Rotavirus is one of the most significant etiological agents of severe gastroenteritis in infants and children below five year of age. Worldwide, gastrointestinal rotavirus infections result in an estimated 453000 deaths in children below five years of age (Tate et al., 2012), with most rotavirus gastroenteritis mortality happening in African countries and South Asian countries (WHO, 2013). The main mode of transmission of this virus is via faeco-oral route, thus incidence of diarrhea induced by Rotavirus in under developed and developing countries is likely to be very high due to poor hygienic condition and insufficient chlorinated water supplies. In Indian socioeconomic fabric, where animals and human infants/children share same habitat, spread of rotaviruses across species is likely to be very common (Kang et al., 2004; Tate et al., 2012; Saluja et al., 2014). There are earlier reports indicating interspecies transmission of rotavirus strain/serotypes (Das et al., 1994; Estes and Greenberg, 2013; Desselberger, 2014).

According to latest estimates, Rotavirus gastroenteritis is responsible for 78500 deaths (About 22% of global mortality), 872315 hospitalizations, 3271187 outpatient visits and 11373098 cases of diarrheic episodes in Indian children below five years of age. Till five years of age, one in two children suffer with diarrheic episode, one in every 8 children have to visit a paediatric hospital for rotavirus gastroenteritis, one in each 31 children will be admitted in hospital and one in each 345 Indian children will die due to rotavirus gastroenteritis (John et al., 2014). Expenditure incurred as direct costs per year for the medical care of rotavirus gastroenteritis in children below 5 years was estimated as INR 4.9 billion and INR 5.38 million in indirect (nonmedical) costs, with a total economic burden of Rs 10.28 billion (John et al., 2014). This high disease and economic burden makes an essential case for considering the use of specific interventions for prevention of rotavirus diarrhoea such as vaccines.

Rotaviruses belong to genus Rotavirus under family Reoviridae. Rotavirus is non-enveloped, 75 nm in diameter and its genome is encapsulated in three layers of capsid. Genome consists of 11 segments of double stranded RNA, with each segment coding for single protein except segment 11 which is proposed to encode for two polypeptides (NSP5, NSP6) (Estes and Greenberg, 2013). The genome is encased in core, composed of VP1, VP2 & VP3. The core is further encapsulated by major capsid protein, VP6. This protein constitutes approximately 51% of capsid proteins (Estes and
Kapikian, 2007). A major titer of antibodies is directed against VP6 protein and this protein is targeted in most of the immunological assays (ELISA, Immunofluorescence and immunochromatography) for Rotaviral detection (Greenberg et al., 1983; Estes and Kapikian, 2007; Desselberger, 2014). On the basis of genetic data of VP6, rotaviruses have been classified into eight groups (A to H) (Matthijnssens et al., 2012).

GARs are further classified into subgroups (SG) I, II, I+II and non-I, non-II based on their reactivity with VP6 specific array of monoclonal antibodies (Greenberg et al., 1983). Subgroup II rotavirus infection has been more commonly reported in human being while subgroup I rotavirus infection has been more frequent in animals. The outermost capsid layer is constituted by two structural proteins: VP7 (Glycoprotein and encoded by segment 7, 8 or 9 of ds RNA genome depending on the strain) & VP4 (Protease sensitive protein and encoded by gene segment 4). A high titer of neutralizing antibody is induced against these proteins during infection and these proteins independently participate in neutralization process. Earlier, monoclonal antibodies were used against these proteins for dual classification of group A Rotaviruses, designated as G- serotype (Glycoprotein VP7 based) & P- serotypes (Protease sensitive VP4 based) but, presently sequence based molecular methods of genotyping are in routine practice. Limited supply of monoclonal antibodies and consistency are the major drawbacks of antibody based serotyping.

Molecular analysis has determined 27 G-types and 37 P-types infecting both human and animals (Matthijnssens et al., 2011; Trojnar et al., 2013). Increasing use of sequencing based genotyping has complemented serotyping methods and is commonly used. According to Rotavirus classification working group, Rotavirus A genome segments VP7-VP4-VP6-VP1-VP2-NSP1-NSP2-NSP3-NSP4-NSP5/6 are referred to as the Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx genotypes, respectively (Matthijnssens et al., 2008). Genotypes G1-G4 have been found as the most common detected G-types whereas rotaviruses strains carrying P[8], P[6] and P[4] genotype have been recognized as the major strains causing rotavirus diarrhea (Gentsch et al., 1996) but during last decade novel genotype G9 has become the third commonly detected genotype in India (Kahn et al., 2012). G12 reported sporadically at the starting of 21st century is also increasing in prevalence. Contrary to this, incidence of G3 and G4 is decreasing continuously in India (Miles et al., 2012; Tiku et al., 2014; Babji et al., 2014)
In addition to VP4, VP7 & VP6 gene, NSP4 gene play important role in pathogenesis of rotavirus. This protein is located mainly in endoplasmic reticulum as a transmembrane protein. It is a multifunctional protein as it acts as intracellular receptor for interaction with VP6 of double layer particles (Taylor et al., 1996). It acts as viroporin for release of calcium from intracellular locations (Hyser et al., 2010, 2012) and elevated intracellular calcium level is required for stabilization of TLPs. This increased level of Ca^{2+} activates a kinase dependent signalling, which resulted in autophagy (Crawford and Estes, 2013). It changes permeability of plasma membrane and most importantly, it acts as an enterotoxin which leads to diarrhea (Ball et al., 1996). Furthermore, NSP4 has been found to provide heterotypic immunity and anti-NSP4 antibodies decreased the severity of rotavirus infection (Ray et al., 2003).

A number of detection methods have been developed in the past for rotavirus diagnosis. Conventionally, rotavirus detection is done by rotavirus antigen detection in faeces using enzyme linked immunosorbent assay (Beards et al., 1989). Other methods of detection of rotavirus rely upon the presence of viral genomic RNA in the stool sample. These methods include direct detection of rotavirus genomic ds RNA by polyacrylamide gel electrophoresis (PAGE), probe hybridization assays and seminested reverse transcription-Polymerase chain reaction (RT-PCR). Nucleic acid based assays are more sensitive than antibody based assay (Wilde et al., 1991). Few RT-Real Time PCR studies based on SYBR green chemistry have been published (Kang et al., 2004; Min et al., 2006) but detection of multiple genotypes with single assay has not been demonstrated. Rotaviruses remain highly stable in the environment for long period of time. This is the reason for high incidence of rotaviral gastroenteritis, especially in winter months. In the present study, a new Real Time PCR based approach for Group A Rotavirus detection will be developed for detection of multiple rotavirus genotypes and its sensitivity will be compared with conventional diagnostic tools, like Enzyme linked immunosorbent assay (ELISA), RNA-PAGE and Reverse Transcription PCR.

Among rotaviruses, group A rotavirus is most common and results in severe diarrheal diseases in infants, neonates and young ones of animals (Midgley et al., 2012). The G-genotypes G1, G2, G3 and G4, has been found most frequently worldwide in combination with P-genotype P[4] & P[8] (Banyai et al., 2012). Continuous reassortment may be reason for rapid emergence of some other strains like G9 and G12. Genotype G9 was first isolated in 1980s by Clark et al. (1987) but its
prevalence was very low until mid 1990s when it was reported from USA and UK (Ramachandran et al., 1988; Iturriza-Gomara et al., 2000). Gradually G9 prevalence increased in many nations including Bangladesh, Nepal and India (Gentsch et al., 2005; Saluja et al., 2014; Santos and Hoshino, 2005; Tiku et al., 2014).

Recent findings from various countries (Bangladesh, India, Nepal, Brazil and other countries) have reported increasing prevalence of another important emerging genotype G12, in combination with various P types such as P[8], P[6], P[9] (Castello et al., 2006; Estes and Kapikian, 2007; Pongsuwananna et al., 2010; Rahman et al., 2007; Santos and Hoshino, 2005). Zoonotic nature of this virus has contributed more genotype diversity and has increased the chances of rapid emergence of rare genotype combinations which may result in evolution of new global strains over the time scale (Li et al., 2008; Urasawa et al., 1992; Varghese et al., 2004). Therefore, regular epidemiological studies are required for monitoring these rare genotype combinations, especially in the countries who are considering introducing Rotavirus immunization in their vaccine programs. These pre-vaccination and post-vaccination studies need to be conducted to monitor the effectiveness of the vaccine strains on these rare strains and whether these strains are capable to escape the host immune response elicited by immunization. Several reports from Asian countries, Australia and Brazil have indicated the presence of genotypes which could not be targeted by the candidate vaccines (Paul et al., 2008; Rahman et al., 2007; Snelling et al., 2011; Gomez et al., 2011).

Sustained endeavors have been made to design potent rotavirus vaccines to decrease the childhood morbidity, mortality and economic losses. In 1998, Rotashield™ was licensed for commercial production but it was found to have strong correlation with intestinal intussusception in children (Simonsen et al., 2005). Therefore, it was withdrawn from the market. Food and Drug Administration (FDA), USA has approved two rotavirus vaccines: Rotarix™ which is a live attenuated human monovalent oral strain (G1P[8]) (Glaxo Smith Kline, USA) and RotaTeq™ which is a live attenuated pentavalent oral oral reassortant strain WC3 (G1, G2, G3, G4, P[5]) (Merck, USA). These vaccines have been found highly efficacious against the major G-P- combinations and available in more than 100 nations for routine vaccination program.
Induction of rotavirus vaccine in national vaccination schedule in developing and poor countries has become main priority for decreasing the rotavirus disease burden. RotaTeq and Rotarix have been found highly effective against the severe rotavirus gastroenteritis in developed countries (Ruiz-Palacios et al., 2006; Vesikari et al., 2006) but their efficacy was comparatively lower in developing countries (Madhi et al., 2010). Both vaccines are available commercially in India but due to high cost, these are being used in private sector only and vaccine coverage has been estimated to be less than 10% (Gargano et al., 2012). An indigenous live attenuated vaccine based on strain 116E, Rotavac™ has cleared clinical phase III trials and will be available commercially in near future. Indian government has announced introduction of rotavirus vaccination in national immunization program in July, 2014. Therefore, molecular surveillance before introduction of this vaccine will be of prime importance for determination of its efficacy and detection of newly emerging strains, if any in this region.

Further, studies on variations in nucleotide sequence and genome diversity analysis may prove to be useful in providing a base line data for molecular characterization of emerging rotavirus strain. Although the prevalence of group 'A' rotavirus in bovines has been reported from Haryana (Minakshi and Pandey, 2002), there is paucity of information on the distribution of human rotavirus genotypes in this state. The present research work is therefore aimed at cloning and sequencing of hyper-variable regions of VP4 and VP7 genes and studying genomic diversity in circulating rotavirus isolates. In addition, variation in amino acids at important epitopes of Haryana rotavirus strains with vaccine strains could affect the efficacy of vaccination. These variations can be determined by comparing these epitopes for both Haryana rotavirus strains as well vaccine strains. Therefore, the present study was designed to gather molecular surveillance data of circulating Haryana strains.