, secreting IgA directed to rotavirus plays a major role (Franco et al. 2006). Although the mechanism is unknown, serum IgG is also related to protection of rotavirus infection. While cell-mediated immunity to rotavirus also exists and CTL epitopes have been identified in viral proteins, its significance in protection against acute rotavirus infection is not clear. Most attempts to develop rotavirus
vaccine have aimed at inducing antibody to rotavirus. Neutralization antibodies to rotavirus are directed to outer capsid proteins VP7 and VP4. Therefore, these proteins are considered as the most important antigens for immunization, to obtain protective immunity to rotavirus. Although rotavirus is not neutralized by antibody to VP6, anti-VP6 IgA antibody is able to inhibit propagation of rotavirus in the infected epithelial cells, by the mechanism designated “expulsion” (Franco et al. 2006). Antibody to NSP4 may reduce severity of diarrhea caused by rotavirus infection; however, it is unable to protect rotavirus infection.

2.6. Clinical manifestation and diagnosis

Typically, rotavirus induced diarrhea is watery, lasts for approximately 5 days, is preceded by sudden onset of vomiting lasting for 2 days and is frequently accompanied by several days of fever and dehydration. The vomiting associated with rotavirus disease has been correlated with the increased level of alpha interferon found in the sera of acutely infected children (Uhnoo et al. 1986). Rotavirus infection can cause severe and prolonged disease in children with primary immunodeficiency, some of who shed virus chronically (Gilger et al. 1992). Complications of rotavirus diarrhea include intussusceptions (Nakagomi et al. 2000), diabetes and seizures (Honeyman et al. 2000). ELISA kits are commonly used to detect rotavirus infection (Gilchrist et al. 1987) and PCR amplification of rotavirus nucleic acid from stool specimen is the most sensitive way to detect group A, B and C rotaviruses (Alfieri et al. 1999; Gouvea et al. 1990).

2.7. Epidemiology

The World Health Organization estimates that 453,000 children <5 years, mostly from developing countries, died of RV infection in 2008, which accounts for 5% of global child death (Tate et al. 2012). Infection with RVs has also resulted in immense economic losses in the livestock industry (Fagiolo, 2005). Nosocomial rotavirus infections occur frequently and it is estimated that one of
every five infections is hospital acquired. Viral epidemics display a seasonal pattern with the peak occurrence during the cooler months of the year but this pattern is more prominent in temperate climates. The frequency of rotavirus infection is the same in both developed and developing countries but mortality is low in the developed countries. In India, it is estimated 78,583 annual deaths by the virus each year (Johna et al. 2014). The seasonal variation of rotavirus diarrhea in India varies in different geographical regions with high incidence in winter months at low relative humidity in north India. In India, a higher prevalence of subgroup II was reported compared to subgroup I. Electropherotyping has also demonstrated that a number of multiple electropherotypes co-circulate at one time in a particular community leading to extensive genomic variation and the appearance of new strains which may become the predominant electropherotype during the peak season (Sharma et al. 2013).

Since the first description of rotavirus in humans in 1973, and their subsequent recognition as a major human and animal pathogen, there have been a large number of studies on the structure, pathogenesis and epidemiology of these viruses. Their clinical relevance, structural complexity and unique morphogenesis strategies have prompted extensive research on these viruses in recent years. Many studies (Gouvea et al. 1990; Falcone et al. 1999; Neog et al. 2011; Mukherjee et al. 2012) illustrated on detection and typing of rotavirus by various serological techniques where antibody based technique like enzyme immunoassay (EIA)/ELISA are widely used.

2.7.1. Serological techniques for rotavirus detection

Standard method for detection of RV antigen in faeces is ELISA that has been adopted by WHO. Woode et al. (1976) reported that the infected piglet excrete $10^7$ to $10^8$ infectious viral particle per gram of faeces which could be efficiently detected by using a number of serological test such as ELISA and AGPT. In Nepal, out of 1315 diarrhoeal stool specimens, RV was detected by
ELISA in 116 (17%) patients less than 5 years of age (Uchida et al. 2006). 308 bovine field samples was analyzed and 79% tested positive by VP7 monoclonal antibody and serotyped as G1,G6,G8,G10 for bovine RV isolates (Lucchelli et al. 1994). Barman et al. in 1998 used polyclonal antibody based ELISA for detection of RV from diarrhoeic piglets and found 26.9% to be positive for rotavirus whereas Nath et al. in 2007 found 128 (22.86%) samples were positive in S-ELISA.

The clinical manifestation of naturally occurring and experimental RV infection in animals has been described by many workers (Bridger and Woode 1975; Pearson and McNulty, 1977; Neog et al. 2006). They have described that the infection is usually asymptomatic, but 8-24% of neonates may have minimal diarrhoea and vomiting associated with fever. The infected piglet excretes up to $10^7$ to $10^8$ infectious particles per gram of faeces (Woode et al. 1976). In human route of transmission is generally by fecal-oral but respiratory route has also been speculated (Singh and Pandey 1990). Neog et al. in 2006 experimentally produced piglet diarrhea in caesarean-derived colostrum-deprived piglets using cell culture propagated group A rotavirus (BRV–201). The virus concentration of $3.2 \times 10^4$ TCID$_{50}$/ml was found optimum for induction of RV infection in piglets. Typical clinical signs developed within 24–48 hours post infection. Histopathological examination revealed villous atrophy, especially in the jejunum and ileum.

### 2.7.2. Molecular techniques for rotavirus detection

The introduction of molecular methods has improved our perception of rotavirus strains that affects the development of rotavirus vaccines and involves viral evolution. Several studies have described detection of rotavirus by various molecular techniques like polyacrylamide gel electrophoresis (PAGE) and reverse transcriptase polymerase chain reaction (RT-PCR) etc.

Through RNA-PAGE, variation is generally seen on the migration of 10$^{th}$ and 11$^{th}$ genomic segment. The isolates are known as short pattern strains whereas the other is known as long pattern strains. All long pattern isolates belong to SG II
and short pattern isolates to SG I (Singh and Pandey, 1990). In Manipur, unusually large number of long pattern human isolates with SG I specificity were reported (Ghosh and Naik, 1989) earlier.

In a survey by Winiarczyk and Grandzki in 1999, ELISA, RT-PCR and native-PAGE was performed for detection of RV in faecal sample where they showed relative sensitivity and specificity of RT-PCR (100%, 98.97%); ELISA (100%, 94.7%) and PAGE (91.3%, 100%), respectively.

Martella et al. in 2001 have reported 20 porcine RV isolated in an outbreak in Northern Italy were all isolates characterized as type G6P[5] by nested-multiplex PCR. Sharma et al. in 2008 screened 1024 faecal samples obtained from hospitalized children in Delhi. Out of which, 500 were found positive by ELISA and 465 were positive by PCR. Kheyami et al. in 2008 detected 160 (87%) out of 984 stool specimen from children below 5 year by PCR.

2.7.3. Rotaviruses Serotyping/Genotyping

Based on the molecular differences, currently, 35 P-types and 27 G-types have been reported, of these, 15 P-types and 12 G-types have been identified in acute infections (Mukherjee et al. 2012). Earlier, Ramachandran et al. in 1996 determined G and P genotypes of group A rotavirus strains obtained from children admitted to diarrhea treatment centers in five Indian cities. Common worldwide strains G1P8, G2P4, G3P8, and G4P8 were indentified among Indian children (33%), whereas strains of P type [6] (G1P6, G2P6, G3P6, G4P6, and G9P6), which primarily infect asymptomatic newborns but are rare in children with diarrhea were common in India (43%). Of these, G9P6, a strain not previously reported was the most prevalent (22%).

In 2008, Broor et al. described that the most common G types reported from India are G1 and G2 and P types are P[4] and P[8]. In addition, a novel neonatal strain P type [11] human rotavirus (116 E) was isolated from neonates in Delhi, the VP4 of which was closely related to the bovine serotype G10P[11] strain B223 and VP7 was closely related to the human serotype G9 strain (Das et
Another neonatal strain G10P[11] was reported from Bangalore (Das et al. 1993). G10P[11] strains also have a high prevalence in calves with diarrhoea, in India (Varshney et al. 2002). The occurrence of these unusual rotavirus strains which are natural reassortants of human and bovine rotaviruses, suggests that reassortment may be an important mechanism for generation of new rotavirus strains. In a study from Kolkata, the predominant genotype was GI P[8] (20%), followed by G2P[4] (15%) and G4P[8] (6%). A number of uncommon genotypes G1 P[4] (4%), G2 P[8] (2.5%), G2 P[6] (0.6%), G4 P[4] (2.5%), and G4 P[6] (1.25%), were also observed by Das et al. in 2002 where 22% of specimens showed mixed infections, and 24 % of the total samples remained untypeable.

Further, in a study from Delhi, rotavirus positive stool specimens were characterized into G and P genotypes by oligo-hybridization and nested PCR (Husain et al. 1996). Of the 60, 44 could be G typed, of which 17 were G1, 13 G2, 5 G3, 4 G4 and 5 had multiple G types. Forty three samples were P typed, of these 23 were P[8], 14 were P[4], 4 were P[6] and 2 had multiple P types. All G2 strains had P[4] genotype. The G1, G3 and G4 strains were characterized into P[6] and P[8] genotypes.

In 2008, Steyer et al. reported genotype G1P[8] was the most prevalent, found in 146/241 human samples (60.6 %). The second most frequently found rotavirus genotype was G9P[8] (57 samples, 23.7 %), followed by G4P[8] (18 samples, 7.5 %) and G2P[4] (11 samples, 4.6 %). There were also some other rotavirus genotypes at lower prevalence, such as G3, G8 and G12 in combination with P[8] and just one strain with the G3P[6] combination. The most prevalent rotavirus genotypes in pigs were G3P[6] (15 samples, 18.5 %), G4P[6] (ten samples, 12.3 %) and G5P[7] (11 samples, 13.6 %). The newly recognized rotavirus genotype P[27] by Khamrin et al. 2007; Martella et al. 2007; Steyer et al. 2007a few years back was found with three different G types: G1, G2 and G4.

In another study by Paul et al. (2008), rotaviruses causing gastroenteritis in children and adults was investigated where overall rotavirus-positive rates in children and adults were 26.4 and 10.1%, respectively. Most frequent G genotype
was G2 (detection rate: 54.0 and 47.6%, respectively), followed by G1 (21.2 and 26.2%, respectively), and G9 (15.9 and 9.5%, respectively). G12 was also detected in five specimens (3.2% in total; four children and one adult). They have also described that the rotaviruses detected during the study were included in a single cluster in phylogenetic dendrograms of VP7 or VP4 genes of individual G/P types. Rotaviruses with two emerging types, G9 and G12, had VP7 genes that were phylogenetically close to those of individual G-types recently reported strains. These findings suggested that genetically identical rotaviruses, including those with the emerging types G9 and G12, were circulating in the population.

Khamrin et al. again in 2009 studied changing distribution of rotavirus G9 genotype where molecular genetic evolutionary analysis of the G9 rotavirus VP7 gene was performed. Phylogenetic analysis demonstrated that all the circulating G9 rotaviruses clustered closely together based on the year of virus isolation. One amino acid change from Threonine to Isoleucine was observed in antigenic region C at position 208 between the G9 strains isolated in 1989 and the strains of 1997-2005. These findings provide the overall picture and genomic data of G9 rotaviruses circulating at the period of study.

2.7.4. Advancement of molecular genotyping in authenticating genetic diversity, inter-species transmission and genetic reassortment.

RV demonstrates tremendous heterogeneity. The observed diversity is due to the fact that proteins are encoded by different RNA segments and thus independent reassortment of different viral genome segment can occur (Kapikian et al. 2001; Armah et al. 2010). Genetic reassortment between strains of RV isolated in a period from 2005-2007 was reported by Sharma et al. in 2008. Steyer et al. in 2008 also studied three important characteristics related to rotaviruses i.e. asymptomatic RV infection in animals, zoonotic transmission and reassortment among RV during the period of 2004-2005. Studies also reported porcine RV serotype antigenically related to human RV serotype 3 and one strain was
antigenically similar to the OSU strain of porcine RV (Serotype 3) (Nagesha and Holmes, 1988).

In 1999, Iturriza-GoMara et al. reported G and P genotypes of 3,601 rotavirus strains collected in the United Kingdom between 1995 and 1999. In 95.4% of the strains, the most common G and P combinations, G1P[8], G2P[4], G3P[8], and G4P[8], were found. A small but significant number (2%) of isolates from the remaining strains were reassortants of the most common co-circulating strains, e.g., G1P[4] and G2P[8]. Rotavirus G9P[6] and G9P[8] strains, which constituted 2.7% of all viruses and were genetically related in their G components, but the P components of the G9P[8] strains were very closely related to those of cocirculating strains of the more common G types (G1, G3, and G4). Thus, they concluded that genetic interaction by reassortment among cocirculating rotaviruses is not a rare event and contributes significantly to their overall diversity.

In 2003, Laird et al. reported that the majority of strains from children in Mexico were globally common types (55.4% of total), while uncommon types represented 5.7%, mixed infections with common types represented 14.8%, and partially or fully nontypeable isolates represented about 24%. They sequenced a subset of strains that were G nontypeable by reverse transcriptase PCR and found surprisingly that two strains having common human rotavirus P genotypes (8 and 6) had serotype G3 and G4 VP7 gene sequences that shared closer homology with canine and porcine strains, respectively, than with human strains, suggesting that these isolates represented reassortants between human and animal rotaviruses.


Globally, the most common human rotavirus strains are G1P[8], G3P[8], G4P[8] and G9P[8] on the Wa-like and G2P[4] on the DS1-like genomic
configuration (Matthijnssens et al. 2009; Banyai et al. 2012). In general, the neutralization antigen combinations of epidemiologically important animal strains are typically different from those identified in humans. For example, in swine, the G3–G5 and G11 VP7 types and the P[6], P[7] and P[13] VP4 types are the most common (Martella et al. 2010). However, some particular antigen combinations, such as G3P[6] and G4P[6], are shared between human and porcine strains (Matthijnssens et al. 2009 & 2011). In addition, other gene variants, including some of the VP6 and NSP4 genotypes, are also shared; a finding based on the postulation of common evolutionary roots of porcine and Wa-like human rotavirus strains (Matthijnssens et al. 2008a).

The overall evolutionary depiction and possible cases of RV strains producing reassortants could be resolved more accurately using the total genomic data of the circulating rotaviruses; studies those have a great impact in the massive vaccine evaluation programs and assessing efficacy of the marketed RV vaccines. In 2007, Khamrin et al. reported a novel and unusual strain of porcine rotavirus (PoRV) CMP034 isolated from a 7-week-old piglet. Phylogenetic analysis of the VP4 sequence revealed that CMP034 was only distantly related to the other 26 P genotypes and was located in a separate branch. Phylogenetic analysis of gene VP7 revealed two major lineages among G2 rotavirus strains based on the host origin. PoRV strain CMP034 clustered exclusively with G2-like PoRV strain 34461-4 in a novel lineage that is distinct from the major G2 human lineage. Moreover, the strain displayed a porcine-like VP6 and NSP5/6 with subgroup I specificity, while bearing an NSP4 with some genetic group B humanlike characteristics. These findings provided an evidence of emergence of novel VP4 genotype P[27].

In 2010, Aung et al. reported a phylogenetic study of human rotavirus where VP7 and VP8* gene sequences of 5 group A human rotaviruses detected and analyzed for their relatedness to rotavirus strains reported globally. VP7 genes of the two G1P[8] strains and the two G2P[4] strains clustered phylogenetically with Indian-Bangladeshi lineages having extremely high sequence identities. In
contrast, a G3P[8] strain exhibited a close relatedness of VP7 gene to G3 rotaviruses currently prevailing in China. VP8* genes of P[4] and P[8] strains clustered with those of Indian and Bangladeshi strains, only the G1 strain was grouped into a rare P[8] subtype, ie, P[8]b (OP354-like P[8]) with close relatedness to the P[8]b strains in eastern India and Thailand. The coexistence in Myanmar of G1/G2 and G3 rotaviruses, which are virtually identical to those predominating in India/Bangladesh and China, respectively, suggests the significant spread of these predominant rotaviruses.

In 2010, Matthijnssens et al. described G11 rotaviruses are believed to be of porcine origin. To investigate the evolutionary relationships of these strains, they analyzed the whole genomes of 2 human G11P[25] strains, 2 human G11P[8] strains, and 3 porcine reference strains. Most of the 11 gene segments of these 7 strains belonged to genotype 1 (Wa-like). However, phylogenetic clustering patterns suggested that an unknown G11P[25] strain with a new I12 VP6 genotype was transmitted to the human population, in which it acquired human genotype 1 gene segments through reassortment, resulting in a human G11P[8] rotavirus strain with an entire human Wa-genogroup backbone. This Wa-like backbone is believed to have caused the worldwide spread of human G9 and G12 rotaviruses.

In a similar study by Grazia et al. (2010), they investigated 2 rare G3P[9] human rotavirus strains isolated from children with diarrhea using full-length genome sequence analysis. The genomes were recognized as assortments of genes closely related to rotaviruses originating from cats, ruminants, and humans. Results suggested multiple transmissions of genes from animal to human strains of rotaviruses. Sequence analysis found all 3 strains to be genetically related to strains of either human or feline origin in the VP7, VP4, and VP6 genes. In contrast, the nonstructural protein (NSP) 4 gene of these viruses resembled that of G2P[4] human strains, suggesting a reassortment between AU-1–like and DS-1–like strains.
In 2011, Matthijnssens et al. reported a study of RNA-RNA hybridization assays and complete genome sequence analyses of feline rotavirus (FRV) and canine rotavirus (CRV) strains. The complete genome analysis suggested the occurrence of reassortment events among strains belonging to this feline, canine and human genogroup. These findings suggest that the human strain has a history of zoonotic transmission and is likely a reassortant among FRV/CRV strains. The detailed phylogenetic analysis indicated that three major genotype constellations exist among FRV, CRV and feline/canine-like HRV strains, and that reassortment and interspecies transmission events contribute significantly to their wide genetic diversity.

Molecular epidemiological studies on bovine rotaviruses revealed that greater than 80% of the bovine isolates across India belong to the G10 P[11] serotype. Evidence of zoonotic transmission was detected in two porcine RV strains with human strain SI-MB6, suggesting an interspecies reassortment event in the past (Steyer et al. 2008) finding two porcine RV strains carried VP7 of probable human origin. P[19] Pig RV strains were also closely related to another human P[19] strain (RMC 321) isolated from India in 1990 (Maneekarn et al. 2006). Characterization of a genotype G5P[7] human RV from Cameron with diarrhoea showed similarity to each from porcine and human RV (Mathew et al. 2004). The P[11], G10 genotype strain was an important genotype of group A bovine rotavirus because of its zoonotic transmission from humans to cattle and also from cattle to humans, as reported by Iturriza-Gomara et al. (2004). In 2013, Badaracco et al. reported that Group A rotavirus (RVA) is one of the main causes of neonatal calf diarrhea worldwide. RVA strains affecting cattle mainly possess combinations of the G6, G10, P[5] and P[11] genotypes. In addition, the G6, G10, P[5] and P[11] bovine RVA strains grouped together with human strains, highlighting their potential for zoonotic transmission. Thus, such studies of RVA circulating in animals raised for consumption and in close contact with humans, contribute to a better understanding of the epidemiology of the RVA infection and evolution.
In 2013, Kindler et al. also described that Rotaviruses are a leading cause of viral acute gastroenteritis in humans and animals. Eight different rotavirus species (A-H) have been defined based on antigenicity and nucleotide sequence identities of the VP6 gene. Here, they reported the first complete genome sequences of rotavirus F (strain 03V0568) and G (strain 03V0567) with lengths of 18,341 and 18,186bp, respectively where phylogenetic analyses indicated a separation of rotaviruses in two major clades consisting of rotavirus A/C/D/F and rotavirus B/G/H. Within these clades, rotavirus F mainly clustered with rotavirus D and rotavirus G with rotavirus B. In addition, differentiation among mammalian and avian rotavirus A strains, host-specific evolution of rotavirus B and C as well as an ancient reassortment event between avian rotavirus A and D are indicated by the phylogenetic data. These results underline the high diversity of rotaviruses as a result of a complex evolutionary history.

2.8. Possible treatments in rotavirus infection

Therapy during rotavirus induced diarrhea is aimed at preventing dehydration, as the disease spontaneously resolves in a few days to 1-2 weeks without any treatment. The standard hydration solution used is that recommended by the World Health Organization. Severe dehydration needs intravenous administration of fluids (International study group 1995; Rautenen et al. 1997). It is also reported that administration of Lactobacillus can stimulate a stronger immune response and shorten the duration of diarrhea (Majamaa et al. 1995). Rececadotril, an enkephalinase inhibitor with antisecretory and antidiarrheal action has been shown to be safe and effective in rotavirus induced diarrhea. Also antagonists for the 5-hydroxytryptamine 3 (5-HT (3)) receptor and vasoactive intestinal peptide (VIP) receptor attenuate rotavirus diarrhea (Kordasti et al. 2004).
2.9. Vaccines for rotavirus

In view of the high burden of RV disease worldwide, a safe and effective RV vaccine is urgently needed, particularly for developing countries. Passive protection of newborns by immunizing their mothers has been demonstrated as oral immunization of mothers increases levels of antibodies against rotaviruses (Pickering et al. 1998). Since animal and human rotaviruses share antigens capable of inducing protection, jennerian vaccines have been attempted (Wyatt et al. 1979). The first RV vaccine to be tested in humans was the live bovine strain RIT4237 (G6 P[1]). In two initial efficacy trials in Finland, the vaccine showed 50 and 58% protective efficacy, but little or no protection was observed in subsequent tests in Gambia, Rwanda and Southwestern USA. In view of the inconsistency of these results, efforts were made to either use naturally attenuated human RV strains or to develop reassortant RV strains bearing a human RV gene for the VP7 protein together with the other 10 genes from a simian or a bovine RV strain (Jacobson et al. 1999; Midthun et al. 1996).

2.9.1. Live reassortant virus vaccines

The first live oral reassortant vaccine was developed by the National Institute of Health (NIH, Bethesda) as a tetravalent mixture of the P[3]G3 rhesus RRV strain and of three independent rhesus-human reassortants formed between the RRV strain and human RV strains of G types 1, 2, and 4, respectively (Midthun et al. 1996). The vaccine (RotaShield™), which was introduced in 1998 in the US market by Wyeth- Lederle Vaccines, was shown to provide 48-68% protection against any RV disease and 64-91% protection against severe disease in different studies. Vaccination was however stopped less than a year later and the vaccine discontinued for use, when several cases of intussusception (IS) following vaccine administration were reported.

More recently, a pentavalent human-bovine (WC3) reassortant (GI, G2, G3, G4 and P[8]) live-attenuated, 3-dose oral vaccine, has been developed by Merck Research Co. This vaccine (RotaTeq™) was tested in a Phase III trial in
several countries including the USA and Finland on more than 70,000 children who were carefully monitored for 2 weeks after each immunization for risks of IS. The vaccine was found to be 74% efficacious in preventing any RV disease and 94% efficacious in preventing severe RV disease. The vaccine was well tolerated with respect to intussusceptions (IS). Another multivalent bovine-human reassortant vaccine has been independently developed by the National Institute of Allergy and Infectious Diseases (NIAID, NIH, Bethesda). Phase II data showed a good immune response and no adverse interference with concomitantly administered childhood vaccines (Clements-Mann et al. 2004). Finally, two naturally occurring human-bovine, neonate-derived, reassortant strains (116E and I321) are under development in India in a consortium with USA partners including CDC and the Children's Vaccine Programme at PATH (Glass et al. 2005a) and in under trial. These strains have a G9, P[11] and G10, P[11] antigenic make-up, respectively.

2.9.2. Live attenuated virus vaccines

In China, a lamb-derived monovalent (G10, P[12]) live-attenuated oral vaccine, developed by the Lanzhou Institute of Biomedical Products, is licensed and used in the private sector. The vaccine is reported to induce neutralizing antibody responses in 60% of vaccines but its efficacy is not known as it was not tested against placebo in a controlled phase III trial. A monovalent (G1, P[8]), live attenuated, 2-dose oral vaccine has been developed by AVANT Immunotherapeutics from a human RV strain and licensed to GSK Biologicals, who have further modified the strain (Jones et al. 2001). The vaccine (Rotarix™) has been tested in Latin American countries and Finland in a phase III trial on more than 63,000 children. No increased attributable risk of IS was reported in the high-risk period up to 30 days post any dose. A human neonatal G3, P[6] strain developed by Bishop and colleagues in Australia, RV3, was propagated in African green monkey kidney cell culture (Bishop et al. 1983). The strain was evaluated as an oral vaccine in 3-month-old infants and found to be safe and well tolerated.
A small Phase II study with three doses of 105 pfu of the vaccine indicated relatively low immunogenicity as measured by serum IgA. However, the vaccine recipients who developed an immune response were protected against clinical disease in the following year (Barnes et al. 2002). Further, Phase II immunogenicity studies are planned with a higher dose of the vaccine.

2.9.3. Other vaccine approaches

Rotavirus VLPs expressed in baculovirus system and delivered as injectable vaccine or an oral vaccine is an approach, which is still in development. Efforts are also underway to produce subunit vaccines comprising a number of RV structural proteins expressed in bacteria. At this time, however, these vaccines are lagging behind the live oral vaccines. Production of RV specific IgA by small intestine lymphocytes in the lamina propria is usually shortlived after either natural infection or oral immunization with a live RV vaccine. Microencapsulation of the vaccine using a combination of anionic polymers and amines has been reported to enhance and prolong the virus-specific sIgA response at the intestinal mucosal surface (Khoury et al. 1995) which hopefully might prolong the efficacy of the vaccine. Another problem with oral immunization is the potential neutralizing effect of antibodies in breast milk and colostrum. This problem also could be bypassed by micro encapsulation of the vaccine (Periwal et al. 1997).

2.10. Assessment of prevailing rotavirus strains in comparison to vaccine strains.

More recent research (Matthijnssens et al. 2011; Miyazaki et al. 2011; Tafte & Chitambar 2012) has occurred in this field where genetic variability study provided functional data for a better understanding of virus evolution particularly with respect to evidence of interspecies transmission of rotaviruses which increases the possibility of emerging new strains and unusual genotypic
combinations. This type of research also focuses the development and monitoring of effective vaccines.

The effectiveness of rotavirus vaccines can be determined by analyzing the amino acid (aa) differences, in the neutralizing epitopes of VP7 and VP4 proteins, between vaccine and the circulating strains. In 2005, Gentsch et al. have described in their work that the development of rotavirus vaccines that are based on heterotypic or serotype-specific immunity has prompted many countries to establish programs to assess the disease burden associated with rotavirus infection and the distribution of rotavirus strains. Strain surveillance helps to determine whether the most prevalent local strains are likely to be covered by the serotype antigens found in current vaccines. After introduction of a vaccine, this surveillance could detect which strains might not be covered by the vaccine. Almost 2 decades ago, studies demonstrated that 4 globally common rotavirus serotypes (G1-G4) represent >90% of the rotavirus strains in circulation. Subsequently, these 4 serotypes were used in the development of reassortant vaccines predicated on serotype-specific immunity. More recently, the application of reverse-transcription polymerase chain reaction genotyping, nucleotide sequencing, and antigenic characterization methods has confirmed the importance of the 4 globally common types, but a much greater strain diversity has also been identified. These studies also identified globally (G9) or regionally (G5, G8, and P2A[6]) common serotype antigens not covered by the reassortant vaccines that have undergone efficacy trials. The enormous diversity and capacity of human rotaviruses for change suggest that rotavirus vaccines must provide good heterotypic protection to be optimally effective.

In 2006, Arista et al. investigated the heterogeneity and evolutionary dynamics evolution of G1 rotavirus strains in a geographically defined population. Analysis of the VP7 gene sequences of G1P[8] human rotavirus strains showed the circulation of a heterogeneous population comprising three lineages and seven sublineages. Increase in the circulation of G1 rotaviruses were apparently associated with the introduction of novel G1 strains that exhibited multiple amino
acid in antigenic regions that are involved in rotavirus neutralization compared to the vaccine strains. The emergence and/or introduction of G1 antigenic variants might be responsible for the continuous circulation of G1 rotaviruses in the local population, with the various lineages and sublineages appearing, disappearing, or cocirculating in an alternate fashion under the influence of immune-pressure mechanisms. Sequence analysis of VP4-encoding genes of the G1 strains revealed that the older strains were associated with a unique VP4 lineage. The introduction of such strains thus might alter the forces and balances that drive rotavirus vaccine to be efficient.

Matthijnssens et al. in 2010 analyzed rotaviruses of genotypes G9 and G12, emerged in the human population and were able to spread across the entire globe in a very short time span. To quantify the VP7 mutation rates of these G9 and G12 genotypes and to estimate their most recent common ancestors, they used a Bayesian Markov chain monte carlo framework. For both the G9 and G12 strains, one particular (sub) lineage was able to disseminate and cause disease across the world. The most recent common ancestors of these particular lineages were dated back to 1989 (1986–1992) and 1995 (1992–1998) for the G9 and G12 genotypes, respectively. These estimates suggested that a single novel RV (e.g., a vaccine escape mutant) can spread worldwide in little more than a decade thus re-emphasizing the need for thorough and continued RV surveillance in order to detect such potential spreading events at an early stage.

In an another work by Matthijnssens et al. in 2010, it was reported that the viral genomes of each of the reassortant strains of RotaTeq were completely sequenced and compared pairwise and phylogenetically among each other and to human rotavirus (HRV) and BRV reference strains. Reassortants G1, G2, G3, and G4 contained the VP7 gene from their corresponding HRV parent strains, while reassortants G1 and G2 also contained the VP3 gene (genotypeM1) from the HRV parent strain. The P1 reassortant contained the VP4 gene from the HRV parent strain and all the other gene segments from the BRVWC3 strain. The human VP7s had a high level of overall amino acid identity (G1: 95–99%, G2: 94–99% G3:
96–100%, G4: 93–99%) when compared to those of representative rotavirus strains of their corresponding G serotypes. The VP4 of the P1 reassortant had a high identity (92–97%) with those of serotype P1A[8] HRV reference strains, while the BRV VP7 showed identities ranging from 91% to 94% to those of serotype G6 HRV strains. Contrasting to other studies, sequence analyses of the BRV or HRV genes confirmed that the fundamental structure of the proteins in the vaccine was similar to those of the HRV and BRV references strains. Their analyses showed that RotaTeq® exhibited a high degree of genetic stability as no mutations were identified in the material of each reassortant, which undergoes two rounds of replication cycles in cell culture during the manufacturing process, when compared to the final material used to fill the dosing tubes. They further described that the infectivity of each of the reassortant strains of RotaTeq®, like HRV strains, did not require the presence of sialic acid residues on the cell surface. The molecular and biologic characterization of RotaTeq® adds to the significant body of clinical data supporting the consistent efficacy, immunogenicity, and safety of RotaTeq®.

In 2011, a study from Belgium by Zeller et al. determined sequences for the VP7 and VP4 outer capsid proteins of representative G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] RVAs circulating in Belgium. The analyses showed that multiple amino acid differences existed between the VP7 and VP4 antigenic epitopes of the vaccine viruses and the Belgian isolates, regardless of their G and P genotypes. However, the highest variability was observed among the circulating G1P[8] RVA strains and the G1 and P[8] components of both RVA vaccines. In particular, RVA strains of the P[8] lineage 4 (OP354-like) showed a significant number of amino acid differences with the P[8] VP4 of both vaccines. In addition, the circulating Belgian G3 RVA strains were found to possibly possess an extra N-linked glycosylation site compared to the G3 RVA vaccine strain of RotaTeq. These results indicate that the antigenic epitopes of RVA strains contained in the vaccines differ substantially from those of the currently circulating RVA strains in
Belgium. Over time, these differences might result in selection for strains that escape the RVA neutralizing-antibody pressure induced by vaccines.

Recently, in 2013, a study by Alam et al. reported G and P type prevalence as G1P [4] = 3(6.81%); G1P [6] = 9(20.45%); G1P [8] = 1(2.27%); G2P [4] = 21(47.72%); G2P [8] = 1(2.27%); G9P [4] = 1(2.27%); G9P [6] = 1(2.27%) and G9P [8] = 7(15.90%). Antigenic characterization was performed by analyzing major epitopes in the immune-dominant VP7 and VP4 gene segments. Although the neutralization conferring motifs were found variable between the circulating strains and the two recommended vaccines strains (RotarixTM and RotaTeqTM), they validated the use of rotavirus vaccine based on the proven and recognized vaccine efficacy across the globe. The findings constitute the report on rotavirus genotype diversity, their phylogenetic relatedness and epidemiology during the pre-vaccination era and support the immediate introduction of rotavirus vaccine in the routine immunization program.

2.11. Scenario of Rotavirus research in India

The work on animal rotavirus in India started two decades back with detection of rotaviruses in feces of bovine calves using conventional test such as immunoprecipitation, counter immuno-electrophoresis, ELISA and polyacrylamide gel electrophoresis with silver staining. Majority of work has been done on cattle and buffalo calves which reveals a high percent of morbidity and mortality in young ones (Mittal et al. 1986; Kapoor, 1991; Gulati et al. 1995; Grover et al. 1998; Wani et al. 2004). Later, Gulati et al. in 1999 isolated group A rotaviruses from fecal samples of diarrheic calves using MA-104 cell lines. Isolates were compared with three standard reference BRVs viz. UK, NCDV and B223, to reveal differences in their genome and protein migration profiles. In 2000 Jindal et al. studied the genomic diversity and prevalence of rotavirus infection by RNA-PAGE and ELISA in an organized dairy farm with overall 27.02% prevalence of rotavirus infection using RNA-PAGE and ELISA both in cow and buffalo calves. Minakshi et al. in 2005 developed VP4 and VP7 gene
specific non-radio-labeled DNA probes for genotyping with overall prevalence of 44.81% of BRVs in Northern India.

In Assam, 26.9% incidence of RV in pigs is recorded by Barman et al. (1998). In 2006 Neog et al. detected 45.49% RV in faecal sample by ELISA and 22.84% was positive by RNA-PAGE. In a study conducted by Nath et al. in the same year, out of 560 faecal samples 22.86% was found positive for RV antigen by Sandwich ELISA and 17.86% as positive by AGPT where they found rotavirus prevalence to be highest in the winter (41.13%) as compared to other seasons of the year. Bora et al. in 2007 detected 51.53% RV in diarrhoeic faecal samples pigs where among the pigs of different organized farms of Assam, the highest occurrence of RV infection was reported in winter (43.58%).

In 2008, Ghosh et al. reported one rare G15P[11] and two rare G15P[21] bovine group A rotavirus strains in diarrhoeic calves in Eastern India. Sequence analysis of the VP8*, VP6, NSP4 and NSP5 genes of the G15P[11] strain confirmed its bovine origin whereas their VP6 genes shared higher nucleotide and amino acid identities with simian strain SA11. All these pointed towards a possible reassortment event of VP6 gene between bovine and simian (SA11-like) strains.

Recently, Chitambar et al. in 2009 reported a rare G1P[19] rotavirus strain from India where they evidenced reassortment between human and porcine rotavirus strains. In 2012, Kulkarni et al. analyzed VP7 and VP4 gene sequence of G1P[8] rotaviruses in comparison to the vaccine strains, where they reported immense genetic diversity within the genome of the circulating rotaviruses. Chitambar et al. again in 2014 studied the circulating trend of Indian rotaviruses where they reported emergence of a rare genotype G9P[4] rotavirus strains among the human population.