Chapter 1
Introduction

1.1. Overview

Gastroenteritis can be defined as a medical condition of inflammation in the gastrointestinal (GI) tract, diarrhoea, vomiting and abdominal cramping leading to severe dehydration and fatality. Acute gastroenteritis causing dehydrating diarrhoea accounts for severe economic loss due to morbidity and mortality in man and animals throughout the year. There are numbers of infectious as well as non-infectious causes for gastroenteritis but Rotaviruses (RV) are the single most, important, viral etiologic agents of severe, acute dehydrating diarrhoea in many mammalian species, including humans, calves and pigs (Miyazaki et al. 2011; Ahmed et al. 2012; Badaracco et al. 2012).

Rotavirus infection is highly contagious and is most common in infants (each three among five children <5 years), but repeated, asymptomatic infections are believed to occur in adults. Infection appears to peak during the winter season, except in countries with tropical or subtropical climates, where the virus is present year around. It is transmitted mainly by the fecal-oral route, via contact with contaminated water source, hands, surfaces and after an incubation period of 1-2 days, the onset of gastroenteritis is sudden. Symptoms can last from 4-5 days and range from diarrhoea and vomiting, fever, occasional abdominal pain, loss of fluids and electrolyte disequilibrium, thus leading to severe dehydration and hospitalization with other secondary complications (e.g. renal failure) including death (Holmes 1988; Lipson et al. 1989). They primarily infect the enterocytes of the small intestine, but it is also found to be associated with Central Nervous System (CNS) complications (Lynch et al. 2003). Rotaviruses are relatively resistant to inactivation, they are stable from pH 3.0 to pH 9.0 (De Boissieu et al. 1993) 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 (Pesavento et al. 2006). For these reasons, rotavirus remains a main concern as candidate for vaccine
development and their evaluation programs mainly in the developing countries including India.

Rotaviruses are the only known virus infecting mammal species that contains 11 dsRNA segments of a size range of 0.6 to 3.3 Kb (Kapikian et al. 2001). They are non-enveloped virus of the family *Reoviridae* with an icosahedrally capsid, 70nm across. The virus particle is comprised of three concentric protein layers surrounding an eleven-segmented dsRNA genome. Of this genome, six segments code for the six structural proteins (VP1, VP2, VP3, VP4, VP6 and VP7) that form the capsid of the virus and five segments encode the remaining six nonstructural proteins (NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6) which are involved in virus replication and present exclusively in infected cells, thus forming the triple layered particle (TLP). Among the TLP concentric layers, the core consist three proteins VP1, VP2, VP3 and an inner capsid protein (second layer) VP6 to form the double layered particle (DLP). The two outermost proteins, VP4, a non-glycosylated protein encoded by RNA segment 4 and VP7, a glycoprotein encoded by RNA segment 9 finally maintain the overall TLP structure by defining the outer shell and spikes of the virion (Fig 1.1A and Fig 1.1B). These two outermost proteins also contain the virus neutralizing antigens that elicit antibodies to block virus cell entry and inhibit virus replication (neutralizing antibodies). It was established that trypsin cleaves the VP4 and leads to the formation of VP5* and VP8* that enhances virus infectivity (Fig 1.2) (Crawford et al. 2001).

When the virus encounters a host, the outermost layer of the RV TLP recognizes the host cell receptor and elicits host-virus interaction through the virus spikes. This facilitates the entry of the virus into the host cell shedding the outer layer and results in a clinical and transcriptional active DLP. The successive DLP infection results in viral pathogenesis. Since, outermost layer of RV DLP comprising inner capsid protein VP6 maintains the overall viral integrity during the process of infection and viral replication; it is the main RV antigen as most of the RV-specific antibodies induced after natural infection and/or immunization
are directed against VP6 (Johansen et al. 1994). Thus, it is considered the most immunogenic rotavirus protein (Rahman et al. 2003).

Therefore, the most antigenic structural RV protein is VP6 followed by VP4 and VP7. The inner capsid protein VP6 plays an important role in maintaining the structure of the capsid by interacting with VP2, VP7 and VP4; whereas VP7 and VP4 is primarily responsible for host-virus interaction.

![Schematic representation of (A) Rotavirus Morphology, (B) RNA segments and its corresponding genes coded by Rotavirus genome (Source: National Institute of Allergy and Infectious Disease, 2010).]

**Fig 1.1** Schematic representation of (A) Rotavirus Morphology, (B) RNA segments and its corresponding genes coded by Rotavirus genome (Source: National Institute of Allergy and Infectious Disease, 2010).
Being segmented in nature, rotavirus genome has an affinity towards reassortment (exchange) of the segments particularly during co-infection of different strains among multiple hosts. Rearrangements are a different type of mutation that has been proven to confer to the virus a selective advantage by improving growth and/or increasing viral stability (Mattion et al. 1990). The RNA segments have the capacity to reassort and replace the normal existing genomic RNA of a non-rearranged virus strain. Thus, the virus genomic rearrangement usually does not impair infectivity rather increases a chance of emerging a new viable strain that may escape vaccine response. Protection against rotavirus would depend on the components of the existing vaccines. Any genetic alteration or mutation within their genome not conforming to the vaccine constituent may lead to non-optimal protection. It is utmost necessary to monitor regularly such genetic alteration among the commonly circulating strains of RVs in a geographically defined locality.
The electrophoretic mobility of the RNA segments from group A RV is characterized by 4 high molecular weight segments, 5 intermediate weight segments grouped in 2 larger and 3 smaller segments and 2 low molecular weight segments. Polyacrylamide gel electrophoresis (PAGE) is a useful tool to classify but other assays like Northern blot and sequencing are important complementary tools for the identification of reassortants that are two different virus strains that interchange one or more RNA segments (Watanabe et al. 2001). Application of such tools to study genomic variability in newly set up laboratory can reconfirm the robustness of the techniques. Further, analysis of rotavirus with rearranged genome aids in the study of vaccine evaluation program.

Rotaviruses are classified serologically into 7 distinct groups, A to G based on cross-reactive group-specific antigenic determinants present on VP6 protein (Estes and Cohen, 1989; Ojeh et al. 1991). Groups A, B and C have been isolated from humans and animals whereas groups D, E, F and G have been isolated from animals only.

The intermediate capsid protein VP6 is also the subgroup-specific antigen. Rotaviruses are further classified into four subgroups i.e., subgroup I, subgroup II, subgroup I and II and non subgroup I and II (Greenberg et al. 1983a).

Rotavirus dsRNA segments fall into four size classes (Fig 1.3) viz. class I (segments 1,2,3,4); class II (segment 5,6); class III (7,8,9) and class IV (10,11). Based on the faster or slower migration of class segments ‘long,’ ‘short,’ and ‘supershort’ electropherotypic patterns are analyzed.
Within the groups, rotaviruses are classified into serotypes using sera directed against VP7 and VP4, the two outer capsid proteins. Classification based on VP7, which is a glycoprotein, is termed ‘G’ serotype and that based on VP4, which undergoes proteolytic cleavage, is termed ‘P’ serotype (Mattion et al. 1994). Epidemiologic surveillance of the G- and P-serotypes to clarify prevalent types are important to consider rotavirus vaccine.

However, serotyping antibodies against all serotypes are not available to conveniently to sero-classify rotaviruses and to classify new strains that do not react with the known serotyping antibodies and in such situation genotyping is utilized (Estes and Cohen, 1989). Genotypes of VP4 and VP7 are established by sequence analysis. G-type is determined by the sequences of the variable regions as well as sequence identity of whole VP7 sequences. At present, numbering system of G type (G1-G16) is identical to that of G-serotype. VP4 is made up of two portions corresponding to VP8* and VP5* cleaved by trypsin which represents the P type. P-type is usually described as the number in bracket, and has different numbering system from P-serotype. Sometimes P-type is described with P-serotype, as “P[8]1A”. Because of the complexity of determining P-
serotype, genetic typing system (P-type) has more developed. VP8* sequence is more divergent than VP5* sequence and contains more P-serotype-specific regions. Therefore, VP8* sequence is generally used for discrimination of P-types.

1.2. Challenges in rotavirus research

Rotavirus (RV) remains a main concern for neonatal diarrhea, overwhelming mainly in the developing countries of Asia and Africa. As per the recent report by Katoch in 2014, South Asian country like India endured significant child deaths of 78,583 children per year. Besides, RV outbreaks have also resulted immense economic losses in the livestock industry and here, group A rotaviruses (RVA) acquire the crest for frequent epidemics in piglets and calves. The viruses, having a segmented genome can exchange genetic material, more likely during mixed/co-infections by human-human or human-animal strains. Since, rotaviral transmission is mainly through fecal-oral route and is known for their environmental resilience, it is tempting to suggest that close association of animals with their human handlers favours an appropriate situation for interspecies/zoonotic transmission. Although without clear evidence of host restriction, animal RV gene segments were detected from humans and vice-versa globally (Martinez et al. 2014). Therefore, such region holds a potential hub for surfacing new infections and provides an evidence for animals to act as a source of virus and/or of genetic material transmission leading to emergence of novel/unusual genotype combinations. Moreover, existing rotavirus vaccines were derived or generated using human group A rotavirus strains circulating in the early to mid-1980s for RotaTeq and the late 1980s for Rotarix. It is possible that due to possible cases of reassortments, the currently circulating strains with unusual genotypes are different from those of the vaccine strains. As a result of vaccine implementation, a varying selective pressure against these different strains could be induced and over time, this might result in reduced vaccine effectiveness. Therefore, the subsequent global spread of these apparent
unusual/novel strains may result in the establishment to the population through
time representing a challenge to the massive vaccine evaluation programs.

1.3. Scope of the present investigation

The national surveillance network programs 2005 accomplished by Indian
council of medical research (ICMR) in collaboration with Centre for Disease
Control and Prevention (CDC) initiated again in 2012 which is ensuring
representative data from various new clinical recruitment sites or hospitals.
Nevertheless, many of the high disease burden areas in northeast India portraying
rural subsistence with lack of sanitary disposal maintenance system and human-
animals close settings remains unexplored.

Fig 1.4 WHO under five world mortality rate due to rotavirus disease per 100,000
populations (<5 years of age) (Source: WHO Bulletin, 2008)
**Fig 1.5** WHO under five mortality rate due to rotavirus disease per 100,000 population (<5 years of age) in India (Source: WHO Bulletin, 2012).

**Fig 1.6** (A) Indian Rotavirus Surveillance Network sites for the first time in collaboration of Indian council of Medical research (ICMR) and Centers for Disease Control and Prevention (CDC) which recruited children <5 years of age hospitalized with acute gastroenteritis between 2005 and 2009. (B) Current and Planned National Rotavirus Surveillance Network established again in 2012 recruiting few new referral laboratories (Source: Katohc, 2014).
Molecular characterization of rotaviruses circulating in different geo-climatic areas of characterized regions is paramount importance to select appropriate candidates for vaccine. Based on these challenges, we explored Barak and Brahmaputra valleys of the state Assam where a systematic study of rotavirus characterization at ground level including man and animals living in close proximity is lacking so far. The region characteristically footed on agro-economy where farm animals are being maintained for household and economic purpose. Here, animals are generally housed indoors in group-housing or straw-lined sheds or pens, which allows easy contact with waste matter for the human handlers and vice versa. These animals are also constantly utilized either for domestic or meat purpose. Thus, the animals along with the dwellers, both are at risk of contracting infection which is maintained in the environment. These animals hereby act as an important host to thrive the infection ensuing possibility of interspecies transmission either by fecal-oral route or through direct consumption in any form that accounts the basis of rotaviral circulation in the whole community. The phenomenon is a feature that is predicted to generate possibly more dangerous virus strains and successively causing serious threat to vaccine failure as the efficacy and effectiveness of rotavirus vaccine may differ depending on the predominant natural strain types. Therefore, during the present research work, we aimed to analyze first the VP6 protein, an antigenic factor of rotavirus with a monoclonal antibody targeted against them so as to assess the degree of infection in human, bovine and porcine. Further, we explored the genotypic pattern of the rotaviruses circulating in such human-animal proximal regions by analyzing the VP4 and VP7 gene sequences. Assessing subsistence of putative cases of interspecies/zoonotic transmission and reassortment events by rotavirus strains were also attempted for the time from the region which was quite evident by the unique animal–human mixing patterns. Moreover, the trends of antigenic co-relation of the recent prevailing rotavirus strains with the vaccines strains were too evaluated during the study.
Since, the above stated scenario clearly depicts the episodes of interspecies transmission is a frequent event of rotaviruses during human-animal or animal-animal mixed infection/co-infection, the human neonates associated with those farm animals were too investigated during the study to demonstrate the instances of genetic reaasortment and possible evolution of any viable new strains. Thus, the present work portrays a complete scenario regarding identification and characterizing rotaviruses from farm animals along with the children of associated animal dwellers from two regions of Assam, the Barak and the Brahmaputra valleys circulating among the farms animals during the period of year 2011-2014.

Objectives of the study

To accomplish the statements of problem discussed above and to satisfy the hypothesis of the present research work, the following objectives have been taken:

- Screen Rotaviral antigen in the faecal samples of human and animal at different geographical locations.

- Analyze Rotaviral nucleic acid detected in human and animal faecal samples.

- Characterize G-types of Rotavirus demonstrated in the faecal samples.

- Study the genetic diversity of Rotavirus detected in human and animals.