Rotavirus vaccines: current prospects and future challenges

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Rotavirus is the most common cause of severe diarrhoea in children worldwide and diarrhoeal deaths in children in developing countries. Accelerated development and introduction of rotavirus vaccines into global immunisation programmes has been a high priority for many international agencies, including WHO and the Global Alliance for Vaccines and Immunizations. Vaccines have been developed that could prevent the enormous morbidity and mortality from rotavirus and their effect should be measurable within 2–3 years. Two live oral rotavirus vaccines have been licensed in many countries; one is derived from an attenuated human strain of rotavirus and the other combines five bovine-human reassortant strains. Each vaccine has proven highly effective in preventing severe rotavirus diarrhoea in children and safe from the possible complication of intussusception. In developed countries, these vaccines could substantially reduce the number and associated costs of child hospitalisations and clinical visits for acute diarrhoea. In developing countries, they could reduce deaths from diarrhoea and improve child survival through programmes for childhood immunisations and diarrhoeal disease control. Although many scientific, programmatic, and financial challenges face the global use of rotavirus vaccines, these vaccines—and new candidates in the pipeline—hold promise to make an immediate and measurable effect to improve child health and survival from this common burden affecting all children.

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Diarrhoea remains one of the most common illnesses of children worldwide.[1] In developing countries, it is the third most common cause of death, with an estimated 2 million deaths per year or 17% of all deaths in children younger than 5 years.[2,3] In developed countries, diarrhoea remains the second most common cause of hospital admission and doctor visits. Although more than 20 different microorganisms (bacteria, parasites, and viruses) cause diarrhoea, one pathogen—rotavirus—is the most frequent causative agent for the most severe disease in children younger than 5 years worldwide. Rotavirus causes 25–55% of all hospital admissions for diarrhoea[4–7] and more than 0·6 million deaths every year.[8] Research has led to development of a new generation of live oral rotavirus vaccines that will soon be introduced into routine programmes for childhood immunisation.[9] These vaccines could potentially reach 60–80% of the children worldwide within a decade, prevent clinical visits and admissions as a result of diarrhoea, and stop deaths in developing countries with the most severe disease.

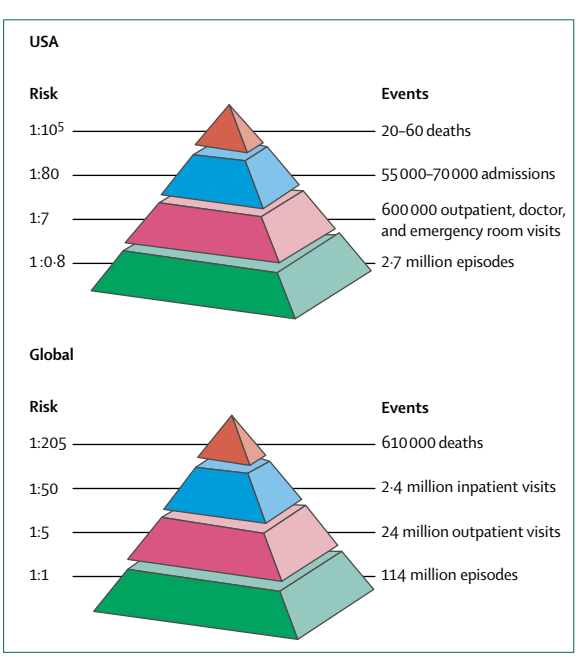

Figure 1: **Rotavirus disease burden and risk by setting**

This Review assesses the global epidemiology of rotavirus disease, describes the 20-year path of scientific development of vaccines ready for public-health practice next year, and discusses the many challenges ahead before we can expect these new vaccines to achieve their full effect on prevention of fatal diarrhoea in children in developing countries. Although introduction of rotavirus vaccines in Europe, North America, Latin America, and Asia is anticipated in the next 1–3 years, the international community is especially interested that these vaccines rapidly become part of childhood immunisation programmes in developing countries.[10] Decisions made today could greatly affect the speed with which these lifesaving vaccines reach those populations where mortality due to this disease is greatest.

Background

Burden of disease

Rotavirus infects all children early in life and although most first infections cause mild diarrhoea, 15–20% need treatment at a clinic, and 1–3% lead to dehydration needing hospitalisation (figure 1). The virus can be identified in 15–35% of children younger than 5 years treated in outpatient settings for diarrhoea and in 25–55% of those hospitalised.[4,11] About 600 000 children die every year from rotavirus, mainly in developing countries, and this figure represents about 5% of all deaths in children younger than 5 years.[8] Mortality is greatest in south Asia and sub-Saharan Africa, with more than 100 000 deaths every year occurring in India alone (see figure 2 for a pictorial representation of the global rate of mortality

from rotavirus diarrhoea).[12] About 1 in 200 children born in these regions will die of rotavirus. Although the estimated number of deaths from diarrhoea has fallen strikingly over the past two decades as a result of improved treatment with oral rehydration therapy and sanitation and water interventions, the number of deaths from rotavirus has not decreased as much.[1,13,14] Although most studies of rotavirus deaths have extrapolated numbers from global rates of diarrhoeal deaths, several investigators have specifically assessed risk factors, and pathological findings of the deceased clearly established rotavirus to be an important causative agent.[15–17]

Pathogenesis and clinical presentation

Rotavirus is transmitted by the faecal-oral route and a small infectious dose (<100 virus particles) facilitates spread from person to person or possibly via airborne droplets.[18] Once ingested, virus not neutralised by stomach acid attaches to the proximal small intestine. During the incubation period of 18–36 h, the virus enters epithelial cells where it first elaborates a potent enterotoxin—NSP4—that can cause diarrhoea, and then goes on after 18–36 h to destroy the epithelial surface leading to blunted villi, extensive damage, and shedding of massive quantities of virus (>10^{12} particles per g) in stools.[19,20] The outcome is a profuse watery diarrhoea with loss of fluid and electrolytes that can last 2–7 days and might lead to severe or fatal dehydration. Aggressive rehydration with oral or intravenous fluids can correct these imbalances and sustain a child until the diarrhoea stops. Rotavirus infection was long thought to be localised exclusively in the small intestine, but studies have detected viral protein and RNA in the blood,[21,22] organs,[23] and cerebrospinal fluid,[24–26] although the clinical implications of these findings remain unclear.

Few clinical or epidemiological features distinguish a child with rotavirus from a child with diarrhoea of any other cause. Newborn babies can be infected in the nursery, but these infections are often asymptomatic, perhaps because of the protective effect of circulating maternal antibodies.[27–30] However, first infections in children 3–24 months of age most often lead to vomiting, then watery diarrhoea that is sometimes accompanied by fever. In temperate climates, rotavirus has a distinct peak in the cooler winter months when it is the predominant pathogen causing up to 70% of hospital admissions for diarrhoea.[31]

Virology and laboratory diagnosis

Rotavirus, is characterised by three shells (an outer capsid, inner capsid, and core) that surround 11 segments of double-stranded RNA.[19] Ten segments encode one protein each, whereas segment 11 encodes two proteins. Two proteins that form the outer capsid—VP7, a glycoprotein (G protein), and VP4, a protease-cleaved protein (P protein)—represent prime targets for the immune system to mount a neutralising antibody response and are key antigens used to characterise strains. Genes encoding the G and P proteins can be characterised by RT-PCR and ten different G genotypes and 11 P genotypes have been identified in strains found in human infections. In mixed infections with rotavirus, gene reassortment could theoretically lead to 110 different G and P combinations, but fortunately, this level of reassortment does not happen and only five strains are commonly detected (P[8], G1; P[4], G2; P[8], G3; P[8], G4; and P[8], G9).[32–37] These strains have become the most important targets for vaccine development. In developing countries compared with the developed world, the diversity of unusual strains is greater, more strains circulate at any time, and as many as 30% of children can be infected with more than one strain, suggesting an alternative mode of transmission, a stronger force of infection, or a higher infectious dose.[32,38,39]

Reassortment—the process by which cells infected with two viruses can yield mixed progeny with gene segments derived from each parent strain—has been used in the laboratory to develop new vaccines.[40] Animal strains from monkeys (rhesus RRV),[41] cows (WC3[42] and UK[43]) and lambs (LLR[44]) that are naturally attenuated for human beings have been cultured with human strains to yield reassortants. The selected progeny viruses contain ten genes from the animal strain, which maintain the property of attenuation, and one gene encoding the outer capsid proteins, representing the common human serotypes. Diagnosis of rotavirus infection is routinely established with inexpensive, straightforward, and sensitive methods of enzyme immunoassays, latex tests, or polyacrylamide gel electrophoresis, which are commercially available.[18,34,45]

Natural history and immunity

Studies of the natural history of rotavirus in Australia,[30] India,[46] and Mexico[47] have demonstrated the protective immunity induced from early infections. In these reports, first infections after the neonatal period were generally symptomatic, few children had severe or moderately severe disease on re-infection, and repeat disease was uncommon after the second infection. These findings provide the scientific rationale to judge that this protective immunity could be induced by live oral vaccines. Despite three decades of research, the mechanism of immunity to rotavirus remains unclear. Local immunity in the gut seems critical but it is hard to measure and is of short duration. Therefore, circulating immunoglobulin A antibodies have been used as the best proxy for vaccine take.[48] However, vaccines that have been marginally immunogenic have nonetheless been very efficacious in field trials, so large clinical trials have become the only secure means to ensure the effectiveness of a vaccine.[49,50] Studies in monkeys suggest that the passive transfer of circulating antibodies can confer protection against infection, a finding that provides the scientific basis for considering the future use of parenteral vaccine.[51] Waning

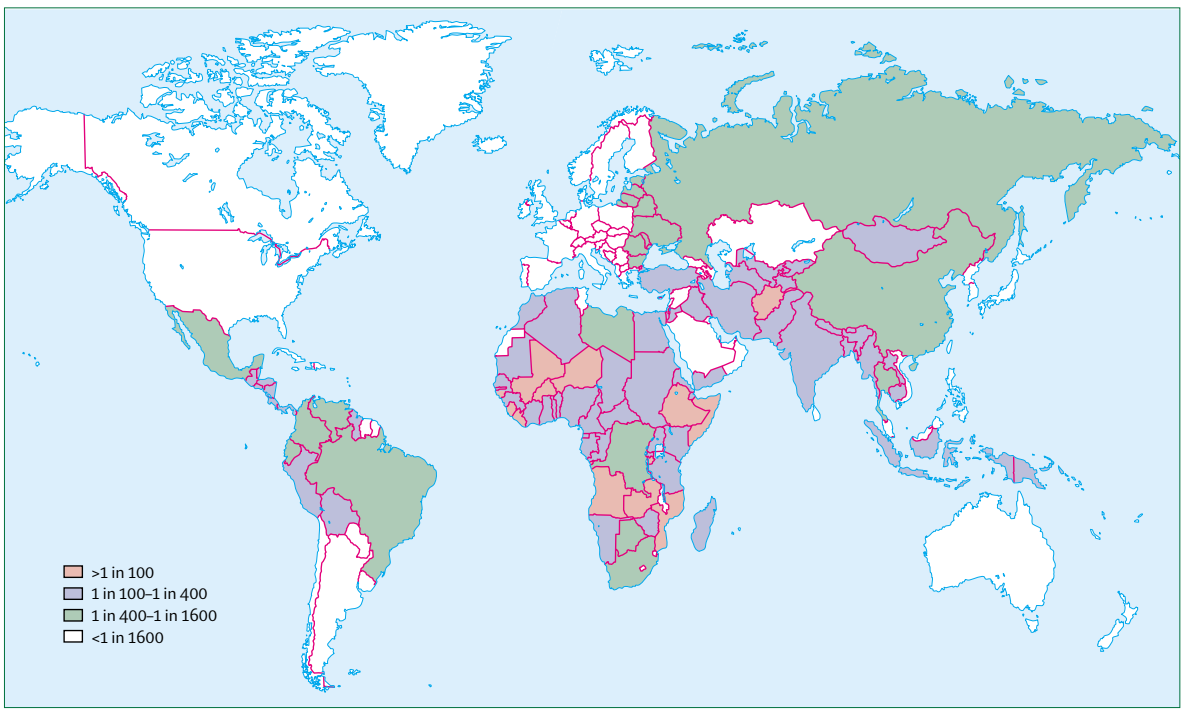

Figure 2: **Map of the world with estimated mortality from rotavirus diarrhoea**
Shading represents risk of death per child by 5 years of age.

immunity leaves elderly people susceptible to rotavirus infections and outbreaks have occurred in nursing homes and hospitals.

Rotavirus vaccines

The large burden of rotavirus disease has led many groups reviewing the need for new vaccines to select rotavirus as a high priority target for accelerated vaccine development. WHO's Diarrhoeal Disease Control Programme was the first to make this recommendation, which has been reaffirmed repeatedly by other international review groups, such as the Institute of Medicine (1985–86),[52] the Children's Vaccine Initiative (1996), the Bill and Melinda Gates Foundation which funded the Children's Vaccine Programme (1999), and the Global Alliance for Vaccines and Immunization (GAVI) (2002).[53]

History

The history of rotavirus vaccine development has been a story of starts and stops, of missteps and advances, of great successes followed by huge disappointments. Every experience has broadened our understanding of how to reach the ultimate goal of developing a safe and effective vaccine. In 1983, Vesikari[54] and colleagues did the first trial of a candidate oral vaccine derived from a bovine strain of rotavirus, RIT 4237. Their results outlined four basic principles of live oral rotavirus vaccination that anticipated many of the subsequent developments in the research area. They established that live oral rotavirus vaccines could be highly effective in shielding infants against severe rotavirus diarrhoea, that protection was greatest against more severe versus milder disease, that animal strains could guard against infection with human strains, and that antibodies were a poor proxy for protection (ie, the poor immune response to the vaccine did not show its high effectiveness in preventing disease in children). The RIT vaccine failed to show consistent efficacy in trials in developing countries and its further development was soon abandoned.

Despite these seminal findings, it still took 15 years to license the first vaccine—RotaShield (Wyeth-Lederle, Pearl River, NY, USA)—a tetravalent rhesus rotavirus vaccine developed by Kapikian at the National Institutes of Health.[41,55] The variable efficacy of monovalent vaccines based on animal strains was improved by development of reassortant strains that had the attenuation properties of the animal strains and individual genes encoding the outer capsid proteins of the common human strains. The tetravalent rhesus vaccine was tested in a series of clinical trials in the USA, Finland, and Venezuela, and was shown to be initially safe and highly effective, preventing more than 90% of admissions for rotavirus diarrhoea in US children and 79% in Venezuelan children.[56–58] The vaccine was licensed by the US Food and Drug Administration in August, 1998, and was immediately recommended for routine immunisation of all children in the USA, and was administered to over 600 000 infants in the first 9 months of the programme.[59,60]

In July, 1999, intussusception, an unexpected complication, was reported to occur in the first 2 weeks after

administration of the first dose of the tetravalent rhesus vaccine.[61] This adverse event led to removal of the vaccine,[62] a long period of reassessment of its risk, and, ultimately, to testing and assessment of a new generation of live oral vaccines. The mechanism of this association has never been elucidated, and the exact risk, which was judged to be about one case of intussusception in 10 000 vaccine recipients, remains controversial.[60,63–66] Subsequent analysis indicated that the level of risk was age-related.[67] The vaccine was offered to children at the time of their routine immunisations at 2, 4, and 6 months of age, but catch-up immunisation was provided any time up to 7 months of age. Most of these cases of intussusception happened in these "catch-up" children who were older than 90 days old at the time of immunisation. The risk in infants who received their vaccine on schedule was subsequently estimated at 1 in 30 000–50 000, a risk 10–20-fold less than that reported when the vaccine was initially withdrawn. Since natural intussusception spares infants in the first 3 months of life, the next generation of vaccines was tested with first doses administered almost exclusively to babies aged less than 90 days.[68] Of note, despite immunisation of 600 000 American children, the year-to-year variability in rates of diarrhoea admission was greater than the effect expected from immunising about a sixth of the US birth cohort. Therefore, the only measure of effect of the vaccine in the post-licensure period was one case-control study that showed the high effectiveness of the vaccine (>90%). (Staat M, University of Cincinnati, personal communication.)

Next generation of rotavirus vaccines

Two vaccines from GlaxoSmithKline (Rotarix) and Merck (RotaTeq) have been licensed in Europe and the USA respectively and in several other countries (table 1).[69–71] These vaccines are based on slightly different principles to achieve broad immunity against the diverse strains of rotavirus in circulation. Rotarix (GlaxoSmithKline, Rixensart, Belgium) was prepared from an individual human strain that replicates well in the intestine and is shed in the stool. RotaTeq (Merck Vaccines, Whitehouse Station, NJ, USA) is a combination of five bovine-human reassortants that replicate poorly in the gut, are administered in a 100-fold higher dose, and are shed in the stool of only around 10% of infants. Both are administered as an oral liquid in buffer as two doses (Rotarix) or three doses (RotaTeq) to babies, with the first dose given at 6–14 weeks of age. Each vaccine is stable at 2–8°C for 2–3 years, and each has been tested in a placebo-controlled trial of more than 60 000 infants to establish its safety against intussusception. Complete data are not yet available for either vaccine on the potential interference with the immune response to oral

	Rotarix (GlaxoSmithKline)	RotaTeq (Merck)
	Monovalent vaccine	Pentavalent vaccine
Original strain	Human rotavirus strain 89-12 P1A[8], G1	Bovine rotavirus strain WC3 P7[5], G6
Final vaccine	RIX4414	Five reassortants; G1_WC3 G2_WC3 G3_WC3 GA_WC3 P1A[8]_WC3
Titre	$10^{5·8}$ focus-forming units	$6·7–12·4×10^{7}$ plaque forming units
Method of attenuation	Passaged 43 times	Animal strains naturally attenuated; passage varies by reassortant 7–69 times
Cell substrate	Vero cells	Vero cells
Presentation and formulation	Dual-compartment device to reconstitute lyophilised virus with buffer	Liquid virus mixed with buffer; no reconstitution needed
Buffer	Calcium carbonate (1 mL)	Citrate phosphate sucrose (2 mL)
Dose regimen	Two oral doses	Three oral doses
	Dose 1: 6–14 weeks of age	Dose 1: 6–12 weeks of age
	Dose 2: ≥4 weeks later	Doses 2 and 3: at about 4–10-week intervals
Shelf life at 2–8°C	36 months	24 months
Vaccine interference		
Diphtheria tetanus pertussis, inactivated polio vaccine, *Haemophilus influenzae* type b vaccine, Hepatitis B vaccine	None	None*
Pneumococcal conjugate†	..	..
Oral polio 1 vaccine†	..	..
Post-dose shedding	>50%	~10%

*Insufficient evidence to confirm absence of interference with pertussis. †Study in progress.

Table 1: **Comparison of monovalent and pentavalent rotavirus vaccines**

polio vaccine; however, neither vaccine obstructs the immune response to other routine childhood vaccines, so they can be coadministered.

Attenuated human vaccine (Rotarix, GlaxoSmithKline)

The GlaxoSmithKline vaccine was developed from a strain of rotavirus (89-12) isolated from a patient in Cincinnati by Ward and Bernstein.[72] Strain 89-12, a representative of the most common serotype in humans, (P1A[8], G1), was first attenuated by passaging 43 times and was then cloned and further passaged in Vero cells and renamed RIX 4414.[71] Infants aged younger than 3 months who received the vaccine did not develop diarrhoea, vomiting, or fever.[73] The licensed vaccine is prepared as a lyophilised powder that is reconstituted with 1 mL of a citrate bicarbonate buffer to a titre of $10^{5,82}$ focus-forming units/dose and is administered orally.

Because of initial uncertainty about possible risk of intussusception and the notion that rotavirus vaccines should ultimately target children in developing countries, GlaxoSmithKline pursued an unusual clinical development plan. Initial trials in Finland showed safety, immunogenicity, and efficacy.[73,74] The assessments revealed that RIX4414 was clinically more attenuated than the parent strain 89-12. The vaccine was next tested in middle-income countries of Latin America (ie, Mexico, Venezuela, and Brazil)[75,76] (table 2) and in Asia (Singapore).[70] It was safe and rates of minor side-effects did not differ between recipients of the vaccine versus placebo. The vaccine showed an efficacy of 70–85% against any rotavirus diarrhoea and 85–93% against severe disease. Additional results from the efficacy trial in Latin America showed significant efficacy (83% [95% CI: 40–97%]) against non-G1 serotypes, that were predominantly G9 strains.[75,76]

Based on these results, GlaxoSmithKline undertook a large, multicentre, safety trial to confirm that the vaccine did not cause intussusception (table 3). 63 000 infants were enrolled from 12 countries of Latin America and in Finland. The endpoint for safety was a case of intussusception occurring within 31 days of receiving the first or second dose of vaccine. Six vaccinated patients and seven placebo recipients developed intussusception in this period, confirming the lack of a causal association at this level of confidence.[77] When the surveillance was done at age 1, the vaccinated patients had substantially fewer intussusception events than recipients of the placebo.[77] The vaccine also was very effective and in a subgroup of about 20 000 children followed up for medical events, it prevented about 85% of all severe rotavirus diarrhoea cases and admissions and reduced admissions for diarrhoea of any cause by 41% (table 2).[77,78]

The regulatory approach to license this vaccine has been innovative. New drugs are generally licensed first in their country of origin, most often in the USA or Europe. This vaccine, however, was first licensed in Mexico and the Dominican Republic in 2004, and has been licensed in more than 35 countries and the European Union.[78]

	Patients enrolled (n)		Outcomes			Effectiveness (95% CI)
	Vaccine	Placebo	Gastroenteritis outcome	In vaccine group	In placebo group	
Monovalent vaccine (Rotarix, GlaxoSmithKline)						
Finland[74]	245	123	Any	13	23	72 (42–87)
			Severe	3	10	85 (42–97)
Brazil, Mexico, Venezuela*[76]	464	454	Any	15	49	70 (46–84)
			Severe	5	34	86 (63–96)
Latin America†[77]	10 159	10 010	Severe	n/a	n/a	84·7 (71·7–92·4)
			Admission			85·0 (69·6–93·5)
Pentavalent vaccine (RotaTeq, Merck)						
USA, Finland[77]	2834	2839	Any	83	315	74·0 (66·8–79·9)
			Severe	1	51	98·0(88·3–100·0)
USA[84]	650	660	Any	15	54	72·5 (50·6–85·6)
			Moderate/severe	10	42	76·3 (52·0–89·4)
			Severe	0	6	100 (13·0–100·0)

*Subset of infants receiving the final dose of vaccine. †Overall reduction of 41% in admissions due to gastroenteritis of any cause.

Table 2: **Results of efficacy trials of two rotavirus vaccines**

Licensure in Brazil and the European Union which have national regulatory authorities meeting WHO standards, will facilitate licensure elsewhere in Latin America. WHO and UNICEF require that a vaccine tendered for global use be licensed in the country of origin. As a result of the European licensure, the vaccine will now be eligible for international procurement by UNICEF for developing countries or by the Pan American Health Organizations revolving fund for Latin American countries. In addition, WHO has recommended that the effectiveness of any new live oral vaccine be shown in poor populations in Africa and Asia before a global recommendation can be made. These studies are just starting and results could take 2–3 years.

Bovine-human pentavalent reassortant rotavirus vaccine (RotaTeq, Merck)

The Merck vaccine was prepared from a bovine strain of rotavirus, WC3, isolated from a calf in Pennsylvania by Clark and Offit at the University of Pennsylvania.[79] The original monovalent bovine strain that was naturally attenuated for human beings showed variable effectiveness in several clinical trials done in the USA, China, and Africa.[79–82] To improve the effectiveness of the vaccine against the diversity of common serotypes, reassortant strains were prepared containing ten genes from the parent bovine strain and an individual capsid gene from the most common human serotypes. Five single gene reassortants, every one containing a gene for a capsid protein for human serotypes G1, G2, G3, G4, and PIA, were combined in a pentavalent vaccine.[69]

This vaccine was formulated as a liquid with its own buffer (citrate phosphorous sucrose) that did not need reconstitution and is delivered in three oral doses beginning at 6 weeks of age. Preliminary clinical trials

	Site	Follow-up period post-vaccination	Number of participants		Intussusception cases			Relative risk (95% CI)
			Vaccinated	Placebo		Vaccinated	Placebo	
Monovalent (Rotarix, GlaxoSmithKline)	Europe, Asia,	31 days	~31 500	~31 500	Total	6	7	~0·86 (0·29–2·55)
	Latin America[77]				Dose 1	1	2	~0·50 (0·05–5·51)
					Dose 2	5	5	~1·00 (0·29–3·45)
	Latin America[78]	1 year	10 159	10 010	Total	4	14	0·28 (0·10–0·81)
Pentavalent (RotaTeq, Merck)	USA, others[84]	42 days	~35 150	~35 150	Total	6	5	~1·20 (0·37–3·93)
					Dose 1	0	1	~0 (0–17·30)
					Dose 2	4	1	~4·00 (0·45–35·79)
					Dose 3	2	3	~0·67 (0·11–3·99)
		1 year	~35 150	~35 150	Total	12	15	~0·80 (0·35–1·71)

Table 3: **Summary of vaccine safety trials focused on intussusception. Risk of intussusception following receipt of rotavirus vaccine or placebo**

showed immunogenicity and effectiveness of different formulations, and a final formulation containing 12×10^7 infectious units per dose was selected.[69,83] This formulation was assessed for safety against intussusception in a field trial done in more than 70 000 infants, mainly in the USA and Finland but with some enrolment in several countries in Central and South America, Europe, and Asia.[84] Intussusception was monitored intensively for 42 days after receipt of any dose and 11 cases of the disorder were identified, six in vaccinated patients versus five in recipients of the placebo. Within 14 days of receipt of any dose of vaccine, one case was identified in each of the vaccine and placebo groups.[84] In a detailed follow-up study, the vaccine showed an efficacy of 86% (95% CI 74–93) against doctor visits, 93% (88–96) against emergency department visits, and 96% (91–98) against admissions for rotavirus diarrhoea. It also protected against the range of rotavirus serotypes in circulation.[84] The vaccine was licensed by the US Food and Drug Administration in February, 2006 and was recommended by the Centers for Disease Control's Advisory Committee on Immunization Practices for the routine immunisation of children in the USA 2 weeks later.

Lanzhou lamb vaccine

The Lanzhou lamb rotavirus vaccine was prepared by Zhi-sheng Bai of the Lanzhou Institute for Biological Products in China. The strain was isolated from a local lamb with diarrhoea, grown in primary calf kidney cells, and licensed as a vaccine in China in 2001.[44] Little data is available about the safety, immunogenicity, and efficacy of this vaccine.[8] The lack of results from a proper clinical trial have cast doubt on its true efficacy.

Other rotavirus vaccines in development

Several vaccine manufacturers in developing countries have stepped forward to make their own rotavirus vaccines, recognising that the disease burden is enormous and that technology to prepare a rotavirus vaccine using traditional tissue culture methods is well established. In India, rotavirus strains identified in nosocomial outbreaks of asymptomatic infections in neonatal units in New Delhi and Bangalore have led to the identification of two candidate strains, 116E [46,86] and I321,[87,88] that have potential properties as vaccines. Both strains do not cause disease in infants, and each protects against severe rotavirus diarrhoea on reinfection. Both are natural human-bovine reassortants and their ability to grow well in the infant gut might derive from the presence of bovine VP4 genes that seem to resist neutralisation from maternal antibodies. Clinical development of these candidate strains might take 4–7 years, but the product will represent a completely Indian vaccine that could be inexpensive to produce.

Another neonatal rotavirus strain, RV3, is being pursued as a vaccine candidate by workers at the Royal Children's Hospital in Melbourne, Australia. Initial safety trials of a sole dose of vaccine showed poor serum immune responses,[89] but in a trial in which three doses of vaccine were administered to infants, those babies who mounted a good immune response seemed to be protected from disease.[90] The researchers are pursuing strategies to increase the titre of this vaccine and to return to clinical trials.

Another bovine reassortant vaccine developed at the National Institutes of Health (USA) contains four strains that are all individual gene reassortants combining the parent strain (UK) with the VP7 gene encoding one of the four major G serotypes.[91] The vaccine proved safe and immunogenic in a quadrivalent formulation when given as three doses to infants and did not interfere with immune responses to other vaccines given concurrently.[43] In a preliminary trial in Finland, the effectiveness of this vaccine was similar to the tetravalent rhesus-human reassortant vaccine but caused less fever.[91] The vaccine strains have been transferred by the National Institutes of Health to vaccine manufacturers in Brazil, China, and India.

The tetravalent rhesus vaccine withdrawn from the USA market because of its association with intussusception, has been acquired by a small biotech company called BioVirx, which plans to manufacture the

vaccine for use in developing countries. Re-analysis of the safety profile of this vaccine suggested that the risk of intussusception could be substantially reduced if the first dose of the vaccine was administered only to infants aged younger than 90 days.[67] Furthermore, despite the complication of intussusception, the vaccine might be more acceptable in developing countries where the risk of a child dying from rotavirus would be more than 100-fold greater than the risk of this rare complication.

Other original approaches to the development of rotavirus vaccines are also being pursued. Rotavirus antigens for parenteral delivery have received some attention, both as virus-like particles prepared in baculovirus,[92–94] expressed antigens,[95] DNA vaccines,[96] and killed virus.[97,98] Every approach has shown some effectiveness and proof of principle in animals but none has yet entered clinical trials.

Challenges: scientific, logistic, financial

Many challenges remain before we can assess the full global effect that rotavirus vaccines can have to prevent severe and fatal cases of rotavirus infection in children. The question remains, will such live oral vaccines work as well in children in the poorest developing countries as they have in middle-income and developed countries where the original trials were done?[10] Unlike parenteral vaccines, live oral vaccines against polio, cholera, and typhoid have not worked equally well in populations in developed and developing country settings. Moreover, the early candidate rotavirus vaccines (RIT4237 WC3, RRV, or RRV-TV) did not do well in three trials done in Africa[82,99,100] or in similar trials in China,[79] Peru,[101–103] and Brazil.[104] It was not apparent whether the vaccines did poorly because of drawbacks in study design and execution (eg, early treatment or poor detection of severe cases during active surveillance) or biological factors of children in these resource-poor settings. Biological factors such as very high titres of maternal antibodies, breastfeeding at the time of vaccination, micronutrient malnutrition, or presence of interfering microorganisms in the infant gut, and companion diseases such as HIV and malaria, could each inhibit the infant's immune response to the vaccine by lowering the effective titre of the oral vaccine or interfering with virus replication.[5,105] Up to now, no trial for efficacy has been done in children in developing countries of Africa or Asia with either of these vaccines that have just been licensed. Until these trials are completed, we will not know whether or not these vaccines will achieve the promise that is anticipated for improvement of child survival.

Another obstacle for the next generation of live oral vaccines will be full assessment of the risk of intussusception. Although initial trials of the new generation of human and bovine-human reassortant vaccines in more than 60 000 children suggest that the complication of intussusception was restricted to the tetravalent rhesus vaccine, this early experience has indelibly coloured perception of the safety of future rotavirus vaccines as well. A full assessment of risk, or lack of risk, will need substantial efforts at post-licensure surveillance, in settings where monitoring the health of large populations can be ensured. Also, even in the absence of any risk, intussusception will happen in the 2 weeks after a dose of either vaccine by chance alone, events that will undoubtedly cause anxiety in parents and doctors and could raise concern even if the number of events is within the statistical boundaries of chance alone.

The issue of financing new vaccines has received lots of discussion in recent years, encouraged by major contributions towards improving vaccine delivery by the Bill and Melinda Gates Foundation, the World Bank, the GAVI, and the proposed International Finance Facility.[106,107] GAVI has taken on the specific role of encouraging increased immunisation of children in the poorest 74 developing countries. By helping to improve vaccine delivery infrastructure and lending support to the purchase of routine vaccines, they hope to have a major effect on improvement of the health of the world's children. GAVI selected two new vaccines, targeting *Streptococcus pneumonia* and rotavirus for accelerated development and introduction, and set aside US$30 million for each to develop the data base on which countries could make an informed decision about vaccine introduction.[108] It remains to be seen whether or not this investment will have the beneficial effect that was anticipated to speed up additional testing of rotavirus vaccines in these developing countries and facilitate their introduction. Much emphasis has been placed on assessment of the value of vaccines since the cost of the next generation of vaccines will be substantially greater than that of those vaccines already included in the universal Programme for Childhood Immunisation. To date, the price of rotavirus vaccine to be procured by UNICEF or the Pan American Health Organizations revolving fund has not been negotiated. This price is the most important single factor for ministers of health and finance in developing countries to determine sustainability after introduction of a new rotavirus vaccine. Although GAVI funding might help the initial introduction phase, other support will be needed for the poorest developing countries to sustain an immunisation programme. The decision by the UK to lend support to the International Financing Facility provides great hope that these life-saving interventions might eventually be financed and reach those children in greatest need.

A key hurdle for decision-makers and paediatricians to consider rotavirus vaccines is the absence of knowledge about the local burden of disease. In most parts of the world, a diagnosis of rotavirus infection is rarely made since it does not alter treatment or encourage prevention. To address this need, regional surveillance networks in Asia,[4] Latin America,[6] Africa,[109] the middle east, and Europe have been organised by Centers for Disease Control and Prevention and WHO, supported by donors

including the Program for Appropriate Technology for Health (Seattle), WHO, and vaccine manufacturers, to undertake sentinel hospital surveillance for rotavirus in major children's hospitals in every region. By providing diagnostic assays and a surveillance protocol to participants in every network, a generation of paediatricians will soon be trained with experience in detecting rotavirus, appreciating its importance and understanding the value of a vaccine.[110] Preliminary data from the first 3 years of surveillance in Asia have already documented that in most countries of the region, more than 40–50% of all children admitted for diarrhoea have rotavirus as their pathogen.[4,111] This pattern is being found in many locations and these data have raised appreciation and awareness of this common childhood disease.

Looking to the future

A new generation of rotavirus vaccines will soon be licensed in many countries and available for more widespread use. Early introduction into the private markets of these countries could lead to appreciable reductions in health-care costs and burden of disease within 2–4 years. Identifying the full value of these vaccines to prevent mortality from rotavirus in developing countries is still several years away and each of the vaccines must first show its effectiveness in poor populations in Africa and Asia. Further studies are also needed to prove the cost-effectiveness of rotavirus vaccination in prevention of overall childhood mortality compared with other health interventions. Although many hurdles remain to ascertain the effectiveness of these vaccines in key target populations and their affordability, and to remove lingering concerns about safety from intussusception, the presence of two candidate vaccines provides an important new instrument to decrease the morbidity and mortality associated with rotavirus diarrhoea.

In 2000, the Millennium Development Group prepared a report to articulate many health-related goals to be targeted by many donor countries and organisations and achieved by the year 2015.[3] A key aim was a reduction by two-thirds of mortality in children aged younger than 5 years from the level in 1990. Inclusion of rotavirus vaccines into the routine schedule for childhood immunisation would be an important addition to prevent the 5% of all childhood deaths caused by rotavirus and as much as 40% of all deaths due to diarrhoeal diseases. Enhanced efforts to bring these vaccines to all children could go a long way to achieve these important and common goals.

Conflict of interest statement

We declare that we have no conflict of interest.

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