ADIS DRUG EVALUATION

BioDrugs 1998 Feb; 9 (2): 155-178
1173-8804/98/0002-0155/$12.00/0

Tetravalent Human-Rhesus Reassortant Rotavirus Vaccine

A Review of its Immunogenicity, Tolerability and Protective Efficacy against Paediatric Rotavirus Gastroenteritis

Rachel H. Foster and *Antona J. Wagstaff*

Adis International Limited, Auckland, New Zealand

Various sections of the manuscript reviewed by:

R.F. Bishop, Department of Gastroenterology and Clinical Nutrition, Royal Children's Hospital, Melbourne, Victoria, Australia; ***R.E. Black,*** Department of International Health, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland, USA; ***M.G. Friedman,*** Department of Virology, Ben Gurion Univerisity of the Negev, Beer Sheva, Israel; ***A. Guarino*** Department of Paediatrics, University 'Federico II' of Naples, Naples, Italy; ***A.C. Linhares,*** General Virology Services, Instituto Evandro Chagas, Belém, Para, Brazil; ***M.B. Rennels,*** Department of Pediatrics, University of Maryland School of Medicine, Baltimore, Maryland, USA; ***R.L. Ward,*** Division of Infectious Diseases, Children's Hospital Medical Center, Cincinnati, Ohio, USA.

Contents

Summary

Synopsis

Tetravalent human-rhesus reassortant rotavirus vaccine (RRV-TV) contains the rhesus rotavirus (RRV) strain MMU 18006, which has serotype G3 specificity, and reassortant rotavirus strains with human serotype G1, G2 and G4 specificity. Rotavirus gastroenteritis in humans is predominantly caused by these 4 serotypes.

RRV-TV 4×10^4, 4×10^5 or 4×10^6 plaque-forming units (PFU) per dose induces seroresponse rates (generally defined as a ≥4-fold increase in antibody titre) of 48 to 93% for IgA against RRV and 49 to 90% for neutralising antibodies to RRV after 1 to 3 doses in infants aged ≥4 weeks. Seroresponse rates for neutralising antibodies to human serotypes G1, G2, G3 and G4 are generally lower (2 to 68%). The rates generally increase with sequential doses, but not necessarily with increased vaccine titre. Seroresponse rates appear to be better in older infants than in neonates or infants aged ≤12 weeks.

RRV-TV is more immunogenic against human G2, G3 and G4 serotypes than the monovalent serotype G1 human-rhesus reassortant rotavirus vaccine (RRV-S1) and tends to be more immunogenic against G1, G2 and G3 serotypes than the human serotype G1 strain vaccine M37.

In most settings, RRV-TV has at least moderate efficacy in reducing the incidence of rotavirus gastroenteritis. Importantly, it protects against severe disease, with efficacy rates of 69 to 100% against very severe rotavirus gastroenteritis in large scale studies in the US, Finland and Venezuela. RRV-TV has similar overall efficacy to RRV-S1, but provides greater protection against gastroenteritis caused by rotavirus strains of serotypes other than G1. The efficacy of RRV-TV is not significantly affected by breast feeding or concurrent use of oral poliovirus vaccine.

The only adverse effect with which RRV-TV has been associated is a mild, transient febrile reaction.

Limited data from the US and Finland suggest that vaccination with RRV-TV could be cost saving.

In conclusion, the incidence of paediatric rotavirus gastroenteritis, particularly severe cases, would be reduced in most settings by the incorporation of RRV-TV into routine childhood immunisation schedules. Further refinements to RRV-TV (and/or development of additional candidate vaccines) may eventually produce even greater protective efficacy. In the meantime, RRV-TV is a significant advance in the prevention of paediatric rotavirus gastroenteritis worldwide.

Overview of Rotavirus Infection

Rotavirus is a double-stranded RNA virus. Human rotavirus gastroenteritis is most commonly caused by group A rotaviruses. Worldwide, the most prevalent glycoprotein antigen-determined (G) serotypes are G1, G2, G3 and G4.

The virus is highly infectious, with transmission occurring primarily via the faecal-oral route. Infection is largely limited to the small intestine. Almost all children are infected with rotavirus by the age of 3 to 5 years, and it is the most common causal agent of severe life-threatening diarrhoea in children and infants worldwide. Dehydration and electrolyte imbalance associated with rotavirus gastroenteritis cause significant mortality in developing countries (more than 800 000 children die each year) and the disease is responsible for a large number of hospitalisations in developed countries. Symptomatic illness occurs most commonly in infants aged ≈3 months to 2 years.

Immunogenicity

Tetravalent human-rhesus reassortant rotavirus vaccine (RRV-TV) is composed

of the rhesus rotavirus (RRV) strain MMU 18006, which shares neutralisation specificity with human rotavirus serotype G3, and 3 reassortant strains with human serotype G1, G2 or G4 specificity.

In immunogenicity trials in healthy infants aged ≥4 weeks, the seroresponse rate (generally defined as a ≥4-fold increase in antibody titre) for IgA against RRV was 48 to 93% (median 74%) after 1 to 3 doses of RRV-TV 4×10^4, 4×10^5 or 4×10^6 plaque-forming units (PFU) per dose. The seroresponse rate was significantly higher than after placebo (0 to 33%; median 11%). For neutralising antibody assays, the greatest seroresponse rates in RRV-TV recipients were to RRV (49 to 90%), whereas those to human serotypes G1, G2, G3 and G4 were lower (2 to 68%). Seroresponse rates to RRV-TV were generally higher after 3 doses than after 1 dose. However, increasing the titre of vaccine dose (rather than the number of doses) was not consistently shown to increase seroresponse rates. RRV-TV 4×10^4 or 4×10^5 PFU was only moderately immunogenic in neonates, and the seroresponse rate was significantly greater in infants aged 16 to 24 weeks than in those aged 6 to 12 weeks in one study in which infants received a single dose of RRV-TV 4×10^5 or 4×10^6 PFU.

Breast feeding did not significantly reduce the immunogenicity of the vaccine in infants aged ≥4 weeks. RRV-TV does not significantly interfere with the immunogenicity of oral poliovirus vaccine (OPV), although a slight reduction in the response to poliovirus serotype 1 has been reported. The IgA seroresponse to RRV is reduced by concurrent use of OPV but the effect does not appear to be clinically significant when 3 doses of RRV-TV are given. Administration of a buffer to prevent inactivation of the acid-labile vaccine is required for the immunogenicity of RRV-TV to be optimal.

RRV-TV (3 doses of 4×10^4 or 4×10^5 PFU/dose) induced similar seroresponse rates for IgA against RRV to those induced by monovalent serotype G1 human-rhesus reassortant rotavirus vaccine (RRV-S1; 3 doses of 4×10^4 or 4×10^5 PFU/dose). However, RRV-TV generally had greater immunogenicity than RRV-S1 against serotype G2, G3 and G4 human rotavirus strains, whereas RRV-S1 had greater immunogenicity against serotype G1. Compared with the human serotype G1 strain vaccine M37 (1 dose of 1×10^4 PFU/dose or 2 doses of 1×10^5 PFU/dose), RRV-TV (1 or 2 doses of 4×10^4 or 4×10^5 PFU/dose) tended to induce higher seroresponse rates for IgA against RRV and for neutralising antibodies to human serotypes G1, G2 and G3.

Protective Efficacy

Data on the efficacy of RRV-TV in preventing paediatric rotavirus gastroenteritis are available from 7 trials involving 8720 infants aged 1 to 6 months from 5 countries. A 3-dose schedule of RRV-TV 4×10^4 PFU/dose in the US and 4×10^5 PFU/dose in the US, Finland and Venezuela reduced the incidence of rotavirus gastroenteritis by 48 to 68% compared with placebo. However, when used at the lower dosage (4×10^4 PFU/dose) the vaccine had a relative efficacy of only 35% in Brazil, and was not significantly protective in Peru. Efficacy was most evident in the first year after vaccination in all studies in which this was analysed.

The vaccine has greater efficacy against more severe disease. In large US, Finnish and Venezuelan studies, the relative efficacy rate of RRV-TV compared with placebo was 69 to 100% for the most severe rotavirus gastroenteritis. Vaccination provided 100% protection against rotavirus gastroenteritis-associated hospitalisation in Finland, and 70% protection in Venezuela. RRV-TV was associated with a reduction in health service use. Some studies showed a marked

reduction in dehydrating rotavirus illness. The efficacy of RRV-TV was not significantly reduced by breast feeding or concurrent administration of OPV.

RRV-TV had similar overall efficacy to RRV-S1. However, RRV-TV was more effective than RRV-S1 against rotavirus gastroenteritis caused by serotypes other than G1.

Pharmacoeconomic Considerations

Depending on the cost of the vaccine and its administration, the introduction of RRV-TV into routine childhood immunisation schedules could be cost saving, according to US and Finnish data. One US cost-effectiveness analysis calculated that the introduction of RRV-TV at $US30 (1993 US dollars) per dose would produce an annual saving of $US79 million ($US78 per case prevented) for the healthcare system and a saving of $US466 million ($US459 per case prevented) for society. Another analysis calculated that RRV-TV reduces the median expected societal cost of rotavirus gastroenteritis by $US11 (1992 US dollars) per infant, and would thus be cost saving provided that the vaccine cost less than this amount. A similar Finnish analysis calculated that RRV-TV reduces costs associated with rotavirus gastroenteritis by 109 Finnish marks (currency year not stated) per infant.

Tolerability

RRV-TV is generally well tolerated at doses of 4×10^4, 4×10^5 or 4×10^6 PFU/dose, but causes a higher incidence of fever (rectal or axillary temperature >38°C) than placebo. The febrile reaction is normally mild (≤39°C) and transient, occurring 3 to 5 days after vaccination and lasting 1 to 2 days. The incidence of fever is not dose related. However, it appears to occur more commonly after the first dose than after subsequent doses, and in older (age 16 to 24 weeks) rather than younger (age 6 to 12 weeks) infants. RRV-TV tended to be associated with a higher incidence of fever than RRV-S1 or M37.

Dosage and Administration

Clinical trial data suggest that ideally a 3-dose schedule of RRV-TV 4×10^5 PFU/dose should be administered to infants between 2 and 7 months of age. RRV-TV can be administered concurrently with other injectable vaccines, but confirmation that there is no clinically significant interference between RRV-TV and OPV is required. Buffering is required to prevent inactivation of the vaccine.

In view of the vast public health burden associated with rotavirus gastroenteritis throughout the world, development of an effective rotavirus vaccine has been given high priority by several public health organisations, including the World Health Organization and the US Institute of Medicine.[1,2] Tetravalent human-rhesus reassortant rotavirus vaccine (RRV-TV) is a live attenuated vaccine that is administered orally. It has been designed to protect against the 4 major serotypes of symptomatic human rotavirus infection, namely serotypes G1, G2, G3 and G4.

This review focuses on the immunogenicity, tolerability and efficacy of RRV-TV in protecting against paediatric rotavirus-associated gastroenteritis.

1. Overview of Rotavirus Infection

1.1 Molecular Biology of Rotavirus

Rotavirus is a double-stranded RNA virus made up of 11 distinct genome segments.[3] Human rotavirus disease is caused predominantly by group A, and less commonly by group B or C, rotaviruses.[4-6] Current vaccines under development are directed at group A viruses only.

The inner capsid of rotavirus contains viral protein (VP) 6, which is the antigen that determines both the group and the subgroup (I or II) and is highly immunogenic.[4,5] The outer capsid contains 2 proteins, VP7 and VP4, which induce neutralising antibody.[3-5] VP7 is a glycoprotein (G) antigen

and VP4 is a protease-sensitive (P) antigen; these determine the G and P serotypes.

Of the 14 G serotypes that have been identified, the most significant in terms of human illness in most parts of the world are G serotypes 1 to 4.[3,7,8] The predominant serotype for the past 2 decades has been G1.[9] However, in some areas, particularly developing countries, G5, G6, G8, G9, G10 and G12 are also important.[3,7,8,10] Of the 20 P types identified by genotyping, the predominant types causing human illness are P[8] and P[4], although P[6], P[9] and P[11] are present in some countries.[3,7-9]

1.2 Immune Response to Infection

During natural rotavirus infection, serum and intestinal IgM, IgG and IgA responses are mounted.[4-6] Cell-mediated responses and nonspecific factors may also contribute to the resolution of infection.[3,4,11,12] Protective immunity appears to correlate approximately with serum and intestinal rotavirus IgA levels, but the exact role (if any) of serum or intestinal serotype-specific neutralising antibodies remains to be clarified.[5,6,11,12] Additional immune factors are also likely to be involved in protection against rotavirus disease.

1.3 Pathogenesis

Transmission of rotavirus occurs mainly via the faecal-oral route, although it may also be spread by the respiratory route.[4-6] The virus replicates within the mature absorptive epithelial cells lining the tips of the villi of the small intestine.[4-6,9,13] The resulting cellular damage is associated with loss of electrolytes and fluid, causing severe watery diarrhoea. Additionally, the nonstructural protein 4 of rotavirus appears to be an enterotoxin.[14]

The infection is largely limited to the mucosal surface, with infectious particles being shed in the faeces. Chronic and/or extraintestinal infection, including infection of the liver and kidney, can occur in immunodeficient individuals.[3,5,6,13]

1.4 Epidemiology

Rotavirus is the most common causal agent of severe, life-threatening diarrhoea in infants and children worldwide.[9,15,16] The virus is ubiquitous and highly infectious; almost all children worldwide are infected by the age of 3 to 5 years.[6,15] The spectrum of disease ranges from asymptomatic infection to severe gastroenteritis. The infection is generally self-limiting and can be treated with supportive care. However, dehydration and electrolyte imbalances can be fatal if adequate replacement therapy is not provided.[4] Recent evidence suggests that oral administration of certain bacteria (e.g. *Lactobacillus*)[17] or immunoglobulins[18] may reduce the severity and/or duration of illness.

More than 800 000 children die each year as a result of rotavirus gastroenteritis, mainly in developing countries.[1,6,9,16] In the US, the mortality rate is only 20 to 40 children per year,[7] but it is estimated that 55 000 to 110 000 children are hospitalised each year with rotavirus gastroenteritis.[7,19-21] It is estimated that 17 810 (5.2 per 1000) children aged <5 years in England and Wales[22] and 18 000 (30 per 1000) children aged <2 years in Venezuela[23,24] are hospitalised for rotavirus gastroenteritis annually.

The peak incidence of symptomatic rotavirus illness occurs in infants aged ≈3 months to 2 years.[5,6,11,13] Very young infants are relatively resistant to rotavirus disease, possibly because of maternally acquired antibodies. Rotavirus does not normally cause severe illness in adults, although asymptomatic or mild infections are common, especially during close contact with an infected infant.[4-6,11,13]

Rotavirus infections peak during the cooler months in temperate climates such as in the US, some parts of Europe and northern Japan.[6,7,25,26] Seasonality of infection seems to be less marked in tropical countries.

2. Immunogenicity of Tetravalent Human-Rhesus Reassortant Rotavirus Vaccine

A fundamental reason for the development of oral, live attenuated vaccines is to mimic the immunological response to natural infection, especially stimulation of local intestinal immunity.[4,11,27]

The rhesus rotavirus (RRV) strain used in RRV-TV is MMU 18006, which shares neutralisation specificity with human rotavirus serotype G3. In addition to the rhesus MMU 18006 strain, RRV-TV contains 3 reassortant rotavirus strains with human G1, G2 or G4 specificity. The reassortant strains were generated by coinfecting cell cultures with MMU 18006 and the relevant human rotavirus strain (strain D for G1 specificity, strain DS-1 for G2 specificity and strain ST-3 for G4 specificity).[27,28] Selection pressure (created by addition of neutralising antibodies to the VP7 of RRV) produced reassortant strains with one VP7 gene encoding the human rotavirus serotype (G1, G2 or G4) and 10 genes from RRV; the reassortants are described as D × RRV, DS-1 × RRV and ST-3 × RRV, respectively.

The immunogenicity of oral RRV-TV has been investigated in healthy infants in a number of placebo-controlled trials, some of which also compared RRV-TV with other vaccines. Vaccination schedules of 1, 2 or 3 doses were evaluated. When multiple doses were given, the doses were separated by a minimum of 2 weeks, and more frequently by 4 to 8 weeks. Most studies aimed for the vaccination schedule to be completed before 30 weeks of age. Vaccination was normally delayed if the infant or a household member had a recent episode of fever, diarrhoea and/or vomiting.

Serum IgA against RRV was measured by enzyme-linked immunosorbent assay (ELISA) before vaccination and after the final dose. Serum levels of neutralising antibodies against RRV and against human rotavirus strains representing the serotypes G1, G2, G3 or G4 were measured by enzyme-linked immunosorbent antigen reduction, fluorescent focus reduction, plaque reduction neutralisation or tube neutralisation assays. Seroresponse was defined as a ≥4-fold increase in serum antibody titre unless otherwise stated.

In considering the immunogenicity of RRV-TV, two important factors must be borne in mind. Firstly, although measurement of serum antibody levels is a standard method of assessing the immunogenicity of a candidate vaccine, this does not reliably or precisely predict protection by vaccines against rotavirus disease in children.[3,12,29-31] In particular, the protective role of serotype-specific neutralising antibodies is still unclear. Secondly, the immunogenicity studies to date have involved multiple potentially confounding variables, including differences in doses and schedules, infant age, concomitant use of other vaccines, breast feeding, buffers and geographical area. Consequently, results from different studies cannot be reliably compared, although data from different studies have been grouped to provide an overview in the following sections.

Results from placebo-controlled trials of the immunogenicity of buffered RRV-TV in infants aged ≥4 weeks are presented in tables I (comparisons with placebo) and II (placebo-controlled comparisons with other rotavirus vaccines). Trials involving neonates or analysing the effect of a specific confounding variable other than dose or schedule [i.e. breast feeding, concomitant use of oral polio vaccine (OPV) and/or buffer] are presented separately rather than being included in the tables.

2.1 Comparisons with Placebo

Significantly more infants who received RRV-TV than those who received placebo demonstrated a seroresponse for IgA against RRV in studies in which statistical analyses were reported. Overall, seroresponse for IgA against RRV was observed in 48 to 93% (median 74%) of infants who received 1 to 3 doses of RRV-TV 4×10^4, 4×10^5 or 4×10^6 plaque-forming units (PFU) per dose, compared with 0 to 33% (median 11%) of placebo recipients (tables I and II).[2,32-43]

In neutralising antibody assays, the highest seroresponse rates in RRV-TV recipients were for

antibodies directed against RRV [range 49 to 90% (median 75.5%); tables I and II]. This suggests that the neutralising antibody response induced by RRV-TV is predominantly directed against the VP4 antigen of the RRV component of the vaccine rather than against the VP7 antigens. Seroresponse rates in RRV-TV recipients for neutralising antibodies against human serotypes were generally low, ranging from 3 to 68% (median 31.5%) for G1, 2 to 48% (median 24%) for G2, 9 to 56% (median 28.5%) for G3 and 2 to 53% (median 25%) for G4.

Many trials identified an inverse correlation between the baseline antibody titre and seroresponse rates for neutralising antibodies and/or IgA, suggesting that maternally acquired antibodies interfere with the immune response to RRV-TV.[2,31,35,38,42-44] This was confirmed by the finding that higher baseline antibody titres reduced the magnitude of postvaccination increase in neutralising antibody titre relative to placebo.[31]

The vaccine does not appear to induce sufficient memory cell development to neutralisation epitopes on VP7 to prime recipients for an enhanced immune response to subsequent natural infection.[30,31]

Viral shedding was detected in stool specimens or rectal swabs from 36 to 89% of RRV-TV recipients 3 to 6 days after a single dose in the 4 studies that assessed this parameter.[32,35,42,43]

2.1.1 Effect of Dose and Schedule

The rate of response to RRV-TV is generally increased by the administration of additional doses. The seroresponse rate for IgA against RRV was significantly higher in the 3-dose group than in the single-dose group in a Peruvian study in which infants were randomised to receive 1 or 3 doses of RRV-TV 4×10^4 PFU/dose or placebo; seroresponse rates were 59, 75 and 24%, respectively (table I).[35] Seroresponse rates for neutralising antibodies to human serotypes remained relatively low in this study (≤36%) even with the additional doses. In Venezuelan studies,[43] a seroresponse rate for IgA against RRV of 76% was achieved with 2 doses of RRV-TV 4×10^5 PFU/dose, compared with 48% with 1 dose, and the second dose increased the rate of neutralising antibody response to human serotypes G1, G3 and G4 (table II). However, with 4×10^4 PFU/dose similar seroresponse rates were seen with 1 and 2 doses.

In the studies presented in tables I and II, seroresponse rates for IgA against RRV were 48 to 74% in infants who received a single dose of RRV-TV, 70 or 76% in those who received 2 doses and 56 to 93% among those who received 3 doses. Seroresponse rates measured after the third dose were higher than after the first dose in the three 3-dose studies that reported rates at both time points.[33,37,38]

Further comparative data are required to determine the optimal immunogenic titre for RRV-TV; it may depend on the population being vaccinated. In a single-dose US study, immunogenicity (IgA against RRV or neutralising antibodies to RRV) was significantly greater with 4×10^6 PFU than 4×10^5 PFU,[2] but this was not replicated in a 3-dose Venezuelan study[33] (table I). Considering the data obtained after a single dose and presented in tables I and II, seroresponse rates for IgA against RRV were 59 to 74% with RRV-TV 4×10^4 PFU, 48 or 49% with 4×10^5 PFU and 69% with 4×10^6 PFU. After 2 doses, the seroresponse rate was 70% with RRV-TV 4×10^4 PFU/dose and 76% with RRV-TV 4×10^5 PFU/dose. After 3 doses, seroresponse rates were 58 to 75% with RRV-TV 4×10^4 PFU/dose, 56 to 93% with 4×10^5 PFU/dose and 79% with 4×10^6 PFU/dose.

The seroresponse rates induced by a single dose of RRV-TV containing 1×10^4 PFU of each component serotype did not differ significantly from those induced by a vaccine containing 1×10^4 PFU each of the serotype G1 (D × RRV) reassortant and RRV (corresponding to serotype G3) plus 5×10^4 PFU each of the serotypes G2 (DS-1 × RRV) and G4 (ST-3 × RRV) reassortants (balanced RRV-TV; table II).[42]

2.1.2 Effect of Infant Age

The immunological immaturity of neonates could theoretically hamper the immunogenicity of

Table I. Immunological response to oral tetravalent human-rhesus reassortant rotavirus vaccine (RRV-TV) compared with placebo. Percentage of infants with a seroresponse after the final vaccine dose in trials in healthy infants aged 1 to 6 months. Seroresponse is defined as a ≥4-fold increase in antibody titre compared with previous or baseline serum samples, unless otherwise stated

Reference (country)	Age at 1st dose (wk)	No. of evaluable participants[a]	RRV-TV dose (PFU)	No. of doses [time between doses (wk)]	ELISA IgA against RRV (% infants with seroresponse)	Neutralising antibody assay[b] (% infants with seroresponse)					Comments
						RRV	hG1 (Wa)[c]	hG2 (DS-1)[c]	hG3 (P)[c]	hG4 (ST-3)[c]	
Dennehy et al.[2] (US)	6-24	182 (total)	4×10^5 [d]	1	49*	67*	9*	5	9*	5	Breast feeding *ad libitum*; OPV separated by ≥2wk; vaccine suspended in buffer[e]
			4×10^6 [d]	1	69*[†]	86*[†]	3*	2	17*	2	
			Placebo	1	0	0	0	0	0	0	
Pérez-Schael et al.[32] (Venezuela)	10-20	18-19	4×10^4 [d]	1	74[f]	74	58	33	42	32	Breast feeding withheld 2h before and 1h after; not stated whether other vaccines administered; large-volume buffer[g] given before vaccination
		58	Placebo	1	10	NR	NR	NR	NR	NR	
Flores et al.[33] (Venezuela)[h]	8-10	29-97	4×10^5 [d]	3 [4-6]	80[f]	88	52	33	38	53	Breast feeding withheld 1h before and after; OPV separated by 2wk; large-volume buffer[g] given before vaccination
		27-92	4×10^6 [d]	3 [4-6]	79[f]	90	47	48	37	47	
		90	Placebo	3 [4-6]	26	NR	NR	NR	NR	NR	
Joensuu et al.[34] (Finland)	7-18	93	4×10^5 [d]	3 [3-12]	89[f,i]	NR	NR	NR	NR	NR	Breast feeding *ad libitum*; received IPV rather than OPV; vaccine suspended in buffer[e]
		98	Placebo	3 [3-12]	7[i]	NR	NR	NR	NR	NR	
Lanata et al.[35] (Peru)	≈8	25-102	4×10^4 [d]	1	59**	NR	36*[j]	24[j]	36**[j]	28*[j]	Breast feeding withheld 1h before and after; received IPV rather than OPV; large-volume buffer[g] given before vaccination
		25-103	4×10^4 [d]	3 [≈4]	75**	NR	36*[j]	16[j]	32**[j]	28*[j]	
		25-102	Placebo	3 [≈4]	24	NR	8	4	0	0	
Linhares et al.[36] (Brazil)	4-8	121	4×10^4 [d]	3 [≈8]	58**	62**	19	19*	16	15	Breast feeding withheld 1h before and after; OPV separated by at least 2wk; large-volume buffer[g] given before vaccination
		40	Placebo	3 [≈8]	33	8	10	5	5	8	
Pérez-Schael et al.[37] (Venezuela)	8-10	38-40	4×10^5 [d]	3 [≈4]	84**	77**	45	33**	28*	10[k]	Breast feeding withheld 1h before and after; OPV separated by 2 to 4wk; large-volume buffer[g] given before vaccination and vaccine suspended in buffer[e]
		50-51	Placebo	3 [≈4]	22	2	22	2	8	10[k]	

Contd next page

Landscape table I (contd)

RRV-TV. Furthermore, the presence of maternally acquired antibodies may interfere with the response to the vaccine.[27]

The results of an Israeli study in neonates suggest that RRV-TV produces a moderate anti-RRV antibody response, but no significant neutralising antibody responses against human serotypes, in this age group.[45] 152 evaluable full-term neonates received a single buffered dose of RRV-TV 4×10^4 PFU at 2 days of age, a buffered dose of RRV-TV 4×10^4 PFU at both 2 days and 6 to 8 weeks of age, or placebo. 36% of the single-dose RRV-TV recipients and 3% of placebo recipients became seropositive for IgA against RRV (titre ≥1 : 50). Fewer than 10% of RRV-TV recipients had a ≥4-fold increase in titre for neutralising antibody to human serotype G1, G2 or G3. The second dose of RRV-TV at 6 to 8 weeks of age did not boost the immunological response; the seropositivity rate for IgA against RRV was 34% in this group. The geometric mean titre (GMT) for neutralising antibody to RRV increased in the vaccinated infants, whereas it declined substantially in the placebo recipients, over the 3 months of the study. GMTs for neutralising antibodies to human serotypes declined in all groups.

Seroconversion rates were not greatly increased by administration of a higher vaccine dose in another study in neonates by the same investigators.[46] Seroconversion for IgA against RRV (criteria not defined) was achieved in 23% of the 47 neonates who received 2 doses of 4×10^4 PFU/dose and 31% of the 46 who received 2 doses of 4×10^5 PFU/dose.

However, measurement of serum antibodies alone may underestimate the response to vaccination in neonates. In a different analysis of the above study, the serum response rate for IgA and/or neutralising antibody to RRV was 50% in neonates who received RRV-TV 4×10^4 PFU/dose and 65% in those who received 4×10^5 PFU/dose, but the response rates increased to 74 and 79%, respectively, when both serum and salivary responses were considered.[47] In this analysis, response was defined as a >1 : 25 titre of IgA against RRV in

Simasathien et al.[38] (Thailand)	≈8	52-94	4×10^4 [d]	3 [≈8]	67**[l]	49**[m]	21*	19	27**	31*	No breast feeding immediately prior to vaccination; received IPV rather than OPV; large-volume buffer[n] given before vaccination
		56-93	Placebo	3 [≈8]	16[l]	14[m]	2	9	4	13	

a In some studies, the number of participants from whom serum samples were taken varied between tests.

b Measured by enzyme-linked immunosorbent antigen reduction, fluorescent focus reduction, plaque reduction neutralisation or tube neutralisation assay.

c The human rotavirus strain against which antibodies were raised is in parentheses, unless otherwise stated.

d 1×10^4 [32,35,36,38] 1×10^5 [2,33,34,37] or 1×10^6 [2,33] PFU each of the serotype G1 (D × RRV), G2 (DS-1 × RRV) and G4 (ST-3 × RRV) reassortants and RRV (corresponding to serotype G3).

e 3ml of sodium citrate-bicarbonate buffer. Where defined, this contained sodium bicarbonate 300 mmol/L or 25.6 μg/L plus sodium citrate 9.6 μg/L or citric acid 33 mmol/L.

f Statistical significance versus placebo not reported.

g 30ml of milk or soy formula containing sodium bicarbonate 400mg.

h Criteria for seroresponse not stated.

i Percentage of infants who became seropositive for rotavirus IgA (≥1 : 25). No infants were seropositive before vaccination.

j Neutralising antibody tests were conducted only in vaccine recipients who had a seroresponse for IgA against RRV.

k Serological response against human serotype G4 strain VA70.

l Percentage of infants with an increase in titre from <1 : 25 to ≥1 : 50 or a ≥4-fold increase.

m Percentage of infants with an increase in titre from <1 : 200 to ≥1 : 400 or a ≥4-fold increase.

n 30ml of soy formula.

Abbreviations and symbols: ELISA = enzyme-linked immunosorbent assay; hG = human serotype G; IPV = injectable inactivated poliovirus vaccine; NR = not reported; OPV = oral poliovirus vaccine; PFU = plaque-forming units; RRV = rhesus rotavirus; * $p \leq 0.05$, ** $p \leq 0.005$ *vs* placebo; † $p \leq 0.05$ *vs* comparator dose.

Table II. Immunological response to oral tetravalent human-rhesus reassortant rotavirus vaccine (RRV-TV) compared with other rotavirus vaccines in placebo-controlled trials. Percentage of infants with a seroresponse after the final vaccine dose in trials in healthy infants aged 1 to 6 months. Seroresponse is defined as a ≥4-fold increase in antibody titre compared with previous or baseline serum samples

Reference (country)	Age at first dose (wk)	No. of evaluable participants[a]	Vaccine and dose (PFU)	No. of doses [time between doses (wk)]	ELISA IgA against RRV (% infants with seroresponse)	Neutralising antibody assay[b] (% infants with seroresponse)					Comments
						RRV	hG1 (Wa)[c]	hG2 (DS-1)[c]	hG3 (P)[c]	hG4 (ST-3)[c]	
Comparisons with the monovalent serotype G1 human-rhesus reassortant rotavirus vaccine RRV-S1											
Bernstein et al.[39] (US)	4-26	155	RRV-TV 4×10^4 [d]	3 [≥2]	74**	88**††	19*	26**††	26**††	28**††	Breast feeding withheld for 30 min before and after; concurrent administration of OPV permitted; large-volume buffer[e] given before vaccination
		153	RRV-S1 4×10^4	3 [≥2]	70**	65**	43**††	9**	7	10*	
		164	Placebo	3 [≥2]	10	2	9	0	9	4	
Rennels et al.[40] (US)	5-25	185	RRV-TV 4×10^5 [d]	3 [≥3]	56[f,g]	90[f,g]	14[f]	31††[f]	29††[f]	14††[f]	Not stated whether breast feeding permitted; concurrent administration of OPV permitted; vaccine suspended in buffer[h]
		175	RRV-S1 4×10^5 [d]	3 [≥3]	65[f]	76[f]	34†[f]	5[f]	2[f]	2[f]	
		193	Placebo	3 [≥3]	2	2	1	0	1	2	
Santosham et al.[41] (US)	6-24	58-217	RRV-TV 4×10^5 [d]	3 [≥3]	93[f]	83[f]	24[f]	24†[f]	26†[f]	19[f]	Not stated whether breast feeding permitted; concurrent administration of OPV permitted; vaccine suspended in buffer[h]
		73-243	RRV-S1 4×10^5	3 [≥3]	88[f]	82[f]	37[f]	7[f]	11[f]	12[f]	
		70-228	Placebo	3 [≥3]	20	7	3	0	6	4	
Comparisons with the neonatal human serotype G1 rotavirus vaccine M37											
Flores et al.[42] (Venezuela)	10-20	23	RRV-TV 4×10^4 [d]	1	74[f,i]	70	39[i]	17[i]	35[i]	35[i]	Breast feeding withheld 2h before and 1h after; not stated whether other vaccines administered; large-volume buffer[e] given before vaccination
		22	RRV-TV balanced [j]	1	86[f,i]	73	41[i]	27[i]	32[i]	32[i]	
		22	M37 1×10^4	1	50[f]	NR	27	9	5	27	
		22	Placebo	1	5	NR	NR	NR	NR	NR	
Pérez-Schael et al.[43] (Venezuela)	10-20	31-32	RRV-TV 4×10^4 [d]	1	63[f,i]	59	22[i]	16[i]	19[i]	19[i]	Not stated whether breast feeding permitted or other vaccines administered; large-volume buffer[e] given before vaccination
		27-30	RRV-TV 4×10^4 [d]	2 [4]	70[f,i]	83	27[i]	10[i]	31[i]	22[i]	
		29-31	M37 1×10^4	1	32*	NR	13	3	13	28	
		29	Placebo	2 [4]	3	NR	NR	NR	NR	NR	

Contd next page

table II (contd)

serum and/or saliva, or a ≥1 : 200 titre of neutralising antibody to RRV in serum. Whether measurement of salivary response accurately reflects the response to vaccine in breast-fed infants needs to be determined, as antibodies derived from colostrum or breast milk may be present.

The effect of age at vaccination was investigated in a study in which infants aged 6 to 12 weeks or 16 to 24 weeks received a single dose of RRV-TV 4×10^5 or 4×10^6 PFU (fig. 1).[2] Seroconversion rates and GMTs (IgA against RRV and neutralising antibody to RRV) were significantly greater in the older than the younger infants when data for both doses were combined ($p \leq 0.05$).

2.1.3 Effect of Breast Feeding

Theoretically, breast feeding could interfere with the immune response to RRV-TV because maternal antibodies and nonspecific inhibitors can be transferred through breast milk. However, the immunological response to RRV-TV 4×10^4 PFU was not significantly lower in infants aged ≥4 weeks who were normally breast fed than in those who were not normally breast fed in studies in which breast feeding was withheld for 0.5 to 2 hours before and after vaccination.[44,48,49] Furthermore, the response to RRV-TV was not impaired in infants who received breast milk in lieu of a buffer immediately before vaccination.[44]

In one of the studies in neonates previously described in section 2.1.2, a nonsignificant trend towards a lower seroresponse rate to RRV-TV 4×10^4 PFU was observed in those who were breast fed compared with those in whom breast feeding constituted ≤10% of their total feeding.[45] The rate of seroresponse according to any assay (IgA against RRV and/or neutralising antibody to RRV and/or human serotypes) was 42% in breast-fed compared with 60% in nonbreast-fed infants. In this study, breast feeding was withheld for 1.5 hours before and after vaccination.

2.1.4 Effect of Concomitant Use of Oral Poliovirus Vaccine

RRV-TV 4×10^4 or 4×10^5 PFU/dose does not have a significant effect on the immune response to OPV, although slight suppression of the re-

Pérez-Schael et al.[43] (Venezuela)	10-20	23-27	RRV-TV 4×10^5 [d]	1	48[f,i]	63[f,i]	38[f,i]	38[f,i]	28[f,i]	19[f,i]	Not stated whether breast feeding permitted or other vaccines administered; large-volume buffer[e] given before vaccination
		24-25	RRV-TV 4×10^5 [d]	2 [4]	76[f,i]	72[f,i]	68[f,i]	36[f,i]	56[f,i]	44[f,i]	
		27-31	M37 1×10^5	1	50[f]	NR	31[f]	16[f]	35[f]	21[f]	
		26-27	M37 1×10^5	2 [4]	44[f]	19[f]	31[f]	8[f]	19[f]	35[f]	
		20-26	Placebo	2 [4]	12	10	13	13	8	0	

a In some studies, the number of participants from whom serum samples were taken varied between tests.

b Measured by enzyme-linked immunosorbent antigen reduction, fluorescent focus reduction, plaque reduction neutralisation or tube neutralisation assay.

c The human rotavirus strain against which antibodies were raised is in parentheses, unless otherwise stated.

d 1×10^4 [39,42,43] or 1×10^5 [40,41,43] PFU each of the serotype G1 (D × RRV), G2 (DS1 × RRV) and G4 (ST-3 × RRV) reassortants and RRV (corresponding to serotype G3).

e 30ml of milk or soy formula containing sodium bicarbonate 400mg.

f Statistical significance versus placebo not reported.

g Statistical significance versus RRV-S1 not reported.

h 3ml of sodium citrate-bicarbonate buffer. Where defined, this contained sodium bicarbonate 300 mmol/L or 25.6 mg/ml plus sodium citrate 9.6 mg/ml or citric acid 33 mmol/L.

i Statistical significance versus M37 not reported.

j 1×10^4 PFU each of the serotype G1 (D × RRV) reassortant and RRV plus 5×10^4 PFU each of the serotypes G2 (DS-1 × RRV) and G4 (ST3 × RRV) reassortants.

Abbreviations and symbols: ELISA = enzyme-linked immunosorbent assay; hG = human serotype G; NR = not reported; OPV = oral poliovirus vaccine; PFU = plaque-forming units; RRV = rhesus rotavirus; * $p \leq 0.05$, ** $p \leq 0.005$ *vs* placebo; † $p \leq 0.05$, †† $p \leq 0.005$ *vs* comparator vaccine.

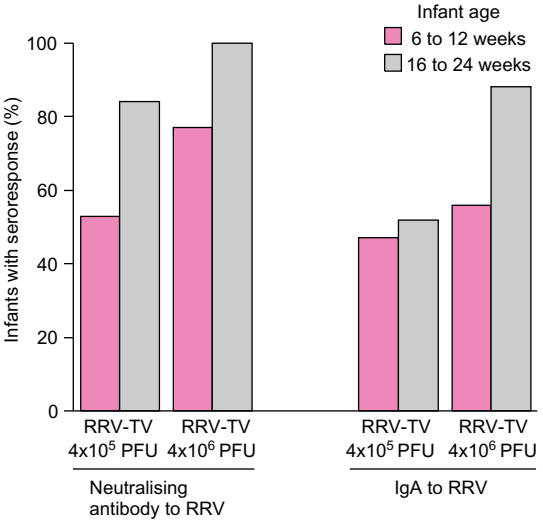

Fig. 1. Effect of age on seroresponse to tetravalent human-rhesus reassortant rotavirus vaccine (RRV-TV).[2] The seroresponse (≥4-fold increase in antibody titre) rate after a single dose of RRV-TV 4×10^5 or 4×10^6 PFU was compared between 71 infants aged 6 to 12 weeks and 51 infants aged 16 to 24 weeks. No statistical analysis was reported for the data as presented, but seroresponse rates were significantly higher in older infants when data for both doses were combined. *Abbreviations:* PFU = plaque-forming units; RRV = rhesus rotavirus.

sponse to poliovirus serotype 1 has been observed in both a US and a Thai study.[49-51]

Concurrent administration of OPV with RRV-TV reduces the IgA seroresponse to RRV, but this may not be clinically important when a full 3-dose schedule is administered (see section 3.1.2).[50,51] Neutralising antibody responses are not significantly affected.

2.1.5 Effect of Buffering

Rotavirus is acid labile, but can survive if the pH of the stomach is buffered.[4] In all of the studies presented in tables I and II, the vaccine was buffered in some way.

The immunogenicity of unbuffered RRV-TV 4×10^4 PFU was compared with that of RRV-TV with small-volume buffer (sodium bicarbonate 64mg + sodium citrate 24mg in 2.5ml water) or large-volume buffer (sodium bicarbonate 400mg in 25ml soybean formula) in a US single-dose study involving 135 infants aged 6 to 16 weeks.[49] The buffer was administered 5 minutes before RRV-TV. All neonates also received OPV. The seroresponse rate for IgA against rotavirus was significantly lower in infants who received unbuffered vaccine (23%) than in those who received vaccine with small-volume buffer (45%) or large-volume buffer (49%).

In a study in which a single dose of unbuffered RRV-TV was administered to 36 infants aged 6 weeks to 4 months, the seroresponse rate (IgA, IgM or IgG against RRV, or neutralising antibodies to RRV or human serotypes) was 22% in those who received 1×10^4 PFU and 61% in those who received 1×10^5 PFU.[52] However, it is difficult to compare these results with those of other studies using buffered vaccine because the vaccine dose was one-quarter of that used elsewhere and IgG and IgM against RRV were measured as well as IgA.

Breast feeding in itself may provide an adequate buffer to prevent acid-mediated inactivation of the vaccine. Seroresponse rates after a single dose of RRV-TV 4×10^4 PFU were not significantly different between infants who were breast fed immediately before vaccination and those who received a buffer.[44]

2.2 Comparisons with Other Rotavirus Vaccines

When compared with a monovalent human-rhesus reassortant rotavirus vaccine (RRV-S1) directed against serotype G1, RRV-TV had similar general immunogenicity, as measured by IgA against RRV, in 3 comparative trials conducted in the US.[39-41] However, RRV-TV had significantly greater immunogenicity against human serotypes G2 and G3 (table II). The immunogenicity of RRV-TV against human serotype G4 was also significantly greater than that of RRV-S1 in 2 of the studies.

RRV-S1 induced higher seroresponse rates than RRV-TV for neutralising antibody to human serotype G1; this was statistically significant in 2 of the 3 studies. The difference may have been because RRV-S1 contained a higher titre of serotype G1 reassortant (4×10^4 or 4×10^5 PFU/dose compared

with 1×10^4 or 1×10^5 PFU/dose in RRV-TV), or it may indicate that interference between the reassortants reduced the immune response to individual strains.[30,31] Such interference between reassortants has been previously reported.[53,54]

RRV-TV 4×10^4 or 4×10^5 PFU/dose was compared with the human serotype G1 strain M37 vaccine 1×10^4 or 1×10^5 PFU/dose in Venezuelan studies, but no statistical analyses of differences in immunogenicity between the vaccines were provided.[42,43] The seroresponse rates for IgA against RRV tended to be higher with RRV-TV than with M37 (table II). The seroresponse rates for neutralising antibody to RRV ranged from 59 to 83% (mean 69%) with RRV-TV, and the seroresponse rates for neutralising antibody to M37 ranged from 33 to 78% (mean 60%) with the M37 vaccine. RRV-TV tended to induce higher seroresponse rates than M37 for neutralising antibodies against human serotypes G1, G2 and G3 (table II). Seroresponse rates were generally similar for neutralising antibody against the human serotype G4 strain ST-3, with which M37 shares VP4 specificity.

3. Protective Efficacy

The efficacy of RRV-TV in preventing rotavirus gastroenteritis was assessed in 7 trials involving a total of 8720 evaluable infants aged 1 to 6 months from 5 countries (table III).[34-37,39-41]

The methodology of these studies is largely described in section 2. The relative efficacy of RRV-TV was calculated as the percentage reduction in the incidence of rotavirus diarrhoea[35-37] or gastroenteritis[34,39-41] in those who received RRV-TV compared with the placebo group, with assessments starting approximately 2 weeks after the third vaccine dose and continuing for up to 2 years. Diarrhoea was defined as 3 or more looser-than-normal stools in a 24-hour period or a single stool with blood. The relationship of diarrhoea or vomiting to rotavirus infection was determined by the presence of rotavirus antigen according to ELISA performed on a stool specimen.

3.1 Comparisons with Placebo

RRV-TV significantly reduced the incidence of rotavirus gastroenteritis in efficacy trials conducted in developed countries. Relative efficacies of 49 to 68%, compared with placebo, were achieved after 3 doses of 4×10^4 PFU/dose in a US study[39] or 3 doses of 4×10^5 PFU/dose in 2 other US studies (one of which involved native American children)[40,41] and a Finnish study.[34]

The efficacy of RRV-TV in trials conducted in developing countries was inconsistent, and the results indicated that a higher titred vaccine is required for adequate efficacy in these areas. The efficacy of 3 doses of RRV-TV 4×10^5 PFU/dose in Venezuela[37] (relative efficacy 48%; table III) was similar to that achieved in the US and Finland. However, when a lower titred vaccine (4×10^4 PFU/dose) was administered in a Peruvian study, neither 1 nor 3 doses was significantly protective.[35] The relative efficacy of RRV-TV 4×10^4 PFU/dose was also low (35%; table III) in a Brazilian study, although the effect was statistically significant compared with placebo.[36]

Bad sanitary conditions, poor nutritional status of infants and/or viral interference in a setting of high transmission of enteric organisms also may have contributed to the poorer performance of RRV-TV in Peru and Brazil.[56] The background level of diarrhoea of any aetiology was considerably higher in these countries (8 and 6 episodes per child-year, respectively, in Peru and Brazil) than in Venezuela (2 episodes per child-year).[24,35-37]

The efficacy of RRV-TV was most evident in the first year after vaccination. The primary efficacy analysis of the study in native American infants was for the first year of surveillance, but follow-up was continued for a second year.[41] During the second year there was a large decrease in the number of rotavirus gastroenteritis episodes in all groups. Under these conditions, RRV-TV did not demonstrate overall protective efficacy, although some protection against the most severe episodes was maintained (relative efficacy 44%). In the Brazilian study, the relative efficacy of RRV-TV declined from 57% in the first year to 12% in

Table III. Prevention of rotavirus gastroenteritis by oral tetravalent human-rhesus reassortant rotavirus vaccine (RRV-TV). Randomised, double-blind, placebo-controlled trials in which healthy infants aged ≤6 months received 1 or 3 doses of vaccine

Reference (country)	No. of evaluable participants [age at first dose (mo)]	Vaccine and dose (PFU) [no. of doses]	Duration of follow-up	Relative efficacy compared with placebo (%)[a]		
				any RV gastroenteritis	moderate/ severe RV gastroenteritis[b]	most severe RV gastroenteritis[b]
Comparisons with placebo						
Joensuu et al.[34] (Finland)	2273 [1-4]	RRV-TV 4×10^5 [c] [3]	1 or 2 RV seasons[d]	68	91	100
Lanata et al.[35] (Peru)	638 [≈2]	RRV-TV 4×10^4 [c] [1]	2 years after last dose	18	36	
		RRV-TV 4×10^4 [c] [3]		24	30	
Linhares et al.[36] (Brazil)	466 [1-2]	RRV-TV 4×10^4 [c] [3]	2 years after 1st dose	35	46	
Pérez-Schael et al.[37] (Venezuela)	2207 [2]	RRV-TV 4×10^5 [c] [3]	19-20mo after last dose	48	47	88
Placebo-controlled comparisons with oral monovalent serotype G1 human-rhesus reassortant rotavirus vaccine (RRV-S1)						
Bernstein et al.[39] (US)	898 [1-6]	RRV-TV 4×10^4 [c] [3]	End of 2nd RV season	57	59	82
		RRV-S1 4×10^4 [3]		40	39	73
Rennels et al.[40] (US)	1187 [1-6]	RRV-TV 4×10^5 [c] [3]	End of 1st RV season	49	68	80
		RRV-S1 4×10^5 [3]		54	56	69
Santosham et al.[41] (US[e])	1051 [1-6]	RRV-TV 4×10^5 [c] [3]	1y[f]	50		69
		RRV-S1 4×10^5 [3]		29		48

a Percentage reduction in the incidence of RV diarrhoea[35-37] or gastroenteritis[34,39-41] relative to placebo.

b Severity of RV gastroenteritis was assessed by scoring systems modified from that of Flores et al.[55] Clinical signs and symptoms such as the duration and frequency of diarrhoea and vomiting, temperature, dehydration and need for hospitalisation were scored to a maximum of 20 points. Moderate/severe episodes were those that scored ≥9 in the studies by Lanata et al.[35], Rennels et al.[40] and Linhares et al.[36], those that scored 9-14 in the studies by Bernstein et al.[39] and Pérez-Schael et al.[37], and those that scored ≥11 in the study by Joensuu et al.[34] Most severe episodes were those that scored ≥15 in Bernstein et al.,[39] Rennels et al.[40], Pérez-Schael et al.,[37] Joensuu et al.[34] and Santosham et al.[41]

c 1×10^4 [35,36,39] or 1×10^5 [34,37,40,41] PFU each of the serotype G1 (D × RRV), G2 (DS-1 × RRV) and G4 (ST-3 × RRV) reassortants and RRV (corresponding to serotype G3).

d Whether follow-up included 1 or 2 RV seasons was dependent on the date of enrolment of the infant into the study.

e Study conducted in native American populations.

f Primary efficacy analysis.

Abbreviations and symbols: PFU = plaque-forming units; RRV = rhesus rotavirus; RV = rotavirus.

the second year.[36] In the Finnish study, vaccine efficacy against any rotavirus gastroenteritis declined from 83% in the first year to 63% in the second, but efficacy against severe episodes was maintained at ≥90% in both years.[34] Only a small decrease in efficacy (from 64% in the first year to 48% in the second year) was observed in a US study.[39]

RRV-TV is effective against rotavirus diarrhoea caused by different serotypes. Protection against illness caused by serotype G1 or G3 was evident in US studies both when serotype G1 predominated[39,40] and when serotype G3 predominated.[41] The efficacy of RRV-TV was similar for rotavirus gastroenteritis caused by serotype G1 or G4 in the Finnish study.[34] In the Peruvian study, there was a trend towards RRV-TV being more effective against serotype G1- than G2-associated illness,[35] but protection against serotype G2 was observed in the Brazilian study.[36]

3.1.1 Efficacy against Severe Disease

Severity of rotavirus gastroenteritis in the efficacy studies was primarily assessed by various

scoring systems modified from that of Flores et al.[55] Clinical signs and symptoms such as the duration and frequency of diarrhoea and vomiting, temperature, dehydration and need for hospitalisation were scored to a maximum of 20 points.

RRV-TV selectively protected against more severe rotavirus disease (table III). In particular, 69 to 100% protection against the most severe episodes of rotavirus gastroenteritis (severity score ≥15) was achieved in US, Finnish and Venezuelan studies,[34,37,39-41] The other studies also identified some indicators of severe disease against which RRV-TV was effective. For instance, in the first year of the Brazilian study, RRV-TV was 84% effective in preventing rotavirus diarrhoea associated with ≥6 liquid stools within 24 hours.[36] Although not significantly protective overall in the Peruvian study, RRV-TV demonstrated significant protective efficacy against rotavirus diarrhoea associated with fever, with vomiting or with ≥6 liquid or semi-liquid stools within 24 hours; relative efficacy rates of 35 to 40% after 3 doses of vaccine were achieved against gastroenteritis episodes associated with these specific characteristics.[35]

Rotavirus is particularly prone to cause dehydrating diarrhoea.[16,57] The protective efficacy of RRV-TV against dehydrating rotavirus diarrhoea was 75% in the Venezuelan study (in which this was the primary end-point),[37] 100% in one of the US studies[40] and 97% in the Finnish study.[34] However, no significant protection against dehydration was seen in the Peruvian[35] or Brazilian[36] studies.

Importantly, RRV-TV provided 100% protection against hospitalisation for rotavirus gastroenteritis in the Finnish study[34] and 70% protection in the Venezuelan study.[37] The number of physician visits was reduced by 69 to 78% in the RRV-TV group compared with the placebo group in US and Finnish studies.[34,39,40] Health service use was reduced by about 20% in RRV-TV recipients in the Peruvian and Brazilian studies, but the difference in use of health services between the vaccine and placebo groups was not statistically significant.[35,36]

The duration of rotavirus diarrhoea was reduced by 75% in RRV-TV recipients compared with placebo recipients in the only study to report this parameter.[39] The vaccine was 71% protective against rotavirus diarrhoea of >4 days' duration in the Venezuelan study[37] and 97% protective against rotavirus diarrhoea of >5 days' duration in the Finnish study.[34]

Further analysis of the data from 2 of the US studies suggests that RRV-TV shifts the spectrum of rotavirus infection from symptomatic to asymptomatic, and from asymptomatic to no infection.[30,31] The severity of gastroenteritis when it developed also tended to be less in vaccinated children.[39]

3.1.2 Effect of Breast Feeding, Oral Poliovirus Vaccine and Infant Age

In line with immunological findings (see section 2.1.3), the protective efficacy of 3 doses of RRV-TV 4×10^4 PFU/dose was not reduced in normally breast-fed infants compared with nonbreast-fed infants.[48] The protective efficacy of 3 doses of RRV-TV 4×10^5 PFU/dose was not reduced by concurrent administration of OPV.[51]

Age at the time of first vaccination did not have a significant effect on the protective efficacy of 3 doses of RRV-TV 4×10^4 PFU/dose in US infants aged ≥4 weeks.[48] When the efficacy of the vaccine was analysed according to the age of the infant at the time of the onset of rotavirus diarrhoea in the Venezuelan study, RRV-TV was more effective in infants aged >12 months compared with younger infants (relative efficacy 61 *vs* 41%; no statistical comparison reported).[37] However, the reverse was observed among native American infants, in whom the vaccine was most effective during the first year of life.[41] The efficacy of RRV-TV in neonates has not been studied.

3.1.3 Transmission of Vaccine Virus

The potential of RRV-TV to induce herd immunity requires further evaluation. Horizontal transmission of vaccine virus was evident in 14% of infants with rotavirus-positive diarrhoea in the Venezuelan study, occurring with similar frequency in placebo and RRV-TV recipients.[37]

It is not known if transmitted vaccine virus stimulated antibody responses, thereby protecting placebo recipients from rotavirus disease. If so, this would have masked the true relative efficacy of the vaccine in the clinical trial, but could be advantageous when the vaccine is used in the community.

3.2 Comparisons with Monovalent Rotavirus Vaccine

As previously described in section 2.2, RRV-TV was compared with the monovalent serotype G1 reassortant vaccine RRV-S1 in 3 large US trials (table III).[39-41] The overall relative efficacy rates for RRV-TV and RRV-S1 did not differ significantly, regardless of the severity of the rotavirus gastroenteritis. However, interesting differences in efficacy between RRV-TV and RRV-S1 were seen when rotavirus-associated diarrhoea was divided into serotype-specific episodes.

In a 2-year study,[39] both vaccines provided significant protection against serotype G1-specific rotavirus gastroenteritis during the first year, when 93% of rotavirus-associated episodes of gastroenteritis were caused by this serotype.[39] The efficacy of RRV-TV against serotype G1 rotavirus gastroenteritis was maintained in the second year, whereas that of RRV-S1 declined substantially (fig. 2). In the second year, 35% of rotavirus-associated episodes of gastroenteritis were caused by rotavirus serotypes other than G1. Importantly, RRV-S1 did not prevent these episodes, whereas RRV-TV had a relative efficacy of 51% compared with placebo against disease caused by serotypes other than G1. These results suggest that RRV-S1 protects only during the first year after vaccination and/or only against serotype G1 infections.

In a 1-year study,[40] the vaccines provided similar protection against serotype G1 rotavirus gastroenteritis, but there was a trend towards RRV-TV being more effective than RRV-S1 against serotype G3 disease (relative efficacy 77 *vs* 45%).[40] Serotype G1 was responsible for 71% and serotype G3 was responsible for 19% of rotavirus gastroenteritis episodes during this study. Similar results were seen in a study in native Americans in which the relative efficacy against serotype G3-associated illness was 53% with RRV-TV and 20% with RRV-S1.[41] As serotype G3 predominated in this study, the overall efficacy of RRV-S1 was low (29%).

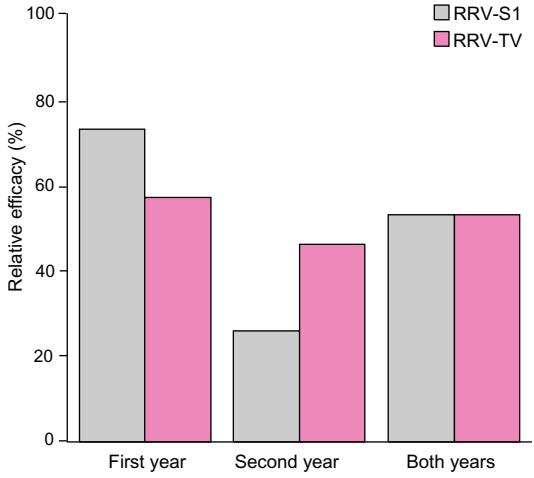

Fig. 2. Relative efficacy of different rotavirus vaccines against serotype G1 rotavirus gastroenteritis in the US.[39] 898 infants aged 4 to 26 weeks received 3 doses of tetravalent human-rhesus reassortant rotavirus vaccine (RRV-TV) 4×10^4 PFU/dose, monovalent human-rhesus reassortant serotype G1 rotavirus vaccine (RRV-S1) 4×10^4 PFU/dose or placebo. Relative efficacy was the percentage reduction in the incidence of serotype G1 gastroenteritis in vaccine recipients compared with placebo recipients during the following rotavirus seasons. *Abbreviation:* PFU = plaque-forming units.

RRV-TV and RRV-S1 were similarly effective in reducing the need for physician visits.[39,40] However, RRV-TV reduced the duration of diarrhoea to a greater extent than RRV-S1 in the 1 study that reported this parameter.[39]

4. Pharmacoeconomic Considerations

The potential economic effect of rotavirus vaccination has been addressed in 2 analyses in the US and 1 in Finland. The results suggest that RRV-TV could be cost saving, depending on the cost of vaccine supply and administration.

An analysis conducted in 1995 investigated the cost effectiveness of routine administration of 3 doses of rotavirus vaccine concurrently with the

diphtheria-tetanus-pertussis (DTP) vaccine in infants younger than 12 months in the US.[21] Calculations were based on the rotavirus vaccination programme being implemented for 1 year and having cost and outcome implications over a 5-year period, using a hypothetical cohort of 4.1 million children from birth to 5 years of age. The analysis was conducted from the perspectives of both the healthcare system (direct outpatient and inpatient medical costs) and society (direct outpatient and inpatient medical costs plus productivity costs attributable to premature loss of life and parental time lost from work).

On the basis of data on RRV-TV from the 2 US efficacy trials (see table III),[39,40] it was assumed that the vaccine would be 50% effective in preventing rotavirus diarrhoea and 75% effective in preventing severe rotavirus diarrhoea. Adverse effects of the vaccine were considered to be negligible and were not considered. The vaccine coverage rate was based on that achieved with the DTP vaccine. The estimated duration of hospitalisation in those who developed severe rotavirus diarrhoea was 3.75 days for unvaccinated infants and 2.5 days for vaccinated infants. Other estimates were derived from published studies and/or national sources. Costs were in 1993 US dollars, discounted at an annual rate of 4%.

At a rotavirus vaccine cost of $US30 per dose, the net saving to the healthcare system was calculated to be $US79 million ($US78 per case prevented). The net savings from the societal perspective would be $US466 million ($US459 per case prevented). The threshold price for the vaccine below which the healthcare system would save money was $US40 per dose using base-case estimates, $US17 per dose using worst-case estimates and $US74 per dose using best-case estimates.

Since the publication of this analysis, the US Centers for Disease Control (CDC) have published new estimates of rotavirus disease burden,[7] and these may reduce the magnitude of the calculated savings associated with RRV-TV. For instance, the annual rate of rotavirus-associated hospitalisation of unvaccinated children aged less than 5 years was estimated in the analysis to be 104 000, whereas the latest CDC estimate is 55 000.

Another pharmacoeconomic analysis[58] was conducted concurrently with one of the US efficacy trials; in this trial, 1187 infants received 3 doses of RRV-TV 4×10^5 PFU/dose, RRV-S1 4×10^5 PFU/dose or placebo (see table III).[40] Data on diagnoses, procedures and resource consumption were collected from the study participants. Standardised costs were then applied to these data to calculate direct medical and nonmedical costs and indirect costs resulting from lost parental work time. As no deaths or long-term morbidity occurred in the trial, costs associated with these outcomes were not considered, but those associated with adverse effects were included. Costs were in 1992 US dollars.

The median expected cost of rotavirus gastroenteritis was reduced by $US11 per infant by RRV-TV and by $US12 per infant by RRV-S1. Thus, vaccination with RRV-TV would be cost saving provided that supply and administration of the vaccine cost <$US11 per infant. Sensitivity analyses showed that RRV-TV could save up to $US40 per infant, or potentially increase costs by $US6 per infant.

Based on these figures, the estimated reduction in total annual cost of rotavirus gastroenteritis with RRV-TV vaccination would be $US45 million when applied to a cohort of 4.1 million children and considered from a societal perspective; the cost of the vaccination programme would then need to be deducted. This cost reduction is considerably less than that calculated in the other analysis (a saving of $US466 million at a vaccine cost of $US30 per dose). Actual savings are likely to lie somewhere between these 2 calculated values. The second analysis was limited by the fact that the study population was not representative of the general US population, that the infants were closely monitored, and that follow-up was only for 1 year.

A cost-benefit analysis[59] was also performed in conjunction with the Finnish efficacy study; in this trial 2398 infants were enrolled to receive 3 doses of RRV-TV 4×10^5 PFU/dose or placebo (see

table III).[34] The pharmacoeconomic analysis was conducted from the societal perspective, with the primary outcome being serious rotavirus gastroenteritis during an average of 1 year's follow-up. Direct and indirect costs associated with vaccination (excluding the cost of the vaccine), treatment of gastroenteritis and treatment of adverse effects were considered.

The net reduction in costs (excluding the cost of vaccine) was 109 Finnish marks [FIM (currency year not stated); FIM1 ≈ $US0.18] per infant vaccinated with RRV-TV. For fully vaccinated infants the net reduction in costs was FIM86 per infant, ranging from FIM10 to 154 when sensitivity analyses were applied. It was estimated that vaccination with RRV-TV could save up to 6 million per year, depending on the cost of the vaccine.

5. Tolerability

This section is based on tolerability data from 15 placebo-controlled trials of RRV-TV involving a total of approximately 10 900 healthy infants aged 1 to 6 months.[2,32-43,49] Approximately 5100 of these infants received RRV-TV (1 to 3 doses of 4×10^4, 4×10^5 or 4×10^6 PFU/dose), whereas the remainder received placebo or comparator vaccines. Reactions to the vaccine were detected by a combination of parental monitoring and active follow-up by study personnel for 5 to 7 days after each vaccine dose.

RRV-TV was generally well tolerated and was not associated with a significantly higher incidence of vomiting or diarrhoea than placebo. However, a mild, transient febrile reaction was noted in most studies. This most commonly manifested as an increase in rectal or axillary temperature to between 38 and 39°C (100.4 to 102.2°F) occurring 3 to 5 days after vaccination and resolving within 2 days. More severe and/or prolonged fever was observed occasionally.

The incidence of fever was significantly higher on at least 1 day in RRV-TV recipients compared with those who received placebo in 9 of the 15 studies reviewed.[33-37,39-42] Averaged over the 13 studies that provided adequate data,[2,32-35,37,39-43,49] the incidence of fever (rectal or axillary temperature >38°C) during the 5 to 7 days of follow-up after the first dose was 20% (median 21.5%) in those who received RRV-TV and 11% (median 8%) in placebo recipients. However, febrile reactions to RRV-TV generally occurred less frequently after subsequent doses than after the first dose.[33-36,39,40,43] The average incidence of fever after the second or third dose was 16% with RRV-TV and 15% with placebo in the 6 studies that provided these data.[33-35,40,41,43]

The incidence of fever after the first dose varied widely between studies, from 7 to 40% in RRV-TV recipients and 0 to 42% in placebo recipients. This may at least partly reflect febrile reactions to other vaccines, since these were administered concurrently in some studies but not in others. For instance, all infants received DTP plus injectable inactivated poliovirus (IPV) vaccine concurrently with RRV-TV or placebo in the study with the highest reported incidence of fever (40% in RRV-TV recipients and 42% in placebo recipients after the first dose).[35] Fever occurred most commonly in the first 24 hours after vaccination in this study, which suggests that it was mainly caused by the DTP/IPV vaccine; fever caused by RRV-TV usually occurs 3 to 5 days after vaccination. The incidence of febrile reaction to RRV-TV does not appear to be dose related at the doses studied (4×10^4, 4×10^5 or 4×10^6 PFU/dose).[2,33,43]

The MMU 18006 rhesus rotavirus strain, which is contained in RRV-TV, causes chronic hepatitis in immunodeficient mice.[60] However, measurement of ALT levels 3 to 5 weeks after a single dose of RRV-TV 4×10^5 or 4×10^6 PFU[2] or 3 doses of 4×10^4 PFU/dose[39] did not detect any significant differences between RRV-TV and placebo recipients.

The extent of reactogenicity to RRV-TV appears to depend on the age of the infant, as has been previously shown with the rhesus rotavirus vaccine.[61] A suggested reason for this is that high levels of maternally acquired antibodies attenuate reactogenicity. The incidence of fever (axillary temperature >38°C) after a single dose of RRV-TV 4×10^5 or 4×10^6 PFU was significantly greater in

infants aged 16 to 24 weeks than in those aged 6 to 12 weeks (17 *vs* 7%).[2]

Only minor adverse events occurred in full-term neonates who received RRV-TV 4×10^4 PFU or placebo 2 days after birth and a second dose of vaccine or placebo at 6 to 8 weeks.[45] Rectal temperature >38°C was recorded in 3 of 183 (1.6%) vaccine recipients and no placebo recipients in the 10 days after the first dose.

In comparative studies, the incidence of adverse events did not differ greatly between those who received RRV-TV and those who received M37[42,43] or RRV-S1,[39-41] although RRV-TV tended to be associated with a higher incidence of fever.

6. Dosage and Administration

As the greatest morbidity and mortality associated with rotavirus gastroenteritis occurs in infants aged <2 years (see section 1.4), the target population for vaccination is young infants. On the basis of present clinical trial data, the most appropriate schedule for vaccination of infants appears to be 3 doses of 4×10^5 PFU/dose, administered between 2 and 7 months of age. The immunogenicity of RRV-TV may be reduced by the presence of maternally acquired antibodies in infants younger than 2 months (see section 2.1.2) and reactogenicity may be greater in older infants (see section 5).

Concurrent administration of RRV-TV with other injectable vaccines such as DTP is possible. Data from the US suggest that OPV has no clinically significant effect on the efficacy of RRV-TV, but no data are available from developing countries. It is not known whether RRV-TV affects the efficacy of OPV.

RRV-TV is administered orally. The vaccine should be buffered to avoid inactivation of the acid-labile virus. In clinical trials, the vaccine was suspended in a sodium citrate-bicarbonate buffer or was given after administration of 25 to 30ml of milk or formula containing sodium bicarbonate 400mg. It is not necessary to withhold breast feeding before vaccination.

7. Place of Tetravalent Human-Rhesus Reassortant Rotavirus Vaccine in the Prevention of Paediatric Rotavirus Gastroenteritis

Rotavirus gastroenteritis is a major public health burden throughout the world. In developing countries, it is associated with substantial mortality (more than 800 000 children per year). In developed countries, rotavirus accounts for a significant proportion of hospitalisations for diarrhoea in infants aged <5 years. In the US alone, the annual cost of rotavirus disease has been estimated at >$US1 billion per year.[7]

The efficacy of the first live attenuated rotavirus vaccines to be tested varied widely. Studies of the rhesus rotavirus vaccine (MMU 18006; serotype G3 specificity) and bovine rotavirus vaccines (WC3 and RIT 4237; serotype G6 specificity) showed moderate or even high levels of protection in some settings,[24,55,61-67] but in others the vaccines failed to provide significant protection against rotavirus gastroenteritis.[68-76] Suggested factors contributing to this variability were the differences in infant ages between studies and differences in serotype specificity between the vaccine and circulating wild-type rotavirus strains. The vaccines did not appear to produce a sufficient heterotypic antibody response to be protective in some situations, particularly in infants aged <6 months who had not been primed by previous infection.[27,77,78]

Although the role of heterotypic versus homotypic immunity is unclear, the possibility that serotype-specific immunity is important in protection against rotavirus gastroenteritis was the impetus for developing the RRV-TV vaccine.[27,78] RRV-TV generally induces a greater immunological response to human rotavirus serotypes G2, G3 and G4 than the serotype G1 reassortant vaccine RRV-S1, and a greater immunological response to G1, G2 and G3 than the human serotype G1 strain M37 vaccine (see section 2.2). Furthermore, RRV-TV provides greater protection than RRV-S1 against rotavirus gastroenteritis caused by serotypes other than G1 (see section 3.2).

An ideal rotavirus vaccine would prevent all cases of rotavirus gastroenteritis. However, a more realistic goal may be the prevention of severe disease in infants aged <2 years, considering that live attenuated vaccines are unlikely to provide better protection than natural infection.[27] In the case of rotavirus infection, prior exposure largely protects against development of severe disease, but protection against reinfection and milder disease is more variable and is often not complete or durable.[11,79-83] RRV-TV is more effective against severe rotavirus gastroenteritis than less severe episodes; efficacy rates of 69 to 100% against very severe disease over a 2-year period have been demonstrated in the US, Finland and Venezuela (see section 3.1.1). However, the overall efficacy of RRV-TV may be less than that of natural infection in some settings. Among placebo recipients in one of the US studies, infection with rotavirus in the first year provided 93% protection against rotavirus illness in the second year,[84] whereas the vaccine had only 57% protective efficacy.[39] Nevertheless, even with a relatively moderate efficacy of 50%, RRV-TV would prevent approximately 1 million cases of rotavirus gastroenteritis per year in the US.[21,39]

It is promising that RRV-TV has demonstrated efficacy in Venezuela as well as developed countries when given as 4×10^5 PFU/dose. However, the poor performance of the vaccine when given in a lower dose in Brazil and Peru highlights the challenges that may be present in some developing countries; even induction of natural immunity is more difficult in these areas.[80,81,83] The presence of other enteric pathogens and/or high maternally acquired antibody levels may interfere with the immunogenicity of RRV-TV.[35,85] Use of high-titred vaccine or optimisation of the vaccination schedule may overcome these hurdles to some extent.[85] Importantly for developing countries where most infants are breast fed, present data suggest that the immunogenicity and efficacy of RRV-TV are not impaired by breast feeding.

The need for buffering of RRV-TV could be a disadvantage in developing countries. However, a reasonably practical small-volume buffer was used successfully in several of the efficacy trials.[34,40,41] There is also some evidence that breast feeding alone may provide an adequate buffer. Alternatively, microencapsulation of the vaccine might eliminate the need for a buffer.[86]

Administering a complete 3-dose schedule of RRV-TV could be logistically difficult in developing countries. Many infants are seen for routine medical examination only at the time of birth.[45] Thus, neonates would be a good target group for vaccination. Immunogenicity studies based on serum samples have not produced highly encouraging results in neonates, but the response to vaccination may have been underestimated (see section 2.1.2). Efficacy trials in this age group would be of interest. Two-dose schedules for developing countries may also warrant further investigation, considering that the immunogenicity of RRV-TV 4×10^5 PFU/dose was similar after 2 and after 3 doses in the large Venezuelan study.[37]

An important issue for the feasibility and cost-effectiveness of RRV-TV in both developing and developed countries is whether it can be incorporated into routine childhood immunisation schedules.[9,21,87] Data from the US suggest that OPV does not interfere with the efficacy of RRV-TV to a clinically important extent. However, OPV reduces the immunogenicity of RRV-TV and it is important to confirm that this does not impair the efficacy of RRV-TV in developing countries, where protective immunity is more difficult to achieve. Data are required to establish whether RRV-TV reduces the clinical efficacy of OPV. Again, this issue is of greatest concern in developing countries, where the potency of OPV tends to be inadequate and the vaccine failure rate for poliovirus serotype 1 tends to be higher and wild poliovirus is still circulating.[88,89]

Data from the US and Finland suggest that under present conditions routine vaccination with RRV-TV could be cost saving in developed countries, depending on the price of the vaccine. The price of the vaccine is the pivotal issue for its use in poor countries, which may have a total per capita health expenditure of only $US5 to $20 per year.[56]

The relative cost effectiveness of rotavirus vaccination may be altered as more effective treatments for rotavirus gastroenteritis, such as immunoglobulin and probiotic therapy, become available.

RRV-TV provides a first step towards the prevention of paediatric rotavirus gastroenteritis; however, several questions remain (table IV), the answers to which will be found only when the vaccine is introduced into routine vaccination schedules. The vaccine may need to be adapted to incorporate other serotypes in order to have optimal efficacy in areas with a significant prevalence of rotavirus serotypes other than G1 to G4 (e.g. Brazil and India).[8]

RRV-TV is a starting point from which even more effective vaccines are likely to evolve as a better understanding of the effectors of protective immunity against rotavirus disease is gained. Human-rhesus reassortant rotavirus vaccines incorporating genes encoding both VP7 and VP4, and similar human-bovine reassortant vaccines, are being investigated.[27] Other strategies being researched include DNA vaccination,[90] live bacterial vectors expressing rotavirus viral proteins,[91] subunit vaccines using baculovirus-expressed virus-like particles,[92] cold-adapted viruses,[27] microencapsulated viruses[93] and inclusion of VP6 and nonstructural protein 4 components.[78,94]

Table IV. Remaining issues associated with tetravalent human-rhesus reassortant rotavirus vaccine (RRV-TV)[87,95]

- Will incorporation of RRV-TV into routine childhood immunisation schedules decrease circulation of rotavirus and produce the expected reduction in disease?
- Will the epidemiological patterns of rotavirus infection be changed by widespread use of RRV-TV?
- Will shedding of the vaccine virus provide protective immunity for unvaccinated individuals?
- Will shedding of the vaccine virus pose a risk for close contacts?
- Is RRV-TV associated with rare, as yet unidentified, risks?
- What is the duration of immunity provided by RRV-TV and will booster doses be needed?
- If vaccine-induced immunity wanes over time, will rotavirus epidemics occur in susceptible older children?
- What are the risk factors for failure of the vaccine when used in the community setting?

In conclusion, incorporation of RRV-TV into routine childhood immunisation schedules has the potential to reduce the incidence of paediatric rotavirus gastroenteritis by approximately 50% in most settings. Importantly, the incidence of the most severe cases could be greatly reduced, and the vaccine is likely to prove cost saving in developed countries. Although a better understanding of the effectors of protective immunity against rotavirus disease is needed to advance rotavirus vaccine research, RRV-TV provides a good basis upon which to make further refinements to increase protective efficacy. RRV-TV is a significant advance in the prevention of paediatric rotavirus gastroenteritis worldwide.

References

1. Institute of Medicine. The prospects for immunizing against rotavirus. In: New Vaccine Development. Establishing priorities. Diseases of importance in developing countries. Vol II. Washington, DC: National Academy Press, 1986: 308-18
2. Dennehy PH, Rodgers Jr GC, Ward RL, et al. Comparative evaluation of reactogenicity and immunogenicity of two dosages of oral tetravalent rhesus rotavirus vaccine. Pediatr Infect Dis J 1996 Nov; 15: 1012-8
3. Estes MK. Advances in molecular biology: impact on rotavirus vaccine development. J Infect Dis 1996 Sep; 174 Suppl. 1: S37-46
4. Christensen ML. Human viral gastroenteritis. Clin Microbiol Rev 1989 Jan; 2 (1): 51-67
5. Blacklow NR, Greenberg HB. Viral Gastroenteritis. N Engl J Med 1991 Jul 25; 325 (4): 252-64
6. Offit PA, Clark HF. Rotavirus. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. 4th ed. Vol 2. New York: Churchill Livingstone, 1995: 1448-55
7. Glass RI, Kilgore PE, Holman RC, et al. The epidemiology of rotavirus diarrhea in the United States: surveillance and estimates of disease burden. J Infect Dis 1996; 174 Suppl. 1: S5-S11
8. Gentsch JR, Woods PA, Ramachandran M, et al. Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. J Infect Dis 1996; 174 Suppl. 1: S30-6
9. Barnes GL, Bishop RF. Rotavirus infection and prevention. Curr Opin Pediatr 1997 Feb; 9: 19-23
10. Gerna G, Sarasini A, Zentilin L, et al. Isolation in Europe of 69 M-like (serotype 8) human rotavirus strains with either subgroup I or II specificity and a long RNA electropherotype. Arch Virol 1990; 112: 27-40
11. Ward RL. Mechanisms of protection against rotavirus in humans and mice. J Infect Dis 1996 Sep; 174 Suppl. 1: S51-8
12. Offit PA. Host factors associated with protection against rotavirus disease: the skies are clearing. J Infect Dis 1996; 174 Suppl. 1: S59-64

13. Greenberg HB, Clark HF, Offit PA. Rotavirus pathology and pathophysiology. Curr Top Microbiol Immunol 1994; 185: 255-83
14. Ball JM, Tian P, Zeng CQ-Y, et al. Age-dependent diarrhea induced by a rotaviral nonstructural glycoprotein. Science 1996 Apr 5; 272: 101-4
15. Kapikian AZ, Flores J, Hoshino Y, et al. Rotavirus: the major etiologic agent of severe infantile diarrhea may be controllable by a 'Jennerian' approach to vaccination. J Infect Dis 1986; 153 (5): 815-22
16. Kapikian AZ, Chancock RM. Rotaviruses. In: Fields BN, Knipe DM, Howley PM, et al., editors. Fields virology. 3rd ed. Vol 2. Philadelphia: Lippincott-Raven, 1996: 1657-705
17. Isolauri E, Kaila M, Mykkänen H, et al. Oral bacteriotherapy for viral gastroenteritis. Dig Dis Sci 1994 Dec; 39 (12): 2595-600
18. Guarino A, Canani RB, Russo S. Development in the treatment of rotaviral gastroenteritis: oral therapy with immunoglobulins and prospects for a vaccine. Clin Immunother 1995 Jun; 3: 476-84
19. Ho M-S, Glass RI, Pinsky PF, et al. Rotavirus as a cause of diarrheal morbidity and mortality in the United States. J Infect Dis 1988 Nov; 158 (5): 1112-6
20. Matson DO, Estes MK. Impact of rotavirus infection at a large pediatric hospital. J Infect Dis 1990 Sep; 162: 598-604
21. Smith JC, Haddix AC, Teutsch SM. Cost-effectiveness analysis of a rotavirus immunization program for the United States. Pediatrics 1995 Oct; 96: 609-15
22. Ryan MJ, Ramsay M, Brown D, et al. Hospital admissions attributable to rotavirus infection in England and Wales. J Infect Dis 1996; 174 Suppl. 1: S12-8
23. Pérez-Schael I. The impact of rotavirus disease in Venezuela. J Infect Dis 1996; 174 Suppl. 1: S19-21
24. Perez-Schael I, Garcia D, Gonzalez M, et al. Prospective study of diarrheal diseases in Venezuelan children to evaluate the efficacy of rhesus rotavirus vaccine. J Med Virol 1990 Mar; 30: 219-29
25. LeBaron CW, Lew J, Glass RI, et al. Annual rotavirus epidemic patterns in North America. Results of a 5-year retrospective survey of 88 centers in Canada, Mexico and the United States. JAMA 1990 Aug 22/29; 264 (8): 983-8
26. Cook SM, Glass RI, LeBaron CW, et al. Global seasonality of rotavirus infections. Bull World Health Organ 1990; 68 (2): 171-7
27. Kapikian AZ, Hoshino Y, Chanock RM, et al. Efficacy of a quadrivalent rhesus rotavirus-based human rotavirus vaccine aimed at preventing severe rotavirus diarrhea in infants and young children. J Infect Dis 1996 Sep; 174 Suppl. 1: S65-72
28. Midthun K, Greenberg HB, Hoshino Y, et al. Reassortant rotaviruses as potential live rotavirus vaccine candidates. J Virol 1985 Mar; 53 (3): 949-54
29. Glass RI, Lang DR, Ivanoff BN, et al. Rotavirus – from basic research to a vaccine. J Infect Dis 1996 Sep; 174 Suppl. 1: S1-2
30. Ward RL, Bernstein DI, Us RVEG. Lack of correlation between serum rotavirus antibody titers and protection following vaccination with reassortant RRV vaccines. Vaccine 1995 Sep; 13: 1226-32
31. Ward RL, Knowlton DR, Zito ET, et al. Serologic correlates of immunity in a tetravalent reassortant rotavirus vaccine trial. J Infect Dis 1997 Sep; 176: 570-7
32. Perez-Schael I, Blanco M, Vilar M, et al. Clinical studies of a quadrivalent rotavirus vaccine in Venezuelan infants. J Clin Microbiol 1990 Mar; 28: 553-8
33. Flores J, Perez-Schael I, Blanco M, et al. Reactogenicity and immunogenicity of a high-titer rhesus rotavirus-based quadrivalent rotavirus vaccine. J Clin Microbiol 1993 Sep; 31: 2439-45
34. Joensuu J, Koskenniemi E, Pang X-L, et al. Randomised placebo-controlled trial of rhesus-human reassortant rotavirus vaccine for prevention of severe rotavirus gastroenteritis. Lancet 1997 Oct 25; 350: 1205-9
35. Lanata CF, Midthun K, Black RE, et al. Safety, immunogenicity, and protective efficacy of one and three doses of the tetravalent rhesus rotavirus vaccine in infants in Lima, Peru. J Infect Dis 1996 Aug; 174: 268-75
36. Linhares AC, Gabbay YB, Mascarenhas JD, et al. Immunogenicity, safety and efficacy of tetravalent rhesus-human, reassortant rotavirus vaccine in Belém, Brazil. Bull World Health Organ 1996; 74: 491-500
37. Pérez-Schael I, Gutinas MJ, Pérez M, et al. Efficacy of the rhesus rotavirus-based quadrivalent vaccine in infants and young children in Venezuela. N Engl J Med 1997 Oct 23; 337 (17): 1181-7
38. Simasathien S, Migasena S, Samakoses R, et al. Vaccination of Thai infants with rhesus-human reassortant tetravalent oral rotavirus vaccine. Pediatr Infect Dis J 1994 Jul; 13: 590-6
39. Bernstein DI, Glass RI, Rodgers G, et al. Evaluation of rhesus rotavirus monovalent and tetravalent reassortant vaccines in US children. JAMA 1995 Apr 19; 273: 1191-6
40. Rennels MB, Glass RI, Dennehy PH, et al. Safety and efficacy of high-dose rhesus-human reassortant rotavirus vaccines – report of the National Multicenter Trial. Pediatrics 1996 Jan; 97: 7-13
41. Santosham M, Moulton LH, Reid R, et al. Efficacy and safety of high-dose rhesus-human reassortant rotavirus vaccine in Native American populations. J Pediatr 1997 Oct; 131 (4): 632-8
42. Flores J, Perez-Schael I, Blanco M, et al. Comparison of reactogenicity and antigenicity of M37 rotavirus vaccine and rhesus-rotavirus-based quadrivalent vaccine. Lancet 1990 Aug 11; 336: 330-4
43. Perez-Schael I, Blanco M, Garcia D, et al. Evaluation of the antigenicity and reactogenicity of varying formulations of the rhesus rotavirus-based quadrivalent and the M37 rotavirus vaccine candidates. J Med Virol 1994 Apr; 42: 330-7
44. Ceyhan M, Kanra G, Seçmeer G, et al. Take of rhesus-human reassortant tetravalent rotavirus vaccine in breast-fed infants. Acta Paediatr 1993 Mar; 82: 223-7
45. Dagan R, Kassis I, Sarov B, et al. Safety and immunogenicity of oral tetravalent human-rhesus reassortant rotavirus vaccine in neonates. Pediatr Infect Dis J 1992 Dec; 11: 991-6
46. Dagan R, Segal B, Kassis I, et al. Safety and immunogenicity of oral rhesus rotavirus tetravalent vaccine (ORRTV) in neonates: comparison of two doses [abstract no. 1096]. 31st Interscience Conference on Antimicrobial Agents and Chemotherapy; 1991 Sep 29-Oct 2; Chicago (IL), 281
47. Friedman MG, Segal B, Zedaka R, et al. Serum and salivary responses to oral tetravalent reassortant rotavirus vaccine in newborns. Clin Exp Immunol 1993; 92 (2): 194-9
48. Rennels MB, Wasserman SS, Glass RI. Comparison of immunogenicity and efficacy of rhesus rotavirus reassortant vaccines in breastfed and nonbreastfed children. Pediatrics 1995 Dec; 96: 1132-6
49. Ing DJ, Glass RA, Woods PA, et al. Immunogenicity of tetravalent rhesus rotavirus vaccine administered with buffer and oral polio vaccine. Am J Dis Child 1991 Aug; 145: 892-7

50. Migasena S, Simasathien S, Samakoses R, et al. Simultaneous administration of oral rhesus-human reassortant tetravalent (RRV-TV) rotavirus vaccine and oral poliovirus vaccine (OPV) in Thai infants. Vaccine 1995 Feb; 13: 168-74
51. Rennels MB, Ward RL, Mack ME, et al. Concurrent oral poliovirus and rhesus-human reassortant rotavirus vaccination: effects on immune responses to both vaccines and on efficacy of rotavirus vaccines. J Infect Dis 1996 Feb; 173: 306-13
52. Pichichero ME, Marsocci SM, Francis AB, et al. A comparative evaluation of the safety and immunogenicity of a single dose of unbuffered oral rhesus rotavirus serotype 3, rhesus/human reassortant serotypes 1, 2 and 4 and combined (tetravalent) vaccines in healthy infants. Vaccine 1993 May; 11: 747-53
53. Wright PF, King J, Araki K, et al. Simultaneous administration of two human-rhesus rotavirus reassortant strains of VP7 serotype 1 and 2 specificity to infants and young children. J Infect Dis 1991 Aug; 164: 271-6
54. Vesikari T, Varis T, Green K, et al. Immunogenicity and safety of rhesus human rotavirus reassortant vaccines with serotype 1 or 2 VP7 specificity. Vaccine 1991 May; 9: 334-9
55. Flores J, Perez-Schael I, Gonzalez M, et al. Protection against severe rotavirus diarrhoea by rhesus rotavirus vaccine in Venezuelan infants. Lancet 1987 Apr 18; I: 882-4
56. Keusch GT, Cash RA. A vaccine against rotavirus – when is too much too much? N Engl J Med 1997 Oct 23; 337 (17): 1228-9
57. Black RE, Merson MH, Huq I, et al. Incidence and severity of rotavirus and Escherichia Coli diarrhoea in rural Bangladesh. Implications for vaccine development. Lancet 1981 Jan 17; I: 141-3
58. Griffiths RI, Anderson GF, Powe NR, et al. Economic impact of immunization against rotavirus gastroenteritis: evidence from a clinical trial. Arch Pediatr Adolesc Med 1995 Apr; 149: 407-14
59. Takala AK, Koskenniemi E, Joensuu J, et al. Economic evaluation of rotavirus vaccination in Finland [abstract no. 420]. Clin Infect Dis 1997 Aug; 25: 432
60. Uhnoo I, Riepenhoff-Talty M, Dharakul T, et al. Extramucosal spread and development of hepatitis in immunodeficient and normal mice infected with rhesus rotavirus. J Virol 1990 Jan; 64 (1): 361-8
61. Gothefors L, Wadell G, Juto P, et al. Prolonged efficacy of rhesus rotavirus vaccine in Swedish children. J Infect Dis 1989 Apr; 159: 753-7
62. Mutz ID, Krainer F, Deutsch J, et al. A trial of RIT-4237 rotavirus vaccine in 1-month-old infants. Eur J Pediatr 1989 Jun; 148: 634-5
63. Vesikari T, Ruuska T, Delem A, et al. Efficacy of two doses of RIT 4237 bovine rotavirus vaccine for prevention of rotavirus diarrhoea. Acta Paediatr Scand 1991; 80: 173-80
64. Vesikari T. Clinical trials of live oral rotavirus vaccines: the Finnish experience. Vaccine 1993; 11 (2): 255-61
65. Clark HF, Borian FE, Bell LM, et al. Protective effect of WC3 vaccine against rotavirus diarrhea in infants during a predominantly serotype 1 rotavirus season. J Infect Dis 1988; 158 (3): 570-87
66. Vesikari T, Isolauri E, D'hondt E, et al. Protection of infants against rotavirus diarrhoea by RIT 4237 attenuated bovine rotavirus strain vaccine. Lancet 1984 May 5; I: 977-80
67. Lanata CF, Black RE, del Aguila R, et al. Protection of Peruvian children against rotavirus diarrhea of specific serotypes by one, two, or three doses of the RIT 4237 attenuated bovine rotavirus vaccine. J Infect Dis 1989 Mar; 159 (3): 452-9
68. Bernstein DI, Smith VE, Sander DS, et al. Evaluation of WC3 rotavirus vaccine and correlates of protection in healthy infants. J Infect Dis 1990; 162 (5): 1055-62
69. De Mol P, Zissis G, Butzler J-P, et al. Failure of live, attenuated oral rotavirus vaccine [letter]. Lancet 1986 Jul 12; II: 108
70. Georges-Courbot MC, Monges J, Siopathis MR, et al. Evaluation of the efficacy of a low-passage bovine rotavirus (strain WC3) vaccine in children in Central Africa. Res Virol 1991; 142: 405-11
71. Rennels MB, Losonsky GA, Young AE, et al. An efficacy trial of the rhesus rotavirus vaccine in Maryland. Am J Dis Child 1990 May; 144: 601-4
72. Santosham M, Letson WG, Wolff M, et al. A field study of the safety and efficacy of two candidate rotavirus vaccines in a Native American population. J Infect Dis 1991; 163: 483-7
73. Senturia YD, Peckham CS, Cordery M, et al. Live attenuated oral rotavirus vaccine [letter]. Lancet 1987 Nov 7; II: 1091-2
74. Vesikari T, Rautanen T, Varis T, et al. Rhesus *Rotavirus* candidate vaccine. Clinical trial in children vaccinated between 2 and 5 months of age. Am J Dis Child 1990 Mar; 144: 285-9
75. Hanlon P, Hanlon L, Marsh V, et al. Trial of an attenuated bovine rotavirus vaccine (RIT 4237) in Gambian infants. Lancet 1987 Jun 13; I: 1342-5
76. Christy C, Madore HP, Pichichero ME, et al. Field trial of rhesus rotavirus vaccine in infants. Pediatr Infect Dis J 1988; 7 (9): 645-50
77. Green KY, Taniguchi K, Mackow ER, et al. Homotypic and heterotypic epitope-specific antibody responses in adult and rotavirus vaccinees: implications for vaccine development. J Infect Dis 1990 Apr; 161: 667-79
78. Glass RI, Gentsch JR, Ivanoff B. New lessons for rotavirus vaccines. Science 1996 Apr 5; 272: 46-8
79. Bishop RF, Barnes GL, Cipriani E, et al. Clinical immunity after neonatal rotavirus infection. A prospective longitudinal study in young children. N Engl J Med 1983 Jul 14; 309 (2): 72-6
80. Mata L, Simhon A, Urrutia JJ, et al. Epidemiology of rotaviruses in a cohort of 45 Guatamalan Mayan Indian children observed from birth to the age of three years. J Infect Dis 1983 Sep; 148 (3): 452-61
81. Grinstein S, Gomez JA, Bercovich JA, et al. Epidemiology of rotavirus infection and gastroenteritis in prospectively monitored Argentine families with young children. Am J Epidemiol 1989 Aug; 130 (2): 300-8
82. Bernstein DI, Sander DS, Smith VE, et al. Protection from rotavirus reinfection: a 2-year prospective study. J Infect Dis 1991 Aug; 164: 277-83
83. Velázquez FR, Matson DO, Calva JJ, et al. Rotavirus infection in infants as protection against subsequent infections. N Engl J Med 1996 Oct 3; 335 (14): 1022-8
84. Ward RL, Bernstein DI, US Rotavirus Vaccine Efficacy Group. Protection against rotavirus disease after natural rotavirus infection. J Infect Dis 1994 Apr; 169: 900-4
85. Vesikari T. Rotavirus vaccines against diarrhoeal disease. Lancet 1997 Nov 22; 350: 1538-41
86. Midthun K, Kapikian AZ. Rotavirus vaccines: an overview. Clin Microbiol Rev 1996 Jul; 9: 423-34
87. Orenstein WA, Hadler S, Kuritsky JN, et al. Rotavirus vaccines – from licensure to disease reduction. J Infect Dis 1996 Sep; 174 Suppl. 1: S118-24
88. Partriarca PA, Wright PF, John TJ. Factors affecting the immunogenicity of oral poliovirus vaccine in developing countries: review. Rev Infect Dis 1991 Sep-Oct; 13: 926-39
89. World Health Organization Collaborative Study Group on Oral Poliovirus Vaccine. Factors affecting the immunogenicity of

oral poliovirus vaccine: a prospective evaluation in Brazil and the Gambia. J Infect Dis 1995 May; 171: 1097-105
90. Hermann JE, Chen SC, Fynan EF, et al. Protection against rotavirus infections by DNA vaccination. J Infect Dis 1996; 174 Suppl. 1: S93-7
91. Martyn J, Wang L, Nagesha H, et al. Immune response to porcine rotavirus VP7 expressed as an OMP a fusion protein in the bacterial outer membrane [abstract]. Vaccines beyond 2000; 1997 Jul 6-9; Gold Coast, Queensland, Australia, P14
92. Conner ME, Zarley CD, Hu B, et al. Virus-like particles as a rotavirus subunit vaccine. J Infect Dis 1996; 174 Suppl. 1: S88-92
93. Khoury CA, Moser CA, Speaker TJ, et al. Oral inoculation of mice with low doses of microencapsulated, noninfectious rotavirus induces virus-specific antibodies in gut-associated lymphoid tissue. J Infect Dis 1995 Sep; 172: 870-4
94. Lang DR, Glass RI, Compans RW. Summary of the Fifth Rotavirus Vaccine Workshop. J Infect Dis 1996 Sep; 174 Suppl. 1: S3-4
95. Henchal LS, Midthun K, Goldenthal KL. Selected regulatory and scientific topics for candidate rotavirus vaccine development. J Infect Dis 1996 Sep; 174 Suppl. 1: S112-7

Correspondence: *Rachel H. Foster*, Adis International Limited, 41 Centorian Drive, Private Bag 65901, Mairangi Bay, Auckland 10, New Zealand.
E-mail:demail@adis.co.nz