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# Rotaviruses: Is their surveillance needed?

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ABSTRACT

Rotaviruses, a major cause of gastroenteritis in children worldwide accounts for around 0.5 million deaths annually. Owing to their segmented genome and frequently evolving capability, these display a wide variation in their genotypes. In addition to commonly circulating genotypes (G1, G2, G3, G4, G9, P[4] and P[8]), a number of infrequent genotypes are being continuously reported to infect humans. These viral strains exhibit variation from one geographical setting to another in their distribution. Though the introduction of vaccines (RotaTeq and Rotarix) proved to be very effective in declining rotavirus associated morbidity and mortality, the number of infections remained same. Unusual genotypes significantly contribute to the rotavirus associated diarrhoeal burden, may reduce the efficacy of the vaccines in use and hence vaccinated individuals may not be benefited. Vaccine introduction may bring about a notable impact on the distribution and prevalence of these viruses due to selection pressure. Moreover, there is a sudden emergence of G2 and G3 in Brazil and United States, respectively, during the years 2006–2008 post-vaccination introduction; G9 and G12 became predominant during the years 1986 through 1998 before the vaccine introduction and now are commonly prevalent strains; and disparity in the predominance of strains after introduction of vaccines and their natural fluctuations poses a vital question on the impact of vaccines on rotavirus strain circulation. This interplay between vaccines and rotavirus strains is yet to be explored, but it certainly enforces the need to continuously monitor these changes in strains prevalence in a particular region. Furthermore, these fluctuations should be considered while administration or development of a vaccine, if rotavirus associated mortality is ever to be controlled.



## 1. Introduction

Rotaviruses (RVs), an important cause of gastroenteritis in various animal species, cause approximately 0.5 million deaths (under the age group of five years) out of 0.8 million total diarrhoea associated child deaths annually [1,2]. Almost all children in the world get infected at least once before they reach the age of 5 and peak incidences occur below the age of 2 years. Furthermore, a recent Global Enteric Multicenter Study (GEMS) reported rotaviruses as the leading cause of diarrhoea in children which included cohort of over 20,000 children from Asia and Africa [2]. Underdeveloped and developing nations of the world are worst affected. Although the mortality rate due to diarrhoea has declined, there is no significant affect on rotavirus associated hospitalizations. In this review, we discuss various aspects of rotavirus focusing on its diversity which is a great challenge for the current vaccination programmes and hence imposing a need for continuous surveillance of this deadly virus.

### 1.1. Structure

Rotaviruses were first observed in 1973 by Bishop and colleagues as a 70 nm particles having a wheel like appearance. These belong to the family *Reoviridae*, have segmented genome (11 segments) of double stranded RNA with size of around 18,550 bp and length of RNA segments range from 667 to 3302 nucleotides. These RNA segments encode 6 structural (VP1, VP2, VP3, VP4, VP6, VP7) and 6 non-structural proteins (NSP1-NSP6). The viral genome is enclosed with a triple layered capsid (Fig. 1). The inner most layer is made up of VP1, VP2 and VP3; the intermediate layer is composed of VP6 protein; and VP4 and VP7 proteins assemble to form outermost shell of the virus. VP6 protein is the basis for classification of rotavirus in various groups (A–G) whereas VP4 and VP7 proteins contain epitopes against homotypic and heterotypic neutralizing antibodies.

### 1.2. Pathogenesis

Rotaviruses are transmitted via feaco-oral route and infect humans and animals by adhering to the epithelial lining of gastrointestinal tract. Various mechanisms [3] which contribute to pathophysiology of rotavirus diarrhoea are: (1) Viral entry causes

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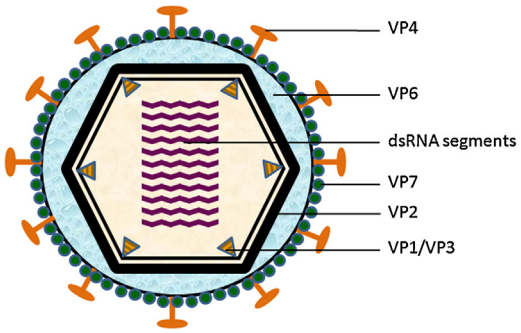


**Fig. 1.** Schematic representation of Rotavirus structure. The major structural proteins i.e. VP1, VP2, VP3, VP4, VP6 and VP7 assemble to form a triple layered structure which encapsulates the segmented RNA genome within it.

destruction of mature enterocytes in the epithelial lining of intestinal villi leading to malabsorption by intestine. However, the latter is basically attributed to enterotoxin NSP4. (2) NSP4 protein mediates the release of $Ca^{2+}$ from endoplasmic reticulum and results in increased intracellular $Ca^{2+}$ concentration [4] that leads to disruption of the cytoskeletal network and cell lysis. (3) NSP4 alters biogenesis and integrity of the tight junctions of enterocytes which consequently results in paracellular flow of water and electrolytes. (4) Additionally, infection also dysregulates $Na^{+}/K^{+}$ pump which is essential for retention of fluid and nutrients by the cells. This dysregulation is because of decreased expression of digestive enzymes following rotavirus infection. (5) Along with malabsorption factor, rotavirus infection also stimulates the secretion of electrolytes and fluid resulting in *secretory* diarrhoea. NSP4 is responsible for activation of enteric nervous system (ENS) which in turn results in increased secretion of electrolytes and water [5]. All these factors collectively contribute to loss of absorption capability of intestine and results in diarrhoea.

### 1.3. Replication

The replication strategy used by rotaviruses is depicted in Fig. 2. Virus has double stranded segmented RNA genome. The segmented nature of genome along with gene reassortment allows large number of combinations resulting in the formation of new reassortment RVs with potential novel antigen combinations that leads to RV diversity generation. Typically, rotaviruses are transmitted by feaco-oral route and infect the enterocytes in the villus of intestine. Rotavirus enters into these cells as triple layered particle (TLP) either by direct penetration or by receptor mediated endocytosis. The virion particles bind to the sialic acid residues on enterocytes with the help of VP8 protein (formed by cleavage of VP4 into VP5 and VP8) [6]. The virion particles are transported in the cytoplasm with the help of early endosomes. The low calcium ($Ca^{2+}$) level in the endosomes results in the uncoating of TLPs and release of the outer most layer of virions yeilding double layered particles (DLPs). Subsequently, DLPs penetrate the endosomal membrane to enter the cytoplasm [7], the place where whole life cycle of rotavirus occurs and this process is facilitated by VP5. In the cytoplasm, DLPs become transcriptionally active and synthesize RNA segments with the help of viral enzymes including RNA dependent RNA polymerase (RdRP). These newly formed positive (+) sense capped mRNA leave the DLP and undergo either translation or replication to synthesize viral proteins or double stranded RNA genome respectively [8]. The replication occurs in electron dense areas of cytoplasm which are located near nucleus and endoplasmic reticulum known as *viroplasms* which are mainly composed of two viral proteins, NSP2 and NSP5 [9]. The viroplasms are the viral factories and contain all the necessary components required for replication and initial packaging of the viral genome. The newly formed progeny DLPs bud into endoplasmic reticulum with the help of NSP4 proteins present on the membrane of endoplasimc reticulum [10]. Here, the DLPs acquire their outermost layer and from fully developed TLPs. The mature triple layered virions leave the cell either by lysis or by trafficking pathway in the case of polarized cells.

## 2. Classification and strain diversity

International Committee on Taxonomy of Viruses (ICTV) divided rotaviruses in seven groups (A–G) on the basis of amino acid sequence of VP6 protein [11]. Out of the seven groups, only group A, B and C rotaviruses are known to infect humans, Group A being the major cause of rotavirus associated morbidity and mortality. Group D, E, F and G rotavirus have never been found to infect humans and are restricted to non-humans, especially aves. Further classification of rotaviruses is done with a G/P-genotyping system that is based on the analysis of (i) **G**lycoprotein VP7 (G type) and (ii) **P**rotease-sensitive protein VP4 (P type) genes by reverse transcription-polymerase chain reaction (i.e., RT-PCR typing) or by cDNA sequencing [12].

Human rotaviruses constitute a diverse group. Until now, 27 G genotypes (G1–G27) and 35 P genotypes (P[1] – P[35]) have been detected [13]. Most commonly isolated G and P types are G1, G2, G3, G4, G9 and P[4], P[8] respectively. The genes encoding VP7 and VP4 proteins segregate independently and give rise to a large number of G-P combinations. Studies reveal the existence of more than 70 different G-P combinations. Out of these, G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] are most commonly identified G-P combinations and accounts for around 74% of rotavirus infections globally [14].

Along with the common strains, a large number of surveillance studies have documented the existence of many rare and uncommon strains in humans. The application of advanced molecular techniques such as RT-PCR and sequencing analysis have resulted in exponential increase in the fraction of uncommon and newly detected strains. The evolution of rotavirus results from four mechanisms: point mutation, interspecies transmission of partial or whole virus, reassortment events during co infection of two different viruses in a common host and gene rearrangement that preferably targets non-structural protein (NSP) coding segment of the genome. These mechanisms work individually or in combination with each other resulting in the diverse group of rotaviruses.

### 2.1. Point mutation

Point mutations (genetic drift) are one of the basis of rotavirus diversity. Rotavirus genome is prone to frequent point mutations and accounts for approximately one mutation per genome replication [15]. These mutations accumulate to generate genetic lineages [16] resulting in neutralizing antibody escape mutants.

### 2.2. Genetic reassortment

Reassortment (antigenic shift) is well explored and established phenomenon resulting in continuous evolution of human rotavirus (HRV). It occurs during co infection of two or more strains in a single cell (Fig. 3A). Rotavirus genome is segmented and facilitates the occurrence of reassortment events. Group A rotaviruses that are responsible for most of the infections in humans belong to two major (Wa-like and DS-1-like) and one minor (AU-1) genotype constellations which are designated as I1-R1-C1-M1-A1-N1-T1-E1-H1, I2-R2-C2-M2-A2-N2-T2-E2-H2 and I3-R3-C3-M3-A3-N3-T3-E3-H3, respectively [17]. Here, each alphabet represents a protein encoding gene segment. The human Wa-like rotavirus strains share a common origin with porcine