Chapter 1

Introduction
Viruses

Viruses have been known as distinct biological entities for a little more than a century. Extrapolating from current knowledge, it can be deduced that some of the recently identified viruses undoubtedly were associated with the earliest precursors of mammals and coevolved with humans, while others entered human population only recently (Flint et al., 2009).

The first report of a virus known to be smaller than any bacterium appeared in 1892. A Russian scientist Dimitrii Ivanowsky observed that the causative agent of Tobacco Mosaic disease was not retained by the unglazed porcelain filters used at that time to remove bacteria from extracts and culture media.

Viruses are distinguished from other microbes due to their simple organization, sub-microscopic, infectious nature, characteristic mode of replication and obligate parasitism. They are responsible for many diseases in plants, animals and humans and hence acquire importance. They perpetuate in nature by transmission to a susceptible host. In human population, viruses cause a wide spectrum of diseases ranging from acute to persistent infections with mild to severe symptoms.

For the past two-three decades the outbreaks and epidemics caused by viruses such as Ebola (Ebola Hemorrhagic Fever), Corona (SARS), Chikungunya (Chikungunya infections) and H5N1 (Avian flu), H1N1 (Swine flu) and Crimean-Congo Hemorrhagic Fever virus (CCHF) have significantly affected the economy of the world. Changing behavioral pattern of viruses towards the host has generated the interest to study the factors that determine and influence the frequency and distribution of diseases. Studies on antigenic and genetic characteristics of viruses and their pathogenesis and virus-host relationship have acquired importance.

Diarrhoeal disease

Diarrhoeal diseases represent a major health problem in developing countries and also to the travellers who visit these countries. Conservative estimates place the global death toll of diarrhoeal diseases at about two million deaths per year (1.7 - 2.5 million deaths), ranking third among all causes of infectious disease deaths worldwide (WHO, 2009). It is a major...
cause of mortality in the younger age group in the developing countries (Kosek et al., 2003; WHO report 2004; UN report 2005). An average morbidity attack rate of 3.2 episodes of diarrhoea per year per child has been reported, but in some settings in developing countries, this number can be as high as 12 episodes per year per child (WHO, 2009).

Etiologies of diarrheal diseases include bacteria, parasites, viruses, toxins, and drugs. The principal bacterial agents of diarrhoeal diseases comprise *Escherichia coli*, *Vibrio cholerae*, *Campylobacter jejuni* and *Shigella* species. Various protozoa include *Giardia*, *Cyclospora*, *Cryptosporidium* species and *Entamoeba histolytica*. Viruses are responsible for a significant proportion of gastroenteritis affecting the individuals of all ages. Viral gastroenteritis ranges from a self-limited watery diarrheal illness (usually <1 wk) associated with symptoms of nausea, vomiting, anorexia, malaise or fever, to severe dehydration resulting in hospitalization or even death. The clinician encounters acute viral gastroenteritis in three settings. The first is sporadic gastroenteritis in infants, which most frequently is caused by rotavirus. The second is epidemic gastroenteritis, which occurs either in semiclosed communities (eg, families, institutions, ships, vacation spots) or as a result of classic food-borne or water-borne pathogens. Most of these infections are caused by caliciviruses. The third is sporadic acute gastroenteritis of adults, which most likely is caused by caliciviruses, rotaviruses, astroviruses, or adenoviruses (http://emedicine.medscape.com/article/176515).

**Rotavirus**

Rotaviruses, the major causative agent of severe, acute gastroenteritis in infants and young children worldwide accounts for about 527,000 deaths (Parashar et al., 2009) annually and about 1/5th of this tragedy is estimated to occur in India. Rotavirus belongs to the family Reoviridae ("Reo" meaning Respiratory Enteric Orphan Virus), since the first member of this family to be discovered was found to inhabit both the respiratory and enteric tracts of man and animal, but to be orphan in the sense that they were not associated with any known disease.
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A mature complete rotavirus particle is about 70nm in diameter (Figure 1). Incomplete particles that have lost the outer capsid are common in fecal specimens. Only the complete 'double shelled' particles have been shown to be infectious. The virus contains an RNA dependent RNA polymerase and other enzymes capable of synthesizing capped but non-polyadenylated mRNA transcripts. Virus replication occurs in the cytoplasm of infected cell.

Rotavirus genome consists of 11 segments of double stranded RNA, which is enclosed in three concentric layers of protein capsid, the outer, intermediate and inner capsids (Prasad et al., 1988; Shaw et al., 1993; Estes, 2001). The gene segments range from 0.6 to 3.3 kilo base pairs in size and code for 6 structural proteins (VP1 - VP4, VP6 and VP7) and 6 non-structural proteins (NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6). Due to the segmented nature of the genome, rotaviruses can undergo genetic reassortment (Estes, 2001).

On the basis of a major inner capsid protein VP6, rotaviruses are classified in seven antigenic groups (A-G) of which groups A, B and C are known to infect humans. Two structural proteins, VP7 (the glycoprotein or G protein) and VP4 (the protease sensitive protein) make up the outer shell, and are the major antigens involved in virus neutralization. Based on these proteins a binary classification system for serotyping / genotyping has been developed. Genotypes of VP4 and VP7 are determined by sequence / hybridization analysis, whereas serotypes are determined by reactivity with either polyclonal or monoclonal antisera (Estes, 2001). To date, 27G and 35 P types of rotavirus strains have been reported (Matthijnssens et al., 2011). A correlation between genotype and serotype has been established for VP7, however not for VP4, though sequence variation between amino acids (aa) 84 and 180 has been suggested for this purpose (Larralde et al., 1991). Three of the rotavirus structural proteins (VP4, VP6 and VP7) have been studied extensively because of their unique biochemical, antigenic and biological properties in rotavirus replication and assembly. Recently a non structural protein, NSP4 known to carry receptors for VP6 and enterotoxigenic property has been also studied for its role in the
morphogenesis of rotavirus and development of rotavirus vaccine candidate (Hyser et al., 2008).

Group A rotavirus infections are common in early years and occur repeatedly throughout life (Bishop, 1994). The incidence of rotavirus disease has been found to be similar in both developed and developing countries (Dennehy et al., 2008). Prevalence rate of rotavirus infection has been reported to be 25-36% in children and about 5-7% in adults, globally (MMWR, 2011). Recent studies on the global prevalence of rotavirus disease estimate that group A rotaviruses cause 39% hospitalizations among children each year (Parashar et al., 2006b). Adults undergo rotavirus infections in a manner similar to children usually with minimal clinical manifestations. Rotavirus infection in immuno-compromised adults can vary from symptom less to severe and sustained infection (Anderson and Weber, 2004). Deaths due to rotaviral infection in hospitalized adults have also been reported (Vasil'ev et al., 1995). Rotavirus infections have been described to occur in different settings such as epidemic and endemic spread, travel related infections, from child to adult and in waterborne outbreaks. The studies conducted in the countries from Europe, America, Asia and Australia have reported 2-44% rates of rotavirus prevalence in adults with gastroenteritis (Timenestsky et al., 1996; del Refugio-Gonzalez-Losa et al., 2001; Banyai et al., 2002; Anderson and Weber, 2004; Faraque et al., 2004; Rubliar-Abreu et al., 2005; Wang et al., 2007). Limited serological studies conducted in adults have detected anti-rotavirus IgM antibodies providing indirect evidence of circulation of rotaviruses in adults (Cox et al., 2003; Ray and Kelkar, 2004a; Ray et al., 2007; Chan et al., 2011). A few studies document the genetic diversity of rotaviruses and occurrence of unusual rotavirus strains in adults that may pose challenges to new vaccine programs (Anderson and Weber, 2004; Fischer et al., 2005; Wang et al., 2007).

Human group B rotavirus (GBR) designated as adult diarrhea rotavirus (ADRV) was first detected in China in the year 1982-1983 during a large-scale epidemic of diarrhea affecting more than one million people (Hung et al., 1984). Since then, several small outbreaks and sporadic infections of GBR have been reported from China (Fang et al., 1989; Yang et al., 1998; Yang et
al., 2004). Other than China, GBR has been detected in sporadic cases of diarrhea from India (Krishnan et al., 1999), Bangladesh (Sanekata et al., 2003) and Myanmar (Aung et al., 2009). GBR infections have also been identified in the outbreaks of gastroenteritis from India (Chitambar et al., 2011).

Group C rotaviruses were first isolated in piglets in 1980 (Saif et al., 1980) and in humans in 1982 (Rodger et al., 1982). Since then, 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; Rasool 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. Since 2000s, studies from different countries on group C rotaviruses 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).

Clinically, profuse diarrhoea, mild fever and vomiting, leading to mild to severe dehydration characterize rotavirus gastroenteritis. Rotavirus is transmitted by the faecal-oral route via contact with contaminated hands, surfaces and objects (Butz et al., 1993) and possibly by the respiratory route (Dennehy et al., 2000). As improved sanitation, hygiene practices and water supply cannot control the spread of rotaviruses, intervention measures such as vaccination acquire importance (Dennehy et al., 2008). To date, two rotavirus vaccines, Rotarix and RotaTeq are available for the control of group A rotavirus infections. These vaccines have shown good safety and protective efficacy records with immunization regimen of two (at 2 and 4 months of age) and three (at 2, 4 and 6 months of age) doses of Rotarix and RotaTeq respectively (Ruis-Palacios et al., 2006; Vesikari et al., 2006a). In developed countries, the efficacy of these vaccines has been reported to be significant against rotavirus gastroenteritis of any severity (74-79%), severe rotavirus gastroenteritis (90-98%) and hospitalization due to rotavirus (96%) (Dennehy, 2008). As against this in the developing countries from Asia and Africa, a low
level (39.0-77.0%) efficacy of these vaccines has been noted (Armah et al., 2010; Zaman et al., 2010; Patel et al., 2011).

A recent review of studies carried out for the assessment of the efficacy of Rotateq (RV5) vaccine, showed effectiveness of RV5 to be around 70-82% and 82% against rotavirus gastroenteritis related deaths and hospitalizations respectively in India (Khoury et al., 2011). Studies on immunogenicity, reactogenicity and safety of Rotarix (RIX4414) vaccine in the Indian infants during 2006 have shown IgA seroconversion rate of 58.3% at one month after receiving the second dose (Narang et al., 2009). Immunogenicity studies of live attenuated oral rotavirus vaccine, 116E in Indian infants conducted during 2006-2008 have shown four-fold increase in IgA titres in 89.7% of infants 28 days after 3 doses of 1x10^5 FFU (Bhandari et al., 2009).

**Figure 1.1: 2D Structure of Rotavirus**