Rotavirus Infection: A Perspective on Epidemiology, Genomic Diversity and Vaccine Strategies

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Abstract For centuries, acute diarrhea has been a major cause of death in young children worldwide, and until 1973, before rotavirus was discovered; no infectious agents could be identified in about 80% of patients admitted to hospital with severe dehydrating diarrhea. Rotaviruses have now been shown to cause 40–50% of severe acute diarrhea in young children worldwide in both developing and developed countries. More than 600,000 young children die and approximately 2.4 million hospitalize annually from rotavirus disease, especially in South-East Asia and sub-Saharan Africa. Two safe and effective vaccines are now licensed in 100 countries but used in 17 countries. Rotarix (GSK) vaccine is derived from single attenuated human rotavirus G1P[8], representative of the most common serotype identified worldwide. RotaTeq (Merck) is a pentavalent mixture of naturally attenuated bovine/human rotavirus reassortants representing G1, G2, G3, G4, and P[8] serotypes. Though these vaccines have already dramatically decreased the morbidity associated with rotavirus in countries where they are widely used, the third generation of vaccines, based on inactivated viruses or recombinant virus like particle are already in pipeline. Continuous surveillance and the genetic and antigenic analysis of the various strains of rotavirus circulating worldwide will aid significantly in assessing the effectiveness of these vaccines and monitor emergence of new strains. Introduction of rotavirus vaccines in national vaccine policy along with

other childhood vaccines may result in significant reduction in mortality in children in poor socioeconomic countries.

Keywords Rotavirus · Vaccine · 23 G genotypes · 32 P genotypes

Introduction

Historically, diarrheal disease has been a common cause of illness and death in young children. For example, approximately 80% of babies admitted to foundling homes in Ireland during the 18th century died from ‘cholera infantum’ during the first 1–2 years of life. In 1900 the death rate of children aged 6–18 months in New York City was calculated as 5603 per 100,000. Even as late as March 1942–April 1943, 109 of 216 children admitted to the West Middlesex Hospital, London with severe diarrhea died, without the identification of an enteric pathogen [37]. Despite a slow appreciation of the need to replace fluid and sodium losses, in 1946–1947, deaths from enteritis in young children aged <15 months still averaged 40 of 70 admissions per week in UK. Although in the past two decades, diarrheal diseases are not a prominent cause of mortality in infants and young children in developed countries, the toll from diarrheal disease in developing countries is staggering. Due to the lack of proper disease management and/or the unavailability of health care facilities in the developing world, rotavirus kills over half a million infants a year, about one child each minute [61]. The death toll has been estimated at 611,000 per year, 82% of which occurs in poor countries [65]. Though rotavirus causes about 10,000 hospital admissions in Australia [18] and 70,000 hospital admissions in the United States,

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Fig. 1 Distribution of rotavirus mortality worldwide (With kind permission, this picture is adapted from Parashar et al. 2003 [Ref. 64])

individually India accounts with the highest number of deaths (>100,000) annually (Fig. 1) [35].

In May 1973, during a study, ultrathin sections of duodenal mucosa from children with acute gastroenteritis were examined, using electron microscopy. Abundant viral particles were identified in the epithelial cells lining the upper villous surface [12]. The virus was identified as being reovirus-like/orbivirus-like, with a close resemblance to viruses already implicated as cause of diarrhea in neonatal mice [1], and in calves [54]. The virus was shed in $\geq 10^{10}$ particles per milliliter in diarrheal feces and could readily be identified (by electron-microscopy of negatively stained fecal extracts) as 70-nm particles [62]. The 70-nm virus from children was initially referred to by several names, including reovirus-like, orbivirus-like, duovirus, infantile gastroenteritis virus, or a 'new' virus. The wheel-like structure seen by electron-microscopy eventually led to agreement to accept the name Rotavirus (rota = Latin for wheel). The rotaviruses comprise the genus Rotavirus within the family *Reoviridae*. Human rotaviruses were quickly linked to previous descriptions in the literature of 'identical' viruses causing severe diarrhea in newborn mice (EDIM virus) [1], newborn calves (NCDV) [54], and a virus identified from a rectal swab of a healthy monkey (SA11) [53]. The clock of rotavirus research in India goes back as early as 1977 when a group of scientists from Christian Medical College Hospital, Vellore discovered rotavirus from the electron microscopy of the stools from children below 2 years of age suffering from acute diarrhea. Rotaviruses have now been shown to be a cause of diarrhea in the young of many mammalian and avian species [35].

Rotavirus vaccines represent an important preventive approach to reducing rotavirus infections and, along with therapeutic interventions such as oral rehydration solution

and zinc supplementation. Efforts to develop a rotavirus vaccine began in 1980s and led in 1998 to the licensing and introduction of a live attenuated rhesus rotavirus vaccine (RotaShield®) in the US market. Unfortunately it was withdrawn from the market in 1999 after reports of increased risk of intussusception among vaccinated infants. Despite this setback, development of other candidate rotavirus vaccines continued. In 2006, two new oral rotavirus vaccines were licensed and introduced in the USA following large-scale safety and efficacy studies in Europe and North and Latin America and later in Asia [78, 99]. These vaccines are now licensed in 100 countries and used in immunization schedule with other EPI vaccines.

Several review articles have presented excellent overviews on rotavirus genomic diversity and importance of development of rotavirus vaccine [52, 59, 66, 82]. The objective of this article is to review the distribution of rotavirus serotypes/genotypes derived from globally collected data between 1973 and 2010 and the present vaccine strategies with special focus on India.

Rotaviruses: Molecular Structure and Genotypes

Rotavirus has an 11-segmented double-stranded RNA genome that is surrounded by three protein shells: a core, an inner capsid, and outer capsid (Fig. 2). The RNA gene segments encode for six structural viral proteins (VP1–VP4, VP6, VP7) and six non-structural proteins (NSP1–NSP6). It is the gene 11, which codes for both NSP5 and NSP6. Based on the two structural proteins, VP7 (a glycoprotein—G protein) and VP4 (a protease-cleaved protein—P protein), forming the outer shell, rotavirus strains are characterized. Group A rotavirus classification based on neutralizing VP7 antigens is termed G-type

Fig. 2 **a** Ultrastructure of rotavirus. **b** Schematic representation of a rotavirus virion (With kind permission, these pictures are adapted and modified from Juana et al. 2007 [Ref. 3])

(since VP7 is a glycoprotein), and VP4 neutralizing antigens is termed P-type (since VP4 is a protease sensitive protein). Genes that encode VP7 and VP4 can segregate separately, resulting in a large amount of antigenic diversity [42, 88]. So far, 23 G-types and 32 P-types of rotavirus have been reported (Tables 1, 2) [24, 57, 94]. Based on G and P type, a particular strain is known by describing its G and P types together, e.g. G1P2A[8], meaning this rotavirus strain belongs to G-type 1, P-serotype 2A and P-genotype 8. There has been complete agreement between the genotype and serotype of G types, but such agreement is not available for P types, due to difficulty in developing serological typing reagents, such as monoclonal antibodies, against VP4. Various combinations of G-and P-type exist in nature giving a huge variety of rotavirus types. There are 45 different combinations of G and P types that have been recognized to date [57].

Epidemiology and Genetic Diversity of Group A Rotaviruses

Like most RNA viruses, the rotavirus genome is very flexible and it is observed that the genome can undergo point mutation at the rate of 5×10^{-5} per nucleotide, 1000 times higher than for most of DNA viruses [13, 72]. In case of the reassortant strains, point mutations occur spontaneously, but can accumulate over time and result in the development of different lineages or sub lineages within types [33, 43, 45]. Genetic rearrangement has been shown to occur both in vitro and in vivo [33, 76]. However, reassortment is considered to be most important and significant factor in the generation of strain diversity. This process occurs in vitro in mixed infections with different strains of Group A rotavirus and has also been used to study gene-protein assignment [17, 76]. Comparison of various unusual strains and comparing their gene homology has provided ample evidence that reassortment also occurs

in vivo resulting in large number of variants co existing in nature [17, 27, 29, 74, 84, 93, 100].

Due to diverse nature of rotavirus and its ever changing genetic character, the epidemiological study and surveillance are vital. Epidemiological studies conducted in various parts of the world have shown a wide variety of rotavirus strains occurring in nature. In a study by Iturriza-Gomara et al. [45], a collection of over 3000 rotavirus isolates collected during 1995–1998 indicated the presence of four common types, G1P[8], G2P[4], G3P[8] and G4P[8], that constituted over 94% of typed isolates [45]. However, uncommon strains like G1P[4], G2P[8], G4P[4], G9P[6] and G9P[8] were found in smaller frequencies. In contrast, an epidemiological survey conducted in India from 1986 to 1988, showed that 70% of strains belonged to the unusual combination of G9P[11] [29, 74]. A survey conducted in 1993 showed equal distribution of the common strain G2P[4] and the unusual strain G9P[6], which were found in 21 and 24% of isolates, respectively in India [4, 74]. In two European studies, 38% of isolates belonged to G1P[8], 52% to G4P[8] and 3% to the unusual combination of G9P[8] [4, 83]. In a North and central American study carried out in 1990–1992, 71% of isolates were of type G1P[8], with 20% of isolates belonging to either G3P[8] or the unusual type G1P[6] [75, 83]. In a similar study carried out a few years later (1996–1997) the emergence of G9P[6] was observed, increasing from 0% (in 1990) to 6% [75]. In Brazil, serotype G5 is commonly isolated in greater numbers, >10% as reported in many studies [39, 51].

In most of the studies from eastern India, G1, G2 and P[8] revealed as the most common genotypes. In a study from Kolkata (1998–2000), the most predominant genotype was G1P[8], followed by G2P[4] and G4P[8]. A number of uncommon genotypes, G1P[4], G2P[8], G2P[6], G4P[4], and G4P[6] were also observed [29]. A novel rotavirus strain G4P[8] was also reported from children with acute diarrhea during that study. In an another study few unusual combinations G1P[6], G2P[8], G2P[6],

Table 1 Diversity of P genotypes (VP4 gene) of group A rotavirus and their probable origin

Genotype	Probable origin	Accession no.	Isolate name
P[1]	Bovine	AB119636	NCDV
P[2]	Simian	M23188	SA11
P[3]	Feline, Canine	D14723	FRV64
P[4]	Human	M58292	L26
P[5]	Bovine	M22306	UK
P[6]	Porcine	M33516	Gottfried
P[7]	Porcine	X13190	OSU
P[8]	Human	L34161	Wa
P[9]	Human	D10970	AU-1
P[10]	Human	M60600	69 M
P[11]	Bovine	D13394	B223
P[12]	Equine	L04638	H-2
P[13]	Porcine	L07886	MDR-13
P[14]	Bovine	AB158430	Sun9
P[15]	Ovine	L11599	Lp14
P[16]	Murine	AF039219	EDIM
P[17]	Avian	AB009632	PO-13
P[18]	Equine	D13399	L338
P[19]	Human	D38054	Mc345
P[20]	Murine	U08424	EHP
P[21]	Bovine	AF237665	Hg18
P[22]	Lapine	AF526374	160/01
P[23]	Porcine	AY768809	34461-4
P[24]	Simian	AY596189	TUCH
P[25]	Human	AY773004	Dhaka6
P[26]	Porcine	DQ061053	134/04-15
P[27]	Porcine	DQ534016	CMP034
P[28]	Human	EU805773	Ecu534
P[29]	Bovine	AB454420	AzuK-1
P[30]	Chicken	EU486956	Ch-2G3
P[31]	Chicken	EU486962	Ch-661G1
P[32]	Porcine	FJ492835	61/07ire

G4P[4], and G4P[6] were detected in 14.3% of cases whereas thirty percent of samples in this study were mixed infections [31]. Eleven specimens collected from Manipur were also genotyped and revealed a very high degree of genomic reassortment during the study. Two G9 strains (RMC321 and ISO-3) were identified with P[19] and P[8] specificities after sequence analysis and revealed that they have much lower homology to the G9 strains (116E, INL1, and G16) isolated earlier from Indian subcontinent, but have much higher homology to isolates from Argentina, Brazil, Malawi, Taiwan, and USA suggesting a separate progenitor for these strains [31]. The genotypic distribution in eastern India, varied remarkably from the earlier study period (1998–2001) with G1 being the most predominant strain followed by G2, G12, G9 and not a single G3 or G4 isolate was detected separately during a surveillance

(2003–2005) where G12 strains emerged as an important pathogen among children in eastern India [80]. In the year 2006, it was also reported that the G9 strains are increasing as an important VP7 genotype among children in eastern India and phylogenetic analysis of these G9 strains revealed clustering within G9 lineage III [81]. While the four major global rotavirus serotypes continue to be detected, a striking feature of most studies carried out over the last decade is the worldwide emergence of serotype G9 and G12.

Emergence of Serotype G9 and G12 Rotaviruses

Serotype G9 is an emerging serotype reported globally in high numbers. This serotype is not new and was first detected in the USA in 1983, but was not isolated again

Table 2 Diversity of G genotypes (VP7 gene) of group A rotavirus and their probable origin

Genotype	Probable origin	Accession no.	Isolate name
G[1]	Human	D16343	KU
G[2]	Human	M11164	S2
G[3]	Simian	M21650	RRV
G[4]	Porcine	X06386	Gottfried
G[5]	Porcine	X04613	OSU
G[6]	Bovine	M12394	NCDV
G[7]	Chicken	X56784	Ch-2
G[8]	Bovine	D01054	A5
G[9]	Human	L14072	116E
G[10]	Bovine	X53403	61A
G[11]	Porcine	M23194	YM
G[12]	Human	M58290	L26
G[13]	Equine	D13549	L338
G[14]	Equine	D25229	CH3
G[15]	Bovine	AF237666	Hg18
G[16]	Murine	U08430	EW
G[17]	Turkey	S58166	Ty-1
G[18]	Pigeon	D82979	PO-13
G[19]	Chicken	AB080738	Ch-1
G[20]	Human	EU805775	Ecu534
G[21]	Bovine	AB454421	AzuK-1
G[22]	Turkey	EU486973	Tu-2E10
G[23]	Pheasant	FN393056	Phea17655

until more than a decade later in 1996. To date, serotype G9 rotavirus strains have been reported in more than ten countries, including Thailand, India, Brazil, Bangladesh, Malawi, Italy, France, United States, United Kingdom and Australia, and is reported as endemic in the US, Bangladesh and Thailand [35, 82, 93]. In India, serotype G9 was first reported in New Delhi in 1986 [38]. There was gradual increase in its prevalence, from 0.0% of strains in 2003 to 9.2% in 2005 [58, 81].

G12 strains were rare in humans and after being first detected in 1987–1988 from Philippines [89], emerged as an important g-genotype in the last few years. In recent years, studies from India, Bangladesh, Nepal, Argentina and other parts of the world have reported increasing incidence of another potentially emerging strain G12, with a variety of P types such as P[6], P[8] and P[9] [6, 8, 19, 35, 58, 67, 68, 70, 71, 73, 80, 82, 89]. During routine surveillance of diarrhea caused by rotavirus in Calcutta, India, the emergence of a novel type of human rotavirus, serotype G12, was reported [80]. Three rare human serotype G12 strains were detected from diarrheic samples from children aged less than 8 months. The VP7 genes of the serotype G12 strains and their deduced amino acid products showed maximum homology (97–99% at the nucleotide level and 98% at the amino acid level, respectively) with those of

two recently reported serotype G12 strains from the United States and Thailand, but lesser homology with those of the prototype G12 strain, L26 [30]. Thereafter this serotype maintained a steady rate of occurrence between 8 and 17% between the year 2001–2008 [58, 80].

The epidemiological characteristics of the introduction of serotype G9 and G12 strains worldwide may suggest that pre-existing immunity towards other rotavirus serotypes was not sufficient in preventing infection and proliferation of these strains [26, 30, 44, 80]. Though the current vaccines seem to provide heterologous protection, long term studies after vaccination in highly endemic areas with lots of strain variations will shed light whether the formulation of rotavirus vaccines used in different parts of world will need regular updating to adapt their composition to that of locally or regionally circulating prevalent strains.

Animal Strains and the Zoonotic Potential of Human Rotavirus Infection

The great diversity of human rotaviruses has been contributed by possible transmission from animal rotaviruses, either as whole viruses [38] or by contributing individual genes during reassortment in mixed infections [60]. Animal

Table 3 Past and current rotavirus vaccines

First generation (animal backbone)

Acronym (trade name)	Backbone	Valency	Antigens	Concept	Status
RIT 4237	Bovine	Single-strain	G6P[6]	derived from the Nebraska calf diarrhea virus strain	Discontinued
RRV-MMU	Rhesus	single-strain	G3P[3]	isolated from a rhesus monkey	Discontinued
WC3 (non-reassortant)	Bovine	single-strain	G6P[5]	low-passage bovine strain from calf in Pennsylvania	Discontinued
LLR	Lamb	single-strain	G10P[12]	Derived from Lanzhou lamb rotavirus	Licensed

Second generation (animal-human reassortants)

Acronym (trade name)	Backbone	Valency	Antigens	Concept	Status
Wa X UK	Bovine-Human	Single-strain	G6P[8]	live oral human(Wa)-bovine (UK) reassortant	Active Phase I
RRV-TV (Rotashield)	Rhesus	Four-strain	G1P[3], G2, G3, G4	Rhesus-human reassortant with G3 rhesus backbone	Withdrawn
Reassortant WC3 (RV5; RotaTeq)	Bovine	Five-strain	G1, G2, G3, G4, P[8]	bovine-human reassortant with G6 bovine backbone	Licensed

Second generation (human attenuated strains)

Acronym (trade name)	Backbone	Valency	Antigens	Concept	Status
M37	Human	Single-strain	G1P[6]	Monovalent live oral human neonatal M37 strain	Discontinued
Rotarix	Human	Single-strain	G1P[8]	Monovalent live oral human 89-12 strain	Licensed
116E	Human	Single-strain	G9P[11]	Monovalent live oral human neonatal 116E strain	Active Phase I

rotaviruses are even more flexible and exhibit a greater number of possible G/P combination than human rotaviruses. There are a number of serotypes that are found both in animals and humans, such as G3 which is found in cats, dogs and monkeys [28, 56], G9 which has been identified in lambs and pigs [4, 74, 75] and P[6] which is found in pigs [50, 74]. Serotype G6, an uncommon human serotype but a common bovine or porcine type, was isolated in Hungary from a child and upon characterization, it was found to possess a VP4 gene of type P[9] with porcine characteristics [7]. In Australia, strain G6P[13], normally found in cattle, was isolated from a hospitalized child suffering from acute gastroenteritis suggesting a possible reassortment between human and animal rotaviruses [63]. In some other cases the genetic relatedness determined through nucleotide sequencing has suggested an animal rotavirus as the likely origin of human isolates [17, 38, 84]. There have been many reports of human rotaviruses with unusual combinations of subgroup, G-P-type, suggesting considerable diversity among rotavirus strains worldwide [25, 29, 35, 36, 82]. Moreover, there are also reports of unusual strains such G5, G8 and G10 occurring locally in certain parts of world [25, 29, 30, 36]. These findings indicate that there is a huge diversity of rotaviruses circulating in humans and, more importantly in the context of public health, animal strains have also been found to be the cause of infections in humans.

Rotavirus Vaccines

Due to the significance of rotavirus infection, its enormous health burden worldwide and, in particular, the staggering death toll in developing countries, the development of an effective rotavirus vaccine has been a major priority. Researchers around the world have been involved in rotavirus vaccine development and the recent licensure of few human rotavirus vaccines was highly anticipated. The current and previous candidate rotavirus vaccines can be categorized in two groups on the basis of their development (Table 3).

First Generation Vaccines

These early rotavirus vaccines were single-animal strains that were naturally attenuated in that they did not cause clinical disease in humans but conferred protection against subsequent infection with human rotavirus strains.

Second Generation Vaccines

(a) animal-human reassortants—these rotavirus vaccines developed subsequent to the first generation vaccines. Human-animal reassortants: these reassortants contain an animal strain which incorporate additional genes from human strains by capitalizing on the viruses' ability to

reassort in vitro; (b) human attenuated strains—these are vaccines developed through attenuation of human rotavirus strains.

Development of Rotavirus Vaccines

Jennerian Approach

The first generation of rotavirus vaccines was developed on the basis of the Jennerian approach where single-strain animal rotaviruses were used as human vaccines—these animal strains were naturally attenuated in that they did not cause clinical disease in humans but conferred protection against subsequent infection with human rotavirus strains [15, 16]. In industrialized countries, clinical trials for three Jennerian vaccines using attenuated animal strains demonstrated good efficacy against severe rotavirus disease [20, 69, 79, 91, 95–98]. In contrast, these vaccines failed to provide protection in challenging impoverished settings where the vaccine would be most critical to saving lives [32, 40, 41, 48, 49, 85]. That said, the encouraging data from developed countries for these early vaccines confirmed that rotavirus vaccines held great promise; moreover, the trials identified early in the course of vaccine development that improvements in performance would be necessary for success of these vaccines in developing country settings. It is important to note that substantial heterogeneity existed among the trials for the first generation vaccines with regard to number of doses administered, age at vaccine administration, outcome measures of severity, and time of follow-up. The development and testing of this first generation Jennerian vaccines is reviewed below.

RIT 4237

The first rotavirus vaccine tested was a bovine strain belonging to serotype G6 (P type 6 specificity), Nebraska calf diarrhea virus, that underwent 147 passages in bovine embryonic kidney cells and seven in African green monkey kidney cells [55]. Efficacy of this vaccine, designated RIT 4237, was 60–89% against severe rotavirus disease in clinical trials in industrialized settings (Finland and US) [79, 95, 96, 98]. In contrast, RIT 4237 provided no protection in poor settings of Rwanda (efficacy = 0%) [32], the Gambia (7%) [41], and US Native Americans (0%) [85]. In Peru, however, vaccine efficacy was 58–75% against the more severe rotavirus illnesses [48]. The studies also highlighted that the vaccine did not induce sufficient protection against milder rotavirus disease in either developed or developing country settings, which was consistent with the observation that under natural

conditions, milder re-infections are not uncommon. Moreover, because RIT 4237 was derived from serotypes G6 and P6, a strain that does not cause human disease, proof of concept was established that a heterologous virus could indeed provide heterotypic protection against severe rotavirus disease.

RRV-MMU

The RRV-MMU vaccine was a G3 strain that was originally isolated from a 3 month-old rhesus monkey with acute diarrhea [87]. RRV-MMU was developed as an alternative to RIT 4237 on the premise that it shared the G3 epitope with one of the four strains causing most of the human rotavirus disease worldwide. The RRV-MMU strain underwent 9 passages in monkey kidney cell cultures and 7 passages in diploid rhesus cells [55]. Clinical trials for the vaccine in industrialized countries resulted in variable efficacy ranging from 12–90% [20, 69, 97]. No protection was observed after one dose of RRV-MMU in an impoverished Native American population in the US [85]. Several findings from these trials were of interest for future vaccine development. First, in the one trial from Venezuela where high efficacy (90%) was observed against severe disease, the predominant circulating strain (G3) was similar to the rhesus vaccine strain, indicating that serotype-specific protection might be of importance for the animal-based rotavirus vaccines, and prompted the addition of more antigens in future modifications of animal based vaccines [69]. Second, the vaccine provided greater protection against severe illness compared to mild and moderate disease. Third, a small immunogenicity trial from Japan indicated that additional booster doses of RRV-MMU may enhance protection against heterotypic strains [92].

WC3 (Non-Reassortant)

This is a vaccine similar to RIT 4237 in that it was derived from a G6 serotype bovine strain (P type 5) that was isolated from a calf with diarrhea in Pennsylvania [21]. The vaccine underwent 12 cell-culture passages prior to evaluation in two efficacy trials. In a trial in the US, three doses of WC3 conferred 87% protection against severe rotavirus disease [91]; in contrast, two doses of WC3 provided substantially lower protection (31%) in a trial conducted in the Central African Republic [40].

LLR

Lanzhou lamb rotavirus (LLR) vaccine (Lanzhou Institute of Biological Products, Lanzhou, China), isolated in 1985 from a local lamb with diarrhoea, grown in primary calf

kidney cells. After 37 generations, the LLR vaccine has proved to consist of monovalent serotype of (P[12]G[10]), group A [5]. This RV vaccine was licensed formally for gastroenteritis prevention (group A rotavirus) among children in China in 2000. Since then nearly 5,000,000 children younger than 5 years old have been immunized. However, little data is available on the vaccine's safety, immunogenicity and efficacy. The vaccine is reported to induce neutralizing antibody responses in 60% of patients but its efficacy is unknown since it was not tested against placebo in a controlled phase III trial [2, 14].

Modified Jennerian Approach

The second generation of vaccines, including currently licensed or candidate rotavirus vaccines, use the modified Jennerian approach where the vaccines are either: (1) reassortant vaccines with the backbone of an animal strain (e.g., RRV-TV, WC3) which incorporate one or more human VP7 or VP4 genes; or (2) attenuated human rotavirus strains such as the common G1 strain or uncommon neonatal strains [15, 16]. Of these second generation vaccines, three have undergone phase III efficacy trials in various developing and developed country settings: RRV-TV (RotaShield); RV1 (Rotarix); and RV5 (RotaTeq). In general, less heterogeneity in study design was observed across these trials because of lessons learned from trials of first generation vaccines with regard to number of doses administered, vaccine titer, age at immunization, outcome measure, and time of follow-up. Similar to the first generation vaccines, in developed countries, the second-generation vaccines demonstrated high efficacy against severe rotavirus disease [47]. In addition, substantial improvements were noted compared with the first generation vaccines with regard to vaccine efficacy in developing countries where a pooled efficacy of 51% was observed [47, 49]. While efficacy of the second generation vaccines was still lower in developing countries than efficacy in industrialized settings, the higher burden of severe disease in developing countries translated into a greater absolute reduction in severe rotavirus disease in the poorest settings [52].

Rhesus-Human Reassortant (RRV-TV; Rotashield)

The human rhesus reassortant vaccine resulted from two strategic improvements to the first generation single-strain RRV-MMU vaccine. Initially, a single reassortant preparation was developed by combining 10 genes from the naturally attenuated RRV-MMU (G3 strain) with a single VP7 gene from commonly encountered human rotaviruses (i.e., G1, G2 or G4) [55]. The reassortants were recovered after coinfection of monkey kidney cell cultures with the

RRV-MMU strain with the human rotavirus strains. Successful development of a reassortant vaccine with broad protection against diverse, epidemiologically important human strains was sought by combining the individual reassortants into a tetravalent RRV (RRV-TV) formulation, with G1, G2 and G4 human strains and G3 rhesus component. RRV-TV was evaluated in both developed and developing country settings. In the early trials, a lower titer was used compared with subsequent trials of RRV-TV. The trials using lower RRV-TV titers yielded good protection (efficacy = 78%) in the US, but efficacy was lower in Brazil (46%) and Peru (30%), a middle and low-middle income country, respectively [47]. In subsequent studies of RRV-TV, a higher concentration (4×10^5) was administered in multiple doses to improve vaccine performance. These trials yielded good efficacy against severe rotavirus disease in the US (80%) and Finland (91%), leading to the introduction of RRV-TV in the routine childhood immunization schedule in the US. Due to these encouraging results, RRV-TV was licensed and introduced in the US; however, RRV-TV was subsequently withdrawn by the manufacturer from the US after a post licensure association was uncovered between the vaccine and a rare adverse event, intussusceptions [59, 66].

Bovine-Human Reassortants (WC3; RotaTeq)

RotaTeq (RV5) is a pentavalent vaccine with five separate reassortant viruses, each one that incorporates either a VP7 (G1, G2, G3, G4) or VP4 (P8) gene from a human rotavirus strain on the RNA backbone of the previously discussed WC3 bovine rotavirus strain [21]. Thus, the naturally attenuated WC3 bovine virus was altered to provide cross-protection against epidemiologically important human rotavirus strains.

In a large clinical trial conducted primarily in the US and Finland, RV5 showed an efficacy of 98% against severe rotavirus gastroenteritis. A substantial reduction (59%) in admissions from all-cause gastroenteritis was also noted in the trial. Protection was good against all G1-4 and G9 serotypes (range 88–100%) [47]. Clinical trials in developing countries of Asia and Africa have recently been completed. RV5 has also been introduced in routine immunization programs of several countries. In the US, field effectiveness of the vaccine (88%–100%) was similar to that observed in the clinical trial.

Human Rotavirus Vaccine (RIX-4414; Rotarix)

Rotarix (RV1) is a single-strain vaccine of P[8]G1 specificity derived from a human rotavirus strain (89-12) which was isolated from a child who was naturally infected with rotavirus gastroenteritis in 1989 [9]. Attenuation of the

virulent 89-12 strain was conducted by serial passages in monkey kidney cell cultures and plaque purification.

RV1 was subsequently evaluated in large clinical trials in middle and high income countries in Latin America, Europe and Africa. Many countries, predominantly in the Region of the Americas, have introduced RV1, and several post-licensure evaluations have been conducted over the time. Of note, the fully heterotypic (non-vaccine) P[4]G2 strain was responsible for all infections in this study, thus confirming the contention that RV1 provides sufficient cross-protection against a broad range of serotypes. Two studies from field settings in Australia also further support that cross-protection occurs after RV1 vaccination, demonstrating that RV1 provided good protection against severe disease from partially heterotypic P[8]G9 (84%) and fully heterotypic P[4]G2 (86%) strains [86]. Rotarix and RotaTeq vaccine were recently found to be contaminated with porcine circovirus viral DNA and were temporarily suspended by US FDA in March 2010. However it was proven that porcine circovirus did not impose any threat to human and thus both vaccines have been declared safe for use.

The effectiveness of the rotavirus vaccines currently available on the international market has also inversely correlated to the childhood mortality levels in the countries where the clinical trials were performed [90]. In general, live oral vaccines have had an inconsistent performance in India and other developing countries where most of the rotavirus disease burden lies. Several third generation rotavirus vaccines are in development because of possible safety issues as well as low efficacy in developing world, with use of live oral attenuated vaccines. Currently groups are pursuing inactivated viruses or recombinant virus like particle approaches. Parenteral immunization with inactive virus has proven effective in animal models. Similarly parenteral or intranasal immunization with recombinant non replicating virus like particles has been effective in animal models and these candidate vaccines are ready for phase I studies in humans.

Candidate Rotavirus Vaccines in India

In spite of recommendation by WHO for implementation of rotavirus vaccine in national vaccination programs, the current vaccines are not affordable in most developing countries. To overcome this problem, vaccine manufacturer's in developing countries are being supported by Program for Appropriate Technology in Health (PATH) - which is an international nonprofit organization. Several candidate rotavirus vaccines are currently being developed in India. One candidate is based on a neonatal rotavirus strain, 116E, and is being developed by Bharat Biotech

International Limited. This strain is a natural reassortant between a human rotavirus virus G9P[11] strain with the VP4 protein from a bovine rotavirus strain and was originally isolated from a neonate with an asymptomatic rotavirus infection [10]. A recent randomized double blinded placebo controlled trial for this strain has demonstrated that the vaccine elicits a strong immune response in Indian children [11]. This study, as well as previous studies, did not find any association with vaccination and adverse events [10, 11]. Phase 3 trials of the 116E vaccine in India are in progress. If successful, this vaccine provides the possibility of a locally developed and tested rotavirus vaccine for the Indian and international markets.

In addition a series of UK bovine strain-based reassortants have been licensed by the NIH, USA to several companies in India, China and Brazil. This vaccine is being formulated as tetravalent G1-G4 of a UK backbone. Earlier this vaccine was shown to be highly immunogenic, efficacious and non reactogenic in Finland. In India, Serum Institute of India and Shantah Biotechnics Ltd are developing this vaccine [22, 23]. With the support of PATH, Phase 1 and 2 clinical trials have been conducted with these vaccines to evaluate safety (<http://www.path.org/publications/detail.php?i=1578>). If either of these vaccine candidates prove to be effective and safe, they could be produced as much lower price for developing nations.

Perspectives

Rotavirus causes a considerable amount of health problems both in developed and developing countries. Hospitalization resulting from acute severe diarrhea in developed countries (India, Nepal, Bangladesh, etc.), lead to significant financial burdens on public health systems and the high level of fatalities. More than 90% of children have experienced at least one episode of rotavirus-related diarrhea by the age of 5 years. Rotavirus infection cannot be prevented simply by adherence to good hygiene and sanitation; therefore prevention of the disease by vaccination is seen as the only practical means of control. In spite of predicted low vaccine efficacy the overall reduction in disease severity and hospitalization rates will have huge impact in preventing ~27,000–44,000 deaths in children <5 years of age annually in India. The cost-effectiveness of a national rotavirus vaccination program in India has been evaluated in two separate studies, which reached similar conclusions [34, 77]. At a vaccine price of US \$1.00 dollar per dose, the target pricing for Indian vaccines, these models estimated an incremental cost effectiveness ratio (ICER) of \$21.41 to \$34 per disability adjusted life year (DALY) which satisfies the WHO criterion for a cost effective intervention where the

incremental cost-effectiveness ratio is less than the country's per capita gross domestic product [46]. Thus, a national rotavirus vaccination program in India would be a highly cost-effective means for reducing the tremendous disease and economic burden of rotavirus GE in the country.

Due to the diverse nature of rotavirus, the genomics study and continuous longitudinal surveillance of rotavirus are essential. There are different serotypes circulating in a community at any given time and the types of strains circulating change in subsequent years. The emergence of new types has been reported worldwide and they pose a serious threat to control of the disease. The global disease burden can only be reduced if the focus is to develop a vaccine which will not only protect against all types of heterologous strains, but will also be affordable and available to the poorest regions of the world which suffer the greatest burden of rotavirus infection.

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