

# CHAPTER TWO

## LITERATURE REVIEW

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### 2.1. Introduction

The Baltimore classification system categorises viruses into seven groups, Group I to VII. Group III consist of dsRNA viruses that are distinguished based on the differences in their viral structure, genome organisation, virus protein coding strategies and nucleotide sequence identities. The dsRNA genome of Group III viruses is segmented thereby enabling them to undergo the reassortment. This is considered to be one of the major evolutionary mechanisms responsible for the genetic diversity within Group III viruses. Group III consist of the *Reoviridae*, *Cystoviridae*, *Birnaviridae*, *Hypoviridae*, *Partitiviridae* and *Totiviridae* families of which *Reoviridae* is the largest family, Table 2.1. The *Reoviridae* family consists of a vast range of viruses that infect invertebrates, vertebrates and plants. Rotaviruses form their own genus in the family *Reoviridae*. Due to the economic impact of rotavirus infections in humans and animals, rotavirus is the most significant pathogen amongst reoviruses. As such, rotaviruses have been studied extensively (Desselberger, 2002, Mertens et al., 2000).

The approximately 70 nm rotavirus particles were first associated with severe diarrhoea in young children through direct visualisation of thin-section electron microscope (EM) of duodenal mucosa biopsy specimens (Bishop et al., 1973). This was followed by identification of similar 70 nm wheel-shaped particles surrounded with spikes in human faeces by Flewett et al. (1973), Bishop et al. (1974) and others (Hamilton et al., 1974, Kapikian et al., 1974). These particles were designated rotaviruses, '*rota*' for wheel in Latin (Flewett et al., 1973). Prior to these reports, 70 nm virus particles similar to those reported by Bishop et al. (1973) in humans were reported in animals in 1963, from intestinal tissues of mice infected with epizootic diarrhoea of infant disease (EDIM) (Adams and Kraft, 1963) and from rectal swabs obtained from vervet monkeys designated as simian agent 11 (SA11) (Malherbe and Harwin, 1963). A virus similar to SA11, the offal (O) agent, was isolated in 1967 from monkey kidney cell culture. The O agent was recovered from the mixed intestinal washings of cattle and sheep (Malherbe and Strickland-Cholmley, 1967). Mebus et al. (1969) isolated 70 nm rotavirus-like particles from faeces collected from symptomatic calves and demonstrated that these agents could cause disease when serially passaged in calves. Fernelius et al. (1972) noted that the morphology of Nebraska calf diarrhoea virus (NCDV) resemble reoviruses after successfully cultivating NCDV in primary

**Table 2.1.** Classification of the Group III (dsRNA) viruses.

Family	Genera	Genome segments	Virion diameter ( total genome size)	Examples of selected types of species	
<i>Cystoviridae</i>	Cystovirus	3	85 nm (approximately bp)	Pseudomonas phage Phi6	
<i>Birnaviridae</i>	Aquabirnavirus	2	70 nm (approximately 6, 000 bp)	Infectious pancreatic necrosis virus	
	Avibirnavirus	2	70 nm (approximately 6, 000 bp )	Infectious bursal disease virus	
	Blosnavirus	2	70 nm (approximately 6, 000 bp )	Blotched snakehead virus	
	Entomobirnavirus	2	70 nm (approximately 6, 500 bp )	Drosophila X virus	
<i>Hypoviridae</i>	Hypovirus	1	50 – 80nm (9, 000 – 13, 000 bp)	Cryphonectria hypovirus 1	
<i>Partitiviridae</i>	Partitivirus	2	30 – 35 nm (approximately 4,000 bp)	Atkinsonella hypoxylon virus	
	Alphacryptovirus	2	30 nm (approximately 4, 000 bp)	White Clover Cryptic Virus 1	
	Betacryptovirus	2	40 nm (approximately 4, 000 bp)	White clover cryptic virus 2	
	Cryspovirus	2	30 – 35 nm (approximately 4, 000 bp)	Cryptosporidium parvum virus 1	
<i>Reoviridae</i> <b>Spinareovirinae</b>	Aquareovirus	11	75 nm (30,500 bp)	Aquareovirus A (Chum Salmon reovirus CS)	
	Coltivirus	12	60 – 80 nm (29, 000 bp)	Colorado tick fever virus (strain Florio)	
	Cypovirus	10	65 nm (25, 000 bp)	Lymantria dispar cypovirus 1	
	Dinovernavirus	9	49 – 50 nm (Not known)	Aedes pseudoscutellaris reovirus	
	Fijivirus	10	65 – 70 nm (27, 000 – 30, 000 bp)	Fiji disease virus	
	Idnoreovirus	10 – 11	70 nm (27, 000 – 30, 000 bp)	Idnoreovirus 1: Diadromus pulchellus	
	Mycoreovirus	11 or 12	80 nm (approximately 23, 000 bp)	mycoreovirus 1: Cryphonectria parasitica mycoreovirus-1	
	Orthoreovirus	10	80 nm (approximately 23, 500 bp)	Mammalian orthoreovirus	
	Oryzavirus	10	70 nm (26, 000 bp)	Rice ragged stunt virus	
	Cardoreovirus	11	55 nm (Not known)	Eriocheir sinensis reovirus	
	<b>Sedoreovirinae</b>	Orbivirus	10	80 nm (19, 200 bp)	Bluetongue virus
		Mimoreovirus	11	90 nm – 95 nm (25, 400 bp)	Micromonas pusilla reovirus
		Phytoreovirus	12	70 nm (26, 000 bp)	Wound tumor virus
		Rotavirus	11	80 nm (approximately 18, 500 bp)	Rotavirus A
Seadornavirus		11	60 nm – 70nm (approximately 21, 000 bp)	Banna virus	
<i>Chrysoviridae</i>		Chrysovirus	4	35 – 40nm (12, 300bp)	Penicillium chrysogenum virus
<i>Endornaviridae</i>	Endornavirus	Linear dsRNA genome	Do not produce virions (14, 000 – 17, 600bp)	Vicia faba endornavirus	
<i>Picobirnaviridae</i>	Picobirnavirus	2	35 – 40 nm (1, 700 – 2, 500 bp)	Human picobirnavirus	
<i>Totiviridae</i>	Giardivirus	Single linear dsRNA	36nm ( 6, 277 bp)	Giardia lamblia virus	
	Leishmanivirus	Single linear dsRNA	33 nm (5, 300 bp)	Leishmania RNA virus 1-1	
	Totivirus	Single linear dsRNA	40 nm (4, 600 – 6, 700 bp)	Saccharomyces cerevisiae virus L-A	
	Victorivirus	Single linear dsRNA	40 nm (4, 600 – 6, 700 bp)	Helminthosporium victoriae virus-190S	

The Table was compiled by adapting information from Nibert and Schiff (2001), Viral zone (<http://www.expasy.org/viralzone>), and the Universal Database for the International Committee on Taxonomy of Viruses (<http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdb/>).

foetal bovine cell culture. Later, the bovine, O, murine and simian agents were found to share group antigens with other human rotaviruses (Kapikian et al., 1976, Flewett et al., 1974). Since then, rotaviruses have been documented as the major cause of severe diarrhoeal disease in young mammals. Furthermore, rotaviruses have also been associated with extra-intestinal infections in humans (Ramig, 2004).

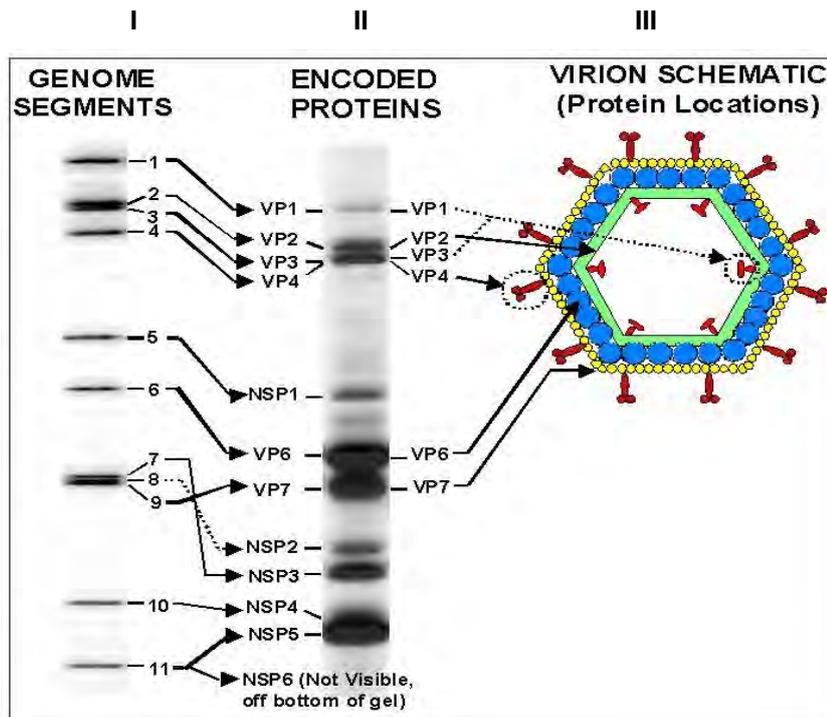
Rotaviruses are associated with both symptomatic and asymptomatic infections. Most symptomatic infections occur in young and elder mammals. The strains circulating in humans and animals differ significantly. Overall, there is a wide rotavirus strain diversity. Rotaviruses can be classified into groups, subgroups, serotypes, genotypes and electropherotypes (Reviewed by Estes and Kapikian, 2007). Several methods have been developed for the detection of rotavirus particles. These include enzyme-linked immunosorbent assays (ELISA) (Flewett et al., 1978), EM (Flewett, 1978), hybridization (Nakagomi et al., 1989a), reverse transcriptase polymerase chain reaction (RT-PCR) (Gouvea et al., 1990a) and many more. Since no pharmaceutical therapy against rotavirus infections are available, clinicians administer supportive therapy in the form of oral rehydration salts (ORS) to patients presenting with acute rotavirus-associated diarrhoea (Glass et al., 2005b). This emphasises the need to fast-track development of intervention measures. Extensive research in the past three decades has focused on vaccine development against rotaviruses, understanding the pathophysiology of rotavirus particles, the pathogenesis of the rotavirus-associated diseases, and the development of therapeutic intervention measures to control rotaviruses infections.

Massive vaccination programmes have led to the eradication of some viral diseases like smallpox and rinderpest (World Health Organization Factsheet, 2006). Efforts to eradicate all cases of poliomyelitis appear to be close to fruition as evidenced by the drop in numbers of diagnosed cases from millions to around a thousand worldwide due to implementing of routine immunisation programmes in most countries (Global Polio Eradication Initiative, 2011). This has encouraged development of vaccines against a number of other pathogens including rotaviruses.

## **2.2. The virus particle, functions of rotavirus proteins and the replication cycle**

### **2.2.1. Architecture of the rotavirus virion**

Although earlier reports indicated that rotavirus particles are 70 nm in diameter (see section 2.1), atomic resolution of complete infections rotavirus particle using X-ray crystallography, single-particle reconstructions of cryo-electron microscopy (cryoEM) or micrograph images taken with



**Fig. 2.1. Diagrammatic representation of the rotavirus particle and its genome coding assignments. (I)** The migration pattern of 11 dsRNA genome segments of rotavirus on a polyacrylamide gel. **(II)** Virus proteins encoded by specific genome segments in section 2.1.I. The proteins were blotted onto a cellulose membrane and detected with rotavirus-specific antibodies. **(III)** Schematic diagram of rotavirus particle showing the cross-section arrangement of viral proteins through the three capsid layers namely: outer (VP4, red; VP7, yellow), inner (VP6, blue) and the inner core (VP2, green). Figure provided by Robert F. Ramig, Ph.D. and used with his permission.

TEM in recent years estimated the diameter of approximately 80 nm for mature particles (Settembre et al., 2011, McClain et al., 2010, Palomares and Ramírez, 2009, Crawford et al., 1994). The mature rotavirus particle contains three concentric capsid layers that surround 11 dsRNA genome segments of approximately 18,500 bp. Each segment encodes a single viral protein except segment 11 which encodes two proteins, Fig 2.1. There are six structural (VP) and six non-structural protein (NSP). The functions of each protein are summarised in Table 2.2.

The three capsid layers are formed by the structural rotavirus proteins. Immediately surrounding the genome is the VP2 layer, designated inner core, which has a triangulation number ( $T$  number) of 1. This layer is composed of asymmetrical units of 60 VP2 dimers (McClain et al., 2010). The monomers in each VP2 dimer form VP2-A and VP2-B conformers that fold into thin comma-shaped plates, Fig. 2.2.IA. VP2-A converges tightly around the five-fold vertices, whereas VP2-B is positioned further back and intercalates between adjacent VP2-A molecules (McClain et al., 2010). VP2 interacts with the viral polymerase complex, which is composed of a single copy of the viral RNA-dependent RNA polymerase (RdRp), VP1, and viral RNA-capping enzyme,

**Table 2.2.** Coding assignment of the genome segments, functions and distinctive properties of rotavirus proteins.

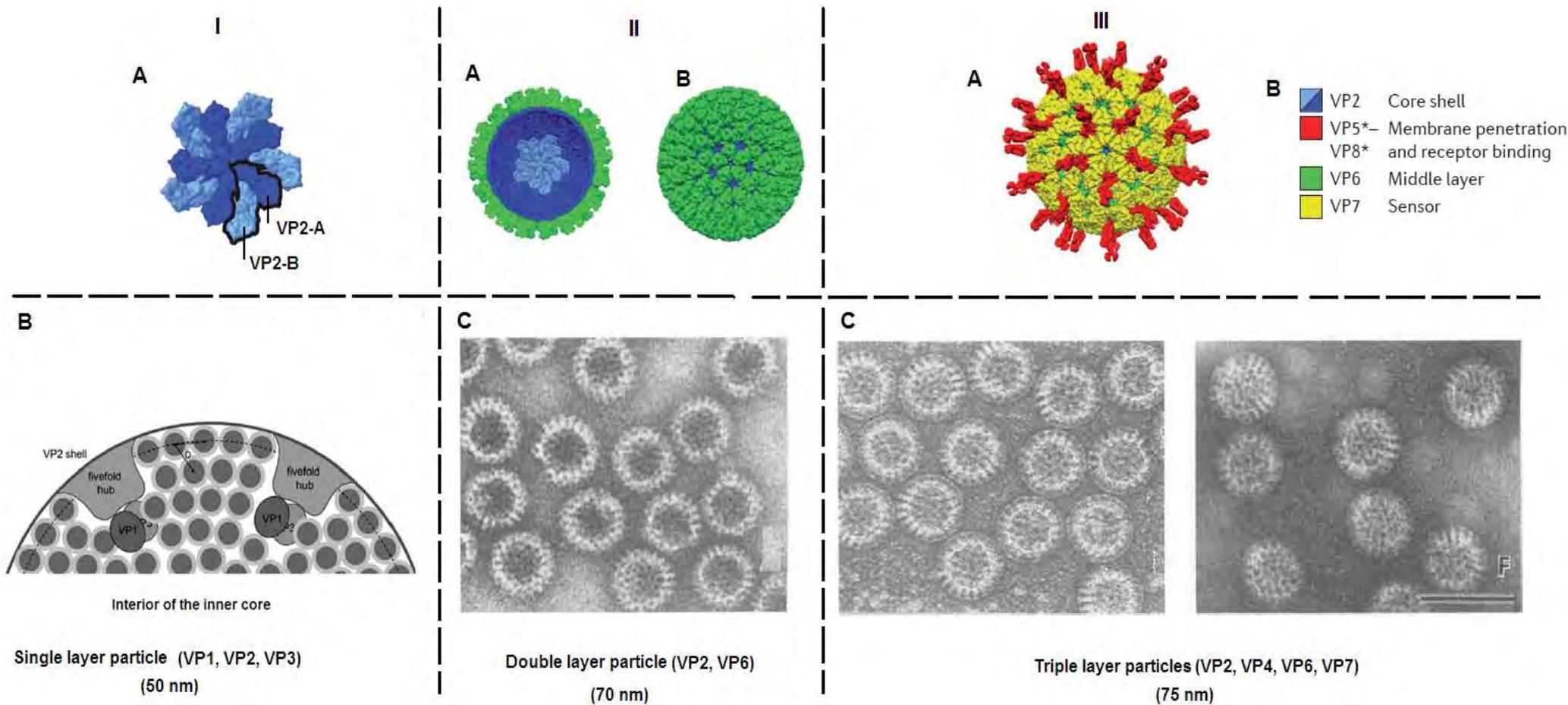
Encoding genome segment	Protein	Molecular weight (kDa) <sup>a</sup>	Number of molecules <sup>b</sup>	Location	Description of the gene product	Function
1	VP1	125	12	Core vertices	RdRp	RdRp, replicase and transferase activities. <sup>c, d</sup>
2	VP2	102	120	Inner shell	Core shell protein	Binds non-specifically to mRNA and serves as a platform for VP1 catalysed minus-strand synthesis. <sup>e</sup>
3	VP3	97	12	Core vertices	Guanylyl-transferase	Guanylyl-transferase and guanyl-transferase, initiates complex for minus-strand synthesis of viral dsRNA. <sup>f</sup>
4	VP4	88	180	Surface spike	Protease-sensitive	Cell attachment, virulence, neutralising antigen. <sup>e</sup>
5	NSP1	59	-	Cytoskeleton	Interferon antagonist	Interferes with the cellular interferon. <sup>g</sup>
6	VP6	45	780	Inner capsid	Intermediate capsid shell	Structural and group-specific antigen, adapter for outer capsid proteins, stabilises the inner core. <sup>e, f</sup>
7	NSP3	37	-	Viroplasm, ER	Translation enhancer	Enhances viral mRNA activity and shuts-off cellular protein synthesis. <sup>h</sup>
8	NSP2	35	-	Viroplasm	NTPase	NTPase, non-specific ssRNA-binding and helix destabilisation activities, RNA encapsidation and RNA packaging. <sup>i</sup>
9	VP7	34 and 38	780	Surface	Glycosylated	Structural and neutralising antigen. <sup>j</sup>
10	NSP4	20	-	Viroplasm, ER	Enterotoxin	Key regulator of viral morphogenesis, outer capsid assembly <sup>k, l</sup> and virulence. <sup>m, n</sup>
11	NSP5 & NSP6	22	-	Viroplasm, ER	Phosphoprotein	Together with NSP2 and NSP3, transports other viral proteins into viroplasm from their various sites of synthesis and nascent virus particles to the ER membrane from the viroplasm, ssRNA and dsRNA binding modulator of NSP2 <sup>p</sup> , autokinase activity of the <i>O</i> -glycosylated phosphoprotein. <sup>q</sup>

<sup>a</sup> The size of the encoded proteins were based on the simian rotavirus strain RVA/Vervet monkey-tc/ZAF/SA11-4F/1958/G3P6[1].

<sup>b</sup> Molecules were calculated per virion from structural studies of purified virions and confirmed by X-ray crystallography studies (McClain et al., 2010, Prasad et al., 1994, Prasad et al., 1990).

The table was compiled from Shaw et al. (1996)<sup>c</sup>, Settembre et al. (2011)<sup>d</sup>, Prasad et al. (1988)<sup>e</sup>, McClain et al. (2010)<sup>f</sup>, Trask et al. (2012)<sup>g</sup>, Montero et al. (Montero et al., 2006)<sup>h</sup>, Taraporewala and Patton, (2001)<sup>i</sup>, Dormitzer et al. (2004)<sup>j</sup>, Au and Siu, (1992)<sup>k</sup>, Trask and Domitzer, (2006)<sup>l</sup>, Barro and Patton, (2005)<sup>m</sup>, Offit et al. (1986)<sup>n</sup>, Estes, (2001)<sup>p</sup>, Afrikanova et al. (1998)<sup>q</sup> reports.

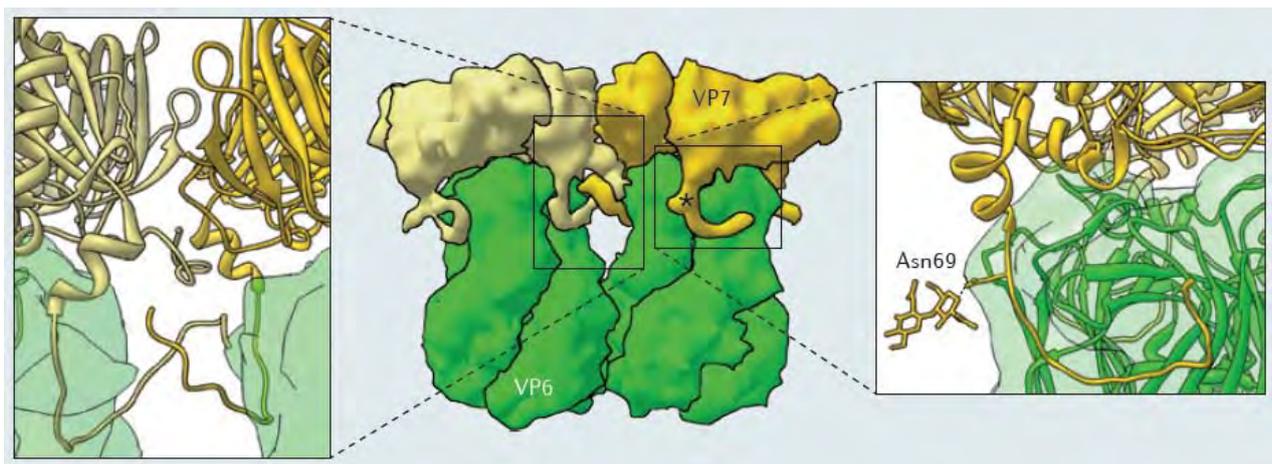
ER, Endoplasmic reticulum; kDa, kiloDalton, RdRp, RNA-dependent RNA polymerase; NTPase, nucleoside triphosphatase.



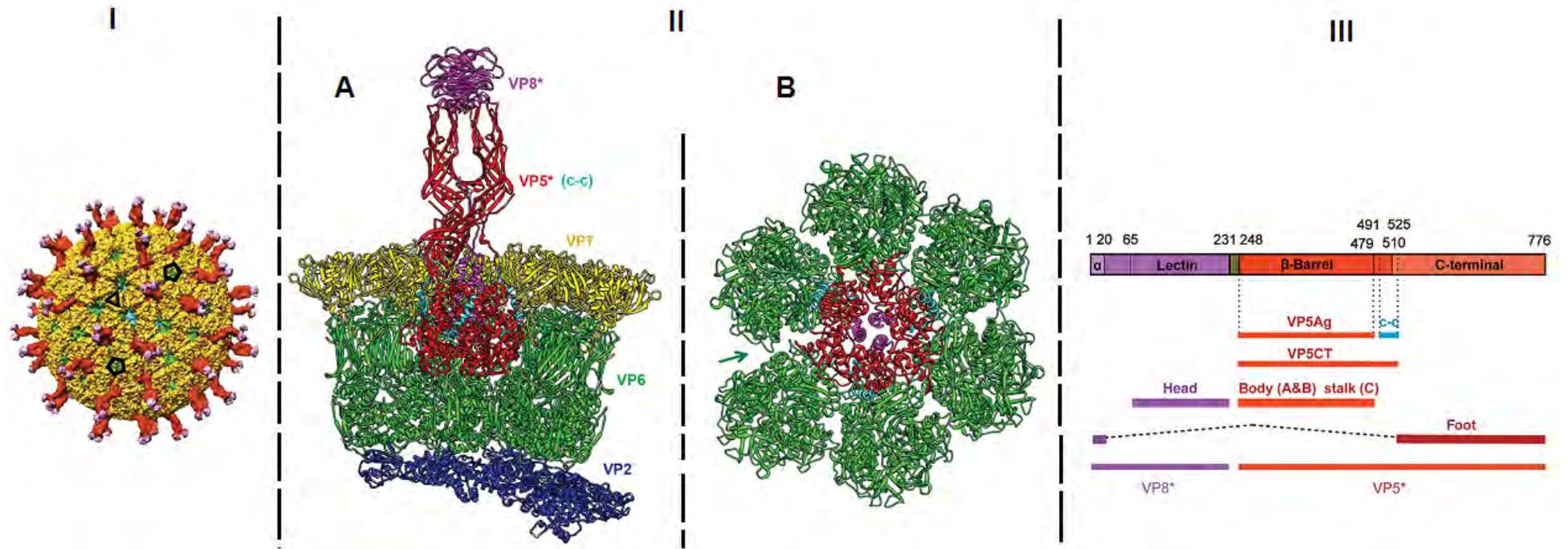
**Fig. 2.2. Structures of rotavirus particles highlighting the arrangement and morphology of the inner, intermediate and outer capsids.** (I) **A.** Outline of a decamer formed by the inner capsid protein illustrating the arrangement of VP2-A and VP2-B subunits of the five-fold-symmetrical  $T = 1$  asymmetrical unit, adapted from Trask et al. (2012) with permission from the publisher. **B.** Schematic cross section of the X-ray crystal structure of the interior of the inner core illustrating dsRNA packing. The dsRNA is coiled around each fivefold axis with approximate hexagonal close packing in cross section. The principal density of the RNA helices is about 20 Å in diameter; the lighter region around each cross section shows the hydration layer, from McClain et al. (2010), with permission from the publisher. (II) **A** and **B.** A continuous shell of VP6 around a cut-away X-ray crystal structure of the subviral particle, from Trask et al. (2012), with permission from the publisher. **C.** Electron micrograph (EM) of double-layer RV-VLPs (dRV-VLPs), from Crawford et al. (1994), with permission from the publisher. (III) **A.** Infectious rotavirus virion coated with the trimeric protein VP7 and decorated with the VP4. **B.** Colours designated for VP2, VP4, VP6 and VP7. **C.** EM micrographs of triple-layer RV-VLPs (tRV-VLP; left) and wild-type triple-layered particles (TLP; right), from Crawford et al. (1994), with permission from the publisher.

VP3, through its flexible interior regions that projects towards the five-fold vertex, Fig. 2.2.IB. It is hypothesised that the five-fold vertices have a single polymerase complex, which is anchored in place via simultaneous interactions with the sub-domains of multiple VP2-A and VP2-B conformers and their N-terminal tethers (McDonald and Patton, 2011b).

VP2 is surrounded by an intermediate layer composed of 260 trimers of VP6, Fig 2.2.IIA. Rotavirus particles composed of the inner core and intermediate capsids are designated as ‘double-layered particles’ (DLPs). Binding of VP6 to VP2 stabilises the very fragile inner shell. VP6 extensively interacts with both inner and outer capsid subunits through its two domains, a distal jelly-roll  $\beta$ -barrel and a proximal  $\alpha$ -helical pedestal (McClain et al., 2010, Mathieu et al., 2001). VP6 also serves as an adaptor for the rotavirus outer capsid proteins (Trask et al., 2012). The 260 trimers of the calcium binding-glycoprotein VP7, lie directly on top of the VP6 trimers. Contact with VP6 is facilitated by the arm-like extensions formed by the VP7 N-termini that also forms lattice with other VP7 trimers, Fig 2.3 (Settembre et al., 2011). This interaction allows gripping of VP7 to the intermediate VP6 layer and reinforces the integrity of the outer-shell. VP7 constitute a thin outer capsid layer with a  $T=13$  (laevo) icosahedral lattice (Dormitzer et al., 1992). Exactly 60 trimeric protease-sensitive VP4 protein spikes, which are 120 Å in length, protrude through the five-fold apices of the VP7 outer-shell, Fig 2.4.I. VP4 stalk from the peripentonal channels of the VP6 layer, Fig. 2.4.II. The rotavirus particle becomes infectious when the VP4 is

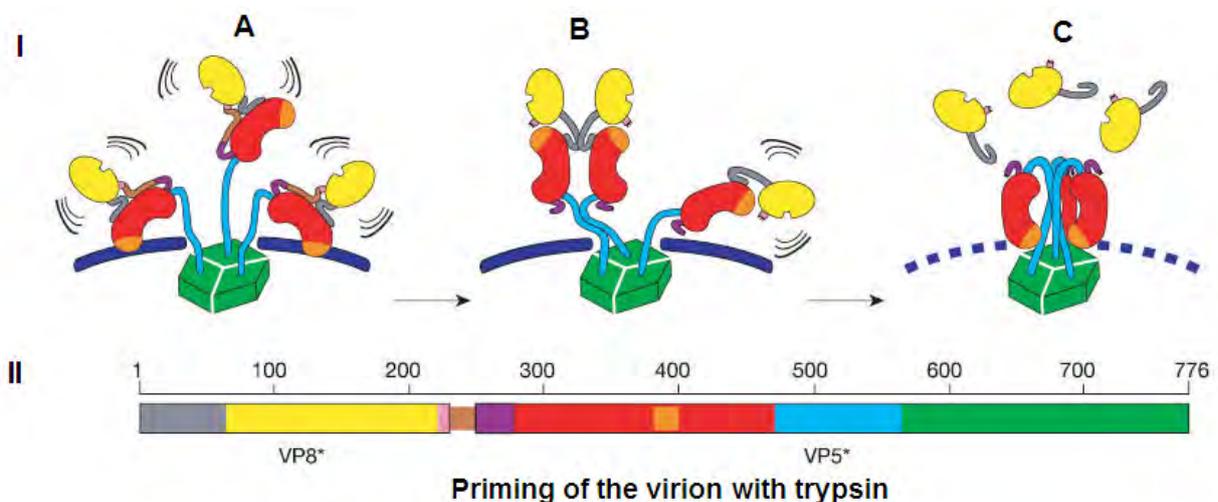


**Fig. 2.3. The grip arm model of VP7 assembly.** The amino terminus of VP7 bolts onto a small projection formed by a loop in the VP6  $\beta$ -jelly roll domain. The amino terminus of mature VP7 begins at residue Gln51 (residues 1–50 are the cleaved signal peptide). Residues 58 – 62 interact with VP6 to effectively extend the  $\beta$ -sheet of VP6 by an additional strand, whereas residues 63–78 curve under the VP6 protrusion and lead up into the main VP7 structure (Chen et al., 2009). A glycosylation site (Asn69) in VP7 that is conserved among many group A rotaviruses is actually in the grip arm and below the virion surface, in asterisk (\*). From (Trask et al., 2012), with permission the publisher.



**Fig. 2.4. CryoEM map and coordinates of VP4 spike protein in the TLP.** VP8\* is in magenta, VP5\* is in red, and VP7 is in yellow. **(I)** Pentagons and triangles represent the two five-fold axes and a three-fold axis, respectively, of selected icosahedral symmetry elements. VP4 spikes occupy six-coordinated positions intervening between the five-folds formed by VP7 trimers. **(II)** Interactions of VP4 with VP6 and VP7 in the TLP. **A.** Cutaway view of the VP4 spike. The VP5\* segment, in cyan, forms the coiled coil in the 'post-entry' conformation. In addition to the  $\alpha$ -helix that runs axially at the periphery of the foot, this segment includes about 15 more C-terminal residues at the interface between globular foot domains of the VP5\* subunits. The foot is anchored at a six-coordinated position in the VP6 layer (green); a VP7 trimer (yellow) caps each trimer of VP6 as shown in Fig. 2.3. **B.** The VP5\* foot and surrounding VP6 trimers, seen from outside the TLP as if the spike model in (A) were tipped towards the viewer. The arrow shows the noticeable six-coordination deviation from local six-fold symmetry. **(III)** Organization of the VP4 polypeptide chain (Dormitzer et al, 2001, 2004). Trypsin cleavage excise residues 232 – 247, resulting in VP8\* and VP5\* subunits, are indicated in green. The segments and domains of VP8\* and VP5\* ( $\alpha$ , lectin,  $\beta$ -barrel, c-c, C-terminal) are shown. The  $\beta$ -barrel domain represents the antigen domain, VP5Ag. VP5CT represents the fragment generated from recombinant VP4 by successive cleavages with chymotrypsin. From Trask et al. (2012), Settembre et al. (2011) and Dormitzer et al. (2004); with permission from the publishers.

post-translationally cleaved at arginine 241 or arginine 247 into VP8\* (28 kDa) and VP5\* (60 kDa) subunits with trypsin-like proteases of the host gastrointestinal tract (Dormitzer et al., 2004), Fig.2.4.III. Proteolysis rigidifies the cleaved VP8\* and VP5\* products that remain non-covalently associated with each other on the mature virion surface (Yeager et al., 1994). The proximal trimeric VP5\* portion of the spike is sandwiched between the VP6 and VP7 layers. Two VP8\* molecules cap the distal ends of the two upright VP5\* subunits and project their extended N-termini down into the base of the spike, whereas the third VP8\* subunit is presumed to dissociate from the virion (Settembre et al., 2011). The VP5\* subunit enables the virion to permeabilise lipid vesicles during the infection process, whereas the galectin-like fold VP8\* subunit is assumed to mediate host cell attachment for many rotavirus strains by attaching to either sialic acid (SA) moieties located on glycolipids (Ciarlet and Estes, 1999), galactose (Jolly et al., 2001) or co-receptors like heat-shock cognate 70 proteins (hsc70) (Guerrero et al., 2002). All rotaviruses require terminal sialic acid (SA) for attachment as attachment receptors, however, SA is not located in the outermost position of the receptor molecule in some cases (Haselhorst et al., 2009). This may suggest that alternative cell surface molecules may also serve as functional receptors for



**Fig. 2.5. Model for VP4 rearrangements during trypsin priming.** The colours in the cartoon (IA – C) match those in the linear diagram (II). The VP7 shell is blue. (I) **A. Uncleaved state:** The wavy lines indicate flexibility. **B. Trypsin-primed state:** The activation region (brown in I) between VP8\* and VP5\* has been removed by digestion. Two clustered molecules form the visible spike. The head is the yellow oval (the VP8\* core), notched at the sialoside-binding cleft. The body includes the red ‘kidney’ (the VP5\* antigen domain), the orange cap (the potential membrane interaction loop), the purple appendage, and part of the grey and cyan tubes. The stalk is the lower part of the cyan tubes. The foot is the green hexagonal prism. **C. Folded-back state:** This state corresponds to the VP5CT crystal structure and may form during membrane penetration. The dashed blue line indicates potential loss of VP7. (II) Linear diagram of structural elements: The VP8\* and VP5\*. Grey, residues M1 – V64; yellow, L65 – L224; pink, P225 – R231; brown, N232 – R247; purple, A248 – Q266; red, Y267 – T381 and A401 – L470; orange, G382 – G400; cyan, I471–unknown; green, unknown–L776. From Dormitzer et al. (2004), with permission from the publisher.

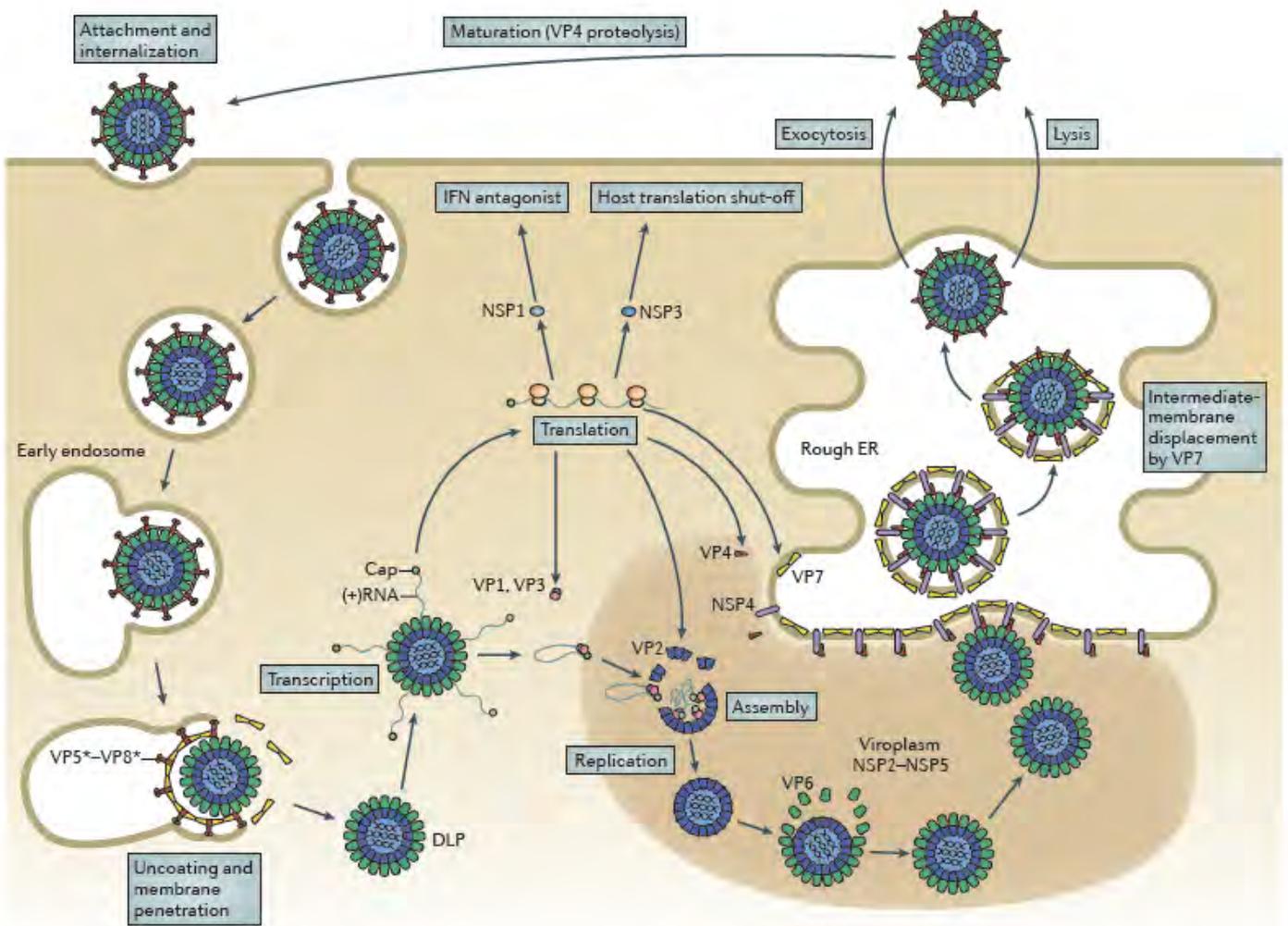
rotaviruses attachment . VP4 is also believed to be fusogenic (Estes and Cohen, 1989). Based on electron microscopy studies, Dormitzer et al. (2004) proposed that VP4 adopts three transformations that are essential for penetration of rotavirus particle into the host cell, Fig. 2.5.

The mature infectious rotavirus particles constituting all three concentric protein layers are designated 'triple layered particles' (TLPs), Fig. 2.2.III. While the structural rotavirus proteins basically form the architecture of the virion, the six non-structural proteins are mostly associated with the viral replication processes (Trask et al., 2012) and virulence as discussed in the following section.

### **2.2.2. The replication cycle**

The current understanding of the rotavirus replication cycle is depicted in Fig. 2.6. Studies in polarized intestinal epithelial cells (Caco-2) coupled with assays involving *in vitro* systems focusing on protein expression and RNA-protein interaction established a platform to understand specific steps of rotaviruses replication (Estes and Cohen, 1989). The invention of the confocal microscope, the discovery of the small interfering RNA (siRNA) (Dector et al., 2002) and inception of expression of recombinant rotavirus proteins in various systems (Reviewed in Palomares and Ramírez, 2009) enabled understanding of the roles of rotavirus proteins in the replication process. However, most of the known information on rotavirus replication was derived from studies in rhesus monkey epithelial and kidney cells lines (MA104 and BS-C1) infected with rotaviruses. *In vitro*, rotavirus replication is rapid in continuous MA104 cell lines. The maximum yield of rotaviruses is achieved with a MOI of 10 – 20 plaque forming units (PFU)/cell after 10 – 12 h at 37 °C or 18 h at 33 °C. Contrary, replication is fairly slow in human intestinal cell lines with viruses only detected between 20 – 24 hours post infection (hpi) (McCrae and McCorquodale, 1987). This suggests that rotavirus adsorption, entry and uncoating vary in different cell lines and between various rotavirus strains. This has contributed to the incomplete understanding of the whole replication process. Most of the replication steps discussed below is based on findings that were derived from studies in MA104 cells.

Mature enterocytes consisting of SA receptors, located at the tip of the villous in the gut, were initially thought to be the natural cell tropism for rotaviruses attachment (Bastardo and Holmes, 1980). However, evidence of extra-intestinal spread of rotavirus suggests that other host cells might also be susceptible to rotavirus infection (Blutt et al., 2003, Cioc and Nuovo, 2002). The outer capsid proteins (VP4 and VP7) are involved in the initial interaction between rotavirus and the host cell. Newly assembled rotavirus virions are not fully infectious. Attachment to the



**Fig. 2.6. The rotavirus replication cycle.** The rotavirus particle attaches to the target cell membrane through its VP8\*. The virion is delivered to the endosome through endocytosis where reduced calcium concentrations trigger loss of the VP7 layer, the uncoating stage. VP5\* mediates membrane penetration thereby releasing the DLP into the cytoplasm. The internal polymerase complex (VP1 and VP3) is activated by uncoating of the outer capsid leading to transcription of capped (+) RNAs from each of the 11 dsRNA genome segments. (+) RNAs serve either as mRNAs for synthesis of viral proteins by cellular ribosomes or as templates for synthesis of (-) RNA during genome replication. Non-structural protein 2 (NSP2) and NSP5 interact to form viroplasms where assembly of subviral particles takes place. Genome packaging begins with the formation of the 'assortment complex' that result from attachment of VP1 and VP3 to the 3' end of the 11 viral (+) RNAs. dsRNA synthesis by VP1 is triggered by condensation of the inner VP2 around the assortment complex. VP6 then assembles onto the nascent core to form the DLP. NSP4 recruits DLPs and VP4 to the cytosolic face of the ER membrane. The DLP-VP4-NSP4 complex buds into the ER through an unknown mechanism. The TLP is formed when the ER membrane is removed followed by assembly of the VP7 which is localised to the ER. The virion is either released through exocytosis or cell lysis to the gut where it is exposed to trypsin-like proteases. Cleavage of VP4 into VP5\* and VP8\* result in a fully infectious virion. DLP, double-layered particle; ER, endoplasmic reticulum; IFN, interferon; TLP, Triple-layered particle. From Trask et al. (2012), with permission.

membrane of the host cell is achieved only with the proteolytically primed VP5\* portion of VP4 (Estes et al., 1981). Post-attachment receptors are critical for efficient rotavirus entry as several

types of host cells can bind to rotaviruses (Ciarlet et al., 2002). Although the virus-host interaction mechanisms that determine the final outcome of infection and pathogenesis are not well understood, it is believed that the infection is initiated by a NA-independent mechanism and preferentially through the basolateral surface of polarized intestinal cells (Ciarlet and Estes, 1999). Rotavirus entry into host cells involves a number of coordinated steps and sequential interactions between several ligands. Several conformational changes of the outer capsid proteins occur that are mainly caused by: a) Trypsin cleavage of VP4 into VP5\* and VP8\* subunits (Dormitzer et al., 2004). VP5\* remains associated with the particle and its lipophilic activity enhances entry of the particles into the cell by accelerated penetration of the membrane (Yoder et al., 2009, Denisova et al., 1999). Exposure of three hydrophobic loops in VP5\*, which are tucked below the VP8\* lobes and nestled into the base of the upright VP5\* subunits, is required for membrane penetration (Kim et al., 2010). This is achieved through a 'fold back' mechanism where the umbrella-shaped VP8\* that has a triple-helical coiled-coil at its core folds backwards onto itself (Dormitzer et al., 2004) (Fig. 2.5). b) Initial interaction between VP8\* and cells containing SA receptors: This allows rotavirus to interact with integrin  $\alpha 2\beta 1$  through the DGE sequence of the VP5\* that binds to  $\alpha 2I$  domain (Graham et al., 2006). Additional post-attachment interactions between VP5\* and VP7 to hsc70 and  $\alpha v\beta 2$   $\alpha 4\beta 1$  integrins have also been reported (Hewish et al., 2000). It is not fully understood how these structural changes in VP5\* result in membrane penetration as rotaviruses are not enveloped which could be used to fuse to the host cell membrane. VP5\* may perforate the cell membrane through its hydrophobic loops in much the same way as reovirus myristoylated penetration protein,  $\mu 1$ , that forms approximately 7 nm pores on model cell membranes (Agosto et al., 2006).

It is assumed that the membrane penetration activity of VP5\* is regulated by VP7. In the absence of VP7, proteolytic processing of VP4 is more extensive which results in the post-penetration state of VP5\* (Dormitzer et al., 2004). The VP7 layer arrests and stabilises VP5\* in the upright spike conformation to allow for viral attachment. Thus, VP7 layer makes it possible for protease to cleave only the region that links VP8\* and the VP5\* subunit (Settembre et al., 2011), Fig. 2.4.III. Although the mechanisms used by rotaviruses to internalise host cells is not well understood, endocytosis and direct membrane penetration have been suggested as probable mechanisms (Estes, 2001). Fukuhara et al. (1987) showed that acidification of the endosome is essential for receptor mediated endocytosis. It is assumed that rotaviruses also employ more than one mechanism to enter host cells and requires low calcium intracellular concentrations similar to other reoviruses and polioviruses (Cohen et al., 1979). Low calcium concentration in the

endosome triggers VP7 disassembly (Chemello et al., 2002) that serves as a signal for VP5\* rearrangement, thereby allowing the virion to penetrate the endosomal membrane (Yoder et al., 2009). NSP4 is indirectly involved in the particle entry process as it affects calcium mobilisation which affects the stability of the outer capsid layer (Tian et al., 1995). Solubilisation of the outer capsid proteins activates trypsin which cleaves VP4 into fragments that have the potential to disrupt the endosomal membrane (Benureau et al., 2005). This then allows entry of the DLPs into the host cell cytosol where transcription is initiated.

The endogenous viral RdRp complexes of VP1 and VP3 which are attached to the inner layer of VP2 are transcriptionally active and exhibit transcriptase, nucleotide phosphohydrolase, guanyltransferase and methylase activities to achieve the synthesis of capped viral mRNAs (McCrae and McCorquodale, 1982, Mason et al., 1980). The virus associated transcriptase is latent in TLPs, but when exposed to chelating agents or heat shocked *in vitro*, it gets activated (Cohen et al., 1979). This may be synonymous to events occurring during natural rotavirus infection where TLPs are known to lose their outer capsid upon entering the host cell resulting in DLPs. Transcription takes place at the five-fold axis of the structurally intact DLPs where 11 species of non-polyadenylated (+) RNAs are synthesised using the (-) RNAs of the dsRNA genome segments as templates (Pesavento et al., 2006, Crawford et al., 2006, Lawton et al., 2000, Gorziglia and Esparza, 1981). The hydrolysable form of adenosine triphosphate (ATP) is essential for initiation and elongation of RNA molecules. Each genome segment is transcribed by a specific polymerase complex and the mature rotavirus transcripts have a 5'-terminal cap structure, 7-methyl-GpppG (Gorziglia and Esparza, 1981). These new transcripts exit the core through the type I channel of the VP2 located opposite to their site of synthesis (McClain et al., 2010, Lawton et al., 1999). There are four tunnels that lead into the catalytic centre of the RdRp that serve as passages for the entry and exit of nucleotides, template RNAs and RNA products (Lu et al., 2008). Rotavirus transcription is fully conservative. One RNA exit tunnel directs nascent (+) RNAs towards VP3 where it acquires a 5'-cap (Chen et al., 1999) followed by extrusion from the viral particle, whereas the other exit tunnel guides the genomic (-) RNA back into the core where it reassociates with the genomic (+) RNA. The nascent rotavirus (+) RNAs made in DLPs act as mRNAs for protein synthesis as well as templates for genome replication (Lu et al., 2008). It is not clear how loss of the outer capsid induces transcription. Potentially, removal of VP7 causes a dilation of the vertex channels in the particle, thereby providing passage for the influx of ions and nucleotides and the efflux of transcripts (Chen et al., 1999). Alternatively, since VP1 is sensitive to conformational changes in VP2, loss of VP7 may transmit structural changes to VP2 through

VP6. This may influence the interaction of VP1 with VP2 that may permit the RdRp to act (Trask et al., 2012). It should be noted that antibody specific to VP6 can also block the transcription activity of DLPs perhaps by narrowing the class I channels (Thouvenin et al., 2001).

Rotavirus utilises the host cellular translation mechanism to synthesise its proteins (Kabcenell and Atkinson, 1985). Translation is facilitated by NSP3 which acts like the poly (A) binding proteins (PABPs) as viral RNA is not polyadenylated. The NSP3 N-terminus interacts with the 3' end of the mRNA, whereas its C- terminal forms a complex by interacting with the eukaryotic translation factor eIF4G-like PABP (Vende et al., 2000). Binding of NSP3 to the viral mRNA offers an added advantage as it serves to transport newly synthesised mRNA to viral proteins for replication (Gonzalez et al., 1998, Poncet et al., 1993).

The dsRNA remains associated with the subviral particles. Free dsRNA is not found in the host cell after synthesis so as not to trigger interferon-induced apoptosis (Sato et al., 2006). The NSPs are associated with numerous roles in rotavirus replication. NSP2, NSP3 and NSP5 have been linked to rotavirus replication and packaging. This was supported by characterisation of VP1, VP2, VP6, NSP2, NSP3 and NSP5 within the replicase complexes in both infected cells and cell-free systems (Helmberger-Jones and Patton, 1986). Although the products of transcription from incoming DLPs accumulate in the cytosol, which are available for translation into viral proteins by host ribosomes, it is believed that the viral replication, selective genome packaging and initial steps of DLP assembly take place within the RNA-dense pseudocrystalline aggregated cytoplasmic inclusion bodies formed by NSP2 and NSP5, known as viroplasm (Trask et al., 2012, Gonzalez et al., 2000). The pre-core containing VP1, VP3, NSP2, NSP3, NSP5 and RNA is formed first followed by the sequential addition of VP2, then VP6, leading to DLP formation (Gallegos and Patton, 1989).

The replication complex is said to be organised around NSP2 due to its octomer structure. The RNA and NSP5 binds to inner grooves of the NSP2 octomer (Jiang et al., 2006). In addition, NSP2 is able to interact with VP2, thereby, acting like an adapter or a molecular motor that facilitates NSP2, VP1 and VP3 interactions during the packaging of mRNA, core assembly and encapsidation (Berois et al., 2003).

Some reports indicate that NSP4 plays several significant roles in rotavirus replication. NSP4 regulates expression of other viral proteins and mRNA synthesis (Lopez et al., 2005), genome encapsidation (Silvestri et al., 2005), and is involved in packaging of rotavirus proteins (Trask et al., 2012, Estes and Cohen, 1989). How equimolar packaging of the rotavirus genome segments is

achieved is not yet clearly understood. Several models have been proposed. One model speculates that pre-core replication-intermediate-precursor-complexes that contain viral mRNA, polymerase and capping enzymes serve as nuclear sites where the VP2 core protein and *T=1* (laevo) core binds and assemble (Patton et al., 2004). The concerted model is based on the ability of rotavirus capsid proteins to self assemble. The core-filling model proposed that mRNA is inserted following the formation of the core or assortment is mediated by RNA–RNA interactions before genome packaging (McDonald and Patton, 2011a). A model proposed by Lu et al. (2008) and Patton (1996) suggest that individual copies of VP1, which are possibly complexed with VP3, initially bind to the 3' ends of the viral (+) RNAs. The former model likely reflect the real mechanism of packaging.

The RdRp, in the 11 catalytically inactive enzyme–RNA complexes, specifically recognises the conserved 4-nucleotide sequence (UGUG) (Lu et al., 2008). It has been proposed that (+) RNAs initiate synthesis of dsRNA by adopting a looped ‘pan handle’ conformation with base-paired 5'- and 3'-end untranslated terminal regions (UTRs) as in influenza viruses (Patton et al., 1996). Assembling of the VP2 layer activates the RdRp that initiates (–) RNA strand synthesis by engaging the polymerase component of polymerase–(+) RNA complexes through the N-termini of VP2 that protrude inwards. These steps result in the synthesis of the dsRNA genome (Patton et al., 1997).

Further studies are still required to understand the encapsidation process of rotavirus mRNA molecules. Rotaviruses exhibit a distinctive morphological feature from any other virus because the subviral particles assemble in the cytoplasmic viroplasm but bud directly through the ER membrane. The decameric assembly of VP2 that activates each VP1 monomer has been hypothesised (Patton et al., 1997). VP2 regulation of RdRp probably prevents premature synthesis of dsRNA thereby allowing the virus to precisely coordinate the stages of RNA packaging, genome replication and core shell assembly (Trask et al., 2012). VP2 is believed to self assemble into a complete closed *T = 1* shell composed of 12 VP2 decamers, resulting in 12 vertices that contain 11 internally tethered polymerase–(+) RNA complexes (McClain et al., 2010).

The specific mechanisms how rotaviruses achieve precise VP6 assembly onto the VP2 core is not well understood. Considering that VP2 and VP6 spontaneously self-assemble into capsid-like structures when expressed in the absence of other rotavirus proteins (Crawford et al., 1994) indicates that there must be a specific mechanism that governs the DLP assembly process, otherwise there would be uncontrolled self-assembly of the structural proteins that would impair genome replication. NSP2 and NSP5 are believed to positively and negatively regulate DLP

formation, respectively, by interacting with subset of structural proteins and (+) RNA (Berois et al., 2003).

Several hypotheses exist on how the DLPs associate with VP4, exit the viroplasm and breach the ER membrane where they acquire a thin glycosylated VP7 layer. In general, NSP4 is a key regulator of the outer capsid assembly (Tian et al., 1996). NSP4 accumulates in the ER near the cytosolic viroplasms. The C-terminal cytoplasmic domain of the NSP4 (aa 161 – 175) acts as an intracellular receptor on the ER membrane (Taylor et al., 1993), whereas its N-terminus extends into the ER lumen, where it forms intramolecular disulphide bonds (Bergmann et al., 1989). NSP4 mediates budding of the particle out of the viroplasm by binding to both the newly made DLPs and VP4 using its C-terminus (Au et al., 1993), thereby acting as a chaperone for the DLP–VP4 pro-virion (Trask and Dormitzer, 2006). This is supported by the observation that DLPs can not bind to ER membrane in the absence of NSP4 (Au et al., 1989). NSP4 binds both the DLP and VP4 through its unstructured C-terminus (Au et al., 1993, O'Brien et al., 2000, Taylor et al., 1993).

Successive interaction of the DLP with surrounding NSP4 tetramers deforms the ER membrane, thus allowing the DLP–VP4–NSP4 complex to bud into the ER, Fig. 2.6. This process gives the maturing particles a transient envelope which is replaced by a thin layer of protein as the particles move towards the interior of the ER (Gonzalez et al., 2000). Thereafter, the membrane is removed and VP7, which is retained in the ER (Stirzaker et al., 1987), assembles onto the particle, thereby locking VP4 into place (Trask and Dormitzer, 2006). Although the incorporation of the inner capsid to the core occurs rapidly, addition of VP4 and VP7 to mature virus particles is slower (lag time of 10 – 15 minutes). It is believed that the membrane associated forms of the proteins are precursors to the VP4 and VP7 of the virion (Kabcenell et al., 1988).

The maturation step is a calcium-dependent process. Rotaviruses produced in the absence of calcium are often DLPs, and budding of the particles through the ER membrane is not possible (Shahrabadi et al., 1987). Since NSP4 is a calcium agonist, it probably mobilises the intracellular calcium through a phospholipase C (PLC) independent mechanism for efficient budding of rotavirus particles during infection (Morris and Estes, 2001). How the envelope is removed or how the outer capsid is assembled on the DLP, remains to be understood.

The final step of the infectious cycle is the egression of the progeny virions through direct cell lysis (Altenburg et al., 1980) or secretion from the apical cell surface as evidenced in polarized and non-polarized kidney epithelial cells (Musalem and Espejo, 1985). Cellular and viral proteins

may also be released if the permeability of the plasma membrane has been drastically altered during the late stages of infection or through the non-classical vesicular transport involving interaction with lipid rafts near the plasma membrane (Musalem and Espejo, 1985).

Despite the knowledge presented above of the rotavirus replication process, several aspects of rotavirus biology are still not well understood. For instance, the exact order of events in genome replication and packaging is not known (Jayaram et al., 2004), the precise control mechanisms that direct packaging and reassortment of RNA segments into viral cores are not known (Desselberger et al., 2009), the pathogenesis of rotavirus infection is not clearly understood. In addition, the determinants of immunological protection against rotavirus infection is not well understood. It is not clear which immunological markers correlate best with protection against rotavirus infection (Molyneaux, 1995). Proper understanding of these mechanisms could assist in developing therapeutics by targeting specific steps of the replication cycle and also rational design of rotavirus vaccines. Reverse genetics systems is the single most powerful state of the art cutting-edge tool in modern virology which can be used to provide the currently missing critical rotavirus biological information. Previously, use of selection-free plasmid-based reverse genetics system in functional studies demonstrated that the Arg-Gly-Asp amino acid motif of foot and mouth disease virus does not function as a ligand for binding in BHK-21 cells (Storey et al., 2007). The system also revealed that the NS1 influenza B mutant viruses are attenuated (Hai et al., 2008). Furthermore, a plasmid-based reverse genetics system has been used to produce a reassortment trivalent formalin inactivated influenza vaccine (Subbarao and Katz, 2004).

Reverse genetics systems have also been developed for *Reoviridae* viruses. Bluetongue virus has been recovered using *in vitro* synthesised T7 mRNA transcripts obtained from cDNA clones (Boyce et al., 2008), whereas a true plasmid-based reverse genetics system has been described for reoviruses that does not require selection steps or a helper virus (Kobayashi et al., 2007). A plasmid-based reverse genetics system that used a helper virus and a T7 promoter has been described for rotavirus (Komoto et al., 2006). This system is not ideal for extensive study of rotavirus biology as it does not allow manipulation of more than two genome segments at the same time, requires several steps that includes neutralisation of VP4 expressed by the KU helper virus and uses a vaccinia virus that may require downstream purification processes. Studies are on-going worldwide to develop a selection-free reverse genetics system that can be used to recover rotaviruses that can be utilised in understanding rotavirus biology and also in formulating rationally designed rotavirus vaccines.

### **2.3. The pathogenesis of rotavirus infection**

Rotaviruses employ several mechanisms to induce diarrhoea. Not one of these mechanisms is completely understood (Tian et al., 1996, Ramig, 2004). Prior to cell lysis, NSP4 may trigger release of  $\text{Ca}^{2+}$  through non-classical pathways. The increased  $\text{Ca}^{2+}$  levels impair the absorptive capacity of the epithelium due to desquamation. This lead to malabsorptive diarrhoea (Reviewed by Ramig, 2004). NSP4 may also induce chloride secretion and reduce glucose absorption thereby causing malabsorption leading to secretory diarrhoea (Huang et al., 2001, Ramig, 2004). As an enterotoxin, NSP4 may also cause diarrhoea by inducing secretion of fluids in the enterocytes through direct stimulation of the enteric nervous system (ENS) (Lundgren et al., 2000). Virus ischemia, which is defined as the intestinal damage caused by the virus infection, is the other proposed mechanism. These factors lead to villous atrophy followed by reactive crypt-cell hyperplasia that impairs the absorptive function of the enterocytes resulting into osmotic diarrhoea as an osmotic bolus is created in the lumen by the undigested fats, carbohydrates and proteins (Osborne et al., 1991). Recently, Hagbom et al. (2011) demonstrated that emesis, which is a hallmark of the rotavirus disease, is caused by serotonin (5-hydroxytryptamine, 5-HT). 5-HT is secreted by enterochromaffin cells (EC) that can be directly infected with and replicate rotaviruses in humans. The 5-HT activates vagal afferent nerves connected to the nucleus of the solitary tract and area postrema in the brain stem structures associated with nausea and vomiting.

### **2.4. Immune response to rotavirus infection and correlates of protection**

The mechanisms of rotavirus immunity in rotavirus infected hosts are at present not well defined. There are no clear correlates of protection (Reviewed in Desselberger and Huppertz, 2011). Immunoglobulin A (IgA) copro-antibodies are thought to be the best proxy to determine rotavirus exposure and vaccine take (Barnes and Bishop, 1997, Bishop et al., 1983, Coulson et al., 1992).

While neonatal infections are often asymptomatic (Bishop et al., 1983), primary rotavirus infections early in life are often severe and mostly elicit a homotypic humoral immune response (Estes and Kapikian, 2007). Subsequent re-infections with rotaviruses are common and often asymptomatic, but often elicit both homotypic and heterotypic antibody responses (Richardson and Bishop, 1990, Chiba et al., 1986, Velazquez et al., 1996). Similarly, rotavirus infections post-vaccination rarely result in severe disease (Offit, 1994). The level of mucosal IgA antibodies is believed to determine the homotypic protection, whereas heterotypic protection depends on the viral infection dose, type of the strain, and has been correlated to the ability of the infecting strain to stimulate detectable rotavirus specific IgA antibodies (Feng et al., 1994).

The rotavirus proteins induce antibodies in different ways. NSP4 is associated with homotypic, VP4 and VP7 with both heterotypic and homotypic immune responses, while VP6 induces strong VP6-specific antibodies that do not neutralise the virus but can protect against primary infection. IgA is believed to protect through intracellular inactivation of the virus following transcytosis (Zhou et al., 2011, Yuan et al., 2004a, Yuan et al., 2004b, Burns et al., 1996).

Since the immunity against severe diarrhoea in humans resulting from series of childhood rotavirus infections often wanes with age, elderly persons become more susceptible to rotavirus re-infection (Glass et al., 2006a). The significance of the systemic presence of IgA, IgG and IgM antibodies towards protection against rotavirus infection in both humans and animals remain to be understood (Desselberger and Huppertz, 2011, Fischer et al., 2005, Ramig, 2004). However, it is known that maternal IgG antibodies may play a role in protecting infants under the age of three months from developing severe diarrhoea caused by rotavirus infections as evidenced by the neutralising activity of antibodies detected from transitional milk and colostrum specimens (Chan et al., 2011, Herrmann et al., 1996, Yang et al., 2001).

## **2.5. The rotavirus classification systems**

### **2.5.1. Family, group and subgroup classification**

Rotaviruses constitute a genus in the *Reoviridae* virus family, Table 2.1. The following morphological and biological properties define the rotavirus genus: a) rotaviruses replicate in the cytoplasm and carry their own viral encoded enzymes necessary for replication; b) rotavirus transcripts assume dual roles of serving as mRNA for protein synthesis and acting as templates for negative strand RNA synthesis; c) the entire replication of rotavirus takes place in viroplasm, therefore, no free dsRNA is found in infected cells; d) subviral DLPs acquire an outer capsid protein by budding through the ER membrane; e) intracellular calcium regulates rotavirus integrity and assembly; f) the mature particles are released through cell lyses; and g) addition of exogenous protease to the culture media activates rotavirus infectivity (Reviewed in Trask et al., 2012, Estes and Kapikian, 2007).

Rotaviruses have been classified into seven major serogroups (A – G) based on the group specific epitopes localised in an immunodominant site of VP6 between amino acid residue 48 and 75 (Kohli et al., 1992). Group A rotaviruses are further classified into four subgroups (I, II, I+II, non I and non II) depending on whether they carry the subgroup specific domain (s) on VP6 detectable by serological assays (Greenberg et al., 1983a, Gary et al., 1982, Greenberg et al., 1982). Although some studies suggest that non-group A rotaviruses do not share subgroup antigens with

group A rotaviruses (Bridger, 1987), the hybrid RV-VLP studies (Kim et al., 2002), restriction endonuclease digestion of a cDNA fragment of the genome segment 6 (Iturriza Gomara et al., 2002b), nucleic acid sequencing and phylogenetic analysis (Iturriza Gomara et al., 2002a) studies suggest that rotaviruses from different groups rotaviruses share VP6 antigenic properties.

### **2.5.2. The electropherotype classification**

The enzyme immune assays (EIAs), which are commonly used to screen for the presence of rotaviruses in clinical specimens, mostly target VP6. Often groups D – G rotaviruses are not detected with these EIAs as they lack the group-specific antigenic domains (Kohli et al., 1992). This was known since the inception of EIAs (Pacini et al., 1988). Therefore, alternative methods to classify rotaviruses that belong to different rotavirus groups were developed (Estes and Kapikian, 2007).

The 11 genome segments of rotaviruses have different migration patterns when subjected to SDS-PAGE due to their differences in size. The term electropherotyping was coined to classify rotaviruses based on their dsRNA profiles (Estes, 2001, Jiang, 1985). Electropherotype classification of group A rotaviruses refers to four-size classes that include: (a) four large genome segments (1 – 4), (b) two medium sized genome segments (5 – 6), (c) three smaller genome segments (7 – 9), and (d) two smallest genome segments (10 – 11) (Greenberg et al., 1983b), refer to Chapter four, Fig. 1b for the patterns. Polyacrylamide gel electrophoresis (PAGE) offers several advantages as it can identify atypical rotaviruses devoid of the known group antigens (Lipson and Kaplan, 1992), can identify novel rotavirus strains through comparison of the mobility of each dsRNA genome segment (Todd et al., 1980) and the electropherotype profiles can be used to monitor rotavirus transmission in epidemiological studies (Steele et al., 1993).

Rotaviruses typically exhibit two distinct dsRNA profiles, long and short electropherotypes. The long pattern presents a genome segment 11 of approximately 664 bp that migrates faster than the other 10 segments. In some strains, genome segment 11 is approximately 816 bp in length, therefore, it migrates between segment 9 and 10 which are approximately 1,062 bp and 751 bp, respectively.

Although electropherotyping is a widely used rotavirus classification technique, it has not been adopted as a taxonomical tool for a number of reasons. PAGE analysis can not identify point mutations, recombination and reassortment events (Estes and Kapikian, 2007). Rotaviruses with similar sizes of the nucleotide sequences in the corresponding dsRNA genome segments do not always exhibit similar patterns, therefore, genetic variations can not be deduced from the

numerous electrophoretic patterns. Furthermore, the inability of PAGE to distinguish rotaviruses beyond the known group A to G is one of its major disadvantages (Nicolas et al., 1984). As such, electropherotyping has been replaced by sequencing procedures for classification and other purposes (Matthijnssens et al., 2011, Matthijnssens et al., 2008a).

### **2.5.3. The dual typing classification systems**

Group A rotaviruses were initially differentiated by serotyping based on the reactivity of their outer capsid proteins, VP4 and VP7, to neutralising antibodies (Nakagomi et al., 1989b, Offit and Blavat, 1986). This formed the basis of the widely adopted binary classification system of rotaviruses where the P types (protease-sensitive) represents VP4, and G types (glycoprotein) designate VP7 (Estes and Kapikian, 2007). Serotypes are complemented by genotypes that are based on the identities between the nucleotide sequences of cognate rotavirus genome segments (Green et al., 1992, Green and Kapikian, 1992, Offit and Blavat, 1986). So far, 27 G and 35 P genotypes have been identified (Matthijnssens et al., 2011). Unlike P types, correlation between G serotypes and genotypes is complete. Therefore, where available, P serotypes and genotypes are designated jointly with genotypes in square brackets, for instance, RVA/Human-tc/USA/DS-1/1976/G2P1B[4] (Matthijnssens et al., 2011). Although the dual typing system has been widely used in most epidemiological and molecular characterisation studies, its use is primarily limited to classifying rotavirus strains. The dual typing system can not determine factors that are involved in viral tropism and virulence of rotavirus strains. Furthermore, some evolutionary pathways like reassortment and recombination followed by all the 11 genome segments of rotaviruses can not be studied because the dual classification is restricted only to outer capsid encoding genome segments (Matthijnssens et al., 2008a).

### **2.5.4. The whole genome classification system**

Previously, the genogroup concept was used to categorise group A rotaviruses into genogroups by making use of RNA-RNA hybridization assays (Nakagomi et al., 1989a). Following the advent of hybridization techniques, researchers could investigate the occurrence of reassortment events between human strains that belong to different genogroups or between human and animal strains which frequently lead to generation of novel rotavirus strains (Nakagomi and Nakagomi, 2002, Nakagomi and Nakagomi, 1989). Human rotaviruses were classified into two major (represented by the Wa and DS-1 reference strains) genogroups and one minor (represented by the AU-1 reference strain) genogroup (Nakagomi et al., 1989a). A high degree of genetic relatedness was noted in rotaviruses of the same genogroup. Although the 11 dsRNA segments segregates

independently, Nakagomi and Nakagomi (1993) proposed that optimum sets of gene constellation among rotaviruses circulating in nature exist. The hybridization probes effectively characterised rotaviruses into three distinct genetic groups which was linked to rotavirus genotypes: (a) genogroup I which contains human rotaviruses with subgroup I specificity and G2P[4] strain; (b) genogroup II that contains subgroup II specific human rotaviruses with G1P[8], G3P[8], G3P[6], G5P[8], and G9P[11] genotypes combinations, and; (c) genogroup III which contains the rhesus rotavirus vaccine strain (G3P[3]). The International Committee on Taxonomy of Viruses (ICTV) has not however accepted ‘genogroup’ as a classification criterion due to several reasons. For instance, reassortment between rotaviruses of different genotypes muddles the genogroup concept. Despite the significant impact of hybridization methods on the understanding of rotavirus epidemiology, performing the assays is labour-intensive. Therefore, more recently partial or complete sequencing of all 11 genome segments was initiated to understand the relationships between rotavirus strains. Pairwise sequence identity profile methods used to differentiate the virus genotypes into well-resolved peaks was, therefore, exploited as a basis for a possible classification systems (Matthijnsens et al., 2008a). Nucleotide sequencing allows direct determination of genetic relationships, direct phylogenetic inferences and understanding the patterns of rotavirus evolution (Matthijnsens et al., 2009, Rahman et al., 2007, Matthijnsens et al., 2006, Tate et al., 2011a).

An extended genotyping classification system for rotaviruses involving all 11 genomic RNA segments was proposed by Matthijnsens et al. (2008a). Maes et al (2009) further proposed a system that speeds up the differentiation of all 11 genome segments for group A rotaviruses and developed a user friendly web-based tool, RotaC (<http://rotac.regatools.be>). All the new guidelines proposed by the RCWG were taken into consideration prior to the launch of RotaC (Maes et al., 2009). RotaC compares phylogenetically the pairwise nucleotide identities of the complete ORF of rotavirus genome segments of the strain under investigation to other complete ORFs of cognate genome segments available in the GenBank database. A strain may be assigned as a prototype of a new genotype only if all the pairwise nucleotide identities between a genome segment of the strain under investigation and the cognate genome segment of all the established genotypes are below the proposed cut-off value (Matthijnsens et al., 2008a). To distinguish different genotypes for each of the 11 genome segments in the proposed full genome classification scheme, nucleotide identity cut-off percentages were defined for each genome segment, Table 2.3. The cut-off percentage value for each of the 11 genome segments was calculated based on the entire ORF only. The RCWG recommended that partial sequence may be used to assign a rotavirus genome

segment to an established genotype only when the following conditions are met: “a) at least 50% of the nucleotide sequence of the ORF should be determined; b) at least 500 nucleotides of the ORF should be determined; and c) identity between the strain under investigation and a strain belonging to an established genotype should be at least 2% above the appropriate cut-off value”, (Maes et al., 2009). Consequently, the annotation G<sub>x</sub>-P<sub>[x]</sub>-I<sub>x</sub>-R<sub>x</sub>-C<sub>x</sub>-M<sub>x</sub>-A<sub>x</sub>-N<sub>x</sub>-T<sub>x</sub>-E<sub>x</sub>-H<sub>x</sub> (where x represents the number of the genotype) representing VP7, VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4, and NSP5 encoding genome segments, respectively, is used to classify rotavirus strains (Matthijssens et al., 2008a). The letters in the annotation represent the description of the proteins encoded by each genome segment, for instance, G for the glycosylated VP7. A description of the proteins encoded by the genome segments is listed in Table 2.2.

**Table 2.3.** Cut-off values of the nucleotide sequence identity percentage defining genotypes for the eleven rotavirus genome segments.

Genome Segment	Size bp	Protein	Cut-off value (%)	Genotype (n) <sup>a, b</sup>
1	3 302	VP1	83	<b>R</b> (6)
2	2 684	VP2	84	<b>C</b> (6)
3	2 591	VP3	81	<b>M</b> (7)
4	2 362	VP4	80	<b>P</b> (35)
5	1 561	NSP1	79	<b>A</b> (16)
6	1 356	VP6	85	<b>I</b> (14)
7	1 104	NSP3	85	<b>T</b> (8)
8	1 059	NSP2	85	<b>N</b> (6)
9	1 062	VP7	80	<b>G</b> (27)
10	751	NSP4	85	<b>E</b> (12)
11	667	NSP5	91	<b>H</b> (8)

The Table was compiled from Matthijssens et al. (2008b).

<sup>a</sup> Notations in bold, used in the whole genome rotavirus classification system, denotes the description of the 11 rotavirus genome segments, Table 2.2.

<sup>b</sup> Current number of genotypes as at the end of this study (April, 2012).

## 2.6. The global epidemiology of human rotaviruses

Each year, an estimated 744 million to 1 billion diarrhoea cases that lead to 2.4 – 5 million deaths occur (Linhares, 2000, Bryce et al., 2005). Diarrhoea is a multifactoral disease. It is caused by several agents ranging from eukaryotic organisms to ingested poisonous particles like toxins. Specific eukaryotes and prokaryotes, directly linked to diarrhoea, include bacteria (*Campylobacter jejuni*, *Escherichia coli*, *Salmonella*, and *Shigella* species), viruses (rotaviruses, adenoviruses, caliciviruses and astroviruses) and parasites (*Entamoeba histolytica*,

*Cyptosporidium parvum* and *Giardia lamblia*) (Desselberger, 2002). Rotaviruses are the major aetiological agents of severe dehydrating diarrhoea. It is estimated that each year rotavirus infections are associated with 111 million gastroenteritis episodes, of which 25 million require medical attention and two million are hospitalised (Parashar et al., 2003). While the mortality of diarrhoeal cases caused by bacteria and parasites are declining with each successive year due to improving sanitation, water quality and better access to healthcare facilities in many developing countries, the incidence of rotavirus infection has remained constant (Parashar et al., 2009, Santos and Hoshino, 2005). Inadequate healthcare facilities is believed to be the major attributer to high mortalities in developing countries as most ill children succumb to severe dehydration before reaching the hospital. Factors prevailing in developing countries like inefficient transport facilities and high illiterate levels or traditional cultural beliefs complicate strategies to save lives of rotavirus infected children (Grimwood and Bines, 2007). These factors, coupled with the short incubation period of the rotavirus disease makes rotavirus diarrhoea lethal in these regions.

Several factors complicate the epidemiology of rotaviruses. Usually, rotaviruses of different G- or P-types co-circulate in any geographic location. In addition, the relative prevalence of G types changes over time in the same region (Ramachandran et al., 1998, Gentsch et al., 1996). The wide rotavirus strain diversity (Matthijnssens et al., 2011, Solberg et al., 2009, Matthijnssens et al., 2008a, Khamrin et al., 2007, Martella et al., 2007) does not only complicate the understanding of the pathological aspects of the rotavirus disease, but also create challenges towards developing intervention measures, for instance, formulation of rotavirus vaccines. In the past three decades, group A rotaviruses bearing G1, G2, G3, G4, G8 and G9 genotypes associated with P[4], P[6] and P[8] genotypes were the most prevalent human strains worldwide. A systemic review of 124 studies from 52 countries by Santos and Hoshino (2005) showed that G1, G2, G3 and G4 in conjunction with P[4] and P[8] constituted 88% of the strains circulating in most developed countries before the 1990s. During that time, genotype G9 strains were considered emerging and were detected in 4.1% of the total rotavirus infections. Since then, numerous studies have reported the emergence and localised prevalence of genotypes like G8, G12, P[6] and P[11] in humans. G8 strains were frequently detected in Africa where its prevalence was similar to the global prevalence of G3 and G4 (Santos and Hoshino, 2005). Furthermore, Steele and Ivanoff (2003) reported the predominance of G1P[6] and G3P[6] strains across Africa between 1996 and 1999. They also noted that the highest rotavirus diversity occurs in West Africa where mosaic viruses comprising of G1/G8 and G1/G3 were characterised. The latest rotavirus strains to emerge amongst the frequently detected strains bear G12 associated with a P[4] or P[6] genotype. G12

rotaviruses were first detected in Philippine children (Urasawa et al., 1990). Currently, G12 strains have been detected worldwide (Martella et al., 2010, Cunliffe et al., 2009, Page et al., 2009, Le et al., 2008, Rahman et al., 2007, Cunliffe et al., 1998).

Several studies suggest that animal-to-human interspecies transmission and genome segment reassortment are the major mechanisms leading to rotavirus strain diversity in humans (Desselberger et al., 2001). Although all known rotavirus strains have been reported in animals, only genotypes G1, G2, G3, G4, G5, G6, G8, G9, G10 and G12 in association with either P[4], P[6], P[8], P[9], P[10], P[11], P[12], P[14] or P[25] have been associated with human infections (Matthijssens et al., 2009, Le et al., 2008, Martella et al., 2008, Matthijssens et al., 2008a, Mphahlele et al., 1999, Cunliffe et al., 1998). The frequent detection of animal-like rotavirus strains in humans and vice versa lead to suspicion of interspecies transmission, for instance, the detection of the porcine-like G5 and G8 bovine-like rotaviruses in Brazilian and Malawian infants, respectively. This was thought to be the consequence of the close proximity between human and animal dwellings in these communities (Santos and Hoshino, 2005, Cunliffe et al., 2001, Alfieri et al., 1996). In addition, antigenic shift arising from genome reassortments, genetic drift arising from nucleotide substitutions and sequence homoplasy, and intragenic recombination are also considered to be important evolutionary mechanisms driving rotavirus strain diversity (Phan et al., 2007a, Phan et al., 2007b, Parra et al., 2004, Gouvea and Brantly, 1995).

Diverse rotavirus strains have been reported in tropical regions (mostly developing countries) compared to temperate regions (mostly developed countries). Whether these variations are influenced by climate, remains to be understood. Rotavirus infections usually peak in cooler and drier months of the year in temperate regions (Gouvea et al., 1990b, Estes and Kapikian, 2007, Steele et al., 2003), whereas rotavirus infections occur throughout the year in the tropics (Cunliffe et al., 1998). Several factors have been proposed that may lead to proliferation of rotavirus disease in developing compared to developed countries. These include the high population densities, high birth rates, crowded living conditions, and many others (Cunliffe et al., 1999). A systemic review of 26 meta-analysis studies conducted in tropical countries by Levy and colleagues (2009) showed a relationship between climate changes and rotavirus infections in the tropics. They also indicated that the highest rates of rotavirus infections occur during cooler and drier months of the year. In a nutshell, Levy and colleagues (2009) suggested that there is a reduction of 10%, 1% and 3% of rotavirus incidence for every temperature decrease by one degree Celsius, one centimetre increase in mean monthly rainfall and every one percent increase in relative humidity, respectively.

Although seven rotavirus serogroups have been described, most of the symptomatic infections in humans are associated with group A rotaviruses. Initially, group B and C rotaviruses were only associated with infections in animals (Kalica et al., 1978). However, sporadic human outbreaks associated with group B rotaviruses have been reported in China amongst older children and adults (Hung et al., 1985, Hung et al., 1984). A group C rotavirus was first isolated in humans in 1982 from an Australian infant (Rodger et al., 1982). Although a few outbreaks reported later in the United Kingdom (Caul et al., 1990, James et al., 1997) and Japan (Matsumoto et al., 1989) were also linked to group C rotaviruses, their clinical presentations were generally asymptomatic and mostly affected children older than five years (Matsumoto, et al. 1989). Since then, sporadic studies also demonstrated the presence of antibodies against group C rotaviruses in both faeces and sera, for instance in Sweden (Nilsson et al., 2000), the United Kingdom (James et al., 1997) and South Africa (Steele and Alexander, 1987).

Although linking specific rotavirus strains to severity of rotavirus disease has not been successful, rotavirus strain diversity poses one of the major challenges in rotavirus vaccine formulation efforts. It is well known that strains circulating in different parts of the world differ (Estes and Kapikian, 2007). Whether the currently licensed rotavirus vaccines will render protection against the emerging rotavirus strains and regionally prevalent strains bearing different genotypes to the vaccine strains, remains to be seen.

## **2.7. Intervention measures against rotavirus infection**

### **2.7.1. Treatment regimens**

The onset of illness suddenly appears after an incubation period of 24 – 48 h. The clinical symptoms of rotavirus infections vary widely. Mortality is caused by watery diarrhoea, vomiting and severe dehydration. The current treatment is exclusively directed at reversing the loss of fluids and electrolytes through rehydration. Oral, subcutaneous or intravenous rehydration is recommended as the primary treatment for rotavirus gastroenteritis (Desselberger, 1999). Although oral immunoglobulins are not used as routine therapeutic regimens, Bass (1997) showed their potential in treating rotavirus infection. Similarly, Garaicoechea et al (2008) demonstrated that immunoglobulins can be employed as an essential tool for the prevention and treatment of rotavirus diarrhoea by achieving broad protection against diarrhoea in mice with llama-derived single-chain antibody fragments directed to rotavirus VP6.

Chinese communities have been using Qiwei Baizhu Powder (QWBZP) as a cheap drug in treating rotavirus diarrhoea for thousands of years. QWBZP improves the absorptive function of

small intestine and alleviates the pathological changes of small intestine induced by rotavirus, thereby shortening the duration of diarrhoeal symptoms in mammals (He et al., 2001). He et al. (2001) showed the ability of QWBZP to inhibit the replication of rotaviruses in monolayers of MA104 cells. Some therapies have been administered to immune-suppressed patients. These include antiviral antibodies in form of oral immunoglobulins and human milk containing rotavirus antibodies (Asensi et al., 2006, Van de Perre, 2003). The WHO recommends reduced osmolarity oral rehydration solution (ORS), addition of *Lactobacillus* GG to ORS and rice-based solution as treatment for rotavirus diarrhoea (Todaro et al., 1995).

### **2.7.2. Prevention and control strategies**

Rotaviruses are highly stable in the environment as they contain a glycoprotein on their outer capsid instead of the envelope and also due to their triple capsid layers (Estes and Cohen, 1989). Treating surfaces with disinfectants like chloroform, ethanol, phenol and formalin that affects haemagglutinin (VP4 in rotaviruses) partly inactivates rotavirus infectivity, thereby, partially halting rotavirus transmission (Estes, 2001). However, it has been shown that treating rotavirus TLPs with 70% or 100% alcohol uncoat the particles into SLPs and often fragments the capsid proteins (Estes et al., 1979)

With each successive year, diarrhoea-associated mortalities caused by bacteria and parasites have drastically been reduced due to improved sanitation measures in most countries (Bryce et al., 2005). However, in both developed and developing countries, the incidence rates of rotavirus infections are still the same (Glass et al., 2006b). This suggests that improving sanitary measures alone may not be sufficient in preventing rotavirus infections which is associated with high mortalities in less than five year old children. Therefore, to achieve the Millennium Development Goal (MDG) number four “to reduce childhood mortality by two-thirds by 2015”, immunization is believed to be the main option.

### **2.8. Rotavirus vaccines**

Generally, children infected with rotavirus earlier in life present with asymptomatic disease after secondary and subsequent rotavirus infections (Kapikian et al., 1996b, Velazquez et al., 1996). Animal rotavirus strains are naturally attenuated in humans, for instance, the rhesus RVA/Simian-tc/USA/RRV/1975/G3P[3] strain (Kapikian et al., 1996a), the bovine RVA/Cow-tc/USA/WC3/1981/G6P[5] strain (Clark et al., 1996) and RVA/Cow-tc/GBR/UK/1973/G6P7[5] strain (Clements-Mann et al., 1999) and the lamb strain RVA/Sheep-wt/CHN/LLR/1985/G10P[12] (Glass et al., 2005b). These two observations formed the fundamental principles

adopted in formulating all licensed live attenuated rotavirus vaccine candidates to date. Infants who receive a dose of the currently available rotavirus vaccines are not necessarily immune to rotavirus infection, but are protected from developing severe dehydrating life-threatening diarrhoea (Glass et al., 2005b).

### **2.8.1. The live-attenuated rotavirus vaccines**

To date, almost all rotavirus vaccine candidates that have at least passed through phase III clinical trials are live-attenuated. These candidates are administered orally, are known to achieve better safety and high efficacy levels against severe diarrhoea, and are designed not to interfere with other EPI vaccines in inducing the immune response (Glass et al., 2006b). Although 27 different G and 35 P genotypes have been described (Matthijnssens et al., 2011), only human rotaviruses with G1, G2, G3, G4, and P[8] genotypes are directly targeted by the two globally licensed rotavirus vaccines, Rotarix<sup>®</sup> and RotaTeq<sup>®</sup>.

Prior to the WHO recommendation for the routine use of Rotarix<sup>®</sup> and RotaTeq<sup>®</sup> (World Health Organization, 2009), several rotavirus vaccines were experimentally designed with mixed outcomes. In 1983, SmithKline undertook the first rotavirus vaccine clinical trials. The candidate, RIT 4237, which was a monovalent strain formulated from RVA/Simian-tc/USA/RRV/1975/G3P[3], demonstrated inconsistent results in developing countries despite achieving high efficacy levels in developed nations. Therefore, further development of RIT 4237 was halted (Vesikari et al., 1984).

The ability of rotaviruses to reassort their genome segments has been exploited in the formulation of human-animal reassortant rotavirus vaccine candidates (Greenberg et al., 1981). In theory, reassortment of the known rotavirus serogroups and genotypes can lead to circulation of approximately 2046 novel progeny rotavirus strains (Chen et al., 1992). However, reassortment can only occur between rotaviruses of the same group that are infecting the same host cell simultaneously (Estes and Kapikian, 2007). *In vitro*, reassortant strains consisting of the circulating human genotypes on their outer capsids and animal strain on their genetic backbone have been engineered (Vesikari et al., 2006b). These candidates offer a number of advantages as their property of attenuation in humans is maintained, and also can effectively induce multivalent protective antibodies (Glass et al., 2006b). A rhesus-human reassortant tetravalent vaccine candidate, RotaShield<sup>®</sup>, was developed by Kapikian et al. (1996a). The rhesus strain RVA/Simian-tc/USA/RRV/1975/G3P[3] was reassorted with genome segments of human strains that encode the outer capsid proteins of the circulating human rotavirus genotypes. RotaShield<sup>®</sup>

was also designed to have a catch-up immunisation program at anytime up to seven months of age beside the normal dosage administered at two, four and six months. RotaShield<sup>®</sup> achieved 90% efficacy and prevented hospital admissions by 79% in clinical trials conducted in the USA and Venezuela. Marketing authority was granted for RotaShield<sup>®</sup> in European countries in 1999 after recommendations and licensure for routine immunisation in all US children in 1998 by Wyeth-

**Table 2.4.** Alternative rotavirus vaccines in developmental phases worldwide.

Institute/Country	Type of the vaccine	Development phase
Bharat Biotech, India	G9P[11] monovalent, live-attenuated	Phase 3
Murdoch Children's Research Institute, Australia	G3P[6], live-attenuated	Preparing Phase 2
POLYVAC, Vietnam	G1P[8], live-attenuated	Completed Phase 1
International Medical Foundation, Germany <sup>a</sup>	Rhesus-human quadravalent, live-attenuated	Phase 2b, Ghana
Shantha Biotechnics and Serum Institute (India), Instituto Butantan (Brazil) and Wuhan Institute of Biological Products, China	Human-bovine reassortant, live-attenuated <sup>b</sup>	Phase 1/2, Finland
Lanzhou Institute of Biological Products, India	G2, G3, and G4 trivalent, live-attenuated <sup>c</sup>	Phase 1
Centre for Disease Control, USA	Inactivated	Proof of concept in animals
Baylor College, USA	Subunit, RV-VLP	Proof of concept in animals
Cincinnati Children's Hospital, USA	Subunit, VP6	Proof of concept in animals
National Institute of Allergy and Infectious Diseases, USA	Subunit, VP8* <sup>d</sup>	Proof of concept in animals
North-West University, South Africa <sup>d</sup>	Reverse genetics, live-attenuated Subunit, RV-VLP	Research and Development

<sup>a</sup> The vaccine which is the original quadravalent RotaShield<sup>®</sup> is being produced by IDT Biologika, a German-based contract manufacturer.

<sup>b</sup> The vaccine was based on a virus developed by Albert Kapikian at the U.S. National Health Institute (Kapikian et al., 1996b). It was licensed in 2006. Clinical trials demonstrated 90% efficacy. Clinical studies were also conducted in Brazil and India in 2010.

<sup>c</sup> Formulation was based on the Lanzhou lamb rotavirus vaccine (World Health Organization., 2001). The G10 was replaced with G2, G3, and G4 to make a trivalent candidate.

<sup>d</sup> Efforts are under way to formulate rotavirus vaccine candidates by developing a reverse genetics approach and also chimaeric RV-VLPs using the rotaviruses with G2, G8, G9, G12, P[4], P[6] and P[8] genotypes.

Lenderle (Glass et al., 2006b). Unfortunately, cases of gut intussusception were reported in at least one in every 10 000 children immunised with RotaShield<sup>®</sup> (Murphy et al., 2001). Although the naturally occurring hospital intussusceptions cases is much higher, estimated at 5.6 per 10 000

in US children (Parashar et al., 2000), the Advisory Committee on Immunization Practice (ACIP) withdrew their recommendation as vaccine-associated side effect of 1: 10,000 frequency is not acceptable. Therefore, RotaShield<sup>®</sup> was withdrawn from the market in July, 1999.

Several other rotavirus vaccine candidates were developed after the suspension of RotaShield<sup>®</sup>, summarised in Table 2.3. The National Institute of Health (NIH), USA, engineered a reassortant vaccine candidate that contained G1, G2, G3 and G4 human rotavirus genotypes on a parental backbone of the bovine RVA/Cow-tc/GBR/UK/1973/G6P7[5] strain. This candidate demonstrated good safety, however, immunogenicity levels were not encouraging (Kapikian et al., 2005). Zhi-Sheng Bai also formulated a vaccine candidate from Lanzhou lamb rotavirus, strain RVA/Sheep-wt/CHN/LLR/1985/G10P[12], that was licensed in China in 2001 (World Health Organization., 2001). In addition, the Indian neonatal strains, RVA/Human-tc/IND/116E/1985/G9P[11] and RVA/Human-tc/IND/I321/XXXX/G10P[11], have also received much attention as potential candidates as infants infected with these two strains do not develop severe diarrhoea during the initial and subsequent infections (Glass et al., 2005a). Similarly, a neonatal RVA/Human-tc/AUS/RV3/1977/G3P2A[6] strain isolated in Australia was reported to induce a good immune response using a three dose regimen (Barnes et al., 1997).

In 1998, Ward and Bernstein formulated the live-attenuated monovalent vaccine, Rotarix<sup>®</sup>, formally known as vaccine 89-12 (Bernstein et al., 1998), then RIX 4414 (De Vos et al., 2004), and now marketed by GlaxoSmithKline (GSK) as a lyophilized powder preparation reconstituted in 1 ml buffer of citrate bicarbonate that can achieve titres of up to  $10^{5.8}$  focus-forming units (Salinas et al., 2005). It was prepared by attenuating the human strain RVA/Human-wt/USA/89-12/1988/G1PA[8] through 33 passages in primary African green monkey kidney (AGMK) cells and then multiple times in Vero cells (Bernstein et al., 1999). Rotarix<sup>®</sup> achieved efficacies of 85 – 93% against severe rotavirus disease, and 75% against hospitalisation from infection with other diarrhoeal pathogens in a large multicentre safety clinical trial involving up to 63, 000 infants (Ruiz-Palacios et al., 2006). Recent data on clinical trials conducted in developed countries is inconsistent. Good safety, immunogenicity and appreciable efficacy has been reported in some countries, for instance, 76.9% efficacy against severe diarrhoea in South Africa (Madhi et al., 2010) and 85 – 93% in Latin America among middle income communities (Ruiz-Palacios et al., 2006). However, efficacy of 49.4% was reported in Malawi (Madhi et al., 2010). Although Rotarix<sup>®</sup> contains only G1P1A[8] serotypes, efficacies of 75% against G2, and exceeding 95% against G3, G4 and G9 rotaviruses has been reported (Vesikari et al., 2007). The US Food and Drug Administration (FDA) approved the use of Rotarix<sup>®</sup> to prevent American children against

severe diarrhoea disease on April 3, 2008. Although PCV1 DNA has been detected in Rotarix<sup>®</sup> (Victoria et al., 2010), Baylis et al. (2011) demonstrated that this DNA does not represent the presence biologically active PCV1 virus particles.

RotaTeq<sup>®</sup> is a bovine-human reassortant pentavalent vaccine marketed by Merck. It contains five reassortant strains of which each comprises of 10 genome segments of the bovine strain RVA/Cow-tc/USA/WC3/1981/G6P[5] and a single genome segment that encodes either a human G1, G2, G3, G4 or P1A[8] outer capsid protein (Heaton et al., 2005). RotaTeq<sup>®</sup> is administered to infants in three doses. The first dose is initiated in children aged between six to ten weeks. It does not require reconstitution as it is a liquid formulation that contains  $12 \times 10^7$  infectious units per dose in citrate phosphate buffer (Clark et al., 2003). RotaTeq<sup>®</sup> has demonstrated good safety and achieved efficacies of 86% against doctors' visits, 93% against emergency department visits, and 96% against hospital admissions in double-blinded random controlled clinical trials exceeding 70,000 children that were conducted in USA and Finland (Vesikari et al., 2006c). ACIP recommended the use of RotaTeq<sup>®</sup> for routine immunisation in US children, whereas licensure for its use was granted by the FDA in 2006 (Glass et al., 2006b).

Rotarix<sup>®</sup> and RotaTeq<sup>®</sup> have been licensed in more than 100 countries at present. Both vaccines were pre-qualified by WHO and were introduced into EPI of several countries. However, better coverage may be achieved if licensure is granted in countries with vaccine regulatory authorities that meet WHO and United Nations Children's Fund (UNICEF) standards such as Brazil that has an approximately 4 million babies per year birth-rate, which is the largest in Latin America, and European Union countries (Glass et al., 2006b). Both vaccines are highly effective in preventing severe rotavirus diarrhoea in children (Linhares and Bresee, 2000, Vesikari et al., 2007, Zaman et al., 2010). In developed countries, these vaccine candidates will reduce the numbers of hospitalisation and associated costs caused by rotavirus diarrhoea. In the developing world, substantial reduction in childhood death from diarrhoea will undoubtedly be achieved through immunisation programmes (Tate et al., 2010). However, the adverse effects that have been associated with both RotaTeq<sup>®</sup> and Rotarix<sup>®</sup> as discussed in section 1.1 could be a setback towards their use in some countries. The relative high cost of these vaccines is another major concern. At the moment, Rotarix<sup>®</sup> and RotaTeq<sup>®</sup> vaccine courses costs US \$120 to \$200, respectively, in industrialised countries, whereas the two-dose course of Rotarix<sup>®</sup> cost more than US \$20 in South Africa in private sector. At the moment, GSK and Merck are coping with the yearly 30 million dose demand from countries using rotavirus vaccines (see names of countries listed in Anonymous, 2010). It is estimated that if all the countries would introduce rotavirus vaccines at a

coverage rate commiserate with that of other vaccines, demand could rise to more than 300 million doses. This may create shortages in rotavirus vaccine supply. Therefore, it is necessary that vaccine developers and suppliers from developing countries to also enter into the market (Anonymous, 2010).

Owing to some of the shortfalls of the live-attenuated vaccines described above, there is renewed interest in developing other alternative vaccines such as non-live candidates to fill the void left by Rotarix<sup>®</sup> and RotaTeq<sup>®</sup>. Table 2.3 summarises the current state of rotavirus vaccines and development. The emphases of the new alternative vaccine candidates under development include safety so that they should be administered to both immune compromised and health children without concerns. Next generations rotavirus vaccines that include RV-VLP and non-live recombinant subunit vaccines may offer safety solutions as they do not contain the genomic material hence can not replicate nor revert to virulence (Conner et al., 1993). If the next generation vaccines can be relatively inexpensive, easier to store and administer, some of the shortfalls of licensed rotavirus candidates might be solved. Technologies are being evaluated that could reduce the need for cold storage, minimize packaging costs and optimize the ease of administration. Adjuvants, which can increase the immune stimulating properties of vaccines, are being considered for some of the inactivated killed vaccines (Johansen et al., 2003). Genetic engineering of rotavirus also presents alternative possibilities in the future design of rotavirus vaccines. Such approaches have resulted into the production of rotavirus antigens that have been used as parenteral delivered vaccines in form of RV-VLPs prepared from baculovirus recombinants in insect cells (Jiang et al., 1999), expressed antigens (Choi et al., 2004), DNA vaccines (Herrmann et al., 1996, Choi et al., 2007), and killed virus (Coffin et al., 1995). Although these experimental rotavirus vaccine candidates have shown some success in animals, verification is needed before embarking on clinical trials in humans.

### **2.8.2. Virus-like particle vaccines**

VLPs are structures that do not contain genomic material but resemble the capsids of native viruses. Recent advances in biotechnology do not only allow propagation of various micro-organisms in tissue cultures, but also *in vitro* manipulation of their genomic materials. Such techniques have enabled production of VLPs that mimic numerous viruses mostly through yeast, bacteria, plant and baculovirus vector expression systems (Matic et al., 2012, Bundy et al., 2008, Crawford et al., 1994, Gavilanes et al., 1982). VLPs have been used in numerous important ways. Due to the tissue tropism of VLPs which is similar to native viruses, VLPs have been proposed as

effective delivery systems to target cells by encapsulating the space left by the viral genomes with compounds such as nucleic materials, drugs and many other compounds (Lee and Wang, 2006, Georgens et al., 2005). VLPs can also be employed for therapeutic and diagnostics purposes where relevant compounds loaded into VLPs can be delivered to target organs or cells (Lee and Wang, 2006). Viral vectored chimaeric VLP could also be applied as therapeutic vaccines for autoimmune diseases and cancer (Chackerian, 2007)

Due to the nanometric dimensions and affinity of VLPs to metals, *in situ* deposition of thin lining of metals like gold, silver, platinum and palladium onto the external surfaces of VLPs suggest potential application of VLPs in nanobiotechnology as nanomaterials such as nanotubes, nanowires, nanocrystals, nanobatteries or memory devices (Plascencia-Villa et al., 2009, Nam et al., 2008). VLPs have also been used extensively in basic research to understand the functional roles of both structural and non-structure proteins of viruses, determining the structures of viruses, understanding the virus–host interactions and many more (Lepault et al., 2001, Mathieu et al., 2001, Labbe et al., 1991). Viruses like Norwalk virus that cannot be cultured *in vitro* have also been studied by using Norwalk-VLPs (Bertolotti-Ciarlet et al., 2002).

The size of VLPs allows easier uptake by dendritic cells, whereas their structure allows them to efficiently display antigenic epitopes which are ideal for activating B cells (Grgacic and Anderson, 2006). The fact that VLPs are not infectious makes them excellent vaccine candidates (Crawford et al., 1994). Therefore, several VLP vaccine candidates against viral diseases have been formulated, of which some have been approved for use, or are in developmental phases. These include the yeast expressed quadrivalent VLP-based vaccine (Gardasil, Merk) against oncogenic human papilloma virus (HPV) types 6/7/16/18 (Grgacic and Anderson, 2006), insect cell baculovirus expressed bivalent VLP-based vaccine (Cervarix, GSK) against HPV types 16/18 (Paavonen et al., 2007), and recombinant vaccines against hepatitis B virus (HBV) produced in yeast systems (Descos, 1990). Other VLP-based vaccines under development and clinical trials include those formulated against infectious diseases caused by ebola virus, hepatitis C virus, human immunodeficiency virus (HIV), influenza virus, marburg virus, norwalk virus, rift valley fever, severe acute respiratory syndrome (SARS) and rotavirus (Chackerian, 2007, National Institute of Allergy and Infectious Diseases, 2007).

Engineering of VLP-based vaccines against rotaviruses is one of the novel strategies for developing alternative second generation subunit rotavirus vaccine candidates (Istrate et al., 2008). The ability of the recombinant structural proteins of rotaviruses to spontaneously assemble

into RV-VLPs *in vitro* has been exploited in preparing candidate vaccines (Crawford et al., 1994). RV-VLPs with only a single protein capsid layer (formed by VP2), dRV-VLPs (formed by VP2 and VP6) and tRV-VLPs (formed by VP2, VP6, VP7 and VP4) have been engineered (Bertolotti-Ciarlet et al., 2003, Crawford et al., 1994, Conner et al., 1993). The BVESs have often been utilised to prepare RV-VLPs that mimic the native rotaviruses. The resultant RV-VLPs have been evaluated as vaccine candidates in animal models through both mucosal and parenterally administration (Bertolotti-Ciarlet et al., 2003, Johansen et al., 2003, Conner et al., 1996, Conner et al., 1993).

Several reasons make RV-VLPs more attractive vaccine candidates compared to the licensed live oral vaccine candidates, for instance: (a) RV-VLPs do not replicate but are antigenically similar to native rotaviruses (Conner et al., 1996), (b) the perceived reduced vaccine-take risk associated with oral vaccines due to the pre-existing maternal antibodies transferred over the placenta and in breast milk (Moon et al., 2010) can be reduced significantly as RV-VLPs can induce immune response through parenteral administration (Ciarlet et al., 1998), (c) the risks associated with the development of chronic excretion after an oral dose administration in children with acquired immunodeficiency or/and congenital diseases is almost zero (Uyungil et al., 2010), and (d) the risks of generating new reassortants in the gut between the vaccine and the wild-type strains as has been reported with live-attenuated strains (Bucardo et al., 2012) is effectively eliminated.

Since the currently licensed rotavirus vaccines can only protect against severe diarrhoea (Vesikari et al., 2006a, Vesikari et al., 2004a), studies that may lead to the formulation of vaccine candidates capable of protecting against rotavirus infection are required to reduce the high mortality rates associated with rotaviruses. The potential of experimental VLP vaccines in protecting against rotavirus infection were evaluated in animal models administered in different combinations (VP2/6, VP2/6/7, VP2/4/6/7, VP8\*-2/6/7). Such studies yielded encouraging results that included the induction of both serum and faecal immunoglobulins through intranasal and intramuscular administration in mice (El-Attar et al., 2009, Bertolotti-Ciarlet et al., 2003, Yuan et al., 2000, Conner et al., 1996). In addition, both homologous (Conner et al., 1996), and heterologous (Crawford et al., 1999) protection against natural infection has been reported in mouse models with VLP rotavirus vaccines as also observed with live-attenuated candidates (Desselberger and Huppertz, 2011).

Most of the reported RV-VLP experimental vaccines were engineered from prototype animal rotaviruses grown in tissue culture, notably from the bovine RVA/Cow-tc/FRA/RF/1982/G6P[1], RVA/Cow-tc/USA/WC3/1981/G6P[5] and RVA/Cow-tc/GBR/UK/1973/G6P7[5] strains, and the

simian RVA/Vervet monkey-tc/ZAF/SA11-4F/1958/G3P6[1] and RVA/Simian-tc/USA/RRV/1975/G3P[3] strains. The prototype Wa human rotavirus strain has also been used, although rarely (Yuan et al., 2001, Yuan et al., 2000). Thus, so far only strains with G1 and G3 outer capsids have been targeted by most of the RV-VLP experimental rotavirus vaccines that have been formulated. As for Rotarix<sup>®</sup> and RotaTeq<sup>®</sup>, only G1, G2, G3 and G4 and P1A[8] genotypes of human rotavirus strains were incorporated in their formulation. Although the rotavirus strains prevailing in developed and developing countries do not always correlate, Rotarix<sup>®</sup> and RotaTeq<sup>®</sup> were recommended for routine use by the WHO in all countries based on the high efficacies reported in some developed countries (Vesikari et al., 2004b, Vesikari et al., 2006c) and significant reduction of the burden of rotavirus disease in Latin American countries (Richardson et al., 2010). The high regional prevalence of G3 (West Africa), G5 (Brazil), G8 (Africa) and G9 (Africa) strains and G12 rotavirus that emerged in the past two decades (Santos and Hoshino, 2005) may pose concerns regarding the effectiveness of the two licensed vaccines. It is not known how well these two licensed vaccines will cross-protect against these emerging strains and strains with different genotypes to those incorporated in the formulation of both Rotarix<sup>®</sup> and RotaTeq<sup>®</sup>. Development or improvements to the current rotavirus vaccines by taking into account the prevalent strains in developing countries may help directly to reduce the mortality rates caused by rotaviruses since most of the rotavirus-associated deaths occurs in these countries (Grimwood and Buttery, 2007).

Comparing the 95 annual rotavirus-associated deaths in USA (Parashar et al., 2006) against 188,000 in South-East Asia (Glass et al., 2005a) or the approximated 232,000 in the sub-Saharan African region (Tate et al., 2011b) clearly calls for the development of rotavirus vaccines that are effective in developing countries. The combined efficacies of 61.7% for Rotarix<sup>®</sup> in Malawi and South Africa (Madhi et al., 2010) compared to 85% in USA and up to 93% in Finland (Ruiz-Palacios et al., 2006), and also the combined RotaTeq<sup>®</sup> efficacy of 51.0% achieved in Kenya and Ghana, 51.0% in Bangladesh and Vietnam (Armah et al., 2010) compared to up to 96% in the USA (Vesikari et al., 2006c) may strengthen the need for further formulation of vaccine candidates applicable to developing countries. In addition to differences in the circulating rotavirus strain between developed and developing countries, several other reasons may contribute to the varied rotavirus vaccine efficacies. Patel et al. (2009) suggested that the differences in maternal antibody titres, breast feeding practices, the quantities of stomach acid, micronutrient malnutrition, the role of the interfering gut flora, acute and chronic disease conditions in children that could impair their immune response elicited by rotavirus vaccine are some of the reasons that may explain why the efficacy of the rotavirus vaccines seems to vary in developed and developing

countries. Therefore, in order to achieve formulation of potent future rotavirus vaccines, all these factors need to be taken into consideration. In this study, recombinant baculoviruses were produced using the genome segments encoding structural proteins of some of the frequently characterised rotavirus strains in Africa and strains that emerged in the past two decades globally. The nucleotide sequences of these strains were obtained through next generation 454<sup>®</sup> pyrosequencing. The recombinant baculoviruses were used to express recombinant rotavirus proteins and prepare chimaeric RV-VLPs that will be evaluated as a vaccine candidate in future.

## 2.8. References

- ADAMS, W. R. & KRAFT, L. M. 1963. Epizootic diarrhea of infant mice: identification of the etiologic agent. *Science*, 141, 359-60.
- AFRIKANOVA, I., FABBRETTI, E., MIOZZO, M. C. & BURRONE, O. R. 1998. Rotavirus NSP5 phosphorylation is up-regulated by interaction with NSP2. *J Gen Virol*, 79 ( Pt 11), 2679-86.
- AGOSTO, M. A., IVANOVIC, T. & NIBERT, M. L. 2006. Mammalian reovirus, a nonfusogenic nonenveloped virus, forms size-selective pores in a model membrane. *Proc Nat Acad Sci USA*, 103, 16496-501.
- ALFIERI, A. A., LEITE, J. P., NAKAGOMI, O., KAGA, E., WOODS, P. A., GLASS, R. I. & GENTSCH, J. R. 1996. Characterization of human rotavirus genotype P[8]G5 from Brazil by probe-hybridization and sequence. *Arch Virol*, 141, 2353-64.
- ALTENBURG, B. C., GRAHAM, D. Y. & ESTES, M. K. 1980. Ultrastructural study of rotavirus replication in cultured cells. *J Gen Virol*, 46, 75-85.
- ANONYMOUS. 9th International Rotavirus Symposium, 2 - 3 August 2010 Johannesburg, South Africa. Sabin Vaccine Institute, 1 - 60.
- 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., SCHODEL, F., CIARLET, M. & 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*, 376, 606-14.
- ASENSI, M. T., MARTINEZ-COSTA, C. & BUESA, J. 2006. Anti-rotavirus antibodies in human milk: quantification and neutralizing activity. *J Pediatr Gastroenterol Nutr*, 42, 560-7.
- AU, K. S., CHAN, W. K., BURNS, J. W. & ESTES, M. K. 1989. Receptor activity of rotavirus nonstructural glycoprotein NS28. *J Virol*, 63, 4553-62.
- AU, K. S., MATTION, N. M. & ESTES, M. K. 1993. A subviral particle binding domain on the rotavirus nonstructural glycoprotein NS28. *Virology*, 194, 665-73.
- AU, K. S. & SIU, Y. L. 1992. Variations in Ca(2+)-mediated activation of Ca(2+)-ATPase and its associated inhibitor in erythrocyte membrane. *Int J Biochem*, 24, 1169-73.
- BARNES, G. L. & BISHOP, R. F. 1997. Rotavirus infection and prevention. *Curr Opin Pediatr*, 9, 19-23.
- BARNES, G. L., LUND, J. S., ADAMS, L., MORA, A., MITCHELL, S. V., CAPLES, A. & BISHOP, R. F. 1997. Phase 1 trial of a candidate rotavirus vaccine (RV3) derived from a human neonate. *J Paediatr Child Health*, 33, 300-4.
- BARRO, M. & PATTON, J. T. 2005. Rotavirus nonstructural protein 1 subverts innate immune response by inducing degradation of IFN regulatory factor 3. *Proc Natl Acad Sci U S A*, 102, 4114-9.
- BASS, D. M. 1997. Interferon gamma and interleukin 1, but not interferon alfa, inhibit rotavirus entry into human intestinal cell lines. *Gastroenterology*, 113, 81-9.
- BASTARDO, J. W. & HOLMES, I. H. 1980. Attachment of SA-11 rotavirus to erythrocyte receptors. *Infect Immun*, 29, 1134-40.

- BAYLIS, S. A., FINSTERBUSCH, T., BANNERT, N., BLUMEL, J. & MANKERTZ, A. 2011. Analysis of porcine circovirus type 1 detected in Rotarix vaccine. *Vaccine*, 29, 690-7.
- BENUREAU, Y., HUET, J. C., CHARPILLENNE, A., PONCET, D. & COHEN, J. 2005. Trypsin is associated with the rotavirus capsid and is activated by solubilization of outer capsid proteins. *J Gen Virol*, 86, 3143-51.
- BERGMANN, C. C., MAASS, D., PORUCHYNSKY, M. S., ATKINSON, P. H. & BELLAMY, A. R. 1989. Topology of the non-structural rotavirus receptor glycoprotein NS28 in the rough endoplasmic reticulum. *The EMBO journal*, 8, 1695-703.
- BERNSTEIN, D. I., SACK, D. A., ROTHSTEIN, E., REISINGER, K., SMITH, V. E., O'SULLIVAN, D., SPRIGGS, D. R. & WARD, R. L. 1999. Efficacy of live, attenuated, human rotavirus vaccine 89-12 in infants: a randomised placebo-controlled trial. *Lancet*, 354, 287-90.
- BERNSTEIN, D. I., SMITH, V. E., SHERWOOD, J. R., SCHIFF, G. M., SANDER, D. S., DEFEUDIS, D., SPRIGGS, D. R. & WARD, R. L. 1998. Safety and immunogenicity of live, attenuated human rotavirus vaccine 89-12. *Vaccine*, 16, 381-7.
- BEROIS, M., SAPIN, C., ERK, I., PONCET, D. & COHEN, J. 2003. Rotavirus nonstructural protein NSP5 interacts with major core protein VP2. *J Virol*, 77, 1757-63.
- BERTOLOTTI-CIARLET, A., CIARLET, M., CRAWFORD, S. E., CONNER, M. E. & ESTES, M. K. 2003. Immunogenicity and protective efficacy of rotavirus 2/6-virus-like particles produced by a dual baculovirus expression vector and administered intramuscularly, intranasally, or orally to mice. *Vaccine*, 21, 3885-900.
- BERTOLOTTI-CIARLET, A., WHITE, L. J., CHEN, R., PRASAD, B. V. & ESTES, M. K. 2002. Structural requirements for the assembly of Norwalk virus-like particles. *J Virol*, 76, 4044-55.
- BISHOP, R. F., BARNES, G. L., CIPRIANI, E. & LUND, J. S. 1983. Clinical immunity after neonatal rotavirus infection. A prospective longitudinal study in young children. *New Engl J Med*, 309, 72-6.
- BISHOP, R. F., DAVIDSON, G. P., HOLMES, I. H. & RUCK, B. J. 1973. Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. *Lancet*, 2, 1281-3.
- BISHOP, R. F., DAVIDSON, G. P., HOLMES, I. H. & RUCK, B. J. 1974. Detection of a new virus by electron microscopy of faecal extracts from children with acute gastroenteritis. *Lancet*, 1, 149-51.
- BLUTT, S. E., KIRKWOOD, C. D., PARRENO, V., WARFIELD, K. L., CIARLET, M., ESTES, M. K., BOK, K., BISHOP, R. F. & CONNER, M. E. 2003. Rotavirus antigenaemia and viraemia: a common event? *Lancet*, 362, 1445-9.
- BOYCE, M., CELMA, C. C. & ROY, P. 2008. Development of reverse genetics systems for bluetongue virus: recovery of infectious virus from synthetic RNA transcripts. *J Virol*, 82, 8339-48.
- BRIDGER, J. C. 1987. Non-group A rotaviruses. In: KAPIKIAN, A. Z. (ed.) *Viral infections of the gastrointestinal tract*. New York: Marcel Dekker.
- BRYCE, J., BOSCHI-PINTO, C., SHIBUYA, K. & BLACK, R. E. 2005. WHO estimates of the causes of death in children. *Lancet*, 365, 1147-52.
- BUCARDO, F., RIPPINGER, C. M., SVENSSON, L. & PATTON, J. T. 2012. Vaccine-derived NSP2 segment in rotaviruses from vaccinated children with gastroenteritis in Nicaragua. *Infect Genet Evol*, April 2 [Epub ahead of print].
- BUNDY, B. C., FRANCISZKOWICZ, M. J. & SWARTZ, J. R. 2008. Escherichia coli-based cell-free synthesis of virus-like particles. *Biotechnol Bioeng*, 100, 28-37.
- BURNS, J. W., SIADAT-PAJOUH, M., KRISHNANEY, A. A. & GREENBERG, H. B. 1996. Protective effect of rotavirus VP6-specific IgA monoclonal antibodies that lack neutralizing activity. *Science*, 272, 104-7.
- CAUL, E. O., ASHLEY, C. R., DARVILLE, J. M. & BRIDGER, J. C. 1990. Group C rotavirus associated with fatal enteritis in a family outbreak. *J Med Virol*, 30, 201-5.
- CHACKERIAN, B. 2007. Virus-like particles: flexible platforms for vaccine development. *Expert review of vaccines*, 6, 381-90.
- CHAN, J., NIRWATI, H., TRIASIH, R., BOGDANOVIC-SAKRAN, N., SOENARTO, Y., HAKIMI, M., DUKE, T., BUTTERY, J. P., BINES, J. E., BISHOP, R. F., KIRKWOOD, C. D. & DANCHIN, M. D. 2011. Maternal antibodies to rotavirus: could they interfere with live rotavirus vaccines in developing countries? *Vaccine*, 29, 1242-7.

- CHEMELLO, M. E., ARISTIMUNO, O. C., MICHELANGELI, F. & RUIZ, M. C. 2002. Requirement for vacuolar H<sup>+</sup>-ATPase activity and Ca<sup>2+</sup> gradient during entry of rotavirus into MA104 cells. *J Virol*, 76, 13083-7.
- CHEN, D., LUONGO, C. L., NIBERT, M. L. & PATTON, J. T. 1999. Rotavirus open cores catalyze 5'-capping and methylation of exogenous RNA: evidence that VP3 is a methyltransferase. *Virology*, 265, 120-30.
- CHEN, D. Y., ESTES, M. K. & RAMIG, R. F. 1992. Specific interactions between rotavirus outer capsid proteins VP4 and VP7 determine expression of a cross-reactive, neutralizing VP4-specific epitope. *J Virol*, 66, 432-9.
- CHEN, J. Z., SETTEMBRE, E. C., AOKI, S. T., ZHANG, X., BELLAMY, A. R., DORMITZER, P. R., HARRISON, S. C. & GRIGORIEFF, N. 2009. Molecular interactions in rotavirus assembly and uncoating seen by high-resolution cryo-EM. *Proc Natl Acad Sci U S A*, 106, 10644-8.
- CHIBA, S., YOKOYAMA, T., NAKATA, S., MORITA, Y., URASAWA, T., TANIGUCHI, K., URASAWA, S. & NAKAO, T. 1986. Protective effect of naturally acquired homotypic and heterotypic rotavirus antibodies. *Lancet*, 2, 417-21.
- CHOI, A. H., BASU, M., MCNEAL, M. M., BEAN, J. A., CLEMENTS, J. D. & WARD, R. L. 2004. Intranasal administration of an Escherichia coli-expressed codon-optimized rotavirus VP6 protein induces protection in mice. *Protein Expr Purif*, 38, 205-16.
- CHOI, A. H., SMILEY, K., BASU, M., MCNEAL, M. M., SHAO, M., BEAN, J. A., CLEMENTS, J. D., STOUT, R. R. & WARD, R. L. 2007. Protection of mice against rotavirus challenge following intradermal DNA immunization by Biojector needle-free injection. *Vaccine*, 25, 3215-8.
- CIARLET, M., CRAWFORD, S. E., BARONE, C., BERTOLOTTI-CIARLET, A., RAMIG, R. F., ESTES, M. K. & CONNER, M. E. 1998. Subunit rotavirus vaccine administered parenterally to rabbits induces active protective immunity. *J Virol*, 72, 9233-46.
- CIARLET, M. & ESTES, M. K. 1999. Human and most animal rotavirus strains do not require the presence of sialic acid on the cell surface for efficient infectivity. *J Gen Virol*, 80 ( Pt 4), 943-8.
- CIARLET, M., LUDERT, J. E., ITURRIZA-GOMARA, M., LIPRANDI, F., GRAY, J. J., DESSELBERGER, U. & ESTES, M. K. 2002. Initial interaction of rotavirus strains with N-acetylneuraminic (sialic) acid residues on the cell surface correlates with VP4 genotype, not species of origin. *J Virol*, 76, 4087-95.
- CIOC, A. M. & NUOVO, G. J. 2002. Histologic and in situ viral findings in the myocardium in cases of sudden, unexpected death. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc*, 15, 914-22.
- CLARK, H. F., BURKE, C. J., VOLKIN, D. B., OFFIT, P., WARD, R. L., BRESEE, J. S., DENNEHY, P., GOOCH, W. M., MALACAMAN, E., MATSON, D., WALTER, E., WATSON, B., KRAH, D. L., DALLAS, M. J., SCHODEL, F., KAPLAN, M. & HEATON, P. 2003. Safety, immunogenicity and efficacy in healthy infants of G1 and G2 human reassortant rotavirus vaccine in a new stabilizer/buffer liquid formulation. *Pediatr Infect Dis J*, 22, 914-20.
- CLARK, H. F., OFFIT, P. A., ELLIS, R. W., EIDEN, J. J., KRAH, D., SHAW, A. R., PICHICHERO, M., TREANOR, J. J., BORIAN, F. E., BELL, L. M. & PLOTKIN, S. A. 1996. The development of multivalent bovine rotavirus (strain WC3) reassortant vaccine for infants. *J Infect Dis*, 174 Suppl 1, S73-80.
- CLEMENTS-MANN, M. L., MAKHENE, M. K., MRUKOWICZ, J., WRIGHT, P. F., HOSHINO, Y., MIDTHUN, K., SPERBER, E., KARRON, R. & KAPIKIAN, A. Z. 1999. Safety and immunogenicity of live attenuated human-bovine (UK) reassortant rotavirus vaccines with VP7-specificity for serotypes 1, 2, 3 or 4 in adults, children and infants. *Vaccine*, 17, 2715-25.
- COFFIN, S. E., KLINEK, M. & OFFIT, P. A. 1995. Induction of virus-specific antibody production by lamina propria lymphocytes following intramuscular inoculation with rotavirus. *J Infect Dis*, 172, 874-8.
- COHEN, J., LAPORTE, J., CHARPILLENNE, A. & SCHERRER, R. 1979. Activation of rotavirus RNA polymerase by calcium chelation. *Arch Virol*, 60, 177-86.
- CONNER, M. E., CRAWFORD, S. E., BARONE, C. & ESTES, M. K. 1993. Rotavirus vaccine administered parenterally induces protective immunity. *J Virol*, 67, 6633-41.

- CONNER, M. E., ZARLEY, C. D., HU, B., PARSONS, S., DRABINSKI, D., GREINER, S., SMITH, R., JIANG, B., CORSARO, B., BARNIAK, V., MADORE, H. P., CRAWFORD, S. & ESTES, M. K. 1996. Virus-like particles as a rotavirus subunit vaccine. *J Infect Dis*, 174 Suppl 1, S88-92.
- COULSON, B. S., GRIMWOOD, K., HUDSON, I. L., BARNES, G. L. & BISHOP, R. F. 1992. Role of coproantibody in clinical protection of children during reinfection with rotavirus. *J Clin Microbiol*, 30, 1678-84.
- CRAWFORD, S. E., ESTES, M. K., CIARLET, M., BARONE, C., O'NEAL, C. M., COHEN, J. & CONNER, M. E. 1999. Heterotypic protection and induction of a broad heterotypic neutralization response by rotavirus-like particles. *J Virol*, 73, 4813-22.
- CRAWFORD, S. E., LABBE, M., COHEN, J., BURROUGHS, M. H., ZHOU, Y. J. & ESTES, M. K. 1994. Characterization of virus-like particles produced by the expression of rotavirus capsid proteins in insect cells. *J Virol*, 68, 5945-52.
- CRAWFORD, S. E., PATEL, D. G., CHENG, E., BERKOVA, Z., HYSER, J. M., CIARLET, M., FINEGOLD, M. J., CONNER, M. E. & ESTES, M. K. 2006. Rotavirus viremia and extraintestinal viral infection in the neonatal rat model. *J Virol*, 80, 4820-32.
- CUNLIFFE, N. A., GONDWE, J. S., BROADHEAD, R. L., MOLYNEUX, M. E., WOODS, P. A., BRESEE, J. S., GLASS, R. I., GENTSCH, J. R. & HART, C. A. 1999. Rotavirus G and P types in children with acute diarrhea in Blantyre, Malawi, from 1997 to 1998: predominance of novel P[6]G8 strains. *J Med Virol*, 57, 308-12.
- CUNLIFFE, N. A., GONDWE, J. S., GRAHAM, S. M., THINDWA, B. D., DOVE, W., BROADHEAD, R. L., MOLYNEUX, M. E. & HART, C. A. 2001. Rotavirus strain diversity in Blantyre, Malawi, from 1997 to 1999. *J Clin Microbiol*, 39, 836-43.
- CUNLIFFE, N. A., KILGORE, P. E., BRESEE, J. S., STEELE, A. D., LUO, N., HART, C. A. & GLASS, R. I. 1998. Epidemiology of rotavirus diarrhoea in Africa: a review to assess the need for rotavirus immunization. *Bull World Health Organ*, 76, 525-37.
- CUNLIFFE, N. A., NGWIRA, B. M., DOVE, W., NAKAGOMI, O., NAKAGOMI, T., PEREZ, A., HART, C. A., KAZEMBE, P. N. & MWANSAMBO, C. C. 2009. Serotype g12 rotaviruses, Lilongwe, Malawi. *Emerg Infect Dis*, 15, 87-90.
- DE VOS, B., VESIKARI, T., LINHARES, A. C., SALINAS, B., PEREZ-SCHAEL, I., RUIZ-PALACIOS, G. M., GUERRERO MDE, L., PHUA, K. B., DELEM, A. & HARDT, K. 2004. A rotavirus vaccine for prophylaxis of infants against rotavirus gastroenteritis. *Pediatr Infect Dis J*, 23, S179-82.
- DECTOR, M. A., ROMERO, P., LOPEZ, S. & ARIAS, C. F. 2002. Rotavirus gene silencing by small interfering RNAs. *EMBO Rep*, 3, 1175-80.
- DENISOVA, E., DOWLING, W., LAMONICA, R., SHAW, R., SCARLATA, S., RUGGERI, F. & MACKOW, E. R. 1999. Rotavirus capsid protein VP5\* permeabilizes membranes. *J Virol*, 73, 3147-53.
- DESCOS, B. 1990. [Vaccination against hepatitis B virus]. *Pediatric*, 45, 173-9.
- DESSELBERGER, U. 1999. Rotavirus infections: guidelines for treatment and prevention. *Drugs*, 58, 447-52.
- DESSELBERGER, U. 2002. Reoviruses. In: GREENWOOD, D., SLACK, R. C. B. & PEUTHERER, J. F. (eds.) *Medical microbiology, a guideline to microbial infections: pathogenesis, immunity, laboratory diagnosis and control* Sixth ed. London: Churchill Livingstone.
- DESSELBERGER, U. & HUPPERTZ, H. I. 2011. Immune responses to rotavirus infection and vaccination and associated correlates of protection. *J Infect Dis*, 203, 188-95.
- DESSELBERGER, U., ITURRIZA-GOMARA, M. & GRAY, J. J. 2001. Rotavirus epidemiology and surveillance. *Novartis Found Symp*, 238, 125-47; discussion 147-52.
- DESSELBERGER, U., MANKTELOW, E., LI, W., CHEUNG, W., ITURRIZA-GOMARA, M. & GRAY, J. 2009. Rotaviruses and rotavirus vaccines. *Br Med Bull*, 90, 37-51.
- DORMITZER, P. R., HO, D. Y., MACKOW, E. R., MOCARSKI, E. S. & GREENBERG, H. B. 1992. Neutralizing epitopes on herpes simplex virus-1-expressed rotavirus VP7 are dependent on coexpression of other rotavirus proteins. *Virology*, 187, 18-32.
- 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, 1053-8.

- EL-ATTAR, L., OLIVER, S. L., MACKIE, A., CHARPILLENNE, A., PONCET, D., COHEN, J. & BRIDGER, J. C. 2009. Comparison of the efficacy of rotavirus VLP vaccines to a live homologous rotavirus vaccine in a pig model of rotavirus disease. *Vaccine*, 27, 3201-8.
- ESTES, M. 2001. Rotaviruses and their replication. In: KNIPE, D. M. & HOWLEY, P. M. (eds.) *Fields virology*. fourth ed. Philadelphia: Lippincott Williams & Wilkins.
- ESTES, M. K. & COHEN, J. 1989. Rotavirus gene structure and function. *Microbiol Rev*, 53, 410-49.
- ESTES, M. K., GRAHAM, D. Y. & MASON, B. B. 1981. Proteolytic enhancement of rotavirus infectivity: molecular mechanisms. *J Virol*, 39, 879-88.
- ESTES, M. K., GRAHAM, D. Y., SMITH, E. M. & GERBA, C. P. 1979. Rotavirus stability and inactivation. *J Gen Virol*, 43, 403-9.
- ESTES, M. K. & KAPIKIAN, A. Z. 2007. Rotaviruses. In: KNIPE, D. M., HOWLEY, P.M., GRIFFIN, & D.E., L., R.A., MARTIN, M.A., ROIZMAN, B., STRAUS, S.E. (eds.) *Fields Virology*. Fifth edition ed. Philadelphia: Lippincott Williams & Wilkins.
- FENG, N., BURNS, J. W., BRACY, L. & GREENBERG, H. B. 1994. Comparison of mucosal and systemic humoral immune responses and subsequent protection in mice orally inoculated with a homologous or a heterologous rotavirus. *J Virol*, 68, 7766-73.
- FERNELIUS, A. L., RITCHIE, A. E., CLASSICK, L. G., NORMAN, J. O. & MEBUS, C. A. 1972. Cell culture adaptation and propagation of a reovirus-like agent of calf diarrhea from a field outbreak in Nebraska. *Arch Gesamte Virusforsch*, 37, 114-30.
- FISCHER, T. K., ASHLEY, D., KERIN, T., REYNOLDS-HEDMANN, E., GENTSCH, J., WIDDOWSON, M. A., WESTERMAN, L., PUHR, N., TURCIOS, R. M. & GLASS, R. I. 2005. Rotavirus antigenemia in patients with acute gastroenteritis. *J Infect Dis*, 192, 913-9.
- FLEWETT, T. H. 1978. Electron microscopy in the diagnosis of infectious diarrhea. *J Am Vet Med Assoc*, 173, 538-43.
- FLEWETT, T. H., BRYDEN, A. S. & DAVIES, H. 1973. Letter: Virus particles in gastroenteritis. *Lancet*, 2, 1497.
- 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*, 2, 61-3.
- FLEWETT, T. H., THOULESS, M. E., PILFOLD, J. N., BRYDEN, A. S. & CANDEIAS, J. A. 1978. More serotypes of human rotavirus. *Lancet*, 2, 632.
- FUKUHARA, N., YOSHIE, O., KITAOKA, S., KONNO, T. & ISHIDA, N. 1987. Evidence for endocytosis-independent infection by human rotavirus. *Arch Virol*, 97, 93-9.
- GALLEGOS, C. O. & PATTON, J. T. 1989. Characterization of rotavirus replication intermediates: a model for the assembly of single-shelled particles. *Virology*, 172, 616-27.
- GARAICOECHA, L., OLIHON, A., MARCOPPIDO, G., WIGDOROVITZ, A., MOZGOVOJ, M., SAIF, L., SURREY, T. & PARRENO, V. 2008. Llama-derived single-chain antibody fragments directed to rotavirus VP6 protein possess broad neutralizing activity in vitro and confer protection against diarrhea in mice. *J Virol*, 82, 9753-64.
- GARY, G. W., JR., BLACK, D. R. & PALMER, E. 1982. Monoclonal IgG to the inner capsid of human rotavirus. *Arch Virol*, 72, 223-7.
- GAVILANES, F., GONZALEZ-ROS, J. M. & PETERSON, D. L. 1982. Structure of hepatitis B surface antigen. Characterization of the lipid components and their association with the viral proteins. *J Biol Chem*, 257, 7770-7.
- GENTSCH, J. R., WOODS, P. A., RAMACHANDRAN, M., DAS, B. K., LEITE, J. P., ALFIERI, A., KUMAR, R., BHAN, M. K. & GLASS, R. I. 1996. Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. *J Infect Dis*, 174 Suppl 1, S30-6.
- GEORGENS, C., WEYERMANN, J. & ZIMMER, A. 2005. Recombinant virus like particles as drug delivery system. *Curr Pharm Biotechnol*, 6, 49-55.
- GLASS, R. I., BHAN, M. K., RAY, P., BAHL, R., PARASHAR, U. D., GREENBERG, H., RAO, C. D., BHANDARI, N., MALDONADO, Y., WARD, R. L., BERNSTEIN, D. I. & GENTSCH, J. R. 2005a. Development of candidate rotavirus vaccines derived from neonatal strains in India. *J Infect Dis*, 192 Suppl 1, S30-5.

- GLASS, R. I., BRESEE, J., JIANG, B., PARASHAR, U., YEE, E. & GENTSCH, J. 2006a. Rotavirus and rotavirus vaccines. *Adv Exp Med Biol*, 582, 45-54.
- GLASS, R. I., BRESEE, J. S., PARASHAR, U., TURCIOS, R., FISCHER, T. K., JIANG, B., WIDDOWSON, M. A. & GENTSCH, J. 2005b. Rotavirus vaccines: past, present, and future. *Arch Pediatr*, 12, 844-7.
- GLASS, R. I., PARASHAR, U. D., BRESEE, J. S., TURCIOS, R., FISCHER, T. K., WIDDOWSON, M. A., JIANG, B. & GENTSCH, J. R. 2006b. Rotavirus vaccines: current prospects and future challenges. *Lancet*, 368, 323-32.
- GLOBAL POLIO ERADICATION INITIATIVE. 2011. *Polio Communications Quarterly Update* [Online]. [http://polioinfo.org/downloads/reports/f\\_IMB\\_Q4-2011\\_20120124\\_Web.pdf](http://polioinfo.org/downloads/reports/f_IMB_Q4-2011_20120124_Web.pdf). [Accessed 18 April 2012].
- GONZALEZ, R. A., ESPINOSA, R., ROMERO, P., LOPEZ, S. & ARIAS, C. F. 2000. Relative localization of viroplasmic and endoplasmic reticulum-resident rotavirus proteins in infected cells. *Arch Virol*, 145, 1963-73.
- GONZALEZ, R. A., TORRES-VEGA, M. A., LOPEZ, S. & ARIAS, C. F. 1998. In vivo interactions among rotavirus nonstructural proteins. *Arch Virol*, 143, 981-96.
- GORZIGLIA, M. & ESPARZA, J. 1981. Poly(A) polymerase activity in human rotavirus. *J Gen Virol*, 53, 357-62.
- GOUVEA, V. & BRANTLY, M. 1995. Is rotavirus a population of reassortants? *Trends Microbiol*, 3, 159-62.
- GOUVEA, V., GLASS, R. I., WOODS, P., TANIGUCHI, K., CLARK, H. F., FORRESTER, B. & FANG, Z. Y. 1990a. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. *J Clin Microbiol*, 28, 276-82.
- GOUVEA, V., HO, M. S., GLASS, R., WOODS, P., FORRESTER, B., ROBINSON, C., ASHLEY, R., RIEPENHOFF-TALTY, M., CLARK, H. F., TANIGUCHI, K. & ET AL. 1990b. Serotypes and electropherotypes of human rotavirus in the USA: 1987-1989. *J Infect Dis*, 162, 362-7.
- GRAHAM, K. L., TAKADA, Y. & COULSON, B. S. 2006. Rotavirus spike protein VP5\* binds alpha2beta1 integrin on the cell surface and competes with virus for cell binding and infectivity. *J Gen Virol*, 87, 1275-83.
- GREEN, K. Y. & KAPIKIAN, A. Z. 1992. Identification of VP7 epitopes associated with protection against human rotavirus illness or shedding in volunteers. *J Virol*, 66, 548-53.
- GREEN, K. Y., SARASINI, A., QIAN, Y. & GERNA, G. 1992. Genetic variation in rotavirus serotype 4 subtypes. *Virology*, 188, 362-8.
- GREENBERG, H., MCAULIFFE, V., VALDESUSO, J., WYATT, R., FLORES, J., KALICA, A., HOSHINO, Y. & SINGH, N. 1983a. Serological analysis of the subgroup protein of rotavirus, using monoclonal antibodies. *Infect Immun*, 39, 91-9.
- GREENBERG, H. B., FLORES, J., KALICA, A. R., WYATT, R. G. & JONES, R. 1983b. Gene coding assignments for growth restriction, neutralization and subgroup specificities of the W and DS-1 strains of human rotavirus. *J Gen Virol*, 64 (Pt 2), 313-20.
- GREENBERG, H. B., KALICA, A. R., WYATT, R. G., JONES, R. W., KAPIKIAN, A. Z. & CHANOCK, R. M. 1981. Rescue of noncultivable human rotavirus by gene reassortment during mixed infection with ts mutants of a cultivatable bovine rotavirus. *Proc Natl Acad Sci U S A*, 78, 420-4.
- GREENBERG, H. B., WYATT, R. G., KAPIKIAN, A. Z., KALICA, A. R., FLORES, J. & JONES, R. 1982. Rescue and serotypic characterization of noncultivable human rotavirus by gene reassortment. *Infect Immun*, 37, 104-9.
- GRGACIC, E. V. & ANDERSON, D. A. 2006. Virus-like particles: passport to immune recognition. *Methods*, 40, 60-5.
- GRIMWOOD, K. & BINES, J. E. 2007. Rotavirus vaccines must perform in low-income countries too. *Lancet*, 370, 1739-40.
- GRIMWOOD, K. & BUTTERY, J. P. 2007. Clinical update: rotavirus gastroenteritis and its prevention. *Lancet*, 370, 302-4.
- GUERRERO, C. A., BOUYSSOUNADE, D., ZARATE, S., ISA, P., LOPEZ, T., ESPINOSA, R., ROMERO, P., MENDEZ, E., LOPEZ, S. & ARIAS, C. F. 2002. Heat shock cognate protein 70 is involved in rotavirus cell entry. *J Virol*, 76, 4096-102.

- HAGBOM, M., ISTRATE, C., ENGBLOM, D., KARLSSON, T., RODRIGUEZ-DIAZ, J., BUESA, J., TAYLOR, J. A., LOITTO, V. M., MAGNUSSON, K. E., AHLMAN, H., LUNDGREN, O. & SVENSSON, L. 2011. Rotavirus stimulates release of serotonin (5-HT) from human enterochromaffin cells and activates brain structures involved in nausea and vomiting. *PLoS Pathog*, 7, e1002115.
- HAI, R., MARTINEZ-SOBRIDO, L., FRASER, K. A., AYLON, J., GARCIA-SASTRE, A. & PALESE, P. 2008. Influenza B virus NS1-truncated mutants: live-attenuated vaccine approach. *J Virol*, 82, 10580-90.
- HAMILTON, J. R., MIDDLETON, P. J. & GALL, D. G. 1974. Letter: Virus particles in duodenal biopsy specimens. *Lancet*, 1, 633.
- HASELHORST, T., FLEMING, F. E., DYASON, J. C., HARTNELL, R. D., YU, X., HOLLOWAY, G., SANTEGOETS, K., KIEFEL, M. J., BLANCHARD, H., COULSON, B. S. & VON ITZSTEIN, M. 2009. Sialic acid dependence in rotavirus host cell invasion. *Nat Chem Biol*, 5, 91-3.
- HE, S. T., HE, F. Z., WU, C. R., LI, S. X., LIU, W. X., YANG, Y. F., JIANG, S. S. & HE, G. 2001. Treatment of rotaviral gastroenteritis with Qiwei Baizhu powder. *World J Gastroenterol*, 7, 735-40.
- HEATON, P. M., GOVEIA, M. G., MILLER, J. M., OFFIT, P. & CLARK, H. F. 2005. Development of a pentavalent rotavirus vaccine against prevalent serotypes of rotavirus gastroenteritis. *J Infect Dis*, 192 Suppl 1, S17-21.
- HELMBERGER-JONES, M. & PATTON, J. T. 1986. Characterization of subviral particles in cells infected with simian rotavirus SA11. *Virology*, 155, 655-65.
- HERRMANN, J. E., CHEN, S. C., FYNAN, E. F., SANTORO, J. C., GREENBERG, H. B., WANG, S. & ROBINSON, H. L. 1996. Protection against rotavirus infections by DNA vaccination. *J Infect Dis*, 174 Suppl 1, S93-7.
- HEWISH, M. J., TAKADA, Y. & COULSON, B. S. 2000. Integrins alpha2beta1 and alpha4beta1 can mediate SA11 rotavirus attachment and entry into cells. *J Virol*, 74, 228-36.
- HUANG, H., SCHROEDER, F., ZENG, C., ESTES, M. K., SCHOER, J. K. & BALL, J. M. 2001. Membrane interactions of a novel viral enterotoxin: rotavirus nonstructural glycoprotein NSP4. *Biochemistry*, 40, 4169-80.
- HUNG, T., CHEN, G. M., WANG, C. G., YAO, H. L., FANG, Z. Y., CHAO, T. X., CHOU, Z. Y., YE, W., CHANG, X. J., DEN, S. S. & ET AL. 1984. Waterborne outbreak of rotavirus diarrhoea in adults in China caused by a novel rotavirus. *Lancet*, 1, 1139-42.
- HUNG, T., FAN, R. L., WANG, C. A., CHEN, G. M., CHOU, D. N., CHANG, J. Q., MCCRAE, M. A., WANG, W. Z., SE, W. Z., DAN, R. & ET AL. 1985. Seroepidemiology of adult rotavirus. *Lancet*, 2, 325-6.
- ISTRATE, C., HINKULA, J., CHARPILLENNE, A., PONCET, D., COHEN, J., SVENSSON, L. & JOHANSEN, K. 2008. Parenteral administration of RF 8-2/6/7 rotavirus-like particles in a one-dose regimen induce protective immunity in mice. *Vaccine*, 26, 4594-601.
- ITURRIZA GOMARA, M., WONG, C., BLOME, S., DESSELBERGER, U. & GRAY, J. 2002a. Molecular characterization of VP6 genes of human rotavirus isolates: correlation of genogroups with subgroups and evidence of independent segregation. *J Virol*, 76, 6596-601.
- ITURRIZA GOMARA, M., WONG, C., BLOME, S., DESSELBERGER, U. & GRAY, J. 2002b. Rotavirus subgroup characterisation by restriction endonuclease digestion of a cDNA fragment of the VP6 gene. *J Virol Methods*, 105, 99-103.
- JAMES, V. L., LAMBDEN, P. R., CAUL, E. O., COOKE, S. J. & CLARKE, I. N. 1997. Seroepidemiology of human group C rotavirus in the UK. *J Med Virol*, 52, 86-91.
- JAYARAM, H., ESTES, M. K. & PRASAD, B. V. 2004. Emerging themes in rotavirus cell entry, genome organization, transcription and replication. *Virus Res*, 101, 67-81.
- JIANG, B., ESTES, M. K., BARONE, C., BARNIAK, V., O'NEAL, C. M., OTTAIANO, A., MADORE, H. P. & CONNER, M. E. 1999. Heterotypic protection from rotavirus infection in mice vaccinated with virus-like particles. *Vaccine*, 17, 1005-13.
- JIANG, J. Y. 1985. [Detection of rotavirus by polyacrylamide gel electrophoresis]. *Zhonghua Yi Xue Za Zhi*, 65, 335-7.

- JIANG, X., JAYARAM, H., KUMAR, M., LUDTKE, S. J., ESTES, M. K. & PRASAD, B. V. 2006. Cryoelectron microscopy structures of rotavirus NSP2-NSP5 and NSP2-RNA complexes: implications for genome replication. *J Virol*, 80, 10829-35.
- JOHANSEN, K., SCHRODER, U. & SVENSSON, L. 2003. Immunogenicity and protective efficacy of a formalin-inactivated rotavirus vaccine combined with lipid adjuvants. *Vaccine*, 21, 368-75.
- JOLLY, C. L., HUANG, J. A. & HOLMES, I. H. 2001. Selection of rotavirus VP4 cell receptor binding domains for MA104 cells using a phage display library. *J Virol Methods*, 98, 41-51.
- KABCENELL, A. K. & ATKINSON, P. H. 1985. Processing of the rough endoplasmic reticulum membrane glycoproteins of rotavirus SA11. *J Cell Biol*, 101, 1270-80.
- KABCENELL, A. K., PORUCHYNSKY, M. S., BELLAMY, A. R., GREENBERG, H. B. & ATKINSON, P. H. 1988. Two forms of VP7 are involved in assembly of SA11 rotavirus in endoplasmic reticulum. *J Virol*, 62, 2929-41.
- KALICA, A. R., SERENO, M. M., WYATT, R. G., MEBUS, C. A., CHANOCK, R. M. & KAPIKIAN, A. Z. 1978. Comparison of human and animal rotavirus strains by gel electrophoresis of viral RNA. *Virology*, 87, 247-55.
- KAPIKIAN, A. Z., CLINE, W. L., KIM, H. W., KALICA, A. R., WYATT, R. G., VANKIRK, D. H., CHANOCK, R. M., JAMES, H. D., JR. & VAUGHN, A. L. 1976. Antigenic relationships among five reovirus-like (RVL) agents by complement fixation (CF) and development of new substitute CF antigens for the human RVL agent of infantile gastroenteritis. *Proc Soc Exp Biol Med*, 152, 535-9.
- KAPIKIAN, A. Z., HOSHINO, Y., CHANOCK, R. M. & PEREZ-SCHAEL, I. 1996a. Efficacy of a quadrivalent rhesus rotavirus-based human rotavirus vaccine aimed at preventing severe rotavirus diarrhea in infants and young children. *J Infect Dis*, 174 Suppl 1, S65-72.
- KAPIKIAN, A. Z., HOSHINO, Y., CHANOCK, R. M. & PEREZ-SCHAEL, I. 1996b. Jennerian and modified Jennerian approach to vaccination against rotavirus diarrhea using a quadrivalent rhesus rotavirus (RRV) and human-RRV reassortant vaccine. *Arch Virol Suppl*, 12, 163-75.
- KAPIKIAN, A. Z., KIM, H. W., WYATT, R. G., RODRIGUEZ, W. J., ROSS, S., CLINE, W. L., PARROTT, R. H. & CHANOCK, R. M. 1974. Reoviruslike agent in stools: association with infantile diarrhea and development of serologic tests. *Science*, 185, 1049-53.
- KAPIKIAN, A. Z., SIMONSEN, L., VESIKARI, T., HOSHINO, Y., MORENS, D. M., CHANOCK, R. M., LA MONTAGNE, J. R. & MURPHY, B. R. 2005. A hexavalent human rotavirus-bovine rotavirus (UK) reassortant vaccine designed for use in developing countries and delivered in a schedule with the potential to eliminate the risk of intussusception. *J Infect Dis*, 192 Suppl 1, S22-9.
- KHAMRIN, P., MANEEKARN, N., PEERAKOME, S., CHAN-IT, W., YAGYU, F., OKITSU, S. & USHIJIMA, H. 2007. Novel porcine rotavirus of genotype P[27] shares new phylogenetic lineage with G2 porcine rotavirus strain. *Virology*, 361, 243-52.
- KIM, I. S., TRASK, S. D., BABYONYSHEV, M., DORMITZER, P. R. & HARRISON, S. C. 2010. Effect of mutations in VP5 hydrophobic loops on rotavirus cell entry. *J Virology*, 84, 6200-7.
- KIM, Y., CHANG, K. O., KIM, W. Y. & SAIF, L. J. 2002. Production of hybrid double- or triple-layered virus-like particles of group A and C rotaviruses using a baculovirus expression system. *Virology*, 302, 1-8.
- KOBAYASHI, T., ANTAR, A. A., BOEHME, K. W., DAN THI, P., EBY, E. A., GUGLIELMI, K. M., HOLM, G. H., JOHNSON, E. M., MAGINNIS, M. S., NAIK, S., SKELTON, W. B., WETZEL, J. D., WILSON, G. J., CHAPPELL, J. D. & DERMODY, T. S. 2007. A plasmid-based reverse genetics system for animal double-stranded RNA viruses. *Cell host & microbe*, 1, 147-57.
- KOHLI, E., MAURICE, L., VAUTHEROT, J. F., BOURGEOIS, C., BOUR, J. B., COHEN, J. & POTHIER, P. 1992. Localization of group-specific epitopes on the major capsid protein of group A rotavirus. *J Gen Virol*, 73 ( Pt 4), 907-14.
- KOMOTO, S., SASAKI, J. & TANIGUCHI, K. 2006. Reverse genetics system for introduction of site-specific mutations into the double-stranded RNA genome of infectious rotavirus. *Proc Nat Acad Sci USA*, 103, 4646-51.
- LABBE, M., CHARPILLENNE, A., CRAWFORD, S. E., ESTES, M. K. & COHEN, J. 1991. Expression of rotavirus VP2 produces empty corelike particles. *J Virol*, 65, 2946-52.

- LAWTON, J. A., ESTES, M. K. & PRASAD, B. V. 1999. Comparative structural analysis of transcriptionally competent and incompetent rotavirus-antibody complexes. *Proc Natl Acad Sci U S A*, 96, 5428-33.
- LAWTON, J. A., ESTES, M. K. & PRASAD, B. V. 2000. Mechanism of genome transcription in segmented dsRNA viruses. *Adv Virus Res*, 55, 185-229.
- LE, V. P., KIM, J. Y., CHO, S. L., NAM, S. W., LIM, I., LEE, H. J., KIM, K., CHUNG, S. I., SONG, W., LEE, K. M., RHEE, M. S., LEE, J. S. & KIM, W. 2008. Detection of unusual rotavirus genotypes G8P[8] and G12P[6] in South Korea. *J Med Virol*, 80, 175-82.
- LEE, L. A. & WANG, Q. 2006. Adaptations of nanoscale viruses and other protein cages for medical applications. *Nanomed Nanotec Biol*, 2, 137-49.
- LEPAULT, J., PETITPAS, I., ERK, I., NAVAZA, J., BIGOT, D., DONA, M., VACHETTE, P., COHEN, J. & REY, F. A. 2001. Structural polymorphism of the major capsid protein of rotavirus. *The EMBO journal*, 20, 1498-507.
- LEVY, K., HUBBARD, A. E. & EISENBERG, J. N. 2009. Seasonality of rotavirus disease in the tropics: a systematic review and meta-analysis. *Int J Epidemiol*, 38, 1487-96.
- LINHARES, A. C. 2000. [Rotavirus infection in Brazil: epidemiology and challenges for its control]. *Cad Saude Publica*, 16, 629-46.
- LINHARES, A. C. & BRESEE, J. S. 2000. Rotavirus vaccines and vaccination in Latin America. *Rev Panam Salud Publica*, 8, 305-31.
- LIPSON, S. M. & KAPLAN, M. H. 1992. Atypical rotavirus genomic patterns identified by polyacrylamide gel electrophoresis. *J Diarrhoeal Dis Res*, 10, 97-100.
- LOPEZ, T., CAMACHO, M., ZAYAS, M., NAJERA, R., SANCHEZ, R., ARIAS, C. F. & LOPEZ, S. 2005. Silencing the morphogenesis of rotavirus. *J Virol*, 79, 184-92.
- LU, X., MCDONALD, S. M., TORTORICI, M. A., TAO, Y. J., VASQUEZ-DEL CARPIO, R., NIBERT, M. L., PATTON, J. T. & HARRISON, S. C. 2008. Mechanism for coordinated RNA packaging and genome replication by rotavirus polymerase VP1. *Structure*, 16, 1678-88.
- 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*, 287, 491-5.
- 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. *New Engl J Med*, 362, 289-98.
- MAES, P., MATTHIJNSSENS, J., RAHMAN, M. & VAN RANST, M. 2009. RotaC: a web-based tool for the complete genome classification of group A rotaviruses. *BMC Microbiol*, 9, 238.
- MALHERBE, H. & HARWIN, R. 1963. The cytopathic effects of vervet monkey viruses. *S Afr Med J*, 37, 407-11.
- MALHERBE, H. H. & STRICKLAND-CHOLMLEY, M. 1967. Simian virus SA11 and the related O agent. *Arch Gesamte Virusforsch*, 22, 235-45.
- MARTELLA, V., BANYAI, K., MATTHIJNSSENS, J., BUONAVOGLIA, C. & CIARLET, M. 2010. Zoonotic aspects of rotaviruses. *Vet Microbiol*, 140, 246-55.
- MARTELLA, V., CIARLET, M., BANYAI, K., LORUSSO, E., ARISTA, S., LAVAZZA, A., PEZZOTTI, G., DECARO, N., CAVALLI, A., LUCENTE, M. S., CORRENTE, M., ELIA, G., CAMERO, M., TEMPESTA, M. & BUONAVOGLIA, C. 2007. Identification of group A porcine rotavirus strains bearing a novel VP4 (P) Genotype in Italian swine herds. *J Clin Microbiol*, 45, 577-80.
- MARTELLA, V., COLOMBRITA, D., LORUSSO, E., DRAGHIN, E., FIORENTINI, S., DE GRAZIA, S., BANYAI, K., CIARLET, M., CARUSO, A. & BUONAVOGLIA, C. 2008. Detection of a porcine-like rotavirus in a child with enteritis in Italy. *J Clin Microbiol*, 46, 3501-7.
- MASON, B. B., GRAHAM, D. Y. & ESTES, M. K. 1980. In vitro transcription and translation of simian rotavirus SA11 gene products. *J Virol*, 33, 1111-21.
- MATHIEU, M., PETITPAS, I., NAVAZA, J., LEPAULT, J., KOHLI, E., POTHIER, P., PRASAD, B. V., COHEN, J. & REY, F. A. 2001. Atomic structure of the major capsid protein of rotavirus: implications for the architecture of the virion. *EMBO J*, 20, 1485-97.

- MATIC, S., MASENGA, V., POLI, A., RINALDI, R., MILNE, R. G., VECCHIATI, M. & NORIS, E. 2012. Comparative analysis of recombinant Human Papillomavirus 8 L1 production in plants by a variety of expression systems and purification methods. *Plant Biotechnol J*, 10: 410-21.
- MATSUMOTO, K., HATANO, M., KOBAYASHI, K., HASEGAWA, A., YAMAZAKI, S., NAKATA, S., CHIBA, S. & KIMURA, Y. 1989. An outbreak of gastroenteritis associated with acute rotaviral infection in schoolchildren. *J Infect Dis*, 160, 611-5.
- MATTHIJNSSENS, J., CIARLET, M., HEIMAN, E., ARIJS, I., DELBEKE, T., MCDONALD, S. M., PALOMBO, E. A., ITURRIZA-GOMARA, M., MAES, P., PATTON, J. T., RAHMAN, M. & VAN RANST, M. 2008a. Full genome-based classification of rotaviruses reveals a common origin between human Wa-Like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. *J Virol*, 82, 3204-19.
- MATTHIJNSSENS, J., CIARLET, M., MCDONALD, S. M., ATTOUI, H., BANYAI, K., BRISTER, J. R., BUESA, J., ESONA, M. D., ESTES, M. K., GENTSCH, J. R., ITURRIZA-GOMARA, M., JOHNE, R., KIRKWOOD, C. D., MARTELLA, V., MERTENS, P. P., NAKAGOMI, O., PARRENO, V., RAHMAN, M., RUGGERI, F. M., SAIF, L. J., SANTOS, N., STEYER, A., TANIGUCHI, K., PATTON, J. T., DESSELBERGER, U. & VAN RANST, M. 2011. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). *Arch Virol*, 156, 1397-413.
- MATTHIJNSSENS, J., CIARLET, M., RAHMAN, M., ATTOUI, H., BANYAI, K., ESTES, M. K., GENTSCH, J. R., ITURRIZA-GOMARA, M., KIRKWOOD, C. D., MARTELLA, V., MERTENS, P. P., NAKAGOMI, O., PATTON, J. T., RUGGERI, F. M., SAIF, L. J., SANTOS, N., STEYER, A., TANIGUCHI, K., DESSELBERGER, U. & VAN RANST, M. 2008b. Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. *Arch Virol*, 153, 1621-9.
- MATTHIJNSSENS, J., POTGIETER, C. A., CIARLET, M., PARRENO, V., MARTELLA, V., BANYAI, K., GARAI COECHEA, L., PALOMBO, E. A., NOVO, L., ZELLER, M., ARISTA, S., GERNA, G., RAHMAN, M. & VAN RANST, M. 2009. Are human P[14] rotavirus strains the result of interspecies transmissions from sheep or other ungulates that belong to the mammalian order Artiodactyla? *J Virol*, 83, 2917-29.
- MATTHIJNSSENS, J., RAHMAN, M., MARTELLA, V., XUELEI, Y., DE VOS, S., DE LEENER, K., CIARLET, M., BUONAVOGLIA, C. & VAN RANST, M. 2006. Full genomic analysis of human rotavirus strain B4106 and lapine rotavirus strain 30/96 provides evidence for interspecies transmission. *J Virol*, 80, 3801-10.
- 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 Å resolution. *J Mol Biol*, 397, 587-99.
- MCCRAE, M. A. & MCCORQUODALE, J. G. 1982. The molecular biology of rotaviruses. II. Identification of the protein-coding assignments of calf rotavirus genome RNA species. *Virology*, 117, 435-43.
- MCCRAE, M. A. & MCCORQUODALE, J. G. 1987. Expression of a major bovine rotavirus neutralisation antigen (VP7c) in *Escherichia coli*. *Gene*, 55, 9-18.
- MCDONALD, S. M. & PATTON, J. T. 2011a. Assortment and packaging of the segmented rotavirus genome. *Trends Microbiol*, 19, 136-44.
- MCDONALD, S. M. & PATTON, J. T. 2011b. Rotavirus VP2 core shell regions critical for viral polymerase activation. *J Virol*, 85, 3095-105.
- MEBUS, C. A., UNDERDAHL, N. R., RHODES, M. B. & TWIEHAUS, M. J. 1969. Further studies on neonatal calf diarrhea virus. *Proc Annu Meet U S Anim Health Assoc*, 73, 97-9.
- MERTENS, P. P. C., ARELLA, M., ATTOUI, H., BELLONCIK, S., BERGOIN, M., BOCCARDO, G., BOOTH, T. F., CHIU, W., DIPROSE, J. M. & DUNCAN, R. 2000. Reoviridae. In: VAN REGENMORTEL, M. H. V., FAUQUET, C. M., BISHOP, D. H. L., CALISHER, C. H., CARSTEN, E. B., ESTES, M. K., S. M. LEMON, MANILOFF, J., MAYO, M. A., MCGEOCH, D. J., PRINGLE, C. R. & WICKNER, R. B. (eds.) *Virus Taxonomy. Seventh Report of the International Committee for the Taxonomy of Viruses*. New York: Academic Press.
- MOLYNEAUX, P. J. 1995. Human immunity to rotavirus. *Journal of medical microbiology*, 43, 397-404.
- MONTERO, H., ARIAS, C. F. & LOPEZ, S. 2006. Rotavirus Nonstructural Protein NSP3 is not required for viral protein synthesis. *J Virol*, 80, 9031-8.

- MOON, S. S., WANG, Y., SHANE, A. L., NGUYEN, T., RAY, P., DENNEHY, P., BAEK, L. J., PARASHAR, U., GLASS, R. I. & JIANG, B. 2010. Inhibitory effect of breast milk on infectivity of live oral rotavirus vaccines. *Pediatr Infect Dis J*, 29, 919-23.
- MORRIS, A. P. & ESTES, M. K. 2001. Microbes and microbial toxins: paradigms for microbial-mucosal interactions. VIII. Pathological consequences of rotavirus infection and its enterotoxin. *Am J Physiol Gastrointest Liver Physiol*, 281, G303-10.
- MPHAHLELE, M. J., PEENZE, I. & STEELE, A. D. 1999. Rotavirus strains bearing the VP4P[14] genotype recovered from South African children with diarrhoea. *Arch Virol*, 144, 1027-34.
- 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. 2001. Intussusception among infants given an oral rotavirus vaccine. *N Engl J Med*, 344, 564-72.
- MUSALEM, C. & ESPEJO, R. T. 1985. Release of progeny virus from cells infected with simian rotavirus SA11. *J Gen Virol*, 66 ( Pt 12), 2715-24.
- NAKAGOMI, O. & NAKAGOMI, T. 1993. Interspecies transmission of rotaviruses studied from the perspective of genogroup. *Microbiol Immunol*, 37, 337-48.
- NAKAGOMI, O., NAKAGOMI, T., AKATANI, K. & IKEGAMI, N. 1989a. Identification of rotavirus genogroups by RNA-RNA hybridization. *Mol Cell Probes*, 3, 251-61.
- NAKAGOMI, T., MATSUDA, Y., OHSHIMA, A., MOCHIZUKI, M. & NAKAGOMI, O. 1989b. Characterization of a canine rotavirus strain by neutralization and molecular hybridization assays. *Arch Virol*, 106, 145-50.
- NAKAGOMI, T. & NAKAGOMI, O. 1989. RNA-RNA hybridization identifies a human rotavirus that is genetically related to feline rotavirus. *J Virol*, 63, 1431-4.
- NAKAGOMI, T. & NAKAGOMI, O. 2002. Genogroup characterization of reemerging serotype G9 human rotavirus strain 95H115 in comparison with earlier G9 and other human prototype strains. *Microbiol Immunol*, 46, 575-8.
- NAM, K. T., WARTENA, R., YOO, P. J., LIAU, F. W., LEE, Y. J., CHIANG, Y. M., HAMMOND, P. T. & BELCHER, A. M. 2008. Stamped microbattery electrodes based on self-assembled M13 viruses. *Proc Nat Acad Sci USA*, 105, 17227-31.
- NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES, N. I. A. I. H. 2007. The Jordan Report, U.S.: Department of Health and Human Services, [www.niaid.nih.gov](http://www.niaid.nih.gov)
- NIBERT, M. L. & SCHIFF, L. A. 2001. Reoviruses and their replication. In: KNIPE, D. M. & HOWLEY, P. M. (eds.) *Fields Virology*. Philadelphia: Lippincott Williams and Wilkins.
- NICOLAS, J. C., POTHIER, P., COHEN, J., LOURENCO, M. H., THOMPSON, R., GUIMBAUD, P., CHENON, A., DAUVERGNE, M. & BRICOUT, F. 1984. Survey of human rotavirus propagation as studied by electrophoresis of genomic RNA. *J Infect Dis*, 149, 688-93.
- NILSSON, M., SIGSTAM, G. & SVENSSON, L. 2000. Antibody prevalence and specificity to group C rotavirus in Swedish sera. *J Med Virol*, 60, 210-5.
- O'BRIEN, J. A., TAYLOR, J. A. & BELLAMY, A. R. 2000. Probing the structure of rotavirus NSP4: a short sequence at the extreme C terminus mediates binding to the inner capsid particle. *Journal of virology*, 74, 5388-94.
- OFFIT, P. A. 1994. Immunologic determinants of protection against rotavirus disease. *Curr Top Microbiol Immunol*, 185, 229-54.
- OFFIT, P. A. & BLAVAT, G. 1986. Identification of the two rotavirus genes determining neutralization specificities. *J Virol*, 57, 376-8.
- OFFIT, P. A., BLAVAT, G., GREENBERG, H. B. & CLARK, H. F. 1986. Molecular basis of rotavirus virulence: role of gene segment 4. *J Virol*, 57, 46-9.
- OSBORNE, M. P., HADDON, S. J., WORTON, K. J., SPENCER, A. J., STARKEY, W. G., THORNBER, D. & STEPHEN, J. 1991. Rotavirus-induced changes in the microcirculation of intestinal villi of neonatal mice in relation to the induction and persistence of diarrhea. *J Pediatr Gastroenterol Nutr*, 12, 111-20.
- PAAVONEN, J., JENKINS, D., BOSCH, F. X., NAUD, P., SALMERON, J., WHEELER, C. M., CHOW, S. N., APTER, D. L., KITCHENER, H. C., CASTELLSAGUE, X., DE CARVALHO, N. S., SKINNER, S. R., HARPER, D. M., HEDRICK, J. A., JAISAMRARN, U., LIMSON, G. A., DIONNE, M., QUINT, W., SPIESSENS, B., PEETERS, P., STRUYF, F., WIETING, S. L.,

- LEHTINEN, M. O. & DUBIN, G. 2007. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. *Lancet*, 369, 2161-70.
- PACINI, D. L., BRADY, M. T., BUDDE, C. T., CONNELL, M. J., HAMPARIAN, V. V. & HUGHES, J. H. 1988. Polyacrylamide gel electrophoresis of RNA compared with polyclonal- and monoclonal-antibody-based enzyme immunoassays for rotavirus. *J Clin Microbiol*, 26, 194-7.
- PAGE, N. A., DE BEER, M. C., SEHERI, L. M., DEWAR, J. B. & STEELE, A. D. 2009. The detection and molecular characterization of human G12 genotypes in South Africa. *J Med Virol*, 81, 106-13.
- PALOMARES, L. A. & RAMÍREZ, O. T. 2009. Challenges for the production of virus-like particles in insect cells: The case of rotavirus-like particles. *Biochem Eng J*, 158-167.
- PARASHAR, U. D., BURTON, A., LANATA, C., BOSCHI-PINTO, C., SHIBUYA, K., STEELE, D., BIRMINGHAM, M. & GLASS, R. I. 2009. Global mortality associated with rotavirus disease among children in 2004. *J Infect Dis*, 200 Suppl 1, S9-S15.
- PARASHAR, U. D., GIBSON, C. J., BRESSE, J. S. & GLASS, R. I. 2006. Rotavirus and severe childhood diarrhea. *Emerg Infect Dis*, 12, 304-6.
- PARASHAR, U. D., HOLMAN, R. C., CUMMINGS, K. C., STAGGS, N. W., CURNS, A. T., ZIMMERMAN, C. M., KAUFMAN, S. F., LEWIS, J. E., VUGIA, D. J., POWELL, K. E. & GLASS, R. I. 2000. Trends in intussusception-associated hospitalizations and deaths among US infants. *Pediatrics*, 106, 1413-21.
- PARASHAR, U. D., HUMMELMAN, E. G., BRESEE, J. S., MILLER, M. A. & GLASS, R. I. 2003. Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Dis*, 9, 565-72.
- PARRA, G. I., BOK, K., MARTINEZ, M. & GOMEZ, J. A. 2004. Evidence of rotavirus intragenic recombination between two sublineages of the same genotype. *J Gen Virol*, 85, 1713-6.
- PATEL, M., SHANE, A. L., PARASHAR, U. D., JIANG, B., GENTSCH, J. R. & GLASS, R. I. 2009. Oral rotavirus vaccines: how well will they work where they are needed most? *J Infect Dis*, 200 Suppl 1, S39-48.
- PATTON, J. T. 1996. Rotavirus VP1 alone specifically binds to the 3' end of viral mRNA, but the interaction is not sufficient to initiate minus-strand synthesis. *J Virol*, 70, 7940-7.
- PATTON, J. T., JONES, M. T., KALBACH, A. N., HE, Y. W. & XIAOBO, J. 1997. Rotavirus RNA polymerase requires the core shell protein to synthesize the double-stranded RNA genome. *J Virol*, 71, 9618-26.
- PATTON, J. T., VASQUEZ-DEL CARPIO, R. & SPENCER, E. 2004. Replication and transcription of the rotavirus genome. *Curr Pharm Des*, 10, 3769-77.
- PATTON, J. T., WENTZ, M., XIAOBO, J. & RAMIG, R. F. 1996. cis-Acting signals that promote genome replication in rotavirus mRNA. *Journal of virology*, 70, 3961-71.
- PESAVENTO, J. B., CRAWFORD, S. E., ESTES, M. K. & PRASAD, B. V. 2006. Rotavirus proteins: structure and assembly. *Curr Top Microbiol Immunol*, 309, 189-219.
- PHAN, T. G., KHAMRIN, P., QUANG, T. D., DEY, S. K., TAKANASHI, S., OKITSU, S., MANEEKARN, N. & USHIJIMA, H. 2007a. Detection and genetic characterization of group A rotavirus strains circulating among children with acute gastroenteritis in Japan. *J Virol*, 81, 4645-53.
- PHAN, T. G., OKITSU, S., MANEEKARN, N. & USHIJIMA, H. 2007b. Evidence of intragenic recombination in G1 rotavirus VP7 genes. *J Virol*, 81, 10188-94.
- PLASCENCIA-VILLA, G., SANIGER, J. M., ASCENCIO, J. A., PALOMARES, L. A. & RAMIREZ, O. T. 2009. Use of recombinant rotavirus VP6 nanotubes as a multifunctional template for the synthesis of nanobiomaterials functionalized with metals. *Biotechnol Bioeng*, 104, 871-81.
- PONCET, D., APONTE, C. & COHEN, J. 1993. Rotavirus protein NSP3 (NS34) is bound to the 3' end consensus sequence of viral mRNAs in infected cells. *J Virol*, 67, 3159-65.
- 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, 476-9.
- PRASAD, B. V., ROTHNAGEL, R., JIANG, X. & ESTES, M. K. 1994. Three-dimensional structure of baculovirus-expressed Norwalk virus capsids. *J Virol*, 68, 5117-25.

- PRASAD, B. V., WANG, G. J., CLERX, J. P. & CHIU, W. 1988. Three-dimensional structure of rotavirus. *J Mol Biol*, 199, 269-75.
- RAHMAN, M., SULTANA, R., AHMED, G., NAHAR, S., HASSAN, Z. M., SAIADA, F., PODDER, G., FARUQUE, A. S., SIDDIQUE, A. K., SACK, D. A., MATTHIJNSSENS, J., VAN RANST, M. & AZIM, T. 2007. Prevalence of G2P[4] and G12P[6] rotavirus, Bangladesh. *Emerg Infect Dis*, 13, 18-24.
- RAMACHANDRAN, M., GENTSCH, J. R., PARASHAR, U. D., JIN, S., WOODS, P. A., HOLMES, J. L., KIRKWOOD, C. D., BISHOP, R. F., GREENBERG, H. B., URASAWA, S., GERNA, G., COULSON, B. S., TANIGUCHI, K., BRESEE, J. S. & GLASS, R. I. 1998. Detection and characterization of novel rotavirus strains in the United States. *J Clin Microbiol*, 36, 3223-9.
- RAMIG, R. F. 2004. Pathogenesis of intestinal and systemic rotavirus infection. *J Virol*, 78, 10213-20.
- RICHARDSON, S. C. & BISHOP, R. F. 1990. Homotypic serum antibody responses to rotavirus proteins following primary infection of young children with serotype 1 rotavirus. *J Clin Microbiol*, 28, 1891-7.
- RICHARDSON, V., HERNANDEZ-PICHARDO, J., QUINTANAR-SOLARES, M., ESPARZA-AGUILAR, M., JOHNSON, B., GOMEZ-ALTAMIRANO, C. M., PARASHAR, U. & PATEL, M. 2010. Effect of rotavirus vaccination on death from childhood diarrhea in Mexico. *New Engl J Med*, 362, 299-305.
- RODGER, S. M., BISHOP, R. F. & HOLMES, I. H. 1982. Detection of a rotavirus-like agent associated with diarrhea in an infant. *J Clin Microbiol*, 16, 724-6.
- RUIZ-PALACIOS, G. M., PEREZ-SCHAEEL, I., VELAZQUEZ, F. R., ABATE, H., BREUER, T., CLEMENS, S. C., CHEUVART, B., ESPINOZA, F., GILLARD, P., INNIS, B. L., CERVANTES, Y., LINHARES, A. C., LOPEZ, P., MACIAS-PARRA, M., ORTEGA-BARRIA, E., RICHARDSON, V., RIVERA-MEDINA, D. M., RIVERA, L., SALINAS, B., PAVIA-RUZ, N., SALMERON, J., RUTTIMANN, R., TINOCO, J. C., RUBIO, P., NUNEZ, E., GUERRERO, M. L., YARZABAL, J. P., DAMASO, S., TORNIEPORTH, N., SAEZ-LLORENS, X., VERGARA, R. F., VESIKARI, T., BOUCKENOOGHE, A., CLEMENS, R., DE VOS, B. & O'RYAN, M. 2006. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *New Engl J Med*, 354, 11-22.
- SALINAS, B., PEREZ SCHAEEL, I., LINHARES, A. C., RUIZ PALACIOS, G. M., GUERRERO, M. L., YARZABAL, J. P., CERVANTES, Y., COSTA CLEMENS, S., DAMASO, S., HARDT, K. & DE VOS, B. 2005. Evaluation of safety, immunogenicity and efficacy of an attenuated rotavirus vaccine, RIX4414: A randomized, placebo-controlled trial in Latin American infants. *Pediatr Infect Dis J*, 24, 807-16.
- SANTOS, N. & HOSHINO, Y. 2005. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Rev Med Virol*, 15, 29-56.
- SATO, A., IIZUKA, M., NAKAGOMI, O., SUZUKI, M., HORIE, Y., KONNO, S., HIRASAWA, F., SASAKI, K., SHINDO, K. & WATANABE, S. 2006. Rotavirus double-stranded RNA induces apoptosis and diminishes wound repair in rat intestinal epithelial cells. *J Gastroent Hepat*, 21, 521-30.
- SETTEMBRE, E. C., CHEN, J. Z., DORMITZER, P. R., GRIGORIEFF, N. & HARRISON, S. C. 2011. Atomic model of an infectious rotavirus particle. *EMBO J*, 30, 408-16.
- SHAHABADI, M. S., BABIUK, L. A. & LEE, P. W. 1987. Further analysis of the role of calcium in rotavirus morphogenesis. *Virology*, 158, 103-11.
- SHAW, A. L., ROTHNAGEL, R., ZENG, C. Q., LAWTON, J. A., RAMIG, R. F., ESTES, M. K. & PRASAD, B. V. 1996. Rotavirus structure: interactions between the structural proteins. *Arch Virol Suppl*, 12, 21-7.
- SILVESTRI, L. S., TORTORICI, M. A., VASQUEZ-DEL CARPIO, R. & PATTON, J. T. 2005. Rotavirus glycoprotein NSP4 is a modulator of viral transcription in the infected cell. *J Virol*, 79, 15165-74.
- SOLBERG, O. D., HASING, M. E., TRUEBA, G. & EISENBERG, J. N. 2009. Characterization of novel VP7, VP4, and VP6 genotypes of a previously untypeable group A rotavirus. *Virology*, 385, 58-67.
- STEELE, A. D. & ALEXANDER, J. J. 1987. Molecular epidemiology of rotavirus in black infants in South Africa. *J Clin Microbiol*, 25, 2384-7.

- STEELE, A. D. & IVANOFF, B. 2003. Rotavirus strains circulating in Africa during 1996-1999: emergence of G9 strains and P[6] strains. *Vaccine*, 21, 361-7.
- STEELE, A. D., MNISI, Y. N., WILLIAMS, M. M., BOS, P. & ASPINALL, S. 1993. Electrophoretic typing of nosocomial rotavirus infection in a general paediatric unit showing the continual introduction of community strains. *J Med Virol*, 40, 126-32.
- STEELE, A. D., PEENZE, I., DE BEER, M. C., PAGER, C. T., YEATS, J., POTGIETER, N., RAMSAROOP, U., PAGE, N. A., MITCHELL, J. O., GEYER, A., BOS, P. & ALEXANDER, J. J. 2003. Anticipating rotavirus vaccines: epidemiology and surveillance of rotavirus in South Africa. *Vaccine*, 21, 354-60.
- STIRZAKER, S. C., WHITFIELD, P. L., CHRISTIE, D. L., BELLAMY, A. R. & BOTH, G. W. 1987. Processing of rotavirus glycoprotein VP7: implications for the retention of the protein in the endoplasmic reticulum. *J Cell Biol*, 105, 2897-903.
- STOREY, P., THERON, J., MAREE, F. F. & O'NEILL, H. G. 2007. A second RGD motif in the 1D capsid protein of a SAT1 type foot-and-mouth disease virus field isolate is not essential for attachment to target cells. *Virus Res*, 124, 184-92.
- SUBBARAO, K. & KATZ, J. M. 2004. Influenza vaccines generated by reverse genetics. *Curr Top in Microbiol*, 283, 313-42.
- TARAPOREWALA, Z. F., CHEN, D. & PATTON, J. T. 2001. Multimers of the bluetongue virus nonstructural protein, NS2, possess nucleotidyl phosphatase activity: similarities between NS2 and rotavirus NSP2. *Virology*, 280, 221-31.
- TATE, J. E., BURTON, A. H., BOSCHI-PINTO, C., STEELE, A. D., DUQUE, J. & PARASHAR, U. D. 2011a. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis*, 12:136-41.
- TATE, J. E., PATEL, M. M., STEELE, A. D., GENTSCH, J. R., PAYNE, D. C., CORTESE, M. M., NAKAGOMI, O., CUNLIFFE, N. A., JIANG, B., NEUZIL, K. M., DE OLIVEIRA, L. H., GLASS, R. I. & PARASHAR, U. D. 2010. Global impact of rotavirus vaccines. *Expert Rev Vaccines*, 9, 395-407.
- TAYLOR, J. A., O'BRIEN, J. A., LORD, V. J., MEYER, J. C. & BELLAMY, A. R. 1993. The RER-localized rotavirus intracellular receptor: a truncated purified soluble form is multivalent and binds virus particles. *Virology*, 194, 807-14.
- THOUVENIN, E., SCHOEHN, G., REY, F., PETITPAS, I., MATHIEU, M., VANEY, M. C., COHEN, J., KOHLI, E., POTHIER, P. & HEWAT, E. 2001. Antibody inhibition of the transcriptase activity of the rotavirus DLP: a structural view. *J Mol Biol*, 307, 161-72.
- TIAN, P., BALL, J. M., ZENG, C. Q. & ESTES, M. K. 1996. Rotavirus protein expression is important for virus assembly and pathogenesis. *Arch Virol Suppl*, 12, 69-77.
- TIAN, P., ESTES, M. K., HU, Y., BALL, J. M., ZENG, C. Q. & SCHILLING, W. P. 1995. The rotavirus nonstructural glycoprotein NSP4 mobilizes Ca<sup>2+</sup> from the endoplasmic reticulum. *J Virol*, 69, 5763-72.
- TODARO, W., WALL, C., EDWARDS, K. & CLEGHORN, G. 1995. Acceptability of a rice-based oral rehydration solution in Port Moresby General Hospital's Children's Outpatient Department. *P N G Med J*, 38, 278-83.
- TODD, D., MCNULTY, M. S. & ALLAN, G. M. 1980. Polyacrylamide gel electrophoresis of avian rotavirus RNA. *Arch Virol*, 63, 87-97.
- TRASK, S. D. & DORMITZER, P. R. 2006. Assembly of highly infectious rotavirus particles recoated with recombinant outer capsid proteins. *J Virol*, 80, 11293-304.
- TRASK, S. D., MCDONALD, S. M. & PATTON, J. T. 2012. Structural insights into the coupling of virion assembly and rotavirus replication. *Nat Rev. Microbiol*.
- URASAWA, S., URASAWA, T., WAKASUGI, F., KOBAYASHI, N., TANIGUCHI, K., LINTAG, I. C., SANIEL, M. C. & GOTO, H. 1990. Presumptive seventh serotype of human rotavirus. *Arch Virol*, 113, 279-82.
- UYGUNGIL, B., BLEESING, J. J., RISMA, K. A., MCNEAL, M. M. & ROTHENBERG, M. E. 2010. Persistent rotavirus vaccine shedding in a new case of severe combined immunodeficiency: A reason to screen. *J Allergy Clin Immunol*, 125, 270-1.
- VAN DE PERRE, P. 2003. Transfer of antibody via mother's milk. *Vaccine*, 21, 3374-6.

- VELAZQUEZ, F. R., MATSON, D. O., CALVA, J. J., GUERRERO, L., MORROW, A. L., CARTER-CAMPBELL, S., GLASS, R. I., ESTES, M. K., PICKERING, L. K. & RUIZ-PALACIOS, G. M. 1996. Rotavirus infections in infants as protection against subsequent infections. *New Engl J Med*, 335, 1022-8.
- VENDE, P., PIRON, M., CASTAGNE, 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. *J Virol*, 74, 7064-71.
- VESIKARI, T., CLARK, H. F., OFFIT, P. A., DALLAS, M. J., DISTEFANO, D. J., GOVEIA, M. G., WARD, R. L., SCHODEL, F., KARVONEN, A., DRUMMOND, J. E., DINUBILE, M. J. & HEATON, P. M. 2006a. Effects of the potency and composition of the multivalent human-bovine (WC3) reassortant rotavirus vaccine on efficacy, safety and immunogenicity in healthy infants. *Vaccine*, 24, 4821-9.
- VESIKARI, T., ISOLAURI, E., D'HONDT, E., DELEM, A., ANDRE, F. E. & ZISSIS, G. 1984. Protection of infants against rotavirus diarrhoea by RIT 4237 attenuated bovine rotavirus strain vaccine. *Lancet*, 1, 977-81.
- VESIKARI, T., KARVONEN, A., KORHONEN, T., ESPO, M., LEBACQ, E., FORSTER, J., ZEPP, F., DELEM, A. & DE VOS, B. 2004a. Safety and immunogenicity of RIX4414 live attenuated human rotavirus vaccine in adults, toddlers and previously uninfected infants. *Vaccine*, 22, 2836-42.
- VESIKARI, T., KARVONEN, A., PRYMULA, R., SCHUSTER, V., TEJEDOR, J. C., COHEN, R., MEURICE, F., HAN, H. H., DAMASO, S. & BOUCKENOOGHE, A. 2007. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. *Lancet*, 370, 1757-63.
- VESIKARI, T., KARVONEN, A., PUUSTINEN, L., ZENG, S. Q., SZAKAL, E. D., DELEM, A. & DE VOS, B. 2004b. Efficacy of RIX4414 live attenuated human rotavirus vaccine in Finnish infants. *Pediatr Infect Dis J*, 23, 937-43.
- VESIKARI, T., KARVONEN, A. V., MAJURI, J., ZENG, S. Q., PANG, X. L., KOHBERGER, R., FORREST, B. D., HOSHINO, Y., CHANOCK, R. M. & KAPIKIAN, A. Z. 2006b. Safety, efficacy, and immunogenicity of 2 doses of bovine-human (UK) and rhesus-rhesus-human rotavirus reassortant tetravalent vaccines in Finnish children. *J Infect Dis*, 194, 370-6.
- 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., KARVONEN, A., WATT, J. P., O'BRIEN, K. L., DINUBILE, M. J., CLARK, H. F., BOSLEGO, J. W., OFFIT, P. A. & HEATON, P. M. 2006c. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *New Engl J Med*, 354, 23-33.
- VICTORIA, J. G., WANG, C., JONES, M. S., JAING, C., MCLOUGHLIN, K., GARDNER, S. & DELWART, E. L. 2010. Viral nucleic acids in live-attenuated vaccines: detection of minority variants and an adventitious virus. *J Virol*, 84, 6033-40.
- WORLD HEALTH ORGANIZATION. 2009. *Global use of rotavirus vaccines recommended* [Online]. [http://www.who.int/mediacentre/news/releases/2009/rotavirus\\_vaccines\\_20090605/en/index.html](http://www.who.int/mediacentre/news/releases/2009/rotavirus_vaccines_20090605/en/index.html). [Accessed 5 November 2009].
- WORLD HEALTH ORGANIZATION FACTSHEET. 2006. *Smallpox* [Online]. <http://www.who.int/mediacentre/factsheets/smallpox/en/>. [Accessed 23rd September 2006].
- WORLD HEALTH ORGANIZATION. 2001. Report of the meeting on future directions for rotavirus vaccine research in developing countries. Geneva: World Health Organization.
- YANG, K., WANG, S., CHANG, K. O., LU, S., SAIF, L. J., GREENBERG, H. B., BRINKER, J. P. & HERRMANN, J. E. 2001. Immune responses and protection obtained with rotavirus VP6 DNA vaccines given by intramuscular injection. *Vaccine*, 19, 3285-91.
- YEAGER, M., BERRIMAN, J. A., BAKER, T. S. & BELLAMY, A. R. 1994. Three-dimensional structure of the rotavirus haemagglutinin VP4 by cryo-electron microscopy and difference map analysis. *EMBO J*, 13, 1011-8.
- YODER, J. D., TRASK, S. D., VO, T. P., BINKA, M., FENG, N., HARRISON, S. C., GREENBERG, H. B. & DORMITZER, P. R. 2009. VP5\* rearranges when rotavirus uncoats. *J Virol*, 83, 11372-7.

- YUAN, L., GEYER, A., HODGINS, D. C., FAN, Z., QIAN, Y., CHANG, K. O., CRAWFORD, S. E., PARRENO, V., WARD, L. A., ESTES, M. K., CONNER, M. E. & SAIF, L. J. 2000. Intranasal administration of 2/6-rotavirus-like particles with mutant *Escherichia coli* heat-labile toxin (LT-R192G) induces antibody-secreting cell responses but not protective immunity in gnotobiotic pigs. *J Virol*, 74, 8843-53.
- YUAN, L., HONMA, S., ISHIDA, S., YAN, X. Y., KAPIKIAN, A. Z. & HOSHINO, Y. 2004a. Species-specific but not genotype-specific primary and secondary isotype-specific NSP4 antibody responses in gnotobiotic calves and piglets infected with homologous host bovine (NSP4[A]) or porcine (NSP4[B]) rotavirus. *Virology*, 330, 92-104.
- YUAN, L., IOSEF, C., AZEVEDO, M. S., KIM, Y., QIAN, Y., GEYER, A., NGUYEN, T. V., CHANG, K. O. & SAIF, L. J. 2001. Protective immunity and antibody-secreting cell responses elicited by combined oral attenuated Wa human rotavirus and intranasal Wa 2/6-VLPs with mutant *Escherichia coli* heat-labile toxin in gnotobiotic pigs. *J Virol*, 75, 9229-38.
- YUAN, L., ISHIDA, S., HONMA, S., PATTON, J. T., HODGINS, D. C., KAPIKIAN, A. Z. & HOSHINO, Y. 2004b. Homotypic and heterotypic serum isotype-specific antibody responses to rotavirus nonstructural protein 4 and viral protein (VP) 4, VP6, and VP7 in infants who received selected live oral rotavirus vaccines. *J Infect Dis*, 189, 1833-45.
- 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., SCHODEL, 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*, 376, 615-23.
- ZHOU, H., GUO, L., WANG, M., QU, J., ZHAO, Z., WANG, J. & HUNG, T. 2011. Prime immunization with rotavirus VLP 2/6 followed by boosting with an adenovirus expressing VP6 induces protective immunization against rotavirus in mice. *Virol J*, 8, 3.