

# Rotavirus Vaccines: an Overview

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## INTRODUCTION

Rotavirus is the leading cause of severe diarrhea disease in infants and young children worldwide. About 600,000 children die every year from rotavirus, with more than 80% of all rotavirus-related deaths occurring in resource-poor countries in south Asia and sub-Saharan Africa (66). Rotavirus-related deaths represent approximately 5% of all deaths in children younger than 5 years of age worldwide.

The virus infects the mature villus epithelial cells of the small intestine, and infection often leads to fever, vomiting, and diarrhea in children. Dehydration and electrolyte disturbances are the major sequelae of rotavirus infection and occur most often in the youngest children. Rotavirus infection is usually localized to the intestine; however, recent studies reported antigenemia or viremia in children with rotavirus diarrhea (11, 12, 17, 18, 90). Rarely, involvement of extraintestinal sites, including the respiratory tract, liver, kidney, lymph nodes, and central nervous system, has been reported (54, 55, 64, 70).

## Disease Burden and Epidemiology

Each year, rotavirus causes approximately 114 million episodes of gastroenteritis requiring home care only, 24 million clinic visits, and 2.4 million hospitalizations in children <5 years of age worldwide. By age 5, nearly every child will have an episode of rotavirus gastroenteritis, 1 in 5 will visit a clinic, 1 in 50 will be hospitalized, and approximately 1 in 205 will die (35). Recent studies indicate that rotavirus causes approximately 39% of childhood diarrhea hospitalizations worldwide (66).

In temperate climates, rotavirus disease occurs during the cooler months. Seasonal patterns in tropical climates are less pronounced, but disease is more common during the drier, cooler months. In the United States, rotavirus causes yearly epidemics of disease from late fall to early spring (Fig. 1). The peak of disease varies by region. In the southwest, the peak rotavirus season is November to December. The peak of the epidemic then travels sequentially across the United States from west to east, concluding in April to May in the northeast (41, 51, 78, 79).

Rotavirus gastroenteritis results in only 20 to 70 childhood deaths per year in the United States (30, 47). However, nearly every child in the United States is infected with rotavirus by 5 years of age, and most will develop gastroenteritis. One child in 7 will require a clinic or emergency room visit, and 1 in 70 will be hospitalized (36, 56). Each year, rotavirus causes more than

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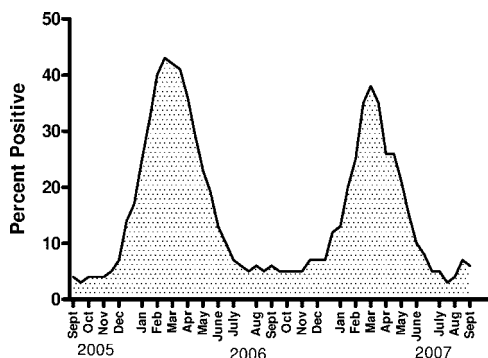


FIG. 1. Seasonal trends in rotavirus activity in the United States from September 2005 through September 2007. These data are from the National Respiratory and Enteric Virus Surveillance System, a voluntary, laboratory-based system organized by the CDC, Atlanta, GA. The National Respiratory and Enteric Virus Surveillance System prospectively monitors seasonal trends in viral activity on a weekly basis.

400,000 physician visits, more than 200,000 emergency room visits, and 55,000 to 70,000 hospitalizations (30). Rotavirus infection is responsible for only 5 to 10% of all gastroenteritis episodes among children <5 years of age in the United States. However, rotavirus causes more severe disease than other pathogens causing gastroenteritis and thus accounts for 30 to 50% of all hospitalizations for gastroenteritis among children aged <5 years and more than 70% of hospitalizations for gastroenteritis during the seasonal peaks of rotavirus disease in the United States (13, 48, 57, 72).

Although severity of disease may differ, rates of rotavirus illness among children in industrialized and resource-poor countries are similar, indicating that clean water supplies and good hygiene have little effect on virus transmission, and further improvements in water or hygiene are unlikely to prevent the disease. In view of the high burden of rotavirus disease, safe and effective rotavirus vaccines are urgently needed, particularly in the resource-poor countries of the world. Such vaccines would have universal application in childhood vaccination programs.

## VIROLOGY

Rotaviruses were discovered in the 1960s in animals. The virus was first described in humans when it was found by electron microscopy in duodenal biopsies from children with acute gastroenteritis (9).

Rotaviruses are 70-nm icosahedral viruses that belong to the family *Reoviridae*. Seven rotavirus serogroups (serogroups A to G) are described. Most human pathogens belong to groups A, B, and C. Group A rotaviruses are the most important from a public health standpoint.

The virus is composed of three protein shells, an outer capsid, an inner capsid, and an internal core, that surround the 11 segments of double-stranded RNA (Fig. 2). For the most part, each gene segment codes for a single protein. When mixed infection with more than one rotavirus strain occurs, the gene segments from the parental viruses may reassort independently, producing reassortants of mixed parentage, a source of viral diversity.

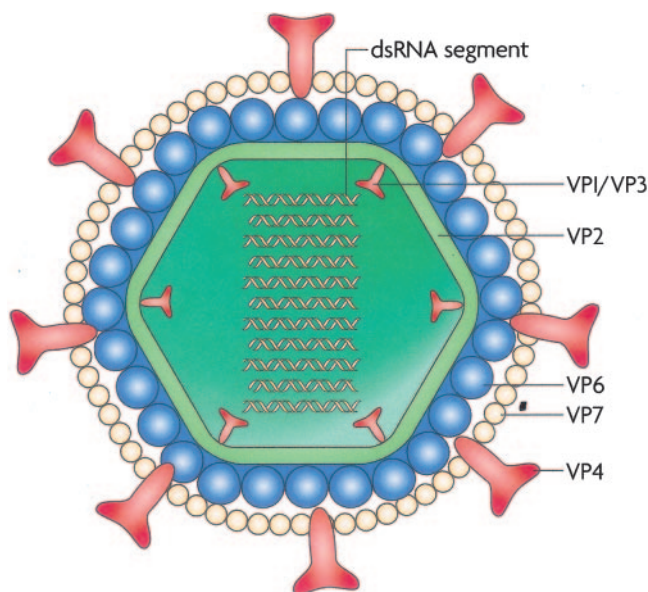


FIG. 2. Schematic representation of a rotavirus virion. The virus is composed of three protein shells, an outer capsid, an inner capsid, and an internal core, that surround the 11 segments of double-stranded RNA. The outer capsid proteins VP4 and VP7 are neutralization antigens and define the P and G serotypes, respectively. VP6, the inner capsid structural protein, is the subgroup antigen. (Reprinted from reference 1 by permission from Macmillan Publishers.)

Four major structural and nonstructural proteins are of interest in vaccine development: VP6, NSP4, VP7, and VP4. VP6, the most abundant viral structural protein, is found in the inner capsid (43). VP6 bears group-specific antigenic determinants. NSP4 is a nonstructural protein and has been shown to be an enterotoxin (2).

VP7 and VP4 are structural proteins found in the outer capsid. These two proteins define the serotype of the virus and are considered to be critical for vaccine development because they are targets for neutralizing antibodies that may provide both serotype-specific and, in some instances, cross-reactive protection (38). The VP7 protein is glycosylated, and serotypes determined by this protein are termed G serotypes. Fourteen G serotypes have been identified.

VP4 is a protease-cleaved protein, and serotypes determined by this protein are termed P serotypes. P types have been difficult to characterize by traditional methods of virus neutralization; therefore, molecular methods have been used to define a genotype based on sequence analysis. These genotypes correlate well with known serotypes, so the genotypes are tentatively designated in brackets (e.g., P1A[8]). Strains are generally designated by their G serotype specificities (e.g., serotypes G1 to G4 and G9).

Human rotaviruses exhibit enormous diversity. The gene segments that encode the G and P proteins can segregate independently, giving rise to strains with at least 42 different P-G serotype combinations (33). However, a small number of rotavirus strains bearing VP7 G serotypes G1 to G4 and G9 and VP4 P genotypes P1B[4], P2A[6], and P1A[8] are predominant worldwide. In a recent study, four G types (G1, G2, G3, and G4) in conjunction with P1A[8] or P1B[4] represented

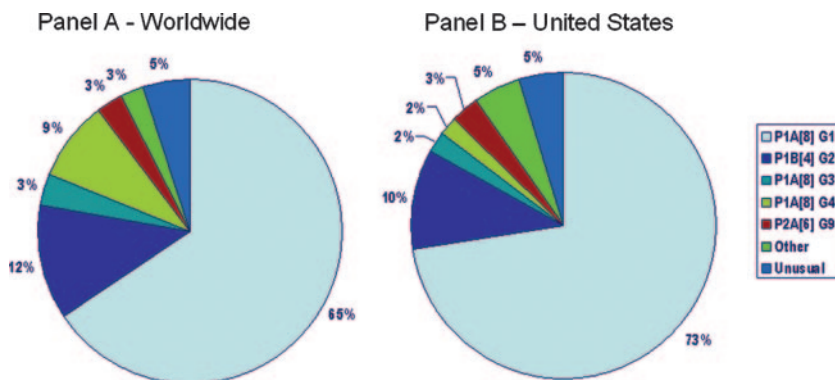


FIG. 3. Distribution of rotavirus serotypes worldwide and in the United States. (A) Global distribution from 1989 to 2004. The G serotypes of >88% of rotavirus strains worldwide are G1, G2, G3, and G4. The P serotype of >80% of rotavirus strains worldwide is P1A[8]. (B) U.S. distribution from 1973 to 2003. The G serotypes of >97% of rotavirus strains in the United States are G1, G2, G3, and G4. The P serotype of >80% of rotavirus strains is P1A[8]. This figure is based on data from reference 74.

over 88% of the strains analyzed worldwide. Serotype G9 viruses associated with P1A[8] or P2A[6] have been emerging since the late 1990s and now represent approximately 4% of global isolates (Fig. 3) (74).

G and P serotype distributions differ geographically. P1A[8]G1 is the globally predominant strain, representing over 70% of rotavirus infections in North America (Fig. 3), Europe, and Australia but only about 30% of the infections in South America and Asia and 23% of those in Africa (74). G9 strains now constitute the predominant strains in some parts of Asia and Africa, and G8 strains are proportionally more frequently isolated in Africa. In South America, G5 strains have emerged in children with diarrhea, and G9 is associated with more severe disease in Latin America (53). Similarly, the distribution of the VP4 P2A[6] antigen differs according to region. P2A[6] strains now constitute over 50% of the strains circulating in Africa, whereas P1A[8] is associated with most rotavirus strains from the rest of the world (76).

Implementation of an effective rotavirus vaccine program will need to take into account the geographical variation of prevalent strains. The continued identification of the most common G and P serotypes for inclusion in vaccines is an important priority. After the introduction of a vaccine candidate, monitoring of circulating strains may be necessary, as vaccine pressure may lead to the selection of novel rotavirus strains.

#### NATURAL PROTECTION

Most symptomatic rotavirus infections occur between 3 months and 2 years of age, with a peak incidence between 7 and 15 months. Rotavirus infections are more likely to be severe in children 3 to 24 months of age than in younger infants or older children and adults (21, 67, 88). Longitudinal studies demonstrated that naturally acquired rotavirus infections provide protection against rotavirus disease upon reinfection and that protection is greatest against the most severe disease outcomes (29, 80). Although children can be infected with rotavirus several times during their lives, initial infection after 3 months of age is most likely to cause severe diarrhea and dehydration.

Most mothers have rotavirus antibody from previous infection that is passed transplacentally, protecting the neonate. As a result, most infected neonates will have asymptomatic or mild disease (8). An exception is the preterm infant, who is at greater risk of severe illness than the term infant because of the lack of transplacental maternal antibodies (62). Exposure of neonates (asymptotically) to rotavirus is associated with a reduced likelihood of their developing severe rotavirus diarrhea later in infancy (6, 8).

After a first natural infection, infants and young children are protected against subsequent symptomatic disease regardless of whether the first infection was symptomatic or asymptomatic. In a study in Mexico, 40% of children were protected against a subsequent infection with rotavirus after a single natural infection, 75% were protected against diarrhea caused by a subsequent rotavirus infection, and 88% were protected against severe rotavirus diarrhea (80). Second, third, and fourth infections conferred progressively greater protection. No child with two previous infections subsequently developed severe rotavirus diarrhea.

Despite three decades of research, the immune correlates of protection from rotavirus infection and disease are not completely understood. The mouse model has been extensively used to investigate the contribution of different components of the immune system in protection (87). These studies have suggested that both humoral and cell-mediated immunity are important in the resolution of ongoing rotavirus infection and in protection against subsequent infection.

Humoral immunity is believed to play an important role in protection. Studies of monkeys have demonstrated that the passive transfer of serum antibodies can provide protection against infection (89). Studies have also demonstrated that the first infection with rotavirus elicits a predominantly homotypic, serum-neutralizing antibody response to the virus, and subsequent infections elicit a broader, heterotypic response (19, 23, 58, 63). Controversy exists as to whether serum antibodies are directly involved in protection or merely reflect recent infection. Review of data from a variety of studies of humans, including challenge experiments with adult volunteers, longitudinal studies of rotavirus infection in young children, and clinical trials of animal and animal-human reassortant rotavi-



rus vaccines in infants, suggests that serum antibodies, if present at critical levels, are either protective themselves or an important and powerful correlate of protection against rotavirus disease, even though other host effectors may play an important role as well (40).

VP6 is the immunodominant antigen in the antibody response to human rotavirus infection (77). Serum immunoglobulin A (IgA) or IgG antibodies against VP6 antigen tested by enzyme immunoassay are regarded as an indicator of rotavirus immunity after infection and vaccination. Serum IgA appears to act intracellularly in rotavirus-infected cells (32). A high level of serum IgA antibody correlates with clinical protection against rotavirus gastroenteritis (81).

Neutralizing antibodies against VP7 and VP4 antigens clearly play a role in protection after natural rotavirus infection (19), but their role in rotavirus vaccine-induced immunity is less clear. The current live oral rotavirus vaccines rely on the concept that immunity to the rotavirus surface antigens is essential or important for vaccine-induced protection. However, vaccines that elicit low levels of serum antibodies have been effective in field trials.

Local immunity in the gut also seems to be important for protection against subsequent infection. The total serum anti-rotavirus IgA level, measured shortly after infection, generally reflects intestinal IgA levels and appears to be the best marker of protection (31). However, gut immunity appears to be of short duration and has been hard to measure.

Since a reliable immune correlate of protection has not been forthcoming from studies of humans, each new vaccine candidate must be tested in large field trials for efficacy.

### GOALS FOR A ROTAVIRUS VACCINE

A realistic goal for a rotavirus vaccine is to duplicate the degree of protection against disease that follows natural infection. Therefore, vaccine program objectives include the prevention of moderate to severe disease but not necessarily of mild disease associated with rotavirus. An effective rotavirus vaccine will clearly decrease the number of children admitted to the hospital with dehydration or seen in emergency departments but should also decrease the burden on the practicing primary care practitioner by reducing the number of office visits or telephone calls due to rotavirus gastroenteritis. Finally, effective rotavirus vaccines are most needed in resource-poor countries, where mortality associated with rotavirus is high.

### VACCINE STRATEGIES

Attenuation of rotaviruses for use as oral vaccines may be achieved in several ways. The most extensively evaluated approach is based on the "Jennerian" concept, involving immunization of infants with animal rotaviruses that are considered to be naturally attenuated for humans (39). More recently, human rotaviruses attenuated by passage in cell culture have been developed and tested (5). Finally, rotaviruses recovered from asymptomatic human neonates, which may be naturally less virulent, are being developed as oral vaccine candidates (4, 34).

## VACCINES BASED ON ANIMAL ROTAVIRUSES

### Previous Strategies

**Monovalent animal rotavirus vaccines.** Research to develop a safe, effective rotavirus vaccine began in the mid-1970s, when investigators demonstrated that previous infection with animal rotavirus strains protected laboratory animals from experimental infection with human rotaviruses (91). Researchers thought that live animal strains that were naturally attenuated for humans, when given orally, might mimic the immune response to natural infection and protect children against disease. Three nonhuman rotavirus vaccines, two bovine rotavirus strains, RIT 4237 (P6[1]G6) and WC3 (P7[5]G6), and a simian (rhesus) rotavirus reassortant vaccine (RRV) strain (P[3]G3), were studied (20, 22, 82). These vaccines demonstrated variable efficacy in field trials and gave particularly disappointing results in developing countries (37, 50). In 2000 and 2001, China introduced a rotavirus vaccine for childhood immunization (52). The LLR vaccine is a monovalent (P[12]G10) live-attenuated oral vaccine that was derived from a lamb strain of rotavirus developed and produced by the Lanzhou Institute of Biological Products. The efficacy of this vaccine is not known, as it was not tested against placebo in a controlled phase III trial.

In view of the inconsistency of protection from monovalent animal rotavirus-based vaccines, vaccine development efforts began to use either naturally attenuated human rotavirus strains or reassortant rotavirus strains bearing a human rotavirus gene for the VP7 protein together with the other 10 genes from an animal rotavirus strain (59). The next generation of vaccines was formulated to include more than one rotavirus G serotype to provide heterotypic as well as homotypic immunity. The ability of rotaviruses to reassort during mixed infections *in vitro* allowed the production of reassortant vaccines, termed the "modified Jennerian" approach (45). Reassortant viruses contain some genes from the animal rotavirus parent and some genes from the human rotavirus parent. VP7 was thought to be important for protection; therefore, human-animal reassortant rotaviruses for use as vaccines included human VP7 genes to provide protective immune responses.

**Human-rhesus RRV (RotaShield).** The first multivalent live oral reassortant vaccine developed was RotaShield (a rhesus rotavirus tetravalent [RRV-TV] vaccine). This tetravalent vaccine contained a mixture of four virus strains representing the most commonly seen G types, G1 to G4: three rhesus-human reassortant strains containing the VP7 genes of human serotypes G1, G2, and G4 strains were substituted for the VP7 gene of the parent RRV, and the fourth strain comprised serotype G3 of rhesus RRV (44). RRV-TV was extensively evaluated in field trials in the United States, Finland, and Venezuela and proved highly effective (80 to 100%) in preventing severe diarrhea due to rotavirus in each of these settings (42, 68, 71, 75). Due to the proven efficacy, the RRV-TV vaccine was licensed in August 1998 for routine use in children in the United States at 2, 4, and 6 months of age (16).

After inclusion of this vaccine in the immunization schedule in the United States and immunization of over 600,000 infants in the first 9 months of the program, several cases of vaccine-associated intussusception were reported (14). The period of

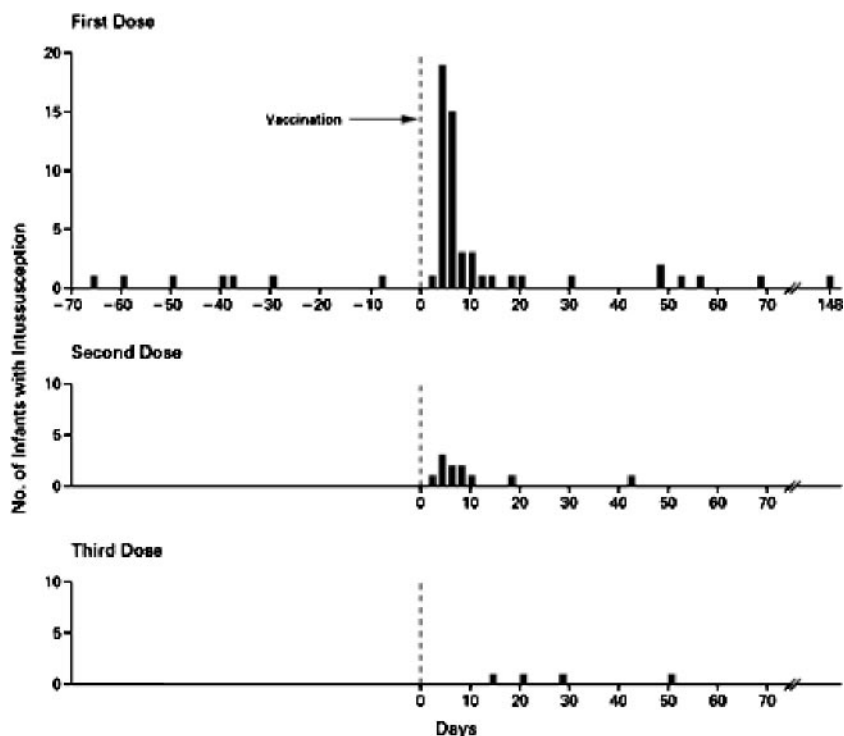


FIG. 4. Interval between vaccination with RRV-TV and the development of intussusception. (Reprinted from reference 60 with permission, copyright ©2007 Massachusetts Medical Society.)

greatest risk of intussusception was shown to be 3 to 10 days after the first of three oral doses (Fig. 4) (49, 60, 61). Although the true overall incidence of this adverse event proved to be difficult to assess, a group of international experts suggested a consensus rate of 1 per 10,000 vaccinated infants (69). The pathogenic mechanisms involved in intussusception following vaccination are currently unknown.

As a consequence of this rare but potentially dangerous adverse effect, Wyeth, the manufacturer, withdrew RotaShield from the market in the United States 14 months after its introduction. Unfortunately, the vaccine was not evaluated in terms of risk-benefit for children in resource-poor countries, as the ongoing trials in Asia (Bangladesh and India) and Africa (Ghana and South Africa) were stopped at that time. Although still licensed, the vaccine has not been tested since then or licensed in other parts of the world.

#### Currently Licensed Vaccine: Human-Bovine Rotavirus Reassortant Vaccine (RotaTeq)

Current human-animal reassortant rotaviruses for use as vaccines include either human VP7 or VP4 genes. Initially, VP7 was thought to be the most important antigen in inducing protection; therefore, human-animal reassortant rotaviruses for use in vaccines such as RRV-TV included only human VP7 genes to provide protective immune responses. More recently, VP4 has also been considered to be important for protection. Human-animal reassortant rotaviruses now include either human VP7 or VP4 genes to provide protective immune responses.

**Derivation.** A pentavalent human-bovine (WC3) reassortant live-attenuated, oral vaccine (RotaTeq) (see Table 1) has been developed by Merck Research Co. This vaccine contains five live reassortant rotaviruses (Fig. 5). Four reassortant rotaviruses express the VP7 protein (G1, G2, G3, or G4) from the human rotavirus parent strain and the attachment protein (P7[5]) from bovine rotavirus parent strain WC3. The fifth reassortant virus expresses the attachment protein (P1A[8]) from the human rotavirus parent strain and the outer capsid protein G6 from the bovine rotavirus parent strain. RotaTeq is administered in three oral doses at 1- to 2-month intervals beginning at 6 to 12 weeks of age.

**Safety, immunogenicity, and efficacy.** RotaTeq was tested in a large phase III trial in 11 countries, with subjects from the United States and Finland accounting for more than 80% of all enrolled subjects (85). The trial included more than 70,000 children and was designed primarily to evaluate vaccine safety with respect to intussusception but also to evaluate the immunogenicity and efficacy of the vaccine with respect to the severity of illness and the number of hospitalizations or emergency department visits for rotavirus gastroenteritis.

The risk of intussusception was evaluated for 42 days after each vaccine dose in the phase III trial. Six cases of intussusception were observed in the RotaTeq group, compared to five cases of intussusception in the placebo group (multiplicity-adjusted relative risk, 1.6). The data did not suggest an increased risk of intussusception in vaccine recipients relative to that for placebo. Among vaccine recipients, there were no confirmed cases of intussusception within the 42-day period

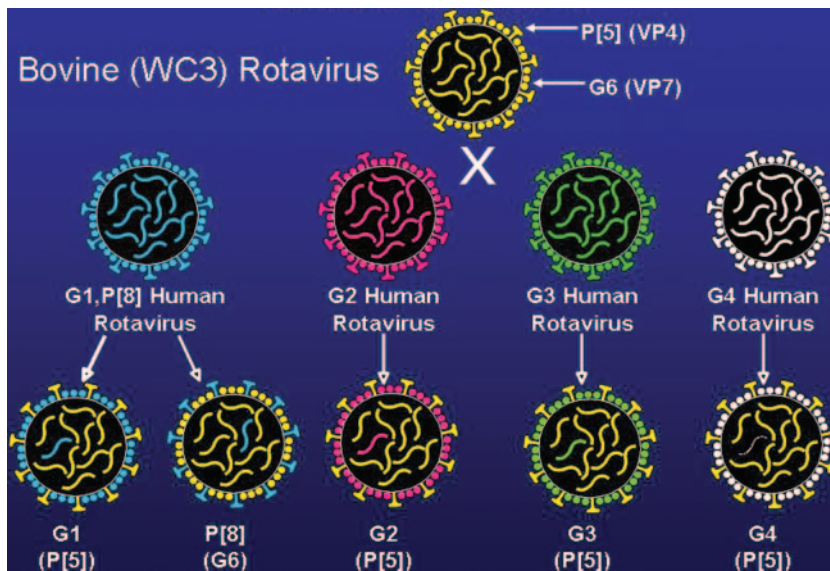


FIG. 5. Human-bovine rotavirus reassortant vaccine (RotaTeq). This vaccine contains five reassortant rotaviruses. Four reassortant rotaviruses express the VP7 protein (G1, G2, G3, or G4) from the human rotavirus parent strain and the VP4 protein (P7[5]) from the bovine rotavirus parent strain. The fifth reassortant virus expresses the VP4 protein (P1A[8]) from the human rotavirus parent strain and the outer capsid protein G6 from the bovine rotavirus parent strain. (Adapted with permission from SLACK Inc. [62a].)

after the first dose, which was the period of highest risk for the previously licensed RRV-TV vaccine. In addition, no evidence of clustering of cases of intussusception was observed within a 7- or 14-day window after immunization for any dose. The overall rate of intussusception is consistent with the expected background rate of intussusception.

Pooled data from the large phase III and two smaller phase III trials showed that in the week following the first dose of RotaTeq, the incidence of fever and irritability did not differ between vaccine and placebo recipients. Diarrhea and vomiting occurred more frequently among vaccine recipients than among placebo recipients (10.4% versus 9.1% and 6.7% versus 5.4%, respectively).

An increase in titer of rotavirus group-specific serum IgA antibodies was used as one of the measures of the immunogenicity of the pentavalent rotavirus vaccine. Serum samples were obtained from a subset of study participants before immunization and approximately 2 weeks after the third dose, and seroconversion was defined as a threefold or greater increase in antibody titer from baseline. Seroconversion rates for IgA antibody to rotavirus were 95% among 189 vaccine recipients, compared to 14% in 161 recipients of the placebo (85).

The efficacy of RotaTeq was evaluated in two phase III trials (10, 85). In these trials, the efficacy of RotaTeq against rotavirus gastroenteritis of any severity after completion of a three-dose regimen was 74%, and that against severe rotavirus gastroenteritis was 98%. RotaTeq also proved to be strongly efficacious in preventing rotavirus gastroenteritis of any severity caused by the predominant G1 serotype (75% efficacy) and the G2 serotype (63% efficacy). There was a trend toward efficacy for the remaining serotypes, but patient numbers were too small to show statistical significance (83% efficacy for G3, 48% efficacy for G4, and 65% efficacy for G9).

The efficacy of RotaTeq in reducing the number of office

visits for rotavirus gastroenteritis and in reducing the number of emergency department visits and hospitalizations for rotavirus gastroenteritis was evaluated in a large study. (85). The efficacy of RotaTeq in reducing the number of office visits for rotavirus gastroenteritis among 5,673 subjects and in reducing the number of emergency department visits and hospitalizations for rotavirus gastroenteritis among 68,038 subjects over the first 2 years of life was evaluated. RotaTeq reduced the incidence of office visits by 86%, emergency department visits by 94%, and hospitalizations for rotavirus gastroenteritis by 96%. Efficacy against all gastroenteritis hospitalizations of any etiology was 59%.

The efficacy of RotaTeq in the second rotavirus season after immunization was 63% against rotavirus gastroenteritis of any severity and 88% against severe rotavirus gastroenteritis (85).

Data on the efficacy of fewer than three doses of RotaTeq are limited. In the large study, the efficacy of RotaTeq in reducing the number of emergency department visits and hospitalizations for rotavirus gastroenteritis was evaluated in children receiving fewer than three doses of vaccine (85). Although the study included more than 68,000 children, the number receiving fewer than three doses of vaccine or placebo was less than 8,600. The estimated rates of reduction in hospitalizations and emergency department visits of one, two, and three doses of vaccine in this study were 29%, 81%, and 95%, respectively (T. Vesikari, D. Matson, P. Dennehy, M. Dallas, R. Itzler, M. Dinubile, and P. Heaton, presented at the 44th Annual Meeting of the Infectious Disease Society of America, Toronto, Canada, October 2006).

RotaTeq was licensed in February 2006 by the Food and Drug Administration (FDA) for use among infants in the United States and is routinely recommended as a three-dose schedule at 2, 4, and 6 months of age (65). The first dose should be administered between 6 and 12 weeks of age, with

subsequent doses administered at 4- to 10-week intervals and all three doses of vaccine administered by 32 weeks of age. Immunization should not be initiated for infants older than 12 weeks because of insufficient data on the safety of the first dose of pentavalent rotavirus vaccine in older infants. The vaccine should also not be administered after 32 weeks of age because of insufficient data on the safety and efficacy of pentavalent vaccine in infants after this age.

In the United States, the postmarketing safety of RotaTeq is being monitored jointly by the Centers for Disease Control and Prevention (CDC) and the FDA through both evaluation of reports to Vaccine Adverse Event Reporting System and active surveillance using data from the Vaccine Safety Datalink. Merck and Co. is also conducting a postmarketing observational study, which will monitor patients for occurrences of intussusception within 30 days of vaccination of 44,000 infants in the United States. Data available to date do not suggest that RotaTeq is associated with intussusception (15). The number of intussusception cases among infants vaccinated with RotaTeq reported to the Vaccine Adverse Event Reporting System does not exceed the number of expected background cases for either the 1- to 7-day period or the 1- to 21-day period after vaccination. In addition, no cases of intussusception were detected within 30 days of vaccination in more than 28,000 infants reported to have received RotaTeq according to the Vaccine Safety Datalink.

As of May 2007, applications for licensure of RotaTeq have been filed in more than 100 countries, including Australia, Canada, the European Union, Asia, and Latin America. Through its partnership with the Rotavirus Vaccine Program at the Program for Appropriate Technology in Health (PATH), Merck plans to conduct clinical trials in Africa and Asia.

### Vaccine Candidates

**Human-bovine rotavirus reassortants.** Another multivalent bovine-human reassortant vaccine has been independently developed by the National Institute of Allergy and Infectious Diseases (NIAID). This bovine rotavirus tetravalent (BRV-TV) vaccine incorporates four reassortant viruses with a single gene for VP7 of either a G1, G2, G3, or G4 human serotype and 10 genes from the bovine rotavirus UK strain (P[7]G6). Phase II data from a study with the BRV-TV vaccine showed a good immune response and no adverse interference with concomitantly administered childhood vaccines (24). Before the withdrawal of the RRV-TV vaccine, placebo-controlled trials of BRV-TV vaccine versus RRV-TV vaccine were conducted in Finland with a total of 510 infants. Two doses of study vaccine or placebo were administered at 3 and 5 months of age. The first dose of RRV-TV vaccine was followed by a significant excess rate of febrile reactions (36%), whereas the rate of fever after the administration of the BRV-TV vaccine did not differ significantly from that in the placebo group. A seroresponse was detected in 97% of BRV-TV vaccine recipients and 94% of RRV-TV vaccine recipients. Both vaccines were equally effective, with 68% to 69% efficacy against any and 88% to 100% efficacy against severe rotavirus gastroenteritis during the first epidemic season (84).

With the emergence of the G9 serotype as an epidemiolog-

ically important serotype and the importance of the G8 serotype in focal areas, the vaccine developers at NIAID are planning to add human-bovine (UK) reassortants with G8 and G9 specificities to the tetravalent vaccine, thereby formulating a hexavalent vaccine for use in developing countries (46). A nonexclusive license for the production of the human-bovine (UK) vaccine is being negotiated with vaccine producers in Brazil, China, and India.

**Naturally occurring human-bovine reassortants.** Various observational studies suggested that neonatal rotavirus infection confers protection against diarrhea due to subsequent rotavirus infection. Two strains obtained from asymptotically infected newborns in Delhi (116E) and Bangalore (I321) have been assessed as vaccine candidates. These strains have P[10]G9 and P[11]G10 antigenic makeups, respectively. Each strain is a naturally occurring human-bovine reassortant; 116E is a human rotavirus with a single gene segment encoding VP4 derived from a bovine rotavirus, and I321 is a bovine strain with two nonstructural gene segments derived from a human strain (25, 27). These vaccine candidates are under development in India in a consortium with partners from the United States including the CDC and the Children's Vaccine Program at PATH (34). A phase I trial of a single dose of either vaccine candidate or placebo in 8-week-old infants was conducted in Delhi (7). That study demonstrated that while both vaccines were safe and well tolerated, strain 116E was superior in its ability to induce an immune response with strain I321 or placebo. In a recent study in three urban slums in Vellore, South India, neonatal G10P[11] infection with a strain resembling the I321 vaccine candidate did not confer protection against subsequent rotavirus infection or diarrhea of any severity in this setting (3). These findings suggest that strain 116E should be further evaluated as a vaccine candidate.

### VACCINES BASED ON HUMAN ROTAVIRUS

#### Currently Licensed Vaccine: Live-Attenuated Human Rotavirus Vaccine (Rotarix)

**Derivation.** A live-attenuated human rotavirus vaccine (strain 89-12) was originally developed in Cincinnati, OH, by tissue culture passage of a wild-type human rotavirus isolate (5). This vaccine is a P1A[8]G1 strain and thus represents the most common of the human rotavirus VP7 and VP4 antigens. The vaccine was further developed by Avant Immunotherapeutics and licensed to GlaxoSmithKline Biologicals, who further modified the vaccine by cloning and tissue culture passaging of the parent 89-12 vaccine strain. The resulting vaccine, RIX4414 (Rotarix) (Table 1), underwent initial trials in Finland, which showed safety, immunogenicity, and efficacy. The assessments revealed that Rotarix was clinically more attenuated than the parent strain 89-12.

**Safety, immunogenicity, and efficacy.** A large-scale, double-blind, placebo-controlled trial of more than 63,000 infants enrolled in 11 Latin American countries and Finland was done to confirm that the vaccine did not cause intussusception (73). The vaccine was administered in two oral doses at 2 and 4 months of age and was well tolerated, with a reactogenicity profile similar to that of the placebo in terms of fever, diarrhea, and vomiting. During a 31-day period after each dose, there



TABLE 1. Comparison of currently licensed rotavirus vaccines

Vaccine <sup>a</sup>	Parent strain and genotype	Formulation	Dose regimen	% Protection against severe rotavirus infection <sup>b</sup>	% Reduction in hospitalization	Association with intussusception	% Vaccine virus shedding
RotaTeq	Bovine rotavirus strain WC3, P7[5]G6	5 reassortants; 4 reassortants with the VP7 gene from G1, G2, G3, or G4 and 1 reassortant with the VP4 P1A[8] gene from the human rotavirus parent strain with the remainder of the genes from the WC3 bovine rotavirus parent	3 oral doses at 2, 4, and 6 mo of age	98	63	No	9
Rotarix	Human rotavirus strain 89-12, P1A[8]G1	No reassortants; RIX4414, a further-passaged human rotavirus 89-12 strain	2 oral doses at 2 and 4 mo of age	85	42	No	<50

<sup>a</sup> RotaTeq, a pentavalent vaccine, is manufactured by Merck. Rotarix, a monovalent vaccine, is manufactured by GlaxoSmithKline.

<sup>b</sup> Different scoring systems were used; therefore, these results are not comparable.

was no increase in intussusception among recipients of vaccine compared with that for placebo. Six vaccinated patients and seven placebo recipients developed intussusception in this period, confirming the lack of a causal association.

A subset of 20,000 infants in this large trial was monitored for efficacy (73). The results demonstrated a protection rate of 85% against severe rotaviral gastroenteritis and 100% protection against the most severe dehydrating rotaviral gastroenteritis episodes. The vaccine also proved to be strongly efficacious in preventing rotavirus gastroenteritis of any severity caused by the predominant G1 serotype (92% efficacy) and serotypes G3, G4, or G9 (88% efficacy). Efficacy against the G2 serotype (41%) was not significant in this large trial.

Although Rotarix was not efficacious against the G2 serotype in the large phase III trial, significant cross-protection against non-G1 and non-P[8] strains was shown using the meta-analysis of efficacy trials, where protection was 81% against the P[4]G2 strain. This finding was confirmed by the recent results of a European trial with two seasons of follow-up. In that study, efficacy against rotavirus gastroenteritis of any severity was 79%, that against severe rotavirus disease was 90%, and that against hospitalization due to rotavirus was 96%. For severe rotavirus gastroenteritis, the vaccine had efficacies of 96% against G1P[8] and 88% against non-G1P[8] RV strains (83).

Rotarix was first licensed in Mexico and the Dominican Republic in 2004. As of May 2007, Rotarix has been approved in 90 countries worldwide. Fifty countries in Latin America, Europe, Asia, and Africa are already using the vaccine, with more than 11 million doses distributed. Brazil, El Salvador, Mexico, Panama, and Venezuela included the rotavirus vaccine in their national vaccination programs. The vaccine is recommended in a two-dose schedule beginning at 6 weeks of age. Rotarix is not yet approved in the United States; however, the manufacturer is in late-stage development discussions with the FDA regarding licensure of the vaccine for the U.S. market.

Clinical data from efficacy and safety trials of Rotarix in Asia and Africa are expected to become available during the next months and years. A large phase III trial (>9,000 infants) currently ongoing in Singapore, Hong Kong, and Taiwan is

expected provide efficacy results by the end of 2007. The phase III trial in Africa (South Africa and Malawi) is under way and has already enrolled more than 50% of the expected subjects. Smaller studies of human immunodeficiency virus-positive infants, preterm infants, and twins have been initiated.

The postmarketing safety of Rotarix will be monitored by the manufacturer according to recently established guidelines issued by the European Union addressing risk management for medical products with the aim to detect and identify risks and to implement strategies that minimize those risks. Rotavirus vaccines will be the first vaccines to follow these new guidelines. The number of reported intussusception cases will be monitored versus the number expected to occur by coincidence following vaccination based on the natural background rate. The manufacturer has also planned a safety study in Mexico in collaboration with the Mexican government. The manufacturer plans to continue monitoring vaccine effectiveness and impact on serotype distribution in Europe and elsewhere along with partners such as the European Rotavirus Network, the CDC, and the World Health Organization (WHO).

#### Vaccine Candidates: Neonatal Rotavirus Strains

Neonatal strains were initially explored as vaccine candidates because they appeared to be naturally attenuated, and a natural history study had shown that asymptomatically infected neonates subsequently had reduced frequency and severity of rotavirus diarrhea. However, a neonatal strain failed to provide protection in a small efficacy study, and this approach was temporarily abandoned (86).

A human neonatal P[6]G3 strain, RV3, developed by Bishop and colleagues in Australia, was evaluated as an oral vaccine in 3-month-old infants and was found to be safe and well tolerated. A small phase II study with three doses of  $10^5$  PFU of the vaccine indicated relatively low immunogenicity as measured by serum IgA levels. However, the vaccine recipients who developed an immune response were protected against clinical disease in the following year (4). Furthermore, phase II immunogenicity studies with a higher dose of the vaccine ( $10^7$  PFU per dose) are planned.



## OTHER VACCINE APPROACHES

Other approaches to the development of rotavirus vaccines are also being pursued. Rotavirus antigens for parenteral delivery have received some attention as virus-like particles prepared in baculovirus, expressed antigens, DNA vaccines, and killed virus. These novel approaches are being pursued using animal models.

## FUTURE CHALLENGES

Postmarketing surveillance studies to monitor the impact of vaccine on circulating viral strains recovered from stool samples will be important to screen for possible vaccine selection pressure and strain replacement. Studies to measure the extent of cross-protection against different rotavirus serotypes, including serotype G9, which is becoming increasingly important across Asia and Africa, and G8, which is gaining prevalence in parts of Africa, will also need to be carried out to ensure that the vaccine protects children in the developing world, where those strains are prevalent.

The implementation of rotavirus immunization programs will require scientists and health officials to work effectively with the media to ensure that the public is informed about both the risks and benefits of the new rotavirus vaccines, particularly since the media may be the public's principal source of such information (Table 1). A balanced portrayal of these risks and benefits can help avert abrupt shifts in media and public reactions that can undermine the success of vaccination programs (26). Accurate information on vaccine risks and benefits will form the foundation of the dialogue that must take place between clinicians, health authorities, legislators, and the public to maintain public trust in rotavirus immunization (28).

The development and introduction of rotavirus vaccines for children in the resource-poor countries of the world have been given high priority by the WHO. Vaccine efficacy, which has already been demonstrated in children in industrialized and middle-income countries, needs to be proven in resource-poor countries in Africa and Asia. The availability of these vaccines will depend on distribution, including the need for a cold chain. The WHO's Initiative for Vaccine Research intends to provide funding for the development of liquid or dry powder formulations of rotavirus vaccines to facilitate the development of rotavirus vaccines that are logistically simple to administer in resource-poor countries, occupy minimal space in the cold chain, can be stored outside of the cold chain for reasonable time periods without a loss of activity, and are compatible with multidose vial formats.

In 2003, the Global Alliance for Vaccines and Immunizations sponsored a new public-private organization, the Rotavirus Vaccine Program, at PATH, whose role is to accelerate the development and introduction of rotavirus vaccines in developing countries. Despite this support, the implementation of rotavirus immunization programs in the developing world will require substantial input from the international donor community. Novel financing strategies will be needed to ensure that new vaccines are affordable and available in the developing world. Decision makers and parents in developing countries need to know about rotavirus disease since, currently, few have heard of the virus, and rotavirus infection is rarely diag-

nosed. Finally, for the global effort toward the prevention of rotavirus disease to be successful, special efforts will be required in India, China, and Indonesia, because one-third of all deaths due to rotavirus disease occur in these countries and because these countries depend almost entirely on vaccines manufactured domestically.

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