CHAPTER 2

LITERATURE REVIEW

Gastroenteritis is commonly referred to as stomach flu or infectious diarrhoea which is often confused and fatal if remain under-diagnosed and untreated because of association of many etiological agents such as bacteria, parasites, and viruses. Diarrhoeal disease is the second leading cause of deaths in children and rotavirus is the common causative agent of diarrhoea worldwide. Enteric viruses infect the upper small intestine and cause non-inflammatory diarrhoea.

2.1. Genome organization

Rotaviruses are positive sense RNA virus; genome is approximately 18,550 base pairs and the size of the individual RNA segments ranged from 0.6 to 3.3 kb [1]. Except for segment 11, which codes for two proteins, NSP5 and NSP6, each segment of the rotavirus genome codes for one protein [2]. Each RNA segment is modified with the addition of 5'-guanylate residues and lacks poly (A) tail and the genome (58-67%) is rich in AU sequence. The consensus sequence at 5' end of rotavirus mRNA is 5'-[GGCA/UA/UUA/UAA/UA/U]-3, while the 3' end has 5'-[A/UUG/UU/GG/UA/GCC]-3'. The length of the 5' and 3' untranslated regions (UTRs) vary in the 11-dsRNA segments. Typically, tail 3' UTRs can be as long as 185 nucleotides (nt), the 5' UTRs are typically less than 50 nt long [3]. The function of rotavirus structural (VP1-4, VP6 & VP7) and non-structural proteins (NSP1-6) (Figure 2.1) are provided in Table 2.1. The NSPs are synthesized only in virus-infected cells. Some of the viral proteins undergo post-translational modifications such as glycosylation, phosphorylation, myristylation and proteolytic cleavage [4]. The conserved sequence of four nucleotides at the 3' end of the viral mRNA is known to function as translation enhancer [5].

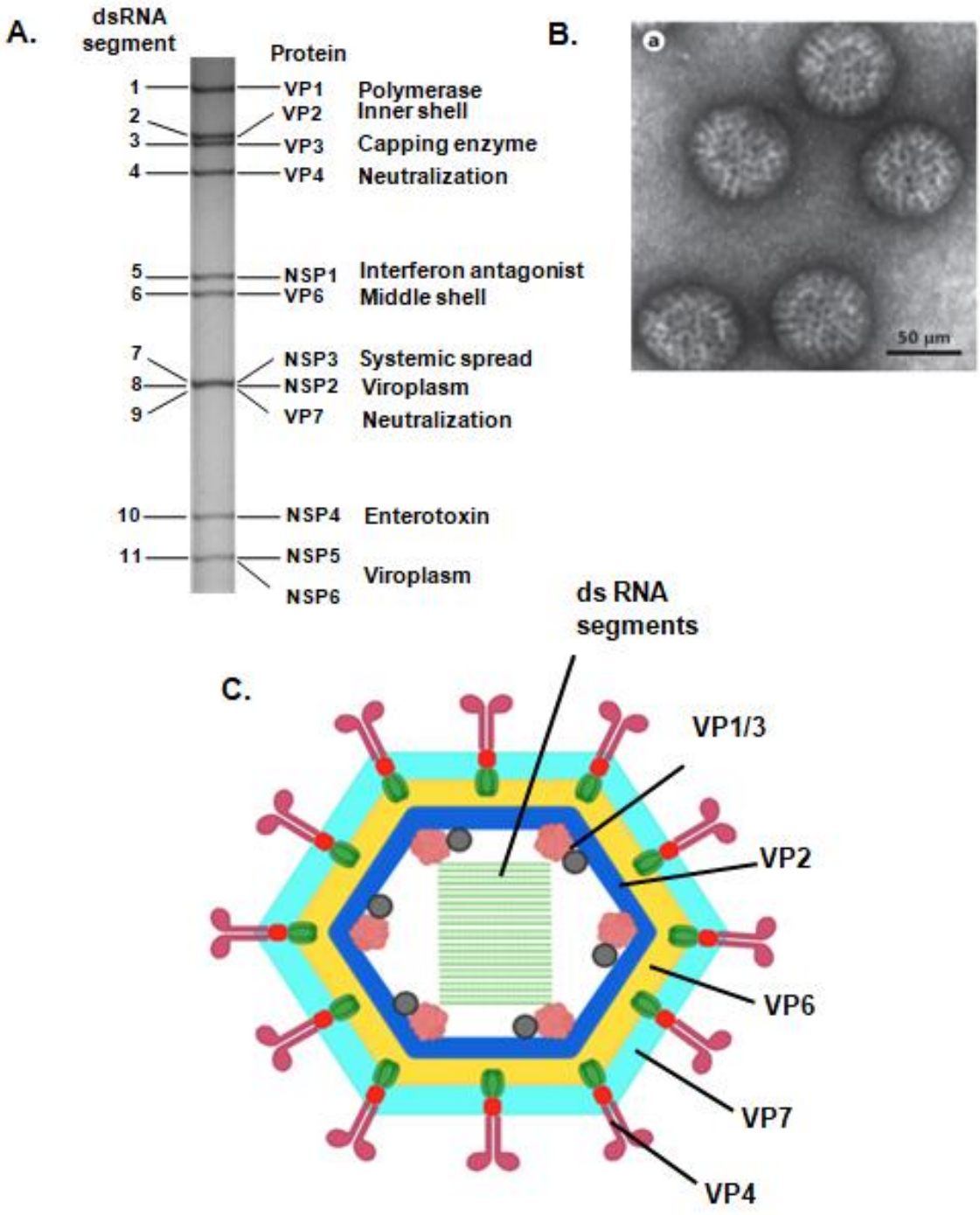

Figure 2.1. Rotavirus genome and structure organization. (A) Migration pattern on on polyacrylamide gel stained with silver nitrate showing the separation of 11 dsRNA segments of group A rotavirus (RVA) genome. The gene segments are numbered on the left and the corresponding encoded proteins are indicated on the right, (B) Electron microscopy image of the rotavirus triple-layered particle (TLP) (Crawford et al. 2017) and (C) Diagrammatic representation of RV structure and its organization.

Table 2.1. Rotavirus structural and non-structural proteins

Genome Segment	Length (bp)	Protein encoded	Size (kDa)	Location on the virus particle	Molecules per virion	Crystal structure (PDB ID)	Distribution of RV proteins in the infected cell	Functions
1	3302	VP1	125	Core	12	2R7Q, 2R7O, 2R7S-X	Viroplasm [69]	mRNA binding, RNA-dependent RNA polymerase
2	2690	VP2	102	Core	120	-	Viroplasm [69]	Nonspecific RNA binding, formation of viroplasm
3	2591	VP3	98	Core	12	6O3V, 6O6B, 5AF2, 4YE2, 4RPT	Viroplasm [69]	Binding of viral mRNA, guanylyltransferase, methyltransferase
4	2362	VP4	86	Outer capsid	180	1SLQ, 1KRI, 4YG3, 2B4I, 4DRR, 3SIS, 2P3J, 2P3I, 3TAY, 2B4H, 3SIT etc....	Cytosol [184]	Hemagglutinin, neutralization, binding and fusion
5	1611	NSP1	58	Nonstructural	-	5JEO, 5JER	Cytosol/nucleus [185]	Binding of viral mRNA, zinc-finger protein
6	1356	VP6	44	Inner capsid	780	1QHD, 3J9S	Viroplasm [69]	Interact with NSP4 at maturation stage
7	1104	NSP3	36	Nonstructural	-	1KNZ, 1LJ2	Cytosol	Binding of viral mRNA
8	1059	NSP2	36	Nonstructural	-	2GU0 (Taraporewala, 2006)	Viroplasm [69,186]	Nonspecific binding of ssRNA, interact with VP1, viroplasm formation
9	1062	VP7	37	Outer capsid	780	3FMG, 2LM7, 2KVL	ER [187,188]	Glycoprotein, neutralizing, calcium binding
10	751	NSP4	20	Nonstructural	-	3MIW, 2O1J, 5Y2E etc	ER and Cytoplasm	Transmembrane glycoprotein, viral enterotoxin
11	667	NSP5	21	Nonstructural	-	-	Viroplasm [69,186]	Binding to poly U and phosphoprotein, interact with NSP2
		NSP6	12	Nonstructural	-	-	Viroplasm [69]	Interact with NSP5

2.2. Structure of rotavirus

Rotaviruses are non-enveloped with triple capsid layer architecture primarily based on T=13 icosahedral symmetry. The RV triple-layered particles (TLPs) are the complete and fully infectious particle measuring 1000 Å in diameter including the spikes. Under the electron microscopy (EM), TLPs appear as wheel-like structures (*latin-rota*) hence the name 'rotavirus' originated [6]. The outermost layer is composed of two structural proteins: VP7 and VP4, VP6 make up the middle layer and inner layer is VP2 proteins.

In the outer layer, 780 copies of VP7 proteins are assembled as 260 trimers and feature 132 aqueous channels (comprising 12 channels of class I, 60 class II, and 60 class III channels) [7]. There are 60 VP4 spikes (180 copies) in a virus particle projected 120Å away from the VP7 layer and each spike is a homotrimer appearing dimeric above the outer layer and while forming a trimeric base anchored inside the capsid layer. The structure of spike protein was resolved by cryo-electron microscopy of the RV particle complexed with monoclonal antibodies specific to VP4 [8,9]. It displays a well-defined structure with VP8* making two globular domains as head, a central body or stalk, and a trimeric VP5* domain anchored under the VP7 layer [10]. The head portion of the spike protein is clearly identified as VP8* on X-ray imaging of proteolytic fragments of VP4, and the remaining body of the spike is identified as VP5* [11,12].

VP6 protein exists as trimers and contains 760 numbers of copies and each VP7 trimer grips the underlying VP6 trimer. The icosahedral lattice in the inner VP2 capsid layer differs remarkably from the middle and outermost layers and exhibits T-1 symmetry [7,13]. Around the five-fold symmetry axis, five dimers form a decamer, and twelve decamers make up the uniform core capsid layer with small pores along the axis [14]. Inside the core, the RNA dependent RNA polymerase (RdRP) complex consists of VP1 and VP3 and faces the class I channels [14,15,16,17].

VP7 layer detachment permits the double layered particle (DLP) to synthesize RNA. Transcriptionally active DLP has been shown to gain internal order as mRNA synthesis progress while inactive DLP remained dynamically disordered [18]. Like other viruses having segmented RNA as genome (e.g., reovirus, orbivirus, and influenza virus), the 5′ and 3′ ends of rotavirus RNAs are highly conserved (uppercase letters) with the sequence as 5′-GGC-poly(A/U) and 5′-aUgugaCC-3, respectively [19].

In a study, unusual interaction of RV proteins (VP1, VP3, NSP2, VP2 and VP6) and RNA in the replication intermediate (RI) complexes were observed during the process of assembly and replication [20]. Using cryo-EM, the native structures of the dsRNA genome of some viruses in the *Reoviridae* family were determined and were estimated to occupy only one to two thirds of the volume available in core particles. These findings raise the fascinating question of the maximum limit of the recombinant RNA packaging capacity of reovirus particles [21].

2.3. Classification

Rotaviruses belong to the family *Reoviridae*, which contains fifteen other distinct genera. The genera share a segmented dsRNA genome as a unifying feature. Rotaviruses are further classified into subgroups and serotypes based on three antigenic specificities such as VP4, VP6, and VP7. In overall, rotaviruses are characterized based on the (1) serologic characteristics: serotyping and dot-blot hybridization, or (2) sequence diversity i.e., genotyping of VP7 (glycosylated, G type) and VP4 (protease sensitive, P type), (3) the migration pattern of the 11th (smallest) segment on the RNA-PAGE: (electropherotying), and (4) genome sequence diversity.

2.3.1 Group

RVs are classified into nine groups, A to I, based on the presence of a group specific antigen called VP6 [22]. RVAs are the most common pathogens of humans and animals. The group B rotaviruses (RVB) are sporadic animal viruses [23] and reported in several large outbreaks infecting adults in China, and sporadically in children and adults in Bangladesh and India [24,25,26,27,28]. Group C rotaviruses (RVC) mainly infect animals but are sporadically caused diarrhoea in children [29] and adults in Argentina [30]. An outbreak of adult diarrhoea in China led to the discovery of a new rotavirus called ADRV-N (novel adult diarrhoea rotavirus) [31,32,33].

2.3.2. Subgroup

Rotavirus subgroup is classified based on antigenic specificity associated with the VP6 protein. RVAs are further classified into Subgroup I, Subgroup II, I+II, non-subgroup I, and non-subgroup II [34,35].

2.3.3. Serotype

Within the groups, rotaviruses are classified into serotypes. This classification, 'P' and 'G' serotyping is done based on reactivity of the sera against the outer capsid proteins VP4 (protease sensitive) and VP7 (glycosylated) [4]. Thus, 15 'G' and 27 'P' serotypes [36,37] have been identified using serotype specific antibodies [36,38]. The P serotype is written as letter 'P' immediately followed by a number, e.g., P1A.

2.3.4. Genotype

Genotyping is initiated because of the unavailability of the serotype specific antibodies to all the serotypes and new strains that do not react with the known serotype antibodies [39]. Genotyping of rotaviruses has now largely been carried out based on sequence analysis of two rotavirus outer capsid genes such as VP4 and VP7. Therefore, a strain with an amino acid (aa) sequence identity of ≥89% is regarded as belonging to the same genotype [40,41]. Thus, 51 'P' and 36 'G' genotypes have been reported [40,36,42]. A general correlation between genotype and serotype was established for VP7 but a lack of consistency was observed for VP4. However, based on sequence variation between aa position 84 and 180, P-type-specific epitopes on VP4 were defined using genotyping [43]. The P genotype is denoted by a number in square brackets [40] (e.g. P[8] or P[6]) and accordingly, the serotype and genotype of VP4 genes are represented as P1A[8] and P1B[4].

Recent developments in next generation sequencing technologies facilitated determination of the sequence of the complete genomes of several strains of rotavirus. Based on the sequence homologies of the 11-segments among the strains, a new genotyping scheme has been proposed in 2008. In this system, the rotavirus genes viz VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 are denoted as Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx (where x=Arabic numbers starting from 1 and G=Glycosylated; P=Protease sensitive; I= Intermediate capsid shell; R=RNA-dependent RNA polymerase; C=Core shell protein; M= Methyl transferase; A=Interferon antagonist; N=NTPase; T=Translation enhancer; E= Enterotoxin; H= Phosphoprotein), respectively. There are now three primary rotavirus genome constellations in humans: Wa-like, DS-1-like, and AU-1-like [44].

2.3.5. Electropherotype

RV genome consists of 11-dsRNA segments and the size of the gene segment ranges from 0.6 to 3.3 kb [45]. The RVAs genome migrates in four clusters (I-IV); cluster I comprise of segments 1-4 with high molecular weight, cluster II, segments 5 and 6, cluster III, segments 7, 8 & 9 and two smaller segments (10 and 11) in cluster IV. A deviation in this general pattern suggests that the rotavirus could be a group A avian virus or a reassortants or a unique RVA or non-group A rotavirus [46]. The RNA migration patterns are reportedly affected by genetic drift and shift or genome re-arrangements [47]. Group A rotaviruses can be characterized into three electropherotypes (E-types) namely, 'long', 'short' and 'super-short' based on the faster, moderate, and slower migration of gene segment 11 on polyacrylamide gels.

2.4. Life cycle

The rotaviruses enter the body through mouth, travel through the alimentary canal and infect the lining of the small intestines. *In vivo*, rotaviruses infect primarily the mature non-dividing enterocytes at the tip of the villus and enteroendocrine cells of small intestine [48]. The infection causes diarrhoea through the lesions on absorptive enterocytes causing malabsorption, NSP4 triggered intestinal secretions and activation of the enteric nervous system (ENS) [49]. RV isolates generally adapt well in primary African green monkey kidney (AGMK) cells and later can efficiently infect continuous cells such as MA104 [50]. Other cell types derived from various origins commonly used in rotavirus experiments are HT-29 (human colorectal adenocarcinoma), CaCo-2 (human colon adenocarcinoma), HepG2 (human liver), BGM (Buffalo green monkey kidney), FRhL-2 (Rhesus monkey lung), LLC-MK2 (Rhesus monkey kidney), and BSC-1, COS-7, CV-1 and Vero derived from African green monkey kidney [50]. The infection and replication cycle are shown in Figure 2.2 accompanied by the description of life cycle.

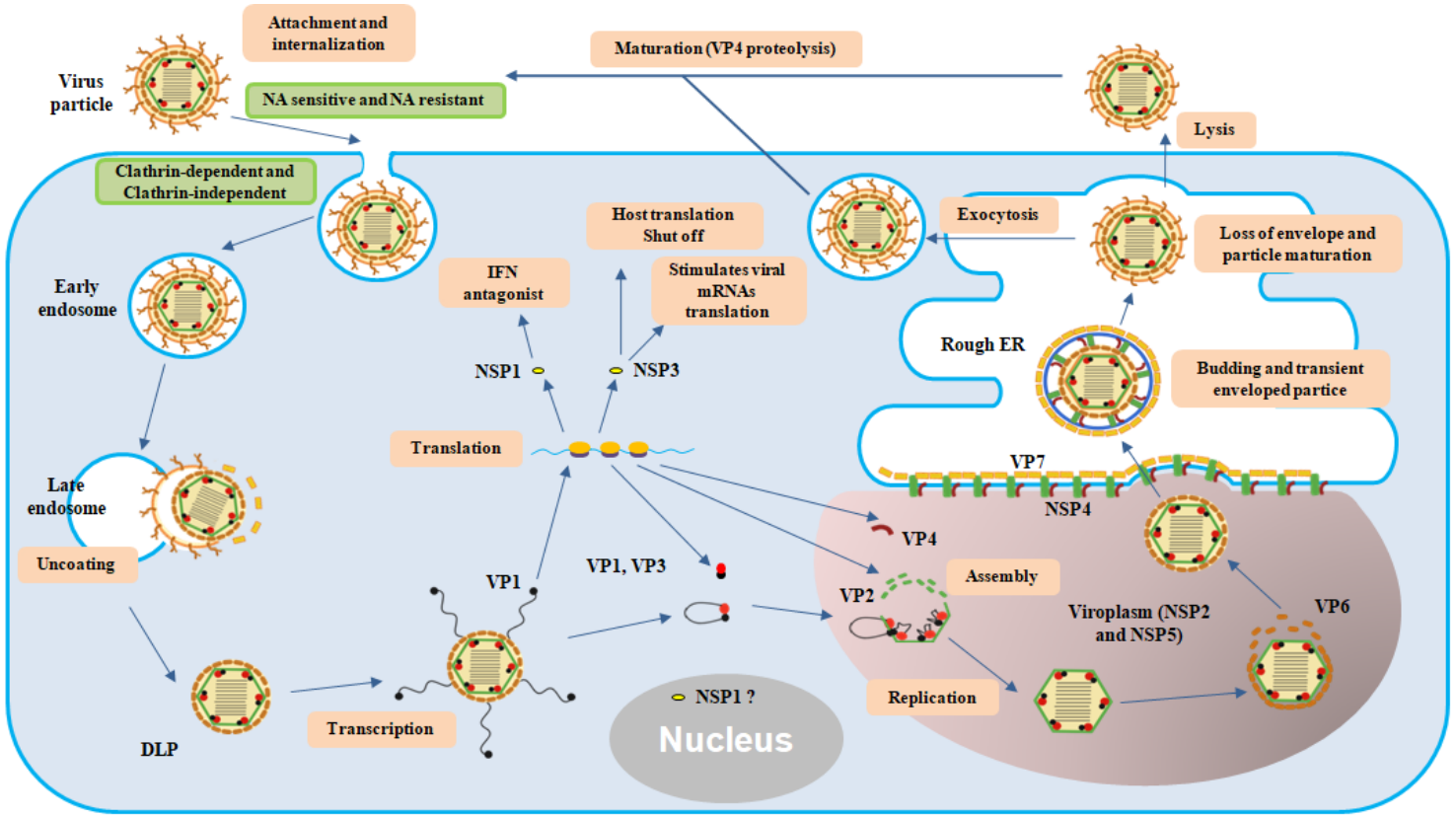

Figure 2.2. Life cycle of rotavirus. RVs attach to glycan receptors and co-receptors on the host cell surface, through interaction with VP8* domain of VP4. Initial attachment of some animal strains of RV involves sialic acid receptors (SA sensitive RVs) while human RV strains like Wa and DS-1, often attach to and enter cells in a sialic acid-independent manner (SA insensitive RVs) [189] where they interact with potential host receptors like histo blood group antigens (HBGA) [55,56,190,]. Cell entry starts with RVs interaction with co-receptors, cellular surface

molecules, such as heat shock protein (Hsc70), gangliosides [57] and integrins [59,60,61,191]. Depending on the strain, RVs are internalized into cells by clathrin-dependent or clathrin-independent and caveolin-independent endocytic pathways [192,193]. Gradient in calcium levels triggers the removal of the outer capsid layer (VP7 and VP4), which releases the transcriptionally active double-layered particle (DLP) into the cytoplasm. Viral (+) RNA is used for translation of the viral proteins and also as a template for RNA synthesis during genome replication. The new virions are assembled, and RNA is then packaged into new DLPs within the electron dense structures called viroplasms which are mainly composed of NSP2 and NSP5. Triple-layered particles (TLPs) assembly takes place by the coordinated action of NSP4 and VP6. DLPs buds into the endoplasmic reticulum (ER) forming transiently enveloped particle and the outer capsid proteins VP4 and VP7 are added onto the DLPs. The envelope is lost when RV particles acquire the outer layer consisting of VP4 and VP7 [91]. Virions are then released from the cells through cell lysis or Golgi-independent non-classical vesicular transport mechanism in polarized epithelial cells.

2.4.1. Rotavirus entry into the cell; attachment, penetration or internalization and vesicular trafficking

Rotavirus entry into the cell is a multi-step process which involves attachment to a receptor, followed by interactions with co-receptors on the cell surface and internalization. The virus displays restricted tropism, binding to a diverse cell line, but efficiently infecting only those of renal or intestinal epithelium origin explicitly of the intestinal villi suggesting that specific host receptors play a crucial role in virus entry into the cell [51]. Trypsin-like protease present in the digestive tract cleaves VP4 into VP5* and VP8* fragments which leads to change in the conformation facilitating the virus particle to invaginate cellular plasma membrane [52]. The probable sites of cleavage are Arg230, Arg241, Arg247 of VP4, mutation of which made it more resistant to trypsin proteolysis than the wild-type protein and cleavage enhances infectivity of rotavirus [53,51]. In the initial attachment, RV strains differ in their utilization of sialic acid (SA) for binding on the cell surface. Accordingly, RVs are classified into two types: SA-sensitive (or NA-sensitive) and SA-insensitive (or NA-resistant). First, RVs interact with cellular receptors (sialic acid as attachment receptors) via VP8* domain VP4 spikes. Several studies have shown some animal RVs engage sialic acid (SA-sensitive strains), while human strains are often SA-insensitive strains e.g., Wa and DS-1 [54]. Certain strains which are SA independent interact with potential host receptors, i.e., histo blood group antigens (HBGA) [54,55,56] and gangliosides [57]. After initial attachment, RVs interact with co-receptors, cellular surface molecules such as several heat shock cognate proteins (Hsc70), and integrins. Integrins (α2β1, αvβ3, αxβ2, α4β1) interact with VP5* or VP7 proteins through the integrin ligand motif [58,59,60,61]. It also has been shown that actin cytoskeletons (Actn4 and Cdc42) have an important role in rotavirus internalization in MA104 cells [62]. A biological membrane represents a physical barrier for viruses to enter living cells. Enveloped viruses enter host cells through the membrane fusion mechanism, while non-enveloped viruses, such as RVs, destabilize the host cell membrane as a means for entry. Probably a peptide in the C-terminal region of VP7 is responsible for permeabilizing biological membrane [63]. To reach the core of the cell, most viruses hijack the cellular endocytic pathway via mechanisms either clathrin or caveolae-mediated endocytosis, or macropinocytosis [64]. Regardless of the route of entry, once inside the cell, all RV strains merge in early endosomes (EE), core components of which are Rab 5 and its effector EEA1. Then the virus enters maturing

endosomes (ME) with the help of endosomal sorting complex required for transport (ESCRT) and form intraluminal vehicles (ILVs). RRV and SA11 strains escape the endosomal network and behave as early penetrating viruses. Other viral strains remain in the late endosomes (LEs) and RVs require CD-M6PR and cathepsins present in LEs to infect cells [65].

2.4.2. Virus particle uncoating and release of plus strand RNA

Under low calcium environment the outer capsid layer proteins VP6 and VP4 are dislodged, exposing VP6 protein coat, thus forming a transcriptionally active DLP. RVs are not fully uncoated, the reason being that the coat is partially resistant to protease digestion thereby protecting degradation by the host defense mechanism [66]. RVs do not utilize the cells replication enzymes, but rather utilize their own transcription complexes (TCs) that consist of VP1 (RdRP), and VP3. VP3 is a capping enzyme and has characteristics of phosphodiesterase, guanylyl transferase and methylase [67]. In the cytoplasm, the particles produce (+) ssRNA transcripts; capped but non-polyadenylated using the negative strand as template, which are then released through the class I channels from the DLP. The transcripts act as template for synthesis of viral proteins and progeny viruses [68]. The segmented nature of the genome allows reassortment of gene segments during replication and packaging of RNA segments leading to development of reassortant strains of rotavirus.

2.4.3. Translation of viral proteins and formation of viroplasm

RVs rely on translation machinery of the host to produce proteins encoded by the viral genome. Viral NSPs are synthesized only in the infected cell but are not assembled in the virion particle. NSPs have an important role in transcription, translation of viral proteins, genome replication and viral particle formation. Synthesis of virus-encoded proteins occurs in the cytoplasm using the (+) strand ssRNA as template. The synthesized proteins form an electron dense structure called viroplasm that serve as the site of virion factories where genome replication and particle assembly take place. The components that interact together and form viroplasm structure are VP1, VP2, VP3, VP6, NSP2, NSP5 and NSP6, as well as (+) ssRNA and dsRNA [69]. Silencing the expression of NSP2 and NSP5 have been linked to significant reduction in size and number of viroplasm and production of infectious progeny virions [68,70,71]. NSP4, a

transmembrane glycoprotein, forms caps on viroplasms and vesicular transport compartments regulated by levels of intracellular calcium [72]. Cellular lipid droplets (LDs) recruited by viroplasms serve as both energy source as well as transport vehicles in the cell and are crucial for rotavirus replication [73,74].

The host mRNAs are polyadenylated at the 3' end and capped at the 5' end. The 5' cap is bound by the translation initiation factor eIF4E, a multipurpose ribosome adaptor responsible for conveying capped and poly-adenylated messages to the ribosome. The poly (A) tail interacts with poly (A)-binding protein (PABP), which then interacts with eIF4G [75]. In contrast to cellular mRNAs, rotaviral mRNAs are not polyadenylated, but are capped at the 5' end. Lack of poly (A) tail would hamper the efficient translation of viral mRNAs due to failure of circularization of the viral mRNAs. Rotaviruses overcome this problem by using the consensus sequence at the 3' end of the viral RNAs which specifically interacts with NSP3 protein. The C-terminal domain of NSP3 interacts with eIF4G with an affinity greater than that of PABP and the N-terminal domain with the consensus sequence, thus assisting the 5' and 3' interaction of the mRNAs. In this way, NSP3 enhances the selective translation of the rotavirus mRNAs by competing with PABP and dislodging it from actively translating host eIF4F complexes [76,77,78]. Competition of NSP3 for eIF4G results in effective inhibition of cellular protein synthesis [79]. RoXaN (rotavirus X protein associated with NSP3), a cellular protein interacts with NSP3 and is present in a ternary complex comprising NSP3, RoXaN and eIF4GI in the infected cell implying RoXaN in translation regulation of rotaviral mRNAs [80]. In contrary, recent studies using siRNAs directed against NSP3 indicated that NSP3 is not required for selective viral mRNA translation [81]. eIF2α becomes phosphorylated and inhibits cell protein synthesis. In uninfected cells, phosphorylation of eIF2α forms the stress granules but in infected cells it is prevented to efficiently translate its mRNA [82]. Interestingly, it was reported that the overall production of viral proteins was largely contributed by secondary transcription products synthesized by the newly assembled virions during rotavirus replication [83].

2.4.4. Synthesis of RV genome

The genome of the progeny virion is synthesized using the positive sense RNA as template which also serves as template for the synthesis of viral proteins. The core proteins function together for replication and producing the 11-capped segments of RV

genome. RdRP alone can recognize viral (+) RNAs, however, requires VP2 that is an essential co-factor which triggers the polymerase to initiate dsRNA synthesis and regions in inner face of the core layer are crucial for viral polymerase activation [84]. The minus strand synthesis is facilitated by the panhandle structure form by the base-pairing between the 5' and 3' consensus sequence of mRNA. The 3' consensus sequence of RVAs contains cis-acting replication signals which extends as a 3'-tail from the panhandles. The RNA packaging involves NSP2 and NSP5; NSP2 possesses NTPase, helix-destabilizing and RNA-binding activity [85,86]. In a recent study, GTPase Rac1 has been shown its importance for maximal viral RNA synthesis [62]. DLPs have transcriptional pores through which synthesized (+) RNA are exited [67].

2.4.5. Packaging of new virion particle

The synthesized structural proteins and viral RNA genome get accumulated in the viroplasm where packaging of the genome and DLP particle assembly take place. Like the chromatin packaging in humans, naked viral dsRNA needs to be packaged compact into the core shells due to its long sequence length. Interactions of the replication complexes (VP1/VP3/ssRNA), a VP2 decamer, NSP2 and NSP5 are believed to coordinate genome replication and core assembly [15]. The negative charge of the viral RNA is counteracted by co-packaging with divalent cations and cellular trivalent cationic compound spermidine [87]. The multi-segmented genome assembly is directed by specific RNA-RNA interactions between the segments. The segments undergo assortment and form ordered complexes that nucleate assembly of core proteins around it [88]. Once core particles are assembled, it is rapidly trans-capsidated by VP6 and form DLPs. Deletion mapping of functional domains had identified assembly domain that are in the amino acid positions, aa122-147 at the amino terminal end of VP6 protein [89]. Hydrophobic effects between VP6 and VP2 have been implicated in providing the stability of the DLPs [90].

2.4.6. Maturation and release from the host cell

For maturation, DLPs from the site of viroplasm are transported to endoplasmic reticulum (ER) by NSP4 and VP6. The rotavirus immature virions transiently acquire lipid envelope inside the lumen of ER and the VP7 outer capsid layer replaces it, which consists of VP7 glycoprotein (260 trimers) and VP4 spike (60 trimers) [91]. Virions are

then released either from the cells through cell lysis pathway or non-classical pathway that bypass goldi.

2.5. Pathogenesis and pathology

Rotavirus enters through oral route and can result in either acute or persistent infections leading to symptomatic or asymptomatic disease. The virus mainly infects the mature enterocytes of small intestine, in addition, several reports have indicated extraintestinal (systemic) involvement in many organs; spleen, liver, heart, kidneys, testes, bladder and respiratory tract [92,93]. The disease pathology has been described as an "iceberg" model whereby the most visible tip is diarrhoea. The rest being extra-intestinal manifestations due to the systemic spread of the infection, such as, seizures in the CNS, autoimmune trigger in individuals with a specific genetic background [94]. The pathogenicity of the virus depends on various host and viral factors. RV infections in children under the age of five are more common worldwide. The damage to the mucosa, membrane lining body cavities, leads to malabsorption. Also, the activity of disaccharidases and peptidases in the microvilli on the surface of epithelial cells decreases and the resulting osmosis from unabsorbed nutrients contributes to diarrhoea. Other consequences include villus ischemia, intestinal secretion stimulated and intracellular Ca^{2+} and Cl^{-} ion mobilization and activation of the ENS and enteric vascular systems which stimulate indirect secretion together contributes to rotavirus pathogenesis [95,96,97]. The mechanism of clearance of infection is complex involving overlapping elements of innate, humoral, and cellular immunity [98]. In symptomatic children with diarrhoea, the median duration of fecal shedding was found to be 24 days in the range of 14 to 51 days. Whereas, in asymptomatic children, it was observed 18 days with the duration of shedding in the range of 8 to 25 days [99].

2.6. Epidemiology

2.6.1. Occurrence, reservoir, transmission, and incubation period of rotavirus

Rotaviruses are highly contagious viruses that do not discriminate between the poor and rich, and infection occurs throughout the globe, with primary reservoir being the gastrointestinal tract and stool of infected humans and non-human mammals. The mode of transmission of the virus is through fecal-oral route, close-contact, and contaminated

food, water, hands, respiratory spread, and fomites [100,101,102,103]. Respiratory mode of transmission of rotavirus has also been proposed [104]. The incubation period is generally 2 to 3 days, while the infective period is 1 to 3 weeks and asymptomatic carriers are very common. After the patient becomes symptomatic, the virus is shed for 6-10 days. The severity of the disease was found correlated with the prolonged period of shedding of rotavirus particles [105].

2.6.2. Seasonality and geographical distribution of rotavirus infection

Seasonality of a disease represents a periodic surge of the disease during a year. Oscillation in the pathogen's effective reproductive cycle reflects its infectiousness, pathogen survival, or host susceptibility [106]. In temperate climates rotavirus infections showed a distinct seasonal pattern with peaks incidence observed mainly in cooler months. However, in tropical countries, RV disease seasonality is less pronounced, although, the infection is more common during the dry winter season [107,108,109].

In a global age distribution study in children under five-year-old, the median age of RV-positive hospital admissions was 38-65 weeks in countries where rotavirus infection was prevalent [110]. There is no clear data to support if male is more prone to RV infection than female or vice-versa. The environmental factors associated with the seasonality of rotavirus infection remain unclear till now. Geographical distribution studies showed that in temperate countries, most likely, seasonality results from unfavorable conditions for virus transmission which could be high relative humidity, higher atmospheric temperatures, and less crowding. Interestingly, a country's income level was described as another possible factor for seasonality of rotavirus infection [111].

2.7. Symptoms

Most clinical features of RV infection are similar to the general symptoms of gastroenteritis which is distinguished by asymptomatic mild watery diarrhoea to acute gastroenteritis and dehydration which leads to death and occur most often in the children. The incubation period is short that ranged from 1 to 3 days followed by abrupt onset of symptoms such as fever, decrease in urination, dry mouth and throat, dizziness, abdominal pain, and vomiting for 24 to 48 hours accompanied by pale watery or loose non-bloody diarrhoea for 3 to 8 days (10 to 20 times per day) and metabolic acidosis,

although prolong episodes have been noted occasionally [112,113]. Two major consequences of rotavirus infection are dehydration and electrolyte disturbances: hypernatremia, being the most common electrolyte disturbance in acute diarrhoeal diseases [114]. Furthermore, respiratory illness signs, infection of the oropharynx and respiratory tract are often found during RV gastroenteritis [115]. Immuno-compromised individuals are at risk of complicated rotavirus gastroenteritis leading to hospitalization [116]. Another emerging and widely discussed feature of RV infection is the antigenemia and extraintestinal manifestations in patients with RV gastroenteritis suggesting RV infection is systemic. Several studies have reported RNA and antigen of the virus, and infectious particles in serum, cerebrospinal fluid (CSF) and extraintestinal tissues in patients with RV acute gastroenteritis (AGE) [117,118,119]. The severity of rotavirus-induced AGE is scored based on seven parameters that are recorded during the visit of patient to Hospital by the clinicians (Table 1.2a and 1.2b). Accordingly, treatment is provided to the severe patients that requires hospitalization [120].

Table 2.2a: Vesikari clinical severity scoring system (VSS). VSS was determined to scale the severity of rotavirus induced acute gastroenteritis based on seven clinical parameters recorded at the hospital.

Parameters Sl. No.	Paramaters / Score	1	2	3
	Diarrhoea			
1	Maximum Number Stools per Day	1-3	4-5	≥6
2	Diarrhoea Durations (Days)	1-4	5	≥6
	Vomiting			
3	Maximum Number Vomiting Episodes per Day	1	2-4	≥5
4	Vomiting Duration (Days)	1	2	≥3
5	Temperature (ºC)	37.1-38.4	38.5-38.9	≥39.0
6	Dehydration	N/A	1-5%	≥6%
7	Treatment	Rehydration	Hospitalization	N/A

*Table adapted from rotavirus clinical trials utilizing the Vesikari clinical severity scoring system (Clark et al., 2004; Ruuska & Vesikari, 1990).

Table 2.2b: Vesikari Clinical Severity Scoring System Severity Rating Scale

Severity Category			
Mild	Moderate	Severe	Maximum Score
<7	7-10	≥11	20

*Table adapted from rotavirus clinical trials utilizing the Vesikari clinical severity scoring system (Clark et al., 2004; Ruuska & Vesikari, 1990).

2.8. Diagnosis

Rotavirus diarrhoea is clinically identical with gastroenteritis caused by other infectious agents (such as norovirus, adenovirus, astrovirus, *Escherichia coli* and *Salmonella spp*.). Rotavirus infection is seasonal in nature and in temperate countries, if a child has diarrhoea in cold season; generally, the causal agent suggested is rotavirus or norovirus. Rapid identification of viruses associated with gastroenteritis is necessary for timely implementation of the effective management strategy to control rotavirus disease outbreaks [49].

Laboratory based diagnosis of rotavirus infection is carried out using stools of the patients. The techniques include electron microscopy (EM) [121], virus isolation in cell culture [122,50], native polyacrylamide gel electrophoresis (Native-PAGE) of viral RNA genome segments [123], enzyme immunoassays (EIAs) [124], passive particle agglutination tests [125], immunochromatographic tests [126], RT-PCR followed by gel electrophoresis [127,128,129,130], and qRT-PCR [131,132,133]. Several multi-pathogen detection assays, point-of-care testing (POCT) are commercially available for simultaneous detection of different enteroviruses associated with acute gastroenteritis [134,135]. For epidemiological surveillance studies, serological and molecular techniques are used for strain characterization [130]. These molecular techniques include full-genome sequencing [22], next-generation sequencing [136,137,138,139,140], Sanger sequencing [141,142], and dot-blot hybridization [143,144].

2.9. Treatment

There is no specific medicine/drug to treat rotavirus infection and treatment focuses on symptom relief. The treatment consists mainly of rehydration (orally or intra-venous); the primary aim is to compensate fluids and electrolytes lost in vomiting and diarrhoea. Oral rehydration can treat mild infections; however, severe rotavirus urgently needs intravenous fluids to overcome the risk of fatality due to dehydration [145]. However, medicine to treat the symptoms is recommended. In addition to fluids and electrolytes, other supplements under investigation are amino acids, lactoferrins, lysozyme, and beneficial probiotics [146,147]. Studies have shown some of the potential anti-viral agents against RV. One such drug is thiazolides, a class of broad-spectrum antiviral drugs by targeting viral morphogenesis and inhibiting viroplasm formation [148]. Gemcitabine, brequinar (BQR) and leflunomide (LFM) targets pyrimidine nucleotide synthesis pathway. The latter two drugs target dihydroorotate dehydrogenase (DHODH) enzyme [149,150]. While resveratrol inhibits viral structural expression and genomic RNA synthesis [151] and ursolic acid inhibits the maturation of viral particles in the ER [152].

2.10. Control and prevention

Improved sanitation and hand hygiene, exclusive breastfeeding, and improved water quality is highly recommended to reduce the chances of getting RV infection. But these

do not significantly reduce the burden of rotavirus and the virus continues to be a threat particularly in children under five-year-old. The incidence of RV diarrhoea was observed 40% (approx.) in high-income and low-income countries in pre-vaccine era and continued to be a burden even in post vaccine era [153]. The difference in rotavirus vaccine efficiency between these countries was found associated with universal access to safe water and sanitation. Vaccination is considered the most reliable preventive measure for rotaviral diarrhoea. Global rotavirus vaccines available worldwide are: 1) RV5 vaccine, RotaTeq (Merck, USA), and 2) RV1 vaccine, Rotarix (GlaxoSmithKline, Belgium). Both are oral and live attenuated vaccines and since licensure in 2006, rotavirus vaccines have been introduced in more than 100 nations [154,155].

RotaTeq is composed of five bovine–human reassortant rotaviruses and is known to confer protection against five common serotypes: G1-G4 with P7[5] and G6P1A[8] genotypes [156]. Rotarix is a vaccine derived from a human-strain 89–12, with G1P[8] genotype [157]. Similar dose and age schedules are followed for these two vaccines in most countries worldwide. Clinical and surveillance studies have reported that the vaccines are safe and effective in preventing severe rotavirus diarrhoea and mortality associated with it and reported positive impact upon public health resources with higher performance in countries with lower child mortality [154,155]. Interestingly, substantial reduction of severe rotavirus disease by ~50-60% was observed in many low-income countries [158,159,160] and shown higher effectiveness in high-income nations [161]. However, the mechanism of vaccines protection from RV infections is not fully understood [162].

Environmental enteropathy (EE) is a disorder in children characterized by intestinal inflammation and reduced gastrointestinal immunity. It has been linked to reduced efficacy of vaccines [163] especially RV5 vaccine and there is possibility that preventing EE may improve vaccine efficacy [164]. In addition, it has been observed that co-administration of different oral polio vaccine (OPV) formulations with oral RV vaccines reduces the effectiveness of the later [165]. Maternal antibodies and genetic variation in the population is another factor that affects vaccine immunity. Further, the effect of IgA antibodies in breast milk on vaccine immunogenicity is not clearly understood [166,167,168,169]. It is evident that asymptomatic infections with specific RV strains in newborns are associated with protection against the disease in adulthood thereby leading

to development of vaccines for rotavirus in the recent year. In India, ROTAVAC is a vaccine for rotavirus developed by the Bharat Biotech International, Hyderabad which is based on rotavirus strain (116E) with G9P[8] genotype, has been licensed in 2015 and included in universal immunization program (UIP) with initial introduction in four states in 2016 and five additional states in 2017 in a phased manner [170,171]. Human Neonatal Rotavirus Vaccine (RV3-BB) (PT Bio Farma, Indonesia/Murdoch Children's Research Institute, Australia) has been developed from a human strain, RV3 (G3P[6]) isolated from a asymptomatic infants in clinical trials in Australia and New Zealand [172] and demonstrated positive cumulative effect irrespective of HBGA status [173]. Other vaccines include the Lanzhou lamb rotavirus (LLR) vaccine in China [172] and the Rotavin-M1 in Vietnam [162,175]. To lower vaccine costs, RotaSIIL is a bovine rotavirus pentavalent vaccine (BRV-PV) comprising of serotypes G1, G2, G3, G4 and G9, developed by the Serum Institute of India Pvt. Ltd has been introduced in India and Nigeria showing efficacy of 66.7% in infants in India [176,177,178]. RotaShield was the first licensed vaccine (Wyeth, USA) which was withdrawn from the market due to cases of intussusceptions (~1 case per 5,000 to 10,000 vaccinated children) [179]. Rotarix and RotaTeq have a good safety record with a minimal associated risk [180]. However, additional studies are required to further investigate intussusceptions events in vaccinated children [181]. To overcome the risk associated with live attenuated vaccines and low efficacy, efforts have been made to develop vaccine formulations that are non-replicating, safe, immunogenic, and temperature insensitive [162]. For examples, some of the under-investigation rotavirus vaccine candidates are inactivated rotavirus vaccine (IRV), vaccine based on expressed VP6 inner capsid protein, virus-like particles (VLPs) and VP8 expressed proteins (NRRV) [182,183].

2.11. Bibliography

[1] Rodger, S. M., Craven, J. A., & Williams, I. (1975). Letter: Demonstration of reovirus-like particles in intestinal contents of piglets with diarrhoea. *Australian Veterinary Journal*, 51(11):536. https://doi.org/10.1111/j.1751-0813.1975.tb06917.x.

[2] Mattion, N. M., Mitchell, D. B., Both, G. W., & Estes, M. K. (1991). Expression of rotavirus proteins encoded by alternative open reading frames of genome segment 11. *Virology*, 181(1):295–304. https://doi.org/10.1016/0042-6822(91)90495-w.

[3] Baybutt, H. N., & McCrae, M. A. (1984). The molecular biology of rotaviruses. VII. Detailed structural analysis of gene 10 of bovine rotavirus. *Virus Research*, 1(7):533–541. https://doi.org/10.1016/0168-1702(84)90011-x.

[4] Mattion, N. M., Cohen, J., and Estes, M. K. (1994). The rotavirus proteins. In ‘‘Viral Infections of the Gastrointestinal Tract’’ (A. Z. Kapikian, Ed.), pages 169–249, Dekker, New York/Basel/Hong Kong.

[5] Patton, J. T., Chizhikov, V., Taraporewala, Z., & Chen, D. (2000). Virus replication. *Methods in Molecular Medicine*, 34:33–66. https://doi.org/10.1385/1-59259-078-0:33.

[6] Flewett, T. H., Davies, H., Bryden, A. S., & Robertson, M. J. (1974). Diagnostic electron microscopy of faeces. II. Acute gastroenteritis associated with reovirus-like particles. *Journal of Clinical Pathology*, 27(8):608–614. https://doi.org/10.1136/jcp.27.8.608.

[7] Jayaram, H., Estes, M. K., & Prasad, B. V. (2004). Emerging themes in rotavirus cell entry, genome organization, transcription and replication. *Virus Research*, 101(1):67–81. https://doi.org/10.1016/j.virusres.2003.12.007.

[8] Prasad, B. V., Burns, J. W., Marietta, E., Estes, M. K., & Chiu, W. (1990). Localization of VP4 neutralization sites in rotavirus by three-dimensional cryo-electron microscopy. *Nature*, 343(6257):476–479. https://doi.org/10.1038/343476a0.

[9] Tihova, M., Dryden, K. A., Bellamy, A. R., Greenberg, H. B., & Yeager, M. (2001). Localization of membrane permeabilization and receptor binding sites on the VP4 hemagglutinin of rotavirus: implications for cell entry. *Journal of Molecular Biology*, 314(5): 985–992. https://doi.org/10.1006/jmbi.2000.5238.

[10] Rodríguez, J. M., Chichón, F. J., Martín-Forero, E., González-Camacho, F., Carrascosa, J. L., Castón, J. R., & Luque, D. (2014). New insights into rotavirus entry machinery: stabilization of rotavirus spike conformation is independent of trypsin cleavage. *PLoS Pathogens*, 10(5):e1004157. https://doi.org/10.1371/journal.ppat.1004157.

[11] Dormitzer, P. R., Sun, Z. Y., Wagner, G., & Harrison, S. C. (2002). The rhesus rotavirus VP4 sialic acid binding domain has a galectin fold with a novel carbohydrate binding site. *The EMBO Journal*, 21(5):885–897. https://doi.org/10.1093/emboj/21.5.885.

[12] Dormitzer, P. R., Nason, E. B., Prasad, B. V., & Harrison, S. C. (2004). Structural rearrangements in the membrane penetration protein of a non-enveloped virus. *Nature*, 430(7003):1053–1058. https://doi.org/10.1038/nature02836.

[13] Li, Z., Baker, M. L., Jiang, W., Estes, M. K., & Prasad, B. V. (2009). Rotavirus architecture at subnanometer resolution. *Journal of Virology*, 83(4):1754–1766. https://doi.org/10.1128/JVI.01855-08.

[14] McClain, B., Settembre, E., Temple, B. R., Bellamy, A. R., & Harrison, S. C. (2010). X-ray crystal structure of the rotavirus inner capsid particle at 3.8 A resolution. *Journal of Molecular Biology*, 397(2):587–599. https://doi.org/10.1016/j.jmb.2010.01.055.

[15] Trask, S. D., McDonald, S. M., & Patton, J. T. (2012a). Structural insights into the coupling of virion assembly and rotavirus replication. *Nature Reviews. Microbiology*, 10(3):165–177. https://doi.org/10.1038/nrmicro2673.

[16] Trask, S. D., Ogden, K. M., & Patton, J. T. (2012b). Interactions among capsid proteins orchestrate rotavirus particle functions. *Current Opinion in Virology*, 2(4):373–379. https://doi.org/10.1016/j.coviro.2012.04.005.

[17] Estrozi, L. F., Settembre, E. C., Goret, G., McClain, B., Zhang, X., Chen, J. Z., Grigorieff, N., & Harrison, S. C. (2013). Location of the dsRNA-dependent polymerase, VP1, in rotavirus particles. *Journal of Molecular Biology*, 425(1):124–132. https://doi.org/10.1016/j.jmb.2012.10.011.

[18] Hauser, Mary & Dearnaley, William & Varano, Ann & Casasanta, Michael & Mcdonald, Sarah & Kelly, Deb. (2019). Cryo-EM Reveals Architectural Diversity in Active Rotavirus Particles. *Computational and Structural Biotechnology Journal*. 17:1178–1183. https://doi.org/10.1016/j.csbj.2019.07.019.

[19] Desselberger, U., & McCrae, M. A. (1994). The rotavirus genome. *Current Topics in Microbiology and Immunology*, 185:31–66. https://doi.org/10.1007/978-3-642-78256-5_3.

[20] Boudreaux, C. E., Kelly, D. F., & McDonald, S. M. (2015). Electron microscopic analysis of rotavirus assembly-replication intermediates. *Virology*, 477:32–41. https://doi.org/10.1016/j.virol.2015.01.003.

[21] Desselberger U. (2020). What are the limits of the packaging capacity for genomic RNA in the cores of rotaviruses and of other members of the Reoviridae?. *Virus Research*, 276:197822. https://doi.org/10.1016/j.virusres.2019.197822.

[22] Matthijnssens, J., Ciarlet, M., McDonald, S. M., Attoui, H., Bányai, K., Brister, J. R., Buesa, J., Esona, M. D., Estes, M. K., Gentsch, J. R., Iturriza-Gómara, M., Johne, R., Kirkwood, C. D., Martella, V., Mertens, P. P., Nakagomi, O., Parreño, V., Rahman, M., Ruggeri, F. M., Saif, L. J., Van Ranst, M. (2011). Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). *Archives of Virology*, 156(8):1397–1413. https://doi.org/10.1007/s00705-011-1006-z.

[23] Chang, K. O., Parwani, A. V., Smith, D., & Saif, L. J. (1997). Detection of group B rotaviruses in fecal samples from diarrheic calves and adult cows and characterization of their VP7 genes. *Journal of Clinical Microbiology*, 35(8):2107–2110. https://doi.org/10.1128/jcm.35.8.2107-2110.1997.

[24] Hung, T., Chen, G. M., Wang, C. A., Fan, Railian, Yong, Ronjan, Chang, Jiaqu, Dan, Robert & Ng, Mon. (1987). Novel Diarrhoea Viruses. *Ciba Found. Symp.* 128:49-62. https://doi.org/10.1002/9780470513460.ch4.

[25] Krishnan, T., Sen, A., Choudhury, J. S., Das, S., Naik, T. N., & Bhattacharya, S. K. (1999). Emergence of adult diarrhoea rotavirus in Calcutta, India. *Lancet (London, England)*, 353(9150):380–381. https://doi.org/10.1016/s0140-6736(05)74954-0.

[26] Penaranda, M. E., Ho, M. S., Fang, Z. Y., Dong, H., Bai, X. S., Duan, S. C., Ye, W. W., Estes, M. K., Echeverria, P., & Hung, T. (1989). Seroepidemiology of adult diarrhea rotavirus in China, 1977 to 1987. *Journal of Clinical Microbiology*, 27(10):2180–2183. https://doi.org/10.1128/jcm.27.10.2180-2183.1989.

[27] Sanekata, T., Ahmed, M. U., Kader, A., Taniguchi, K., & Kobayashi, N. (2003). Human group B rotavirus infections cause severe diarrhea in children and adults in Bangladesh. *Journal of Clinical Microbiology*, 41(5):2187–2190. https://doi.org/10.1128/JCM.41.5.2187-2190.2003.

[28] Su, C. Q., Wu, Y. L., Shen, H. K., Wang, D. B., Chen, Y. H., Wu, D. M., He, L. N., & Yang, Z. L. (1986). An outbreak of epidemic diarrhoea in adults caused by a new rotavirus in Anhui Province of China in the summer of 1983. *Journal of Medical Virology*, 19(2):167–173. https://doi.org/10.1002/jmv.1890190210.

[29] Jiang, B., Dennehy, P. H., Spangenberger, S., Gentsch, J. R., & Glass, R. I. (1995). First detection of group C rotavirus in fecal specimens of children with diarrhea in the United States. *The Journal of Infectious Diseases*, 172(1):45–50. https://doi.org/10.1093/infdis/172.1.45.

[30] Castello, A. A., Argüelles, M. H., Villegas, G. A., Olthoff, A., & Glikmann, G. (2002). Incidence and prevalence of human group C rotavirus infections in Argentina. *Journal of Medical Virology*, 67(1):106–112. https://doi.org/10.1002/jmv.2198.

[31] Caul, E. O., Ashley, C. R., Darville, J. M., & Bridger, J. C. (1990). Group C rotavirus associated with fatal enteritis in a family outbreak. *Journal of Medical Virology*, 30(3):201–205. https://doi.org/10.1002/jmv.1890300311.

[32] Nagashima, S., Kobayashi, N., Ishino, M., Alam, M. M., Ahmed, M. U., Paul, S. K., Ganesh, B., Chawla-Sarkar, M., Krishnan, T., Naik, T. N., & Wang, Y. H. (2008). Whole genomic characterization of a human rotavirus strain B219 belonging to a novel group of the genus Rotavirus. *Journal of Medical Virology*, 80(11):2023–2033. https://doi.org/10.1002/jmv.21286.

[33] Yang, J. H., Kobayashi, N., Wang, Y. H., Zhou, X., Li, Y., Zhou, D. J., Hu, Z. H., Ishino, M., Alam, M. M., Naik, T. N., & Ahmed, M. U. (2004). Phylogenetic analysis of a human group B rotavirus WH-1 detected in China in 2002. *Journal of Medical Virology*, 74(4):662–667. https://doi.org/10.1002/jmv.20222.

[34] Greenberg, M. T., Siegel, J. M., & Leitch, C. J. (1983). The nature and importance of attachment relationships to parents and peers during adolescence. *Journal of Youth and Adolescence*, 12(5):373–386. https://doi.org/10.1007/BF02088721.

[35] Iturriza Gómara, M., Wong, C., Blome, S., Desselberger, U., & Gray, J. (2002). Molecular characterization of VP6 genes of human rotavirus isolates: correlation of genogroups with subgroups and evidence of independent segregation. *Journal of Virology*, 76(13):6596–6601. https://doi.org/10.1128/jvi.76.13.6596-6601.2002.

[36] Hoshino, Y., & Kapikian, A. Z. (1996). Classification of rotavirus VP4 and VP7 serotypes. *Archives of Virology*. Supplementum, 12:99–111. https://doi.org/10.1007/978-3-7091-6553-9_12.

[37] Rao, C. D., Gowda, K., & Reddy, B. S. (2000). Sequence analysis of VP4 and VP7 genes of nontypeable strains identifies a new pair of outer capsid proteins representing novel P and G genotypes in bovine rotaviruses. *Virology*, 276(1):104–113. https://doi.org/10.1006/viro.2000.0472.

[38] van Doorn, L. J., Kleter, B., Hoefnagel, E., Stainier, I., Poliszczak, A., Colau, B., & Quint, W. (2009). Detection and genotyping of human rotavirus VP4 and VP7 genes by reverse transcriptase PCR and reverse hybridization. *Journal of Clinical Microbiology*, 47(9):2704–2712. https://doi.org/10.1128/JCM.00378-09.

[39] Estes MK, Cohen J (1989). Rotavirus gene structure and function. *Microbiol Rev,* 53:410–49.

[40] Estes M. K. (1996). Advances in molecular biology: impact on rotavirus vaccine development. *The Journal of Infectious Diseases*, 174(1):S37–S46. https://doi.org/10.1093/infdis/174.supplement_1.s37.

[41] Gorziglia, M., Nishikawa, K., Hoshino, Y., & Taniguchi, K. (1990). Similarity of the outer capsid protein VP4 of the Gottfried strain of porcine rotavirus to that of asymptomatic human rotavirus strains. *Journal of Virology*, 64(1):414–418. https://doi.org/10.1128/JVI.64.1.414-418.1990.

[42] Zhao, L., Shi, X., Meng, D., Guo, J., Li, Y., Liang, L., Guo, X., Tao, R., Zhang, X., Gao, R., Gao, L., & Wang, J. (2021). Prevalence and genotype distribution of group A rotavirus circulating in Shanxi Province, China during 2015-2019. *BMC Infectious Diseases*, 21(1):94. https://doi.org/10.1186/s12879-021-05795-4.

[43] Larralde, G., & Gorziglia, M. (1992). Distribution of conserved and specific epitopes on the VP8 subunit of rotavirus VP4. *Journal of Virology*, 66(12):7438–7443. https://doi.org/10.1128/JVI.66.12.7438-7443.1992.

[44] Matthijnssens, J., & Van Ranst, M. (2012). Genotype constellation and evolution of group A rotaviruses infecting humans. *Current opinion in virology*, 2(4):426–433. https://doi.org/10.1016/j.coviro.2012.04.007.

[45] Kapikian A. Z. (2001). A rotavirus vaccine for prevention of severe diarrhoea of infants and young children: development, utilization and withdrawal. *Novartis Foundation Symposium*, 238:153–179. https://doi.org/10.1002/0470846534.ch10.

[46] Estes, M. K., Kang, G., Zeng, C. Q., Crawford, S. E., & Ciarlet, M. (2001). Pathogenesis of rotavirus gastroenteritis. *Novartis Foundation Symposium*, 238:82–100. https://doi.org/10.1002/0470846534.ch6.

[47] Desselberger U. (1996). Genome rearrangements of rotaviruses. *Archives of Virology*. Supplementum, 12:37–51. https://doi.org/10.1007/978-3-7091-6553-9_5.

[48] Lundgren, O., & Svensson, L. (2001). Pathogenesis of rotavirus diarrhea. *Microbes and Infection*, 3(13):1145–1156. https://doi.org/10.1016/s1286-4579(01)01475-7.

[49] Crawford, S. E., Ramani, S., Tate, J. E., Parashar, U. D., Svensson, L., Hagbom, M., Franco, M. A., Greenberg, H. B., O'Ryan, M., Kang, G., Desselberger, U., & Estes, M. K. (2017). Rotavirus infection. *Nature Reviews. Disease Primers*, 3:17083. https://doi.org/10.1038/nrdp.2017.83.

[50] Arnold, M., Patton, J. T., & McDonald, S. M. (2009). Culturing, storage, and quantification of rotaviruses. *Current Protocols in Microbiology*, Chapter 15:Unit–15C.3. https://doi.org/10.1002/9780471729259.mc15c03s15.

[51] Guerrero, C. A., Méndez, E., Zárate, S., Isa, P., López, S., & Arias, C. F. (2000). Integrin alpha(v)beta(3) mediates rotavirus cell entry. *Proceedings of the National Academy of Sciences of the United States of America*, 97(26):14644–14649. https://doi.org/10.1073/pnas.250299897.

[52] Rodríguez, J. M., Chichón, F. J., Martín-Forero, E., González-Camacho, F., Carrascosa, J. L., Castón, J. R., & Luque, D. (2014). New insights into rotavirus entry machinery: stabilization of rotavirus spike conformation is independent of trypsin cleavage. *PLoS pathogens*, 10(5):e1004157. https://doi.org/10.1371/journal.ppat.1004157.

[53] Trask, S. D., Wetzel, J. D., Dermody, T. S., & Patton, J. T. (2013). Mutations in the rotavirus spike protein VP4 reduce trypsin sensitivity but not viral spread. *Journal of General Virology*, 94(Pt 6):1296–1300. https://doi.org/10.1099/vir.0.050674-0.

[54] Liu, Y., Huang, P., Tan, M., Liu, Y., Biesiada, J., Meller, J., Castello, A. A., Jiang, B., & Jiang, X. (2012). Rotavirus VP8*: phylogeny, host range, and interaction with histo-blood group antigens. *Journal of Virology*, 86(18):9899–9910. https://doi.org/10.1128/JVI.00979-12.

[55] Hu, L., Crawford, S. E., Czako, R., Cortes-Penfield, N. W., Smith, D. F., Le Pendu, J., Estes, M. K., & Prasad, B. V. (2012). Cell attachment protein VP8* of a human rotavirus specifically interacts with A-type histo-blood group antigen. *Nature*, 485(7397):256–259. https://doi.org/10.1038/nature10996.

[56] Ramani, S., Cortes-Penfield, N. W., Hu, L., Crawford, S. E., Czako, R., Smith, D. F., Kang, G., Ramig, R. F., Le Pendu, J., Prasad, B. V., & Estes, M. K. (2013). The VP8* domain of neonatal rotavirus strain G10P[11] binds to type II precursor glycans. *Journal of Virology*, 87(13):7255–7264. https://doi.org/10.1128/JVI.03518-12.

[57] Martínez, M. A., López, S., Arias, C. F., & Isa, P. (2013). Gangliosides have a functional role during rotavirus cell entry. *Journal of Virology*, 87(2):1115–1122. https://doi.org/10.1128/JVI.01964-12.

[58] Coulson, B. S., Londrigan, S. L., & Lee, D. J. (1997). Rotavirus contains integrin ligand sequences and a disintegrin-like domain that are implicated in virus entry

into cells. *Proceedings of the National Academy of Sciences of the United States of America*, 94(10):5389–5394. https://doi.org/10.1073/pnas.94.10.5389.

[59] Graham, K. L., Halasz, P., Tan, Y., Hewish, M. J., Takada, Y., Mackow, E. R., Robinson, M. K., & Coulson, B. S. (2003). Integrin-using rotaviruses bind alpha2beta1 integrin alpha2 I domain via VP4 DGE sequence and recognize alphaXbeta2 and alphaVbeta3 by using VP7 during cell entry. *Journal of Virology*, 77(18):9969–9978. https://doi.org/10.1128/jvi.77.18.9969-9978.2003.

[60] Zárate, S., Romero, P., Espinosa, R., Arias, C. F., & López, S. (2004). VP7 mediates the interaction of rotaviruses with integrin alphavbeta3 through a novel integrin-binding site. *Journal of Virology*, 78(20):10839–10847. https://doi.org/10.1128/JVI.78.20.10839-10847.2004.

[61] Gutiérrez, M., Isa, P., Sánchez-San Martin, C., Pérez-Vargas, J., Espinosa, R., Arias, C. F., & López, S. (2010). Different rotavirus strains enter MA104 cells through different endocytic pathways: the role of clathrin-mediated endocytosis. *Journal of Virology*, 84(18):9161–9169. https://doi.org/10.1128/JVI.00731-10.

[62] Trejo-Cerro, O., Aguilar-Hernández, N., Silva-Ayala, D., López, S., & Arias, C. F. (2019). The actin cytoskeleton is important for rotavirus internalization and RNA genome replication. *Virus Research*, 263:27–33. https://doi.org/10.1016/j.virusres.2019.01.003.

[63] Elaid, S., Libersou, S., Ouldali, M., Morellet, N., Desbat, B., Alves, I. D., Lepault, J., & Bouaziz, S. (2014). A peptide derived from the rotavirus outer capsid protein VP7 permeabilizes artificial membranes. *Biochimica et Biophysica Acta*, 1838(8):2026–2035. https://doi.org/10.1016/j.bbamem.2014.04.005.

[64] Yamauchi, Y., & Helenius, A. (2013). Virus entry at a glance. *Journal of cell science*, 126(Pt 6):1289–1295. https://doi.org/10.1242/jcs.119685.

[65] Arias, C. F., Silva-Ayala, D., & López, S. (2015). Rotavirus entry: a deep journey into the cell with several exits. *Journal of Virology*, 89(2):890–893. https://doi.org/10.1128/JVI.01787-14.

[66] Greenberg, H. B., & Estes, M. K. (2009). Rotaviruses: from pathogenesis to vaccination. *Gastroenterology*, 136(6):1939–1951. https://doi.org/10.1053/j.gastro.2009.02.076.

[67] Periz, J., Celma, C., Jing, B., Pinkney, J. N., Roy, P., & Kapanidis, A. N. (2013). Rotavirus mRNAS are released by transcript-specific channels in the double-layered viral capsid. *Proceedings of the National Academy of Sciences of the*

United States of America, 110(29):12042–12047. https://doi.org/10.1073/pnas.1220345110.

[68] Silvestri, L. S., Taraporewala, Z. F., & Patton, J. T. (2004). Rotavirus replication: plus-sense templates for double-stranded RNA synthesis are made in viroplasms. *Journal of virology*, 78(14):7763–7774. https://doi.org/10.1128/JVI.78.14.7763-7774.2004.

[69] Patton, J. T., Silvestri, L. S., Tortorici, M. A., Vasquez-Del Carpio, R., & Taraporewala, Z. F. (2006). Rotavirus genome replication and morphogenesis: role of the viroplasm. *Current Topics in Microbiology and Immunology*, 309:169–187. https://doi.org/10.1007/3-540-30773-7_6.

[70] Vascotto, F., Campagna, M., Visintin, M., Cattaneo, A., & Burrone, O. R. (2004). Effects of intrabodies specific for rotavirus NSP5 during the virus replicative cycle. *The Journal of general virology*, 85(Pt 11):3285–3290. https://doi.org/10.1099/vir.0.80075-0.

[71] Campagna, M., Eichwald, C., Vascotto, F., & Burrone, O. R. (2005). RNA interference of rotavirus segment 11 mRNA reveals the essential role of NSP5 in the virus replicative cycle. *The Journal of general virology*, 86(Pt 5):1481–1487. https://doi.org/10.1099/vir.0.80598-0.

[72] Berkova, Z., Crawford, S. E., Trugnan, G., Yoshimori, T., Morris, A. P., & Estes, M. K. (2006). Rotavirus NSP4 induces a novel vesicular compartment regulated by calcium and associated with viroplasms. *Journal of Virology*, 80(12):6061–6071. https://doi.org/10.1128/JVI.02167-05.

[73] Cheung, W., Gill, M., Esposito, A., Kaminski, C. F., Courousse, N., Chwetzoff, S., Trugnan, G., Keshavan, N., Lever, A., & Desselberger, U. (2010). Rotaviruses associate with cellular lipid droplet components to replicate in viroplasms, and compounds disrupting or blocking lipid droplets inhibit viroplasm formation and viral replication. *Journal of Virology*, 84(13):6782–6798. https://doi.org/10.1128/JVI.01757-09.

[74] Crawford, S. E., Utama, B., Hyser, J. M., Broughman, J. R., & Estes, M. K. (2013). Rotavirus exploits lipid metabolism and energy production for replication. In American Society for Virology Annual Meeting, Pennsylvania State University, University Park, PA (p. 74).

[75] Hershey, J.W.B., and W.C. Merrick. (2000). The pathway and mechanism of initiation of protein synthesis. Translational Control of Gene Expression. N.

Sonenberg, J.W.B. Hershey, and M.B. Mathews, editors. Cold Spring Harbor Press, Cold Spring Harbor, NY. 33–88.

[76] Piron, M., Delaunay, T., Grosclaude, J., & Poncet, D. (1999). Identification of the RNA-binding, dimerization, and eIF4GI-binding domains of rotavirus nonstructural protein NSP3. *Journal of Virology*, 73(7):5411–5421. https://doi.org/10.1128/JVI.73.7.5411-5421.1999.

[77] Piron, M., Vende, P., Cohen, J., & Poncet, D. (1998). Rotavirus RNA-binding protein NSP3 interacts with eIF4GI and evicts the poly(A) binding protein from eIF4F. *The EMBO Journal*, 17(19):5811–5821. https://doi.org/10.1093/emboj/17.19.5811.

[78] Vende, P., Piron, M., Castagné, N., & Poncet, D. (2000). Efficient translation of rotavirus mRNA requires simultaneous interaction of NSP3 with the eukaryotic translation initiation factor eIF4G and the mRNA 3' end. *Journal of Virology*, 74(15):7064–7071. https://doi.org/10.1128/jvi.74.15.7064-7071.2000.

[79] Padilla-Noriega, L., Paniagua, O., & Guzmán-León, S. (2002). Rotavirus protein NSP3 shuts off host cell protein synthesis. *Virology*, 298(1):1–7. https://doi.org/10.1006/viro.2002.1477.

[80] Vitour, D., Lindenbaum, P., Vende, P., Becker, M. M., & Poncet, D. (2004). RoXaN, a novel cellular protein containing TPR, LD, and zinc finger motifs, forms a ternary complex with eukaryotic initiation factor 4G and rotavirus NSP3. *Journal of Virology*, 78(8):3851–3862. https://doi.org/10.1128/jvi.78.8.3851-3862.2004.

[81] Montero, H., Arias, C. F., & Lopez, S. (2006). Rotavirus Nonstructural Protein NSP3 is not required for viral protein synthesis. *Journal of Virology*, 80(18):9031–9038. https://doi.org/10.1128/JVI.00437-06.

[82] Montero, H., Rojas, M., Arias, C. F., & López, S. (2008). Rotavirus infection induces the phosphorylation of eIF2alpha but prevents the formation of stress granules. *Journal of Virology*, 82(3):1496–1504. https://doi.org/10.1128/JVI.01779-07.

[83] Papa, G., Venditti, L., Braga, L., Schneider, E., Giacca, M., Petris, G., & Burrone, O. R. (2020). CRISPR-Csy4-Mediated Editing of Rotavirus Double-Stranded RNA Genome. *Cell Reports*, 32(13):108205. https://doi.org/10.1016/j.celrep.2020.108205.

[84] McDonald, S. M., & Patton, J. T. (2011). Rotavirus VP2 core shell regions critical for viral polymerase activation. *Journal of Virology*, 85(7):3095–3105. https://doi.org/10.1128/JVI.02360-10.

[85] Gratia, M., Sarot, E., Vende, P., Charpilienne, A., Baron, C. H., Duarte, M., Pyronnet, S., & Poncet, D. (2015). Rotavirus NSP3 Is a Translational Surrogate of the Poly(A) Binding Protein-Poly(A) Complex. *Journal of Virology*, 89(17):8773–8782. https://doi.org/10.1128/JVI.01402-15.

[86] Patton, John. (2001). Rotavirus RNA Replication and Gene Expression. *Novartis Foundation Symposium*. 238:64-77; discussion 77. https://doi.org/10.1002/0470846534.ch5.

[87] Desselberger, U., & Gray, J. (2013). Viral gastroenteritis. *Medicine* (Abingdon, England : UK ed.), 41(12):700–704. https://doi.org/10.1016/j.mpmed.2013.09.009.

[88] Borodavka, A., Desselberger, U., & Patton, J. T. (2018). Genome packaging in multi-segmented dsRNA viruses: distinct mechanisms with similar outcomes. *Current Opinion in Virology*, *33*:106–112. https://doi.org/10.1016/j.coviro.2018.08.001.

[89] Affranchino, J. L., & González, S. A. (1997). Deletion mapping of functional domains in the rotavirus capsid protein VP6. *Journal of General Virology*, 78(Pt 8):1949–1955. https://doi.org/10.1099/0022-1317-78-8-1949.

[90] Charpilienne, A., Lepault, J., Rey, F., & Cohen, J. (2002). Identification of rotavirus VP6 residues located at the interface with VP2 that are essential for capsid assembly and transcriptase activity. *Journal of Virology*, 76(15):7822–7831. https://doi.org/10.1128/jvi.76.15.7822-7831.2002.

[91] Estes MK, Greenberg HB (2013). Rotaviruses. In: Knipe DM, Howley PM, et al, editors. *Fields Virology*. Philadelphia: Kluwer/Lippincott, Williams and Wilkins. p. 1348–401.

[92] Blutt, S. E., Matson, D. O., Crawford, S. E., Staat, M. A., Azimi, P., Bennett, B. L., Piedra, P. A., & Conner, M. E. (2007). Rotavirus antigenemia in children is associated with viremia. *PLoS Medicine*, 4(4):e121. https://doi.org/10.1371/journal.pmed.0040121.

[93] Tsukida, K., Goto, M., Yamaguchi, N., Imagawa, T., Tamura, D., & Yamagata, T. (2018). Rotavirus gastroenteritis-associated urinary tract calculus in an infant. *The Turkish Journal of Pediatrics*, 60(6):769–770. https://doi.org/10.24953/turkjped.2018.06.025.

[94] Gómez-Rial, J., Sánchez-Batán, S., Rivero-Calle, I., Pardo-Seco, J., Martinón-Martínez, J. M., Salas, A., & Martinón-Torres, F. (2018). Rotavirus infection beyond the gut. *Infection and Drug Resistance*, 12:55–64. https://doi.org/10.2147/IDR.S186404.

[95] Estes, M. K., Kang, G., Zeng, C. Q., Crawford, S. E., & Ciarlet, M. (2001). Pathogenesis of rotavirus gastroenteritis. *Novartis Foundation Symposium*, 238:82–100. https://doi.org/10.1002/0470846534.ch6.

[96] Lundgren, O., Peregrin, A. T., Persson, K., Kordasti, S., Uhnoo, I., & Svensson, L. (2000). Role of the enteric nervous system in the fluid and electrolyte secretion of rotavirus diarrhea. *Science (New York, N.Y.)*, 287(5452):491–495. https://doi.org/10.1126/science.287.5452.491.

[97] Boshuizen, J. A., Reimerink, J. H., Korteland-van Male, A. M., van Ham, V. J., Koopmans, M. P., Büller, H. A., Dekker, J., & Einerhand, A. W. (2003). Changes in small intestinal homeostasis, morphology, and gene expression during rotavirus infection of infant mice. *Journal of Virology*, 77(24):13005–13016. https://doi.org/10.1128/jvi.77.24.13005-13016.2003.

[98] Weisenberg E. Rotavirus. PathologyOutlines.com website. https://www.pathologyoutlines.com/topic/smallbowelrotavirus.html. Accessed on December 9th, 2020.

[99] Mukhopadhya, I., Sarkar, R., Menon, V. K., Babji, S., Paul, A., Rajendran, P., Sowmyanarayanan, T. V., Moses, P. D., Iturriza-Gomara, M., Gray, J. J., & Kang, G. (2013). Rotavirus shedding in symptomatic and asymptomatic children using reverse transcription-quantitative PCR. *Journal of Medical Virology*, 85(9):1661–1668. https://doi.org/10.1002/jmv.23641.

[100] Butz, A. M., Fosarelli, P., Dick, J., Cusack, T., & Yolken, R. (1993). Prevalence of rotavirus on high-risk fomites in day-care facilities. *Pediatrics*, 92(2):202–205. https://doi.org/10.1542/peds.92.2.202.

[101] Dennehy P. H. (2000). Transmission of rotavirus and other enteric pathogens in the home. *The Pediatric Infectious Disease Journal*, 19(10):S103–S105. https://doi.org/10.1097/00006454-200010001-00003.

[102] Kiulia, N. M., Netshikweta, R., Page, N. A., Van Zyl, W. B., Kiraithe, M. M., Nyachieo, A., Mwenda, J. M., & Taylor, M. B. (2010). The detection of enteric viruses in selected urban and rural river water and sewage in Kenya, with special

reference to rotaviruses. *Journal of Applied Microbiology*, 109(3):818–828. https://doi.org/10.1111/j.1365-2672.2010.04710.x.

[103] Van Zyl, W. B., Page, N. A., Grabow, W. O., Steele, A. D., & Taylor, M. B. (2006). Molecular epidemiology of group A rotaviruses in water sources and selected raw vegetables in southern Africa. *Applied and Environmental Microbiology*, 72(7):4554–4560. https://doi.org/10.1128/AEM.02119-05.

[104] Coffin, S. E., & Clark, S. L. (2001). Induction of intestinal rotavirus-specific antibodies in respiratory, but not gut, lymphoid tissues following mucosal immunization of mice with inactivated rotavirus. *Virology*, 291(2):235–240. https://doi.org/10.1006/viro.2001.1180.

[105] Kang, G., Iturriza-Gomara, M., Wheeler, J. G., Crystal, P., Monica, B., Ramani, S., Primrose, B., Moses, P. D., Gallimore, C. I., Brown, D. W., & Gray, J. (2004). Quantitation of group A rotavirus by real-time reverse-transcription-polymerase chain reaction: correlation with clinical severity in children in South India. *Journal of Medical Virology*, 73(1):118–122. https://doi.org/10.1002/jmv.20053.

[106] Naumova EN, MacNeill IB (2006). Seasonality assessment for biosurveillance systems. In: Balakrishnan N, Auget JL, Mesbah M, editors. Advances in statistical methods for the health sciences: applications to cancer and aids studies, genome sequence analysis, and survival analysis. 1st ed. Boston, MA: Birkhaeuser.

[107] Armah, G. E., Mingle, J. A., Dodoo, A. K., Anyanful, A., Antwi, R., Commey, J., & Nkrumah, F. K. (1994). Seasonality of rotavirus infection in Ghana. *Annals of Tropical Paediatrics*, 14(3):223–229. https://doi.org/10.1080/02724936.1994.11747721.

[108] Jagai, J. S., Sarkar, R., Castronovo, D., Kattula, D., McEntee, J., Ward, H., Kang, G., & Naumova, E. N. (2012). Seasonality of rotavirus in South Asia: a meta-analysis approach assessing associations with temperature, precipitation, and vegetation index. *PloS ONE*, 7(5):e38168. https://doi.org/10.1371/journal.pone.0038168.

[109] Levy, K., Hubbard, A. E., & Eisenberg, J. N. (2009). Seasonality of rotavirus disease in the tropics: a systematic review and meta-analysis. *International Journal of Epidemiology*, 38(6):1487–1496. https://doi.org/10.1093/ije/dyn260.

[110] Hasso-Agopsowicz, M., Ladva, C. N., Lopman, B., Sanderson, C., Cohen, A. L., Tate, J. E., Riveros, X., Henao-Restrepo, A. M., Clark, A., & Global Rotavirus Surveillance Network and Rotavirus Age Study Collaborators (2019). Global

Review of the Age Distribution of Rotavirus Disease in Children Aged <5 Years Before the Introduction of Rotavirus Vaccination. *Clinical Infectious Diseases: an official publication of the Infectious Diseases Society of America*, 69(6):1071–1078. https://doi.org/10.1093/cid/ciz060.

[111] Patel, M. M., Pitzer, V. E., Alonso, W. J., Vera, D., Lopman, B., Tate, J., Viboud, C., & Parashar, U. D. (2013). Global seasonality of rotavirus disease. *The Pediatric Infectious Disease Journal*, 32(4):e134–e147. https://doi.org/10.1097/INF.0b013e31827d3b68.

[112] Staat, M. A., Azimi, P. H., Berke, T., Roberts, N., Bernstein, D. I., Ward, R. L., Pickering, L. K., & Matson, D. O. (2002). Clinical presentations of rotavirus infection among hospitalized children. *The Pediatric Infectious Disease Journal*, 21(3):221–227. https://doi.org/10.1097/00006454-200203000-00012.

[113] Alkali, B. R., Daneji, A. I., Magaji, A. A., & Bilbis, L. S. (2015). Clinical Symptoms of Human Rotavirus Infection Observed in Children in Sokoto, Nigeria. *Advances in Virology*, 2015:890957. https://doi.org/10.1155/2015/890957.

[114] Gubbari, Keerthana. (2019). Electrolyte disturbance in rotaviral diarrhea and other acute diarrheal diseases in children under 5 years. *International Journal of Contemporary Pediatrics*. 6(5):2077-2080. http://dx.doi.org/10.18203/2349-3291.ijcp20193728.

[115] Zheng, B. J., Chang, R. X., Ma, G. Z., Xie, J. M., Liu, Q., Liang, X. R., & Ng, M. H. (1991). Rotavirus infection of the oropharynx and respiratory tract in young children. *Journal of Medical Virology*, 34(1):29–37. https://doi.org/10.1002/jmv.1890340106.

[116] Bruijning-Verhagen, P., Nipshagen, M. D., de Graaf, H., & Bonten, M. (2017). Rotavirus disease course among immunocompromised patients; 5-year observations from a tertiary care medical centre. *The Journal of Infection*, 75(5):448–454. https://doi.org/10.1016/j.jinf.2017.08.006.

[117] Alfajaro, M. M., & Cho, K. O. (2014). Evidences and consequences of extra-intestinal spread of rotaviruses in humans and animals. *Virusdisease*, 25(2):186–194. https://doi.org/10.1007/s13337-014-0197-9.

[118] Jalilvand, S., Marashi, S. M., Tafakhori, A., & Shoja, Z. (2015). Extraintestinal Involvement of Rotavirus Infection in Children. *Archives of Iranian Medicine*, 18(9):604–605.

[119] Rivero-Calle, I., Gómez-Rial, J., & Martinón-Torres, F. (2016). Systemic features of rotavirus infection. *The Journal of Infection*, 72 (Suppl):S98–S105. https://doi.org/10.1016/j.jinf.2016.04.029.

[120] Ruuska, T., & Vesikari, T. (1990). Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. *Scandinavian Journal of Infectious Diseases*, 22(3):259–267. https://doi.org/10.3109/00365549009027046.

[121] Flewett, T. H., Bryden, A. S., Davies, H., Woode, G. N., Bridger, J. C., & Derrick, J. M. (1974). Relation between viruses from acute gastroenteritis of children and newborn calves. *Lancet (London, England)*, 2(7872):61–63. https://doi.org/10.1016/s0140-6736(74)91631-6.

[122] Ward, R. L., Knowlton, D. R., & Pierce, M. J. (1984). Efficiency of human rotavirus propagation in cell culture. *Journal of Clinical Microbiology*, 19(6):748–753. https://doi.org/10.1128/jcm.19.6.748-753.1984.

[123] Herring, A. J., Inglis, N. F., Ojeh, C. K., Snodgrass, D. R., & Menzies, J. D. (1982). Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. *Journal of Clinical Microbiology*, 16(3):473–477. https://doi.org/10.1128/jcm.16.3.473-477.1982.

[124] Herrmann, J. E., Blacklow, N. R., Perron, D. M., Cukor, G., Krause, P. J., Hyams, J. S., Barrett, H. J., & Ogra, P. L. (1985). Enzyme immunoassay with monoclonal antibodies for the detection of rotavirus in stool specimens. *The Journal of Infectious Diseases*, 152(4):830–832. https://doi.org/10.1093/infdis/152.4.830.

[125] Cevenini, R., Rumpianesi, F., Mazzaracchio, R., Donati, M., Falcieri, E., & Lazzari, R. (1983). Evaluation of a new latex agglutination test for detecting human rotavirus in faeces. *The Journal of Infection*, 7(2):130–133. https://doi.org/10.1016/s0163-4453(83)90527-3.

[126] Khamrin, P., Tran, D. N., Chan-it, W., Thongprachum, A., Okitsu, S., Maneekarn, N., & Ushijima, H. (2011). Comparison of the rapid methods for screening of group a rotavirus in stool samples. *Journal of Tropical Pediatrics*, 57(5):375–377. https://doi.org/10.1093/tropej/fmq101.

[127] Wilde, J., Yolken, R., Willoughby, R., & Eiden, J. (1991). Improved detection of rotavirus shedding by polymerase chain reaction. *Lancet (London, England)*, 337(8737):323–326. https://doi.org/10.1016/0140-6736(91)90945-l.

[128] Gouvea, V., Glass, R. I., Woods, P., Taniguchi, K., Clark, H. F., Forrester, B., & Fang, Z. Y. (1990). Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. *Journal of Clinical Microbiology*, 28(2):276–282. https://doi.org/10.1128/jcm.28.2.276-282.1990.

[129] Gentsch, J. R., Glass, R. I., Woods, P., Gouvea, V., Gorziglia, M., Flores, J., Das, B. K., & Bhan, M. K. (1992). Identification of group A rotavirus gene 4 types by polymerase chain reaction. *Journal of Clinical Microbiology*, 30(6):1365–1373. https://doi.org/10.1128/jcm.30.6.1365-1373.1992.

[130] Gómara, M. I., Green, J., & Gray, J. (2000). Methods of rotavirus detection, sero- and genotyping, sequencing, and phylogenetic analysis. *Methods in molecular medicine*, 34:189–216. https://doi.org/10.1385/1-59259-078-0:189.

[131] Freeman, M. M., Kerin, T., Hull, J., McCaustland, K., & Gentsch, J. (2008). Enhancement of detection and quantification of rotavirus in stool using a modified real-time RT-PCR assay. *Journal of Medical Virology*, 80(8):1489–1496. https://doi.org/10.1002/jmv.21228.

[132] Jothikumar, N., Kang, G., & Hill, V. R. (2009). Broadly reactive TaqMan assay for real-time RT-PCR detection of rotavirus in clinical and environmental samples. JIN2@cdc.gov. *Journal of Virological Methods*, 155(2):126–131. https://doi.org/10.1016/j.jviromet.2008.09.025.

[133] Gautam, R., Esona, M. D., Mijatovic-Rustempasic, S., Ian Tam, K., Gentsch, J. R., & Bowen, M. D. (2014). Real-time RT-PCR assays to differentiate wild-type group A rotavirus strains from Rotarix(®) and RotaTeq(®) vaccine strains in stool samples. *Human Vaccines & Immunotherapeutics*, 10(3):767–777. https://doi.org/10.4161/hv.27388.

[134] Reddington, K., Tuite, N., Minogue, E., & Barry, T. (2014). A current overview of commercially available nucleic acid diagnostics approaches to detect and identify human gastroenteritis pathogens. *Biomolecular Detection and Quantification*, 1(1):3–7. https://doi.org/10.1016/j.bdq.2014.07.001.

[135] Gray, J., & Coupland, L. J. (2014). The increasing application of multiplex nucleic acid detection tests to the diagnosis of syndromic infections. *Epidemiology and Infection*, 142(1):1–11. https://doi.org/10.1017/S0950268813002367.

[136] Mlera, L., Jere, K. C., van Dijk, A. A., & O'Neill, H. G. (2011). Determination of the whole-genome consensus sequence of the prototype DS-1 rotavirus using sequence-independent genome amplification and 454® pyrosequencing. *Journal of*

Virological Methods, 175(2):266–271. https://doi.org/10.1016/j.jviromet.2011.05.004.

[137] Minami-Fukuda, F., Nagai, M., Takai, H., Murakami, T., Ozawa, T., Tsuchiaka, S., Okazaki, S., Katayama, Y., Oba, M., Nishiura, N., Sassa, Y., Omatsu, T., Furuya, T., Koyama, S., Shirai, J., Tsunemitsu, H., Fujii, Y., Katayama, K., & Mizutani, T. (2013). Detection of bovine group a rotavirus using rapid antigen detection kits, rt-PCR and next-generation DNA sequencing. *The Journal of Veterinary Medical Science*, 75(12):1651–1655. https://doi.org/10.1292/jvms.13-0265.

[138] Masuda, T., Nagai, M., Yamasato, H., Tsuchiaka, S., Okazaki, S., Katayama, Y., Oba, M., Nishiura, N., Sassa, Y., Omatsu, T., Furuya, T., Koyama, S., Shirai, J., Taniguchi, K., Fujii, Y., Todaka, R., Katayama, K., & Mizutani, T. (2014). Identification of novel bovine group A rotavirus G15P[14] strain from epizootic diarrhea of adult cows by de novo sequencing using a next-generation sequencer. *Veterinary Microbiology*, 171(1-2):66–73. https://doi.org/10.1016/j.vetmic.2014.03.009.

[139] Dennis, F. E., Fujii, Y., Haga, K., Damanka, S., Lartey, B., Agbemabiese, C. A., Ohta, N., Armah, G. E., & Katayama, K. (2014). Identification of novel Ghanaian G8P[6] human-bovine reassortant rotavirus strain by next generation sequencing. *PloS ONE*, 9(6):e100699. https://doi.org/10.1371/journal.pone.0100699.

[140] Libonati, M. H., Dennis, A. F., Ramani, S., McDonald, S. M., Akopov, A., Kirkness, E. F., Kang, G., & Patton, J. T. (2014). Absence of genetic differences among G10P[11] rotaviruses associated with asymptomatic and symptomatic neonatal infections in Vellore, India. *Journal of Virology*, 88(16):9060–9071. https://doi.org/10.1128/JVI.01417-14.

[141] Green, K. Y., Sears, J. F., Taniguchi, K., Midthun, K., Hoshino, Y., Gorziglia, M., Nishikawa, K., Urasawa, S., Kapikian, A. Z., & Chanock, R. M. (1988). Prediction of human rotavirus serotype by nucleotide sequence analysis of the VP7 protein gene. *Journal of Virology*, 62(5):1819–1823. https://doi.org/10.1128/JVI.62.5.1819-1823.1988.

[142] Fischer, T. K., & Gentsch, J. R. (2004). Rotavirus typing methods and algorithms. *Reviews in Medical Virology*, 14(2):71–82. https://doi.org/10.1002/rmv.411.

[143] Yamakawa, K., Oyamada, H., & Nakagomi, O. (1989). Identification of rotaviruses by dot-blot hybridization using an alkaline phosphatase-conjugated

synthetic oligonucleotide probe. *Molecular and Cellular Probes*, 3(4):397–401. https://doi.org/10.1016/0890-8508(89)90019-4.

[144] Flores, J., Green, K. Y., Garcia, D., Sears, J., Perez-Schael, I., Avendaño, L. F., Rodriguez, W. B., Taniguchi, K., Urasawa, S., & Kapikian, A. Z. (1989). Dot hybridization assay for distinction of rotavirus serotypes. *Journal of Clinical Microbiology*, 27(1):29–34. https://doi.org/10.1128/jcm.27.1.29-34.1989.

[145] Parashar, U. D., Nelson, E. A., & Kang, G. (2013). Diagnosis, management, and prevention of rotavirus gastroenteritis in children. *BMJ (Clinical research ed.)*, 347:f7204. https://doi.org/10.1136/bmj.f7204.

[146] Hojsak, I., Snovak, N., Abdović, S., Szajewska, H., Misak, Z., & Kolacek, S. (2010). Lactobacillus GG in the prevention of gastrointestinal and respiratory tract infections in children who attend day care centers: a randomized, double-blind, placebo-controlled trial. *Clinical Nutrition (Edinburgh, Scotland)*, 29(3):312–316. https://doi.org/10.1016/j.clnu.2009.09.008.

[147] Sullivan, A., & Nord, C. E. (2002). The place of probiotics in human intestinal infections. *International Journal of Antimicrobial Agents*, 20(5):313–319. https://doi.org/10.1016/s0924-8579(02)00199-1.

[148] La Frazia, S., Ciucci, A., Arnoldi, F., Coira, M., Gianferretti, P., Angelini, M., Belardo, G., Burrone, O. R., Rossignol, J. F., & Santoro, M. G. (2013). Thiazolides, a new class of antiviral agents effective against rotavirus infection, target viral morphogenesis, inhibiting viroplasm formation. *Journal of Virology*, 87(20):11096–11106. https://doi.org/10.1128/JVI.01213-13.

[149] Chen, S., Ding, S., Yin, Y., Xu, L., Li, P., Peppelenbosch, M. P., Pan, Q., & Wang, W. (2019). Suppression of pyrimidine biosynthesis by targeting DHODH enzyme robustly inhibits rotavirus replication. *Antiviral Research*, 167:35–44. https://doi.org/10.1016/j.antiviral.2019.04.005.

[150] Chen, S., Wang, Y., Li, P., Yin, Y., Bijvelds, M. J., de Jonge, H. R., Peppelenbosch, M. P., Kainov, D. E., & Pan, Q. (2020). Drug screening identifies gemcitabine inhibiting rotavirus through alteration of pyrimidine nucleotide synthesis pathway. *Antiviral Research*, 180:104823. https://doi.org/10.1016/j.antiviral.2020.104823.

[151] Huang, H., Liao, D., Zhou, G., Zhu, Z., Cui, Y., & Pu, R. (2020). Antiviral activities of resveratrol against rotavirus in vitro and in vivo. *Phytomedicine:*

International Journal of Phytotherapy and Phytopharmacology, 77:153230. https://doi.org/10.1016/j.phymed.2020.153230.

[152] Tohmé, M. J., Giménez, M. C., Peralta, A., Colombo, M. I., & Delgui, L. R. (2019). Ursolic acid: A novel antiviral compound inhibiting rotavirus infection in vitro. *International Journal of Antimicrobial Agents*, 54(5):601–609. https://doi.org/10.1016/j.ijantimicag.2019.07.015.

[153] Patel, M. M., Glass, R., Desai, R., Tate, J. E., & Parashar, U. D. (2012). Fulfilling the promise of rotavirus vaccines: how far have we come since licensure? *The Lancet. Infectious Diseases*, 12(7):561–570. https://doi.org/10.1016/S1473-3099(12)70029-4.

[154] Burnett, E., Parashar, U. D., & Tate, J. E. (2020). Real-world effectiveness of rotavirus vaccines, 2006-19: a literature review and meta-analysis. *The Lancet. Global health*, 8(9):e1195–e1202. https://doi.org/10.1016/S2214-109X(20)30262-X.

[155] Clark, A., van Zandvoort, K., Flasche, S., Sanderson, C., Bines, J., Tate, J., Parashar, U., & Jit, M. (2019). Efficacy of live oral rotavirus vaccines by duration of follow-up: a meta-regression of randomised controlled trials. *The Lancet. Infectious Diseases*, 19(7):717–727. https://doi.org/10.1016/S1473-3099(19)30126-4.

[156] Vesikari, T., Matson, D. O., Dennehy, P., Van Damme, P., Santosham, M., Rodriguez, Z., Dallas, M. J., Heyse, J. F., Goveia, M. G., Black, S. B., Shinefield, H. R., Christie, C. D., Ylitalo, S., Itzler, R. F., Coia, M. L., Onorato, M. T., Adeyi, B. A., Marshall, G. S., Gothefors, L., Campens, D., Rotavirus Efficacy and Safety Trial (REST) Study Team (2006). Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *The New England Journal of Medicine*, 354(1):23–33. https://doi.org/10.1056/NEJMoa052664.

[157] Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, Abate H, Breuer T, Clemens SC, Cheuvart B, Espinoza F, Gillard P, Innis BL, Cervantes Y, Linhares AC, López P, Macías-Parra M, Ortega-Barría E, Richardson V, Rivera-Medina DM, Rivera L, Salinas B, Pavía-Ruz N, Salmerón J, Rüttimann R, Tinoco JC, Rubio P, Nuñez E, Guerrero ML, Yarzábal JP, Damaso S, Tornieporth N, Sáez-Llorens X, Vergara RF, Vesikari T, Bouckenooghe A, Clemens R, De Vos B, O'Ryan M; Human Rotavirus Vaccine Study Group (2006). Safety and efficacy of an

attenuated vaccine against severe rotavirus gastroenteritis. *The New England Journal of Medicine*, 354(1):11–22. https://doi.org/10.1056/NEJMoa052434.

[158] Armah, G. E., Sow, S. O., Breiman, R. F., Dallas, M. J., Tapia, M. D., Feikin, D. R., Binka, F. N., Steele, A. D., Laserson, K. F., Ansah, N. A., Levine, M. M., Lewis, K., Coia, M. L., Attah-Poku, M., Ojwando, J., Rivers, S. B., Victor, J. C., Nyambane, G., Hodgson, A., Schödel, F., Neuzil, K. M. (2010). Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. *Lancet (London, England)*, 376(9741):606–614. https://doi.org/10.1016/S0140-6736(10)60889-6.

[159] Madhi, S. A., Cunliffe, N. A., Steele, D., Witte, D., Kirsten, M., Louw, C., Ngwira, B., Victor, J. C., Gillard, P. H., Cheuvart, B. B., Han, H. H., & Neuzil, K. M. (2010). Effect of human rotavirus vaccine on severe diarrhea in African infants. *The New England Journal of Medicine*, 362(4):289–298. https://doi.org/10.1056/NEJMoa0904797.

[160] Zaman, K., Dang, D. A., Victor, J. C., Shin, S., Yunus, M., Dallas, M. J., Podder, G., Vu, D. T., Le, T. P., Luby, S. P., Le, H. T., Coia, M. L., Lewis, K., Rivers, S. B., Sack, D. A., Schödel, F., Steele, A. D., Neuzil, K. M., & Ciarlet, M. (2010). Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. *Lancet (London, England)*, 376(9741):615–623. https://doi.org/10.1016/S0140-6736(10)60755-6.

[161] Leshem, E., Moritz, R. E., Curns, A. T., Zhou, F., Tate, J. E., Lopman, B. A., & Parashar, U. D. (2014). Rotavirus vaccines and health care utilization for diarrhea in the United States (2007-2011). *Pediatrics*, 134(1):15–23. https://doi.org/10.1542/peds.2013-3849.

[162] Angel, J., Steele, A. D., & Franco, M. A. (2014). Correlates of protection for rotavirus vaccines: Possible alternative trial endpoints, opportunities, and challenges. *Human Vaccines & Immunotherapeutics*, 10(12):3659–3671. https://doi.org/10.4161/hv.34361.

[163] Harris, V. C., Armah, G., Fuentes, S., Korpela, K. E., Parashar, U., Victor, J. C., Tate, J., de Weerth, C., Giaquinto, C., Wiersinga, W. J., Lewis, K. D., & de Vos, W. M. (2017). Significant Correlation Between the Infant Gut Microbiome and

Rotavirus Vaccine Response in Rural Ghana. *The Journal of Infectious Diseases*, 215(1):34–41. https://doi.org/10.1093/infdis/jiw518.

[164] Becker-Dreps, S., Vilchez, S., Bucardo, F., Twitchell, E., Choi, W. S., Hudgens, M. G., Perez, J., & Yuan, L. (2017). The Association Between Fecal Biomarkers of Environmental Enteropathy and Rotavirus Vaccine Response in Nicaraguan Infants. *The Pediatric Infectious Disease Journal*, 36(4):412–416. https://doi.org/10.1097/INF.0000000000001457.

[165] Emperador, D. M., Velasquez, D. E., Estivariz, C. F., Lopman, B., Jiang, B., Parashar, U., Anand, A., & Zaman, K. (2016). Interference of Monovalent, Bivalent, and Trivalent Oral Poliovirus Vaccines on Monovalent Rotavirus Vaccine Immunogenicity in Rural Bangladesh. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, 62(2):150–156. https://doi.org/10.1093/cid/civ807.

[166] Chilengi, R., Simuyandi, M., Beach, L., Mwila, K., Becker-Dreps, S., Emperador, D. M., Velasquez, D. E., Bosomprah, S., & Jiang, B. (2016). Association of Maternal Immunity with Rotavirus Vaccine Immunogenicity in Zambian Infants. *PloS ONE*, 11(3):e0150100. https://doi.org/10.1371/journal.pone.0150100.

[167] Ali, A., Kazi, A. M., Cortese, M. M., Fleming, J. A., Moon, S., Parashar, U. D., Jiang, B., McNeal, M. M., Steele, D., Bhutta, Z., & Zaidi, A. K. (2015). Correction: Impact of Withholding Breastfeeding at the Time of Vaccination on the Immunogenicity of Oral Rotavirus Vaccine-A Randomized Trial. *PloS ONE*, 10(12):e0145568. https://doi.org/10.1371/journal.pone.0127622.

[168] Kazi, A. M., Cortese, M. M., Yu, Y., Lopman, B., Morrow, A. L., Fleming, J. A., McNeal, M. M., Steele, A. D., Parashar, U. D., Zaidi, A., & Ali, A. (2017). Secretor and Salivary ABO Blood Group Antigen Status Predict Rotavirus Vaccine Take in Infants. *The Journal of Infectious Diseases*, 215(5):786–789. https://doi.org/10.1093/infdis/jix028.

[169] Armah, G., Lewis, K. D., Cortese, M. M., Parashar, U. D., Ansah, A., Gazley, L., Victor, J. C., McNeal, M. M., Binka, F., & Steele, A. D. (2016). A Randomized, Controlled Trial of the Impact of Alternative Dosing Schedules on the Immune Response to Human Rotavirus Vaccine in Rural Ghanaian Infants. *The Journal of Infectious Diseases*, 213(11):1678–1685. https://doi.org/10.1093/infdis/jiw023.

[170] Bhandari, N., Rongsen-Chandola, T., Bavdekar, A., John, J., Antony, K., Taneja, S., Goyal, N., Kawade, A., Kang, G., Rathore, S. S., Juvekar, S., Muliyil, J., Arya,

A., Shaikh, H., Abraham, V., Vrati, S., Proschan, M., Kohberger, R., Thiry, G., Glass, R., India Rotavirus Vaccine Group (2014). Efficacy of a monovalent human-bovine (116E) rotavirus vaccine in Indian infants: a randomised, double-blind, placebo-controlled trial. *Lancet (London, England)*, 383(9935):2136–2143. https://doi.org/10.1016/S0140-6736(13)62630-6.

[171] Reddy, S., Nair, N. P., Giri, S., Mohan, V. R., Tate, J. E., Parashar, U. D., Gupte, M. D., Arora, R., Kang, G., & Indian Intussusception Surveillance Network (2018). Safety monitoring of ROTAVAC vaccine and etiological investigation of intussusception in India: study protocol. *BMC Public Health*, 18(1):898. https://doi.org/10.1186/s12889-018-5809-7.

[172] Chen, M. Y., Kirkwood, C. D., Bines, J., Cowley, D., Pavlic, D., Lee, K. J., Orsini, F., Watts, E., Barnes, G., & Danchin, M. (2017). Rotavirus specific maternal antibodies and immune response to RV3-BB neonatal rotavirus vaccine in New Zealand. *Human Vaccines & Immunotherapeutics*, 13(5):1126–1135. https://doi.org/10.1080/21645515.2016.1274474.

[173] Boniface, K., Byars, S. G., Cowley, D., Kirkwood, C. D., & Bines, J. E. (2020). Human Neonatal Rotavirus Vaccine (RV3-BB) Produces Vaccine Take Irrespective of Histo-Blood Group Antigen Status. *The Journal of Infectious Diseases*, 221(7):1070–1078. https://doi.org/10.1093/infdis/jiz333.

[174] Li, J., Zhang, Y., Yang, Y., Liang, Z., Tian, Y., Liu, B., Gao, Z., Jia, L., Chen, L., & Wang, Q. (2019). Effectiveness of Lanzhou lamb rotavirus vaccine in preventing gastroenteritis among children younger than 5 years of age. *Vaccine*, 37(27):3611–3616. https://doi.org/10.1016/j.vaccine.2019.03.069.

[175] Dang, D. A., Nguyen, V. T., Vu, D. T., Nguyen, T. H., Nguyen, D. M., Yuhuan, W., Baoming, J., Nguyen, D. H., Le, T. L., & Rotavin-M1 Vaccine Trial Group (2012). A dose-escalation safety and immunogenicity study of a new live attenuated human rotavirus vaccine (Rotavin-M1) in Vietnamese children. *Vaccine*, 30(1):A114–A121. https://doi.org/10.1016/j.vaccine.2011.07.118.

[176] Isanaka, S., Guindo, O., Langendorf, C., Matar Seck, A., Plikaytis, B. D., Sayinzoga-Makombe, N., McNeal, M. M., Meyer, N., Adehossi, E., Djibo, A., Jochum, B., & Grais, R. F. (2017). Efficacy of a Low-Cost, Heat-Stable Oral Rotavirus Vaccine in Niger. *The New England Journal of Medicine*, 376(12):1121–1130. https://doi.org/10.1056/NEJMoa1609462.

[177] Coldiron, M. E., Guindo, O., Makarimi, R., Soumana, I., Matar Seck, A., Garba, S., Macher, E., Isanaka, S., & Grais, R. F. (2018). Safety of a heat-stable rotavirus vaccine among children in Niger: Data from a phase 3, randomized, double-blind, placebo-controlled trial. *Vaccine*, 36(25):3674–3680. https://doi.org/10.1016/j.vaccine.2018.05.023.

[178] Kawade, A., Babji, S., Kamath, V., Raut, A., Kumar, C. M., Kundu, R., Venkatramanan, P., Lalwani, S. K., Bavdekar, A., Juvekar, S., Dayma, G., Patil, R., Kulkarni, M., Hegde, A., Nayak, D., Garg, B. S., Gupta, S., Jategaonkar, S., Bedi, N., Maliye, C., … Kulkarni, P. S. (2019). Immunogenicity and lot-to-lot consistency of a ready to use liquid bovine-human reassortant pentavalent rotavirus vaccine (ROTASIIL - Liquid) in Indian infants. *Vaccine*, 37(19):2554–2560. https://doi.org/10.1016/j.vaccine.2019.03.067.

[179] Murphy, T. V., Gargiullo, P. M., Massoudi, M. S., Nelson, D. B., Jumaan, A. O., Okoro, C. A., Zanardi, L. R., Setia, S., Fair, E., LeBaron, C. W., Wharton, M., Livengood, J. R., & Rotavirus Intussusception Investigation Team (2001). Intussusception among infants given an oral rotavirus vaccine. *The New England Journal of Medicine*, 344(8):564–572. https://doi.org/10.1056/NEJM200102223440804.

[180] Tate, J. E., Burton, A. H., Boschi-Pinto, C., Parashar, U. D., & World Health Organization–Coordinated Global Rotavirus Surveillance Network (2016). Global, Regional, and National Estimates of Rotavirus Mortality in Children <5 Years of Age, 2000-2013. *Clinical Infectious Diseases: an official publication of the Infectious Diseases Society of America*, 62(2), S96–S105. https://doi.org/10.1093/cid/civ1013.

[181] Yen, C., Healy, K., Tate, J. E., Parashar, U. D., Bines, J., Neuzil, K., Santosham, M., & Steele, A. D. (2016). Rotavirus vaccination and intussusception - Science, surveillance, and safety: A review of evidence and recommendations for future research priorities in low- and middle-income countries. *Human Vaccines & Immunotherapeutics*, 12(10):2580–2589. https://doi.org/10.1080/21645515.2016.1197452.

[182] Kirkwood, C. D., Ma, L. F., Carey, M. E., & Steele, A. D. (2019). The rotavirus vaccine development pipeline. *Vaccine*, 37(50):7328–7335. https://doi.org/10.1016/j.vaccine.2017.03.076.

[183] Sartorio, M., Folgori, L., Zuccotti, G., & Mameli, C. (2020). Rotavirus vaccines in clinical development: Current pipeline and state-of-the-art. *Pediatric Allergy and Immunology: official publication of the European Society of Pediatric Allergy and Immunology*, 31(24):58–60. https://doi.org/10.1111/pai.13167.

[184] Nejmeddine, M., Trugnan, G., Sapin, C., Kohli, E., Svensson, L., Lopez, S., & Cohen, J. (2000). Rotavirus spike protein VP4 is present at the plasma membrane and is associated with microtubules in infected cells. *Journal of virology*, 74(7):3313–3320. https://doi.org/10.1128/jvi.74.7.3313-3320.2000.

[185] Samantha K. Murphy, Michelle M. Arnold. Rotavirus NSP1 localizes in the nucleus to disrupt PML nuclear bodies during infection. *bioRxiv*, 619932. https://doi.org/10.1101/619932.

[186] Dhillon, P., & Rao, C. D. (2018). Rotavirus Induces Formation of Remodeled Stress Granules and P Bodies and Their Sequestration in Viroplasms To Promote Progeny Virus Production. *Journal of virology*, 92(24):e01363-18. https://doi.org/10.1128/JVI.01363-18.

[187] López, T., Camacho, M., Zayas, M., Nájera, R., Sánchez, R., Arias, C. F., & López, S. (2005). Silencing the morphogenesis of rotavirus. *Journal of virology*, 79(1):184–192. https://doi.org/10.1128/JVI.79.1.184-192.2005.

[188] González, R. A., Espinosa, R., Romero, P., López, S., & Arias, C. F. (2000). Relative localization of viroplasmic and endoplasmic reticulum-resident rotavirus proteins in infected cells. *Archives of virology*, 145(9):1963–1973. https://doi.org/10.1007/s007050070069.

[189] Liu, Y., Huang, P., Tan, M., Liu, Y., Biesiada, J., Meller, J., Castello, A. A., Jiang, B., & Jiang, X. (2012). Rotavirus VP8*: phylogeny, host range, and interaction with histo-blood group antigens. *Journal of virology*, 86(18):9899–9910. https://doi.org/10.1128/JVI.00979-12.

[190] Huang, P., Xia, M., Tan, M., Zhong, W., Wei, C., Wang, L., Morrow, A., & Jiang, X. (2012). Spike protein VP8* of human rotavirus recognizes histo-blood group antigens in a type-specific manner. *Journal of virology*, 86(9):4833–4843. https://doi.org/10.1128/JVI.05507-11.

[191] Hussein, H. A., Walker, L. R., Abdel-Raouf, U. M., Desouky, S. A., Montasser, A. K., & Akula, S. M. (2015). Beyond RGD: virus interactions with integrins. *Archives of virology*, 160(11):2669–2681. https://doi.org/10.1007/s00705-015-2579-8.

[192] Arias, C. F., Silva-Ayala, D., & López, S. (2015). Rotavirus entry: a deep journey into the cell with several exits. Journal of virology, 89(2), 890–893. https://doi.org/10.1128/JVI.01787-14.

[193] Díaz-Salinas, M. A., Romero, P., Espinosa, R., Hoshino, Y., López, S., & Arias, C. F. (2013). The spike protein VP4 defines the endocytic pathway used by rotavirus to enter MA104 cells. Journal of virology, 87(3), 1658–1663. https://doi.org/10.1128/JVI.02086-12.

[194] Clark, H. F., Bernstein, D. I., Dennehy, P. H., Offit, P., Pichichero, M., Treanor, J., Ward, R. L., Krah, D. L., Shaw, A., Dallas, M. J., Laura, D., Eiden, J. J., Ivanoff, N., Kaplan, K. M., & Heaton, P. (2004). Safety, efficacy, and immunogenicity of a live, quadrivalent human-bovine reassortant rotavirus vaccine in healthy infants. The Journal of pediatrics, 144(2), 184–190. https://doi.org/10.1016/j.jpeds.2003.10.054.

[195] Crawford, S. E., Ramani, S., Tate, J. E., Parashar, U. D., Svensson, L., Hagbom, M., Franco, M. A., Greenberg, H. B., O'Ryan, M., Kang, G., Desselberger, U., & Estes, M. K. (2017). Rotavirus infection. *Nature reviews. Disease primers*, 3:17083. https://doi.org/10.1038/nrdp.2017.83.

2.12. Book chapter manuscript:

Yengkhom Damayanti Devi, Chongtham Shyamsunder Singh and Nima D. Namsa. Rotaviruses and method of molecular characterization. *Introduction for Introduction to General Virology* (Elsevier). CH0045. (Under publication)