LACONICALLY REVIEWED CONCEPTS PERTAINING THE ROTAVIRUS: A SEVERE DIARRHEA CAUSING MICROBE

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Abstract

Nigeria and developing countries are suffering from infectious diseases due to a mixture of determinants. Parable, rotavirus is a major concern that causes a lot of deaths and hospitalization among children under five of age. Therefore, it is imperative to have more understanding of the rotavirus. A literature review to form concepts were made under the following headings: Historical background of the Rotaviruses, General Characteristics of Rotaviruses, Classification of Rotaviruses, Structure of Rotavirus particle, Rotavirus protein, Non- structural proteins, Structure of Rotavirus genome, Genetic reassortment of Rotaviruses, Mechanisms of Rotavirus genetic diversity, Rotavirus infection, Rotavirus replication, Transmission of Rotaviruses, Incubation period of Rotaviruses, Pathogenesis of Rotavirus infection, Signs and symptoms of Rotavirus infection, Immunity to Rotaviruses, Laboratory diagnosis of Rotaviruses, Epidemiology of Rotaviruses, Treatment of rotavirus infection, Antiviral therapy, Other Therapies, Management of Rotavirus infection, Prevention of Rotavirus infections, General Preventive Measures, Hospital Infection Control Measures. This information would invariably help in gaining understanding the basics of Rotavirus that causes diarrhea in children.

Keywords: children, death, diarrhea, infection, reassortment, rotavirus

1. Introduction

Rotavirus is a leading cause of severe diarrheal disease and was responsible in 2022 for about 700,000 global deaths in children less than 5 years of age. In 2022, more than 14% of such deaths occurred in Nigerian children (Ukazu, 2022). Global estimates suggest rotavirus is responsible for between 36 and 45% of hospitalizations for diarrhea among children ages less than 5 years (Troeger et al., 2018).

Rotaviruses, of the family *Reoviridae*, are icosahedral viruses approximately 75 nm in diameter that comprises three protein layers, giving them a distinct wheel-like appearance under the electron microscope. The viral capsid encloses 11 segments of double-stranded RNA, each of which codes for a single viral protein, except for gene 11, which is bicistronic (Dorsey et al., 2017). There are nine species of the genus, referred to as A, B, C, D, F, G, H, I and J. Rotavirus A, the most common species, causes more than 90% of rotavirus infections in humans (Suzuki, 2019). There are six viral proteins (VPs) that form the virus particle (virion). These structural proteins are called VP1, VP2, VP3, VP4, VP6 and VP7. In addition to the VPs, there are six nonstructural proteins (NSPs) that are only produced in cells infected by rotavirus. These are called NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6 (Kirkwood, 2010).

Group A rotavirus is commonly classified based on the binary system of two outer capsid structural proteins, VP7 (glycoprotein G) and VP4 (protease sensitive P), which can independently stimulate the production of neutralizing antibodies throughout infection (Patton, 2012; Moussa et al., 2017). The prevalence of the individual G-types and P-types varies between, and within, countries and years (*Beards* et al., 1984). There are 41 G- types and 57 P- types already identified globally, but in infection of humans only a few combinations of G and P types predominates: G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and G12P[8] (RCWG, 2021).

In 2009, the World Health Organization (WHO) recommended the introduction of rotavirus vaccine in to the immunization programs in all countries, especially those with high incidence of diarrhea (Troeger et al., 2018). By the end of 2019, a total of 108 countries had administered rotavirus vaccines, and 10 countries have currently been approved by Gavi for the introduction of rotavirus vaccine in the near future (Sadiq et al., 2022). Despite this success, several barriers to global vaccine implementation exist, including cost and sub-optimal efficacy in low-income countries (Estes and Kapikian, 2007). The segmented genome facilitates re-assortment between strains, allowing both intra- and inter-Geno group re-assortment (Kirkwood, 2010). Continued genetic variation by sequential point mutations and zoonotic transmission of novel animal strains also increases the genetic diversity within circulating rotavirus strains causing human infection (Estes and Kapikian, 2007).

Meanwhile, there are documented reports in Nigeria and elsewhere of rotavirus strains isolated from humans and animals that share genetic and antigenic features of rotaviruses from other species considered to be naturally derived from in vivo re-assortment (Adah et al., 2001; Palombo, 2003). Natural re-assortments appear to be detected more frequently in developing countries than in developed world (Sadiq et al., 2022). This has been attributed to the low levels of hygiene and poor immunological defense in infants which facilitate mixed infections and hence more re-assortment. In addition, more close contact among humans, livestock and other animals in developing countries makes the possibility of emergence of virulent rotavirus strains very high as a result of gene re-assortment (Nobumchi et al., 2003; Abdullahi et al., 2023; Umar et al., 2023).

Rotavirus diarrhea assumes a special importance in developing countries such as Nigeria, but is not routinely diagnosed in most Nigerian hospitals. Despite recent studies, the existing data are scarce for some African countries like Nigeria, a country which continues to be among the top 10 countries with greatest burden of Rotavirus Infection (Ibrahim et al., 2021). Epidemiological studies of rotavirus infection reveal a greatest degree of diversity of rotavirus strains in West African countries such as Nigeria (Aminu et al., 2008). This calls for stronger need of strain surveillance of rotavirus in developing countries. Therefore, it is imperative to have more understanding of the rotavirus. A literature review of related concepts was made as the main aim of this conceptual review paper.

2. Methods

The methods used in this work was the literature review to make concepts that are related to rotavirus causing diarrhea in children. The literature review is an emerging tool in knowledge and research that entail to give much benefits. It lays a foundation for further research and making suitable hypothesis. Additionally, it informs the researchers with enough understanding of the course of prospective research and what has already been done in areas of studies.

3. Results and Discussion

3.1 Historical background of the Rotaviruses

In 1943 a filterable agent in the faeces of children with infectious diarrhoea also caused scours (livestock diarrhoea) in cattle was discovered. Three decades later, preserved samples of the agent were shown to be rotavirus (Mebus et al., 1976). In the intervening years, a virus in mice was shown to be related to the virus causing scours (Rubenstein et al., 1971; Woode et al., 1976). Therefore, rotavirus was discovered in 1973 at Melbourne, Australia during electron microscopic examination of thin sections of duodenal mucosa from infants that died from severe diarrhoea (Bishop, 1996).

The name rotavirus was suggested after observing that, when viewed through an electron microscope, a rotavirus particle looks like a wheel which refer to as rota in Latin (Flewett et al., 1974). The name was officially recognised by the International Committee on Taxonomy of Viruses four years later (Matthews, 1979). In 1976, related viruses were described in several other species of animals (Woode et al., 1976). These viruses, all causing acute gastroenteritis, were recognised as a collective pathogen affecting humans and other animals worldwide (Flewett and Woode, 1978). Rotavirus serotypes were first described in 1980 (Beards and Brown, 1988) and in the following year, rotaviruses from humans were first grown in cell cultures derived from monkey kidneys, by adding trypsin (an enzyme found in the duodenum of mammals and now known to be essential for rotavirus to replicate) to the culture medium (Urasawa et al., 1981).The ability to grow rotaviruses in culture accelerated the pace of research, and by the mid-1980s the first candidate vaccines were being evaluated (Ward and Bernstein, 2009). Since then, it has been identified as the single most important cause of severe diarrhoea in young children and the young of animals (Kapikian, 2001). Within five years of its discovery, rotavirus was again established as the leading cause of diarrhoea in infants and young children worldwide, accounting for approximately one third of cases of severe diarrhoea requiring hospitalization (Kapikian et al., 1996). As more investigations of the etiologic agents of severe infant diarrhoea continued, it became more apparent that, rotavirus is an important etiologic agent of diarrhoea of infants and young children causing about 35% to 50% of the hospitalizations for this form of gastroenteritis during the first 2 years of life (Kapikian, 2001).

Today, the virus has been found in almost every country of the world where a search for the agent has been conducted as demonstrated by the volume of information in the literature on this agent (Estes and Cohen, 1989; Kapikian et al., 1996; burnett, 2017).

3.2 General Characteristics of Rotaviruses

Rotaviruses, of the family *Reoviridae*, are icosahedral viruses approximately 75 nm in diameter that comprise three protein layers, giving them a distinct wheel-like appearance under the electron microscope. The viral capsid encloses 11 segments of double-stranded RNA, each of which codes for a single viral protein, except for gene 11, which is bicistronic (Dorsey et al., 2017). Five major rotavirus groups (A-E) have been identified on the basis of genetic relatedness and immunologic reactivity of the inner capsid protein VP6. Group A and C rotaviruses can be grown in culture, but no system has been developed for culturing group B viruses (Kirkwood, 2017; WHO, 2022).

Matured virus particles are non-enveloped and possess a multi-layered icosahedra protein capsid, composed of an outer layer and a core; Viral Particles contain an RNA-dependent RNA polymerase and other enzymes capable of producing Capped RNA transcripts. The viral replication occurs in the cytoplasm of infected cells. The viruses are capable of genetic reassortment; Virus cultivation in vitro is facilitated by treatment with proteolytic enzymes which enhances infectivity by cleavage of the outer capsid polypeptide VP4.Virus Particles are formed by budding in to the endoplasmic reticulum. Mature virion particles are liberated from infected cells by cell lysis (Assumpta, 2008;IVAC, 2020).

3.3 Classification of Rotaviruses

Rotaviruses are part of the genus Rotavirus, which is one of the 15 genera of Reoviridae family, subdivided into the sub-families of the Sedoreovirinae (with genera Rotavirus, Orbivirus, Phytoreovirus, Cardoreovirus, Mimoreovirus and Seadornavirus) and the Spinareovirinae (with genera Coltivirus, Orthoreovirus, Aquareovirus, Oryzavirus, Dinovernavirus, Cypovirus, Fijivirus, Mycoreovirus and Idnoreovirus) (Kirkwood, 2010).

According to the International Committee on Taxonomy of Viruses (ICTV), rotavirus can be classified into 9 distinct groups (from A to J), as well as 4 specific subgroups within the group A. Groups A–C can be found in both humans and animals, while rotaviruses of groups D–G are limited exclusively to animals (Suzuki, 2019). As group A is the most important for human infection and disease, it has been classified further using various approaches. Two outer capsid proteins, VP4 (the protease-cleaved protein or P protein) and VP7 (the glycoprotein or G protein) are the determinants of the viral serotype classification, known as P-serotypes and G-serotypes (Kompithra et al., 2014).

Furthermore, this group is also classified according to the migration pattern of the RNA genome segments during polyacrylamide gel electrophoresis, whole genome RNA hybridization patterns (Geno groups), as well as nucleotide sequence analyses or genotypes (Halloran et al., 2000; Lazarus et al., 2018).

The diversity of rotaviruses, their ability to exchange genome segments encoding antigenic determinants, and changing diagnostic technology have led to evolving serology and classification systems. Rotavirus strains have been classified serologically into serogroups, subgroups, G serotypes, and P serotypes and genetically into electropherotypes, genogroups, G genotypes, and P genotypes. To organize the increasing volume of whole-genome rotavirus sequence data, in 2008 the Rotavirus Classification Working Group (RCWG) developed a nucleotide sequence–based complete genome classification system for group A rotavirus (Philip, 2015). Serogroup A is subdivided into subgroups I, II, I+II, and non-I non-II on the basis of monoclonal antibody recognition of antigenic determinants on VP6 (Patton, 2012).

Early attempts at genetic classification grouped rotaviruses according to RNA genome electropherotype, which distinguished group A strains as "long," "short," and "supershort" on the basis of the electrophoretic mobility of genome segments 10 and 11. Whole rotavirus genome analysis indicates that there are two main genogroups of human rotaviruses, each of which contains viruses with overall genome similarity (Phan et al., 2017).

In the RCWG genetic classification, a separate genotype is assigned to each of the 11 genome segments. Gx, P[x], Ix, Rx, Cx, Mx, Ax, Nx, Tx, Ex, and Hx designate the genes that encode VP7, VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4, and NSP5/6, respectively. The RCWG has proposed a strain-naming convention in which strains are named "RV serogroup/species of origin/country of identification/common name/year of identification/G- and P-type *(*Suzuki, 2019).

The prevalence of the individual G-types and P-types varies between, and within, countries and years (Phan et al., 2017). There are 41 G- types and 57 P- types already identified globally, but in infection of humans only a few combinations of G and P types predominates: G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and G12P[8] (RCWG, 2021)**.**

3.4 Structure of Rotavirus particle

When Rotaviruses are examined by Negative stain electron microscopy, intact virus particles resemble a wheel, with short spokes and a well-defined rim (Dennehy, 2000). The name rotavirus (from Latin '*Rota',* Meaning wheel) was suggested on the basis of these characteristics. The type's particles observed by Electron Microscopy are: Double-shelled, single-shelled, and core. Double – shelled particles are 76.5nm in diameter, single-shelled particles 70.5nm and cores are 50nm.Rotavirus Particles possess icosahedra symmetry. The distinctive features of the virus structure are the presence of 132 large channels spanning both shells and linking the outer surface with the inner core. Sixty spikes at, at least 4.5nm in length and each with a knob at its distal end have been shown to extend from the smooth surface of the shell (Pun, 2010; GAVI, 2020). The rotavirus genome of 11 segments of dsRNA is contained within virus core capsid. The total genome contains about 18522 base pairs. The structural proteins present in core particles (VP1, VP2 and VP3) are obvious. Non-structural proteins play a scaffolding role. Rotaviruses are the only mammalian agents known to contain 11 segments of dsRNA. The genome of Group A Viruses is composed of four high molecular weight dsRNA segments (Segments 1 to 4), five middle-sized segments (segments 5 to 9) including a distinct triplet of segments (segment 7 to 9) and two smaller segments (segments 10 and 11). The Rotavirus genes code for structural proteins found in virus particles and for non-structural particles found in infected cells but not in mature particles. The consensus is that the protein products (VP1 to VP4, VP5, VP6, VP7and VP8) of six genome segments are structural proteins found in the virus particles.VP1 is part of the inner core of the virus and one of the three proteins comprising the innermost of the three viral layers (Vende et al.,2002). It is the RNA-Dependent, RNA Polymerase for rotavirus a core replication intermediate and associates with VP2 at its icosahedra vertices. VP2 Protein is main structural component of the inner most layer and is encoded by genome segment Z.VP2 is the only structural proteins shown to possess nucleic acid (dsRNA, ssRNA and dsDNA)

binding activity when evaluated by RNA overlay protein blot assay (Assumpta, 2008). It associates with VP1 and VP3 at its 12 Vertices and is a replication intermediate. The third part of the inner core of the virus acts as the mRNA capping enzyme. It also associates with VP2 and is a replication intermediate. VP3 is encoded by genome segment 6 and is a minor structural protein that migrates with the outer capsid protein. VP4 is the protein product of genome segment 4 and it is non glycosylated outer capsid protein and haemagglutinin in many virus strains. It is also antigenic and induces neutralizing antibodies. The specific structure of this protein is used to determine the rotavirus P serotypes as well as host specificity, virulence and protective immunity (Laird et al., 2003; UNICEF, 2020). VP5 is cleaved from the outer capsid protein VP4 in the presence of trypsin. It remains bound to virion post cleavage and can be bound by neutralizing antibodies made to VP4.It is membrane associated and functions to permeably host cell membrane to facilitate cell invasion (Golantsova et al., 2004). VP6 is encoded by genome segment 6 and is the major structural protein virus particles located on the outer surface of single-shelled particles (Assumpta, 2008). The specificity of this protein is used to determine the A-G groupings, and 1, 11 sub-groupings of rotavirus. It has also been linked to the enterotoxin NSP4. It can induce neutralizing antibodies and determines the G serotype. It is also a highly variable portion of the virus capable of reassortment and possible crossover with animal strains of the virus (Maunula and Bonsdorff, 2003).

VP7 also has associations with heat shock cognate protein (hsc 70), and some integrands, both related to viral entry of the cell (Graham et al., 2003). VP8 is the second cleavage product of VP4. Like VP5, remains virion associated post cleavage and is bound by VP4 neutralizing Antibodies. It functions to bind sialic acid and acts as the virus hem agglutinin (Golantsova et al., 2004).

Rotavirus has six non-structural proteins which are NSP1-NSP6. They include NSP1 which binds interferon regulatory factor (IRF) 3 and may inhibit interferon response during rotavirus infection (Graff, 2002). In conjunction with NSP5, NSP2 is involved in synthesis and packaging of viral RNA and creation of viroplasms. NSP2 is a replication intermediate (Vende et al., 2002).

NSP3, a36KD protein binds viral mRNA at the end and promotes viral protein synthesis. It also represses host cell protein synthesis (Padilla-Noriega et al., 2002).

NSP4 has been shown to act as an entero toxin and cause diarrhoea during infection. There is also correlation between VP6 Virus subgroup and NSP4 genotype. This phosphor protein works with NSP2 in RNA synthesis and Packaging, and to induce viroplasms. It is also a replication intermediate. Little information is available on NSP6 but is associated with NSP5 and its functions (Iturriza Gomara, 2003).

The virion core contains several enzymes needed for transcription and capping of viral RNAs. Rotaviruses are stable to heat at 50°C, to a 3.0–9.0 range of pH, and to lipid solvents, such as ether and chloroform, but they are inactivated by 95% ethanol, phenol, and chlorine (Angel et al., 2009).

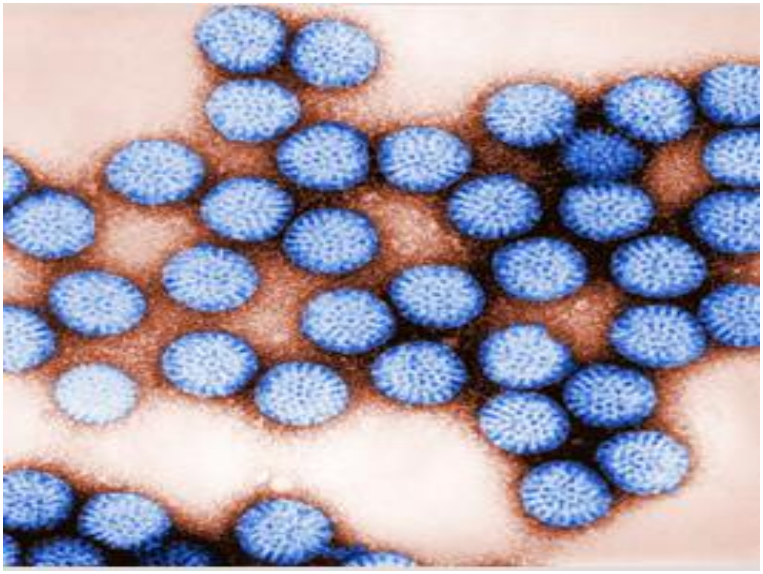

Plate 1. Electron micrograph of rotavirus from stool sample (Pun, 2010).

Rotavirus protein

The genome of rotaviruses consists of 11 unique double helix molecules of RNA (dsRNA) which are 18,555 nucleotides in total. each helix, or segment, is a gene, numbered 1 to 11 by decreasing size.. Each gene codes for one protein, except genes 9, which codes for two. The RNA is surrounded by a three-layered icosahedral protein capsid. viral particles are up to 76.5nm in diameter and are not enveloped (Pesavento et al., 2006).

Structural protein

There are six viral proteins (VPs) that form the virus particle (virion). These structural proteins are called VP1, VP2, VP3, VP4, VP6 and VP7 (Kirkwood, 2010). The RNA genome consists of 11 segments of double-stranded RNA coding for 12 proteins. RNA segments codes for 6 structural proteins VP1, VP2, VP3, VP4, VP6, and VP7. Outer capsid proteins VP4 and VP7 carry epitopes important in neutralizing activity, with VP7 glycoprotein being the predominant antigen (Patton, 2001).

The minor core proteins comprise of VP1 (which functions as the viral polymerase), VP2 (the main scaffolding protein), VP3 (the capping enzyme), VP6 is encoded by genome segment 6 and is the major structural protein virus particles located on the outer surface of single-shelled particles and is the single most abundant rotavirus protein and which interacts with the core protein VP2 and the outer capsid proteins VP7 and VP4 (Sagar, 2021).

At least six of the twelve proteins encoded by the rotavirus genome bind RNA (Patton, 1995). The role of these proteins play in rotavirus replication is not entirely understood; their functions are thought to be related to RNA synthesis and packaging in the virion, mRNA transport to the site of genome replication, and mRNA translation and regulation of gene expression (Patton, 2001; Dan Brennan, 2020).

VP1 is located in the core of the virus particle and is an RNA polymerase enzyme. In an infected cell this enzyme produces mRNA transcripts for the synthesis of viral proteins and produces copies of the rotavirus genome RNA segments for newly produced virus particles (Angel et al., 2007).

VP2 forms the core layer of the virion and binds the RNA genome.VP3 is part of the inner core of the virion and is an enzyme called guanylyl transferase. This is a capping enzyme that catalyses the formation of the 5' cap in the post-transcriptional modification of mRNA. The cap stabilises viral mRNA by protecting it from nucleic acid degrading enzymes called nucleases (Patton, 2001).

VP4 is on the surface of the virion that protrudes as a spike. It binds to molecules on the surface of cells called receptors and drives the entry of the virus into the cell.VP4 has to be modified by the protease enzyme trypsin, which is found in the gut, into VP5* and VP8* before the virus is infectious. VP4 determines how virulent the virus is and it determines the P-type of the virus (Angel et al., 2007)

VP6 forms the bulk of the capsid. It is highly antigenic and can be used to identify rotavirus species. This protein is used in laboratory tests for rotavirus A infection.VP7 is a glycoprotein that forms the outer surface of the virion. Apart from its structural functions, it determines the G-type of the strain and, along with VP4, is involved in immunity to infection (Suzuki, 2019).

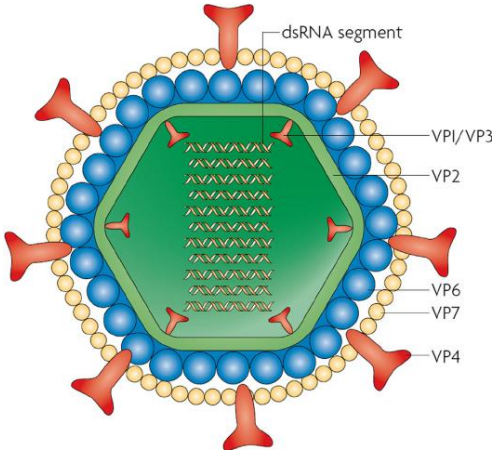

Plate 2. Structure of Rotavirus showing structural proteins (Angel et al., 2007)

Non- structural proteins

The virus genome also encodes for six non-structural proteins NSP1, NSP2, NSP3, NSP4, NSP5, NSP6. The viral mRNAs contain 5′-methylated cap structures but lack polyA tail. Instead, rotavirus mRNAs have at their 3′ end a consensus sequence (UGACC) that is conserved in all 11 viral genes (Jiang et al., 2010; Sagar, 2021).

The replication of rotavirus is a complex process that is orchestrated by an exquisite interplay between the rotavirus non-structural and structural proteins. Subsequent to particle entry and genome transcription, the non-structural proteins coordinate and regulate viral mRNA translation and the formation of electron-dense viroplasms that serve as exclusive compartments for genome replication, genome encapsidation and capsid assembly. In addition, non-structural proteins are involved in antagonizing the antiviral host response and in subverting important cellular processes to enable successful virus replication (Liya et al., 2012; HME, 2020).

NSP1, the product of gene 5, is a non-structural RNA-binding protein. NSP1 also blocks the interferon response, the part of the innate immune system that protects cells from viral infection. NSP1 causes the proteasome to degrade key signalling components required to stimulate production of interferon in an infected cell and to respond to interferon secreted by adjacent cells. Targets for degradation include several IRF transcription factors required for interferon gene transcription *(*Arnold, 2016).

NSP2 is an RNA-binding protein that accumulates in cytoplasmic inclusions (viroplasms) and is required for genome replication. In conjunction with NSP5, NSP2 is involved in synthesis and packaging of viral RNA and creation of viroplasms. NSP2 is a replication intermediate (Taraporewala and Patton, 2004).

NSP3 is bound to viral mRNAs in infected cells and it is responsible for the shutdown of cellular protein synthesis. NSP3 inactivates two translation initiation factors essential for synthesis of proteins from host mRNA. First, NSP3 ejects poly (A)-binding protein (PABP) from the translation initiation factor eIF4F. PABP is required for efficient translation of transcripts with a 3' poly (A) tail, which is found on most host cell transcripts. Second, NSP3 inactivates eIF2 by stimulating its phosphorylation. Efficient translation of rotavirus mRNA, which lacks the 3' poly(A) tail, does not require either of these factors (Poncet et al., 1993; López and Arias, 2012; Gratia et al., 2016).

NSP4 is a viral enterotoxin that induces diarrhoea and was the first viral enterotoxin discovered (Hyser and Estes, 2009; Burnett et al., 2018). NSP4 has been shown to act as an entero toxin and cause diarrhea during infection. There is also correlation between VP6 Virus subgroup and NSP4 genotype. This phosphor protein works with NSP2 in RNA synthesis and Packaging, and to induce viroplasms. It is also a replication intermediate. Little information is available on NSP6 but is associated with NSP5 and its functions (Gratia et al., 2016).

NSP5 is encoded by genome segment 11 of rotavirus A. In virus-infected cells NSP5 accumulates in the viroplasm (Afrikanova et al., 1996).

NSP6 is a nucleic acid binding protein and is encoded by gene 11 from an out-of-phase open reading frame (Rainsford and McCrae, 2007).

TABLE 1. Structural and Non-structural proteins of Rotaviruses and their functions (Gratia et al., 2016).

RNA Segment (Gene)	Size (base pairs)	Protein	Molecular weight kDa	Location	Copies per particle	Function
1	3302	VP1	125	At the vertices of the core	<25	RNA-dependent RNA polymerase
2	2690	VP2	102	Forms inner shell of the core	120	Stimulates viral RNA replicase
3	2591	VP3	88	At the vertices of the core	<25	methyltransferase mRNA capping enzyme
4	2362	VP4	87	Surface spike	120	Cell attachment, virulence
5	1611	NSP1	59	Nonstructural	0	5'RNA binding, interferon antagonist
6	1356	VP6	45	Inner Capsid	780	Structural and species-specific antigen
7	1104	NSP3	37	Nonstructural	0	Enhances viral mRNA activity and shut-offs cellular protein synthesis
8	1059	NSP2	35	Nonstructural	0	NTPase involved in RNA packaging
9	1062	$VP7^1$ $VP7^2$	38 and 34	Surface	780	Structural and neutralisation antigen
10	751	NSP4	20	Nonstructural	0	Enterotoxin
11	667	NSP5 NSP6	22	Nonstructural	0	ssRNA and dsRNA binding modulator of NSP2, phosphoprotein

3.5. Structure of Rotavirus genome

The RNA genome consists of 11 segments of double-stranded RNA coding for 12 proteins. RNA segments codes for 6 structural proteins VP1, VP2, VP3, VP4, VP6, and VP7. Outer capsid proteins VP4 and VP7 carry epitopes important in neutralizing activity, with VP7 glycoprotein being the predominant antigen. The minor core proteins comprise of VP1 (which functions as the viral polymerase), VP2 (the main scaffolding protein), VP3 (the capping enzyme), VP6 which is the single most abundant rotavirus protein and which interacts with the core protein VP2 and the outer capsid proteins VP7 and VP4. (Sagar, 2021). The virus genome also encodes for six non-structural proteins NSP1, NSP2, NSP3, NSP4, NSP5, NSP6.The viral mRNAs contain 5′-methylated cap structures but lack polyA tail. Instead, rotavirus mRNAs have at their 3′ end a consensus sequence (UGACC) that is conserved in all 11 viral genes. The RNA is surrounded by a three-layered icosahedral protein capsid. Viral particles are up to 76.5 nm in diameter and are not enveloped (Angel et al., 2007; Carl et al., 2019).

At least six of the twelve proteins encoded by the rotavirus genome bind RNA. The role of these proteins play in rotavirus replication is not entirely understood; their functions are thought to be related to RNA synthesis and packaging in the virion, mRNA transport to the site of genome replication, and mRNA translation and regulation of gene expression (Tate et al., 2016).

The glycoprotein VP7 defines the G serotypes and the protease-sensitive protein VP4 defines P serotypes. Because the two genes that determine G-types and P-types can be passed on separately to progeny viruses, different combinations are found A whole genome genotyping system has been established for rotavirus A, which has been used to determine the origin of atypical strains (Sagar, 2021).

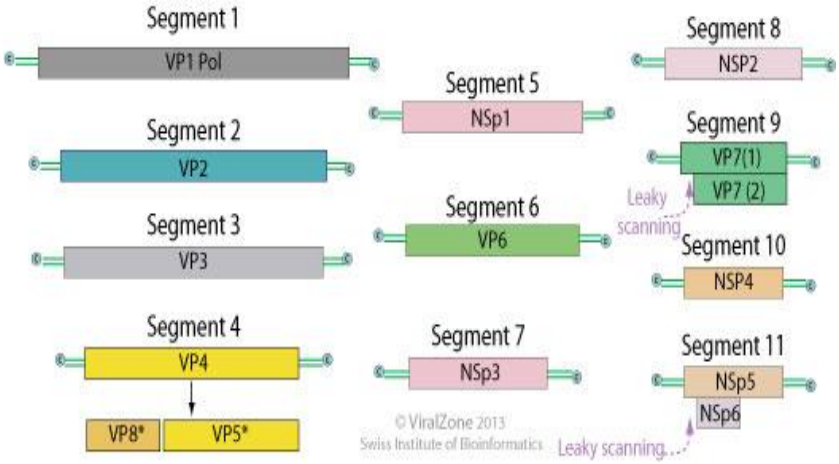

Figure 1. Structure of Rotavirus genome segments (Sagar, 2021)

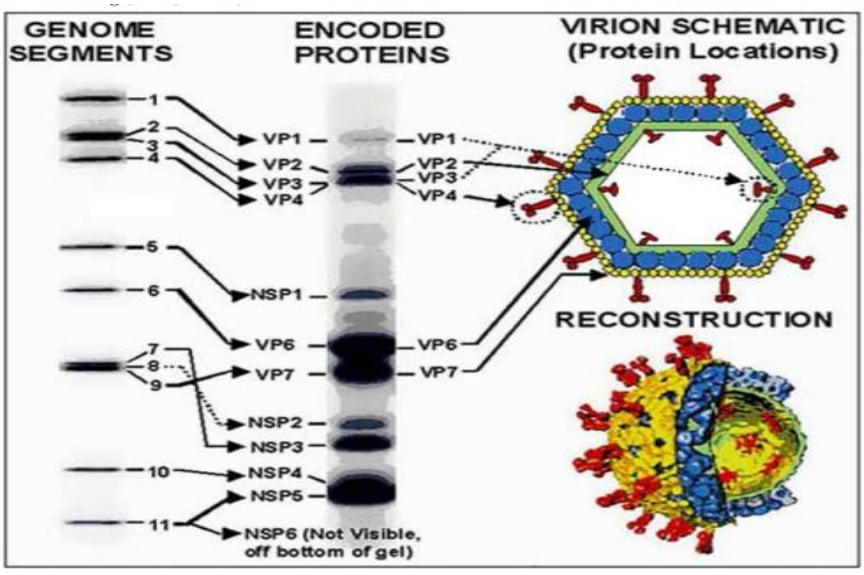

Figure 2. Structure of Rotavirus genome (Sagar, 2021).

3.6 Genetic Reassortment of Rotaviruses

Rotaviruses (RVs) can evolve through the process of reassortment, whereby the **11** double-stranded RNA genome segments are exchanged among strains during co-infection. However, reassortment is limited in cases where the genes or encoded proteins of co-infecting strains are functionally incompatible (Tate et al., 2016).

Reassortment of segmented RNA viruses is a mechanism by which cognate genome segments are exchanged in progeny viruses upon infection of a single cell by two or more closely related virus strains (Iturriza-Gómara et al., 2001).

When these viruses contain different cogent genes, new genotype constellations may emerge. Reassortment of the surface antigens could result in a large number of antigen combinations. However, while over 80 G–P antigen combinations have been identified so far, only six globally common G–P combinations occur in human, globally most common strains, even though many G and P types are expressed by circulating strains (Dóró et al., 2014).

The reason behind this phenomenon is unclear. One explanation operates with genetic or phenotypic incompatibility between some variants of the neutralizing antigen specificities (Iturriza-Gómara et al., 2001).

An alternative scenario is that there is in vivo selection of combinations that result in masking of VP4 or VP7 neutralization epitopes. Strains hiding their neutralization epitopes after reassortment may evoke a weaker neutralizing antibody response, thus enhancing their fitness over less advantageous antigen combinations (Bányai et al., 2017).

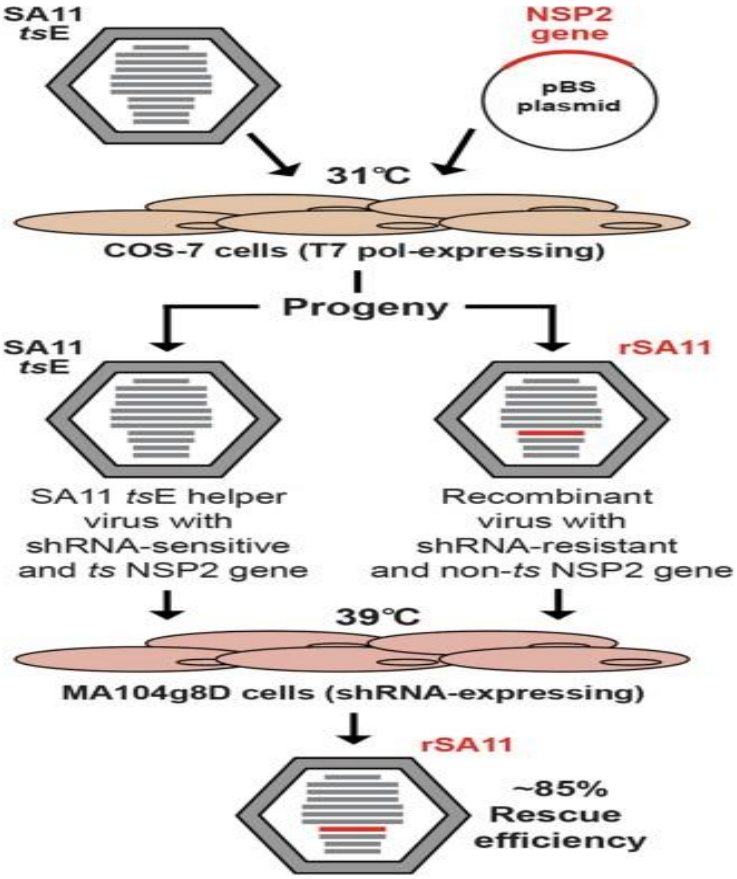

Figure 3. Genetic reassortments in rotavirus (Tate et al., 2016)

3.7 Mechanisms of Rotavirus genetic diversity

Human RVs display a considerable genetic diversity. Among different RV groups (A, B, or C), sequence identity of individual cognate RNA segments (all the viral protein genes) are generally less than 60% (Souvik and Nomubichi, 2011) Even within a group, such as RV-A, high levels of genetic

diversity have been observed among human RV strains (Matthijnssens, 2008). Moreover, the 11 RV gene segments exhibit different levels of sequence diversity, with some genes found to be more conserved than others. For example, in RV-A from humans and animals, the VP1 and VP2 genes were shown to be highly conserved, while the NSP1 gene was found to be the highly divergent (Arguelles et al., 2000). Among the 11 RV-A RV gene segments, the diversity of the genes encoding the outer capsid proteins, VP7 and VP4, have been studied extensively. These proteins contain neutralization antigens and define independent serotype specificity, (i.e., G serotype [VP7] and P serotype [VP4]), which were originally determined by serological tests with antisera or monoclonal antibodies (Souvik and Nomubichi, 2011). Currently, differences among these serotypes can be predicted by comparative analysis of their VP7 or VP4 gene sequences (Ahmed et al., 2004). In the deduced amino acid sequence of VP7, several variable regions (VR1-VR9) which have diverse sequences among different G serotypes have been identified. Among these regions, antigenic epitopes associated with the G serotype are shown to be located primarily in three regions (defined as VR5, VR7, VR8) (Matthijnssens, 2008). Similarly, most of variable regions corresponding to the P serotype-specific neutralization epitopes have been mapped in the deduced amino acid sequence of the VP8* subunit (the smaller product of VP4 following trypsin cleavage) than in the relatively conserved VP5* subunit of VP4 (Neuzil et al., 2010). Therefore, in these outer capsid proteins, sequence diversity is considered to be more pronounced in specific portions, i.e., antigenic regions, possibly due to accumulation of mutations which might have occurred primarily through immune pressure in the host (Arnold, 2016).

To date, genetic diversity of RV has been attributed to at least four mechanisms; point mutation, reassortment, rearrangement, and intragenic recombination. Among these mechanisms, point mutation results in changes in the gene sequence that may affect the function of the viral protein, while reassortment has an impact on the genome constellation (Sagar, 2021)

As in other RNA viruses, point mutation is believed to occur frequently in human RV-A. Through multiple passages of an RV-A strain in MA104 cells, overall mutation rate of gene segment 11 was calculated to be a maximum value of 5×10^{-5} per replicated base (Blackhall et al., 1996). Mutation rate of RV-A in a community was revealed by Matthijnssens and co-workers for the two emerging genotypes G9 and G12 using numerous sequence data, including those of the initially identified strains and globally distributed recent strains for more than 10 years (Matthijnssens et al., 2010). The estimated mutation rates (nucleotide substitutions/site/year) of the VP7 gene were 1.87×10^{-3} for G9 RVs and 1.66×10^{-3} for G12 RVs, which are comparable with some RNA viruses such as respiratory syncytial virus B (G protein gene, 1.9×10^{-3}) and measles virus (H gene, 5.0×10^{-4}) (Zlateva et al., 2005). For the RV-B, evolutionary rates based on VP7 genes have been estimated as 7.9×10^{-4} in China and 1.57×10^{-3} in Bangladesh, which are slightly lower than those of RV-A G9 and G12 strains (Yang et al., 2004).

Reassortment is an exchange/substitution of RNA segment(s) between different RV strains, which occurs during mixed infection of RVs in individuals (natural conditions), as well as in experimental conditions (in vitro). Although reassortant viruses can be generated readily via mixed infection of RVs, it is also evident that the reassortment of RNA segment(s) is influenced by many factors, including parental virus strains, host (cellular) factors, and environmental factors (Kobayashi et al., 2003). Incidence of reassortment is considered to be affected by frequency of co-infection in the population and the genetic diversity of RV strains circulating in the population. Numerous molecular epidemiologic studies revealed that some human RV-A strains might have arisen from reassortment between human and animal RV strains (Martella et al., 2010). Long-term surveillance of the RV-A genotype revealed the emergence and global spread of RV strains that had been generated by reassortment, as represented by G9 RVs (Laird et al., 2003).

Rearrangement represents concatemerization or deletion that occurs within single RNA segments, causing a radical change in the size of the RNA segment (Desselberger, 1996). One of the well-characterized forms of rearrangement is a head-to-tail sequence duplication, which has been observed for RNA segments coding for NSP1, NSP3–5 and VP6. The *NSP5* genes of RVs showing 'short' RNA pattern is typical to G2P[4] strains, and 'supershort' RNA patterns (the 10th RNA segment) represent

commonly observed RNA segments with rearrangement, although some of them were not caused simply by the partial sequence duplication (Matthijnssens et al., 2006). Rearranged RNA segments have been detected in RVs isolated from immuno-deficient children, as well as apparently immuno-competent humans and animals. RV variants with rearranged gene segments have been isolated in vitro through serial passages of RV strain at high multiplicity of infection. Some possible mechanisms, including template switching of RNA-dependent RNA polymerase have been presented for occurrence of rearrangement (Alam et al., 2008). In order to explain the partial duplication in a head-to-tail fashion observed for dsRNA viruses including RV, the "loop model" was proposed by Matthijnssens and co-workers (Matthijnssens et al., 2006). In this model, it is assumed that the 3´-end of the negative strand which has passed through the catalytic core of the RNA polymerase may be inserted into the polymerase again from its template entrance, forming a loop, before completion of transcription from the negative strand. In addition to the partial duplication, a unique form of rearranged gene segment that contained a truncated NSP2 gene inserted into the NSP5 gene has also been described (Cao et al., 2008).

Intragenic recombination of RV RNA segments, i.e., recombination between cognate RNA segments from different strains, has been scarcely reported until recently. However, a few recent studies described evidence for the occurrence of this type of genetic alteration, despite it probably being an extremely rare event. VP7 genes that contain portions derived from different G types or from different lineage of a single G type were reported for human RV-A (Phan et al., 2007). Similarly, the VP7 gene with human and porcine-like portions was identified in a porcine RV-C strain (Martella et al., 2007).

3.8 Rotavirus infection

Rotaviruses infect cells in the villi of the small intestine. They multiply in the cytoplasm of enterocytes and damage their transport mechanisms, the infection prevents the absorption of water, causing a net secretion of water and loss of ions, which together result in watery diarrhea (Ibrahim et al., 2021).

The NSP4 protein of rotavirus acts in a toxin-like manner to promote calcium ion influx into enterocytes, the release of neuronal activators, and a neuronal alteration in water absorption. The loss of fluids and electrolytes can lead to severe dehydration and even death if therapy does not include electrolyte replacement (Shamshul et al., 2013).

Diarrhea caused by rotaviruses may also be due to impaired sodium and glucose absorption as damaged cells on villi are replaced by non- absorbing immature crypt cells. Damaged cells may slough into the lumen of the intestine and release large quantities of viruses, which appear in the stool (Saheed, 2019). Viral excretion usually lasts from 2 to 12 days in otherwise healthy patients but may be prolonged in those with poor nutrition and immuno-compromised patients. Immunity to infection requires the presence of antibody, primarily immunoglobulin A (IgA), in the lumen of the gut. Antibodies to the VP7 and VP4 neutralize the virus (Sagar, 2021)

Babies and very young children who have rotavirus infections need to be watched closely; because they can become dehydrated very quickly. Dehydration occurs when the body loses water more quickly than it is replaced. (Shamshul et al., 2013).

Rotavirus infects not only Children but also adults and rotavirus may occur repeatedly in humans from birth to old age. Young children are the most vulnerable and the prevalence of infection varies according to age (Shamshul et al., 2013; Charles et al., 2021).

Among the disease causing viruses, rotavirus is the most important etiological agent worldwide and is implicated in severe dehydrating diarrhea requiring hospitalization. People in developing countries are at particular risk due to contaminated drinking water. While in industrialized areas, these may occur in immigrants or people with weakened immune system (Alkali et al., 2016; Tate et al., 2016)

3.9. Rotavirus replication

The attachment of the virus to the host cell is initiated by VP4, which attaches to molecules, called glycans, on the surface of the cell. The virus enters cells by receptor mediated endocytosis and form

a vesicle known as an endosome. Proteins in the third layer (VP7 and the VP4 spike) disrupt the membrane of the endosome, creating a difference in the calcium concentration. This causes the breakdown of VP7 trimers into single protein subunits, leaving the VP2 and VP6 protein coats around the viral dsRNA, forming a double-layered particle (DLP) (Rodríguez and Luque, 2019). The eleven dsRNA strands remain within the protection of the two protein shells and the viral RNA-dependent RNA polymerase creates mRNA transcripts of the double-stranded viral genome. By remaining in the core, the viral RNA evades innate host immune responses including RNA interference that are triggered by the presence of double-stranded RNA (Arnold, 2016). During the infection, rotaviruses produce mRNA for both protein biosynthesis and gene replication. Most of the rotavirus proteins accumulate in viroplasm, where the RNA is replicated and the DLPs are assembled. In the viroplasm the positive sense viral RNAs that are used as templates for the synthesis of viral genomic dsRNA are protected from siRNA-induced RNase degradation (Silvestri et al., 2004). Viroplasm is formed around the cell nucleus as early as two hours after virus infection, and consists of viral factories thought to be made by two viral non-structural proteins: NSP5 and NSP2. Inhibition of NSP5 by RNA interference in vitro results in a sharp decrease in rotavirus replication. The DLPs migrate to the endoplasmic reticulum where they obtain their third, outer layer (formed by VP7 and VP4). The progeny viruses are released from the cell by lysis (Jayaram et al., 2004).

Therefore, the rotavirus replication cycle can be summarised in to the following stages: (1) attachment of the virus to the host cells, which is mediated by VP4 and VP7 (2) penetration of the cell by the virus and uncoating of the viral capsid (3) plus strand ssRNA synthesis (this acts as the mRNA) synthesis, which is mediated by VP1, VP3 and VP2 (4) formation of the viroplasm, viral RNA packaging and minus strand RNA synthesis and formation of the double-layered virus particles (5) virus particle maturation and release of progeny virions (Rodríguez and Luque, 2019).

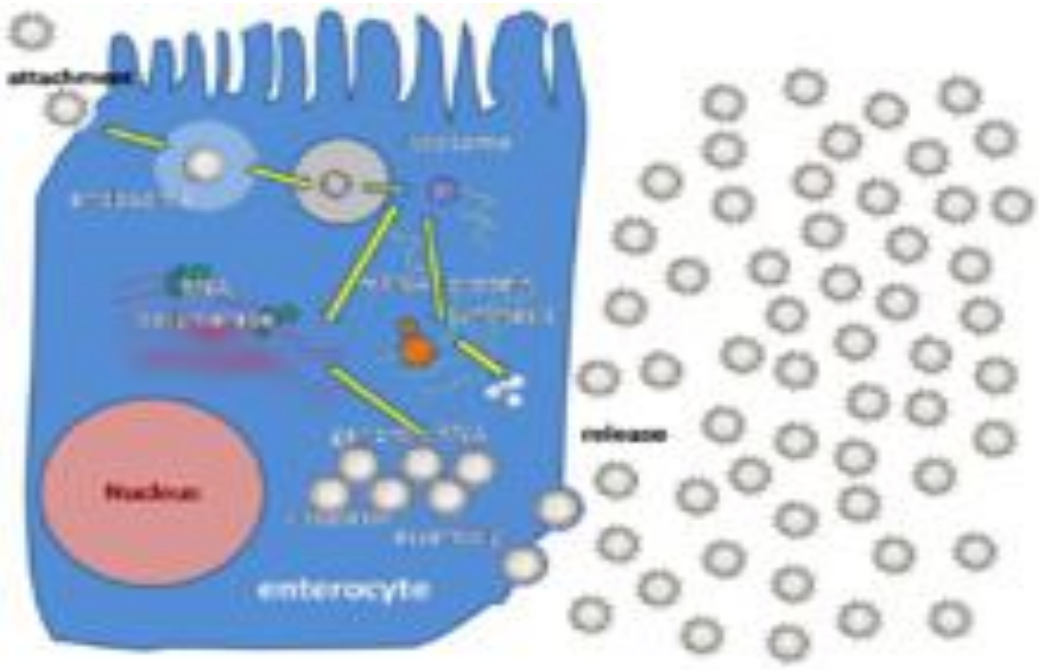

Figure 4. A simplified drawing of the rotavirus replication cycle (Ruiz et al., 2009)

3.10 Transmission of Rotaviruses

Rotavirus is transmitted from person to person through the faecal-oral route. This occurs when the viruses found in stool of infected child is swallowed by another child, in other words children become infected if they put their fingers in their mouth after touching something such as toys, books and clothing that has been contaminated by stool of an infected person, this usually happen when children forget to wash their hands after using the toilet or before eating (Chin, 2000; Ward and Bernstein, 2009).

Health care and child care workers also spread the disease if they don't wash their hands after changing diapers, frequent hand washing is the best tool to limit the spread of rotavirus infection. Rotavirus may

also be transmitted through intake of faecally contaminated water or food which usually occur when infected food handlers who prepare, salad, sandwiches, carrots and other foods that requires no cooking can spread the disease. It may also be transmitted by respiratory droplets that people sneeze, cough, drip, or exhale (Dennehy, 2000; Wang et al., 2015). Nosocomial transmission is frequent in pediatric wards and hospitals with poor sewage water treatment and sanitation (Gentsch, 2005).

Rotaviruses are ubiquitous in the animal kingdom; therefore, interspecies transmission and, more importantly exchange of genetic material between animal and human strains through gene reassortment can lead to the emergence of novel rotavirus strains of epidemiological significance (Uchida et al., 2006). The risk of zoonotic transmission is higher in areas with farms under intensive or extensive management. Zoonotic rotavirus strains are capable of causing not only asymptomatic infection but also mild to severe diarrhea in humans (Palombo, 2002: Varghese e t al., 2004; Gentsch, 2005).

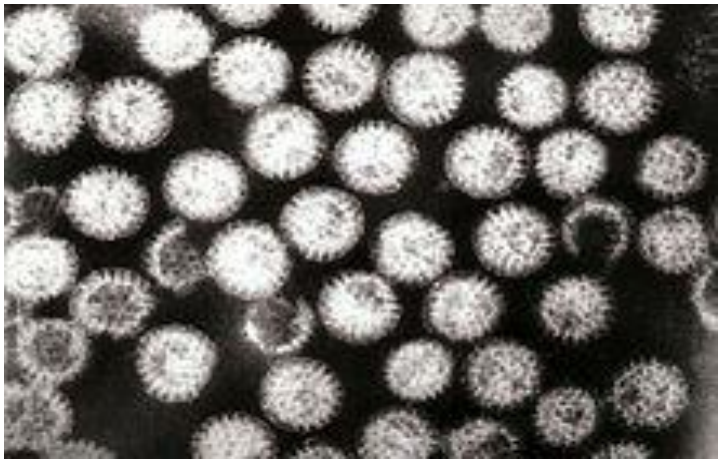

Plate 3. Rotaviruses in the faeces of an infected child (Rodríguez and Luque, 2019).

3.11 Incubation period of Rotaviruses

The time between becoming infected and developing symptoms is about 24 to 72 hours (1 to 3 days). While the time during which an infected person can infects others (Infectious period). Children can spread rotavirus 2 days before and up to eight days after develop diarrhea (Saheri et al., 2014).

It takes 1 to 3 days for a child who is exposed to the virus to start having symptoms. Vomiting is often the first symptom. Usually a fever and diarrhea follow. Most children with rotavirus have very watery diarrhea that seems like a large amount for a baby or small child. The most severe diarrhea lasts 4 to 8 days. But episodes of diarrhea can last longer after your child starts feeling better. In small children diarrhea can last for a few weeks. Diarrhea, especially when it occurs along with vomiting, can quickly lead to dehydration in babies and young children who have rotavirus. For this reason, it is important to keep feeding your child and to watch him or her closely for signs of dehydration (Ibrahim et al., 2021).

3.12 Pathogenesis of Rotavirus infection

Rotaviruses infect mainly the epithelial cells that line the upper part of the small intestine, causing cell death and decreased production of digestive enzymes. These changes gradually return to normal over a number of weeks (Dorsey et al., 2017). The virus replicates in the cytoplasm of epithelial cells of small intestine villi (Holland, 1990; Murphy et al., 1999). As the multiplication progresses, the mature enterocytes are sloughed off and immature cells from crypts take over to the villus surface. This creates a sudden change in the ratio of absorption and secretion leading to fluid accumulation in the intestinal lumen (Holland, 1990; Chauhan and Singh, 1992; Murphy et al., 1999; Ramig, 2004).

The new cells lack efficient functional activities which compromise glucose absorption, sodium transport and secretion of lactase (Steele et al., 2004). The loss of mature erythrocytes also results in depletion of bicarbonates, sodium, potassium, chloride and water which gradually leads to acidosis. The acidosis is further aggravated by the microbial fermentation of undigested milk in neonates. Also milk ingestion by neonates having reduced intestinal lactase may further exacerbate the osmotic deregulation. (Hall et al., 1993; Steele et al., 2004).

Rota-viral diarrhea may also be caused by mal-absorption resulting from progressive destruction of mature erythrocytes, activation of the enteric nervous system caused by vasoactive agents released by damaged cells, or by secretion of a viral enterotoxin (NSP4), which alters calcium-dependent cell permeability and elevates the chloride secretion (Estes, 2003; Desselberger et al., 2005; Chauhan et al., 2008).

Rotaviruses replicate mainly in the gut and infect enterocytes of the villi of the small intestine, leading to structural and functional changes of the epithelium. There is evidence in humans, and particularly in animal models of extra intestinal dissemination of infectious virus to other organs and macrophages (Greenberg and Estes, 2009).

The diarrhoea is caused by multiple activities of the virus (Ramig, 2004). Malabsorption occurs because of the destruction of gut cells called enterocytes. The toxic rotavirus protein NSP4 induces age- and calcium ion-dependent chloride secretion, disrupts SGLT1 (sodium/glucose cotransporter 2) transporter-mediated reabsorption of water, apparently reduces activity of brush-border membrane disaccharides, and activates the calcium ion-dependent secretory reflexes of the enteric nervous system (Hyser and Estes, 2009; Fix et al., 2015).

The elevated concentrations of calcium ions in the cytosol (which are required for the assembly of the progeny viruses) is achieved by NSP4 acting as a viroporin. This increase in calcium ions leads to autophagy (self-destruction) of the infected enterocytes (Hyser et al., 2010). NSP4 is also secreted. This extracellular form, which is modified by protease enzymes in the gut, is an enterotoxin which acts on uninfected cells via integrin receptors, which in turn cause and increase in intracellular calcium ion concentrations, secretory diarrhoea and autophagy (Berkova et al., 2006).

The vomiting, which is a characteristic of rota viral enteritis, is caused by the virus infecting the enterochromaffin cells on the lining of the digestive tract. The infection stimulates the production of 5' hydroxytryptamine (serotonin). This activates vagal afferent nerves, which in turn activates the cells of the brain stem that control the vomiting reflex (Hagbom et al., 2012).Healthy enterocytes secrete lactase into the small intestine; milk intolerance due to lactase deficiency is a symptom of rotavirus infection, which can persist for weeks. A recurrence of mild diarrhoea often follows the reintroduction of milk into the child's diet, due to bacterial fermentation of the disaccharide lactose in the gut (Ouwehand and Vesterlund, 2003; Farnworth, 2008; Babji et al., 2013).

The loss of fluids and electrolytes can lead to severe dehydration and even death if therapy does not include electrolyte replacement. Diarrhea caused by rotaviruses may also be due to impaired sodium and glucose absorption as damaged cells on villi are replaced by nonabsorbing immature crypt cells. Damaged cells may slough into the lumen of the intestine and release large quantities of viruses, which appear in the stool. Viral excretion usually lasts from 2 to 12 days in otherwise healthy patients but may be prolonged in those with poor nutrition and immunocompromised patients (Sagar, 2021)

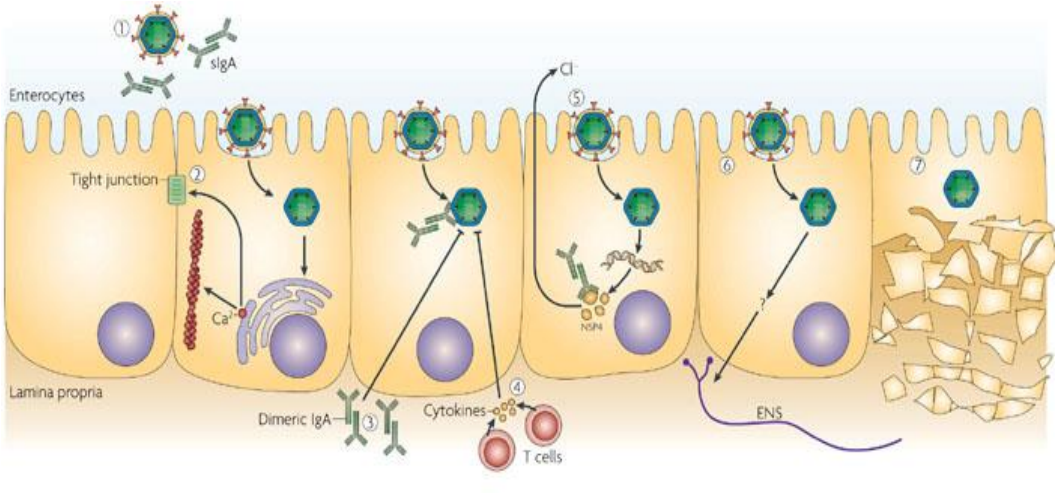

Figure 5. Electron micrograph of a rotavirus infected enterocyte (Sagar, 2021)

3.13 Signs and symptoms of Rotavirus infection

Rotavirus enteritis is a mild to severe disease characterised by nausea, vomiting, watery diarrhoea and low-grade fever. Once a child is infected by the virus, there is an incubation period of about two days before symptoms appear (HochWald and Kivela, 1999). The period of illness is acute. Symptoms often start with vomiting followed by four to eight days of profuse diarrhoea. Dehydration is more common in rotavirus infection than in most of those caused by bacterial pathogens, and is the most common cause of death related to rotavirus infection. (Maldonado and Yolken, 1990). Rotavirus A infections can occur throughout life: the first usually produces symptoms, but subsequent infections are typically mild or asymptomatic, as the immune system provides some protection. Consequently, symptomatic infection rates are highest in children under two years of age and decrease progressively towards 45 years of age. The most severe symptoms tend to occur in children six months to two years of age, the elderly, and those with immunodeficiency. Due to immunity acquired in childhood, most adults are not susceptible to rotavirus; gastroenteritis in adults usually has a cause other than rotavirus, but asymptomatic infections in adults may maintain the transmission of infection in the community (Offit, 2001; Ramsay and Brown, 2000; Anderson and Weber, 2004). There is some evidence to suggest blood group secretor status and the predominant bacteria in the gut can impact on the susceptibility to infection by rotaviruses (Rodríguez-Díaz et al., 2017). Therefore, the typical symptoms include watery diarrhea, fever, abdominal pain, and vomiting, leading to dehydration. Vomiting is usually of short duration and may occur before or after the onset of diarrhea. Diarrhea appears as watery, green or yellow but does not contain mucus. Rotavirus diarrhea is self-limiting and patients recover completely within 5-10 days. It causes gastroenteritis in adults (Saheed, 2019).

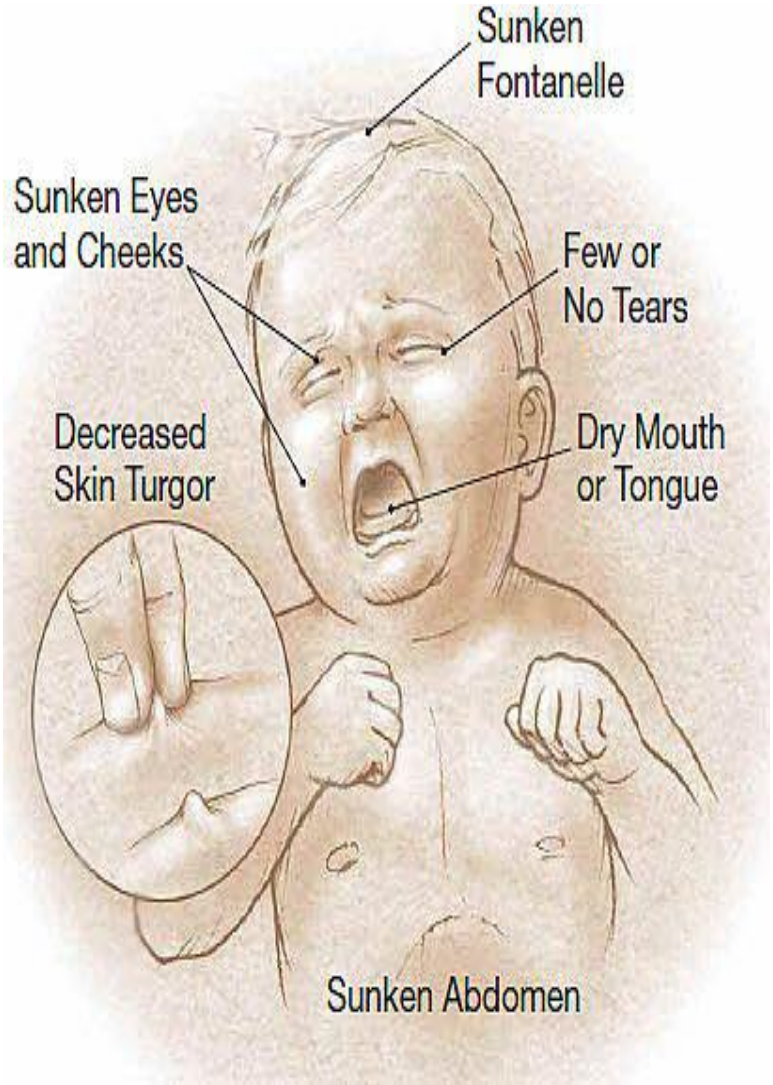

Figure 6. Some physical signs of rotavirus infections on an infected baby (Saheed, 2019)

3.14 Immunity to Rotaviruses

It has been observed that the infected infants that recover from diarrhea may return to normal body weight within 10-28 days' post infection. The CD8+cytotoxic T lymphocytes (CTL) have been suggested to have crucial roles in clearing rotavirus infection (Dharakul et al., 1990; Chahan et al., 2008).The CD4+helper T cells (Tμ) also play a vital in the successful clearance of rotavirus by inducing B cell response (Malik et al., 2005).Thus both B and T lymphocytes are involved in immune generation against rotaviruses for which b cells secrete rotaviruses specific IgA and IgG antibodies, while CTL directly clear the virus from the host. Also, the primary infection in neonates generates rotavirus-specific memory B and T cells, which help to reduce the severity during subsequent infections. Besides the role of B and T cells mature erythrocytes also secrete antibacterial compounds, which are important in preventing secondary bacterial invasion (Chauhan, et al., 2008).

Rotaviruses elicit both B and T cell immune responses. Antibodies to the rotavirus VP4 and VP7 proteins neutralise viral infectivity in vitro and in vivo. Specific antibodies of the classes IgM, IgA and IgG are produced, which have been shown to protect against rotavirus infection by the passive transfer of the antibodies in other animals. Maternal trans-placental IgG might play a role in the protection neonates from rotavirus infections, but on the other hand might reduce vaccine efficacy (Ward and Bernstein, 2009; Vega et al., 2012; Mwila et al., 2017).

Following infection by rotaviruses there is a rapid innate immune response involving types I and III interferons and other cytokines (particularly Th1 and Th2 (Gandhi et al., 2017) which inhibit the replication of the virus and recruit macrophages and natural killer cells to the rotavirus infected cells. The rotavirus dsRNA activates pattern recognition receptors such toll-like receptors that stimulate the production of interferon (Villena et al., 2016). The rotavirus protein NSP1 counteracts the effects of type 1 interferon by suppressing the activity of the interferon regulatory proteins IRF3, IRF5 and IRF7. (Gandhi et al., 2017; Holloway and Coulson, 2013; Villena et al., 2016).

The levels of IgG and IgA in the blood and IgA in the gut correlate with protection from infection. Rotavirus specific serum IgG and IgA at high titres (e.g. >1:200) have been claimed to be protective and there is a significant correlation between IgA titres and rotavirus vaccine efficacy (Offit, 1994).

3.15 Laboratory diagnosis of Rotaviruses

Rotavirus can be detected in stool specimens from children with gastroenteritis by several techniques, including electron microscopy, polyacrylamide gel electrophoresis antigen detection assays reverse transcription polymerase chain reaction (RT-PCR), and virus isolation. Diagnosis of rotavirus was initially by electron microscopy, with and without agglutination by immune sera. Large numbers of rotavirus particles (up to 10^{11}/g faeces) are excreted during the acute phase of infection, and children with severe diarrhea seem to excrete a greater number of viruses (Kang et al., 2004). Polyacrylamide gel electrophoresis detects rotavirus RNA extracted directly from stool specimens; the electrophoretic migration pattern of the 11 segments of the double stranded RNA genome permits analysis of the relatedness of circulating strains (Herring et al., 1982; Groome, 2016).

Children with gastroenteritis are not routinely tested for rotavirus because the results do not alter treatment. When testing is performed, antigen detection tests—including commercially available enzyme linked immuno-sorbent assays (ELISAs) and immuno-chromatographic assay are widely used. Most of these tests have high sensitivity and specificity (90–95%) (Thomas et al., 1988). RT-PCR is widely used in research laboratories to detect the viral genome (Iturriza Gómara et al., 2004). It provides data on the VP7 and VP4 genotypes that form the basis of binary classification (G and P type, respectively) of rotavirus strains (Lazzerini and Ronfani, 2013).

3.16 Epidemiology of Rotaviruses

Rotavirus is the leading cause of severe gastroenteritis in infants and young children worldwide; it was reported to be responsible for about 128,500 deaths in 2016, with over 70%of cases occurring in sub-Saharan Africa (Jonesteller et al., 2017). Rotavirus causes approximately 258 million episodes

of gastroenteritis requiring home care and about 24 million cases requiring medical attention (Troeger, et al., 2018). Rotavirus associated mortality has drastically reduced with 528,000 deaths (range, 465,000–591,000) in 2000 to 215,000 (range, 197,000–233,000) in 2013, of which 75% occur in Africa and Asia. India and Nigeria accounted for 22% and 14%, respectively (Ibrahim et al., 2021). Six countries India, Nigeria, Congo, Ethiopia, China, and Pakistan account for more than half of the global mortality burden of rotavirus diarrhea (Payne et al., 2016).

Rotavirus is endemic worldwide; the infection is associated with high rates of morbidity throughout the world and high rates of mortality in developing countries. In the United States, almost every child is infected at some time before they reached the age of 5 years. Each year in the United States, rotavirus infections result in 500,000 emergency department or clinic visits, and 49,000 hospital admissions, but few deaths. Worldwide, more than 600,000 deaths are attributed to rotavirus each year (Ball et al., 1996; Troeger, et al., 2018).

Rotavirus A, which accounts for more than 90% of rotavirus gastroenteritis in humans, is endemic worldwide. Each year rotaviruses cause millions of cases of diarrhoea in developing countries, almost 2 million of which result in hospitalisation In 2013, an estimated 215,000 children younger than five died from rotavirus infections, 90 percent of whom were in developing countries (Parashar et al., 2006). Almost every child has been infected with rotaviruses by age five. Rotaviruses are the leading single cause of severe diarrhoea among infants and children, is responsible for about a third of the cases requiring hospitalisation and causes 37% of deaths attributable to diarrhoea and 5% of all deaths in children younger than five (Leshem et al., 2014; O'Ryan, 2009). Boys are twice as likely as girls to be admitted to hospital for rotavirus infections. In the pre-vaccination era, rotavirus infections occurred primarily during cool, dry seasons. The number attributable to food contamination is unknown (Atchison et al., 2010; Levy et al., 2009; Koopmans and Brown, 1999).

Outbreaks of Group A Rotavirus diarrhoea are common among hospitalised infants, young children attending day care centres, and elderly people in nursing homes. An outbreak caused by contaminated municipal water occurred in Colorado in 1981(Hopkins et al., 1984; Anderson and Weber, 2004; Sassi et al., 2015). During 2005, the largest recorded epidemic of diarrhoea occurred in Nicaragua. This unusually large and severe outbreak was associated with mutations in the rotavirus A genome, possibly helping the virus escape the prevalent immunity in the population. A similar large outbreak occurred in Brazil in 1977 (Linhares et al., 1981; Bucardo et al., 2007; Wen et al., 2012).

Rotavirus B, also called adult diarrhoea rotavirus or ADRV, has caused major epidemics of severe diarrhoea affecting thousands of people of all ages in China. These epidemics occurred as a result of sewage contamination of drinking water. Rotavirus B infections also occurred in India in 1998; the causative strain was named CAL. Unlike ADRV, the CAL strain is endemic. To date, epidemics caused by rotavirus B have been confined to mainland China, and surveys indicate a lack of immunity to this species in the United States. Rotavirus C has been associated with rare and sporadic cases of diarrhoea in children, and small outbreaks have occurred in families (Hung et al., 1984; Fang et al., 1989; Penaranda et al., 1989; Kelkar and Zade, 2004; Ahmed et al., 2004; Moon et al., 2011).

Rotaviruses are ubiquitous worldwide, with 95% of children infected by 3 to 5 years of age. Rotaviruses are one of the most common causes of serious diarrhea in young children worldwide, affecting more than 18 million infants and children and accounting for close to 1600 deaths per day resulting from dehydration. In North America, outbreaks occur during the autumn, winter, and spring. More severe disease occurs in severely malnourished children (WHO, 2016).

Group A rotavirus is the most important cause of severe diarrhea in childhood causing 20% of fatal diarrhea in young children (Parashar et al., 2006). The annual estimates of rotavirus related mortality in children less than 5 years, show about 453000 children died worldwide due to rotavirus (Tate et al., 2010).

According to Odimayo et al., (2008), in Nigeria, is an important causative agent of diarrhea in Children under the age of 5 years, with 80% of rotavirus-positive cases being in children under 24 months of age (Odimayo et al., 2008).

3.17 Treatment of rotavirus infection

Treatment of gastroenteritis is supportive to correct the loss of water and electrolytes that may lead to dehydration, acidosis, shock, and death. In selected clinical situations, anti-rotaviral immunoglobulin therapy has been used as prophylaxis against, and as treatment of rotavirus gastroenteritis. The mainstay of therapy consists of oral rehydration with fluids of specified electrolyte and glucose composition. Intravenous rehydration therapy is reserved for patients with severe dehydration, shock, or reduced level of consciousness (Sagar, 2021).

Antiviral therapy

Development of chemotherapeutic agents for treating gastrointestinal viruses has made little headway. One major obstacle is the fact that animal models show that a good deal of viral replication occurs prior to the onset of overt symptoms. Although a number of investigations have been carried out in tissue culture models of rotavirus replication, no agents are currently in clinical trials. Ribavirin, a broad-spectrum antiviral, was originally identified as having anti rotavirus activity in tissue culture but no measurable effect in the mouse model of rotavirus infection (Beards et al., 1989; Pesavento et al., 2006). Isoprinosine and a number of other modified nucleoside analogues have shown an ability to prevent viral RNA and protein synthesis in tissue culture, but no experiments in animal models have been reported (Kirkwood, 2010). Flavins, found in tea extracts have also been shown to possess some in vitro anti rotaviral activity (Bányai et al., 2017).

Although rotavirus is sensitive to interferons *in vitro* (Bishop, 2009), neither type 1 nor type 2 interferons seem to be effective in ameliorating disease or viral replication in the murine model (Bernstein, 2009).

Although experimental, some efforts have been to treat rotaviral illness by passively transferring antibodies. Human serum immunoglobulin administered orally to children with chronic rotavirus infection was able to associate with rotavirus in the gut, resulting in formation of large immune complexes rather than free virus. Antigen shedding was significantly reduced after antibody therapy, although the virus was not eliminated in all cases (Guarino et al., 1991). Enteric administration of antibody has shown some efficacy in normal children. A single dose of human immunoglobulin preparation that had a high titre against rotavirus (1:800-1:3,200) significantly speeded the rate of virus clearance and recovery from diarrhea in children infected with rotavirus (Guarino et al., 1994). However, a similar study showed no significant effect of orally administered immunoglobulin. Bovine immunoglobulin against rotavirus has been produced by hyper immunizing cattle with human and simian rotaviruses. Either the milk of hyper immunized cattle or serum immunoglobulins re-suspended in milk formula significantly reduced the incidence of rotavirus illness in children from 3 to 7 months old when orally administered. Children receiving immunoglobulins had significantly diminished symptoms upon becoming infected, compared with children receiving placebo formula (Hammarstrom, 1999).

An alternative means of producing anti rotavirus immunoglobulins is to harvest specific immunoglobulins form the yolks of eggs laid by chickens immunized with rotavirus. Egg yolks contain large quantities of specific immunoglobulin following intramuscular immunization of hens, and the antibody Y stable for long periods of time. Oral dosing of mice and calves with these immunoglobulins significantly reduces overall disease (Mackow et al., 1994). In mice, this protection was directly associated with reduced rotavirus antigen distribution within the intestinal tract, suggesting that replication of the virus has been inhibited. Recent human trials in Bangladesh suggested that such egg yolk preparations may have therapeutic value in human infants as well (Linhares et al., 1996).

Other Therapies

A number of reports have suggested that bacteria may be useful in the treatment of rotavirus-induced gastroenteritis.Feeding two bacterial strains, *Bifidobacterium bifidum* and *Streptococcus thermophilus*, to hospitalized infants aged 6 to 24 months reduced the incidence of diarrheal illness and rotavirus

shedding, suggesting that some mechanisms of protection from infection was in operation. Feeding another bacterial strain, *Lactobacillus casei* strain GG, to infected children appears to shorten diarrheal illness, presumably by restoring the microflora of the intestine and reversing intestinal osmotic and chemical imbalances (Guandalini et al., 2000). In addition, when administered in the acute diarrheal phase, *L casei* appears to stimulate the immune response to rotavirus, particularly the production of IgA, and may significantly enhanced protective immunity to subsequent infection. A quite recent meta-analysis of the use of lactobacillus species for treatment of rotavirus diarrhea suggested a slightly positive effect (Kaila et al., 1992).

Other therapies which have been studied for rotavirus diarrhea include bismuth subsalycylate which had mild efficacy and a new experimental encephala's inhibitor which in preliminary studies reduced rotavirus diarrhea by 50% without significant side effects. Nonspecific antidiarrheal such as loperimide are not suggested for infants with rotavirus disease (Cezard et al., 2001).

Because immunotherapy is relatively expensive and the opportunity to treat quite brief, it remains to be seen what the practical utility of these interventions is. Obviously, in the rare cases of chronic rotavirus infection in severely immuno-compromised individuals, passive immunotherapy may play a bigger role in treatment (Dorsey et al., 2017).

3.18 Management of Rotavirus infection

Treatment of acute rotavirus infection is nonspecific and involves management of symptoms and, most importantly, management of dehydration. If untreated, children can die from the resulting severe dehydration. Depending on the severity of diarrhoea, treatment consists of oral rehydration therapy, during which the child is given extra water to drink that contains specific amounts of salt and sugar. In 2004, the World Health Organisation (WHO) and UNICEF recommended the use of low-osmolarity oral rehydration solution and zinc supplementation as a two-pronged treatment of acute diarrhoea (Sachdev, 1996; Alam and Ashraf, 2003; Diggle, 2007; WHO, 2012).

Some infections are serious enough to warrant hospitalisation where fluids are given by intravenous therapy or nasogastric intubation, and the child's electrolytes and blood sugar are monitored. Rotavirus infections rarely cause other complications and for a well-managed child the prognosis is excellent. Probiotics have been shown to reduce the duration of rotavirus diarrhoea and according to the European Society for Pediatric Gastroenterology "effective interventions include administration of specific probiotics such as *Lactobacillus rhamnosus* or *Saccharomyces boulardii*, diosmectite or racecadotril (Patel et al., 2007; Ramig, 2007; Guarino et al., 2014; Ahmadi et al., 2015; Saghi et al., 2021).

There is no cure for rotavirus. Fortunately, most people develop an immune response that is eventually adequate to clear the virus from the body. However, while this natural response develops, the virus wreaks havoc on the body. Considering that the majority of people affected are young infants, the disease state can be particularly dangerous (Ahmadi et al., 2015).

The most common symptom is diarrhea and this alone can cause severe dehydration and electrolyte imbalance. For example, sodium concentrations in extracellular fluid have to be maintained between 135 and 150 mmol/liter for proper functioning. When a patient experiences diarrhea these levels can drop significantly. Therefore, treatments for rotavirus are supportive, aimed at re-hydration to prevent the severe effects of dehydration. Antidiarrheal medicines are not recommended because they may prolong infection (Saheri et al., 2014).

Due to the absence of pharmaceutical agents to treat rotavirus and the self-limiting nature of the viral infection of the gastrointestinal tract in most normal individuals, the key therapeutic goal is to prevent severe dehydration leading to electrolyte imbalance, shock, and death. Dehydration and complications arising from dehydration are most prevalent in rotavirus infection and are the major cause of mortality in infected children. Rehydration can be performed orally or, in patients with severe vomiting or in shock, by intravenous fluid administration. For oral rehydration, the WHO recommends a standard oral rehydration solution containing 3.5g of sodium chloride, 2.5g of sodium bicarbonate, 1.5g of potassium chloride, and 20g of glucose per liter. An alternative formulation that contains less sodium (40-60 vs. 90

meq/L) is available in the United States. Intravenous rehydration can be achieved using standard saline solutions containing glucose and potassium. In addition to rehydration therapy, it is recommended that efforts be made to improve the nutritional condition of the patient, as malnutrition exacerbates symptoms and slows recovery from diarrheal illness. Several studies have shown that re-feeding promptly after rehydration leads to quicker resolution of illness (WHO, 2019; NIPC, 2021).

To prepare an Oral Rehydration Salts ORS solution at home, if ORS packets are not available, mix an oral rehydration solution using one of the following recipes; depending on ingredients and container availability. Recipe for making a 1 litre ORS solution using Sugar, Salt and Water. Clean Water - 1 litre - 5 cupful (each cup about 200 ml.), Sugar - Six level teaspoons, Salt - Half level teaspoon, Stir the mixture till the sugar dissolves (WHO, 2019).

The home-made solution is adequate in most causes. If the diarrhoea is severe, ask your chemist for a special packet of Oral Rehydration Salts. Follow the instructions on the packet carefully (Simonsen et al., 2001; WHO, 2022).

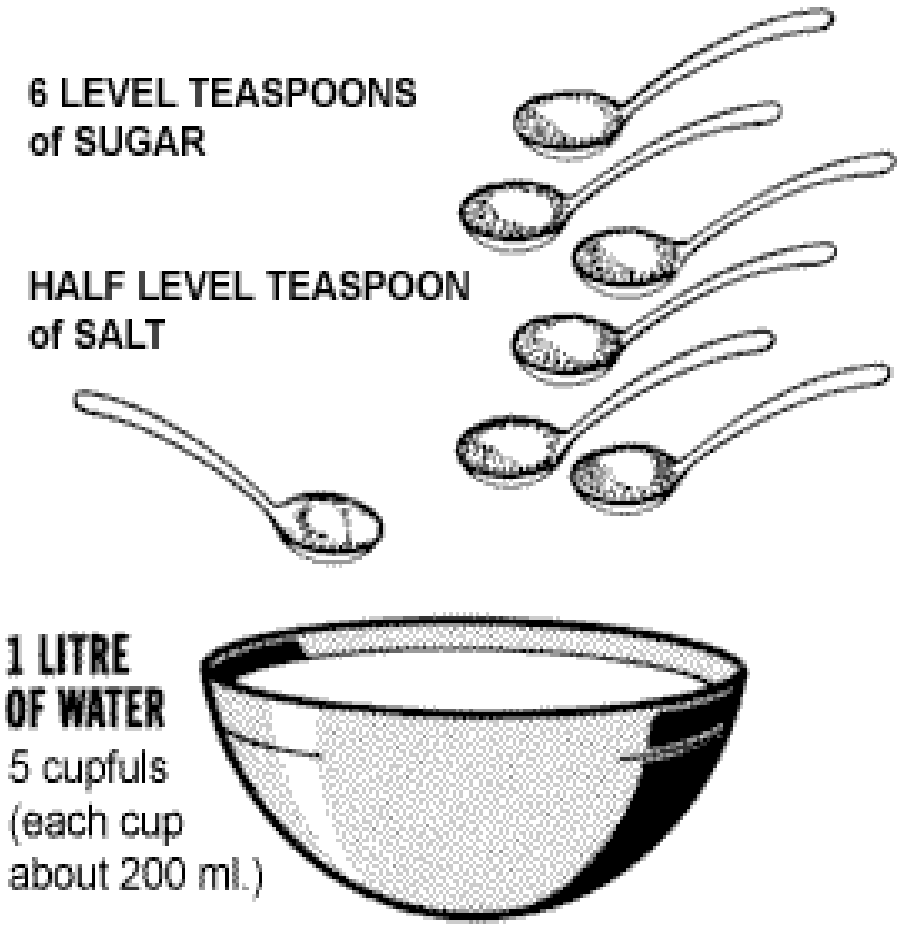

Figure 7. Illustration on how to prepare an Oral Rehydration Salts (ORS**)** (WHO, 2022).

Drink sips of the ORS (or give the ORS solution to the conscious dehydrated person) every 5 minutes until urination becomes normal (it's normal to urinate four or five times a day). Adults and large children should drink at least 3 quarts or litres of ORS a day until they are well. If you are vomiting, continue to try to drink the ORS. Your body will retain some of the fluids and salts you need even though you are vomiting. Remember to take sips of liquids slowly. Chilling the ORS may help. If you have diarrhea, continue to drink the ORS. The fluids will not increase the diarrhea. Someone with symptoms of severe dehydration needs to go to an emergency room or other health care facility to get intravenous fluids (fluids given directly into the veins through a needle) if possible. If able to drink, he or she should also drink the ORS. During or after treatment of dehydration, whatever is causing the diarrhea, vomiting, or other symptoms should also be treated (WHO, 2019; Smee et al., 2018).

3.19 Prevention of Rotavirus infections

Rotaviruses are highly contagious and cannot be treated with antibiotics or other drugs. Because improved sanitation does not decrease the prevalence of rotaviral disease, and the rate of hospitalisations remains high despite the use of oral rehydrating medicines, the primary public health intervention is vaccination (Sarmukaddam and Garad, 2006; Bernstein, 2009*).*In 1998, a rotavirus vaccine was licensed for use in the United States. Clinical trials in the United States, Finland, and Venezuela had found it to be 80 to 100% effective at preventing severe diarrhoea caused by rotavirus A, and researchers had detected no statistically significant serious adverse effects (Kapikian, 2001*).*The manufacturer, however, withdrew it from the market in 1999, after it was discovered that the vaccine may have contributed to an increased risk for intussusception, a type of bowel obstruction, in one of every 12,000 vaccinated infants (Bines, 2005*).* The experience provoked intense debate about the relative risks and benefits of a rotavirus vaccine. In 2006, two new vaccines against rotavirus A infection were shown to be safe and effective in children and in 2009, the WHO recommended that rotavirus vaccine be included in all national immunisation programmes (Doro et al., 2014; Tate et al., 2010).

General Preventive Measures

Vaccination plays an important role in preventing the infant mortality associated with rotavirus diarrhea. Sanitary waste disposal, effective hand washing and hygienic practices are other important measures. Although breastfeeding decreases the severity of rotavirus diarrhea, it does not prevent it (Abrahamson et al., 1991; Doresey et al., 2017).

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The incidence and severity of rotavirus infections has declined significantly in countries that have acted on this recommendation (Giaquinto et al., 2011; Jiang et al., 2010; Parashar et al., 2016). A 2014 review of available clinical trial data from countries routinely using rotavirus vaccines in their national immunisation programs found that rotavirus vaccines have reduced rotavirus hospitalisations by 49–92 percent and all cause diarrhoea hospitalisations by 17–55 percent. In Mexico, which in 2006 was among the first countries in the world to introduce rotavirus vaccine, diarrhoeal disease death rates dropped during the 2009 rotavirus season by more than 65 percent among children age two and under. In Nicaragua, which in 2006 became the first developing country to introduce a rotavirus vaccine, severe rotavirus infections were reduced by 40 percent and emergency room visits by a half. In the United States, rotavirus vaccination since 2006 has led to drops in rotavirus-related hospitalisations by as much as 86 percent. The vaccines may also have prevented illness in non-vaccinated children by limiting the number of circulating infections (Tate and Parashar, 2014; Richardson et al., 2010; Patel et al., 2007; Patel et al., 2013; Gasparinho et al., 2017).

In developing countries in Africa and Asia, where the majority of rotavirus deaths occur, a large number of safety and efficacy trials as well as recent post-introduction impact and effectiveness studies of Rotarix and RotaTeq have found that vaccines dramatically reduced severe disease among infants. In September 2013, the vaccine was offered to all children in the UK, aged between two and three months, and it is expected to halve the cases of severe infection and reduce the number of

children admitted to hospital because of the infection by 70 percent. In Europe, hospitalisation rates following infection by rotaviruses have decreased by 65% to 84% following the introduction of the vaccine. Globally, vaccination has reduced hospital admissions and emergency department visits by a median of 67% (Nelson et al., 2009; WHO, 2012; Neuzil et al., 2010; Karafillakis et al., 2015; Parashar et al., 2016; Burnett et al., 2017).

3.20. Hospital Infection Control Measures

The 1996 CDC guidelines recommend contact isolation including private rooms and cohorting of patients if private rooms are not available. Isolation is to be continued during the entire duration of illness. The 1996 CDC guidelines also mandate the use of gloves, proper hand washing and use of gowns if there is a possibility of soiling with infectious material. Patient transport should be done for essential purposes only and daily environmental cleaning is required (Dorsey et al., 2017; Shaheen et al., 2017). The patient care equipment should be dedicated or cleaned between each use. Age <6 y or diapered or incontinent patients need isolation. Empiric precautions are also mandated in patients with diarrhea syndrome, before the diagnosis of rotavirus diarrhea is made. Rotavirus is often resistant to the commonly used chemical disinfectants and antiseptics. However, in experimental studies Lysol spray has been effective. Hence disinfection of environmental surfaces with Lysol or other alcohol-containing disinfecting agents might decrease the amount of virus on surfaces in an infected child's room and decrease nosocomial transmission of rotavirus. Since hand-washing with plain soap is ineffective and only spreads the virus on the entire surface of the hand, disinfection of hands with 90% ethanol solution along with hand-washing or use of waterless hand-washing agents containing at least 70% alcohol may help limit rotavirus transmission (Gezard et al., 2001; Murphy et al., 2001; Smee et al., 2018).

4. **Conclusion**

Nigeria as a leading player in developing countries is suffering from infectious diseases due to a mixture of determinants. For example, prevalence of rotavirus infection is a major concern that causes a lot of deaths and hospitalization among children under five of age. Therefore, it is imperative to have more understanding of the rotavirus. A literature reviews to form concepts that help in gaining more about the rotavirus and ways to prevent its dissemination were made. Considering the reviewed works, the following recommendations were suggested:

i. Rotavirus detection in diarrhea cases should be included in routine laboratory tests for effective diagnosis and treatment of the infection.

ii. Parents/guardians and teachers in daycare centers should be enlighten on the mode of transmission of rotaviruses in their various places and how they can prevent children from rotavirus diarrhea.

iii. Government, as a matter of urgency should include rotavirus vaccines in routine immunization to children fewer than 5 years of age and should provide a supportive care in the management of the disease in the state.

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